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ORIGINAL PAPER

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Mutations in wheat *starch synthase II* genes and PCR-based selection of a SGP-1 null line

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Abstract Wheat (*Triticum aestivum L.*) starch synthase II, which is also known as starch granule protein 1 (SGP-1), plays a major role in endosperm starch synthesis. The three SGP-1 proteins, SGP-A1, B1, and D1, are produced by three homoeologous *SSII* genes, *wSSII-A*,*B*, and *D*. Lines carrying null alleles for each SGP-1 protein have previously been identified. In this report, the mutations occurring in each *wSSII* gene were characterized, and PCR-based DNA markers capable of detecting the mutations were developed. In the null *wSSII-A* allele, a 289 bp deletion accompanied by 8 bp of filler DNA was present near the initiation codon. A 175 bp insertion occurred in exon 8 of the null *wSSII-B* allele. The insertion represented a recently discovered miniature inverted-repeat transposable element (MITE) named *Hikkoshi* that was first found in a wheat *waxy* gene. A 63 bp deletion was found at the region surrounding the junction of the fifth exon and intron of the null *wSSII-D* allele. Based on this information, we designed primer sets to enable us to conduct allele-specific amplifications for each locus. The applicability of these primer sets for breeding programs was demonstrated by reconstructing a line lacking all three SGP-1 proteins using marker-assisted selection. These markers will also be useful in breeding programs aimed at obtaining partial mutants missing one or two SGP-1 proteins.

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Introduction

Starch consists of a mixture of amylopectin, an α-1,4 linked glucan polymer that is highly branched via α-1,6 linkage, and amylose, an essentially a linear or lightly branched polymer. Starch synthase catalyzes the elongation of these α-1,4 glucan chains. Wheat endosperm has four classes of starch synthase: granule-bound starch synthase I (GBSSI), and starch synthases I (SSI), II (SSII), and III (SSIII). GBSSI, a 60 kDa exclusively granule-bound protein, is solely responsible for amylose synthesis (Mu-Forster et al. 1996), and also appears to be involved in the elongation of long chains in amylopectin (Denyer et al. 1996). Although wheat SSI, II, and III are thought to be involved in amylopectin synthesis, the roles of these enzymes have not been clearly elaborated. These starch synthases are differentially distributed in wheat endosperm tissue. While SSI found both in the soluble and granule-bound fractions of endosperm starch (Denyer et al. 1995; Li et al. 1999a), SSIII is found exclusively in the soluble fraction (Li et al. 2000). SSII is found in both fractions early in endosperm development, but is found only in the granule-bound fraction in the mid- to late-endosperm developmental stages (Li et al. 1999b).

The starch granule proteins (SGP) of wheat include a homoeologous set of proteins, SGP-A1, SGP-B1, and SGP-D1, which are encoded by loci located on the group seven chromosomes (Yamamori and Endo 1996). Li et al. (1999b) showed that these SGP-1 proteins correspond to the wheat starch synthase II (wSSII) enzymes, and cDNAs for the three homoeologous genes, designated as *wSSII-A*,*B*, and *D*, have been cloned (Li et al. 1999b; Gao and Chibbar 2000). By identifying lines missing each of the SGP-1 proteins and using these lines in crosses, Yamamori et al. (2000) were able to produce a line lacking all three SGP-1 proteins. The starch granules from this line showed high apparent amylose levels and significant alterations in granule morphology and amylopectin

chain length distribution, suggesting that the SSII enzyme of wheat has a major effect on amylopectin synthesis. In other higher plants such as pea (Craig et al. 1998), barley (Morell et al. 2003), and maize (Zhang et al. 2004) a lack of SSII has similar effects on apparent amylose content and amylopectin characteristics.

Maize lines with mutations in the gene encoding SSIIa (*sugary2*) produce starch that is useful for food and industrial purposes (Zhang et al. 2004), and in rice, the major gene that separates the economically important *indica* and *japonica*-type cultivars maps to the locus encoding SSIIa (Umemoto et al. 2002). Thus, the unique amylopectin characteristics and high apparent amylose levels of starch from null SGP-1 wheat lines may allow the development of new uses for this starch in food industries, and breeding programs to produce new null SGP-1 commercial varieties are underway. However, the identification of null lines by SDS-PAGE analysis of SGP, which is the only screening method presently available, is not practical in breeding programs employing large population numbers. PCR-based marker-assisted selection (MAS) of wheat lines lacking one or two GBSS proteins has been successfully used in wheat breeding programs (Nakamura et al. 2002), and low amylose lines with good noodle-making quality have been obtained. The availability of similar markers for the null *wSSII* alleles could greatly accelerate breeding programs. Here, we identify the lesions present in previously identified lines lacking each SGP-1 protein (Yamamori and Endo 1996), and describe co-dominant markers for each *SSII* gene of wheat.

Materials and methods

Plant materials

Common wheat (*Triticum aestivum* L.) cultivar Chinese Spring (CS) was used to obtain the sequences of the *wSSII* genes located on chromosomes 7A, 7B, and 7D. The CS nullisomic/tetrasomic (NT) lines (Sears and Miller 1985) for homoeologous group seven chromosomes, N7AT7D, N7BT7D, and N7DT7B, were used to determine the chromosome position of each gene. Chosen 57 (C57), Kanto 79 (K79), and Turkey 116 (T116), which carry null mutations in *wSSII-A*, *-B*, and *-D* genes, respectively, were used as sources for null alleles. C57 and T116 were obtained from the gene bank of the National Institution of Agrobiological Science (NIAS).

Genomic DNA preparation

Wheat genomic DNA was extracted from young leaf tissue using a DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA) or a Nucleon Phytopure Plant DNA Extraction kit (Amersham Biosciences, Little Chalfont, UK) according to the manufacturer's instructions.

Table 1 Primers for cloning of genomic sequences

Primer name	Sequence
SSII Fow1	5′-GGGGGCCGTTCGTACGTACCC
SSII Rev1	5′-CGAAATTTGAGCCGGACGAC
SSII Fow2	5′-AGGGATCCCGTCAAGACGCTCG
SSII Rev2	5′-GTGCCGTGTGCCAATC
SSII Fow3	5′-CATGCGGCGGTGTCCCTTATG
SSII Rev3	5′-TCACCACTGGTACTTGGCCTTGA

Primers and PCR amplification

Primers for cloning *wSSII* genes were designed based on *Aegilops tauschii* Coss. genomic DNA sequences (accession no. AY133248) (Li et al. 2003), and *T. aestivum* Ss2a-1 (accession no. AJ269502), Ss2a-2 (accession no. AJ269503), and SS2a-3 (accession no. AJ269504) cDNA sequences (Gao and Chibbar 2000). The primer sequences are given in Table 1. Each gene was amplified in three segments using the primer sets SSII Fow1/Rev1, Fow2/Rev2, and Fow3/Rev3. These primer sets are capable of producing fragments from all three *wSSII* genes, such that each amplification product contained three sequences. Amplification products were cloned, sequenced, and grouped into three contigs based on overlapping regions. The contigs were then aligned with previously reported *wSSII* sequences (Li et al. 1999b; Gao and Chibbar 2000).

Typically, 20 μl of PCR reaction mixture contained 5.0 pmol primers, 20.0 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, and 0.5 U of LA Taq DNA polymerase (Takara BIO, Kyoto, Japan) in supplemented buffer. The PCR cycle consisted of a 5 min denaturation at 98°C, followed by 40 cycles of 30 s at 98°C, 30 s at 64°C, and 2 min at 74°C, then a final 15 min extension at 74°C. PCR products were analyzed by electrophoresis using 0.8% agarose gels.

Cloning and sequence analysis

Amplified PCR products were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA, USA). After sequencing, insert sequences were aligned using ATGC software (GENETYX Corporation, Tokyo, Japan).

PCR identification of mutations

The primers used for the identification of each mutated allele are listed in Table 2. Primers were designed to produce distinguishable fragments from wild type and null alleles. Each 20 μl reaction included 10.0 pmol primers, 20.0 ng genomic DNA, 2.25 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.5 U LA Taq DNA

Table 2 Primers for the detection of mutations occurring in each *wSSII* gene

Genes	Primer name	Sequence
wSSII-A	SSII AF1	5′-GCGTTTACCCCACAGAGC
	SSII AR1	5′-ACGCGCCATACAGCAAGTCATA
wSSII-B	SSII BF1	5′-ATTTCTTCGGTACACCATTGGCTA
	SSII BR1	5′-TGCCGCAGCATGCC
wSSII-D	SSII DF1	5′-GGGAGCTGAAATTTTATTGCTTATTG
	SSII DR1	5′-TCGCGGTGAAGAGAACATGG

polymerase in supplemented buffer. The PCR cycle consisted of an initial 5 min denaturation at 98°C, followed by 40 cycles of 30 s at 98°C, 30 s at 65°C, and 1 min at 74°C, and a final 5 min extension at 74°C. PCR products were analyzed on 3% agarose gels.

SDS-PAGE analysis

Preparation of starch granules from mature seeds and SDS-PAGE analysis were conducted according to our previous work (Nakamura et al. 1992).

Results

Sequencing of *wSSII* genes

To obtain genomic sequences for *wSSII-A*, *-B*, and *- D*, we designed PCR primers (Table 1) based on sequences of previously isolated cDNA clones (Li et al. 1999a, 1999b; Gao and Chibbar 2000) and a *SSII* genomic DNA sequence from *Ae. tauschii* (Li et al. 2003). The *Ae. tauschii* clone (*ATSSII*) contained more 5′-sequence information than the cDNAs and was therefore particularly useful in designing primers for the upstream regions. Since *Ae. tauschii* is the D genome donor of common wheat, it is likely that the *ATSSII* sequence shares a high level of homology with *wSSII* sequences, particularly with *wSSII-D1*. Each gene was amplified from CS in three overlapping fragments that together covered the area from the 5′ untranslated region past the translation termination codon. The aligned sequences were grouped into three contigs with lengths of 6898, 6811, and 7010 bp. The three contigs shared sequence similarities of 82%–87%. Of the three contigs, the 7010 bp contig showed the highest similarity (99.8%) to *ATSSII*. Only 17 mismatches occurred between the two sequences, five of which were located in exons and 12 in introns (data not shown). Therefore, the 7010 bp contig represented the genomic sequence of *wSSII-D* derived from the D genome (Accession number AB201447).

The exon–intron structure of all three contigs seemed to match well with the exon–intron structure of *Ae. tauschii* (Li et al. 2003), allowing us to deduce the positions of the exons in the three sequences. The coding sequences of the three contigs shared more than 96% similarity. The predicted coding sequences of the 6898 and 6811 bp contigs matched exactly with those of Ss2a-2 and Ss2a-3, respectively, while the deduced coding sequence of the 7010 bp contig differed from that of Ss2a-1 by a single nucleotide mismatch. The deduced amino acid sequences of the coding region did not match as well with the sequences presented by Li et al. (1999b), which were obtained from a cDNA library made from the cultivar Wyuna. However, based on similarity levels between the deduced amino acid sequences of the 6898 and 6811 bp contigs to the deduced amino acid sequences of the *wSSII-A* and *wSSII-B* cDNAs, the 6898 bp contig was designated as the *wSSII-A* gene (Accession number: AB201445) and the 6811 bp contig as the *wSSII-B* gene (accession no. AB201446).

Sequences of mutated *wSSII* genes

Yamamori and Endo (1996) used SDS-PAGE analysis to identify mutants that lack one of the three SGP-1 proteins and crossed the three partial SGP-1 mutants to produce a null SGP-1 line. The same single null lines, C57, K79, and T116, were used in this study, and seed samples were analyzed by SDS-PAGE to reconfirm that they lacked SGP-A1, -B1, and -D1, respectively (Fig. 1). Genomic sequences of *wSSII-A1* from C57, *wSSII-B1* from K79, and *wSSII-D1* from T116 were acquired using the same primers that were used for the wild type genes (Table 1). Sequences of 6619, 6986, and 6946 bp were obtained for null *wSSII-A*, *B*, and *D* genes, respectively.

By comparing these sequences with sequences from the wild type genes, a 289 bp deletion including the initiation codon plus an insertion of 8 bp of filler DNA was identified in the null *wSSII-A* allele (Fig. 2a). In the *wSSII-B* gene of K79, an insertion of 175 bp occurred in the middle of exon 8 (Fig. 2b). The null *wSSII-D* allele carried a 63 bp deletion that spanned the junction between exon 5 and intron 5 (Fig. 2c). Besides these major differences, no mismatches were detected between the wild type and null *wSSII-B* alleles, while a two-nucleotide insertion and a one-nucleotide deletion were observed in the seventh introns of the null *wSSII-A* and *-D* alleles, respectively.

PCR-based DNA markers for detection of *wSSII* alleles

Primer sets for all three genes were developed based on the sequences of the wild type and null alleles (Table 2

and Fig. 3). Co-dominant markers, capable of distinguishing among heterozygous, homozygous null, and homozygous wild type plants, were obtained by mapping primers to either side of a particular mutation. To ensure that the primers were gene-specific and did not amplify fragments from homoeologous loci, the primer set for each gene was tested against the nullisomic-tetrasomic line missing that gene.

With the primer set SSII AF1 and AR1, a single fragment of about 450 bp was amplified from CS, N7BT7D, and N7DT7B, which carry wild type SSII alleles, while a fragment of less than 200 bp was amplified from C57, which carries the null *wSSII-A* allele (Fig. 4a). Moreover, no amplification occurred with N7AT7D, indicating that the amplification products were derived only from the *wSSII* gene on chromosome 7A.

The SSII BF1 primer was designed to hybridize to a sequence found only in the *wSSII-B* alleles (Table 2), therefore the SSII BF1/BR1 primer set should be completely gene-specific. As expected, a single fragment of 671 bp was amplified from genomic DNA of CS, N7AT7D, and N7DT7B, while no amplification occurred with N7BT7D (Fig. 4b). The amplification of a single, larger fragment of approximately 850 bp from K79 was indicative of the 175 bp insertion present in the null *wSSII-B* allele.

For the identification of the *wSSII-D* mutation, the primers SSII DF1 and DR1 were designed from the *wSSII-D* sequence, although both primers shared some homology with the other two *wSSII* genes. DF1 primer mismatch with *wSSII-A* and *-B* genes was 4 out of 26 nucleotides, and DR1 primer mismatch was 5 out of 20 nucleotides. However, with this primer set, only a single product of 558 bp was amplified from CS, N7AT7D, and N7BT7D, while no product was amplified from N7DT7B (Fig. 4c). When the DNA of T116 was used as template, a single fragment of about 500 bp was produced. Thus, this primer set is specific enough to amplify only the targeted fragments from *wSSII-D*. For each primer set, sequencing of amplification products from both wild type and mutated alleles confirmed that the correct DNA fragments were amplified.

Selection of *wSSII* null lines using PCR markers

The PCR markers described in Table 2 were used for MAS of null *wSSII* lines. Initially, crosses were made between K79 (null SGP-B1) and C57 (null SGP-A1), and between K79 and T116 (null SGP-D1). The F_2 progeny from each cross were screened with the appropriate markers, allowing the identification of KC1, which carries null alleles at the *wSSII-A* and - *B* loci, and KT1, which carries null alleles at the *wSSII-B* and - *D* loci (Fig. 5a, b). Next, we crossed KC1 and KT1, and screened 96 F_2 plants from this cross using all three markers. Three plants that were homozygous null at all three *wSSII* loci were identified; the marker pattern for one of these plants (SSIIm) is shown in Fig. 5b. Starch granules were isolated from SSIIm seed, and the granule-bound protein was subjected to SDS-PAGE analysis (SSIIm, Fig. 5c). The three SGP-1 proteins were missing from the starch granules, confirming that the selected plant was null at all *wSSII* loci, and demonstrating that MAS as described here is a viable method for selection of wheat lines missing some or all SGP-1 proteins. SSIIm starch granules also had lower levels of SGP-2 and -3 proteins, as was previously observed in the SGP-1 mutant produced by Yamamori et al. (2000). The co-dominant markers developed in this study were also capable of distinguishing plants that were heterozygous at specific *wSSII* loci. This is demonstrated in Fig. 5b with plant KC2, which is heterozygous at *wSSII-A* and -*B*, and KT2, which is heterozygous at *wSSII-B* and -*D*.

Discussion

In this study, we obtained genomic sequences for *wSSII-A*, - *B*, and - *D*, and characterized the null alleles of these three genes. Mutations were identified in the coding regions of all three *wSSII* genes. The null *wSSII-A* allele from the line C57 carried a 289 bp deletion accompanied by an 8 bp insertion of filler DNA (Figs. 2a and 3b). Filler DNA is frequently associated with spontaneous deletions (Wessler et al. 1990; Li et al. 1997; Grant et al. 1998), and is often derived from nearby sequences.

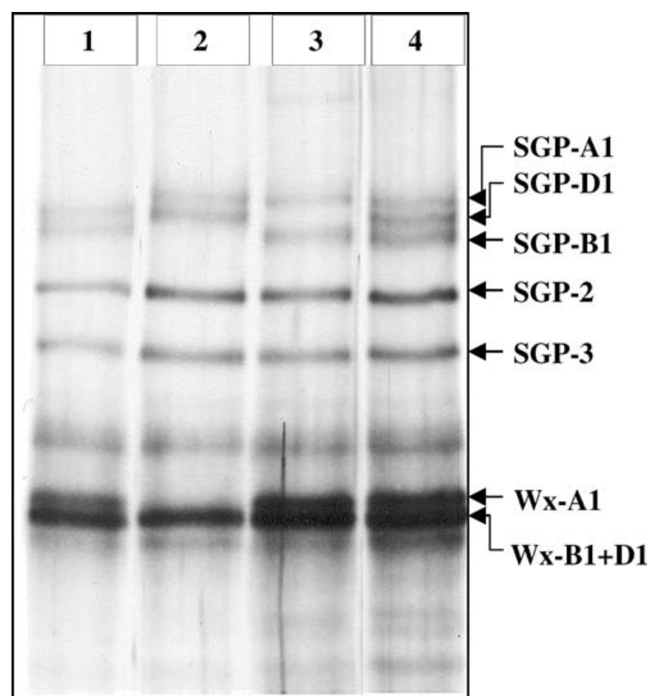

Fig. 1 Occurrence of SGP-1 proteins in wheat lines. Endosperm starch granule proteins were analyzed by SDS-PAGE. *Lane 1* Chosen 57 (*null wSSII-A*), *Lane 2* Kanto 79 (*null wSSII-B*), *Lane 3* Turkey 116 (*null wSSII-D*), and *Lane 4* Chinese Spring (*wild type*). SGP-1, SGP-2, and SGP-3 are products from the *SSII*, *BEII*, and *SSI* genes, respectively. Kanto 79 also lacks Wx-A1 and D1 proteins

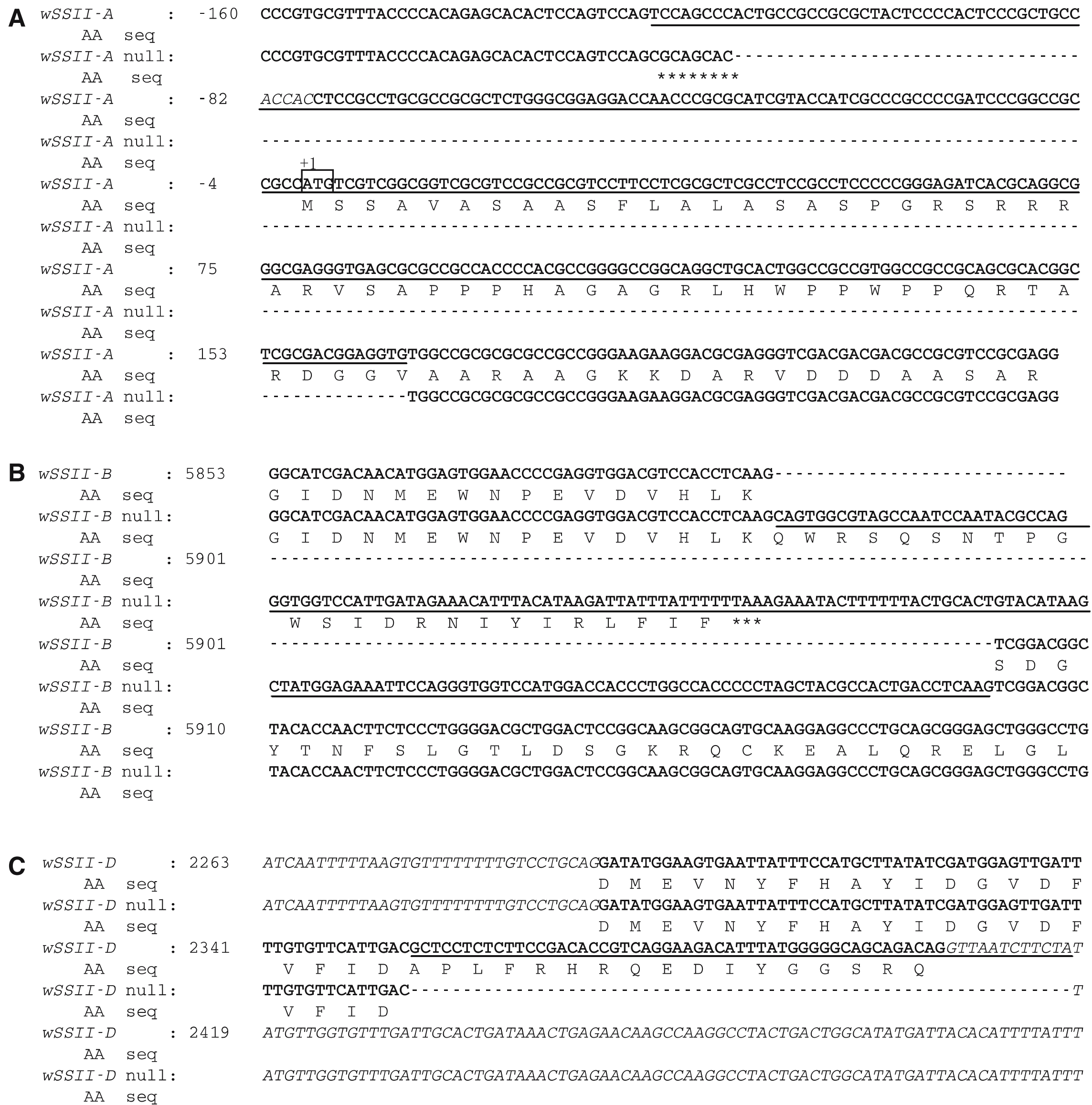

Fig. 2 Nucleotide and deduced amino acid (AA) sequence alignments of wild type and mutated *wSSII* genes. The areas surrounding the mutations are presented. Intron sequences are shown in *italics* and exon sequences are shown in **bold**. The numbering at the left begins with the first nucleotide of the initiation codon. **A** Alignment of wild type and null *wSSII-A* alleles from −60 to +258. In the null allele, a 289 bp deletion that includes the initiation codon is underlined, and an 8 bp filler DNA sequence is indicated by *asterisks*. **B** Alignment of wild type and null *wSSII-B* alleles. The area shown is a portion of exon 8. An insertion in the null allele is underlined and a termination codon within the insertion is indicated by *asterisks*. **C** Alignment of wild type and null *wSSII-D alleles*. A 63 bp deletion occurring at an intron–exon junction in the null allele is underlined

However, the origin of the filler DNA in the null *wSSII-A* allele is uncertain, since it does not match perfectly to any sequence close to the deletion. The deletion in this gene caused the loss of the translational initiation codon and the surrounding sequence; therefore, functional protein cannot be produced from this allele. The null *wSSII-D* allele of T116 carried a 63 bp deletion that included an exon–intron junction (Fig. 2c), suggesting that normal splicing between exons 5 and 6 cannot occur. The null *wSSII-B* allele carries a 175 bp insertion in the middle of exon 8 (Figs. 2b and 3b). The sequence of this insertion showed a high similarity (88%) with that of the miniature inverted-repeat transposable element (MITE), *Hikkoshi* (Saito et al. 2004, 2005). If transcription and translation of the *Hikkoshi* sequence in exon 8 occurs, the translation product would terminate prematurely at a stop codon within this MITE (Fig. 2b). Consequently, the abnormal peptide would be 197 amino acids shorter than wild-type *wSSII-B*, and 24 amino acids at the C-terminus would be altered.

Hikkoshi was first identified in the null *Wx-A1* alleles carried by a number of varieties from Turkey (Saito et al. 2004). The 12 bp terminal inverted repeats (TIRs) were identical in the elements found in the *Wx-A1* and the *wSSII-B* alleles, but the 8 bp target site duplications, which flank the TIRs and are derived from host DNA, differ between the two elements. There was little

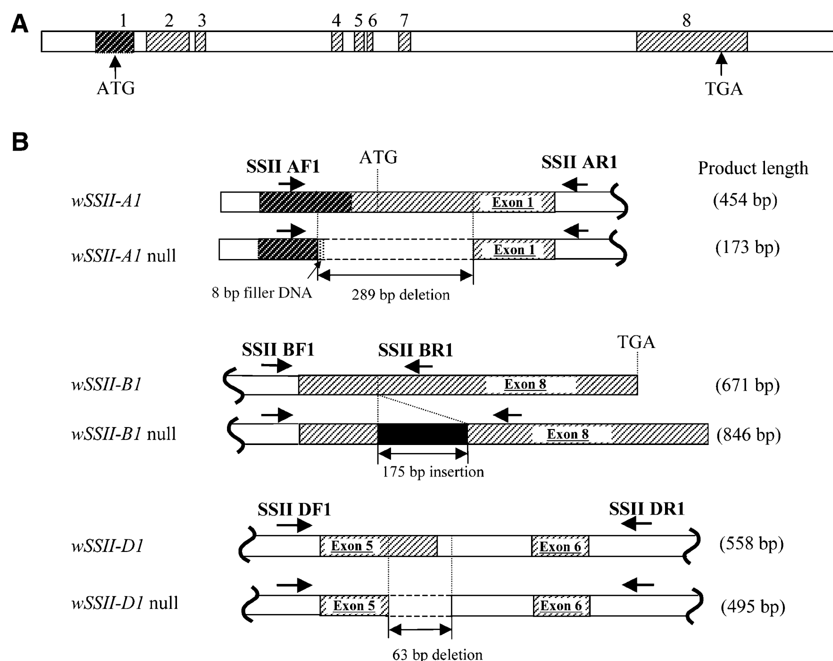

Fig. 3 Diagram of *wSSII* genes and primer positions. **a** *wSSII* genomic structure based on *Ae. tauschii* data (Li et al. 2003). Hatched boxes represent exons 1–8, and white boxes represent introns. **b** Mutations in *wSSII* genes. *Arrows* show the positions of primers. *Broken lines* indicate the deletions in null *wSSII-A* and - *D* alleles, and a *filled box* indicates the insertion in the null *wSSII-B* allele. The fragment size obtained using the indicated primer set is shown in brackets at the right of each allele. In both **a** and **b**, the putative 5'-untranslated region in the first exon is shown as a *box with dark cross stripes*, since the transcription start site has not yet been determined

sequence similarity between the insertion sites in the *Wx-A1* and *wSSII-B* genes, and we could not identify a shared site preference. The Turkey varieties carrying a *Hikkoshi* MITE in the *Wx-A 1* gene represent domesticated wheat lines collected outside of Erzurum in Turkey. The pedigree of K79, which carries the null *wSSII-B*, did not include progenitors related to the varieties from Turkey. This, together with the presence of *Hikkoshi* in the CS genome (our unpublished data) suggests *Hikkoshi* MITEs are present in wheat varieties from throughout the world.

Although Yamamori et al. (2000) have characterized the endosperm starch from a line missing all three SGP-1 proteins, such data is not available for lines missing one or two SGP-1 proteins. Each member of a set of three homoeologous genes in hexaploid wheat does not necessarily contribute equally to phenotype. For example, Yamamori and Quynh (2000) demonstrated that the effect of each of the wheat *GBSS1* genes on amylose synthesis varied, and individual combinations of null and wild type loci resulted in significantly different amylose contents. It is likely that a similar situation occurs with the *wSSII* genes, and lines null at one or two loci may have unique starch characteristics. Recently, efforts to introduce partial or full null SGP-1 characteristics into new cultivars have begun in Japanese wheat breeding programs, and effective screening methods are in demand. Using *wSSII* primers, we were able to identify both wild type and null alleles of *wSSII-A*, *-B*, and - *D* under a single set of PCR conditions (Fig. 4). Furthermore, we were able to select a *wSSII* null line (Fig. 5b). The profile of SGP in this line (Fig. 5c) was similar to that of the SGP-1 null line reported by Yamamori et al. (2000), with both lines showing not only a lack of SGP-1 but also greatly reduced levels of SSI and BE 2 proteins. This indicates that the PCR

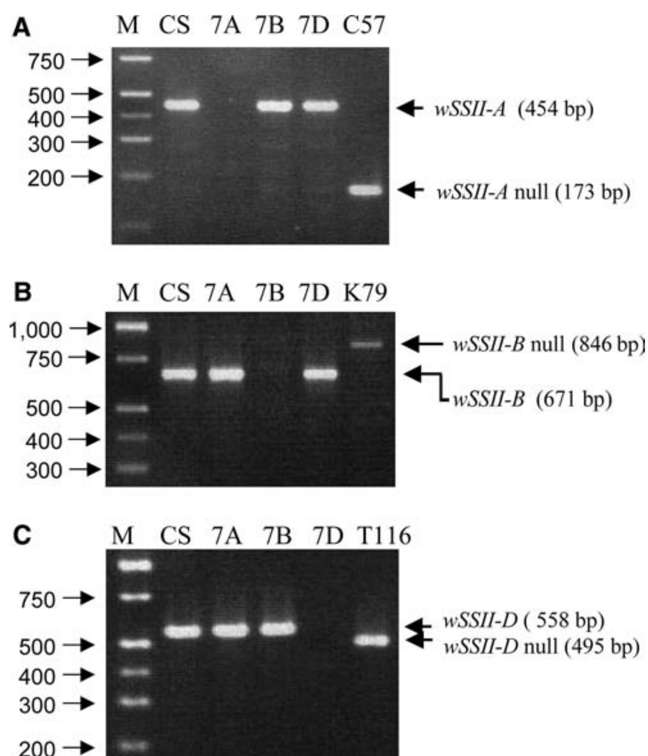

Fig. 4 PCR detection of *wSSII* alleles. Primer sequences are shown in Table 2. *M*, Molecular size marker; *CS*, Chinese Spring; *7A*, N7AT7D; *7B*, N7BT7D; *7D*, N7DT7B; *C57*, Chosen 57; *K79*, Kanto 79; *T116*, Turkey 116. PCR products were analyzed on 3% agarose gels. **A** Detection of wild type and null *wSSII-A* alleles using SSII AF1 and SSII AR1 primers. **B** Wild type and null *wSSII-B* alleles using SSII BF1 and SSII BR1 primers. **C** Wild type and null *wSSII-D* alleles using SSII DF1 and SSII DR1 primers

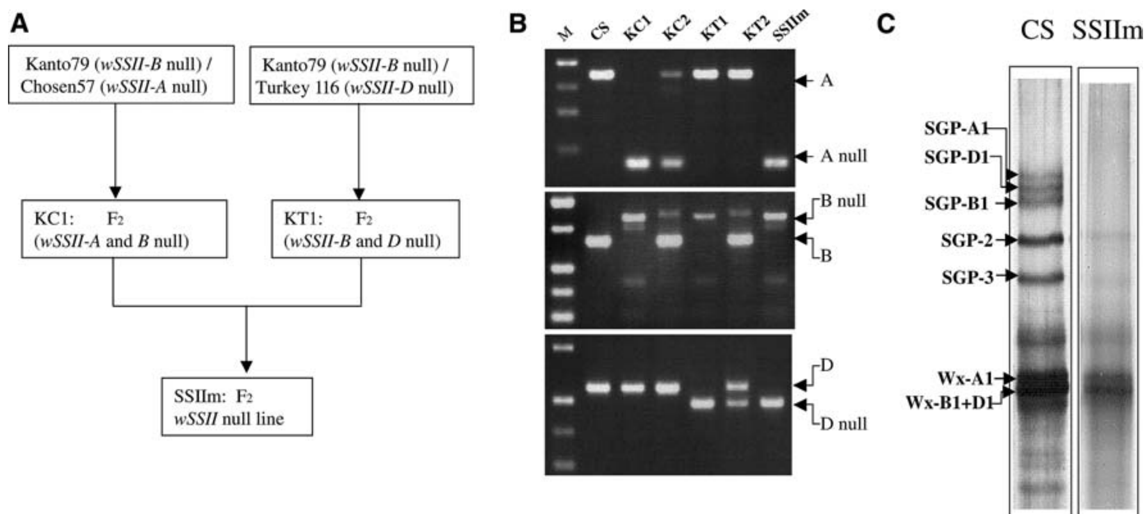

Fig. 5 Production of a null SGP-1 line. **A** Scheme for the production of a *wSSII* null line (SSIIm). K79 (null *wSSII-B*) and C57 (null *wSSII-A*) were crossed, and F_2 plants with null *wSSII-A* and - *B* alleles were selected by PCR. F_2 plants possessing null *wSSII-B* and *-D* alleles were obtained from a cross between K79 and T116 (null *wSSII-D*). The selected plants were crossed and a null *wSSII* line was identified among the F_2 progeny. **B** Selection of partial null lines from each cross using DNA markers. 5 µl of PCR product was loaded in each lane. *Lane M*, molecular marker; *CS*, Chinese Spring (wild type control); *KC1*, F_2 plant from the cross K79/C57 (null at *wSSII-A* and *-B*); *KC2*, F_2 plant from the cross K79/C57 (heterozygous at *wSSII-A* and *-B*); *KT1*, F_2 plant from the cross K79/T116 (null at *wSSII-B* and *-D*); *KT2*, F_2 plant from the cross K79/T116 (heterozygous at *wSSII-B* and *-D*); *wSSIIm*, triple null F_2 plant from the cross KC1/KT1. **C** SDS-PAGE analysis of starch granule proteins from Chinese Spring (CS) and a *wSSII* triple null line (SSIIm)

selection system can successfully replace SDS-PAGE selection methods.

Although the SDS-PAGE method of screening is very effective, only MAS can allow the clear identification of heterozygotes (Fig. 5b), which can save a great deal of time and effort in the introgression of mutated alleles into adapted lines. In addition, for the average lab, handling large numbers of samples is probably more efficiently performed by PCR than by SDS-PAGE. Finally, the availability of markers for other traits, such as grain texture (Gautier et al. 1994; Tranquilli et al. 1999), plant height (Ellis et al. 2002), or gluten strength (Ma et al. 2003) may allow simultaneous selection for several traits of interest. Knight (2003) and Dubcovsky (2004) have reviewed recent progress in the use of MAS in public wheat breeding programs. The availability of suitable DNA markers is an important component of MAS and "perfect markers" derived from the gene controlling the desired traits are preferred (Thro et al. 2004). The markers described here represent new perfect markers suitable for public breeding programs.

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References

Craig J, Lloyd JR, Tomlinson K, Barber L, Edwards A, Wang TL, Martin C, Hedley CL, Smith AM (1998) Mutations in the gene encoding starch synthase II profoundly alter amylopectin structure in pea embryos. Plant Cell 10:413–426

Denyer K, Hylton CM, Jenner CF, Smith AM (1995) Identification of multiple isoforms of soluble and granule-bound starch synthase in developing wheat endosperm. Planta 196:256–265

Denyer K, Clarke B, Hylton C, Tatge H, Smith AM (1996) The elongation of amylose and amylopectin chains in isolated starch granules. Plant J 10:1135-1143

Dubcovsky J (2004) Marker-assisted selection in public breeding programs. In: Thro AM, Parrott W, Udall JA, Beavis WD (eds) The wheat experience. Genomics and plant breeding: the experience of the initiative for future agricultural and food systems. Crop Sci 44:1895–1898

Ellis H, Spielmeyer W, Gale R, Rebetzke J, Richards A (2002) 'Perfect' markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. Theor Appl Genet 105:1038–1042

Gao M, Chibbar RN (2000) Isolation, characterization, and expression analysis of starch synthase IIa cDNA from wheat (*Triticum aestivum* L.). Genome 43:768–775

Gautier MF, Aleman ME, Guirao A, Marion D, Joudrier P (1994) *Triticum aestivum* puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression. Plant Mol Biol 25:43-57

Grant MR, McDowell JM, Sharpe AG, de Torres Zabala M, Lydiate DJ, Dangl JL (1998) Independent deletions of a pathogen-resistance gene in *Brassica* and *Arabidopsis*. Proc Natl Acad Sci USA 95:15843–15848

Knight J (2003) Crop improvement: a dying breed. Nature 421:568–570

Li D, Blakey CA, Dewald C, Dellaporta SL (1997) Evidence for a common sex determination mechanism for pistil abortion in maize and in its wild relative *Tripsacum*. Proc Natl Acad Sci USA 94:4217–4222

Li Z, Rahman S, Kosar-Hashemi B, Mouille G, Appels R, Morell MK (1999a) Cloning and characterization of a gene encoding wheat starch synthase I. Theor Appl Genet 98:1208–1216

Li Z, Chu X, Mouille G, Yan L, Kosar-Hashemi B, Hey S, Napier J, Shewry P, Clarke B, Appels R, Morell MK, Rahman S (1999b) The localization and expression of the class II starch synthases of wheat. Plant Physiol 120:1147–1155

Li Z, Mouille G, Kosar-Hashemi B, Rahman S, Clarke B, Gale KR, Appels R, Morell MK (2000) The structure and expression of the wheat starch synthase III gene. Motifs in the expressed gene define the lineage of the starch synthase III gene family. Plant Physiol 123:613–624

Li Z, Sun F, Xu S, Chu X, Mukai Y, Yamamoto, Ali S, Rampling L, Kosar-Hashemi B, Rahman S, Morell MK (2003) The structural organisation of the gene encoding class II starch synthase of wheat and barley and the evolution of the genes encoding starch synthases in plants. Funct Integr Genomics 3:76–85

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica 134:51–60

Morell MK, Kosar-Hashemi B, Cmiel M, Samuel MS, Chandler P, Rahman S, Buleon A, Batey IL, Li Z (2003) Barley *sex6* mutants lack starch synthase IIa activity and contain a starch with novel properties. Plant J 34:173–185

Mu-Forster C, Huang R, Powers JR, Harriman RW, Knight M, Singletary GW, Keeling PL, Wasserman BP (1996) Physical association of starch biosynthetic enzymes with starch granules of maize endosperm: granule-associated forms of starch synthase I and starch branching enzyme II. Plant Physiol 111:821–829

Nakamura T, Yamamori M, Hidaka H, Hoshino T (1992) Expression of HMW Wx protein in Japanese common wheat (*Triticum aestivum* L.) cultivars. Jpn J Breed 42:681–685

Nakamura T, Vrinten P, Saito M, Konda M (2002) Rapid classification of partial waxy wheats using PCR-based markers. Genome 45:1150–1156

Saito M, Konda M, Vrinten P, Nakamura K, Nakamura T (2004) Molecular comparison of *waxy* null alleles in common wheat and identification of a unique null allele. Theor Appl Genet 108:1205–1211

Saito M, Yonemaru J, Ishikawa G, Nakamura T (2005) A candidate autonomous version of the wheat MITE *Hikkoshi* is present in the rice genome. Mol Gen Genomics 273:404–414

Sears ER, Miller TE (1985) The history of Chinese spring wheat. Cereal Res Commun 13:261–263

Thro AM, Parrott W, Udall JA, Beavis WD (2004) Genomics and plant breeding: the experience of the initiative for future agricultural and food systems. Crop Sci 44:1914–1919 (in Symposium Discussion)

Tranquilli G, Lijavetzky D, Muzzi G, Dubcovsky J (1999) Genetic and physical characterization of grain texture-related loci in diploid wheat. Mol Gen Genet 262:846–850

Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y (2002) Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theor Appl Genet 104:1–8

Wessler S, Tarpley A, Purugganan M, Spell M, Okagaki R (1990) Filler DNA is associated with spontaneous deletions in maize. Proc Natl Acad Sci USA 87:8731–8735

Yamamori M, Endo TR (1996) Variation of starch granule proteins and chromosome mapping of their coding genes in common wheat. Theor Appl Genet 93:275–281

Yamamori M, Quynh NT (2000) Differential effects of Wx-A1, -B1 and -D1 protein deficiencies on apparent amylose content and starch pasting properties in common wheat. Theor Appl Genet 100:32–38

Yamamori M, Fujita S, Hayakawa K, Matsuki J, Yasui T (2000) Genetic elimination of a starch granule protein, SGP-1, of wheat generates an altered starch with apparent high amylose. Theor Appl Genet 101:21–29

Zhang X, Colleoni C, Ratushna V, Sirghie-Colleoni M, James MG, Myers AM (2004) Molecular characterization demonstrates that the *Zea mays* gene *sugary2* codes for the starch synthase isoform SSIIa. Plant Mol Biol 54:865–879