

Relationship between plant height and *Fusarium* head blight resistance for the QTL on the wheat chromosome 2DS, *QFhs.kibr-2DS*

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ABSTRACT

The FHB resistance QTL region of wheat chromosome 2DS (*QFhs.kibr-2D*) flanks the reduced height gene *Rht8*, which might influence initial infection of FHB under the field conditions. However, it is suggested the existence of other potential resistance gene(s) within this QTL region beside the primary plant height effect by *Rht8/rht8* alleles on the FHB resistance and DON accumulation. The results of QTL analysis using doubled haploid lines (DHLs) derived from Sumai3 and Gamanya suggest that MRP (multidrug resistance-associated protein) is a possible candidate for *QFhs.kibr-2D*, which has an additional effect for type II resistance and DON content, and acts independently of *Rht8*. The Gamanya allele for the MRP associated with the QTLs for both type II resistance and low-level DON accumulation, and showed additive effect on the *Fhb1* of Sumai 3. On the other hand, the short culm plant with *Rht8* of Sumai 3 tended to suffer from the severe damage to initial infection, which explained the QTL for FHB field response with epistatic effects on the type II resistance and low level DON accumulation. Relationship between *Rht8* genotype, one of the possible genes controlling plant height among the DH population, and *QFhs.kibr-2D* was examined by drifting of the QTLs in each group of *Xgwm261* allele, corresponding to the *Rht8* genotype. Influence of the plant height on *QFhs.kibr-2D* was also evaluated in the same manner as the *Rht8* genotype between two culm length groups of the DHLs. From the results of the MRP analysis and the possible effects of *Rht8*, we postulate that *QFhs.kibr-2D* is a resistance gene complex consisting of morphological traits controlled by *Rht8* for type I resistance and a specific gene(s) to control type II resistance by detoxification of DON, like MRP.

INTRODUCTION

Three types of genetic resistance to *Fusarium* head blight (FHB) in wheat have been described: resistance to initial penetration as type I resistance, to fungal spread within plant tissues as type II (Schroeder and Christensen 1963), and resistance based on the ability to degrade the mycotoxin deoxynivalenol (DON) (Miller et al. 1985). The type I resistance can be easily influenced by environmental conditions at the time of FHB infection and by the morphological features of spikes

(Mesterházy 1995). For example, dwarf genotypes have a trend of suffering more severe infection with FHB than tall genotypes under natural conditions, but genotypes of different plant height classes were similarly susceptible after artificial inoculation. Using a comparative genomic approach, we have identified the quantitative trait loci (QTLs), *QFhs.kibr-2D* for multiple traits of FHB resistance and for controlling DON accumulation, which is closely linked to the SSR locus *Xgwm261* and the semi-dwarf allele *Rht8* (Handa et al. 2008). One multidrug resistance-associated protein (*MRP*) gene was mapped at *QFhs.kibr-2D* and suggested that *MRP* could be associated with type II resistance and reduced DON accumulation. In this paper, we discuss the relationship between plant height and FHB resistance for the QTL on the wheat chromosome 2DS, *QFhs.kibr-2DS*.

MATERIALS AND METHODS

A segregating population of 118 DHLs developed from the F₁ cross between Sumai 3 and Gamanya with the wheat x maize system was used to evaluate plant height and FHB resistance, for QTL analysis, and DON content analysis (Ban and Suenaga 1998, 2000). Sumai 3 is a Chinese FHB-resistant variety and Gamanya is a highly susceptible variety from Australia.

The DHLs and their parents, Sumai 3 and Gamanya, were planted in randomized block plots with two replications of 1-m-long double rows at CIMMYT, Mexico in 2006. The macroconidia solution of *F. graminearum*, which was a mixture of 6 isolates screened at the CIMMYT field based on their pathogenicity and DON toxigenicity, was prepared with a 1x10⁵ conidia/ml concentration as the spray inoculum of FHB. The corn grain spawn that had formed efficient perithecia was spread over the field at tillering and post flowering stages to enhance the FHB natural infection. Each wheat line was inoculated with 30 ml of the macroconidia solution using CO₂ pressure backpack sprayers at anthesis and three days after the first inoculation. To suffer uniform disease pressure, the moisture in the experimental field was kept by the sprinkler spray for 15 min every 2 hours during the day time. The harvested spikes were threshed mechanically, and the DON contents of the yielded seeds were analysed by the ELISA method (RIDASCREEN[®] FAST DON Kit, R-Biopham AG, Germany). The weight of

100 grains in each line was measured to calculate an average DON content/grain.

RESULTS AND DISCUSSION

In the DH population, a significant QTL for plant height, composed of culm and spike length, was detected on 2DS between *Xgwm269* and *Xwms815* with maximum LOD score 8.5 and 26% of total phenotypic variance explained across the year ($P=0.001$). In addition, other QTLs opportunistically detected on chromosome 3B (LOD=2.5, 9%), 5B (LOD=2.5, 9%) with Gamenya alleles, and 5DS (LOD=3.4, 12%), 6AL (LOD=2.5, 9%), 7BS (LOD 2.2, 8%) with Sumai 3 alleles. The QTL on 2DS was located near the *Rht8* semi-dwarfing gene locus closely linked to *Xgwm261*. The Sumai 3 allele at this QTL decreased plant height by about 10 cm, which indicates that it possesses the semi-dwarf allele *Rht8*.

Using this DH population developed from the F_1 cross of Sumai 3 and Gamenya, a susceptible variety, we also found the QTL for FHB field resistance at the position of *Fhb1* on chromosome 3BS as well as other seven genomic regions on 2B, 2DL, 2DS, 5AL, 5BS, 5DS and 7AS associated with type I resistance and six genomic regions on 1BS, 2DS, 3BS, 4AL, 5AS and 6DS associated with type II resistance (Xu et al. 2001; Ban, unpublished results). The QTL linked with *Xgwm261* on 2DS named as *QFhs.kibr-2D* showed a positive effect on both type I and II resistances to FHB. Interestingly, both alleles from Gamenya, FHB highly susceptible variety, and Nobeokabouzu-komugi, highly resistant variety, contributed to *QFhs.kibr-2D* as counterparts of Sumai 3 allele in two DH populations derived from Sumai 3 x Gamenya and Nobeokabouzu-komugi x Sumai 3 (Xu et al. 2001; Ban, unpublished results). These results suggest that *QFhs.kibr-2D* for the FHB/DON resistance linked with *Xgwm261* on 2DS is masked with other susceptible genes under the genetic background of highly susceptible varieties, therefore we cannot observe its effect on the FHB resistance. We identified and mapped a multidrug resistance-associated protein (*MRP*) gene at *QFhs.kibr-2D* and suggested that *MRP* could be associated with type II resistance and reduced DON accumulation (Handa et al. 2008).

Table 1. Coefficient of correlation among the plant height (culm and spike length) and the FHB resistance/ DON accumulation in the grains.
(n=81, * significant at $P<0.001$ level)

	Culm length	Spike length	Type I	Type II
Spike length	0.62*			
Type I resist.	-0.64*	-0.39*		
Type II resist.	-0.32*	-0.36*	0.45*	
DON accum.	-0.18	-0.15	0.42*	0.37*

The DHLs varied in their culm length between 78 and 131 cm (Ave. 105.2 ± 0.05), although both parents did not have so much difference between their plant heights in

the field. The short culm length showed negative effects on FHB field response and type II resistance ($r=-0.64$ and -0.32 , $P<0.001$) whereas showed no correlation with DON accumulation ($r=-0.18$, $P=0.15$) as shown in Table 1. The culm length distributed continuously with two peaks indicating several different loci to control plant height of both parents and the DHLs (Fig. 1).

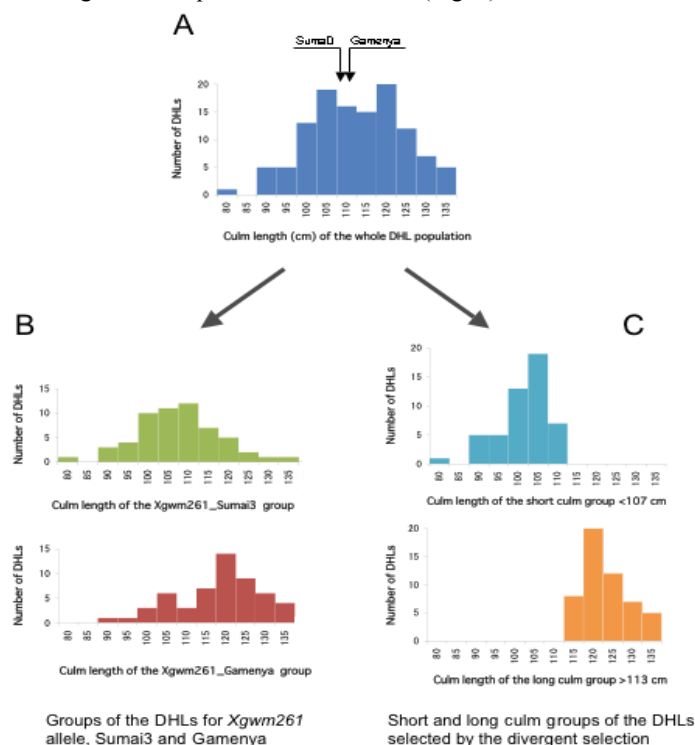


Fig. 1. Frequency distribution of the DHLs for the culm length in 5cm steps, A: whole DHL population, B: two groups separated on their allele for *Xgwm261* corresponding *Rht8/rht8* genotype of Sumai3/Gamenya, C: two groups selected for short or long culm length by the divergent selection with statistically significant at 95% confidence interval.

Relationship between *Rht8* genotype, one of the possible genes controlling plant height among the DHL population, and *QFhs.kibr-2DS* was examined by drifting of the QTLs in each group of *Xgwm261* allele, corresponding to the *Rht8* genotype (Table 2). Influence of the plant height on the FHB/DON-QTLs was also evaluated in the same manner as the *Rht8* genotype between two culm length groups of the DHLs (Table 2). For this analysis, we selected two discontinuous culm length groups of the DHLs by the divergent selection; short group (<107 cm) and long group (>113 cm), which showed a significant difference at 95% confidence interval (Fig. 1). The FHB-QTL for field response on 2DS disappeared in the group with Sumai 3 allele for *Xgwm261* and also in both culm length groups of the DHLs. However the field response QTL associating with *Xgwm539* on 2DL was effective independently with the plant height. On the other hand, in the group with Gamenya allele for *Xgwm261* regarding *rht8*, the QTLs for type II resistance and DON content were still detected associating with *MRP* allele even in the flanking condition with *Xgwm261* and *Rht8* alleles. And also these QTLs were not so influenced by culm length, different from the case of FHB field response. These

results suggest that existence of other potential resistance gene(s) within *QFhs.kibr-2D* region beside the plant height effect (probably controlled by *Rht8*). The *QFhs.kibr-2D* region of wheat chromosome 2DS analysed here flanks the reduced height gene *Rht8*, which could influence initial infection of FHB under field conditions (Table 2). However, we had the results to suggest the existence of other potential resistance gene(s) within *QFhs.kibr-2D* region beside the primary plant height effect by *Rht8/rht8* alleles on the FHB resistance and DON accumulation. The results suggest a possibility that MRP has an additional effect for type II resistance and DON content, which acts independently of *Rht8*. The Gamanya allele for the MRP associated with the QTLs for both type II resistance and low-level DON accumulation (Table 2), and additive effect on the *Fhb1* of Sumai 3 (data not shown here). Our hypothesis that the MRP homologue is a gene for *QFhs.kibr-2D* coincides with a QTL for controlling DON level and type II resistance found by Yang et al. (2005). On the other hand, the short culm plant with *Rht8* of Sumai 3 tended to suffer from the severe damage to initial infection, which explained *QFhs.kibr-2D* for FHB field response with epistatic effects on the type II resistance and low level DON accumulation. From the results of the MRP analysis and the possible effects of *Rht8*, we postulate that the FHB resistance QTL on 2DS is a resistance gene complex consisting of morphological traits controlled by *Rht8* for type I resistance and a specific gene(s) to control type II resistance by detoxification of DON, like MRP.

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Table 2. *QFhs.kibr-2D* for FHB resistance and low level of DON accumulation in the harvest grains by using marker regression analysis with additive regression model by MapManagerQTX in each group classified by the *Xgwm261* allele regarding with *Rht8* or the culm length (low as < 107 cm and high as > 113 cm) in the DH population derived from Sumai 3 x Gamanya.

Trait	Group of the DH lines (number of lines)		Chr.	Associated loci	LOD ^a	Phenotypic variance (%)	P	Additive regression coefficient ^b
Field response to FHB	Whole DH population (118)		2DS	<i>Xgwm261</i>	3.69	13	0.0000	-9.33
				<i>MRP</i>	5.21	18	0.0000	-10.88
			2DL	<i>Xgwm539</i>	1.95	7	0.0027	6.75
	<i>Xgwm261</i> allele	Sumai 3 (57)	2DL	<i>Xgwm539</i>	1.13	9	0.0222	7.73
		Gamanya (54)	2DS	<i>MRP</i>	1.91	15	0.0030	-16.64
	Culm length	Low group (50)	2DL	<i>Xgwm157</i>	1.24	11	0.0173	8.95
<i>Xgwm539</i>				1.15	10	0.0209	8.89	
High group (52)		2DL	<i>Xgwm157</i>	1.26	11	0.0158	5.04	
			<i>Xgwm539</i>	2.04	16	0.0022	5.97	
FHB type II resistance	Whole DH population (118)		2DS	<i>Xgwm296</i>	3.34	14	0.0001	-8.76
				<i>Xgwm261</i>	5.05	20	0.0000	-10.59
				<i>MRP</i>	6.42	25	0.0000	-11.77
	<i>Xgwm261</i> allele	Sumai 3 (57)	none	-	-	-	-	-
		Gamanya (54)	2DS	<i>MRP</i>	1.65	14	0.0057	-12.84
	Culm length	Low group (50)	2DS	<i>Xgwm261</i>	1.74	17	0.0048	-10.74
<i>MRP</i>				1.76	17	0.0045	-11.52	
<i>MRP</i>				1.21	11	0.0182	-7.34	
DON accumulation/ grain	Whole DH population (118)		2DS	<i>Xgwm261</i>	2.62	12	0.0005	-10.89
				<i>MRP</i>	3.02	14	0.0002	-11.46
	<i>Xgwm261</i> allele	Sumai 3 (57)	none	-	-	-	-	-
		Gamanya (54)	2DS	<i>MRP</i>	2.17	22	0.0016	-19.08
	Culm length	Low group (50)	2DL	<i>Xgwm301</i>	1.82	18	0.0038	13.18
		High group (52)	2DS	<i>MRP</i>	1.13	13	0.0223	-10.02

a. The putative QTLs with the LOD value more than 1.0 were selected.

b. Positive/negative values means the contribution of the Sumai 3/ Gamanya allele, respectively.