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Variations for Fusarium head blight resistance associated with genomic diversity in different sources of the resistant wheat cultivar 'Sumai 3'

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a serious disease of wheat (*Triticum aestivum* L.) associated with contamination by the mycotoxin deoxynivalenol (DON). The FHB-resistant wheat cultivar 'Sumai 3' has been used extensively around the world. The existence of variation in FHB resistance among 'Sumai 3' accessions has been discussed. In this study, genetic variation among 'Sumai 3' accessions collected from six countries were identified using SSR markers; our results demonstrate unique chromosome regions in Sumai 3-AUT and Sumai 3-JPN ('Sumai 3' accessions from Austria and Japan, respectively). Field evaluation indicated strong resistance to FHB in Sumai 3-AUT. The polymorphic rate (number of polymorphic markers/number of available markers × 100) based on a DArT array was 12.5% between the two 'Sumai 3' accessions. Genotyping for DNA markers flanking FHB-resistant quantitative trait loci (QTLs) revealed genetic variations for the QTL regions on 5AS and 2DS; however, no variation was observed for the QTL regions on 3BS and 6B. Thus, the variation in FHB resistance among 'Sumai 3' accessions in the field is due to genetic diversity.

Key Words: Fusarium head blight, wheat, genotyping.

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is one of the most destructive diseases of wheat (*Triticum aestivum* L.). FHB causes critical yield losses and serious problems with food hygiene due to contamination with mycotoxins such as trichothecenes (*e.g.*, nivalenol or deoxynivalenol [DON]), T-2 toxin, and zearalenone (Bai and Shaner 1994, McMullen *et al.* 1997, Wegulo 2012). DON is a major threat to animal production and human health. The Joint FAO/WHO Expert Committee on Food Additives has conducted risk assessments of various mycotoxins, and specific maximum levels have been determined for DON contamination in several regions and countries. The use of host plant resistance is the most economical and environmentally sound method for solving the agricultural and food hygiene problems posed by mycotoxins (Zhang *et al.* 2008). Therefore, utilizing resistant wheat varieties will help to control FHB. Resistance to FHB in wheat has been classified into three types: resistance to the initial infection (type 1), resistance to spreading within a spike (type 2) (Schroeder and Christensen 1963), and resistance to mycotoxin degradation (type 3) (Miller *et al.* 1986). Considerable effort has been expended by wheat breeders and researchers to determine the level of resistance to FHB in wheat to identify appropriate cultivars for crossing; the cultivars studied include 'Sumai 3' (Bai and Shaner 2004) and 'Wangshuibai' (Lin *et al.* 2004, 2006, Zhou *et al.* 2004) from Asia for type 2 resistance, 'Nobeokabouzu-komugi' from Asia for type 1 resistance (unpublished data), and 'Frontana' (Steiner *et al.* 2004) from South America for type 3 resistance. The inheritance of FHB resistance in wheat is complex; numerous quantitative trait loci (QTLs) affecting FHB resistance have been identified in wheat (Buerstmayr *et al.* 2009, Holzapfel *et al.* 2008). Accumulation of these resistant alleles at QTLs is the most practical approach for enhancing FHB resistance in locally adapted cultivars.

The FHB-resistant spring wheat cultivar 'Sumai 3' was developed in China by crossing two moderately susceptible parents, 'Funo' and 'Taiwanxiaomai' (Bai and Shaner 1994). This phenomenon could explain that FHB resistance was accomplished by transgressive segregation using an appropriate combination of alleles at QTLs (Schweiger *et al.* 2013, Suzuki *et al.* 2012). In other words, a single QTL associated with FHB resistance might not help to enhance it (Pumphrey *et al.* 2007). 'Sumai 3' exhibits high-level FHB resistance and other important agronomic traits (Bai and Shaner 1994); therefore, it has been utilized, along with its

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derivatives, in breeding programs globally. The alleles at QTL responsible for FHB resistance in 'Sumai 3' are located on chromosomes 3BS, 5AS, and 6B (Anderson *et al.* 2001, Buerstmayr *et al.* 2002, 2003, Cuthbert *et al.* 2007). The locus on 3BS (syn. *Fhb1*) has been shown to have the greatest effect on type 2 resistance. The QTL on 3BS may encode a DON-glucosyl-transferase or regulate the expression of such an enzyme (Lemmens *et al.* 2005). The QTL on 5A (*Qfhs.ifa-5A*) derived from 'Sumai 3' may confer type 1 resistance rather than type 2 resistance (Buerstmayr *et al.* 2003). Anderson *et al.* (2001) indicated that the QTLs on 3B and 6B were responsible for FHB resistance in 'Sumai 3'. Cuthbert *et al.* (2007) identified *Fhb2* as one gene controlling FHB field resistance on chromosome 6BS. DNA markers located on chromosomes 3BS and 5AS have been extensively used for QTL pyramiding in breeding programs (Anderson 2007). Due to the lack of full marker coverage in wheat D genome maps, few QTLs have been reported for the D genome (Buerstmayr *et al.* 2009) and none have been reported for 'Sumai 3'.

Using pentaploids generated from reciprocal crosses between 'Sumai 3' and FHB-susceptible tetraploids, Gilbert *et al.* (2000) reported that the D genome of 'Sumai 3' did not contribute to FHB resistance. Zhou *et al.* (2002) stated that the D genome from 'Sumai 3' increased the DON concentration by evaluating substitution lines derived from crosses between monosomic Chinese Spring lines with 'Sumai 3'. Furthermore, 'Sumai 3' has been reported to have a susceptible allele at QTL (*Qfhs.kibr-2DS*) on 2DS (Basnet *et al.* 2012, Handa *et al.* 2008). Handa *et al.* (2008) used a comparative genomic approach to identify QTL on 2DS, the alleles of which control susceptibility to FHB in 'Sumai 3'; they identified a candidate gene encoding a multidrug resistance-associated protein (MRP) in the susceptible cultivar 'Gamenya'. These reports indicate that the descendants of 'Sumai 3' might also include the susceptible factor. To enhance FHB resistance through QTL pyramiding, susceptible alleles on QTL should be eliminated.

Gilbert *et al.* (2000) reported possible residual heterozygosity in 'Sumai 3' seed stocks, while Bai *et al.* (2003) showed a difference (0.8%) by DNA fingerprinting between two individual 'Sumai 3' accessions stored in the US (Aberdeen, ID) and Nanjing, China. Given the suggested variation among 'Sumai 3' accessions, the results of QTL analysis should be questioned. However, it remains unclear whether the reported genetic variation in 'Sumai 3' confers FHB resistance. Thus, in this study, genotypic variations in 'Sumai 3' accessions from six countries were detected using simple sequence repeat (SSR) markers, and a field evaluation of FHB resistance was carried out using 'Sumai 3' accessions of different origins. Further, the relationship between variations in FHB resistance and genetic diversity was examined based on the genotypes at the QTLs for FHB resistance detected in 'Sumai 3'.

Materials and Methods

Plant materials

Japanese 'Sumai 3' (hereafter, Sumai 3-JPN; Accession No. 21770, Conservation ID 24141) was obtained from the National Institute of Agrobiological Sciences Genebank MAFF (Ibaraki, Japan). Morphologic segregation of anthocyanin pigmentation in the coleoptiles and anthers was observed in Sumai 3-JPN (unpublished data). Plants with green coleoptiles and yellow anthers did not segregate these colors in their offspring, while other plants with purple coleoptiles and anthers segregated colors at 3 : 1 (purple : yellow) in the next generation. The segregated Sumai 3-JPN was separated and fixed by purifying selection at the National Agriculture and Food Research Organization/Kyushu Okinawa Agricultural Research Center (NARO/KARC). Segregants with green coleoptiles and yellow anthers were named Sumai 3-JPNy, and those with purple coleoptiles and anthers were named Sumai 3-JPNp (Supplemental Fig. 1). Other 'Sumai 3' accessions originating from Austria, Canada, China, Iran and the US (Sumai 3-AUT, -CAN, -CHN, -IRN and -USA, respectively) were obtained from the Scab Resistance Screening Nursery (SRSN) coordinated by Dr. L. Gilchrist (CIMMYT) (Table 1). The standard cultivars 'Gamenya' (susceptible) and 'Nobeokabouzu-komugi' (resistant) obtained from the NARO/KARC (Fukuoka, Japan) were used for disease evaluation and DNA fingerprinting (Table 1).

Phenotypic evaluation of FHB resistance in the 'Sumai 3' accessions

As a preliminary study, three 'Sumai 3' accessions (Sumai 3-AUT, -JPNy, and -JPNp), 'Gamenya', and 'Nobeokabouzu-komugi' were evaluated for FHB resistance at CIMMYT in 2006 according to the procedure of Handa *et al.* (2008) with the following modifications. A field experiment was conducted by randomized block experiments with three replications (plots) in the experimental field of CIMMYT, El Batan, Mexico in 2006. Plots were inoculated with the help of precision CO_2 backpack sprayers for liquid inoculum (50,000 conidia/ml) at a rate of 39 ml per meter. A programmable misting system maintains a humid microclimate, which is favorable for disease development, working from 9 am to 8 pm, with 10 minutes of spraying every hour. Disease notes were taken 1 month (31 days) after inoculation. The average number of infected spikelets out of ten was defined as the incidence, and the absolute number of infected spikelets per spike was used to indicate the severity of disease. DON contents (ppm) of the yielded seeds were analyzed by ELISA and the weight of 100 grains in each line was measured to obtain its proportion per grain (ng/grain). Each trait observed among the three Sumai 3 accessions was conducted by ANOVA and the Tukey-Kramer multiple-comparison test. In the subsequent FHB evaluation, a field experiment was carried out in 2009 and 2010 at NARO/KARC using 'Sumai 3' accessions (Sumai

Table 1. Origins of the wheat sources used for the FHB field tests and genotyping

Name of accession	Origin	Stock information	SSR genotyping[a]	FHB field evaluation[a]			Genotypic evaluation[a]	
				CIMMYT 2006	NARO/ KARC 2009	NARO/ KARC 2010	DArT	FHB related
Sumai 3-AUT	Austria	MV-99 VTESTFUS from IFA (Hungarian origin)	+	+	+	+	+	+
Sumai 3-CAN	Canada	Y99-00 INT-98 from CRC-AAFC	+					
Sumai 3-CHN	China	MV-99 VTEATFUS from CIMMYT, Fusarium program	+					
Sumai 3-IRN	Iran	MV-99 SELFFUSIN from Gorgan, Iran	+					
Sumai 3-JPN	Japan	No. 21770 Conservaiton ID 24141 from NIAS Genebank MAFF	+**	+**	+*	+*	+*	+*
Sumai 3-USA	USA	Y99-00 INT-98 from Univ. of Minnesota	+					
Gamenya	Japan	NARO/KARC, Japan	+	+	+	+	+	
Nobeokabouzu-komugi	Japan	NARO/KARC, Japan	+	+	+	+	+	

[a] +, Sources of the wheat used in this study.
*, Sumai 3-JPNy.
**, Sumai3-JPNy and -JPNp.

3-AUT and -JPNy) and the references 'Gamenya' and 'Nobeokabouzu-komugi'. The field experiment was conducted using randomized blocks with two replications (plots) in a field at NARO/KARC. To induce FHB infection, we conducted grain spawn and spray inoculations using liquid inoculum (200,000 conidia/ml) at a rate of 60ml per plot. To induce FHB proliferation, we used sprinkler irrigation to water the field for 2 min at 30–45 min intervals between 8:30–18:00 and at 60 min intervals between 18:00–8:30. The FHB symptom was scored as the severity of FHB (infection + spread) 3 and 4 weeks after inoculation (WAI), and the ratio of *Fusarium*-damaged kernels (FDK) and DON concentration in wheat grains were calculated according to Kubo *et al.* (2012). LC/MS/MS was performed for the wheat samples in 2010 to obtain more precise measurements of the DON concentration. Each trait observed between Sumai 3-AUT and -JPNy was analyzed by t-test in respective years.

Genotyping of the 'Sumai 3' accessions using DNA markers

Genomic DNA was extracted from the wheat accessions by the CTAB method (Murray and Thompson 1980) with modifications, and genotyped by PCR to identify genetic polymorphisms. Genotyping was conducted using SSR markers to detect allelic differences and Diversity Arrays Technology (DArT) for genome-wide analysis by DArT Pty. Ltd. (Yarralumla, ACT, Australia). In addition, specific DNA markers linked to FHB-related QTLs were applied to examine the relationship with FHB resistance. SSR-based DNA fingerprinting was used to characterize the allelic differences among 'Sumai 3' (Sumai 3-AUT, -CAN, -CHN, -IRN, -JPNy, -JPNp and -USA) with 'Gamenya' and 'Nobeokabouzu-komugi' as references. A total of 242 SSR markers were used, including Gwm (Röder *et al.* 1998) and BARC (Song *et al.* 2005), which were distributed throughout the wheat genome according to a linkage map of doubled haploid lines derived from the F_1 cross of 'Sumai 3' and 'Gamenya' (Handa *et al.* 2008) or their reported position. The DArT-GBS array (version 1.0) was applied to the selected 'Sumai 3' accessions (Sumai 3-JPNy and -AUT) based on a previous field evaluation. The DArT-GBS array was performed as described by Akbari *et al.* (2006) and Wenzl *et al.* (2004). The presence or absence of each marker was determined on the basis of signals from labeling and image analyses. The DArT marker data were assigned a value of 1 or 0 (*i.e.*, present or absent), as described by Akbari *et al.* (2006). To identify genetic polymorphisms associated with FHB resistance, flanking markers with FHB-related QTLs were selected and applied to the selected 'Sumai 3' accessions (Sumai 3-AUT and -JPNy) with 'Gamenya' and 'Nobeokabouzu-komugi' as references. Flanking DNA markers at QTLs on chromosomes 3BS, 5AS, and 6B detected in 'Sumai 3' were utilized. Simultaneously, the QTL on 2DS, where 'Sumai 3' had susceptible alleles, was also surveyed. The selected markers included UMN 10 from 3BS (Liu *et al.* 2008), and *Xbarc186*, *Xbarc180*, *Xgwm304*, *Xwmc705*, and *Xgwm293* from 5A (Buerstmayr *et al.* 2002, 2003, Kollers *et al.* 2013). *Xgwm133*, *Xwmc179*, *Xwmc398*, *Xwmc397*, *Xbarc101*, and *Xgwm644* were from 6B (Anderson *et al.* 2001, Cuthbert *et al.* 2007, Shen *et al.* 2003). Four SSR markers (*Xgwm261*, *Xwmc503*, *Xwmc112*, and *Xwms0815*) and three sequence-tagged site (STS) markers (J06R, *TaMRP-D1*, and J06F) located on 2DS QTL were applied (Handa *et al.* 2008, He *et al.* 2013, unpublished data). MRP was identified as a candidate 2DS locus; thus, full-length MRP cDNA (*TaMRP-D1*) from 'Gamenya' and 'Sumai 3' was isolated and used to design specific primers (unpublished data). The STS marker *TaMRP-D1* was utilized to distinguish 'Sumai 3' (susceptible allele) from 'Gamenya' (resistant allele).

Table 2. Number of SSR markers showing polymorphisms compared with Sumai 3-CHN as the original

Chromosome	No. of markers	Sumai 3 accessions						Nobeokabouzu-komugi	Gamenya
		USA	CAN	IRN	AUT	JPNy	JPNp		
1A	8	–	–	–	2	–	1	2	4
1B	13	–	–	–	1	–	–	2	9
1D	9	–	–	–	–	–	1	3	4
2A	18	–	–	–	6	–	6	3	12
2B	18	–	–	–	1	–	3	5	11
2D	18	–	–	–	6	–	7	6	7
3A	10	–	–	–	2	–	3	5	6
3B	19	–	–	–	1	–	2	2	12
3D	9	–	–	–	–	–	–	3	4
4A	10	–	–	–	1	–	–	4	5
4B	4	–	–	–	–	–	–	1	–
4D	2	–	–	–	–	–	–	–	–
5A	21	–	–	–	2	1	11	8	13
5B	22	–	–	–	6	–	–	11	13
5D	7	–	–	–	2	–	1	–	4
6A	8	–	–	–	1	–	2	2	5
6B	8	–	–	–	–	–	4	–	3
6D	14	–	–	–	1	–	4	3	5
7A	12	–	–	–	–	–	1	4	7
7B	7	–	–	–	–	–	1	2	3
7D	5	–	–	–	–	–	–	1	3
Total	242	0	0	0	32	1	47	67	130
Polymorph (%)		0.0	0.0	0.0	13.2	0.4	19.4	27.7	53.7

Results

Characterization of allelic differences in 'Sumai 3' accessions of different origins

DNA fingerprinting with SSR markers was used to characterize the allelic differences among 'Sumai 3' accessions of different origins by comparing the estimated number of detected polymorphic markers to that for Sumai 3-CHN. The alleles in Sumai 3-USA, -CAN, and -IRN were identical to those in Sumai 3-CHN, whereas significant genetic variation was observed in Sumai 3-AUT and -JPNp (Table 2). Notably, a high polymorphic rate was found in Sumai 3-AUT and -JPNp (13.2 and 19.4%), respectively; a much lower rate was found in Sumai 3-JPNy (0.4%) (Table 2). A chromosome-wise comparison found strong variation on 5A in Sumai 3-JPNp and on 5B in Sumai 3-AUT (Table 2). Clear allelic variation was identified among the 'Sumai 3' accessions; in particular, Sumai 3-AUT and -JPNp showed high-level variation.

Phenotypic evaluations of FHB resistance in 'Sumai 3' accessions

A significant difference in DON accumulation, converted by the amount in one grain (ng/ grain), was observed among three Sumai 3 accessions; however, no significant differences was detected in disease incidence, severity and DON accumulation, calculated as ppm (Table 3). In a subsequent experiment, Sumai 3-AUT and -JPNy were evaluated for FHB resistance in the field at NARO/KARC over a two-year period. Sumai 3-AUT had significantly fewer FDK than Sumai 3-JPNy in the 2009 trial (Table 4). A significant difference in symptoms was also observed between Sumai 3-AUT and -JPNy in 3 and 4 WAI. The level of resistance exhibited by Sumai 3-AUT was comparable to that exhibited by 'Nobeokabouzu-komugi' during the experimental period in both years (Table 4).

Table 3. Field evaluation of the incidence, severity and DON accumulation at CIMMYT in 2006

	Incidence	Severity	DON (ppm)	DON (ng/grain)
Sumai 3-JPNy	50.5	5.2	0.6	15.4 a
Sumai 3-JPNp	36.7	6.7	0.1	2.4 b
Sumai 3-AUT	45.6	5.9	0.2	4.0 b
Nobeokabouzu-komugi	80.0	10.8	0.4	6.0
Gamenya	100.0	76.1	9.7	106.0

Values within a column followed by different letters are significantly different according to the Tukey-Kramer multiple-comparison test among the three 'Sumai 3' accessions ($P < 0.01$).

Genotypic characterization of Sumai 3-AUT and -JPNy

A total of 10,832 DArT markers were utilized in this study. These markers revealed genome-wide genetic variation between Sumai 3-AUT and -JPNy (12.5%; Table 5). Additional specific markers linked to FHB-related QTLs identified genetic variations between Sumai 3-AUT and -JPNy. Eighteen markers (except *Xgwm133*) were used to identify genetic polymorphisms (Table 6). Polymorphisms in FHB-related markers were identified on 5AS (*Qfhs.ifa-5A*) and 2DS (*Qfhs.kibr-2DS*), although no variation was observed in 3BS (*Fhb1*) or 6B (*Fhb2*) (Table 6). Only two markers (*Xgwm304* and *Xgwm293*) showed polymorphisms on chromosome 5AS (Table 6). Regarding the QTL region on 2DS, where 'Sumai 3' was reported to have a susceptible

Table 4. Field evaluation of the FHB symptom score (3 and 4 WAI), percentage of FDK, and DON accumulation at the NARO/KARC between 2009 and 2010

	2009				2010			
	3 WAI[a]	4 WAI	FDK[b] (%)	DON[c] (ppm)	3 WAI	4 WAI	FDK (%)	DON (ppm)
Sumai 3-JPNy	1.3	3.0	61.9	8.1	3.3	4.7	47.3	13.5
Sumai 3-AUT	0.2*	1.0**	24.2*	1.1	1.0**	2.5*	22.5	10.1
Nobeokabouzu-komugi	0.2	1.0	23.2	0.6	0.2	4.0	26.3	11.0
Gamenya	7.5	8.7	95.8	15.8	8.2	8.7	84.7	38.2

A *t*-test was conducted to data for Sumai 3-AUT and -JPNy in respective years.
[a] FHB symptom score at 3 weeks after inoculation.
[b] *Fusarium*-damaged kernels.
[c] Deoxynivalenol concentration. It was assessed by ELISA in 2009 and by LC/MS/MS in 2010.
* and ** show significant difference between Sumai 3-AUT and -JPNy ($P < 0.05$ and $P < 0.001$, respectively).

Table 5. Estimation of the polymorphic ratio using DArT markers. The polymorphic ratio was calculated as the number of polymorphic markers/the number of available markers × 100

	Sumai 3-JPNy	Sumai 3-AUT	Gamenya	Nobeokabouzu-komugi
Sumai 3-JPNy	–	12.5	27.3	19.1
Sumai 3-AUT	–	–	26.5	22.0
Gamenya	–	–	–	26.0
Nobeokabouzu-komugi	–	–	–	–

allele, polymorphisms were detected in all of the markers except *Xwms0815*, which was 20 cM (unpublished data) from the markers located in the center of the QTL. A genetic polymorphism was detected in *Xgwm261* (Table 6). Notably, the band pattern for Sumai 3-AUT corresponded to that of the possibly resistant allele of *TaMRP-D1*; this is a prospective QTL (Table 6).

Discussion

In this study, morphological variants of Sumai 3-JPN with distinct anthocyanin pigmentation in the coleoptiles and anthers were observed (Supplemental Fig. 1). Due to the observed segregation ratio of morphologic colors, three genotypes (yellow homozygous, purple heterozygous, and purple homozygous) of anthocyanin pigmentation in Sumai 3-JPN were assumed to exist. Sumai 3-JPN is believed to have been introduced into Japan by the Director-General for Technical Affairs, MAFF, from the Chinese Academy of Agricultural Sciences in 1976 (unpublished data). After purifying selection, SSR genotyping revealed the identity of Sumai 3-USA, -CAN, -IRN, and -CHN, while Sumai 3-AUT and -JPNp were identified as variants (Table 2). Some of the original 'Sumai 3' stocks in China may not have included fixed plants (personal communication, Dr. Shi, Jiangsu Academy of Agricultural Sciences, China). The accessions (Sumai 3-USA, -CAN, and -IRN) may have derived from the same source, which was identified as being in China, while Sumai 3-JPN could not be extracted from the identified source. Sumai 3-AUT originated in China and reached Austria via Hungry after being transported to

Table 6. Polymorphisms detected using markers linked with FHB-resistant QTL between Sumai 3-AUT and -JPNy

Chr.	Marker type	Marker name	Reference	Poly.
3BS	STS	UMN10	Liu *et al.* 2008	–
5AS	SSR	*Xbarc180*	Buerstmayr *et al.* 2003	–
		Xgwm293	Buerstmayr *et al.* 2002, 2003	+
		Xgwm304		+
		Xbarc186	Buerstmayr *et al.* 2002	–
		Xwmc705	Kollers *et al.* 2013	–
6B	SSR	*Xwmc398*	Cuthbert *et al.* 2007	–
		Xgwm133		ND
		Xgwm644	Shen *et al.* 2003, Cuthbert *et al.* 2007	–
		Xwmc397	Cuthbert *et al.* 2007	–
		Xwmc179		–
		Xbarc101	Anderson *et al.* 2001	–
2DS	SSR	*Xgwm261*	Handa *et al.* 2008	+
		Xwmc503	unpublished data	+
	STS	J06R		+
		TaMRP-D1	He *et al.* 2013	+
		J06F	unpublished data	+
	SSR	*Xwmc112*		+
		Xwms0815	Handa *et al.* 2008	–

+, Allelic polymorphisms.
–, No polymorphisms.
ND, No bands were detected.

CIMMYT in Mexico through Brazil (personal communication, Dr. Kohli and Dr. Gilchrist, CIMMYT). However, whether Sumai 3-AUT derived from a mixed source in China or was the result of a complex history and frequent transport remains unclear. The level of genetic variation in these variants (Sumai 3-AUT, 13.2%; Sumai 3-JPNp, 19.4%) was lower than that in 'Nobeokabouzu-komugi' and 'Gamenya' (27.7 and 53.7%, respectively), and thus they can be considered 'Sumai 3' derivatives. Compared to the reported genetic variation (0.8%) between accessions of 'Sumai 3' in the US and China (Bai *et al.* 2003), Sumai 3-JPNy was nearly identical to Sumai 3-CHN, with 0.4% polymorphism. Bai *et al.* (2003) reported lower percentages

of variation within varieties (0.8% in 'Sumai 3' and 1.5% in 'Ning 7840') compared to the 5.2% observed in landrace 'Wangshuibai'. In our study, we identified 'Sumai 3' accessions from the US and China that were identical, which is contrast to the results of Bai *et al.* (2003). This discrepancy can be explained by the source of the accessions used in the study.

During the FHB field evaluation conducted at CIMMYT, increased DON accumulation in Sumai 3-JPNy was observed (Table 3), although DON concentrations in ppm could not always explain the differences in genotypes. Since symptoms were evaluated in all spikes, DON concentration should be measured using the same unit (ng/grain) to compare differences in grain number and weight between accessions. Variations in Sumai 3-AUT and -JPNy with regard to FHB resistance were further evaluated since they are commonly studied strains. For example, FHB-resistant cv. 'CM-82036' derived from 'Sumai 3' in Austria is often used and genetically analyzed (Buerstmayr *et al.* 2002, 2003, Lemmens *et al.* 2005, Miedaner *et al.* 2006, Schweiger *et al.* 2013, Steiner *et al.* 2009), and Sumai 3-JPNy is the source of the DH population, which contains susceptible alleles at QTL on 2DS (Handa *et al.* 2008). Subsequent FHB field evaluation at NARO/KARC demonstrated stronger disease resistance in Sumai 3-AUT than in Sumai 3-JPNy. Location effects were thought to explain differences in FHB symptoms between CIMMYT and NARO/KARC. According to the results of DON accumulation, we found that the NARO/KARC trial was performed under increased disease pressure. The observed phenotypic variation was assumed to be based on genetic differences.

DNA fingerprinting provided information on genome-wide genetic variation, specific polymorphisms related to FHB resistance, and allelic differences among 'Sumai 3' accessions. DArT markers identified 12.5% of the variation between Sumai 3-AUT and -JPNy, which showed very different levels of FHB resistance in field trials (Tables 4, 5). The observed difference of 12.5% is likely associated with the resistance of Sumai 3-AUT to FHB. Genotyping for FHB-related QTLs confirmed the allelic identities between Sumai 3-AUT and -JPNy on 3BS and 6B, but not on 5AS and 2DS (Table 6). Thus, the polymorphisms detected in 5AS and 2DS may explain the increased resistance of Sumai 3-AUT to FHB compared to Sumai 3-JPNy. Since QTLs are known to contribute to FHB resistance (11–20.5% from 5AS, 14–25% from 2DS; Buerstmayr *et al.* 2002, 2003, Handa *et al.* 2008), these regions may play a role in the enhanced FHB resistance in Sumai 3-AUT. Further analysis of the 2DS QTL using the STS marker *TaMRP-D1* clearly revealed a resistant allele in Sumai 3-AUT (Table 6). *TaMRP-D1* is thought to function as a detoxifier in cells by transporting glutathione-conjugated mycotoxins (Handa *et al.* 2008), which could explain the increased resistance of Sumai 3-AUT to FHB. *TaMRP-D1* is common in the 13th SRSN (He *et al.* 2013), but other regions also showed polymorphisms on QTLs, which were reported in different cultivars (*e.g.*, 'Wangshuibai'and 'Ernie'; data not shown). Thus, further studies are required to identify the gene responsible for FHB resistance in Sumai 3-AUT.

Overall, we identified that similarities and dissimilarities exist among 'Sumai 3' accessions. This study is the first to suggest that the inconsistencies identified among 'Sumai 3' accessions in terms of their field responses to FHB are associated with genetic diversity. These findings suggest that the genotypes of crossing parents should be considered when discussing the results of QTL analyses from a bi-parental cross. Sumai 3-AUT with higher FHB resistance did not carry a susceptible haplotype for the QTL on 2DS, suggesting that replacement of the susceptible allele with a resistant allele could improve FHB resistance levels in Sumai 3-JPNy. In previous crosses using ordinary 'Sumai 3', the offspring were introduced into this susceptible allele on the 2DS locus along with resistant alleles on other QTLs. Improved screening using DNA markers for FHB-resistant genes and the replacement of susceptible alleles on the QTL with alternative resistant alleles could coordinate optimal gene pyramiding and the introduction of additional FHB resistance genes into adapted lines.

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