Identification of *R* gene genotypes in Japanese wheat cultivars

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INTRODUCTION

Pre-harvest sprouting (PHS) of bread wheat has been reported frequently in countries with wet weather at harvesting time. It deteriorates flour quality and results in substantial losses of crop yield and price. Induction of high level of grain dormancy appears to be one of the main components of PHS tolerance (Mares 1987).

Red grain colour of wheat has been well known to be associated with the development of seed dormancy and affects flour brightness due to the contamination of red pigment in milling process (Flintham 2000). Grain colour is controlled by genotypes of the three red grain colour genes (*R*) on chromosome 3A, 3B, and 3D. Identification of the *R* genotype of wheat lines is an important requirement of breeding for PHS tolerance and flour quality. However, it is laborious and time-consuming to identify *R* genotype of many wheat strains by progeny test of the cross with the strains with known *R* genotype. Recently, Himi and Noda (2005) reported that the red grain colour gene of wheat was a Myb-type transcription factor and its recessive alleles showed altered nucleotide sequences, which allow identifying the *R* genotypes using PCR method.

In this study, we attempted to identify *R* genotype of Japanese wheat cultivars with the PCR method.

MATERIALS AND METHODS

160 Japanese cultivars bred from 1929 to 2003 were grown in a growth chamber and genomic DNA was extracted from 2-week seedlings, using modified CTAB methods (Murray and Thompson 1980).

R genotypes were identified the PCR methods with the primer sets and PCR conditions used by Himi and Noda (2005)

RESULTS AND DISCUSSIONS

Expected PCR products of *R-B1a and b* alleles and *R-D1a and b* alleles were amplified by the primer sets for *R-B1* and *R-D1*. In *R-A1* PCR products unexpected from two known *R-A1a* alleles were observed in white-grained wheat cultivars, which have foreign strain in their pedigree. Thus, additional sequence variations were detected in the *R-A1* locus. The *R* genotypes estimated by the primer sets were in accord with the grain colour of the cultivars examined except cultivars with new *R-A1* allele. In Japanese wheat cultivars, the primer sets for the *R* genes are useful to identify the *R* genotypes.

The frequency of *R-A1b* allele (functional, dominant) was 86.6 % in Japanese cultivars (Fig.1). The frequency of *R-A1a* alleles (non-functional, recessive) and new *R-A1* alleles was 7.8% and 5.6% (9 cultivars), respectively. Most of Japanese cultivars have *R-A1b* genotype.

The frequency of *R-B1b* (functional, dominant) and *R-B1a* was 61.4% and 38.6%, respectively (Fig.2). The frequency of *R-D1b* (functional, dominant) and *R-D1a* was 64.6% and 35.4%, respectively (Fig.3). The genotypes of *R-B1* alleles and *R-D1* alleles were identified in all Japanese cultivars examined.
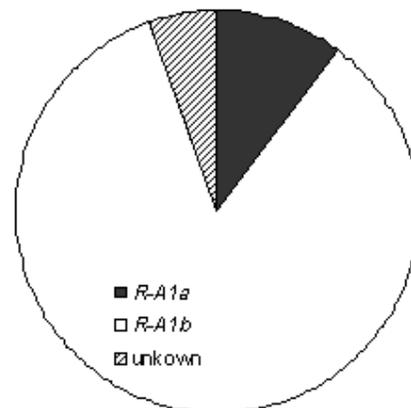

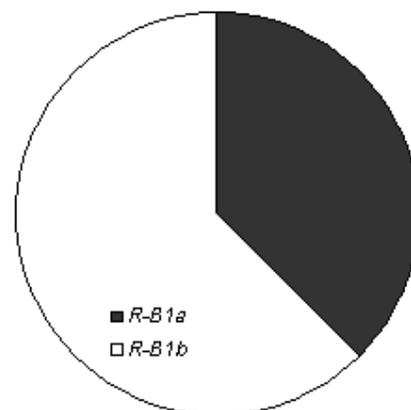Fig.1 Frequencies of *R-A1* alleles (a, b and new allele) in 160 Japanese wheat cultivars.

Fig.2 Frequencies of *R-B1* alleles in 160 Japanese wheat cultivars.
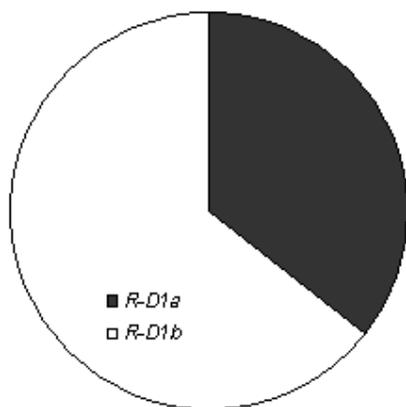
Fig.3 Frequency of *R-D1* alleles in 160 Japanese wheat cultivars.

Comparing the frequencies of *R-B1b* and *R-D1b* alleles (61.4% and 64.6%) with those of *R-A1b* (86.6%), *R-A1b* appears to contribute more to grain colour than the other two alleles, *R-B1b* and *R-D1b*.

Cultivars with three dominant *R* genes are the most frequent genotype (33.1%) (Fig.4). Total frequency of cultivars with two dominant *R* genes was 47.1%. The cultivars with *R-A1b-R-B1b-R-D1a* and *R-A1b-R-B1a-R-D1b* genotypes were 19.7% and 23.6%, respectively. On the other hand, the frequency of cultivars with *R-A1a-R-B1b-R-D1b* was only 3.9%. The cultivars with one dominant *R* allele were 11.8%.

The results show that most Japanese wheat cultivars have 2 or 3 dominant *R* genes. This suggests that wheat lines with high number of functional *R* alleles have been selected for unintentionally in Japan, as a result of frequent rainfalls at harvest time and PHS resistance is a prime breeding target.
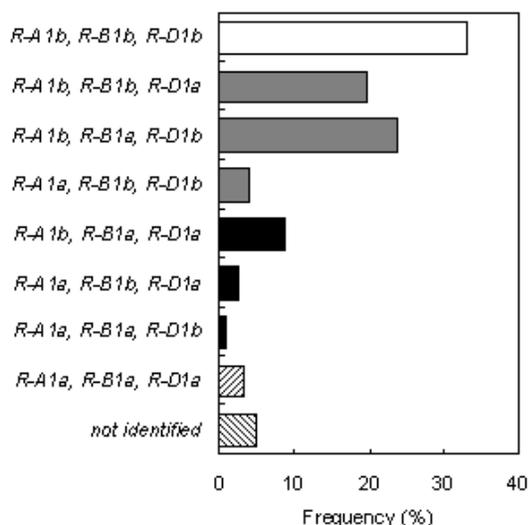

Fig.4 Frequencies of Japanese wheat cultivars with different *R* genotypes.

R-A1b was used at significantly higher frequency than *R-B1b* and *R-D1b*. It is not clear at present whether *R-A1b* can contribute more to grain colour and PHS resistance than the other alleles. It is also possible that chromosome 3A might carry genes which characterize Japanese wheat varieties.

In conclusion, we have shown that we could identify the *R* genotypes of wheat cultivars with the PCR method. In future, this technique can be used to further improve the characteristics associated with grain colour in wheat breeding.

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