The establishment of PCR-based specific markers for *Starch Synthase II* genes in wheat

Liu CL[1], Chen GS[1], Liu XY, Peng G, Xu J, You MS, Li BY[*] and Liu GT
[1]Beijing Key Laboratory of Crop Genetic Improvement, Key Laboratory of Crop Genomics & Genetic Improvement, Ministry of Agriculture, Key Laboratory of Crop Heterosis & Utilization, Ministry of Education; China Agricultural University(CAU), Beijing 100094, China

ABSTRACT

Starch composition in wheat endosperm is a major factor determining starch quality. In common wheat, starch consists of around 25% amylose and 75% amylopectin, but, the enzymes catalyzing synthesis of amylose or amylopectin may be absent in some cultivars . These mutant cultivars can be used to breed new cultivars in which amylose content is higher or lower than those in common varieties. There are three starch synthase IIs encoded by three homoeologous alleles, SSII-A1, SSII-B1, and SSII-D1 in common wheat. These enzymes play important roles in amylopectin synthesis. In this study, five specific pairs of PCR primers were developed for SSII alleles, based on the wheat ssII gene sequences, and the number of primer pairs for ssII-a1, ssII-b1, ssII-d1 genes were 2, 2 and 1, respectively. The specificity of these primers was validated on Chinese spring and its 6 nulli-tetrasomic lines.
Keywords: Wheat; Starch SynthaseII; PCR-based marker

INTRODUCTION

Starch consists of around 25% amylose and 75% amylopectin and accounts for about 65% dry weight of common wheat (*Triticum aestivum* L.) endosperm (Davis, 1994; Rahman, 1994). Amylose is a linear or less branched α-1,4 linked glucan polymer, and amylopectin is a high branched polymer connected by small amylose *via* α-1,6 glycosidic bonds. The molecular weights of amylose and amylopectin are 10^4-10^5 D and 10^7-10^8 D, respectively. There are two types of starch granules, A-type granule and B- type granule. A-type granule has a plate or lens form in diameter from 15nm to 30nm. It is 3% of all starch granules in number and accounts for more than 70% of total starch in weight. B-type granule has a ball or polygon shape with diameter from 2.8nm to 9.9nm. it is 97% of all starch granules in number and accounts for less than 30% of total starch in weight (Morrison and Gadan,1987; Peng et al, 1999; Gains et al, 2000).

Four groups of biosynthetic enzymes, ADPG-pyrophosphorylase, starch synthase, starch branching enzyme and debranching enzyme are involved in starch synthesis in plastids (Denyer et al, 1995; Smith et al, 1997). In wheat endosperm, there are four kinds of starch synthases, granule-bound starch synthasel (GBSSl), starch synthase II (SSII), starch synthase II (SSII) and starch synthase II (SSII,)(Li et al, 1999).

GBSS I, also called waxy protein, plays major roles in amylose synthesis and long chain synthesis of amylopectin. The *GBSS I* locus has three alleles, *Wx-A1* (7AS), *Wx-B1* (4AL) and *Wx-D1* (7DS) and thus three allelic waxy proteins are produced in hexaploid wheat, (Denyer et al, 1996; Vrinten and Nakamura, 2000). If chromosome deletions or gene mutations happen at 3 Wx alleles, waxy wheat in which amylose content is less than 1% will be generated (Nakamura et al, 1995; Zhao and Sharp et al, 1998).

SSII is involved in amylopectin synthesis and in common wheat, there are 3 homoeologous proteins encoded by SSII-A1, SSII-B1, and SSII-D1 alleles located on 7AS, 7BS and 7DS. The molecular weights of SSII-A1, B1, and D1 are 115KD, 100KD and 108KD, respectively (Denyer et al, 1995; Yamamori and Endo, 1996). A few of wheat varieties such as Chosen 57 (null SSII-A1), Kanto 79(null SSII-B1), and Turkey 116(SSII-D1) lack SSII proteins.. A SSII null line displaying a high amylose content and altered amylopectin structure was also bred (Yamamori and Endo, 1996; Yamamori et al, 2000; Shimbata et al, 2005).

SDS-PAGE has been an effective method in SSII mutant selection, but its repeatability was low in our experiments. PCR-based marker assisted selection has been a highly effective selection method in wheat breeding. Many PCR-based markers have been developed for GBSS I gene detection. Shimbata et al (2005) developed 3 PCR-based markers to select a SSII null line. The markers, however, produced unspecific amplifications in Chinese varieties that we tested. To solve the problem, 4 new specific PCR-based markers for SSII were developed in this study.

MATERIAL AND METHODS

2.1 Experimental Materials

Chinese spring and its six nulli-tetrasomic lines (N7AT7B, N7AT7D, N7BT7A, N7BT7D, N7DT7A and N7DT7B) were used to determine the specificity of PCR primers designed for *ss* genes .

2.2 DNA extraction

Fresh leaves were cut from seedling, frozen with liquid nitrogen and then ground to powder. Genomic DNA was extracted using CTAB method.

2.3 Primer design

The DNA sequences of *ssII-a1*(genebank accession: AB201445), *ssII-b1*(genebank accession: AB201446) and *ssII-d1*(genebank accession: AB201447) were reported by Shimbata *et al* previously. PCR primers for *ssII* genes were designed with DNAman (version: 6.0) software,and the primers' sequences were listed in table1.

2.4 PCR amplification

PCR amplification was carried out in a 10μl reaction system consisting of 1μl 10× buffer, 1μl 25mM dNTPs, 0.25U Taq DNA polymerase, 1μl 2.5mM forward and reversed primer, 25ng DNA template. The reaction was initiated by one cycle at 94 ^{o}C for 5 min; then 35 cycles of 94 ^{o}C for 35s, 57.5 ^{o}C or 52 ^{o}C (for primer B1) for 35s, 72 ^{o}C for 35s; One cycle of 72 ^{o}C for 5min. The PCR products were separated by electrophoresis in 8% polyacrylamide gel at 180V for 3h and visualized by silver staining.

RESULTS

Four pairs of primers were designed to amplify SSII gene fragments from Chinese Spring and its six nulli-tetrasome lines. Primer A1 and A2 were found to be specific for *ssII-a1* allele. With primer pairs A1 and A2, there was no PCR amplification from N7AT7B and N7AT7D lines, while a specific 307bp (for A1) or 508bp (for A2) DNA fragment was amplified from Chinese Spring and the other four nulli-tetrasome lines(fig.1 a). Fig. 1b showed that Primer pairs B1 and B2 were specific for *ssII-b1* allele .

DISCUSSION

Starch quality is important in wheat bread making and cooking. Obtaining high amylose content wheat mutants is a new trend in wheat breeding, but high amylose content mutants in wheat are rare in nature. Artificial mutagenesis methods such as ultraviolet radiation, spaceflight and chemical mutagens may be used to accelerate the process. A null SSII line was reported and its amylose content in starch was 30.8–37.4 %. Further analysis showed that SSII was not the restrictive enzyme in amylopectin synthesis (Yamamori et al.,2000). Other null SSII lines should be created to advance the research of SSII effects on starch phenotypes. The marker assisted selection for SSII-A1, B1 and D1 genes can be undertaken by using the specific primers designed in this study to accelerate the breeding progress for amylose content.

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Table1 specific PCR primers for SSII genes in common wheat

primer name	Forward primer sequence	reversed primer sequence
A1	5'-TGTCTCTTGCCTGCTGATAAG-3'	5'-TGTCTTTGAGCCACCCAT-3'
A2	5'-TGTCTCTTGCCTGCTGATAAG-3'	5'-ACACGACCAAATGTCTCTGAT-3'
B1	5'-ATCCACCAACCCAACG-3'	5'-GCTACGCACCACAAGAGT-3'
B2	5'-CGCAGGTATGCTGGTAG-3'	5'-CGTAGTATCACAGATGGTCTC-3'

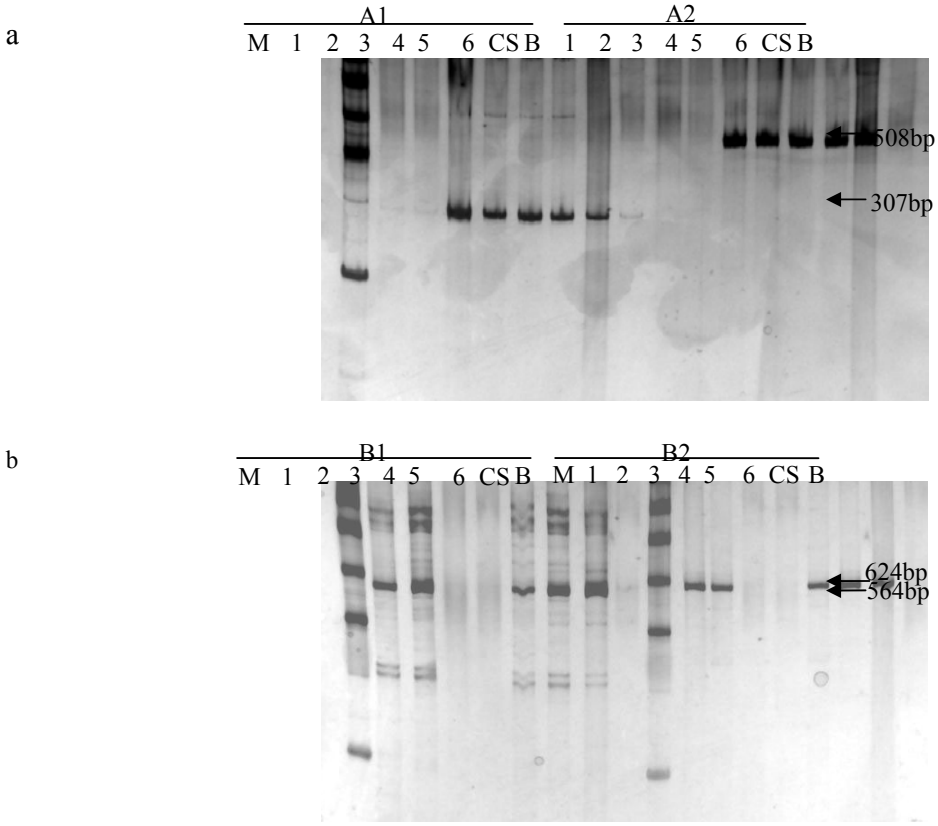

Fig.1 PCR results of 4 pairs of primers. M, molecular size marker; 1, N7AT7B; 2, N7AT7D; 3, N7BT7A; 4, N7BT7D; 5, N7DT7A; 6, N7DT7B; CS, Chinese Spring; B, Blank test. Primer pair A1 and A2 produced a 307bp and 508bp segment respectively except for N7A lines (picture a), and primer pairB1 and B2 produced a 564bp and 624bp segment respectively except for N7B lines (picture b).