Wheat glume colour controlled by *Rg1* locus is useful as a phenotypic marker to select *Glu-B3* alleles encoding LMW glutenin subunits.

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INTRODUCTION

Common wheat (*Triticum aestivum* L.) seed storage proteins present in the endosperm of the grain are composed of two major fractions, gliadin and glutenin. These are important determinants of wheat flour and dough quality. Glutenin can be further classified into high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits. The HMW glutenin subunits (HMW-GSs) are encoded by the *Glu-A1*, *Glu-B1* and *Glu-D1* alleles on the long arm of wheat chromosomes 1A, 1B and 1D, respectively (Payne et al. 1987). While the LMW glutenin subunits (LMW-GSs) are encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3* alleles on the short arm of these chromosomes (Jackson et al. 1983). Recent studies have clarified that Japanese slightly-low-amylose wheat cultivars (*Wx-B1b*) with *Glu-B3g* allele on 1BS encoding LMW-GS often have optimum dough strength as well as higher sensory quality for Japanese white salted noodle (Ikeda *et al*. 2005). Extra-strong wheat cultivars 'Glenlea' (Canada) and 'KS831957' (U.S.A) have the *Glu-B3g* gene. In order to incorporate efficiently the *Glu-B3g* allele into elite breeding lines, marker-assisted selection (MAS) using DNA markers of *Glu-B3* alleles (D'Ovidio *et al.* 1997, Maruyama-Funatsuki *et al.* 2005, Ikeda *et al*. 2006) should be a quite powerful tool in wheat breeding programs. Whereas, there are some difficulties to apply MAS in the breeding programs because MAS is still somewhat troublesome for breeders as well as cost consuming. In this research, we studied from the view points of breeders, the effectiveness of 'glume colour' controlled by *Rg1* locus on the short arm of chromosome 1B as a phenotypic marker to select *Glu-B3* alleles.

MATERIALS AND METHODS

Plant materials

Three kinds of common wheat recombinant inbred lines (RILs) of F_4 generation developed in our breeding program at Aichi Prefecture Agricultural Research Centre (AARC), derived from the crosses between 'Kinuiroha' and 'Kinunonami', 'Iwainodaichi' and 'Kinunonami', 'Haruibuki' and 'Minaminokaori', consist of 147, 64 and 97 RILs, respectively, were used. In each cross, genotypes of *Glu-B3* and *Rg1* loci as well as the 'glume colour' of the seed parent are both different from those of the pollen parent (Table 1).

Table 1. Genotypes of LMW-GS gene locus *Glu-B3* and glume colour of common wheat cultivars used for cross parent.

Cultivar	*Glu-B3* allele	Glume colour	Genotype of *Rg1* locus[1]
Kinuiroha	*Glu-B3i*	Red	*Rg1* (*Rg-B1b*)[2]
Kinunonami	*Glu-B3g*	White	*rg1* (*Rg-B1a*)
Iwainodaichi	*Glu-B3i*	Red	*Rg1* (*Rg-B1b*)
Haruibuki	*Glu-B3j*	White	*rg1* (*Rg-B1a*)
Minaminokaori	*Glu-B3i*	Red	*Rg1* (*Rg-B1b*)

[1] Genotypes of *Rg1* locus was estimated from glume colour

[2] New designation proposed by Khlestkina *et al*. (2006)

Genotypic and phenotypic evaluation

The genotype of *Glu-B3* locus of each RIL was determined by the band patterns of DNA markers for *Glu-B3* alleles developed by Maruyama-Funatsuki *et al*. (2005) and Ikeda *et al*. (2006). DNA of each RIL was extracted from young leaves of five individuals per line, and the bulk DNA of each RIL was evaluated. While, the genotype of *Rg1* locus of each RIL was determined by phenotyping 'glume colour' (dark-red, segregating, white) of each RIL in the breeding field at ripening stage. For each RIL, 25 individuals per line were evaluated.

RESULTS

Cosegregation between *Glu-B3* and *Rg1* loci

In the 147 F_4 RILs derived from the cross between 'Kinuiroha' (*Glu-B3i* and red glume) and 'Kinunonami' (*Glu-B3g* and white glume), all of the 64 RILs with red glume had *Glu-B3i* allele. While 91.5% (43/47) RILs with white glume had *Glu-B3g* allele. Besides, 88.8% (32/36) RILs with segregating glume colour were heterozygous for *Glu-B3* alleles (Table 2). In the 64 F_4 RILs derived from the cross between 'Iwainodaichi' (*Glu-B3i* and red glume) and 'Kinunonami' (*Glu-B3g* and white glume), 92.9% (26/28) RILs with red glume had *Glu-B3i* allele. While 93.8% (15/16) RILs with white glume had *Glu-B3g* allele. Besides, 95.0% (19/20) RILs with segregating glume colour were heterozygous for *Glu-B3* alleles (Table 3). In the 97 F_4 RILs derived from the cross between 'Haruibuki' (*Glu-B3j* and white glume) and 'Minaminokaori' (*Glu-B3i* and red glume),

88.1% (37/42) RILs with red glume had *Glu-B3i* allele. While all of the 25 RILs with white glume had *Glu-B3j* allele. Besides, All of the 30 RILs with segregating glume colour were heterozygous for *Glu-B3* alleles (Table 4). As results, tight cosegregation between *Glu-B3* and *Rg1* loci (glume colour) were found in all of the three kinds of RILs.

Table 2. Cosegregation between *Glu-B3* allele and glume colour controlled by *Rg1* locus observed in the 147 F_4 RILs derived from the cross between 'Kinuiroha' and 'Kinunonami'.

Phenotypes of glume colour	No. of RILs classified by the genotype of *Glu-B3* allele		
	Glu-B3g	Heterogenic	*Glu-B3i*
Red	0	0	**64**
Segregating	4	**32**	0
White	**43**	1	3

Table 3. Cosegregation between *Glu-B3* allele and glume colour controlled by *Rg1* locus observed in the 64 F_4 RILs derived from the cross between 'Iwainodaichi' and 'Kinunonami'.

Phenotypes of glume colour	No. of RILs classified by the genotype of *Glu-B3* allele		
	Glu-B3g	Heterogenic	*Glu-B3i*
Red	0	2	**26**
Segregating	1	**19**	0
White	**15**	0	1

Table 4. Cosegregation between *Glu-B3* allele and glume colour controlled by *Rg1* locus observed in the 97 F_4 RILs derived from the cross between 'Haruibuki' and 'Minaminokaori'.

Phenotypes of glume colour	No. of RILs classified by the genotype of *Glu-B3* allele		
	Glu-B3j	Heterogenic	*Glu-B3i*
Red	0	5	**37**
Segregating	0	**30**	0
White	**25**	0	0

Genotypes of *Glu-B3* allele and phenotypes of glume colour of newly bred common wheat cultivars and their parents

To confirm the tight cosegregation between glume colour and *Glu-B3* alleles observed in the three kinds of RILs, we studied the glume colour and *Glu-B3* alleles of eight newly bred Japanese common wheat cultivars as well as their seed and pollen parents. Among new cultivars with *Glu-B3g* allele (Type 1, 2 ,3), type 1 and type 2 cultivars have parents that genotypes of *Glu-B3* and *Rg1* loci as well as the 'glume colour' of the seed parents are both different from those of the pollen parents. The glume colours of all of the type 1 and type 2 cultivars with *Glu-B3g* allele were the same as those of parents with *Glu-B3g* allele (Table 5). As a result, tight cosegregation between *Glu-B3* and *Rg1* loci (glume colour) were also found in newly bred common wheat cultivars with *Glu-B3g* allele.

DISCUSSION

From these results, we applied 'glume colour' controlled by *Rg1* locus as a phenotypic marker to select *Glu-B3g* allele in the breeding program of AARC and confirmed the tight linkage relationship between the two loci.

Finally, we clarified that wheat glume colour controlled by *Rg1* locus on 1BS is quite useful as a phenotypic marker which is more convenient and less laborious than DNA markers to select *Glu-B3* alleles. Using this 'glume colour marker', breeders can select elite breeding lines with desirable genotype of *Glu-B3* alleles with accuracy in wheat breeding fields just only by observing the 'glume colour' of each line or individual without any troublesome procedures of MAS using DNA marker(s) or SDS-PAGE analysis in the laboratories.

Using this 'glume colour' marker, breeders can select wheat elite lines with *Glu-B3g* allele and strengthen dough properties as well as exploit undesirable wheat with *Glu-B3j* allele (null) for Japanese salted noodle.

We can use the 'glume colour' maker under the condition that the genotypes of *Glu-B3* and *Rg1* loci (glume colour) of the seed parent are both different from those of the pollen parent.

In Japan, since an elite line 'Kanto107' with low-amylose-content as well as *Glu-B3g* allele and white glume has been one of the major cross parents for wheat quality breeding, most of the new cultivars with *Glu-B3g* allele have white glume. Besides, an extra-strong wheat cultivar 'Hanamanten' with *Glu-B3g* has red glume. The red glume and *Glu-B3g* allele of 'Hanamanten' are derived from its seed parent, 'KS831957'. In rare case, an elite line for Japanese salted noodle, 'Kanto 105' with *Glu-B3g* has red glume.

Blanco et al. reported that the map distance between *Glu-B3* and *Rg1* loci were 1.6cM in tetraploid durum wheat. We are now conducting a series of linkage analysis to elucidate precisely the linkage between *Glu-B3* alleles and Rg1 locus using some F_2 and F_3 populations of common wheats.

In this study, all of the genes which control wheat glume colour should be *Rg1* gene on 1BS because of the tight linkage between glume colour and *Glu-B3* alleles on 1BS. We also are interested in the geographic distribution of genes controlling wheat glume colour (*Rg1*, *Rg2*, *Bg* etc.) among the world

ACKOWLEDGEMENTS

This study was conducted in AARC with special assignment of the Ministry of Agriculture, Forestry and Fisheries, Japan.

Table 5. Genotypes of *Glu-B3* allele and glume colour of newly bred common wheat cultivars and their parents.

Type	Newly bred cultivars			Seed parents				Pollen parents		
	Name	Glume colour	*Glu-B3*	Name	Glume colour	*Glu-B3*		Name	Glume colour	*Glu-B3*
1	Kinunonami	White	*g*	Kanto 107	White	*g*	×	Bandouwase	Red	*i*
	Tsurupikari	White	*g*	Bandouwase	Red	*i*	×	Kanto 107	White	*g*
	Ayahikari	White	*g*	Kanto 107	White	*g*	×	Kinuiroha	Red	*i*
2	Hanamanten	Red	*g*	KS831957	Red	*g*	×	Saikai 179	White	*d*
3	Kinuazuma	White	*g*	Kanto 107	White	*g*	×	kanto 105	Red	*g*
	Nebarigoshi	White	*g*	Kanto 107	White	*g*	×	Chihokukomugi	White	*g*
4	Chikugoizum	White	*i*	Kanto 107	White	*g*	×	Asakazekomugi	White	*i*
	Nishihonami	White	*i*	Kanto 107	White	*g*	×	Minaminokomugi	White	*i*

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