コムギ赤かび病抵抗性の遺伝学的研究

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Studies on the Genetics of Resistance to Fusarium Head Blight Caused by *Fusarium graminearum* Schwabe in Wheat (*Triticum aestivum* L.)

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Synopsis

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Fusarium head blight (FHB) caused by *Fusarium* species, is one of the most destructive diseases of wheat, in areas where the weather is warm and humid after the heading of wheat. No accession has yet been found to be completely immune to FHB, although the resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives. The results of repeated screening of genetic resources led to the identification of several resistant cultivars. However, the resistance of these cultivars has not been satisfactory under conditions that are optimal for the pathogen. It is necessary to identify more effective genetic resources among relatives of wheat for the breeding of FHB-resistant wheat. Furthermore, lack of knowledge of the mode of inheritance of the resistance has hampered significant progress in the breeding of FHB-resistant wheat cultivars for a long time. In this study, wheat relatives of indigenous Japanese *Agropyron* (*Elymus*) were screened for resistance to FHB to identify more effective genetic resources. The criteria for genetic analysis of the reaction to FHB in doubled haploid lines (DHLs) and recombinant inbred lines (RILs) were developed. The genetic constitution of resistance to FHB in resistant wheat cultivars, Sumai 3 and its derived line Saikai 165, using DHLs and RILs was then clarified, and a linkage analysis of the resistance genes was conducted. Furthermore, the author tried to analyze quantitative trait loci (QTLs) associated with resistance to FHB in DHLs using random amplified polymorphic DNA (RAPD) markers to evaluate the effect of minor genes. Finally, the relationship between the characteristics of FHB caused by *F. grainearum* and resistance mechanisms in wheat was analyzed. The results suggested that breeding strategies including pyramiding of resistance genes and introgression of additive minor genes in an adapted background may enable to make rapid progress in the breeding of FHB-resistant wheat.

Key words: *Triticum aestivum* L., Fusarium head blight, *Fusarium graminearum, Elymus,* doubled haploid, recombinant inbred, resistance gene, QTL analysis.

Contents

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Introduction

Plant diseases caused by *Fusarium* species are widespread throughout the world and occasionally destructive. Most genera of cultivated plants are affected by diseases caused by *Fusarium* species, including all the members of the Gramineae.[83] *Fusarium* species cause diseases such as head blight, seedling blight, stem and leaf bleach and root rot in small grain cereals.[62, 80] Fusarium head blight (FHB), caused by *Fusarium* species, is one of the most destructive diseases of wheat, where the weather is warm and humid after the heading of wheat. It affects heads (ears or spikes) of wheat, and reduces grain yield and quality due to grain discoloration and shriveling. FHB also produces mycotoxins harmful to animals and human health, such as deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, zearalenone (ZEA) and their derivatives, in the grain.[6, 26, 44, 101, 114, 115, 125]

FHB has been recorded in most of the wheat-growing areas of the world, and has been designated as wheat scab, head blight, Fusarium blight and ear blight.[74, 83] These reports have shown that up to 17 causal fungi have been associated with FHB, although the most common fungi linked with FHB are *Fusarium graminearum* Schwabe (*Gibberella zeae* (Schw.) Petch, formerly known as *G. saubinetii* (Mont.) Sacc.), *F. culmorum* (W. G. Smith) Sacc., *F. avenaceum* (Fr.) Sacc., *F. poae* (Peck) Wollenw. apud Lewis and *Microdochium nivale* (Fr.) Samuels & Hallet (telemorph *Monographella nivalis* (Schaffnit) E. Müller, formerly classified as F. *nivale* (Fr.) Ces.). The predominant species recently causing FHB worldwide has been *F. graminearum*.[1, 41, 43, 47, 48, 53, 55, 56, 81, 106]

Recent articles have reviewed the history of FHB outbreaks and their economic importance.[8, 55, 83] In the mid-to lower Yangtze River Valley of China, outbreaks of FHB have occurred on average every two to four years for the last 30 years and have reduced wheat yields 30 to 40%.[4] Losses of the Hungarian wheat crop due to FHB were estimated at 40 to 50% in 1970 (Kükedi 1972).[83] Investigations in Germany from 1987 to 1995 and in France since 1995 showed an increase in the incidence of *Fusarium* spp.[57] In 1993, FHB struck the tri-state areas of Minnesota, North Dakota, and South Dakota (United States), and the Canadian prairie province of Manitoba, which is one of the major wheat and malting barley production areas in North America affecting 4 million hectares. Yield and quality losses in this region were estimated at one billion dollars, making it one of the greatest losses due to any plant disease in North America in a single year.[24, 55] The worst outbreaks occurred in 1945-46, 1978, 1985, and 1993 in Argentina, and the highest estimated losses of wheat grains reached 50%.[31] The first serious epidemic in Uruguay occurred in 1977, and severe FHB epidemics tend to occur approximately every 11 years.[2] In western Japan, FHB affected 20 to 90% and 10 to 25% of wheat production areas in an average year in the 1960s and 1990s, respectively. Historically, outbreaks of FHB occurred in 1963 and 1998 in Japan. FHB struck almost all areas in Japan, covering more than 400,000 hectares (71.5% of wheat production), and estimated yield losses reached 53.7% in 1963. This outbreak of FHB devastated the production of wheat and barley in western Japan. Farmers in western Japan, particularly in the Kyushu district, which is one of the main wheat production regions in Japan, experienced serious epidemics of FHB in 1998, with damage equivalent to 20 to 100% decrease in wheat production.[107, 108]

Symptoms of FHB infection caused by *F. graminearum* on wheat initially appear as small, water-soaked brownish spots at the base or in the middle of the glume (Fig. 1-H). Water soaking and discoloration spreads in all directions from the point of infection. A salmon-pink to red fungal growth may be seen along the edge of the glumes or at the base of the spikelet. Premature death or bleaching of spikletes is a common symptoms (Fig. 1-A, B and D-G). Infected grains, which shrink and become white to pale pink in color with a floury discolored interior, are referred to as "tombstone" kernels (Fig. 1-L). At harvest time, FHB eventually develops blue-black perithecia on infected spikelets, giving the "scabbed" appearance (Fig. 1-C).[74, 83)]

Early epidemics of FHB occur through wind and rain-splash dissemination of ascospores, which are initially released from perithecia by rain or heavy dew.[76, 88)] The wind-borne conidia of FHB infect wheat spikes during a rain event. Infection of FHB usually occurs at the time of flowering, or shortly after, through extruded or retained anthers.[5, 23, 87)] Warm and humid conditions are essential for FHB inoculum to germinate and grow by infecting wheat spikes.[74-76, 83)] Hence, the environmental conditions around flowering time and during the ripening period become one of the limiting factors of FHB epidemics.

To reduce the risk of an FHB epidemic, several measures of control may be employed, including the use of cultural control techniques, the growth of resistant cultivars and the use of fungicides or biological antagonists.[74-76, 83, 118)] Because of the ubiquitous nature and wide host range of *Fusarium* species, adequate cultural control by crop rotation with non-host crops alone is not effective. However, crop rotation, coupled with plowing to bury infested crop residues and weed hosts, can reduce the source of primary inoculation.[8)] FHB was more severe in wheat following maize and/or chisel plow and no-tillage treatment in Minnesota, USA.[24, 25)] The highest disease score was also recorded in the no-tillage system and following maize cropping in Argentina.[31, 118)] Chemical control of FHB is inconsistent with their effectiveness in this situation, although triazole fungicide tebuconazole appeared to be one of the more effective fungicides along with prochloraz.[2, 31, 83, 61)] Benomyl, thiophanate ethyl and propiconazole have also been widely used recently by Japanese farmers, but tolerance of *F. graminearum* to benomyl and thiophanate ethyl has been reported.[94, 122)] Treatment cost and difficulty in determining the optimum application time also make these methods of control less attractive to farmers.[8)] Control of FHB of wheat by fungicide application is neither practical nor sustainable.

The best way to prevent wheat from being affected by this disease is to develop resistant cultivars. Previous studies have indicated that the resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives.[9, 67, 120)] No accession, however, has yet been found to be completely immune to FHB.[35, 52, 57, 99, 112)] Furthermore, it is known that durum wheats (*Triticum turgidum* L. *durum* Desf.) are consistently more susceptible to FHB caused by *F. graminearum* and *F. culmorum* than common wheat.[83, 64)] It has also been demonstrated that resistant wheat germplasm could be divided into three gene pools: winter wheats from Eastern Europe, spring wheats from China and Japan, and spring wheats from Brazil and Italy.[52, 100)] Repeated screening of genetic resources led to the identification of several cultivars of spring wheat, such as Shinchunaga, Nobeokabouzu-komugi and Nyubai from the Japanese gene pool.

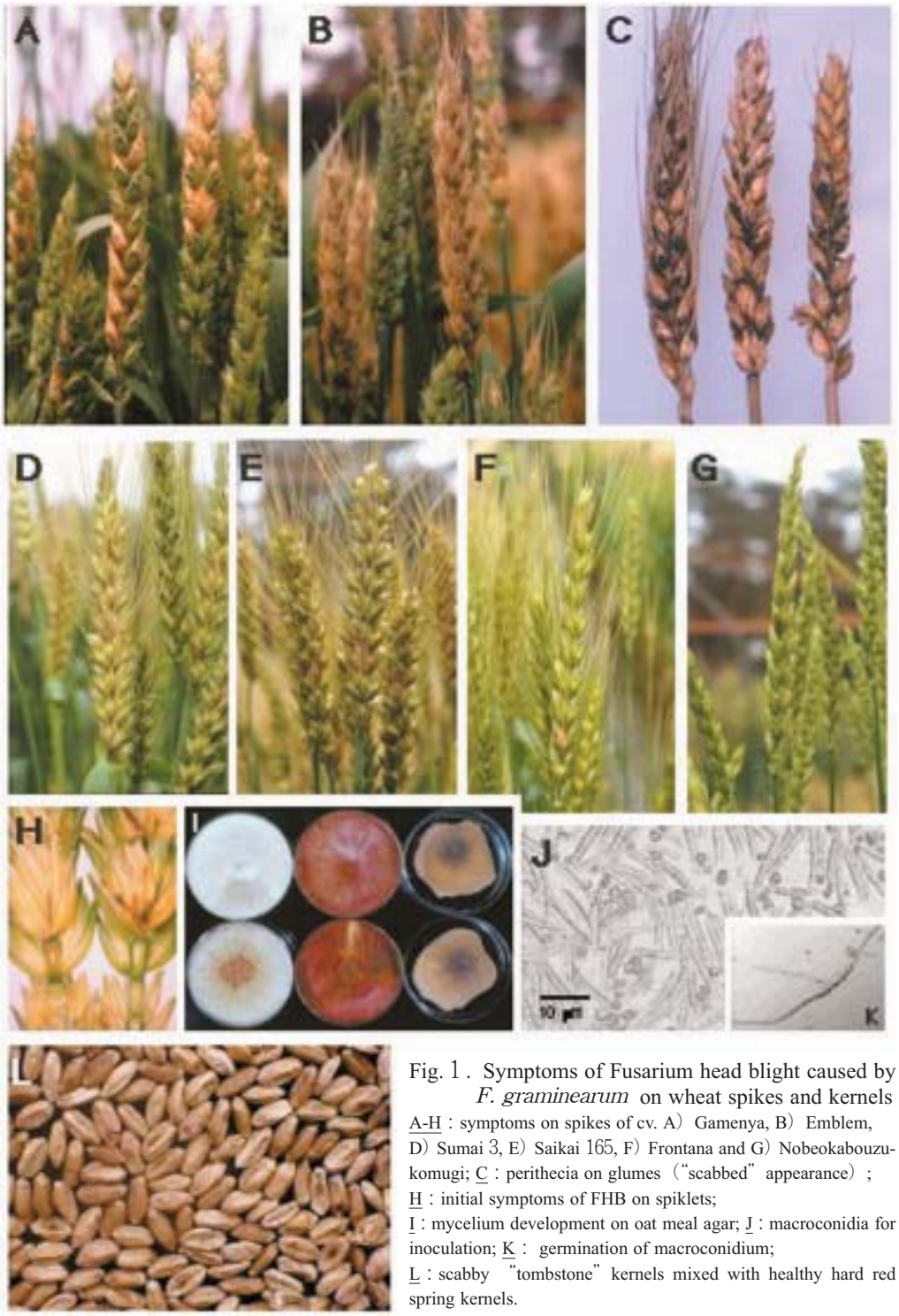

Fig. 1. Symptoms of Fusarium head blight caused by *F. graminearum* on wheat spikes and kernels

A-H : symptoms on spikes of cv. A) Gamenya, B) Emblem, D) Sumai 3, E) Saikai 165, F) Frontana and G) Nobeokabouzu-komugi; C : perithecia on glumes ("scabbed" appearance); H : initial symptoms of FHB on spiklets; I : mycelium development on oat meal agar; J : macroconidia for inoculation; K : germination of macroconidium; L : scabby "tombstone" kernels mixed with healthy hard red spring kernels.

Sumai 3, Ning 7840 and CItr 11028 were identified as cultivars resistant to FHB caused by *F. graminearum* from the Chinese gene pool.[8, 9, 12, 29, 31, 35, 76] Of the idntified wheats, Nobeokabouzu-komugi is highly resistant to FHB.[31, 35, 41, 60, 64, 123] The spike shape of Nobeokabouzu-komugi, which carries long and drill-formed spikes with hard glumes, seems to be responsible for the high resistance to FHB infection in breeding fields (H. Gocho, personal communication). Unfortunately, this cultivar possesses unfavorable agronomic characteristics such as late maturity, lodging and poor quality of flour. One Chinese wheat cultivar, Sumai 3, is considered to be the most useful genetic source for resistance to FHB in Japan. It is also used extensively as a breeding parent elsewhere in the world, resulting in the production of many resistant lines such as the "Ning" selections in China.[52, 100, 119, 126] Similarly, the resistant line Saikai 165 was developed from a cross between Sumai 3 and a leading Japanese cultivar Asakaze-komugi (moderately resistant; MR).[9, 30] Several cultivars resistant to FHB have been developed, but their resistance level is not satisfactory under conditions that are optimal for the pathogen. The best introductions and derived lines break down under high-inoculum and high-humidity conditions because wheat has no immunity to FHB.[29] Therefore, it is necessary to identify more effective materials and additional resistance genes in wild relatives and to transfer them to wheat. Wild species of Triticeae have been sources of resistance genes for wheat breeding in the past.[29, 86] Species of *Agropyron* that are indigenous to Japan (*Elymus*) are well adapted to a humid climate that differs considerably from the climate in the region from which wheat originated.[70] Screening them, therefore, should reveal a considerable variability for resistance to FHB. In the current study, germplasm was screened for resistance to FHB to identify more effective genetic resources among wheat relatives of *Agropyron* (*Elymus*) indigenous to Japan.

Three types of genetic resistance to FHB in common wheat (*Triticum aestivum* L.) have been described: resistance to initial penetration (Type Ⅰ),[91] resistance to fungal spread within plant tissues (Type Ⅱ)[91] and resistance based on the ability to degrade the mycotoxin (Type Ⅲ).[65] It is also recognized that the resistance involves active, passive and/or tolerance mechanisms.[59] Active resistance involves physiological processes, and passive resistance morphological features, such as avoidance, when genotypes allow the plant to escape infection during its most susceptible stage. However, the lack of knowledge of the mode of inheritance of the resistance has hampered significant progress in the breeding of FHB-resistant wheat cultivars for a long time. What the environmental condition influence FHB-infection causes breeders and researchers trouble to access stable reaction of wheat to FHB. Hence, it is necessary to carry out experiments using several replications and preferably under various environments. Although it is essential to develop methods to evaluate the reaction to FHB within individuals of segregated populations to analyze the mode of inheritance of the resistance, it may be more effective to use fixed lines derived from segregated populations. Doubled haploid lines (DHLs) and recombinant inbred lines (RILs) developed from F_1 plants are useful tools for assessing the reaction and evaluating genetic variation due to FHB, because such lines are homozygous and the number of replications can be reduced. This allows selection for resistance to FHB in multi-environmental tests with maximum genetic variance between homogeneous entries and, therefore, a precise estimation of the

genotypic value. DHLs and RILs in wheat have been developed for studies on the genetics of resistance to FHB.[10, 14, 15] For the second objective of this study, we developed criteria for genetic analysis of reactions of DHLs and RILs to FHB.

Inheritance of resistance to FHB in wheat has been studied extensively over the last five decades. Various studies have determined the mode of inheritance and number of genes involved in the resistance, depending on the materials and methods used. However, the genetic constitution of sources for resistance to FHB originating from different gene pools has not yet been clarified.[52, 100] A difference in four resistance genes between Ning 7840 and Frontana was only recently revealed using RILs, showing that the two resistant parents harbor two unique dominant genes each, but the chromosomal location of these resistance genes is not known.[117] Resistance of wheat to FHB is expressed as a quantitative character with a low heritability in the early generations.[35] Hence, analyses of quantitative trait loci (QTLs) associated with resistance to FHB have been conducted. Using monosomic and backcross reciprocal monosomic analysis to study the inheritance of resistance, several researchers reported that five or more chromosomes were associated to enhance the resistance to FHB.[17, 50, 126, 127] Five genomic regions of both parents containing putative QTLs associated with Type II resistance to FHB were detected using molecular markers in a population of RILs from the cross Sumai 3(resistant) / Stoa (moderately susceptible).[3]

It is essential to study the genetics of the resistance to FHB, including the identification of the genes for resistance to FHB in several gene pools, so that different genes can be combined to improve the overall resistance of wheat, and to perform a linkage analysis of the resistance genes. Analysis of QTLs associated with resistance to FHB could be used to determine the number of genes involved, to assess gene action and interaction, and to investigate the relation between FHB resistance and other agronomic traits.[32, 64] The objectives of the present study are therefore as follows:

1. Screening of germplasm for resistance to Fusarium head blight (FHB) in indigenous Japanese species of *Agropyron* (*Elymus*),
2. Development of criteria for genetic analysis of the reaction to FHB in wheat,
3. Studies on the genetic mode of resistance to FHB and linkage analysis of resistance genes using doubled haploid lines (DHLs) and recombinant inbred lines (RILs) in wheat.

Finally, the genetics of resistance to FHB was examined to identify the relationship between the characteristics of FHB and resistance mechanisms in wheat and thus to develop FHB-resistant wheat.

Ⅱ. Screening of Germplasm for Resistance to Fusarium Head Blight (FHB) in Indigenous Japanese Species of *Agropyron* (*Elymus*).

1. Introduction

Several commercial cultivars and parental lines of wheat with high resistance to FHB have been developed, for example, Sumai 3 and Saikai 165. However, the resistance of these cultivars is not satisfactory under conditions that are optimal for the pathogen. It is necessary to identify more effective genetic resources among relatives of wheat to breed of FHB-resistant wheat.

Species of *Agropyron* indigenous to Japan (*Elymus*) are well adapted to a humid climate that differs considerably from the climate in the region from which wheat originated.[70] Therefore, such species might be useful as genetic resources for introducing FHB resistance into wheat.[120] In this study, 17 accessions of four indigenous Japanese species of *Agropyron*, collected in northern Kyushu (Japan), were examined to determine their resistance to FHB.

2. Materials and methods

Accessions of the indigenous Japanese species of *Agropyron*, listed in Table 1, were collected from nine sites in northern Kyushu, Japan. Plants were grown in pots under natural conditions in Chikugo, Fukuoka prefecture. Levels of resistance to FHB were estimated at the flowering stage and compared with those of the six wheat cultivars listed in Table 1. One strain of *Fusarium graminearum* Schwabe, strain G87-36B, was used to estimate FHB resistance. It was derived from a single spore that had been isolated from an FHB-affected wheat spike in the field.[9]

Individual plants of each accession were transferred to a humid chamber, which was kept at 25℃ and 100% humidity, with a day length of 14 h and a light intensity of 1,000 lux, for infection with the fungus. On the first and second days, the spikes at the flowering stage were sprayed with a conidial suspension of G87-36B obtained from a suspension culture[45] that had been adjusted to approximately 40 conidia per 200-fold magnified microscopic field. After three days under humid conditions, each plant was transferred to another chamber where the temperature was maintained at 23℃, with 80 to 90% humidity and a 14 h day length with light at 30,000 lux to favor development of the disease. The diseased parts of the florets and rachis became brownish or whitish in color. Two aspects of resistance were evaluated two weeks after the first inoculation: 1) the extent of penetration of spikelets by FHB (*i. e.* the frequency of diseased spikelets／total number of spikelets); and 2) the extent of fungal spread within rachis internodes (*i. e.* the number of diseased rachis internodes／total number of diseased spikelets). Top and bottom spikelets were excluded from the analysis. These parameters were found to be useful for evaluating the resistance level to FHB of wheat varieties.[49, 50, 71] Both parameters, calculated for each spike, were transformed by angular transformation and averaged for each accession to give indices of resistance to penetration and spreading. Analysis of variance was performed for these indices, then the statistically significant difference was evaluated by the Tukey-HSD test.[105]

In this study, the accessions of indigenous Japanese species of *Agropyron* were assigned into the genus *Elymus*, as described in previous studies.[22, 69, 82] A single exception was *A. mayebaranum*, which is a natural hybrid of *E. humidus* and *E. tsukushiensis.*

Table 1. Accessions of indigenous Japanese species of *Agropyron* (*Elymus*) and wheat cultivars used in this study

Species	Accession	Source		
Elymus humidus (Ohwi et Sakamoto) Osada (2n=42, SHHYY) [1]	AG.91-1	Kue, Chikugo, Fukuoka Prefecture		
	AG.91-2	Kue, Chikugo, Fukuoka Prefec.		
	AG.91-3	Kue, Chikugo, Fukuoka Prefec.		
	AG.91-33	Izumi, Chikugo, Fukuoka Prefec.		
	AG.91-35	Izuminishi, Chikugo, Fukuoka Prefec.		
	AG.91-37	Izuminishi, Chikugo, Fukuoka Prefec.		
	AG.91-38	Izuminishi, Chikugo, Fukuoka Prefec.		
	AG.91-39	Izuminishi, Chikugo, Fukuoka Prefec.		
	AG.91-41	Nagasaki, Chikugo, Fukuoka Prefec.		
E. tsukushiensis Honda var. transiens (Hack.) Osada (2n=42, SSHHYY) [1]	AG.91-7	Kue, Chikugo, Fukuoka Prefec.		
	AG.91-8	Tachibana, Yame, Fukuoka Prefec.		
	AG.91-16	Wakamiya, Kurate, Fukuoka Prefec.		
	AG.91-18	Wakamiya, Kurate, Fukuoka Prefec.		
	AG.91-20	Tonda, Kitakyushu, Fukuoka Prefec.		
	AG.91-31	Kanzaki, Kurate, Fukuoka Prefec.		
E. racemifer (Steud.) Tsvel. (2n=28, SSYY) [1]	AG.91-24	Chiyomaru, Onga, Fukuoka Prefec.		
Agropyron maebaranum var. *intermedium* Hatusima	AG.91-36	Izumi, Chikugo, Fukuoka Prefec.		
Triticum aestivum ssp. vulgare (2n=42, AABBDD)	cv.	Nobeokabouzu-komugi	(VR) [2]	Accessions of KNAES[3]
		Sumai 3	(R)	
		Norin 61	(MR)	
		Chinese Spring	(S)	
		Emblem	(VS)	
		Gamenya	(VS)	

[1] Sakamoto and Muramatsu (1966), Dewey (1984).

[2] Resistance level to Fusarium head blight in the field: (VR) very resistant; (R) resistant; (MR) moderately resistant; (S) susceptible; (VS) very susceptible.

[3] Kyushu National Agricultural Experiment Station, MAFF, Japan.

3. Results and discussion

The index of resistance to penetration of spikelets by FHB in each accession is shown in Table 2. The index varied among the accessions, and no accession was completely resistant to *F. graminearum*. The variance of the index was statistically significant, with a negligible effect (F=2.96**; Table 3). It appears that the spikes were randomly infected with spores of *Fusarium*, with resultant large standard errors in the index for each of the accessions. Due to the favorable conditions for infection in the humid chamber during inoculation, all the spikelets of the moderate to very susceptible cultivars of wheat were infected with FHB. Among the accessions of indigenous Japanese species of *Agropyron*, AG.91-35 of *E. humidus* expressed a statistically significant higher level of resistance to penetration than the resistant wheat cultivar, Nobeokabouzu-komugi. AG.91-24 of *E.racemifer* showed the same level of resistance as Nobeokabouzu-komugi and a higher level than Sumai 3. The other four accessions of *E. humidus* and *E. tsukushiensis* (*i.e.*, AG.91-7, AG.91-37, AG.91-20 and AG.91-41) were as resistant as

Sumai 3. These accessions seem to be suitable genetic sources of resistance to penetration by FHB. By contrast, all the spikelets on every spike of four accessions, namely, AG.91-38, AG.91-16, AG.91-18 and AG.91-36, were infected, as well as the susceptible wheat cultivars. Wheat pollen plays an important role in the initial penetration of FHB.[46, 83] Although AG.91-36 of A. *mayebaranum* was completely sterile, it was highly susceptible to penetration. The spores of Fusarium can easily remain in the glumes of AG.91-36 as a result of the opening of florets caused by sterility and, thus, subsequent invasion of AG.91-36 by FHB should be facilitated. Cleistogamy might be one factor that protects plants from fungal penetration, but no such tendency was observed among the resistant accessions. Further studies are required to evaluate the additive effects of genetic cleistogamy, as reported in several varieties of durum and common wheat,[19, 92] on the genetic background of resistant accessions.

Table 2. Indices of resistance to penetration of spikelets by Fusarium head blight for accessions of indigenous Japanese species of *Agropyron* and common wheat, obtained after inoculation with *Fusarium graminearum* G87-36B

Accession	Species[1] tested	No.of spikes	Index of resistance to penetration	S.E.[2]	Tukey-HSD test[3]
AG.91-35	Eh	10	62.87	25.90	a
AG.91-24	Er	7	68.57	6.27	ab
AG.91-7	Et	10	76.24	13.18	bc
AG.91-37	Eh	10	77.17	17.62	bc
Nobeokabouzu-komugi	Ta	15	77.42	14.87	bc
AG.91-20	Et	10	77.53	16.63	bc
AG.91-41	Eh	12	80.78	18.21	c
AG.91-31	Et	10	81.97	11.16	cd
AG.91-33	Eh	10	84.34	12.63	cd
AG.91-8	Et	10	84.47	12.20	cd
AG.91-1	Eh	10	84.53	11.54	cd
AG.91-2	Eh	9	84.57	10.93	cd
AG.91-39	Eh	10	84.92	16.06	cd
AG.91-3	Eh	5	85.86	9.26	cd
Sumai3	Ta	10	85.74	9.54	cd
AG.91-38	Eh	6	90.00	0.00	d
AG.91-16	Et	4	90.00	0.00	d
AG.91-18	Et	5	90.00	0.00	d
AG.91-36	Am	10	90.00	0.00	d
Norin61	Ta	10	90.00	0.00	d
Chinese Spring	Ta	6	90.00	0.00	d
Emblem	Ta	10	90.00	0.00	d
Gamenya	Ta	7	90.00	0.00	d

[1] Eh, *E. humidus;* Et, *E. tsukushiensis;* Er, *E. racemifer;* Am, *A. maebaranum;* Ta, *T. aestivum*.
[2] Standard error.
[3] Data with the same letter are not significantly different according to the Tukey-HSD test (p=0.05).

Table 3. Results of analysis of variance(ANOVA) for indices of resistance to penetration and spreading by Fusarium head blight in accessions of indigenous Japanese species of *Agropyron* and common wheat inoculated with *Fusarium graminearum* G87-36B

Source of variance	df	SS	F	
Index of resistance to penetration				
Total	208			
Accessions	22	469.89	2.96	$p<0.01$
Error	186	158.53		
Index of resistance to spreading				
Total	208			
Accessions	22	7794.02	131.88	$p<0.01$
Error	186	59.10		

The extent of spreading within rachis internodes by FHB also varied among the accessions (F =131.88**; Tables 3 and 4). The index of resistance to spreading is a suitable parameter for expressing the level of FHB resistance in the field of the six wheat cultivars. All the accessions of indigenous Japanese species of *Agropyron* that we examined, with the exception of AG.91-36, had indices of resistance to spreading that were statistically similar to those of Nobeokabouzu-komugi or Sumai 3. Weng and Liu[120] reported that *E. tsukushiensis* and *E. racemifer*, collected in China, also exhibited a high resistance to the spread of FHB within a spike, but they did not examine *E. humidus* in their study. It is remarkable that there was no spreading of FHB within the rachis internodes by the fungus was detected at all in all the strains of *E.humidus* with the exception of AG.91-1, in accession AG.91-20 of *E. tsukushiensis*, and in *E. racemifer* (Table 4). Therefore, these species of *Agropyron* seem to be valuable genetic sources of resistance to invasion by FHB, if such resistance could be introduced into wheat.

Three types of genetic resistance to FHB in wheat have been described: resistance to initial penetration (Type Ⅰ),[91] resistance to fungal spread within plant tissues (Type Ⅱ),[91] and resistance based on the ability to degrade the mycotoxin (Type Ⅲ).[65] Three accessions, AG.91-38, AG.91-16 and AG.91-18, exhibited a strong resistance to spreading by FHB (Table 4). However, they were as susceptible as susceptible cultivars of wheat to penetration by FHB (Table 2). By contrast, AG.91-8 exhibited a reverse response to penetration and spreading by FHB (Tables 2 and 4). From these examples, it appears that two aspects of resistance, resistance to penetration (Type Ⅰ) and resistance to spreading (Type Ⅱ), are controlled by different genetic systems in the accessions of indigenous Japanese species of *Agropyron*, as well as in resistant wheat cultivars[65, 117]. Therefore, we should use germplasm associated with maximizing both aspects of resistance to FHB (e.g., AG.91-35 and AG.91-24) as genetic resources for introducing resistance to FHB in wheat (Fig. 2).

Table 4. Indices of resistance to fungal spreading of rachis internodes by Fusarium head blight for accessions of indigenous Japanese species of *Agropyron* and common wheat, obtained after inoculation with *Fusarium graminearum* G87-36B

Accession	Species[1] tested	No.of spikes	Index of resistance to spreading	S.E.[2]	Tukey-HSD test[3]
AG.91-2	Eh	9	0.00	0.00	a
AG.91-3	Eh	5	0.00	0.00	a
AG.91-33	Eh	10	0.00	0.00	a
AG.91-35	Eh	10	0.00	0.00	a
AG.91-37	Eh	10	0.00	0.00	a
AG.91-38	Eh	6	0.00	0.00	a
AG.91-39	Eh	10	0.00	0.00	a
AG.91-41	Eh	12	0.00	0.00	a
AG.91-20	Et	10	0.00	0.00	a
AG.91-24	Er	7	0.00	0.00	a
AG.91-7	Et	10	1.95	6.16	a
AG.91-1	Eh	10	2.22	7.02	a
Nobeokabouzu-komugi	Ta	15	2.92	6.12	a
AG.91-31	Et	10	3.00	6.32	a
AG.91-16	Et	4	3.08	6.16	ab
Sumai3	Ta	10	5.68	13.54	ab
AG.91-18	Et	5	8.16	7.66	ab
AG.91-8	Et	10	10.59	9.49	b
AG.91-36	Am	10	20.14	8.39	c
Norin61	Ta	10	43.13	20.97	d
Chinese Spring	Ta	6	83.93	14.88	e
Emblem	Ta	10	85.51	9.53	e
Gamenya	Ta	7	90.00	0.00	e

[1] Eh, *E. humidus;* Et, *E. tsukushiensis;* Er, *E. racemifer;* Am, *A. maebaranum;* Ta, *T. aestivum*.
[2] Standard error.
[3] Data with the same letter are not significantly different according to the Tukey-HSD test (p=0.05).

Although crossing of strains of *E. humidus* with wheat has been attempted, hybrids have yet to be obtained. However, F_1 hybrids and backcross offspring were obtained from crosses between indigenous Japanese species of *Agropyron* and both tetra-and hexaploid wheats.[70, 120] Furthermore, disomic and double monosomic addition lines with high resistance to FHB have been developed.[121] Similarly, hybrids between *E. humidus* and hexaploid wheat have been obtained,[70] and disomic addition lines of *E. racemifer* have been developed.[111] It was also reported that the backcross offspring of these crosses inherited resistance to FHB from the *Agropyron* species, which are promising as crossing parents. Therefore, it may be possible to develop hybrids and to transfer FHB-resistance genes into wheat from the accessions of indigenous Japanese *Agropyron* selected in this study and, possibly, other relatives of wheat.[93] In summary, the accessions of indigenous Japanese species of *Agropyron* identified in this study that exhibit enhanced resistance to both penetration and spreading by FHB should be useful as genetic resources for breeding FHB-resistant wheat.

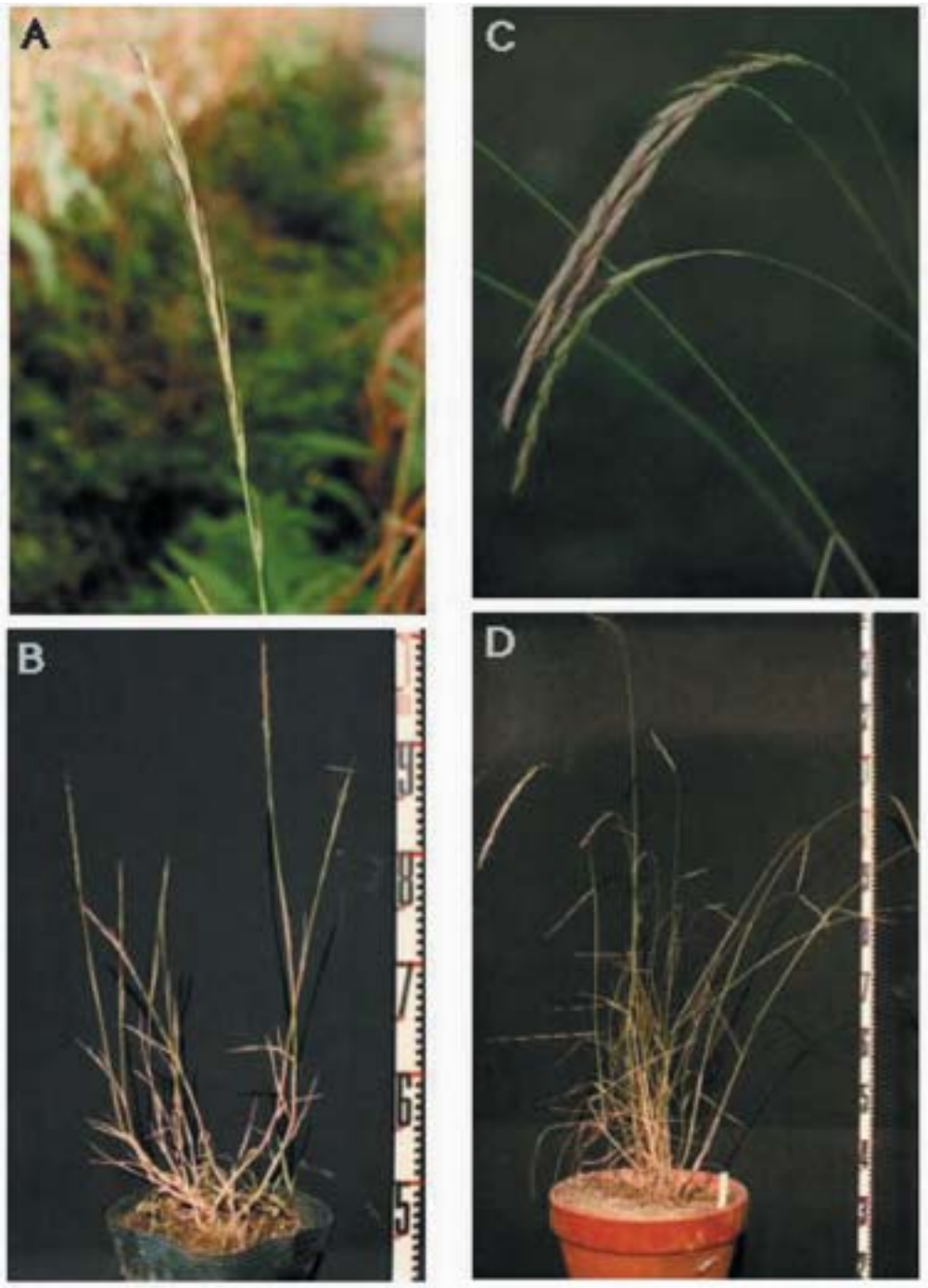

Fig. 2. Head and plant type of accessions of *Agropyron* indigenous to Japan (*Elymus*) selected in this study
A and B: *Elymus humidus* (AG.91-35). C and D: *Elymus racemifer* (Ag.91-24).

Ⅲ. Development of Criteria for Genetic Analysis of Reaction to FHB in Wheat.

1. Introduction

Because warm and humid conditions are essential for FHB inoculum to germinate and grow by infecting wheat spikes,[74-76, 83] it is difficult to compare the level of resistance to FHB among cultivars with various heading dates. To set the heading date of cultivars, testing methods adjusting sowing time and long day treatment in a vinyl film green house or greenhouse were developed for artificial inoculation of FHB.[40, 71] Furthermore, the method using "cut-spike" inoculation in a glass chamber was provev effective for evaluating the variation of the resistance in wheat cultivars and, determining the condition of spikes at the time of FHB infection.[9, 113] Although both methods have been used to evaluate hybrid populations or accessions of genetic resources, it is painstaking and times consuming to apply them to individuals of segregating populations or to a large number of samples at once. In contrast, field trials with artificial inoculation and mist irrigation are more convenient for screening in practical breeding.[65, 59, 112] In this section, I designed field trials with artificial inoculation and mist irrigation to evaluate the resistance levels of wheat cultivars to FHB. Effectiveness of the trials is discussed, then criteria for genetic analysis of reaction to FHB in wheat were developed.

2. Materials and methods

Plant materials for assessing reaction to FHB

The 16 wheat cultivars listed in Table 5 were used as standard cultivars for checking the uniformity of epidemics of FHB and reaction to FHB based on FHB severity in the field. Response levels of the 16 cultivars were evaluated over three years in breeding fields.[9, 12, 30, 36, 40, 71, 72]

Inoculum of FHB

The strain of *Fusarium graminearum* ,G87-36B, used to evaluate the reaction of plant materials to FHB was derived from a single spore isolated from an FHB-affected wheat spike in the field.[9] Macroconidia of G87-36B were prepared using solid medium methods.[71] G87-36B was cultured for four to five days on oat meal agar (oat meal 50 g, sucrose 20 g, agar powder 15 g, and water 1 l). The aerial hyphae on the media were removed by brushing, then the cultures were exposed for three days to fluorescent light. Numerous macroconidia were produced on the surface of the cultures. Subsequently, the macroconidia were suspended in water and adjusted to approximately 50 conidia per 200-fold magnified microscopic field.

Table 5. Standard cultivars used for resistance tests to Fusarium head blight (FHB) caused by *Fusarium graminearum*

Cultivar	Days to heading[+)]	Level of resistance to FHB[++)]	Pedigree
Nobeokabouzu-komugi	160	Very resistant (VR)	Japanese local cultivar
Sumai3	143	(VR) - Resistant (R)	Funo/Taiwanmai
Saikai165	138	Resistant (R)	Sumai3/Asakaze-komugi
Shinchunaga	140	Resistant (R)	Selected from Nakanaga, Japanese local cultivar
Tokai63	141	Resistant (R)	Norin26/Shinchunaga
Asakaze-komugi	137	Moderately resistant (MR)	Hiyoku-komugi/Shirogane-komugi
Shirogane-komugi	138	Moderately resistant (MR)	Shirasagi-komugi/Saikai104
Minamino-komugi	140	Moderately resistant (MR)	Saikai 13/Fujimi-komugi
Norin61	141	Moderately resistant (MR)	Fukuoka-komugi18/Shinchunaga
Norin PL-4	141	Moderately resistant (MR)	Nobeokabouzu-komugi/Sumai3
Norin27	153	Susceptible (S)	Igachikugo-oregon/Jarl Weizen
Chinese Spring	156	(S) - Very susceptible (VS)	Chinese cultivar
Chugoku141	138	Very susceptible (VS)	Norin72/Chugoku113/Saikai135/Omase-komugi
Norin12	147	Very susceptible (VS)	Shirodaruma/Velvet
Gamenya	147	Very susceptible (VS)	Gabo*6/Mentana W1124//Gabo*2/Kenya117AW1347
Emblem	151	Very susceptible (VS)	Ghurka/Pusa4//2*Insignia

[+)] Average of field data in 1994, 1995 and 1996.
[++)] Refer to Nakagawa (1955), Ban and Gocho (1988), Fujita et al. (1988), Ban (1991), Gocho et al. (1992) and Hirai et al. (1993).

Inoculation methods and disease assessment

The reaction of the plant materials to FHB was examined in a field equipped with a sprinkler system in both 1995 and 1996 (Fig. 3-A). The 16 standard cultivars were also examined in a greenhouse, where humidity and night temperature were controlled to promote disease development (Fig. 3-B). The experiment was conducted as a randomized block design with two replications. For each treatment, 10 plants per line were grown at 20 cm spacing per plot. In 1996, four plots of the 16 standard cultivars were replicated in both the field and greenhouse. After heading, simulated rainfall (ca. 13 mm/day) in the form of a fine mist every 2 h was provided intermittently to keep the spikes wet until maturity. The six-rowed barley cultivar Kashimamugi, which is very susceptible to FHB and characterized by early maturity,[12] was planted in every fifth row as a disease spreader. When the spreader reached the heading stage, the macroconidia suspension of *F. graminearum* "G87-36B" was spread through the sprinkler. From the ripening stage to full maturation of each line, FHB severity of representative sets of five spikes was scored three times during three weeks to obtain a mean disease response based on the scoring by the visual scale shown in Fig. 4. Mean values of FHB severity were compared among the 16 standard cultivars using analysis of variance (ANOVA) and least significant difference tests (LSD).

Fig. 3. Field (A) and greenhouse (B) trials for disease assessment of Fusarium head blight caused by *F. graminearum*
Simulated rainfall in the form of a fine mist was provided intermittently to keep wheat spikes wet after heading until maturity (A and B).
Macroconidia of *F. graminearum* (G87-36B) were spread through the sprinkler (A) or dried oat meal agar, on which macroconidia were formed (*see* Fig. 1-I), hanging over the wheat materials (B).

3. Results

Differences in days to heading of the 16 standard cultivars grown in the greenhouse in 1995 and 1996 ranged from four to eight days; the mean difference was 0.7 days (Table 6). Because it was seldom cool throughout the growing season in 1996, heading of all the standard cultivars in the field was delayed from 1 to 16 days (mean 10.2 days) compared with 1995. In contrast with the greenhouse, heading in the field experiment was delayed by a mean of 3.1 days in 1995 and 12.6 days in 1996.

Fig. 4. Diagram of Fusarium head blight (FHB) severity on spikes for evaluating the resistance to FHB caused by *F. graminearum*

*Description : 0%, no disease symptoms; 5%, single floret is diseased; 10%, single spikelet is diseased; 20%, 30%, 40% and 50%, of spikelets and rachis internodes diseased; 60%, 70%, 80% and 90%, of spikelets and excess number of rachis internodes diseased; 100%, spikes are completely diseased.

Results of the ANOVA indicated that the mean FHB severity varied among the 16 standard cultivars over fields and years (Table6). Variance for cultivars was 11 fold for environmental factors, including variance for fields and years (Table 7-A). In the greenhouse, variance for cultivars was 18 times greater than variance for years (Table 7-B). The difference in FHB severity of each cultivar between years caused a large variance in the field (Table 7-C). The ANOVA between fields demonstrated that the variance for cultivars was significant in 1995 (Table 7-D), although the variance for fields was also significant in 1996 (Table 7-E). Differences in the FHB severity were caused mainly by genetic variations of cultivars. Several cultivars apparently escaped infection in the field test in 1996.

FHB severity among the 16 standard cultivars was compared using the LSD test (Table 6). The results reflected the previously reported levels of response to FHB, except for the results of the 1996 field tests because the early flowering cultivars tended to escape infection. It was still possible, however, to distinguish very resistant and susceptible wheats among the standard cultivars.

Table 6. Fusarium head blight (FHB) severity and range for the 16 standard cultivars tested in the greenhouse and field (1/2)

Cultivar	Level of resistance[+]	Tests in the greenhouse,1995				Tests in the greenhouse,1996			
		Days to heading[++]	FHB severity (%) [+++]	Range	LSD[++++]	Days to heading	FHB severity (%)	Range	LSD
Nobeokabouzu-komugi	VR	29	2.5	0-5	a	29	3.8	0-5	a
Sumai3	VR-R	17	7.5	5-10	ab	13	16.3	5-20	ab
Tokai63	R	12	20.0	20-20	bc	11	20.0	10-30	bc
Saikai165	R	10	25.0	20-30	c	8	22.5	20-30	bc
Shinchunga	R	11	25.0	30-30	c	9	25.0	20-30	bc
Asakaze-komugi	MR	5	25.0	20-30	c	6	25.0	20-30	bc
Shirogane-komugi	MR	5	30.0	20-30	c	7	22.5	20-30	bc
Norin61	MR	11	30.0	30-30	c	11	27.5	20-30	bc
Norin PL-4	MR	15	35.0	30-40	c	11	32.5	30-40	c
Minamino-komugi	MR	8	35.0	30-40	c	9	35.0	20-40	c
Norin27	S	17	70.0	70-80	d	23	62.5	60-80	d
Chinese Spring	S-VS	17	85.0	70-70	de	25	60.0	50-80	d
Chugoku141	VS	5	75.0	70-100	de	7	70.0	50-80	de
Emblem	VS	17	90.0	90-90	e	21	80.0	70-90	ef
Gamenya	VS	17	90.0	90-90	e	17	87.5	80-100	f
Norin12	VS	17	100.0	100-100	e	17	92.5	80-100	f

Table 6. (2/2)

Cultivar	Level of resistance	Tests in the field,1995				Tests in the field,1996			
		Days to heading	FHB severity (%)	Range	LSD	Days to heading	FHB severity (%)	Range	LSD
Nobeokabouzu-komugi	VR	29	2.5	0-5	a	39	3.8	0-5	a
Sumai3	VR-R	19	7.5	5-10	ab	30	5.0	0-10	a
Tokai63	R	12	20.0	20-20	bc	23	12.5	5-30	abc
Saikai165	R	19	20.0	20-20	bc	20	5.0	0-10	a
Shinchunga	R	11	30.0	30-30	cd	27	7.5	10-30	ab
Asakaze-komugi	MR	6	25.0	20-30	cd	17	6.3	5-10	a
Shirogane-komugi	MR	6	25.0	20-30	cd	18	5.0	5-5	a
Norin61	MR	13	30.0	30-30	cd	22	22.5	0-20	bcd
Norin PL-4	MR	18	35.0	30-40	d	27	22.5	20-30	bcd
Minamino-komugi	MR	8	35.0	30-40	d	20	13.8	5-30	abc
Norin27	S	23	75.0	70-80	ef	31	65.0	50-90	e
Chinese Spring	S-VS	29	70.0	70-70	e	39	30.0	20-50	d
Chugoku141	VS	5	85.0	70-100	fg	18	23.8	10-40	cd
Emblem	VS	22	90.0	90-90	gh	36	72.5	70-80	e
Gamenya	VS	20	90.0	90-90	gh	28	95.0	90-100	f
Norin12	VS	22	100.0	100-100	h	30	95.0	90-100	f

[+] See Table 5.
[++] Days from 1 April.
[+++] Mean value of FHB severity on five representative spikes from two and four replicated plots in 1995 and 1996.
[++++] The values of FHB severity with the same letter are not significantly different (p=0.05).

Table 7. Analysis of variance (ANOVA) for FHB severity of the 16 standard cultivars

Source	d.f.	Mean square	F-value
A. Cultivars vs. Environment+			
Cultivars	15	11065.80	110.30**
Environment	11	1022.40	10.19
Error	165	100.33	
B. Between years (1995 and 1996) in the greenhouse			
Cultivars	15	5254.93	92.17***
Years	5	284.79	5.00***
Erro	75	57.01	
C. Between years (1995 and 1996) in the test field.			
Cultivars	15	1314.38	10.72***
Years	5	6016.39	49.07***
Error	75	122.60	
D. Between the fields (greenhouse and test field) in 1995.			
Cultivars (C)	15	4256.56	90.81**
Fields (F)	1	1.56	0.03
C x F	15	1.56	0.03
Error	32	46.88	
E. Between the fields (greenhouse and test field) in 1996.			
Cultivars (C)	15	7124.79	85.90***
Fields (F)	1	4753.13	57.31***
C x F	15	303.96	3.66***
Error	96	82.94	

+ Location-year combinations.
** $P < 0.01$; *** $P < 0.001$.

4. Discussion

Epidemics of FHB are initiated by ascospores, which are released from perithecia by rain or heavy dew, through wind and rain-splash dissemination.[76, 88] The wind-borne conidia of FHB infect wheat spikes when it is raining. Infection with FHB usually occurs at flowering time or shortly therafter, through the extruded or retained anthers.[5, 23, 87] Warm (above 15℃) and humid conditions are essential for spores to germinate and to infect wheat spikes.[74-76, 83] Humid conditions for 5 h at 25 to 26℃ or 10 h at 15.9 to 19.0℃ also result in optimum infection of spring wheat.[71] Hence, the environmental conditions around flowering time and during the ripening period are limiting factors of FHB epidemics. Variability in these factors t causes unstable reactions of wheat to FHB. Therefore, it is difficult to compare levels of resistance to FHB among cultivars with various heading dates.

In the present study, the FHB severity of 16 standard cultivars was stable in the greenhouse, where humidity and temperature were controlled to favor FHB development. However, under field conditions, there were some differences in the response between 1995 and 1996. Escape from infection seemed to be involved in some instances. The field trial with artificial inoculation and mist irrigation was more convenient for evaluating a large number of samples with replications, although information about environmental influences on FHB infection and development in the

field is limited.[58] Therefore, the improved field inoculation resulted in a more accurate estimation of the resistance to FHB in wheat. The parallel and independent use of several isolates differing in aggressiveness, the accurate timing of inoculation and its evaluation contributed to that improvement. This improvement, however, still required evaluating the exact levels of resistance involving various components of resistance. Therefore, the assessment of the reaction by visual evaluation of FHB severity was adopted in the present study as a convenient method to evaluate the level of field resistance to FHB. It is generally assumed that the stability of reaction to FHB is related to the level of resistance, the most resistant genotypes being the most stable, and the most susceptible ones tending to show more unstable reactions under different epidemic conditions.[59]

The difference in FHB severity among the 16 standard cultivars reflected the level of resistance to FHB previously reported in the present study. It was possible to distinguish the level of resistance to FHB (very resistant or susceptible) among the standard cultivars based on the reaction to FHB for evaluating FHB severity.

Ⅳ. Studies on Genetic Mode of Resistance to FHB and Linkage Analysis of Resistance Genes Using Doubled Haploid Lines (DHLs) and Recombinant Inbred Lines (RILs) in Wheat.

1. Genetic analysis of resistance to FHB in Chinese wheat cultivar Sumai 3 and the Japanese cultivar Saikai 165.

1) Introduction

Inheritance of resistance to FHB in wheat has been studied extensively over the last five decades. Various studies have determined the mode of inheritance and numbers of genes involved in resistance depending on the materials and methods used. The genetic constitution of sources for resistance to FHB originating from different gene pools, however, has not yet been clarified.[52, 100] A difference in four resistance genes between Ning 7840 and Frontana was only recently revealed using recombinant inbred lines (RILs), indicating that the two resistant parents harbor two unique dominant genes each[117]. Furthermore, the chromosomal location of the resistance genes is not known. It is essential to identify the genes for resistance to FHB in the gene pools, so that different genes can be combined to improve the overall resistance of wheat.

The objectives of the present study were to analyze the genetic constitution and to conduct a linkage analysis of resistance to FHB in Sumai 3 and its derived line, Saikai 165, using doubled haploid lines (DHLs) and RILs.

2) Materials and methods

Plant materials for assessment of reaction to FHB

DHLs and RILs, together with the respective parents, were examined to assess the frequency distribution of FHB severity. DHLs were developed from two crosses, Sumai 3 (very resistant to resistant; VR-R) / Gamenya (very susceptible; VS) and Sumai 3 / Emblem (VS), by applying the wheat x maize system.[109, 110] These lines were abbreviated to DHL (Sumai 3 / Gamenya or Sumai 3 / Emblem). Second and third generations propagated through selfing of DHLs, abbreviated as DH_2 and DH_3, were used to assess the reaction to FHB. F_6 and F_7 populations of RILs were developed from the cross Emblem / Saikai 165 by single seed descent. Saikai 165 is one of the FHB-resistant lines derived from the cross of Sumai 3 / Asakaze-komugi (Table 5).

Inoculum of FHB and inoculation method

One strain of *Fusarium graminearum* Schwabe, G87-36B, was also used as inoculum. The inoculation method, including experimental design and scoring methods, followed those previously described in the Chapter Ⅲ.

Disease assessment

The FHB severity of each DHL or RIL was averaged in each generation across years. The results were compared using combined scores of a parent-offspring relationship, excluding lines tested in a single generation, to deduce balanced values for each DHL and RIL across years and generations. Finally, reactions to FHB in DHLs and RILs were classified into three groups using the criteria shown in Table 8: resistant (R), moderately resistant (MR) and susceptible (S). These criteria used for assessment were based on the mean values and ranges of FHB severity of 16 standard cultivars in the field (Table 6).

Table 8. Criteria used to assess of reaction to Fusarium head blight (FHB) in doubled haploid lines and recombinant inbred lines

FHB severity[+)]	Reaction	Description
0-10%	Resistant (R)	*Equally resistant or more resistant to FHB than Sumai 3 in the field test (in 1995 and 1996). *Equally resistant or more resistant to FHB than Saikai 165 in the field test (in 1996).
11-50%	Moderately resistant (MR)	*Intermediate level of reaction between R and S. *Less than 50% of spikelets and rachis internodes diseased.
51-100%	Susceptible (S)	*Equally susceptible or more susceptible to FHB than standard cultivars with the level of susceptible or very susceptible. *50% or more of spikelets and excess number of rachis internodes are diseased.

[+)] Mean value of FHB severity for five representative spikes with two replicated plots.

Allelism test for awnedness

Sumai 3, which is fully awned, has the genotype *hd b1 b2* for awnedness. Gamenya is tip-awned, and the genotype for awnedness was unknown. Segregation of awnedness in 103 F_2 plants and F_3 populations derived from the cross, Chinese Spring／Gamenya, permitted the identification of the genotype for awnedness in Gamenya. Chinese Spring, which is awnless, has the genotype *Hd b1 B2*.[54)]

3) Results

Distribution of heading dates can be summarized as follows: in DHL (Sumai 3／Gamenya) from 13 April to 6 May 1995 (DH_2) and from 18 April to 10 May 1996 (DH_3) ; in DHL (Sumai 3／Emblem) from 18 April to 29 April, 1995 (DH_2) and from 25 April to 15 May 1996 (DH_3). There was no consistent relationship between days to heading and FHB severity. In both populations of DHLs, days to heading of the DH_3 lines were delayed in 1996 compared with the DH_2 lines in 1995.

The frequency distribution of FHB severity in both populations of DHLs was mainly continuous and within the limits of the parents (Figs. 5 and 6). Because there were high correlation coefficients in FHB severity between generations across years ($r=0.75$ and 0.60), FHB severity was compared with a parent-offspring relationship (excluding lines tested in a single generation)

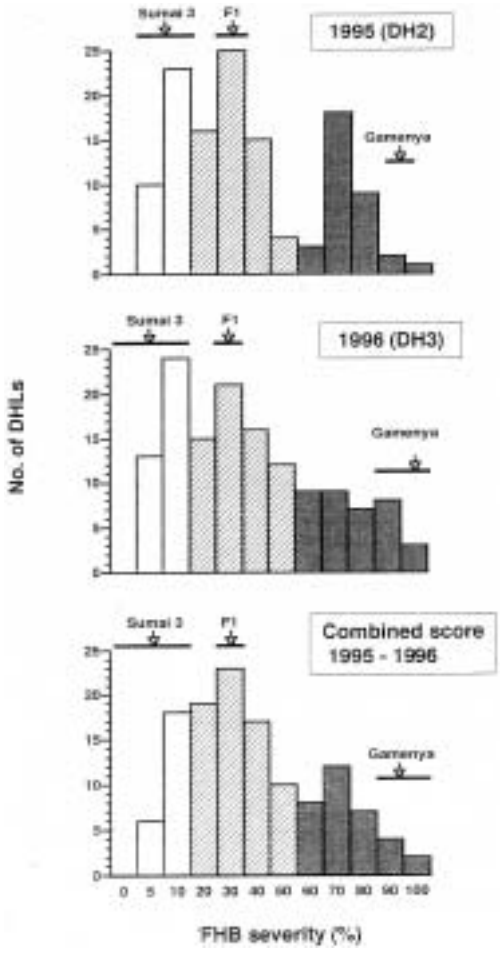

Fig. 5. Frequency distribution of Fusarium head blight (FHB) severity and reaction to FHB in doubled haploid lines (DHLs) developed from the cross between Sumai 3/Gamenya

Reaction to FHB:
Resistant; Moderately resistant;
Susceptible.
: Mean values and ranges of FHB severity of the parents and F_1.

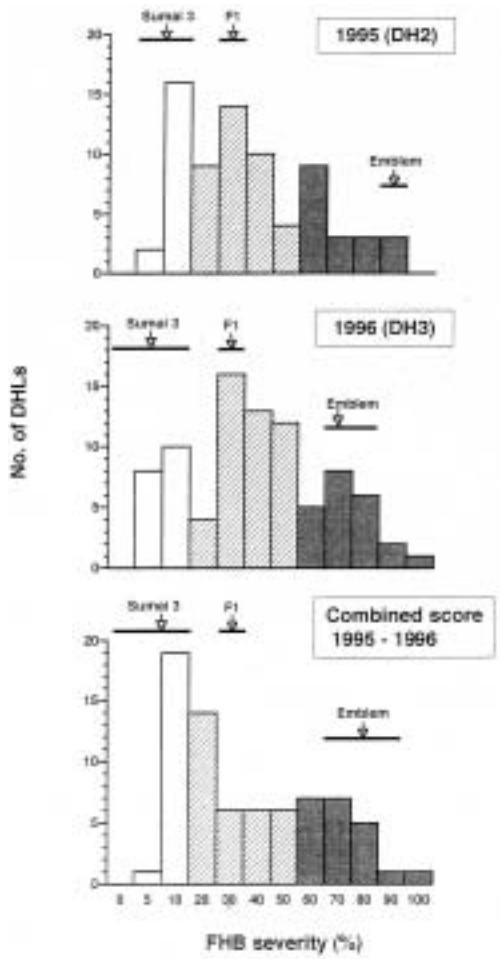

Fig. 6. Frequency distribution of Fusarium head blight (FHB) severity and reaction to FHB in doubled haploid lines (DHLs) developed from the cross between Sumai 3/Emblem

Reaction to FHB:
Resistant; Moderately resistant;
Susceptible.
: Mean values and ranges of FHB severity of the parents and F_1.

to evaluate the balanced value of each DHL across years and generations. Segregation of DHLs for reaction to FHB classified as R, MR and S fitted a tri-modal distribution (Table 9), although it was not clearly distinguished in DH_3 of DHL (Sumai 3/ Gamenya) and combined data of both DHLs (Fig. 5 and 6). Chi-square tests indicated that the segregation fitted a 1 R : 2 MR : 1 S ratio in both DHLs, indicating that the resistance of Sumai 3 was controlled by two major genes, each

Table 9. Segregation for reaction to Fusarium head blight (FHB) in doubled haploid lines (DHLs) and recombinant inbred lines (RILs) developed from crosses between FHB-resistant and susceptible cultivars

Line (cross) Year (generation)	Reaction to FHB+ R	MR	S	Total	χ² 1:2:1 for DHLs 7(R+MR):1S for RILs	Correlation coefficient of FHB severity++
DHL (Sumai3/Gamenya)						
1995 (DH_2)+++	33	60	33	126	0.286 (P= 0.1-0.9)	
1996 (DH_3)	37	64	36	137	0.606 (P= 0.1-0.9)	r=0.75
Combined95-96++++	24	69	33	126	2.429 (P= 0.1-0.9)	
DHL (Sumai3/Emblem)						
1995 (DH_2)	18	37	18	73	0.014 (P>0.95)	
1996 (DH_3)	18	45	21	84	0.629 (P=0.9-0.95)	r=0.60
Combined 95-96	20	32	21	73	1.137 (P=0.1-0.9)	
RIL (Emblem/Saikai 165)						
1996 (F_6)	31	48	10	89	0.130 (P=0.9-0.95)	r= 0.92
1996 (F_7)	39	51	12	102	0.050 (P=0.9-0.95)	
Combined F_6-F_7	25	53	11	89	0.002 (P>0.95)	

Resistant parents: Sumai3 and Saikai165. Susceptible parents: Gamenya and Emblem.

+R, resistant; MR, moderately resistant; S, susceptible.

++Correlation coefficient of FHB severity between generations across years.

+++DH_2, DH_3: Second and third generations through selfing of DHLs.

++++Combined scores of each line with a parent-offspring relationship, except for the lines tested in a generation.

expressing the reaction of MR to FHB, with additive effects.

In the RIL (Emblem/Saikai 165) population tested in 1996, FHB severity was also distributed continuously in both F_6 and F_7 (Fig. 7). Heading dates ranged from 18 April to 13 May 1996 in F_6 and 18 April to 20 May 1996 in F_7. Segregation for reaction to FHB in both RILs deviated from 1 R : 2 MR : 1 S, but corresponded to a three-gene model of 7(R+MR) : 1 S. The ratio observed in the average number of F_6 and F_7 lines was 78 (R+M) : 11 S (χ^2=0.002, P>0.95; Table 9). Furthermore, the high correlation of FHB severity between the F_6 and F_7 generations (r =0.92) indicated that the residual heterozygosity caused by a dominance effect in the RILs was negligible.

In both DHL populations, there was a relationship between the reaction to FHB and awnedness (Table 10). Fully awned lines were more resistant than tip-awned lines. The awned character in both populations segregated into a 1 : 1 ratio for fully awned : tip-awned or awnless phenotypes. The F_1 plants were tip-awned, indicating that awnedness in these DHLs was controlled by one of the suppressor genes *B1* or *B2*.[54]

A weak relationship was also detected between the reaction to FHB and culm length in both DHLs with coefficients of r=-0.57 and -0.43, suggesting that shorter plants are likely to experience FHB infection due to the moist conditions in the test block.

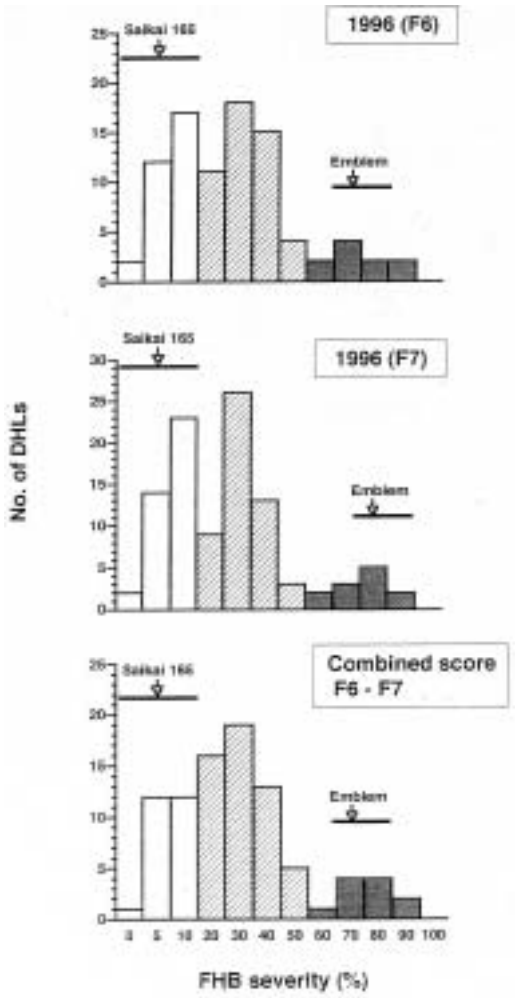

Fig. 7. Frequency distribution of Fusarium head blight (FHB) severity and reaction to FHB in recombinant inbred lines (RILs) developed from the cross between Emblem／Saikai 165

Reaction to FHB:

Resistant; Moderately resistant; Susceptible.

: Mean values and ranges of FHB severity of the parents and F_1.

Table10. Relationship between reaction to Fusarium head blight (FHB) and awnedness (Awn) in DHLs developed from crosses of Sumai 3／Gamenya and Sumai 3／Emblem

Cross of DHLs	Reaction to FHB+				χ^2 1:1for Awn 1:2:1 for FHB	Recombination value++
	R	MR	S	Total		
Sumai3 (fully awned) ／Gamenya (tip-awned)						
Fully awned	20	42	9	71	χ^2 (Awn) =2.0322	15.1±3.3%
Tip-awned	4	27	24	55	χ^2 (FHB) =2.4292	
Total	24	69	33	126	χ^2 (linkage) =16.810	
Sumai3 (fully awned) ／Emblem (tip-awned)						
Fully awned	14	18	6	38	χ^2 (Awn) =0.123	21.4±4.3%
Tip-awned	6	14	15	35	χ^2 (FHB) = 1.137	
Total	20	32	21	73	χ^2 (linkage) =8.2603	

+Combined data from 1995 (DH_2) and 1996 (DH_3).

++Calculated with the column of moderately resistant (MR) for the reaction to FHB omitted.

Allelism test for awnedness

In the cross Gamenya／Chinese Spring, there were two fully awned F_2 plants in a population of 103 plants. This segregation ratio was consistent (P=0.1-0.9) with the expected segregation ratio of 1 (fully awned) : 63 (tip-awned and awnless), indicating differences between the parents at all three loci for awnedness. Gamenya has therefore the genotype *hd B1 b2* and it can be assumed that one of the FHB resistance genes of Sumai 3 is linked in repulsion to *B1*, which is located on the long arm of chromosome 5A. After the plants with a moderately resistant (MR) reaction to FHB were eliminated, recombination values between the resistance level to FHB and awnedness were estimated, at 15.12 ± 3.31% in DHL (Sumai 3／Gamenya) and 21.43 ±4.32% in DHL (Sumai 3／Emblem), respectively.

4) Discussion

Resistance to FHB in wheat is expressed as a quantitative character with low heritability in the early generations.[35] Thus, it is necessary to conduct experiments with several replications and preferably under a range of environments, which is difficult in segregated populations. DHLs and RILs developed from F_1 plants are useful genetic materials for assessing the reaction to FHB. Because such lines are homozygous, the number of replications can be reduced and dominance effects can be excluded. Although the weather conditions in 1996 were unusual, it was cooler than normal years throughout the growing season, and different from the condition in 1995, FHB severity of the DHLs was correlated between years (r=0.75 and 0.60).

Previous investigators reported that the mode of inheritance of resistance to FHB was adequately described by an additive-dominance model, with additive gene action being the essential factor of resistance.[51, 97, 98, 104] Transgressive segregation for resistance to FHB caused by *F. graminearum* and *F. culmorum* often occurs when wheats with varying levels of resistance to FHB are crossed. It is assumed that the resistant parents differed in one or two resistance genes.[52, 99, 117] The numbers of genes were estimated to be three, with epistatic effect, in Japanese wheat,[72] and fewer than five in Chinese wheat for response to *F. graminearum*,[7, 123] and to vary between one and six in winter wheat for the response to *F. culmorum.*[98] Two or three resistance genes are considered to contribute to the resistance in the Brazilian cultivar Frontana.[96, 117] In contrast, some researchers have speculated that the resistance to FHB is conditioned by polygenes.[36] In the present study, we observed that the FHB resistance of Sumai 3 was controlled by two major genes with additive effects using DHLs. These findings are consistent with a previous study of Sumai 3[123] and Ning 7840, derived from Sumai 3, using random F_2-derived F_7 lines.[117]

Furthermore, a relationship between the reaction to FHB and awnedness was detected. Fully awned lines were more resistant than tip-awned lines. A similar relationship was obtained for the response of winter wheat to *F. culmorum*[97]. One of the FHB resistance genes of Sumai 3 appears to be linked to the *B1* gene in the repulsion phase with recombination values of 15.1 ± 3.3% and 21.4 ± 4.3% (Table 10). Due to this linkage, we assumed that the resistance gene is located on the long arm of chromosome 5A. A monosomic analysis of the resistance to FHB in Sumai 3 indicated that at least five chromosomes, 1B, 2A, 5A, 6D and 7D, carried genes for resistance to

the spread of FHB in spikes.[126] Further studies are required to determine the locations of other resistance genes.

The resistance of Saikai 165 to FHB was derived from Sumai 3, and thus the genetic constitution for resistance was identical to that of Sumai 3.[30] The results of the present study, however, indicate that Saikai 165 carries three resistance genes. Therefore, at least one of the resistance genes of Saikai 165 must have been introduced from Asakaze-komugi, which is moderately resistant. The segregation ratio for the reaction to FHB in the RILs fitted a three-gene model with a ratio of 7(R+M) : 1 S. Assuming that the three resistance genes have additive effects of similar magnitude, the RILs should have segregated into 4 R : 3 MR : 1 S. The observed segregation was 25 R : 53 MR : 11 S, however, based on the combined score between F_6 and F_7 lines, fitting well to 2 R : 5 MR : 1 S (χ^2=0.456, P>0.95). These results suggest that the resistance gene introduced from Asakaze-komugi expresses a weak resistance or has no additive effect on the FHB response. The Japanese wheat cultivar, Shinchunaga, was a parent of Asakaze-komugi.[79] Shinchunaga is resistant to FHB and has a high general combining ability and good agronomic traits, as does Sumai 3. Shinchunaga is considered to be one of the main genetic sources for moderate resistance to FHB in many Japanese commercial wheats.[42, 78, 124] Another Japanese genetic source of resistance is Nobeokabouzu-komugi, which shows an outstanding resistance to FHB, but its genetic constitution in terms of FHB resistance has not yet been determined. Further comparative studies of Nobeokabouzu-komugi might explain the variation among resistance genes in the Japanese gene pool.

Three types of genetic resistance to FHB in wheat have been described: resistance to initial penetration (Type Ⅰ),[91] resistance to fungal spread within plant tissues (Type Ⅱ),[91] and resistance based on the ability to degrade the mycotoxin (Type Ⅲ).[65] It was reported that Frontana, representing the Brazilian gene pool for FHB resistance, carries two or three genes for Type Ⅱ resistance, and Ning 7840, representing the Chinese pool, also carries two genes for Type Ⅱ resistance. All four genes are different.[96, 117] We detected two resistance genes in Sumai 3 from China and one or more new genes in a Japanese cultivar representing the Japanese gene pool. Field tests of the DHLs and RILs based on FHB severity mainly evaluate Type Ⅱ resistance. Based on the natural infection with FHB, field tests enable assessment of Type Ⅰ resistance. Field observations suggest that the two resistance genes of Sumai 3 are associated with both types of resistance. It is necessary to identify FHB resistance genes from various gene pools in order to incorporate them into a leading cultivar.

Mesterházy[57] reported that the genetic basis for resistance to *F. graminearum* and *F. culmorum* was identical in winter wheat cultivars. Snijders[100] also reported that spring wheat genotypes resistant to FHB caused by *F. graminearum* were also resistant to *F. culmorum.* No evidence has been presented for strain-specific resistance in wheat to *F. graminearum* or *F. culmorum,*[57, 104] or to other *Fusarium* species.[83,113] It would be useful to test the postulated genetic constitution of our materials with regard to other *Fusarium* species. We are currently performing studies to identify the chromosomal locations of genes using molecular markers. The information obtained might provide a better understanding of the genetic resistance to FHB, and thereby enhance the

likelihood of improved resistance levels in wheat using marker-assisted selection.

2. Analysis of quantitative trait loci (QTLs) associated with resistance to FHB.

1) Introduction

Resistance to FHB is expressed as a quantitative character and is affected by environmental conditions. Experiments with several replications and under various environments are essential to obtain an accurate evaluation. This is difficult to achieve in a breeding program. Molecular markers used as indirect selection markers may enable us to overcome this problem, resulting in a more convenient selection of desired genotypes at quantitative trait loci (QTLs) for resistance to FHB.[64] The first report using molecular markers, restriction fragment length polymorphism (RFLP), to identify genomic regions associated with resistance to Fusarium disease dealt with maize.[27] Using RFLP markers and composite interval mapping, 10 QTLs associated with resistance to FHB, 11 QTLs associated with deoxynivalenol (DON) accumulation, and four QTLs associated with kernel discoloration (KD) caused by *F. graminearum* were identified in barley.[85] Markers flanking these QTLs should be useful for introgressing resistance to FHB, DON accumulation, and KD into elite barley cultivars. Random amplified polymorphic DNA (RAPD) could also allow the description of QTL regions in the genome that can not be studied by RFLP alone. In this chapter, QTLs associated with resistance to FHB were analyzed conducted and screening of RAPD markers associated with resistance to FHB using DHLs is discussed.

2) Materials and methods

Assessment of reaction and resistance to FHB in DHLs

DHLs were successfully developed from the F_1 cross, Fukuho-komugi (moderately susceptible: MS) / Oligo culm (very susceptible: VS), through the wheat x maize system,[109] beside developing DHLs from the F_1 crosses combining the resistant cultivar Sumai 3 was difficult.[14] In 1993, we examined the resistance to FHB of 110 DHLs in a field equipped with a sprinkler system using an inoculation method including the experiment described in Chapter 3. At the ripening stage of each line, we scored the FHB incidence and severity of five representative spikes three times to obtain a stable reaction. FHB severity was scored according to the extent of fungal spread of rachis internodes (*i.e.*, the number of diseased rachis internodes / total number of diseased spikelets) as described in Chapter 2. This parameter, calculated for each spike, was transformed by angular transformation and averaged for each accession to produce an index of resistance to invasion of FHB (IRIF) in order to analyze Type Ⅱ resistance to FHB. The level of resistance of each DHL was then determined using the index shown in Table 11.

Table11. Criteria used in assessing of level of Type Ⅱ resistance to FHB in DHLs

Index of resistance to invasion of FHB (IRIF)	Level of Type II resistance
0-15	Very resistant (VR)
16-25	Resistant (R)
26-30	Moderately resistant (MR)
31-40	Medium (M)
41-50	Moderately susceptible (MS)
51-60	Susceptible (S)
61-90	Very susceptible (VS)

Screening of random amplified polymorphic DNA (RAPD)

Genomic DNA of 110 DHLs was extracted from three to four weeks old seedlings by the potassium acetate and SDS methods (T.Ishige, personal communication). Polymerase chain reaction was performed using genomic DNA as the template. Each reaction comprised: 1 unit Taq polymerase (Promega), 50 to 70 ng genomic DNA, 0.4 mM oligonucleotide primer (10-mer Kits, Operon Technologies), 200 mM each dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0) and 0.1% Glycerol.[16] The temperature and cycling parameters were as follows: pre-heating 2 min at 94℃, 60 cycles of 1 min at 94℃, 1 min 40℃ and 2 min at 72℃, and one final step of 7 min at 72℃. Linkage groups and recombination values of RAPD markers and other markers, *i. e.* red glume color, esterase isozyme, hairy glume and black awn (Ban and Suenaga 1993), were calculated using the computer program "MAPL".[116]

The association between the level of resistance to FHB and RAPD markers was evaluated by comparing the means of IRIF between two groups of DHLs, classified according to each marker type, with a *t*-test.

3) Results

Incidence of FHB in most of the DHLs, both parents and F_1 plants was detected in over 60% of the spikes. However, FHB severity of many DHLs was too unstable to evaluate the frequency of diseased spikelets. Hence, five representative spikes of each line were used to score IRIF. The frequency distribution of 110 DHLs in terms of IRIF fitted a normal distribution, indicating that IRIF was a quantitative character (Fig. 8). The index values of the parents were 49.7 in Fukuho-komugi and 77.0 in Oligo culm. The F_1 plants showed intermediate levels of IRIF (61.7). Transgressive segregation was detected in the DHLs with R, MR, M and VS levels of resistance to FHB, suggesting that the genetic constitution for FHB resistance genes differs between Fukuho-komugi and Oligo culm.

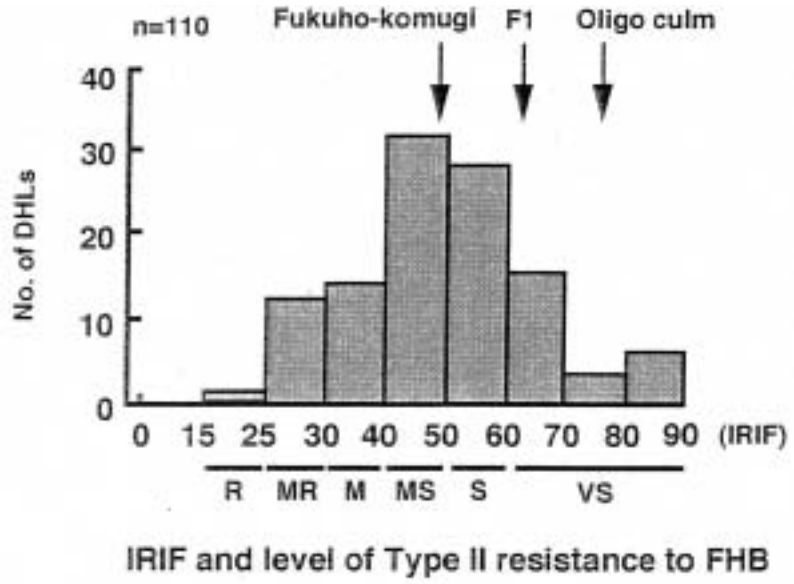

Fig. 8. Frequency distribution in relation to index of resistance to invasion of FHB (IRIF) and level of TypeII resistance to FHB in doubled haploid lines (DHLs) lines developed from a cross between Fukuho-komugi／Oligo culm

Polymerase chain reaction was first performed to survey the extent of polymorphism between the parents of DHLs. Out of 501 oligonucleotide primers, 50 primers (10%) yielded a polymorphism between Fukuho-komugi and Oligo culm with a total of 65 polymorphic bands. Consequently, segregation of these 65 RAPD markers was investigated in DHLs. The segregation ratios of these RAPD markers fitted a 1 : 1 ratio, except for one marker, OPAN-19 $_{800}$, which showed a distorted segregation ($\chi^2 = 7.127^{**}$) in favor of Fukuho-komugi type.[77] A linkage map containing 18 apparent linkage groups of loci for 53 RAPD markers (82%) and four other markers was constructed from the data of 110 DHLs (Fig. 9).

The 110 DHLs were then classified into two groups based on the parental types with respect to each RAPD marker. Classification of DHLs by four RAPD markers, OPZ-10 $_{680}$, OPAG-18 $_{340}$, OPAF-06 $_{345}$ and OPW-13 $_{435}$, revealed significant differences in the averaged IRIF between the two groups at the 5% significance level (Fig. 10). Three RAPD markers were linked with recombination values of 6.4 and 11.8%. Values of averaged IRIF in each group of DHLs classified by each RAPD marker reflected the level of resistance to FHB. This suggests that the RAPD markers selected were associated with susceptibility to FHB.

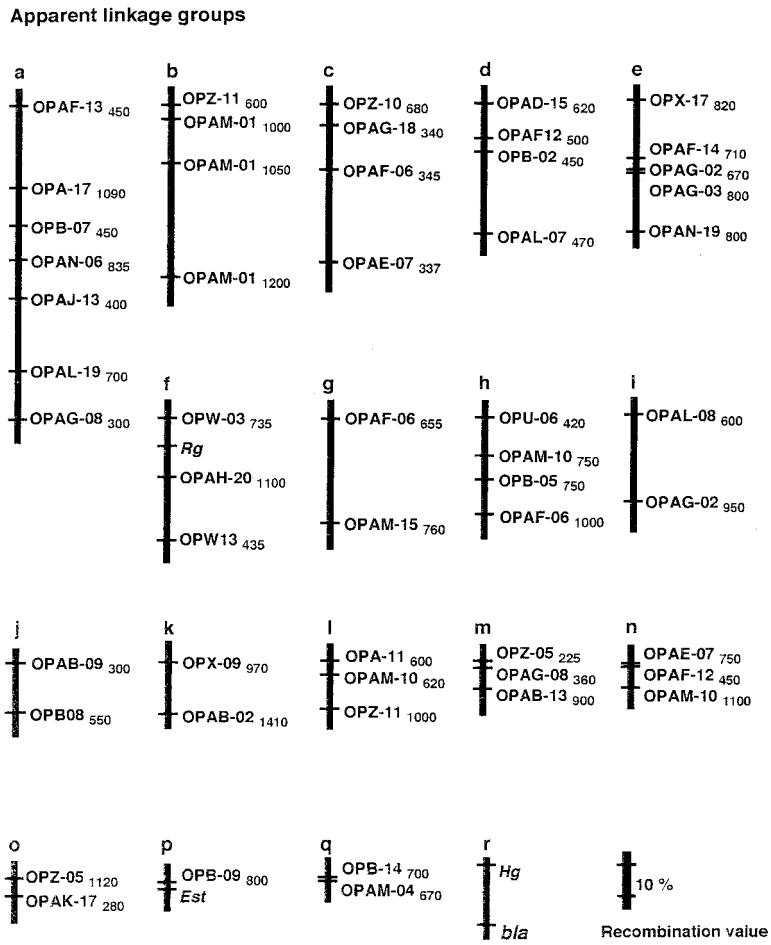

Fig. 9. Linkage map of 53 RAPD markers and four other markers in DHLs developed from a cross between Fukuho-komugi／Oligo culm
Rg: Red glume color. *Est*: Esterase isozyme. *Hg*: Hairy glume. *bla*: Black awn (one of two recessive genes; Ban&Suenaga, 1993).

Distorted segregation of the four markers associated with IRIF was detected in the R and MR levels of resistance to FHB (Table 12). Because lines with a type of RAPD marker similar to that of Oligo culm mainly segregated into R and MR classes, the resistance to FHB associated with the four RAPD markers is considered to be carried by Oligo culm, the susceptible parent. However, three linking markers on provisional "linkage group c" amplified the DNA bands when Fukuho-komugi was used as a template, indicating that the lack of the template of Fukuho-komugi results in the increase of FHB resistance. Therefore, these RAPD markers seem to be associated with a latent susceptible modifier carried by Fukuho-komugi.

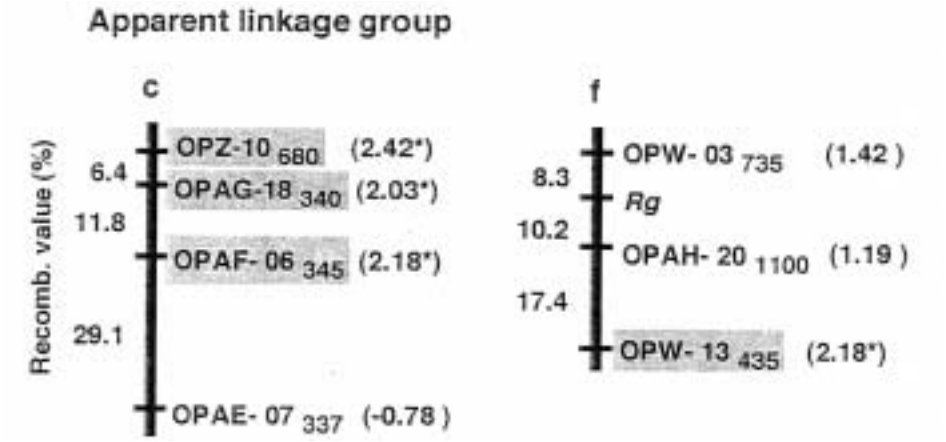

Fig.10. Linkage groups of RAPD markers significantly associated (*; $P<0.05$) with index of resistance to invasion of FHB (IRIF) in DHLs developed from F1 plants between Fukuho-komugi (P1) / Oligo culm (P2)
: markers associated with IRIF. *Rg* : Red glume color.
( ) : *t*-values of "P1 type-P2 type".

Table12. Segregation of RAPD marker types, Fukuho-komugi type/Oligo culm type' associated with susceptibility to FHB in doubled haploid lines developed from the cross Fukuho-komugi(MS)/Oligo culm(VS)

Linkage group RAPD marker	c			f
	OPZ-10_{680}	OPAG-18_{340}	OPAF-06_{345}	OPW-13_{435}
Level of Type II resistance				
R+MR (13) †	1/12*	1/12*	3/10*	3/10*
M (13)	7/6	8/5	8/5	6/7
MS (31)	17/14	17/14	16/15	3/18
S (27)	7/20*	10/16	12/15	12/15
VS (26)	16/10	17/9	16/10	16/10

†No.of DHLs, * Significantly different with 1:1 ratio at 5% level.

4) Discussion

The present study analyzed DHLs developed from the cross combination between susceptible parents to FHB. A high incidence of FHB was detected among the materials, but the reaction to FHB of each spike varied. This made it difficult to evaluate the extent of penetration of the spikelets by FHB, which indicates the resistance to initial penetration(Type Ⅰ). Our study assume that the most susceptible genotypes tend to exhibit more unstable reactions under different epidemic conditions.[59] One of the parents, Oligo culm, carryies a trait of tiller inhibition and it segregated in DHLs. We suggest that this trait resulted in a large difference in heading and

flowering time of each spike even in a plant, and different conditions of FHB infection due to the unstable reaction.

Evaluation of the resistance using IRIF explains the level of Type Ⅱ resistance to FHB. Transgressive segregants of 26 lines with R, MR and M levels of resistance were detected in this 110-DHL population using the parental values as standards. I contrast, seven lines with a VS level of resistance also segregated over the level of Oligo culm. We suggest that the unique configurations of resistance genes are causing the skewed distribution to the low IRIF in this cross.

Molecular markers are useful tools for tagging the resistance genes or QTLs and conducting indirect assisted selection. QTL analysis using 65 RAPD markers in the DHLs developed from the cross of Fukuho-komugi x Oligo culm enabled us to detect four RAPD markers associated with IRIF. These RAPD markers were individually selected by the t-test. Hence, we suggest that the specific regions of provisional group 3 of the wheat genome are associated with Type Ⅱ resistance to FHB. As a result, it is possible to detect the latent susceptible modifier of Fukuho-komugi using three of them. One RAPD marker associated with IRIF (OPW-13 $_{435}$,) was linked with *Rg* for the red glume color on provisional linkage group f. The loci for *Rg* are located on homoeologous group 1; *i*. *e*. *Rg-1* on 1BS, *Rg-2* on 1DL and *Rg-3* on 1AS.[54] Therefore, one of the genomic regions associated with Type Ⅱ resistance to FHB from Oligo culm is located on a chromosome of homoeologous group 1, although no association between *Rg* and IRIF was detected.

Using monosomic and backcross reciprocal monosomic analysis to study the mode of inheritance of the resistance, several researchers reported that five or more chromosomes are associated to enhance the resistance to FHB.[17, 50, 126, 127] Based on published data, chromosomes 6D, 6B, 5A and 7A have frequently been associated with the resistance to FHB in several wheat cultivars.[17] These monosomic analyses revealed that chromosomes were associated with the reaction to FHB as quantitative character, but loci, harboring resistance genes controlling the reaction, and their chromosomal location have not been determined. Five genomic regions of both parents containing putative QTLs associated ($P<0.01$) with Type Ⅱ resistance to FHB were detected using molecular markers, amplified fragment length polymorphism (AFLP) and RFLP in a population of RILs from the cross Sumai 3 (resistant) / Stoa (moderately susceptible),[3] Three of the QTLs were derived from Sumai 3, and the most significant genomic region associated with resistance to FHB in this population was located on the short arm of chromosome 3. The AFLP markers flanking the genomic region explained 16.2% of the variation of the resistance. We may thus be able to detect putative genomic regions associated with resistance to FHB, including differences in genome constitution, even in the analysis of QTLs using molecular markers. Useful tools of molecular markers like microsatellites must be developed to analyze the genome constitution and QTLs in hexaploid wheat.[89] Further comparative studies of several cross combinations may enable us to explain the variation among genomic regions including QTLs associated with resistance to FHB. Studies on genetics with both quantitative and qualitative analyses are considered to be effective not only for tagging the resistance genes but also for estimating the interaction between resistance genes in the selection process with marker assistance.

V. General Discussion

For optimum economic control, genetic resistance should be used to develop resistant cultivars. However, it is difficult to improve the resistance since *Fusarium* species causing FHB are saprophytic pathogens exhibiting a broad host range, with small host-specific variation compared with the variation in general pathogenicity.[28, 57, 83, 104, 113] Furthermore, no accession among wheat, barley and their wild relatives has yet been identified to be completely immune to FHB. Since species of *Agropyron* that are indigenous to Japan (*Elymus*) are well adapted to a humid climate, they were collected and examined for use as genetic sources for resistance to FHB in terms of two components of resistance, *i. e.* initial penetration (Type Ⅰ resistance) and hyphal spreading (Type Ⅱ resistance). Almost all the accessions of Agropyron possessed both types of resistance to a degree equal to or greater than that resistant wheat, Nobeokabouzu-komugi and Sumai 3, but no accession was free from infection with FHB. Variance for the index of Type Ⅱ resistance among the accessions was larger than that of Type Ⅰ resistance under the controlled conditions used in this study, and the index of Type Ⅱ resistance reflected well the levels of FHB resistance among wheat cultivars. As the accessions demonstrated a unique reaction to each type of resistance, we concluded that the resistance to penetration and spreading in Japanese *Agropyron* are controlled separately by different genetic systems, as in the case of wheat.[91, 117] In contrust, several accessions of *E. humidus*, *E. tsukushiensis* and *E. racemifer* with complete Type Ⅱ resistance were identified to explain the immunity to hyphal spreading within a plant. Their Type Ⅱ resistance should be useful for increasing the resistance level of wheat by introducing a resistance gene(s) to wheat. Initial penetration of FHB can be easily influenced by environmental conditions at the time of FHB infection and by the morphological features of spikes.[59] For example, dwarf genotypes were more severely infected with FHB than tall genotypes under natural conditions, but genotypes of different plant height classes were similarly susceptible after artificial inoculation. In contrast to our results, it was also reported that genotypes with awns were more susceptible to FHB when tested under natural epidemic conditions in the field; but this trait did not influence head blight severity in artificial inoculation. Compact, erect spikes were found to be more susceptible to FHB than loose, nodding spikes.[58] It is also known that cultivars with hairy glume or retaining anther wreckage are more susceptible.[76] Hence, we assumed that Type Ⅰ resistance may involve escape from FHB infection by a passive mechanism. As a result, Type Ⅱ resistance can be improved more easily in practically all breeding programs. Meanwhile, avoidance by physiological defensive reactions may increase Type Ⅰ resistance. Transgenic wheat plants into which antifungal protein genes obtained from plant and microbial sources had been introduced, which disturbed the germination of FHB spores, exhibited lower level of spreading of FHB symptoms than the susceptible control.[38] Therefore, improving protection by Type Ⅰ resistance, including both components of the escape and physiological defensive reactions, may prevent FHB infection, germination of FHB spores, and colonization of FHB. It is necessary to combine Type Ⅰ and Ⅱ resistances in FHB-resistant wheat. Two accessions of *E. humidus* and *E. racemifer*, AG.91-35 and AG.91-24, were selected as genetic resources associated with maximizing both Type Ⅰ and Ⅱ

resistances.

Resistance to FHB expressed under natural conditions integrates all the reactions controlled by different mechanisms of genetic resistance. This study suggested that evaluating wheat reactions to FHB under conditions similar to those of natural behavior could be applied to practical breeding. We therefore conducted field trials with mist irrigation to study the genetic resistance of Sumai 3 and Saikai 165 for the reaction of DHLs and RILs. The frequency distribution of FHB severity in DHLs and RILs was generally continuous within the limit of each parent, which suggested that the resistance to FHB was a quantitative trait. However, the classification of DHLs and RILs in terms of the reaction to FHB showed that Sumai 3 harbors two major genes and Saikai 165 harbors three major genes for resistance to FHB. We believe that the resistance of these cultivars can mainly be attributed to two or three major genes with additive effects, and that the variation of FHB severity may be governed by several minor genes.

Inheritance of resistance to FHB in wheat was described by the additive-dominance model with additive gene action being the essential factor of resistance based on the results of diallel analysis.[51, 97, 98] It was also reported that the resistance was generally expressed by partial dominance effects and that the general combining ability played a major role in the resistance. Since transgressive segregation of resistance to FHB has often been observed in various cross combinations, we assume that the number of resistance genes was low, five or less. Accumulation of resistance genes has been shown to be possible. Obviously, transgressive segregation became the source of resistance to FHB in the improved Chinese cultivars: *e. g.*. Sumai 3 was developed from the cross Funo (MS)／Taiwanxiaomai (MS).[52] At this stage, it is essential to identify major genes for resistance to FHB in the various gene pools and to analyze the genetic constitution in resistant cultivars so that the genes can be combined to improve the overall resistance of wheat. A difference in the four resistance genes of the Sumai 3 derivative, Ning 7840, and the Brazilian cultivar Frontana revealed that the two resistant parents harbor two unique dominant genes each.[117] This study has also revealed that Saikai 165 carried three resistance genes introduced from Sumai 3 and the Japanese gene pool. Meanwhile, a difference in the genetic constitution for resistance genes between Nobeokabouzu-komugi and Sumai 3 was observed using their DHLs.[15] The results showed that Nobeokabouzu-komugi also harbored two major genes for resistance to FHB caused by *F. graminearum* and that one of them was identical to that of Sumai 3. These results indicate that the three resistant cultivars of spring wheat from different gene pools, Sumai 3(China), Frontana (Brazil) and Nobeokabouzu-komugi (Japan), possess two unique dominant genes each, and four or five genes could be available among them. However, genetic resources that combine good agronomic characters with resistance to FHB are scarce. Further comparative studies of genetic constitutions for resistance to FHB among genetic resources may enable us to explain the variation among resistance genes in various gene pools. The use of QTL analysis is important in genetic and breeding studies to confirm the presence of minor genes and their effects associated with resistance to FHB in terms of interaction and suitable combinations among the resistance genes.

Chromosomal location of the FHB resistance genes is also necessary to improve FHB-resistant

wheat by integrating resistance genes. A relationship between the reaction to FHB and awnedness was detected in DHLs from the cross Sumai 3／Gamenya and Sumai 3／ Emblem. One of the resistance genes of Sumai 3 appeared to be located on the long arm of chromosome 5A, linked to the *B1* gene in the repulsion phase. It was pointed out that chromosomes 6D, 6B, 5A and 7A have frequently been associated with resistance to FHB in several wheat cultivars.[17] Meanwhile, it is also known that durum wheats (*Triticum turgidum* L. *durum* Desf.) are consistently more susceptible to FHB caused by *F. graminearum* and *F. culmorum* than common wheat.[64, 83] The importance of the D genome in conferring resistance to FHB[34] has been emphasized. Synthetic bread wheats, which were developed from crosses between *Triticum turgidum* and *T. tauschii* (*Aegilops squarrosa*), and derived lines have been developed at CIMMYT to increase the genetic diversity.[68] Types Ⅰ and Ⅱ resistances to FHB were present in some synthetic bread wheats, and the incorporation of different types of resistance from various sources into well-adapted bread wheat genotypes is now being considered.[33]

Reaction to mycotoxins produced by *Fusarium* species is also considered to be another component of resistance to FHB. Miller *et al*.[65] reported that cultivars of wheat, rye and triticale resistant to FHB contained low concentrations of deoxynivalenolenol(DON), produced by *F. graminearum,* in the kernels, whereas susceptible ones contained much higher concentrations. It was also reported that, using a suspension culture of embryo callus, the resistant wheat cultivar Frontana was able to degrade 18% radioactive (^{14}C) DON into two breakdown products and a possible DON glycoside, while the susceptible cultivar Casavant degraded only 5% ^{14}C DON into the possible glycoside. This ability to degrade DON was described as TypeⅢ resistance to FHB.[65, 66] The resistant cultivars Sumai 3 and Nobeokabouzu-komugi also were reported to have genotypes with the ability to degrade DON in addition to their very high resistance to FHB.[6, 65, 119] Furthermore, based on a coleoptile bioassay, Wang and Miller[119] reported that FHB-resistant cultivars Frontana and Sumai 3 could tolerate high mycotoxin concentrations, compared with susceptible cultivars, with no effect on growth. They postulated that, in these resistant cultivars, peptidyl tansferase enzyme was modified so that the cultivars became tolerant to DON. This tolerance to mycotoxins was reported as TypeⅣ resistance.[119] Another type of resistance occurs at the seedling stage and in the spike.[59] Using mutants of *F. graminearum,* Desjardins *et al.*[21] reported that trichothecene-nonproducing (*Tr*5-) mutants were less virulent than trichothecene-producing (*Tr*5+) parental and revertant strains in their ability to cause FHB in field-grown wheat. This evidence indicates that trichothecenes, including DON, were virulence or aggressiveness factors in FHB, although *Tr*5- mutants of *F. graminearum* colonized wheat spikes. In *Fusarium* isolates producing both DON and nivalenol (NIV), the pathogenicity correlates much better with total trichothecene production (DON+NIV) than with the production of DON alone.[63]It was also mentioned that DON accumulation is not only a consequence of the disease, but it might also be a component of the aggressiveness.

Furthermore, it was reported that the trichothecene target site in plants is likely to be associated with protein synthesis, but this hypothesis remains to be confirmed in plants in an *in vitro* translation system.[21, 39] Snijders and Krechting[102] reported that DON appears to be transported

from the infected chaff to the young kernel and the pathogen then colonizes the kernel. In the resistant line, DON translocation from chaff to kernel was inhibited, and minimal fungal colonization occurred, suggesting that this phenomenon is based on a membrane-based trichotecene tolerance associated with Type II resistance. This supports the evidence of systematic fungal invasion of FHB into wheat, which was investigated anatomically.[75] *F. graninearum* initially invaded the lemma through stomata, then hyphae destroyed the tissues of the lemma and palea prior to extension. Shortly thereafter, hyphae reached the young kernel, and invasion occurred through the parenchyma or pericarp near the embryo with the presence and colonization of a large number of hyphae as a prerequisite. Another report of enhanced resistance to *F. graminearum* in transgenic wheat plants, into which pathogenesis-related (PR) protein genes, a rice thaumatin-like protein (TLP) gene (*tlp*) and a rice chitinase gene (*chi11*) had been introduced, showed that the delay in FHB symptom development (Type Ⅱ resistance) due to TLP over-expression in susceptible wheat cultivar was significant, although the effects were quantitative rather than qualitative.[18] Chitinase acting in the cell-wall polysaccharide (chitin) may contribute to Type Ⅰ resistance, whereas TLPs, which are considered to alter the membrane permeability and ／or cellular signal transduction cascades,[18] may enhance TypeⅣ resistance. These data suggest that the ability of DON degradation (TypeⅢ resistance) and tolerance to DON (TypeⅣ resistance) play an important role in quantitatively enhancing the disturbance of hyphal growth (TypeⅡ resistance) and of fungal colonization (Type Ⅰ resistance) with synergistic effects. This suggests a relationship between FHB characteristics and resistance in wheat (Fig. 11).

In addition to the work involving cultivar tolerance to DON, there were reports regarding the tolerance of kernel weight reduction to FHB.[37, 59, 64, 103] It was indicated that the host plant was able to withstand the effects of parasitic infection and disease, which, if they had occurred at equivalent levels in other plants of the same and similar species, would have considerably impaired growth or yield.[20] Careful investigations of field trials also revealed that an additional factor of resistance to kernel infection was present in wheat.[59, 63] Therefore, some genotypes display a significantly lower kernel infection than postulated from FHB-infected values.

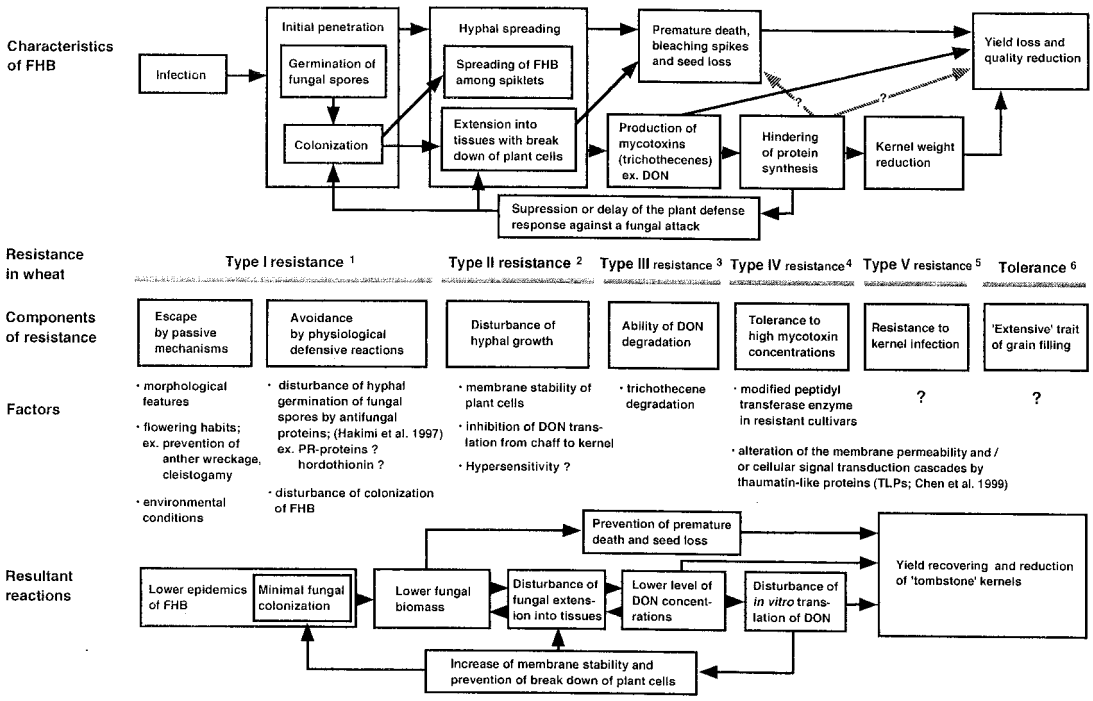

Fig.11. Postulated relationships between characteristics of Fusarium head blight (FHB) caused by *F. graminearum* and resistance mechanisms in wheat
(1, 2 Schroeder and Christensen 1963;[3] Miller et al. 1985;[4] Wang and Miller 1988;[5, 6] Mesterházy 1995, and Mesterházy *et al*. 1999)

The most serious lack of knowledge in the resistance to FHB deals with host resistance and pathogen aggressiveness. Analysis of QTLs associated with each type of resistance and tolerance could be used to determine the number of genes involved, and to reveal the components and factors of resistance to FHB in wheat. Breeding strategies using pyramiding resistance genes and introgression of additive minor genes in an adapted background have been proposed.[52, 95] Further studies on resistance mechanisms and genetic control combined with breeding stratrgies should contribute to rapid progress in the breeding of FHB-resistant wheat cultivars.

VI.Conclusion

Fusarium head blight (FHB) caused by *Fusarium* species, is one of the most destructive diseases of wheat in areas where the weather is warm and humid after the heading of wheat. The optimum way to prevent wheat infection is to develop resistant cultivars. No accession has yet been found to be completely immune to FHB, although the resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives. Repeated screening of genetic resources, led to the identification of several resistant cultivars. However, the resistance of these cultivars has not been satisfactory under conditions that are optimal for the pathogen. It is thus necessary to identify more effective genetic resources among relatives of wheat for the breeding of FHB-resistant wheat. Furthermore, lack of knowledge of the mode of inheritance of the resistance has hampered significant progress in the breeding of FHB-resistant wheat cultivars for a long time.

In this study, I screened germplasm for resistance to FHB to identify more effective genetic resources among wheat relatives of indigenous Japanese *Agropyron* (*Elymus*). I developed criteria for genetic analysis of the reaction to FHB in doubled haploid lines (DHLs) and recombinant inbred lines (RILs). Finally, I clarified the genetic constitution of resistance to FHB in resistant wheat cultivars, Sumai 3 and its derived line Saikai 165 using DHLs and RILs, and conducted a linkage analysis of the resistance genes. Furtherore, I tried to analyze quantitative trait loci (QTLs) associated with resistance to FHB in DHLs using random amplified polymorphic DNA (RAPD) markers to evaluate the effect of minor genes.

1. Four species of indigenous Japanese *Agropyron*, *Elymus humidus* Osada (=*Agropyron humidum*), *E. tsukushiensis* Honda var. *transiens* (=*A. tsukushiense*), *E. racemifer* Tsvel. (=*A. ciliare*) and *A. mayebaranum* var. *intermedium* Hatusima were collected and evaluated for their resistance to FHB after inoculation with a conidial suspension of *Fusarium graminearum* Schwabe at the flowering stage. The resistance to the initial penetration of FHB (Type Ⅰ) and to fungal spread of rachis internodes (Type Ⅱ) was evaluated in each accession compared with six wheat cultivars. The results demonstrated that AG.91-35 of *E. humidus* and AG.91-24 of *E. racemifer* possessed a higher resistance to penetration than the resistant wheat cultivars Nobeokabouzu-komugi and Sumai 3.
2. All the accessions of the indigenous Japanese species of *Agropyron* examined, with the exception of *A. mayebaranum,* exhibited a resistance to invasion statistically similar to that of Nobeokabouzu-komugi or Sumai 3. In all the accessions of *E. humidus,* with only one exception, no fungus spread from the infected spikelets to the rachis internodes. Since the accessions exhibited a unique reaction to each type of resistance, I concluded that Type Ⅰ resistance and Type Ⅱ resistance in Japanese *Agropyron* are controlled separately by different genetic systems.
3. I designed field trials with mist irrigation to study the genetic resistance of Sumai 3 and Saikai 165 for the reaction of DHLs and RILs. It was possible to distinguish the level of resistance to FHB (very resistant or susceptible) among 16 standard cultivars based on the reaction to FHB by evaluating FHB severity. I then developed the criteria shown in Table 8

for genetic analysis of the reaction to FHB in DHLs and RILs.

4. The frequency distribution of DHLs derived from two F_1 crosses, Sumai 3 (very resistant to resistant; VR-R) / Gamenya (very susceptible; VS) and Sumai 3 / Emblem (VS), fitted a 1 : 2 : 1 (resistant : moderately resistant : susceptible) ratio well for the reaction to FHB in the field. This suggested that the resistance of Sumai 3 was controlled by two major genes with additive effects.

5. A relationship between the reaction to FHB and awnedness was detected in DHLs from the crosses Sumai 3 / Gamenya and Sumai 3 / Emblem. One of the resistance genes of Sumai 3 appeared to be located on the long arm of chromosome 5A, linked to the *B1* gene in the repulsion phase with recombination values of 15.1 ± 3.3% and 21.4 ± 4.3%, respectively.

6. Segregation for the reaction to FHB in the RILs, derived from the cross Emblem x Saikai 165, deviated from 1 R : 2 MR : 1 S. It better fit rather three-gene model with 7 (R+MR) : 1 S as the expected ratio, indicating that three resistance genes controlled the resistance of Saikai 165.

7. QTLs associated with Type Ⅱ resistance to FHB in DHLs developed from the cross Fukuho-komugi (moderately susceptible) / Oligo culm (very susceptible) were analyzed using RAPD markers. Transgressive segregants of 26 lines with R, MR and M levels of resistance were detected in this population of 110 DHLs, using the parental values as standards. In contrast, seven lines with a VS level of resistance also segregated over the level of Oligo culm. This suggested that the unique configurations of resistance genes cause the skewed distribution to the low IRIF in this cross.

8. From the results of QTL analysis using 65 RAPD markers in DHLs developed from the cross of Fukuho-komugi x Oligo culm, four RAPD markers, OPZ-10_{680}, OPAG-18 340, OPAF-06_{345} and OPW-13_{435}, associated with Type Ⅱ resistance were identified. Three RAPD markers were linked with recombination values of 6.4 and 11.8%. These RAPD markers were individually selected by the *t*-test. Hence, this suggested that the specific regions of provisional group 3 of the wheat genome are associated with Type Ⅱ resistance to FHB. As a result, it may be possible to detect the latent susceptible modifier of Fukuho-komugi using three of them.

9. Finally, the relationship between the characteristics of FHB caused by *F. grainearum* and resistance mechanisms in wheat was analyzed based on the results of this study and data from the literature for the resistance to FHB (Fig.11). It is suggested that breeding strategies including pyramiding of resistance genes and introgression of additive minor genes in an adapted background may enable rapid progress in the breeding of FHB-resistant wheat.

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Ⅷ. References

1) ABLOBA, I. B. (1997) Resistance to head blight in various types of wheat and triticale in Krasnodar environment. *Cereal Research Communications* 25: 715-720.

2) ACKERMANN, M. D. and M. M. KOHLI (1977) Research of Fusarium head blight of wheat in Urugay. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAB (eds.), Fusarium Head Scab: Global Status and Future Prospects, pp. 13-18. CIMMYT, Mexico, D. F.

3) ANDERSON, J., B. L. WALDRON, B. MORENO-SEVILLA, R. W. STACK, and R. C. FROHBERG (1998) Detection of *Fusarium* head blight resistance QTL in wheat using AFLPs and RFLPs. In: SLINKARD, A. E. (ed.), Proceedings of the 9th International Wheat Genetics Symposium (Saskatoon, Canada), vol.1, pp.135-137. University Extension Press, Univ. of Saskatchewan, Saskatoon.

4) ANONYMOUS (1989) Special supplement: Workshop on Fusarium head blight and related mycotoxins, March 30-31 1989. In: KOHLI, M. M. (ed.), Taller sobre la fusariosis de la espiga en America del Sur, pp. 127-144. CIMMYT, Mexico, D. F.

5) ATANASSOFF, D. (1920) Fusarium blight (scab) of wheat and other cereals. *Journal of Agricultural Research 20* : 1-32.

6) ATANASSOV, Z., C. NAKAMURA, N. MORI, C. KANEDA, H. KATO, Y. JIN, T. YOSHIZAWA, and K. MURAI (1994) Mycotoxin production and pathogenicity of *Fusarium* species and wheat resistance to Fusarium head blight. *Canadian Journal of Botany 72*: 161-167.

7) BAI, G., C. ZHOU, C. QIAN, S. XIA, Y. GE, and Z. CHENG (1990) Genetic analysis on resistant genes to scab extension in wheat. In: Advances in researches on resistance to disease in major crops. pp. 171-177. Jiangsu Sci. & Tec. Pub., Jiangsu.

8) BAI, G., and G. SHANER (1994) Scab of wheat: Prospects for control. *Plant Disease 78*: 760-766.

9) BAN, T. (1991) Efficiency of 'cut-spike' inoculation method for evaluation of scab resistance in wheat. *Japanese Journal of Breeding 41* (*Suppl.2*): 404 (in Japanese).

10) BAN, T. (1992) Analysis of resistance to *Fusarium* head blight in wheat populations derived by single seed descent methods. *Japanese Journal of Breeding 42* (*suppl.2*): 390-391 (in Japanese).

11) BAN, T. (1997) Evaluation of resistance to *Fusarium* head blight in indigenous Japanese species of *Agropyron* (*Elymus*). *Euphytica 97*: 39-44.

12) BAN, T., and H. GOCHO (1988) Estimation of scab resistance in barley and wheat genetic resources. *Japanese Journal of Breeding 38* (*suppl.2*) : 410-411 (in Japanese).

13) BAN, T., and K. SUENAGA (1993) Genetic analysis of black awn character detected in *Triticum aestivum cv.* Oligo culm (preliminary report). *Japanese Journal of Breeding 43* (*suppl.2*): 108 (in Japanese).

14) BAN, T., and K. SUENAGA (1997) Inheritance of resistance to *Fusarium* head blight caused by *F. graminearum* in wheat. *Cereal Research Communications* 25: 727-728.

15) BAN, T., K. SUENAGA, and M. N. INAGAKI (1998) Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in wheat. II. Resistance of Japanese local variety 'Nobeokabouzu-komugi'. *Japanese Journal of Breeding 48* (*suppl.2*) : 258 (in Japanese).

16) BAN, T., K. SUENAGA, E. NIWATA, and S. KURODA (1996) Screening of RAPD markers associated with Pre-Harvest Sprouting resistance in wheat. In: NODA, K., and D. J. MARES (eds.), Pre-Harvest Sprouting in Cereals 1995, Abashiri, Japan, pp. 151-156. CASJO, Osaka.

17) BUERSTMAYR, H., M.LEMMENS, and P. RUCKENBAUER (1997) Chromosomal location of Fusarium head blight resistance genes in wheat. *Cereal Research Communications 25*: 731-732.

18) CHEN, W. P., P. D. CHEN, D. J. LIU, R. KYNAST, B. FRIEBE, R. VELAZHAHAN, S. MUTHUKRISHNAN, and B. S. GILL (1999) Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. *Theoretical and Applied Genetics 99*: 755-760.

19) CHHABRA, A. K., and S. K. SETHI (1991) Inheritance of cleistogamic flowering durum wheat (*Triticum durum*). *Euphytica 55*: 147-150.

20) CLARKE, D. D. (1986) Tolerance of parasites and disease on plants and its significance in host-prasite interactions. In: INGRAM, D. S., and P. H. WILLIAMS (eds.),Advances in Plant Pathology, Vol. 5, 161-197. Academic Press Inc., London.

21) DESJARDINS, A. E., R. H. PROCTOR, S. P. MCCORMIK, and T. M. HOHN (1997) Reduced virulemce of trichothecene antibiotics-nonproducing mutants of *Gibberella zeae* in wheat field tests. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAB (eds.), Fusarium Head

Scab: Global Status and Future Prospects, pp. 29-34. CIMMYT, Mexico, D. F.

22) DEWEY, D. R. (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: GUSTAFSON, J. P. (ed.), Gene Manipulation in Plant Improvement, pp. 209-279. Plenum Press, New York and London.

23) DICKSON, J. G., H. JOHANN, and G. WINELAND (1921) Second progress report on the fusarium blight (scab) of wheat. *Phytopathology 11*: 35.

24) DILL-MACKY, R. (1997) Fusarium head blight: Recent epidemics and research efforts in the upper midwest of the United States. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAB (eds.), Fusarium Head Scab: Global Status and Future Prospects, pp. 1-6. CIMMYT, Mexico, D. F.

25) DILL-MACKY, R., and R. K. JONES (1997) The effect of previous crops and tillage on *Fusarium* head blight of wheat. *Cereal Research Communications 25*: 711-712.

26) EHLING, G., A. COCKBURN, P. SNOWDON, and H. BUSCHHAUS (1997) The significance of the Fusarium toxin deoxynivalenol (DON) for human and animal health. *Cereal Research Communications 25*: 443-447.

27) ENRICO, M., P., L. GIANFRANCESCHI, G. TARAMINO, R. TARCHINI, P. ANGELINI, M. DANI, and G. BINELLI (1993) Mapping quantitative trait loci (QTLs) for resistance to *Gibberella zeae* infection in maize. *Molecular and General Genetics 241*: 11-16.

28) VAN EEUWIJK, F. A., Á. MESTERHÁZY, C. I. KLING, P. RUCKENBAUER, L. SAUR, H. BÜRSTMAYR, M. LEMMENS, L. C. P. KEIZER, N. MAURIN, and C. H. A. SNIJDERS (1995) Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum, F. graminearum* and *F.nivale* using a multiplicative model for infection. *Theoretical and Applied Genetics 90*: 221-228.

29) FEDAK, G., K. C. ARMSTRONG, R. C. SINHA, J. GILBERT, J. D. PROCUNIER, J. D. MILLER, and R. PANDEYA (1997) Wide crosses to improve Fusarium blight resistance in wheat. *Cereal Research Communications 25*: 651-654.

30) FUJITA, M., T. BAN, K. UJIHARA, and R. YOSHIKAWA (1988) Efficiency of scab resistance in wheat breeding. *Japanese Journal of Breeding 38 (suppl.2)*: 412-413 (in Japanese).

31) DE GALICH, M. T. V. (1997) Fusarium head blight in Argentina. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. McNAB (eds.), Fusarium Head Scab: Global Status and Future Prospects, pp. 19-28. CIMMYT, Mexico, D. F.

32) GEIGER, H. H., T. MIEDANER, and H. G. WELZ (1995) Genetic analysis of quantitative disease resistance. In: Proc. XIII Int. Plant Protection Congr. 2-7 July 1995. (The Hague, The Netherlands), *European Journal of Plant Pathology (Special issue)*, 805 (Abstr.).

33) GILCHRIST, L., S. RAJARAM, A. MUJEEB-KAZI, M. VAN GINKEL, H. VIVAR, and W. PFEIFFER (1997) Fusarium scab screening program at CIMMYT. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAB (eds.), Fusarium Head Scab: Global Status and Future Prospects, pp. 7-12. CIMMYT, Mexico, D. F.

34) GILBERT, J., T. AUNG, and J. D. PROCUNIER, K. (1997) Influence of the D genome in Fusarium head blight resistance. *Cereal Research Communications 25*: 647-649.

35) GOCHO, H. (1985) Wheat breeding for scab resistance. *Wheat Information Service 60*: 41.

36) GOCHO, H., T. HIRAI, and T. KASHIO (1992) Breeding of "Wheat Norin PL-4" a new germplasm with resistance to scab (*Gebberella zeae* (Schw.) Petch). *The Bulletin of Kyushu National Agricultural Experiment Station 27*: 317-331 (in Japanese with English summary).

37) GRIFFEY, C. A., J. CHEN, and T. PRIDGEN (1998) Assessment and selection for scab resistance in soft red winter wheat. In: SLINKARD, A. E. (ed.), Proceedings of the 9th International Wheat Genetics Symposium (Saskatoon, Canada), vol. 3, pp.273-275. University Extension Press, Univ. of Saskatchewan, Saskatoon.

38) HAKIMI, S., J. LIANG, Y. WU, C. ROSENBERGER, S. CASTRO, and D. SHAH (1997) Gene therapy for plants. *Cereal Research Communications 25*: 745-747.

39) HARRIS, L. J., A. E. DESJARDINS, R. D. PLATTNER, P. NICHOLSON, G. BUTLER, J. C. YOUNG, G. WESTON, R. H. PROCTOR, and T. M. HOHN (1999) Possible role of trichithecene mycotoxins in virulence of *Fusarium graminearum on maize. Plant Disease 83*: 954-960.

40) HIRAI, T., H. GOCHO, and T. KASHIO (1993) A new method for testing scab resistance by controlling heading time of standard wheat varieties. *The Bulletin of Kyushu National Agricultural Experiment Station 28*: 1-15 (in Japanese with English summary).

41) IRETA, M. J., and L. S. GILCHRIST (1994) Fusarium head scab of wheat (*Fusarium graminearum Schwabe*). Wheat Special Report No. 21b, CIMMYT, Mexico, D. F., P. 25.

42) ITO, H., H. INAMURA, S. ISHIKAWA, C. SUDO, and M. SHIBATA (1966) Pedigree of wheat cultivars. In: Agricultural administration investigative committee (ed.), System Agriculture Encyclopedia, Vol.2, pp. 180-181. Agricultural administration investigative committee, Tokyo (in Japanese).

43) JIANG, G. L., Z. X. CHEN, and Z. S. WU (1997) Comprehensive population improvement of scab resistance and agronomic trait in wheat. *Cereal Research Communications 25*: 663-665.

44) JONES, R. K. (1999) Quality parameters in small grains from Minnesota affected by Fusarium head blight. *Plant Disease 83*: 506-511.

45) KANATANI, R., and K. TAKEDA (1991) A method for mass sporulation in a scab disease pathogen (*Fusarium graminearum Schwabe*). *Nogaku Kenkyu 62*:177-189 (in Japanese with English summary).

46) KITANI, K., and Y. INOUE (1957) On the primary occurrence of the wheat scab and its developing process. *The Bulletin of Shikoku National Agricultural Experiment Station 3*: 125-138.

47) KOIZUMI, S., H. KATO, R. YOSHINO, N. HAYASHI, and M. ICHINOE (1991a) Distribution of causal Fusaria of wheat and barley scab in Japan. Annual *Phytopathology Society of Japan 57*:165-173.

48) KOIZUMI, S., H. KOMADA, H. KATO, R. YOSHINO, M. ICHINOE, and N. HAYASHI (1991b) Isolation of wheat and barley scab pathogens from wheat and barley field soil in Japan. *Annual Phytopathology Society of Japan 57*:263-267.

49) LI, Y., and Y. YU (1990a) A diallel analysis on resistance index to scab in seven wheat cultivars. In: Advances in Researches on Resistance to Diseases in Major Crops, pp. 185-192.

Jiangsu Sci. & Tec., Jiangsu (in Chinese with English summary).

50) LI, Y., and Y. YU (1990b) Monosomic analysis for scab resistance index in wheat cultivar "WZHHAS". In: Advances in Researches on Resistance to Diseases in Major Crops, pp. 193-196. Jiangsu Sci. & Tec., Jiangsu (in Chinese with English summary).

51) LIN, Y., Z. YANG, and Z. WU (1992) Genetic analysis of resistance to scab (*Gibberella zeae*) in wheat varieties from different regions. *Acta Agriculture Shanghai 8* :31-36.

52) LIU, Z. Z., and Z. Y. WANG (1991) Improved scab resistance in China: Sources of resistance and problems. In: SAUNDERS, D. A. (ed.), Wheat for the Nontraditional, Warm Areas, pp. 179-188. CIMMYT, Mexico, D. F.

53) MAULER-MACHNIK, A., and A. SUTY (1997) New findings on the epidemiology, importance and control of Fusarium ear blight on wheat. *Cereal Research Communications 25* : 705-709.

54) MCINTOSH, R., G. E. HART, and M. D. GALE (1993) Catalogue of gene symbols for wheat. In: LI, Z. S., and Z. Y. XIN (eds.), Proceedings of the 8th International Wheat Genetics Symposium (Beijing, China), pp. 1333-1500. China Agric. Sci. Press, Beijing.

55) MCMULLEN, M., R. JONES, and D. GALLENBERG (1997) Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease 81* : 1340-1348.

56) MESTERHÁZY, Á. (1983) Breeding wheat for resistance to *Fusarium graminearum and F. culmorum. Z Pflzüchtng 91* : 295-311.

57) MESTERHÁZY, Á. (1987) Selection of head blight resistant wheats through improved seedling resistance. *Plant Breeding 98* :25-36.

58) MESTERHÁZY, Á. (1989) Progress in breeding of wheat and corn genotypes not susceptible to infection by *Fusaria* . In: CHELKOWSKI, J. (ed.), *Fusarium* Mycotoxins, Taxonomy and Pathogenicity, pp. 357-386. Elsevier, Amsterdam.

59) MESTERHÁZY, Á. (1995) Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding 114* : 337-386.

60) MESTERHÁZY, Á. (1997) Methodology of resistance testing and breeding against *Fusarium* head blight in wheat and results of the selection. *Cereal Research Communications 25* : 631-637.

61) MESTERHÁZY, Á., and T. BARTOK (1997) Effect of chemical control on FHB and toxin contamination of wheat. *Cereal Research Communications 25* : 781-783.

62) MESTERHÁZY, Á., and K. KOVÁCS (1986) Breeding corn against Fusarial stalk rot, ear rot and seedling blight. *Acta Phytopathologica et Entomologia Hungarica 21* : 231-249.

63) MESTERHÁZY, Á., T. BARTOK, C. G. MIROCHA, and R. KOMORÓCZY (1999) Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breding. *Plant Breeding 118* : 97-110.

64) MIEDANER, T. (1997) Breeding wheat and rye for resistance to *Fusarium diseases. Plant Breeding 116* : 201-220.

65) MILLER, J. D., J. C. YOUNG, and D. R. SAMPSON (1985) Deoxynivalenol and Fusarium head blight resistance in spring cereals. *Journal of Phytophathology 113* : 359-367.

66) MILLER, J. D., and P. G. ARNISON (1986) Degradation of deoxynivalenol by suspension

cultures of the Fusarium head blight resistant wheat cultivar Frontana. *Canadian Journal of Plant Pathology 8*: 147-150.

67) MUJEEB-KAZI, A., M. BERNARD, G. T. BEKELE, and J. L. MIRANDA (1983) Incorporation of alien genetic information from *Elymus giganteus* into *Triticum aestivum*. In: SAKAMOTO, S. (ed.), Proceedings of the 6th International Wheat Genetics Symposium (Kyoto, Japan), pp. 223-231. Maruzen Co., Ltd., Kyoto.

68) MUJEEB-KAZI, A. (1995) Chapter 3 and 4, Interspecific Crosss: Hybrid production and Utilization. In: MUJEEB-KAZI, A., and G. P. HETTEL (eds.), Utilizing Wild Grass Biodiversity in Wheat Improvement:15 Years of Wide Cross Research at CIMMYT. CIMMYT Research Report No.2. : CIMMYT, Mexico, D. F., P. 140.

69) MURAMATSU, M. (1994) Triticeae and Bambuseae species distributed in Japan and their characteristics as genetic resources. *The Scientific Reports of the Faculty of Agriculture, Okayama University 83*: 65-90 (in Japanese with English summary).

70) MURAMATSU, M., S. KANETA, R. IKEDA, T. UETSUKI, and K. TAKAHASHI (1983) Hybridization of Japanese indigenous *Agropyron* (*Roegneria*) species with hexaploid wheat and cytogenetics of some of the F_1, BC_1 and amphiploid plants. In: SAKAMOTO, S. (ed.), Proceedings of the 6th International Wheat Genetics Symposium (Kyoto, Japan), pp. 1041-1048. Maruzen Co., Ltd., Kyoto.

71) NAITO, H., S. MOGI, H. GOCHO, and T. HIRAI (1984) A simple method for evaluation of varietal resistance to scab caused by *Gibberella zeae* Petch in wheat. *The Bulletin of Kyushu National Agricultural Experiment Station 23*: 355-386 (in Japanese with English summary).

72) NAKAGAWA, M. (1955) Studies on ear-scab resistance of wheat plants. 2. Genetical factors affecting on the inheritance of ear-scab disease of wheat *plants. Japanese Journal of Breeding 5* :15-22.

73) NAKAGAWA, M. (1956) Studies on Ear-scab resistance on wheat varieties. *Journal of Agricultural Researches in Tokai-Kinki Region 7* :58-61.

74) NISHIKADO, Y. (1958a) Studies on the control of wheat scab (I). *Nougaku-Kenkyu 45*: 59-86 (in Japanese).

75) NISHIKADO, Y. (1958b) Studies on the control of wheat scab (II). *Nougaku-Kenkyu 45*: 141-158 (in Japanese).

76) NISHIKADO, Y. (1958c.) Studies on the control of wheat scab (III). *Nougaku-Kenkyu 45*: 159-224 (in Japanese).

77) NIWATA, E., K. SUENAGA, and S. KURODA (1994) Segregation of RAPD markers among wheat doubled haploid lines derived from wheat x maize crosses. *Japanese Journal of Breeding 43* (*suppl.2*) : 197 (in Japanese).

78) NONAKA, S. (1983) History of wheat breeding in Japan. In: SAKAMOTO, S. (ed.), Proceedings of the 6th International Wheat Genetics Symposium (Kyoto, Japan), pp. 593-599. Maruzen Co., Ltd., Kyoto.

79) NONAKA, S., Y. YOSHIDA, S. KITAHARA, M. TSURU, S. TAYA, and H. ARAKI (1979) A New wheat cultivar 'Asakaze-komugi'. *The Bulletin of Kyushu National Agricultural*

Experiment Station. 20: 221-237 (in Japanese with English summary).

80) NYVALL, R. F., J. A. PERCICH, and C. J. MIROCHA (1999) Fusarium head blight of cultivated and natural wild rice (*Zizania palustris*) in Minnesota caused by *Fusarium graminearum and associated Fusarium spp. Plant Disease 83*:159-164.

81) OBST, A., L. J., GLEISSENTHALL, and R. BECK (1997) On the etiology of *Fusarium* head blight of wheat in south Germany-Preceding crops, wheather conditions for inoculum production and head infection, proneness of the crop to infection and mycotoxin production. *Cereal Research Communications 25*: 699-703.

82) OSADA T. (1989) Illustrated Grasses of Japan. Heibonsya Ltd., Tokyo (in Japanese).

83) PARRY, D. W., P. JENKINSON, and L. MCLEOD (1995) *Fusarium* ear blight (scab) in small grain cereals - a review. *Plant Pathology 44*: 207-238.

84) PARRY, D. W., P. JENKINSON, J. LIGGITT, A. HILTON, and J. A. CLEMENT (1997) Significance and control of Fusarium ear blight (scab) in winter wheat. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAB (eds.), Fusarium Head Scab: Global Status and Future Prospects. pp. 115-119. CIMMYT, Mexico, D. F.

85) DE LA PENA, R. C., K. P. SMITH, F. CAPETTINI, G. J. MUEHLBAUER, M. GALLO-MEAGHER, R. DILL-MACKY, D. A. SOMERS, and D. C. RASMUSSON (1999) Quantitative trait loci associated with resistance to Fusarium head blight and kernel discoloration in barley. *Theoretical and Applied Genetics 99*: 561-569.

86) PIENARR, R. V. De. (1990) Wheat x *Thinopyrum* hybrids. In: BAJAJ, Y. P. S. (ed.), Biotechnology of Agriculture and Forestry vol. 13b Wheat. Springer Verlag, Berlin, Heidelberg.

87) PUGH, G. W., H. JOHANN, and J. G. DICKSON (1933) Factors affecting infection of wheat heads by *Gibberella saubinetii. Journal of Agricultural Research, 46*: 771-797.

88) REIS, E. M. (1991) Integrated disease management-The changing concepts of controlling head blight and spot blotch. In: SAUNDERS, D. A. (ed.), Wheat for the Nontraditional, Warm Areas, pp. 165-177. CIMMYT, Mexico, D. F.

89) RÖDER, M. S., V. KORZUN, K. WENDEHAKE, J. PLASCHKE, M-H, TIXIER, P. LEROY, and M. W. GANAl (1998) A microsatellite map of wheat. *Genetics, 149*: 2007-2023.

90) SAKAMOTO, S., and M. MURAMATSU (1966) Cytogenetic studies in the tribe Triticeae. II. Tetraploid and hexaploid hybrids of *Agropyron. The Japanese Journal of Genetics 42*: 155-168.

91) SCHROEDER, H. W., and J. J. CHRISTENSEN, 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae. Phytopathology 53*: 831-838.

92) SETHI, S. K., and A. K. CHHABRA, (1990) Cleistogamy in wheat. *RACHIS* (*ICARDA*) *9*: 34-36.

93) SHARMA, H. C., and B. S. GILL (1983) Current status of wide hybridization in wheat. *Euphytica 32*: 17-31.

94) SHIBATA, M., and K. TAKEDA (1989) Tolerance to a fungicide 'Topsin' in barley head blight fungi (*Fusarium roseum*). *Japanese Journal of Breeding 39* (*Suppl.1*) : 190-191 (in

Japanese).

95) SINGH, R. P., and M. VAN GINKEL (1997) Breeding strategies for introgressing diverse scab resistances into adapted wheats. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAb (eds.), Fusarium Head Scab: Global Status and Future Prospects. pp. 86-92. CIMMYT, Mexico, D. F.

96) SINGH, R. P., H. MA, and S. RAJARAM (1995) Genetic analysis of resistance to scab in spring wheat cultivar Frontana. *Plant Disease 79*: 238-240.

97) SNIJDERS, C. H. A. (1990a) Diallel analysis of resistance to head blight caused by *Fusarium culmorum* in winter wheat. *Euphytica 50*: 1-9.

98) SNIJDERS, C. H. A. (1990b) The inheritance to head blight caused by *Fusarium culmorum in winter wheat. Euphytica 50*: 11-18.

99) SNIJDERS, C. H. A. (1990c) Response to selection in F_2 generation of winter wheat for resistance to head blight caused by *Fusarium culmorum. Euphytica 50*: 163-169.

100) S*nijders*, C. H. A. (1990d) Genetic variation for resistance to Fusarium head blight in bread wheat. *Euphytica 50*: 171-179.

101) SNIJDERS, C. H. A. (1990e) Fusarium head blight and mycotoxin contamination of wheat, a review. *Netherlands Journal of Plant Pathology* pp. 187-198.

102) SNIJDERS, C. H. A., and C. F. KRECHTING (1992) Inhibition of deoxynivalenol translocation and fungal colonization in Fusarium head blight resistant wheat. *Canadian Journal of Botany 70*: 1570-1576.

103) SNIJDERS, C. H. A., and J. PERKOWSKI (1990) Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. *Phytopathology 80*: 566-570.

104) SNIJDERS, C. H. A., and F. A. VAN EEUWIJK (1991) Genotype x strain interactions for resistance to *Fusarium* head blight caused by *Fusarium culmorum* in winter wheat. *Theoretical and Applied Genetics 81*: 239-244.

105) SPJOTVOLL, E., and M. R. STOLINE (1973) T-method of multiple comparison to include the cases with unequal sample sizes. *Journal of the American Statistical Association 68*: 976-978.

106) STACK, R. W., R. C. FROHBERG, and H. CASPER (1997) Reaction of spring wheats incorporating Sumai# 3-derived resistance to inoculation with seven *Fusarium species.Cereal Research Communications 25*: 667-671.

107) Statistics and Information Department, Ministry of Agriculture, Forestry and Fisheries (1964) The 40 th Statistical yearbook of Ministry of Agriculture, Forestry and Fisheries, Japan, 1962-'63. pp. 118-125. Association of Agriculture and Forestry Statistics, Tokyo.

108) Statistics and Information Department, Ministry of Agriculture, Forestry and Fisheries (1999) The 74 th Statistical yearbook of Ministry of Agriculture, Forestry and Fisheries, Japan, 1997-'98. Association of Agriculture and Forestry Statistics, Tokyo (in press).

109) SUENAGA, K., and K. NAKAJIMA (1989) Efficient production of haploid wheat (*Triticum aestivum*) through crosses between Japanese wheat and maize (*Zea mays*). *Plant Cell Reports 8*:263-266.

110) SUENAGA, K., A. R. MORSHEDI, and N. L. DARVEY (1997) Haploid production of Australian

wheat (*Triticum aestivum* L.) cultivars through wheat x maize (*Zea mays* L.) cross. *Australian Journal of Agricultural Research 48*:1207-1211.

111) TAKATA, K., and M. MURAMATSU (1992) Genetic studies on the Triticeae, Gramineae II. Production of disomic addition lines of *Elymus ciliaris chromosomes. Japanese Journal of Breeding 42* (*Suppl.2*) : 368- 369 (in Japanese).

112) TAKEDA, K., and H. HETA (1989) Establishing the testing method and search for the resistant varieties to Fusarium head blight in barley. *Japanese Journal of Breeding 39*: 203-216 (in Japanese with English summary).

113) TAKEDA, K., R. KANATANI, and C. L. ZHANG (1992) Varietal variation of the scab disease resistance in wheat (*Triticum aestivum*) evaluated by the 'cut-spike' inoculation method. *Japanese Journal of Breeding 42*: 649-656.

114) TANAKA, T., A. HASEGAWA, S. TAMAMOTO, U. S. LEE, Y. SUGIURA, and Y. UENO (1988) Worldwide contamination of cereals by *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. *Journal of Agricultural and Food Chemistry 36*: 979-983.

115) UENO, Y., N. SATO, K. ISHII, K. SAKAI, H. TSUNODA, and M. ENOMOTO (1973) Biological and chemical detection of trichothecene mycotoxins of *Fusarium* Species. *Applied Microbiology 25*: 699-704.

116) UKAI, Y., R. OHSAWA, A. SAITO, and T. HAYASHI (1995) MAPL: A package of computer programs for construction of DNA polymorphism linkage maps and analysis of QTL. *Breeding Science 45*: 139-142.

117) VAN GINKEL, M., W. VAN DER SCHAAR, and Z. YANG (1996) Inheritance of resistance to scab in two wheat cultivars from Brazil and China. *Plant Disease 80*: 863-867.

118) WANG, Y. Z. (1997) Epidemiology and management of wheat scab in Chaina. In: DUBIN, H. J., L. GILCHRIST, J. REEVES, and A. MCNAB (eds.), Fusarium Head Scab: Global Status and Future Prospects, pp. 29-34. CIMMYT, Mexico, D. F.

119) WANG, Y. Z., and J. D. MILLER (1988) Screening techniques and sources of resistance to Fusarium head blight. In: KLATT, A. R. (ed.), Wheat Production Constraints in Tropical Environments. pp. 239-250. CIMMYT, Mexico, D. F.

120) WENG, Y., and D. LIU (1989) Morphology, scab resistance and cytogenetics of intergeneric hybrids of *Triticum aestivum* L. with *Roegneria* c. Koch (*Agropyron*) species. *Scientia Agric. Sinica 22*: 1-7 (in Chinese with English summary).

121) WENG, Y. Q., L. F. WU., P. D. CHEN, and D. J. LIU (1993) Development of alien addition lines of wheat scab resistance from *Roegneria kamoji* C. Koch. In: LI, Z. S., and Z. Y. XIN (eds.), Proceedings of the 8th International Wheat Genetics (Beijing, China), pp. 365-368. China Agric. Sci. Press, Beijing.

122) WU, J., and K. TAKEDA (1992) Further report on the tolerance to a fungicide 'Topsin' in barley head blight fungus (*Fusarium graminearum* Schwabe). *Japanese Journal of Breeding 42* (*Suppl.1*) : 586-587 (in Japanese).

123) YANG, Z. (1994) Breeding for resistance to Fusarium head blight of wheat in the mid-to lower

Yangtze River Valley of China. Wheat Special Report No. 27. CIMMYT, Mexico, D. F., P. 16.

124) YOSHIDA, H., M. KAMIO, and K. KAWAGUCHI (1983) Evaluation of cultivars for early matureness in the Japanese wheat breeding. In: SAKAMOTO, S. (ed.),Proceedings of the 6th International Wheat Genetics Symposium (Kyoto, Japan), pp. 601-612. Maruzen Co., Ltd., Kyoto.

125) YOSHIZAWA, T.,and Y. JIN (1995) Natural occurrence of acetylated derivatives of deoxynivalenol and nivalenol in wheat and barley in Japan. *Food Additives and Contaminants 12*: 689-694.

126) YU, Y. J. (1982) Monosomic analysis for scab resistance and yield components in the wheat cultivar Soo-moo-3. *Cereal Research Communications 10*:185-190.

127) YU, Y. (1990) Genetic analysis for scab resistance in four wheat varieties PHJZM, HHDTB, CYHM and YGFZ. In: Advances in Researches on Resistance to Diseases in Major Crops, pp. 197-205. Jiangsu Sci. & Tec., Jiangsu (in Chinese with English summary).

コムギ赤かび病抵抗性の遺伝学的研究

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要　　旨

Fusarium 菌がイネ科植物の開花期に穂を侵す赤かび病は，コムギにおいても出穂後に温暖で湿潤な環境条件になる世界中のほとんどの地域で例外なく発生し，甚大な被害を引き起こす重用病害である。赤かび病の防除には耕種的防除や農薬による防除が試みられているが，抵抗性品種を育成するのが最も経済的で効果的である。近縁野生種を含めコムギ品種の中では赤かび病の抵抗性程度に差が認められるが，いずれも環境によって左右される量的な抵抗性であり免疫性を示すものは見つかっていない。これまでに選抜されている抵抗性の品種も，降水量の多い年では赤かび病の大きな被害に遭い実用的に問題がある。更に高度抵抗性のコムギ品種を育成するためにはより有効な遺伝資源を探索する必要がある。一方で，赤かび病は気象条件により日和見的な発生をするため抵抗性を効率的に選抜するのが難しく，また抵抗性の遺伝様式が明らかにされていないため抵抗性品種の育成が大きく立ち遅れている。そのため本研究では，抵抗性品種育成に役立てるため，コムギ赤かび病抵抗性の遺伝学的研究を行った。

より有効な遺伝資源を探索するために，日本産のカモジグサから赤かび病抵抗性に有効な遺伝資源を探索して抵抗性を調べた。ミズタカモジ，カモジグサ，アオカモジ，タリホノオオタチカモジの４種の日本産カモジグサを採集し，赤かび病菌の*Fusarium graminearum* を開花期に接種して，赤かび病の侵入に対する抵抗性（TypeⅠ）と穂軸への菌糸の伸展に対する抵抗性（TypeⅡ）を検定した。その結果，TypeⅠについてミズタカモジのAG.91-35とアオカモジのAG.91-24が，コムギ抵抗性品種の延岡坊主小麦や蘇麦３号 と同等以上の抵抗性を示した。またタリホノオオタチカモジを除く全ての日本産カモジグサは，TypeⅡに関してもコムギ抵抗性品種と同等の抵抗性を示し，特にほとんどのミズタカモジが高度抵抗性で穂軸への赤かび病の伸展が全く見られなかった。供試した材料がTypeⅠとTypeⅡの抵抗性について異なった反応を示すことから，両抵抗性は異なった遺伝子により調節されているものと考えた。

次に，抵抗性品種の蘇麦３号とその派生系統である西海165号の赤かび病抵抗性の遺伝分析を行うために，今までに抵抗性程度が明らかになっているコムギの16品種を標準にして，人工降雨装置を用いた圃場検定法と赤かび病被害に対する反応を評価する判定基準を設定した。この方法を用いて，標準の16品種では赤かび病被害程度からその抵抗性レベルを評価することが可能であり，遺伝分析に適用できる検定法が確立できた。

コムギが赤かび病に対して量的な抵抗性を示すために個体レベルでの遺伝分析が困難であり，本研究では蘇麦３号と西海165号を抵抗性親にして育成した半数体倍加系統（DHLs）と組換型近交系統（RILs）を用いて抵抗性の遺伝様式を調べた。そして抵抗性遺伝子の連鎖分析を行った。蘇麦３号（抵抗性；極強～強）とGamenya（極弱）およびEmblem（極弱）のF_1から育成したDHLsは，赤かび病に対する反応（抵抗性R，中間型MR，罹病性S）について１：２：１に分離しており，このことから蘇麦３号の抵抗性が相加的な効果を持つ２個の主働遺伝子に支配されることを明らかにした。これらの組合せのDHLsでは，赤かび病に対する反応と芒の有無とに関連が認められ，蘇麦３号の抵抗性遺伝子の１つが５A染色体の長腕に座乗する*B1* 遺伝子と相反の関係で，組換価15.1±3.3%ないしは 21.4±4.3%で連鎖することを明らかにした。西海165号とEmblemのF_1から単粒系統法で育成したRILsの分離は，1R：２MR：１Sの分離比から歪み，３遺伝子支配のモデルである７（R＋MR）：１Sの分離比に適合していた。このことから西海165号は赤かび病抵抗性に関して３個の主働遺伝子をもつと結論した。

更に微働遺伝子の効果を評価するためにフクホコムギ（抵抗性；やや弱MS）とOligoculm（極弱VS）のF_1から育成したDHLsを材料に，random amplified polymorphic DNA（RAPD）を染色体地図情報の分子マーカーとして用い，赤かび病に対するTypeⅡの抵

抗性に関与する量的遺伝子座（QTL）の分析を試みた。110系統のDHL集団の中で26系統が両親よりも抵抗性の強（R），やや強（MR），中（M）である超越分離が認められ，一方，罹病性親のOligo culmよりも病徴が激しい７系統が分離した。このことから，この組み合わせではTypeⅡの抵抗性に関して両親が１～２個の異なる遺伝子をもつと考えた。65個のRAPDマーカーを用いたQTL分析の結果，TypeⅡの抵抗性に関与するOPZ-10 $_{680}$, OPAG-18 $_{340}$, OPAF-06 $_{345}$および OPW-13 $_{435}$の４個のマーカーが個別にt検定により選び出された。そのうち３個のRAPDマーカーは，それぞれ6.4%および11.8%の組換価で連鎖していた。この３個のRAPDマーカーが座乗する染色体には赤かび病のTypeⅡの抵抗性に関与するQTL領域があると考えられた。そして，今回選抜したRAPDマーカーを用いて，フクホコムギが潜在的にもつ罹病性の修正遺伝子を説明できることを示した。

最後に，本研究の結果とこれまでのコムギ赤かび病抵抗性に関する報告をまとめて赤かび病の病徴および発病現象とコムギの抵抗性メカニズムについてのモデルを提唱し，実用的な赤かび病抵抗性のコムギ品種育成を加速するための，遺伝学的な研究の方向について考察を行った。

キーワード：Triticum aestivum L，赤かび病、Fusarium graminearum，Elymus，半数体倍加系統，組換型近交系，抵抗性遺伝子，ＱＴＬ解析。