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REVIEW

Evaluation of *Fusarium* Head Blight Resistance in Wheat and the Development of a New Variety by Integrating Type I and II Resistance

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Abstract

Fusarium head blight (FHB), which is caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch], is a devastating disease affecting wheat (*Triticum aestivum* L.) spikes, which considerably reduces grain yield and quality and produces mycotoxins that are harmful to humans and animals, especially in warm and humid climates, such as that of west Japan. Therefore, an important target of breeding programs is to improve FHB resistance in wheat. In general, wheat cultivars bred in west Japan exhibit high resistance to FHB worldwide. FHB resistance in wheat can be divided into resistance to initial infection (type I) and resistance to spread within the spike (type II). To improve FHB resistance in west Japanese wheat, we first improved the methods used to evaluate type I and II resistance separately. We then analyzed factors related to type I and II resistance using diverse materials. Finally, we selected a wheat line, 'Norin PL-9,' into which we integrated type I and II resistance, and applied for registration as the wheat parental line. The knowledge and materials developed in this study are useful for breeding FHB resistance in wheat where FHB outbreak is problematic. This review draws on a series of related studies.

Discipline: Plant breeding
Additional key words: cleistogamy, mycotoxin, quantitative trait loci (QTL), scab

Introduction

Fusarium head blight (FHB) is mainly caused by *Fusarium graminearum* and *F. culmorum*, and is one of the most serious diseases of wheat (*Triticum aestivum* L.) worldwide[37, 44], especially in warm and humid environments[40] like Japan. FHB tends to occur on wheat spikes, impairing grain yield[28] and quality[6]. In addition, the contamination of mycotoxins due to FHB, such as deoxynivalenol (DON), is harmful to animals and humans[45]. Global climate change predictions indicate the potential for increased risk of FHB, which, in turn, would adversely affect wheat production and food safety in certain global regions[10, 26].

In Japan, DON contamination was detected in about half of 7746 wheat samples collected in 2002–2006. Furthermore, more than 1.1 mg kg^{-1} DON (provisional limit regulated by the Ministry of Health, Labour and Welfare of Japan) was detected in 3.3% of the samples[36]. The Kyushu region, one of the major areas of wheat production in Japan, is prone to large-scale FHB damage, according to "Crop Statistics" published by the Ministry of Agriculture, Forestry and Fisheries of Japan, making FHB control in wheat production a significant challenge in Japan.

Control of FHB depends on chemical/agronomic application and the genetic improvement of host plants[3]. Although FHB may be partially controlled by the application of fungicides[32, 51] and/or cultural practices[14], their effectiveness is limited. Genetic improvement of FHB resistance is considered the most effective method of control. Consequently, researchers in Japan have been working on breeding FHB-resistant wheat strains since the 1960s[5, 17, 33, 34], alongside many other countries[29, 30, 41]. Recently, there have been several reviews on the genetic improvement of FHB resistance in wheat[3, 9, 18, 24, 25, 30, 31, 36, 38]. However, since there

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is no known source of complete FHB resistance and almost all sources exhibit only partial resistance, continued efforts to increase FHB resistance are required to safeguard stable wheat production and food security.

Resistance to FHB is divided into resistance to initial infection (type I) and resistance to fungal spread from an infected floret along the rachis (type II)[41]. In type I resistance, several quantitative trait loci (QTLs) have been detected in recombinant inbred lines (RILs) and doubled haploid lines (DHLs)[11, 16, 23, 47, 48, 56, 59]. Since this type of resistance is more difficult to assess than others, less related information is available[9]. In comparison, the Chinese 'Sumai 3' spring wheat line and its derivatives (such as 'Saikai 165')[5] are well-known examples of type II resistance. For example, in 'Sumai 3', QTLs for FHB resistance have been found on the short arms of chromosomes 3B (3BS)[1, 4, 7, 8, 50, 55, 61].

We considered that the integration of type I and II resistance might result in greater wheat resistance to FHB than that shown by currently available wheat cultivars. First, we improved on existing methods to screen type I and II resistance separately to evaluate FHB resistance in detail. Second, factors related to type I and II resistance were analyzed using diverse materials (RILs derived from the cross between type I and II resistant varieties). Finally, we selected a line, 'Norin PL-9,' to integrate type I and II resistance, and applied for registration as the wheat parental line. This study reviews a series of studies regarding this topic.

Fig. 1. Evaluation of type I resistance using pot plants in a glass house with a sprinkler system

At the flowering stage of each variety, about 10 mL of suspension per pot containing a concentration of 5×10^5 spores mL^{-1} of *F. graminearum* was sprayed onto spikes in the evening (16:00–18:00). The plants were then incubated overnight in a glasshouse at 18–25℃, and 90–100% humidity. Spore suspension application and incubation were repeated the following day, whereupon the plants were again placed in a glasshouse at 18–25℃, which was equipped with a sprinkler system intermittently producing fine mist to moisten the spikes. The mist was generated continuously for 1.5 min at 10-min intervals on sunny days and for 1.5 min at 15-min intervals on cloudy days, or for 1 min at 20-min intervals on rainy days from 8:30–18:00, and for 1 min at 20-min intervals at night (18:00–8:30).

Methods to evaluate FHB resistance in wheat

1. Type I resistance

Type I resistance was evaluated using the method of Yoshida et al.[58]. Briefly, at the flowering stage (termed Zadoks growth stage, ZGS: 64–65)[60] of each pot plant, 10 mL of a macroconidial suspension containing 5×10^5 spores mL^{-1} of the H-3 *F. graminearum* isolate (National Institute of Agrobiological Sciences, NIAS, Genebank MAFF No. 101551) was sprayed onto the spikes of each plant, whereupon the plants were incubated overnight in a greenhouse at 18–25℃, with 90–100% humidity. The following day, the macroconidial suspension was reapplied, and the plants were re-incubated overnight in the greenhouse. Subsequently, an intermittent sprinkler system was used to mist the plants, and keep the spikes moist (Fig. 1). Seven days after infection (DAI), we visually examined each plant to determine the percentage of infected spikelets. In addition, we graded the severity of infection on a 0–9 scale, according to the method of Patton-Ozkurt et al. (US Wheat and Barley Scab initiative, http://www.scabusa.org/pdfs/ptt/cowger_type1-screening_protocol.pdf). Since FHB infection is easily influenced by environmental conditions such as temperature and humidity, the method used here provided stable conditions for the infection to develop, allowing type I resistance to be evaluated.

2. Type II resistance

To evaluate type II resistance, point-inoculation is generally conducted on pot plants[3]. Usually, plants are inoculated at anthesis, and the spread of FHB is determined based on the evaluation of a whole spike at 21 or 28 DAI[2, 34]. We improved this method using detached spikes and growth chambers to save both space and time[19]. As shown in Fig. 2A, 10 μL of the suspension (1×10^5 spores mL^{-1}) was poured into the 1st or 2nd floret of the 4th, 8th, and 12th spikelets respectively from the neck of the spikes during the early flowering stage (ZGS 60–61). As the source of inoculation, suspensions of macroconidia from the *F. graminearum* isolate 'DON-5' (NIAS Genebank, MAFF No. 240559) were used. Detached 60-cm-long spikes were then fixed vertically in the growth chamber at 20℃ and 90–100% humidity (Fig. 2B). The cut ends

Fig. 2. Evaluation of type II resistance using detached spikes in a growth chamber under conditions of controlled temperature and humidity

A: Total of 10 µL of suspension of macroconidia of *F. graminearum* (1×10^5 spores mL^{-1}) was poured into the first or second florets of 4th, 8th, 12th, and 16th spikelets from the neck of spikes at the flowering stage of each variety, as indicated by the red arrows. B: Detached spikes of 60 cm length, into which the suspension was poured, were fixed vertically in the growth chamber. Cited from Kubo and Kawada[19].

of the stems were soaked in running water during the test. The layout of the experiment was completely randomized with 2 replications and 3 spikes tested in each replication of each variety. The spread of FHB was evaluated by the degree of browning in the inoculated spikelets, and in the rachis connected to the inoculated spikelets (Fig. 3).

3. Fusarium damaged kernels and mycotoxin accumulation

To investigate the accumulation of mycotoxins of selected materials, we performed randomized block field experiments in the experimental fields[19, 20]. Before sowing, the soil was fertilized with 30 kg ha^{-1} N, 26 kg ha^{-1} P_2O_5, and 26 kg ha^{-1} K_2O. Twenty seeds of each materials were sown 70 cm apart in a row. A 2-m-high net surrounded the field to protect the seedlings from the wind. To induce FHB infection, we conducted grain spawn and spray inoculations, as described previously[39]. For grain spawn inoculation, corn (*Zea mays* L.) or barley (*Hordeum vulgare* L.) that has been colonized with the 'H-3', 'DON-5', 'DON-1' (NIAS Genebank MAFF No. 24055), 'NIV-1' (MAFF No. 240547), and 'NIV-7' (MAFF No. 240552) isolates of *F. graminearum* was spread throughout the field (400 L ha^{-1}), when the plants were near the boot stage (ZGS 41). For the spray inoculation, 60 mL of a macroconidia suspension containing 2×10^5 spores mL^{-1} of the 'H-3' *F. graminearum* isolate was sprayed onto the plant spikes at the flowering stage (ZGS 64-65) in each plot. After inoculation with the spray, we used a sprinkler irrigation system to water the field for 2 min at 30–45 min intervals between 8:30–18:00, and at 60-min intervals between 18:00–8:30 for 3 weeks (Fig. 4). After harvesting the mature plants (ZGS 92), the grain was threshed and sieved with a 2.0 mm grid, whereupon the frequency of *Fusarium*-damaged kernels (FDKs) was calculated as the number of damaged kernels among the total (100–200) examined per plot. We defined damaged kernels as those that were shriveled, lightweight, and chalky white, or occasionally pink[12]. To measure the DON concentration, the kernels were ground with a laboratory mill, and then tested with a commercial competitive enzyme immunoassay kit (RIDASCREEN® DON, R-Biopharm AG, Darmstadt, Germany).

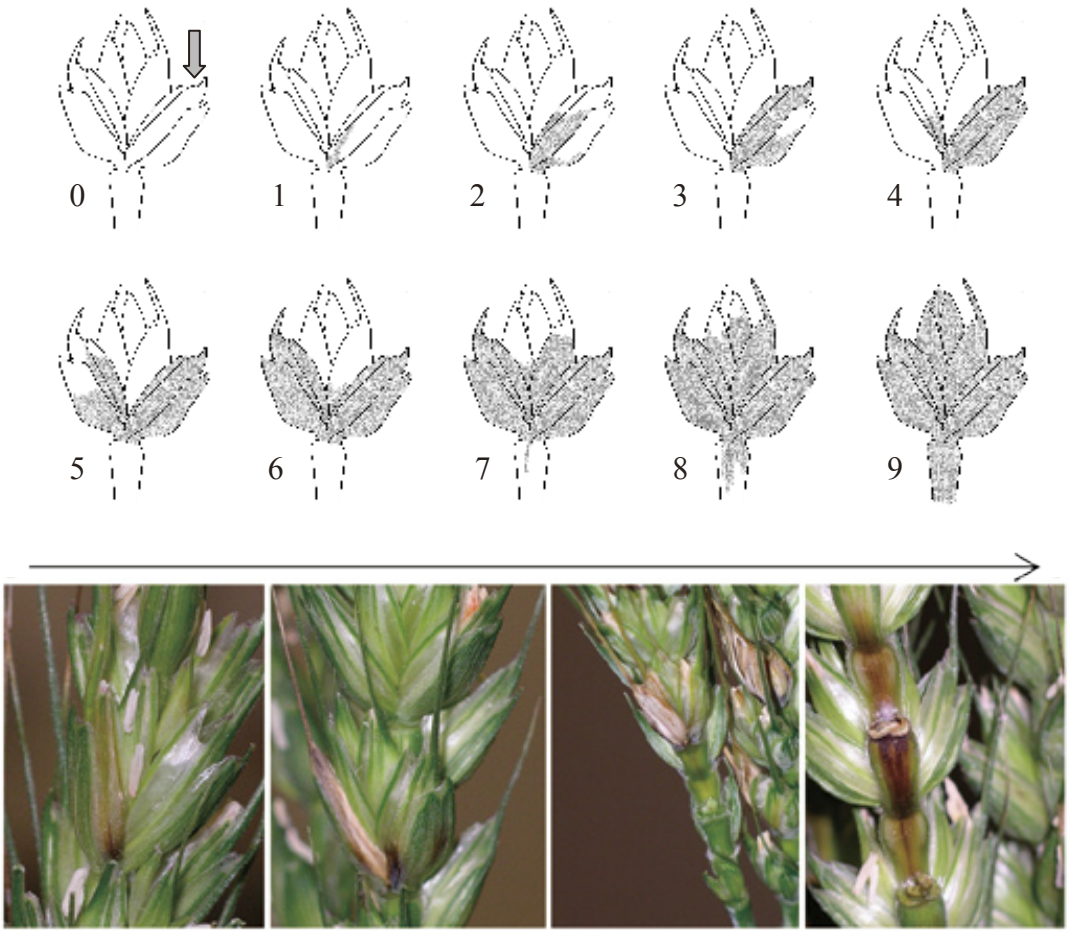

Fig. 3. Scores indicating FHB spread from point inoculations obtained in this study

The arrow indicates the florets into which the suspension was poured.

0, no browning; 1, 0–33% browning of inoculated floret; 2, 33–66% browning of inoculated floret; 3, 66–100% browning of inoculated floret; 4, 0–33% browning of spikelet; 5, 33–66% browning of spikelet; 6, 66–100% browning of spikelet; 7, 0–33% browning of rachis; 8, 33–66% browning of rachis; 9, 66–100% browning of rachis. Cited from Kubo and Kawada[19].

Evaluation of genotypic difference and analyses of related factors in FHB type I and II resistance

1. Type I resistance

We evaluated the type I resistance of F_6 and F_7 RILs, which were derived from a cross between wheat varieties 'U24' and 'Saikai 165' using pot plants in a greenhouse. 'U24' was collected from the Xinjiang-Uygur Autonomous Region of China in 1989, and has the cleistogamous (CL, closed-flowering) characteristic[15, 49]. 'Saikai 165' is a FHB-resistant chasmogamous (CH, chasmogamous) cultivar that was bred from 'Sumai 3 (the international genetic resource of FHB resistance[1, 4, 8])' and 'Asakazekomugi' (a Japanese elite cultivar) at the NARO Kyushu-Okinawa Agricultural Research Center (NARO/KARC; Chikugo, Fukuoka, Japan), aiming to increase FHB resistance in Japanese commercial wheat cultivars. 'Saikai 165' has a shorter culm and earlier growth characteristics than 'Sumai 3'. This experiment revealed a significant difference in type I resistance among RILs (Table 1). Although the interaction between year (generation) and genotype was significant, the F-value of the interaction was smaller than that of the genotype effect. Furthermore, CL RILs showed significantly higher type I resistance than CH RILs (Fig. 5). This result is consistent with previous studies. For example, the wheat cultivar 'Goldfield', which has a narrow flower opening, is highly resistant to FHB infection[16]. In addition, CL cultivars of barley were found to be more resistant to FHB infection than CH cultivars[57]. Since anthers are required for initial FHB infection[13, 38], the lack of anther extrusion in these cultivars may have inhibited

FHB infection. In a comparison between parents of the RILs, the cleistogamous variety 'U24' showed a lower type I resistance score than 'Saikai 165' but the difference was small (Table 1). This was partly due to the high type II resistance of 'Saikai 165' (described later) affecting the type I resistance score, thus hindering detection of the difference.

2. Type II resistance

We first evaluated type II resistance in 23 Asian wheat varieties and breeding lines using detached spikes and growth chambers. Type II resistance differed significantly among the 23 varieties (Table 2). 'Sumai 3' and its derivatives showed significantly higher type II resistance than other cultivars. Other authors have also shown that

Fig. 4. Evaluation of Fusarium damaged kernels and mycotoxin accumulation in the experimental field
To induce FHB infection, when the plants were near the boot stage (ZGS 41), corn (*Zea mays* L.) or barley (*Hordeum vulgare* L.) that was colonized with *F. graminearum* isolates was spread throughout the field (400 L ha^{-1}). In each plot, 60 mL of a macroconidia suspension containing 2×10^5 spores mL^{-1} of *F. graminearum* isolate was sprayed onto the spikes of the plants at the flowering stage (ZGS 64-65). After inoculation with the spray, we used sprinkler irrigation to water the field for 2 min at 30-45 min intervals between 8:30-18:00, and at 60-min intervals between 18:00-8:30 for about 3 weeks.

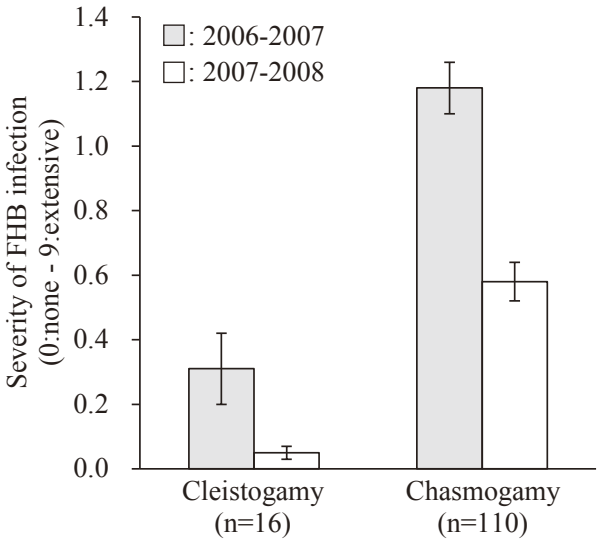

Fig. 5. Comparison of resistance to FHB infection (type I) between cleistogamous and chasmogamous RILs
The bar indicates SE. The difference in type II resistance between cleistogamous and chasmogamous RILs was significant at $P < 0.01$ for both 2006–2007 and 2007–2008 seasons. Data were cited from Kubo et al.[20].

Table 1. Resistance to FHB infection (type I) and the spread (type II) in 'Saikai 165' and 'U24' and their RILs

	Type I resistance			Type II resistance		
	2006-2007	2007-2008	Combined	2007-2008	2008-2009	Combined
Parents						
Saikai 165	0.07	0.13	0.10	4.45	3.33	3.89
U24	0.07	0.00	0.04	6.37	7.09	6.73
RILs						
Mean ± SE	1.07 ± 0.05	0.51 ± 0.03	0.79 ± 0.03	5.14 ± 0.11	5.89 ± 0.10	5.51 ± 0.08
Range	0.00 – 4.83	0.00 – 2.83	0.00 – 3.42	1.60 – 8.78	2.59 – 9.00	2.47 – 8.76
CV (%)	95	127	114	35	27	32
LSD ($P < 0.05$)	1.20	0.60	0.67	1.77	1.83	1.26
ANOVA[1)]						
Genotype (G)	3.619**	7.397**	6.210**	7.297**	4.799**	9.503**
Year (Y)	-	-	166.621**	-	-	85.891**
Y*G	-	-	2.563**	-	-	2.527**

[1)] Values indicate the 'F-value', ** shows significance at $P < 0.01$. Data were cited from Kubo et al.[20].

Table 2. Resistance to FHB spread (type II) in 23 wheat varieties

Variety	Pedigree	Registered year as the cultivar	Soft / Hard	2006-2007	2007-2008	Combined
Akakabikei 2	Nobeokabozukomugi / Sumai 3		Soft	2.95	4.06	3.51
Akakabikei PL 106	Sumai 3 / Asakazekomugi		Soft	2.45	4.33	3.39
Akakabikei PL 13	Sumai 3 / Asakazekomugi		Soft	2.40	4.89	3.65
Akakabikei PL 33	Sumai 3 / Asakazekomugi		Soft	2.75	4.62	3.69
Akakabikei PL 4	Sumai 3 / Asakazekomugi		Soft	1.80	3.84	2.82
Asakazekomugi	Hiyokukomugi / Shiroganekomugi	1979	Soft	2.75	6.00	4.38
Bandowase	Kanto 66 / Hiyokukomugi	1990	Soft	3.00	4.89	3.95
Chikugoizumi	Kanto 107 / Asakazekomugi	1993	Soft	2.55	6.44	4.50
Iwainodaichi	Aki 9 / Saikai 168	1999	Soft	2.70	4.73	3.72
Minaminokaori	Pampa INTA / Saikai 167	2003	Hard	6.40	5.11	5.76
Nishinokaori	Kitamiharu 42 / Saikai 157	1999	Hard	2.90	5.61	4.26
Norin 61	Fukuokakomugi 18 / Shinchunaga	1943	Soft	4.10	5.28	4.69
Saikai 165	Sumai 3 / Asakazekomugi		Soft	1.90	4.17	3.04
Saikai 185	Saikai 171 / Asakazekomugi		Soft	2.05	5.06	3.56
Saikai 188	Chikugoizumi / Hakei 92-97		Soft	3.35	4.89	4.12
Saikai 189	Chikugoizumi / Chugoku 143		Soft	4.15	6.28	5.22
Saikai 190	Chikugoizumi / Saikai 182		Soft	2.40	5.05	3.73
Shinchunaga	Pure selection from Chunaga		Soft	2.75	4.61	3.68
Shiroganekomugi	Shirasagikomugi / Saikai 104	1974	Soft	2.60	5.72	4.16
Sumai 3	Chinese cultivar		Soft	2.30	4.00	3.15
Tokai 63	Norin 26 / Shinchunaga		Soft	3.05	4.28	3.67
Towaizumi	Chikugoizumi / Akakabikei PL 106	2006	Soft	3.35	4.67	4.01
Norin PL-4	Nobeokabozukomugi / Sumai 3		Soft	2.35	3.28	2.82
LSD ($P < 0.05$)				1.31	1.34	0.91
ANOVA[1)]						
Genotype (G)				4.781**	3.055**	4.822**
Year (Y)				-	-	105.755**
Y*G				-	-	2.687**

[1)] Values indicate the 'F-value', ** shows significance at $P < 0.01$. Data were cited from Kubo and Kawada[19].

FHB resistance of 'Sumai 3' mainly depends on type II resistance[1, 4, 8]. Our results supported those of earlier studies. In 'Sumai 3,' QTLs for FHB resistance were found on the short arms of chromosomes 3B (3BS) and 5A (5AS)[1, 4, 7, 8, 50, 55, 61]. Therefore, we also evaluated the type II resistance of F_7 and F_8 RILs, which were derived from the cross between wheat varieties 'U24' and 'Saikai 165' (originating from 'Sumai 3'), and analyzed the effect of the 2 QTLs. Type II resistance differed significantly among the RILs of both generations (Table 1). Although the interaction between generation and line was significant, the F-value of the interaction was smaller than that of line effect. From these results, type II resistance was considered heritable. DNA marker analyses revealed that the amplicons of the markers linked to QTLs located on 3BS and 5AS were the same size in both 'Saikai 165' and 'Sumai 3'. The severity of FHB spread in RILs, while the 'Saikai 165' genotype in 3BS was significantly lower than that in RILs with the 'U24' genotype (Fig. 6). The positive effect of these QTLs on FHB resistance is consistent with the results of many previous studies conducted under various genetic backgrounds and environments[1, 8, 27, 31, 35, 42, 50, 52, 62]. Our findings confirmed that the genotype of QTL, which is located on 3BS of 'Saikai 165' and 'Sumai 3,' is vital for increasing FHB resistance in wheat.

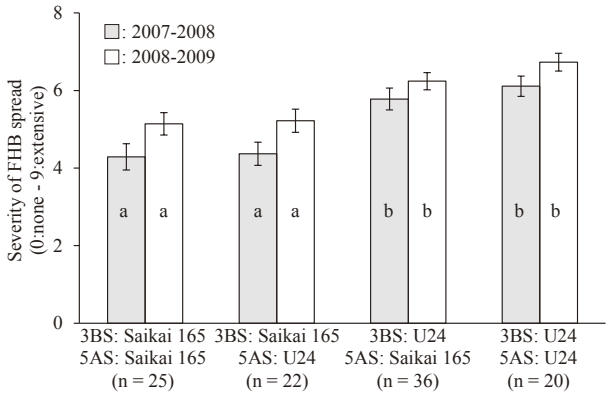

Fig. 6. Comparison of resistance to FHB spread (type II) between FHB-resistant QTL genotypes in RILs

□, 2007–2008; □, 2008–2009. The bar shows SE. The same letters in the figure do not differ significantly, according to the Ryan-Einot-Gabriel-Welsch multiple range test ($P < 0.05$) in each growing season. Data were cited from Kubo et al.[20].

Table 3. Resistance to FHB infection (type I) of the 'Norin PL-9'

	2006-2007	2007-2008	Combined
Norin PL-9	0.07	0.00	0.04
U24	0.07	0.00	0.04
Saikai 165	0.07	0.13	0.10
Norin 61	0.83	1.33	1.08
Minaminokaori	2.33	0.13	1.23
Towaizumi	0.40	0.07	0.24
Sumai 3	0.00	0.07	0.04
LSD ($P < 0.05$)	0.82	0.41	0.44
ANOVA[1)]			
Genotype (G)	9.680**	12.609**	12.109**
Year (Y)	-	-	6.639**
G*Y	-	-	8.424**

[1)] Values indicate the 'F-value', ** and * show significance at $P < 0.01$ and $0.01 \leqq P < 0.05$, respectively. Data were cited from Kubo et al.[22].

Selection of line integrating type I and II resistance

From the above results, we selected a line integrating type I and II resistance to FHB from the RILs derived from the cross between 'U24' and 'Saikai 165'. This line was named 'Norin PL-9', for which registration was applied in 2011[22]. Norin PL-9 has a cleistogamous characteristic, which increases its resistance to initial infection from FHB (type I resistance). We found that resistance was higher in 'Norin PL-9' compared to chasmogamous commercial cultivars such as 'Norin 61' and 'Minaminokaori' (Table 3). 'Norin PL-9' also has the 'Saikai 165' (resistant) genotype in QTL located on 3BS, which is related to its resistance in the spread of FHB (type II resistance). The resistance of 'Norin PL-9' was similar to 'Saikai 165' (Table 4). 'Norin PL-9' could potentially introduce multiple factors related to FHB resistance to wheat materials in breeding programs. However, 'Norin PL-9' has poor resistance to lodging compared with 'Saikai 165' and commercial cultivars, because of its greater culm length (Table 5). Therefore, lines of suitable culm length should be selected for the breeding process when using 'Norin PL-9' as the parent. In addition, plants with halfway-cleistogamy (a condition where anthers peek through between the palea and lemma during grain filling) emerge in progenies derived from 'U24,' which is a cleistogamous parent of 'Norin PL-9' (Fig. 7). Such plants have poor type I resistance to FHB compared to those with chasmogamy and complete cleistogamy (Fig. 8). Skinnes et al. also demonstrated that even low anther extrusion is correlated with susceptibility to FHB infection[43]. Therefore, careful long-term observation from flowering to grain filling is required to select plants with complete cleistogamy[21].

Future research

The ultimate aim of genetically improving FHB resistance in wheat would be to decrease mycotoxin contamination, hence increasing food safety. Unfortunately, 'Norin PL-9,' which integrates cleistogamous type I and II resistance, had a similar level of DON as 'Saikai 165', which is the donor of type II resistance (Table 6). This result may indicate that cleistogamy has little effect on mycotoxin accumulation and/or that 'Norin PL-9' has higher mycotoxin accumulation per fungal cell (i.e. it may be classified as "type III resistance")[29]. Further analysis involving the investigation of near isogenic lines on the flowering type under various environmental conditions is required to determine the effect of cleistogamy on mycotoxin accumulation. To address type III resistance, we have recently acquired data on varietal difference (unpublished). The continued development of studies on the genetic analyses of type III resistance might help reduce mycotoxin contamination in wheat. In addition, studies in west Japan should be focused on expanding analyses to introduce additional identified QTLs (2DL, 4BS, 5AS, 6BS, etc.) obtained from 'Sumai 3' and 'Wuhan-1'[1, 7, 12, 46, 53, 54] to 'Norin PL-9' and other cultivars.

Table 4. Resistance of the 'Norin PL-9' to FHB spread (type II)

	Genotype of QTL on 3BS		Severity of FHB spread		
	Xgwm533	*Xgwm493*	2006-2007	2007-2008	Combined
Norin PL-9	Sumai 3	Sumai 3	2.71	3.50	3.10
U24	not Sumai 3	not Sumai 3	5.83	7.04	6.44
Saikai 165	Sumai 3	Sumai 3	2.13	4.17	3.15
Norin 61	not Sumai 3	Sumai 3	4.09	5.28	4.68
Minaminokaori	not Sumai 3	Sumai 3	6.41	5.44	5.93
Towaizumi	Sumai 3	Sumai 3	3.34	4.67	4.00
Sumai 3	Sumai 3	Sumai 3	2.27	4.00	3.14
LSD (P < 0.05)			1.49	1.45	0.94
ANOVA[1)]					
Genotype (G)			15.796**	6.232*	19.629**
Year (Y)			-	-	18.642**
G*Y			-	-	3.159*

[1)] Values indicate the 'F-value', ** and * show significance at $P < 0.01$ and $0.01 \leqq P < 0.05$, respectively. Data were cited from Kubo et al.[22].

Table 5. Agricultural characteristics of 'Norin PL-9'

	Heading day (month. day)	Maturing day (month. day)	Culm length (cm)	Panicle length (cm)	Panicle number (m^{-2})	Yield (kg a^{-1})	1000 grain weight (g)
Norin PL-9	4.11	6.02	108	11.0	483	43.0	33.0
Saikai 165	4.06	5.30	82	8.1	577	50.3	32.3
Norin 61	4.09	5.31	94	8.5	482	58.9	37.0
LSD (P < 0.05)	-[1)]	0.02	7	1.5	128	13.4	5.8
ANOVA[2)]	-	37.000*	127.000**	42.096*	6.782 ns	13.124 ns	7.066 ns

[1)] There was no statistical analysis because each variety has the same data in all replications. [2)] Values indicate the 'F-value', ** and * show significance at $P < 0.01$ and $0.01 \leqq P < 0.05$, respectively. ns, not significant. Evaluation was conducted in a yield test field of NARO/KARC during 2009–2010, with a randomized block design of 2 replications. 'U24' could not be included in this study because its heading and maturity is up to 7 days later than 'Norin PL-9'. Data were cited from Kubo et al.[22].

Table 6. Fusarium damaged kernels and mycotoxin accumulation of 'Norin PL-9'

	FDK (%)				DON (ug g^{-1})			
	2007-2008	2008-2009	2009-2010	Combined	2007-2008	2008-2009	2009-2010	Combined
Norin PL-9	22.1	18.0	28.1	22.7	3.42	2.20	7.00	4.21
U24	75.0	78.6	96.3	83.3	27.50	31.30	32.40	30.40
Saikai 165	23.8	30.0	30.7	28.2	4.40	3.20	2.70	3.43
Norin 61	20.5	34.4	47.2	34.0	4.30	4.10	5.10	4.50
Minaminokaori	24.5	34.6	39.5	32.9	4.90	6.50	5.00	5.47
Towaizumi	19.8	21.8	36.2	25.9	3.90	3.20	3.90	3.67
LSD (P < 0.05)	14.6	9.2	10.4	6.1	3.58	2.96	3.13	1.69
ANOVA[1)]								
Genotype (G)	29.129**	55.435**	58.846**	111.905**	93.511**	145.210**	130.940**	324.132**
Year (Y)	-	-	-	21.703**	-	-	-	2.208 ns
G*Y	-	-	-	1.779 ns	-	-	-	2.344**

[1)] Values indicate the 'F-value', ** and * show significance at $P < 0.01$ and $0.01 \leqq P < 0.05$, respectively. ns, not significant. Data were cited from Kubo et al.[22].

Fig. 7. Progenies derived from 'U24,' which is the cleistogamous parent of 'Norin PL-9' with halfway-cleistogamy

A, anther extrusion score: 2 (0: cleistogamy; 5: chasmogamy); B, anther extrusion score: 3.

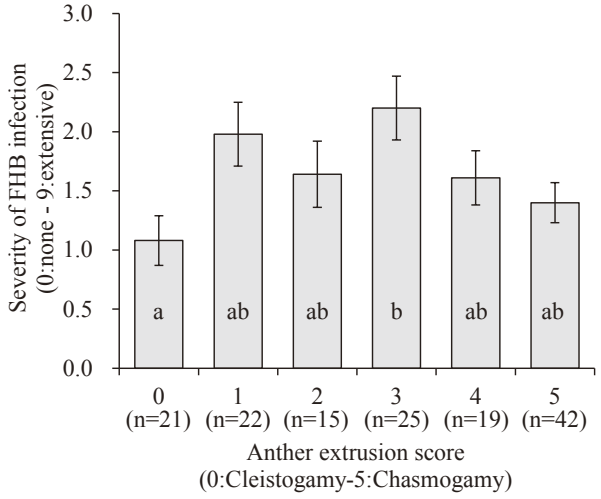

Fig. 8. Relationship between the degree of anther extrusion and FHB infection in double haploid lines derived from 'Sumai 3'/'Saikai 165'//'U24'

The bar shows SE. The same superscripted letters in the figure do not differ significantly according to the Ryan-Einot-Gabriel-Welsch multiple range test ($P < 0.05$).

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