Application Note

Marker-assisted selection (MAS) of wheat lines for udon noodle production

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The QIAxcel® system was used for analysis of PCR products generated for marker-assisted selection in wheat. The high-throughput capacity of the system allowed large numbers of plants to be quickly and reliably analyzed, making it highly suitable for plant breeding applications.

Introduction

The development of wheat varieties begins with a cross between 2 varieties or lines. The plants grown from the seeds of the cross are considered to be the first generation (F_1); the suitability and productivity of the subsequent generations in multiple regions are examined from the F_8 generation onwards for several years. When a variety is found to be superior, it becomes certified and can be grown on a farm. Since a generation requires approximately one year in the field, the development of a variety traditionally requires approximately 10 years.

The QIAxcel system.

Due to recent progress in genomic research, this breeding approach is undergoing a major change. Rather than selecting traits based on phenotypes, the genes or gene regions that control the traits can be used to design markers, and selection is based on studies of these markers using a method called marker-assisted selection (MAS). The use of DNA markers in MAS enables the selection of plants with the targeted traits by analyzing results from F_2 individuals. Thus, MAS is a breakthrough technology that changes the process of variety development from the traditional field-based format to a laboratory-based format. As a representative example, we present here a DNA marker selection system for the viscoelasticity trait in udon noodles. Viscoelasticity of udon noodles improves as the amylose content of the starch decreases. Amylose synthesis in seed starch is controlled by the *Wx* gene family. In wheat, amylose synthesis is influenced by genes from the A, B, and D genomes (*Wx*-A1, *Wx*-B1, and *Wx*-D1 genes). ▷

When no *Wx* gene products are present, the wheat line is referred to as "waxy wheat" which is not suitable for producing udon noodles. When only 1 or 2 gene products are missing (partially waxy wheat), the amylose content of the wheat is reduced and the wheat is more suitable for making udon noodles.

Selection of a partial waxy line based on the actual amylose content is problematic because measuring amylose content is difficult and imprecise and measurements must be obtained from several generations of plants. Therefore, we developed the DNA markers listed in Table 1 for PCR analysis to select partial waxy wheat plants (1). An effective method for multiple selections requires a high-throughput system. Our laboratory used a simple DNA extraction method in conjunction with the QIAxcel system to efficiently select lines from many breeding sites throughout the country.

Materials and methods

Genomic DNA from Chinese Spring (CS, wild type), Mochi Otome (MO, mutations in all 3 *Wx* genes), and a heterozygous plant (H) was prepared for the detection of mutations. PCR amplification was performed using primers for the detection of mutations in *Wx* genes (Table 1), and the amplified products were analyzed on the QIAxcel system with the QIAxcel DNA Screening Kit and the AM420 method.

Table 1. Sizes of fragments that are specific PCR markers for mutants of *Wx* alleles

		Amplification product (bp)	
Gene	**Primer**	**Wild type**	**Mutant**
***Wx*-A**	AFC	389	370
	AR2	(408, 410)	(408, 410)
***Wx*-B**	BDFL	425	none
	BRD	(455, 497)	(455, 497)
***Wx*-D**	BDFL	2307	1731
	DRSL	—	—

Fragments amplified from other *Wx* genes are indicated in parentheses.

Results and Discussion

Representative results are shown in Figure 1. Clearly distinguishable separation was obtained for the PCR-amplified fragments, as seen with the 370 bp and 389 bp fragments in Figure 1A. The PCR products for *Wx*-A1 and *Wx*-D1 alleles (Figures 1A and 1C, respectively) are co-dominant, and the assessment of heterozygosity using the gel image generated by the QIAxcel system is easy and straightforward. BDFL and BRD primers (*Wx*-B gene) amplify a 425 bp fragment that is present in the wild type but not the mutant (Figure 1B). In the electropherograms, the height of the *Wx*-B peak from the heterozygote (Figure 1D) is half the height of the peak from the homozygous wild type (Figure 1E), clearly demonstrating differences in gene dosage. The throughput capacity of the QIAxcel system allowed simultaneous analysis of up to 96 samples to be performed (Figure 2).

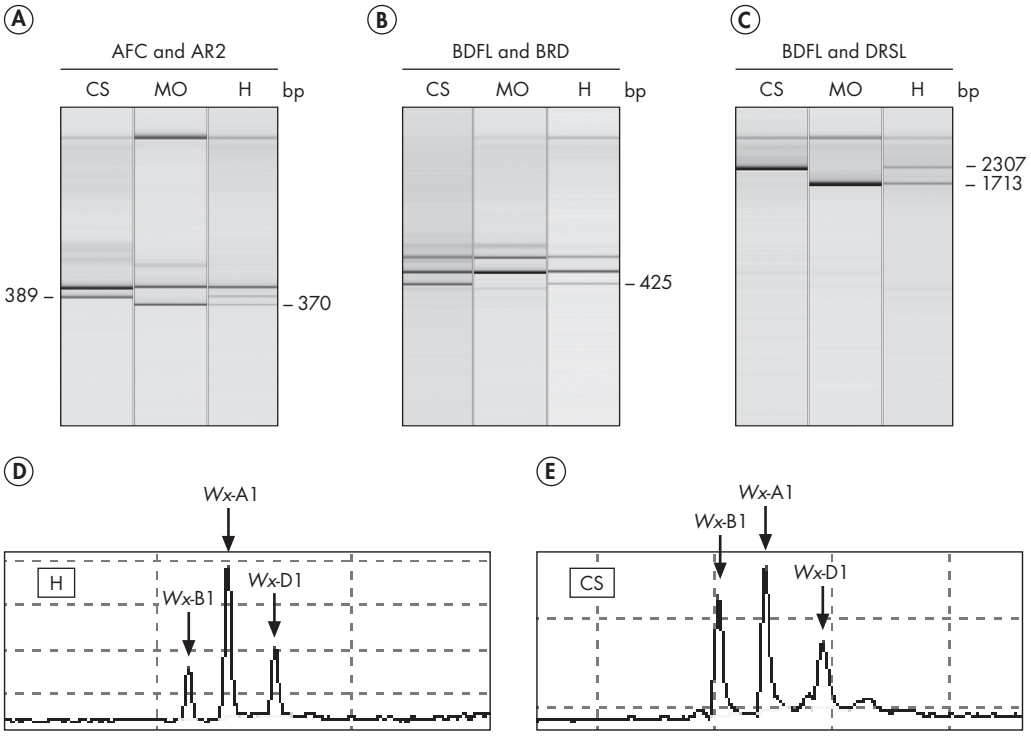

Figure 1. *Wx* gene analysis in a segregating F_3 generation of wheat. Genomic DNA was isolated from wild type (CS), Mochi- Otome (MO), and heterozygous (H) plants and amplified using primers for the **A.** *Wx*-A1, **B.** *Wx*-B1, and **C.** *Wx*-D1 genes. PCR products were analyzed on the QIAxcel system using the QIAxcel DNA Screening Kit. The sizes of expected fragments are listed in Table 1. Electropherogram views of fragments amplified from **D.** heterozygous and **E.** wild type plants using BDFL and BDR primers.

Figure 2. Analysis of Wx-A1 gene types in a segregating F_3 generation of wheat. **W:** wild type; **N:** mutant; **H:** heterozygote.

Conclusions

- The high-throughput capacity of the QIAxcel system provides an efficient way to handle large numbers of plants from breeding programs and is highly suitable for the wheat breeding industry.
- Selection of wild type, mutant, and heterozygous plants is feasible and simple using the QIAxcel system.

References

1. Nakamura, T., Vrinten, P., Saito, M., and Konda, M. (2002) Rapid classification of partial waxy wheats using PCR-based markers. Genome **45**, 1150.

Ordering Information

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