

Empirical verification of heterogeneous DNA fragments generated from wheat genome-specific SSR primers

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Chen, Q.-J., Zhang, L., Yuan, Z., Yan, Z., Zheng, Y., Sun, G. and Liu, D. 2008. **Empirical verification of heterogeneous DNA fragments generated from wheat genome-specific SSR primers.** *Can. J. Plant Sci.* **88**: 1065–1071. Due to the high polymorphisms between synthetic hexaploid wheat (SHW) and common wheat, SHW has been widely used in genetic studies. The transferability of simple sequence repeats (SSR) among common wheat and its donor species, *Triticum turgidum* and *Aegilops tauschii*, and their SHW suggested the possibility that some SSRs, specific for a single locus in common wheat, might appear in two or more loci in SHWs. This is an important genetic issue when using synthetic hexaploid wheat population and SSR for mapping. However, it is largely ignored and never empirically well verified. The present study addressed this issue by using the well-studied SSR marker *Xgwm261* as an example. The *Xgwm261* produced a 192 bp fragment specific to chromosome 2D in common wheat Chinese Spring, but generated a 176 bp fragment in the D genome of *Ae. tauschii* AS60. Chromosomal location and DNA sequence data revealed that the 176 bp fragment also donated by 2B chromosome of durum wheat Langdon. These results indicated that although a single 176 bp fragment was appeared in synthetic hexaploid wheat Syn-SAU-5 between Langdon and AS60, the fragment contained two different loci, one from chromosome 2D of AS60 and the other from 2B of Langdon which were confirmed by the segregating analysis of SSR *Xgwm261* in 185 plants from a F₂ population between Syn-SAU-5 and Chinese Spring. If *Xgwm261* in Syn-SAU-5 was considered as a single locus in genetic analysis, distorted segregation or incorrect conclusions would be yielded. A proposed strategy to avoid this problem is to include SHW's parental *T. turgidum* and *Ae. tauschii* in SSR analysis as control for polymorphism detection.

Key words: Synthetic hexaploid wheat, microsatellite, segregation distortion, *Xgwm261*, transferability

Chen, Q.-J., Zhang, L., Yuan, Z., Yan, Z., Zheng, Y., Sun, G. et Liu, D. 2008. **Vérification empirique des fragments d'ADN hétérogènes issus des amorces SSR spécifiques au blé.** *Can. J. Plant Sci.* **88**: 1065–1071. La génétique recourt largement au blé hexaploïde synthétique (BHS) à cause de son grand polymorphisme avec le blé commun. Le fait qu'on puisse transférer les SSR du blé commun et des espèces donatrices *Triticum turgidum* et *Aegilops tauschii* au BHS laisse croire que certains SSR, spécifiques à un locus donné du blé commun, se retrouvent sur deux locus ou plus du BHS. Il s'agit d'un point important à tenir compte en génétique, lorsqu'on utilise le BHS et des SSR pour cartographier le génome. Pourtant, on le fait rarement et la chose n'a jamais été vérifiée de manière empirique. L'étude devait combler cette lacune en prenant le marqueur SSR bien connu *Xgwm261* comme exemple. Le marqueur *Xgwm261* produit un fragment de 192 bp spécifique au chromosome 2D du blé commun Chinese Spring et un fragment de 176 bp du génome D d'*Ae. tauschii* AS60. L'emplacement du marqueur sur le chromosome et les données sur la séquence de l'ADN révèlent que le fragment de 176 bp vient aussi du chromosome 2B du blé dur Langdon. On en conclut que le fragment de 176 bp qui apparaît dans le BHS Syn-SAU-5, entre Langdon et AS60, comprend deux locus, le premier du chromosome 2D de AS60 et le second du chromosome 2B de Langdon, ce que confirme l'analyse par ségrégation du SSR *Xgwm261* chez 185 plants de la F₂ d'un croisement entre Syn-SAU-5 et Chinese Spring. Si le SSR *Xgwm261* de Syn-SAU-5 était considéré comme émanant d'un locus unique dans une analyse génétique, il en ressortirait des conclusions erronées. Les auteurs proposent une façon d'éviter le problème, en l'occurrence inclure les parents *T. turgidum* et *Ae. tauschii* du BHS comme témoins dans l'analyse des SSR pour déceler le polymorphisme.

Mots clés: Blé hexaploïde synthétique (BHS), microsatellite (SSR), distorsion par ségrégation, *Xgwm261*, transférabilité

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Abbreviations: CS, Chinese Spring; LDN, Langdon; QTL, quantitative trait loci; SDL, segregation-distortion loci; SHW, synthetic hexaploid wheat; SSR, simple sequence repeats

Bread or common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD), one of the most remarkable allopolyploids, was formed by spontaneous hexaploidization after the intercrossing between *T. turgidum* and *Ae. tauschii* (Kihara 1944; McFadden and Sears 1944). Limited genotypes of donor species were involved in the origin of common wheat. Due to the evolution bottleneck, numerous genetic variations in the ancestral *T. turgidum* or *Ae. tauschii* are not represented at the hexaploid level (Reif et al. 2005; Warburton et al. 2006). The creation of synthetic hexaploid wheats (SHW) by chromosome doubling of *T. turgidum* × *Ae. tauschii* hybrids (McFadden and Sears 1944), which is analogous to the origin of hexaploid wheat, has been presenting a promising method to unlock the diversity conserved in the progenitors of wheat (Hoisington et al. 1999; Van Ginkel and Ogonnaya 2007). In addition to their usefulness in breeding, due to the high polymorphisms in SHWs, they have been widely used to construct populations for genetic analysis.

Microsatellite or simple sequence repeat (SSR) markers are widely used for genetic studies and breeding programs in common wheat. The molecular genetic maps of common wheat contain over 1500 SSRs, some of them were mapped to single locus and specific to individual chromosome or chromosome arm (Bryan et al. 1997; Röder et al. 1998, 2004; Pestsova et al. 2000; Somers et al. 2004). SSRs from common wheat have been shown to give amplification in many related or alien species and vice versa, which is known as “transferability” (Pestova et al. 2000; Sourdille et al. 2001; Guyomarc’h et al. 2002a,b; Sharma et al. 2002; Kuleung et al. 2004; Adonina et al. 2005; Zhang et al. 2004, 2005, 2007a). Comparison of SSR patterns among newly synthesized hexaploid wheat (SHW) and its donor *T. turgidum* and *Ae. tauschii* indicated that some SSR markers located on the D genome of common wheat could amplify the SSR sequences from both the A/B genome of *T. turgidum* and D genome of *Ae. tauschii*. The amplified fragments were also detected in SHWs. These results suggested that these SSR markers specific to D genome in common wheat were probably non-specific to D genome in SHWs. Similarly, some SSR primers specific to A/B genome in common wheat were probably nonspecific to the A/B genome in SHWs (Zhang et al. 2004, 2005, 2007a). Given that the wheat genome is so large and complex, it can be hypothesized that the chance of amplifying non-specific sequences is very high. This hypothesis needs to be verified by more effective methods, such as DNA sequencing and chromosome location analysis of the transferable fragments.

The well-studied SSR *Xgwm261*, a (CT/GA)_n marker in common wheat (Röder et al. 1998) is of particular interest to breeders because of its association with plant height. The *Xgwm261* on the short arm of chromosome 2D is linked to the *Rht8* with a genetic distance of 0.6 cM between them (Korzun et al. 1998; Röder et al. 1998). At locus *Xgwm261*, besides a number of rare

alleles, alleles of 204, 192, 174, and 164 bp were presented in most varieties of common wheat (Worland et al. 1998, 2001; Chebotar et al. 2001; Ahmad and Sorrells 2002; Manifesto and Suárez 2002; Liu et al. 2005; Ellis et al. 2007). In our study, a set of well-designed experiments clearly indicated that *Xgwm261*, specific for a single locus on 2DS in common wheat, appeared in two loci in a SHW.

MATERIALS AND METHODS

Plant Materials

The durum wheat (*T. turgidum* ssp. *durum*) cv. Langdon (LDN) and its disomic substitution line 2D (2B), in which a pair of chromosome 2B in cv. Langdon were replaced by a pair of homoeologous chromosome 2D of common wheat Chinese Spring, were developed by Joppa and Williams (1983). The Langdon lines were provided by Dr. L. R. Joppa, Fargo, ND, North Dakota State University, USA. *Ae. tauschii* accession AS60 with desirable crossability (Liu et al. 2002) was used for crossing with Langdon lines. Common wheat lines Chinese Spring (CS) and Chinese Spring nullisomic 2B-tetrasomic 2D (CS N2BT2D) were obtained from E. R. Sears, University of Missouri, Columbia, MO. CS N2BT2D has four copies of chromosome 2D and has zero copies of chromosome 2B.

Production and Cytological Observation of Synthetic Wheat

The durum wheat lines Langdon and Langdon 2D(2B) as female parents were crossed with *Ae. tauschii* (accession AS60). The emasculation and pollination techniques described by Liu et al. (1999) were followed. No embryo rescue technique or hormone treatments were applied when producing the F₁ hybrids. F₁ seeds were germinated in Petri dishes before transplanting into the field. Spikes of the F₁ and F₂ hybrid plants were randomly selected for further investigation. These selected spikes were bagged before flowering to prevent cross-contamination.

Somatic chromosomes were counted using squashed root-tip preparations (Zhang et al. 2007b). Root tips (1–3 cm long) were cut from the seedlings and pretreated in ice water at 0–4°C for 20–24 h, then fixed in Carnoy’s fixative I (three parts of 95% ethanol to one part of glacial acetic acid) overnight. Feulgen staining was applied to the fixed root tips to visualize chromosomes. The F₂ hybrid plants with $2n = 42$ chromosomes were selected. These plants were grown 10 cm apart with 60-cm row spacing in the experimental field of Triticeae Research Institute of Sichuan Agricultural University at Dujiangyan City, Sichuan Province, China.

SSR Analysis

Total genomic DNA was isolated from young fresh leaves using 2 × CTAB method according to Zhang et al. (2004). The SSR *Xgwm261* primer sequences were:

5'-CTCCCTGTACGCCTAAGGC-3' and 5'-CTCGCGCTACTAGCCATTG-3' (Röder et al. 1998). PCR reaction contains: 1 unit ExTaq polymerase (TaKaRa, Dalian, China), 1 × ExTaq buffer (Mg²⁺ free), 1.5 mM MgCl₂, 0.2 mM dNTPs, 200 nM of each primer, and 50–100 ng template DNA in 20 μL final volumes. Thermocycling conditions were: initial denaturation at 94°C for 5 min; 38 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C; and a final extension at 72°C for 5 min. PCR was performed in a MJ Research thermocycler (Watertown MA). The amplified fragments were separated by electrophoresis in a denaturing 6% polyacrylamide gel and visualized with the silver-staining method (Zhang et al. 2004).

Cloning and Sequence Analysis

The target fragments were cut from the polyacrylamide gel and boiled in 20 μL ddH₂O for 5 min and then centrifuged at 4000 × g for 10 min. The upper liquid was used to conduct the second PCR reaction using the same program as above. The final *Xgwm261* PCR products were separated on 3.0% agarose gels and the expected fragments were purified from gels using E.Z.N.A.® Gel Extraction Kit (OMIGA, USA.). The purified PCR products were cloned into pMD18-T vector using cloning kit from TaKaRa Biotechnology (Dalian) Co. Five positive clones from each plant materials were identified, and commercially sequenced by the SunBio-tech company (Beijing, China). Sequence alignments were conducted using the DNAMAN 6.0 Demo software (Lynnon Biosoft).

Genetic Analysis on F₂ Population

The F₂ population was developed from the cross between common wheat Chinese Spring and new synthetic hexaploid wheat Syn-SAU-5. Randomly chosen 185 F₂ individuals were analyzed using marker *Xgwm261*. SSR bands on silver staining gels were calculated based on the band size. The examined plants were classified into three groups: (I) with 176 bp band, (II) with 192 bp band, (III) with both 176 bp and 192 bp bands. Chi-square analysis was performed with SPSS software version 11.0 (SPSS Inc., Chicago, IL) to assess the goodness-of-fit of marker *Xgwm261* between observed and expected segregation ratio in the segregating population. A *P* value less than 0.01 was regarded as highly significant.

RESULTS

Production of Synthetic Hexaploid Wheat, Nullisomic 2B-tetrasomic 2D

In 2004, LDN and LDN 2D(2B) were pollinated with *Ae. tauschii* accession AS60. Triploid F₁ seeds were obtained from both crosses without embryo rescue. Averaged crossability was 3.16% for the LDN × AS60 and 0.87% for the LDN 2D(2B) × AS60. Most of the F₁ seeds were germinated and produced vigorously growing

F₁ plants. As expected, the F₁ hybrid plants had 21 chromosomes, and showed tough tenacious brown glumes, which were obviously inherited from *Ae. tauschii*. All the triploid F₁ hybrids were highly fertile and gave high seed set. In 2005, the seed set rate was 36.87% (755 seeds from 2048 florets) for hybrids of LDN-*Ae. tauschii* and 65.32% (290 seeds from 444 florets) for the F₁ hybrids of LDN 2D(2B)-*Ae. tauschii*.

Chromosome numbers were checked for 34 randomly selected F₂ seeds of LDN-*Ae. tauschii*. Twenty-nine had 42 chromosomes. Ten out of eleven LDN 2D(2B)-*Ae. tauschii* F₂ seeds had 42 chromosomes. The high frequency of selfed seed set rate and F₂ seeds with 42 chromosomes indicated that unreduced gametes in the LDN lines × AS60 hybrids are common. Previous cytological observations on male gametogenesis have shown that meiotic restitution is responsible for the production of functional unreduced male gametes, resulting in spontaneously amphidiploids with high selfed seed-settings in LDN-*Ae. tauschii* hybrids, which is controlled by the gene from LDN (Xu and Joppa 1995, 2000; Jauhar et al. 2000; Jauhar 2003; Matsuoka and Nasuda 2004).

Newly SHW lines with 2*n* = 42, derived from LDN × *Ae. tauschii*, were obtained. Of which, the SHW line Syn-SAU-5 was further crossed with common wheat Chinese Spring to produce F₂ population. SHW lines with 2*n* = 42 chromosomes from LDN 2D(2B)-*Ae. tauschii* should be nullisomic 2B-tetrasomic 2D, which has four copies of chromosome 2D, two from Chinese Spring and two from *Ae. tauschii* line AS60, and has zero copies of chromosome 2B. The synthetic nullisomic 2B-tetrasomic 2D is abbreviated as Syn-SAU N2BT2D.

SSR Analysis of the Newly Synthetic Wheats and Their Parents

Common wheat cultivar Chinese Spring (CS) has a 192 bp *Xgwm261* allele (Worland et al. 2001) which was also observed in CS in this study (Fig. 1), but absent in CS N2DT2B. This result indicated that SSR *Xgwm261* was only located on chromosome 2D, and specific to chromosome 2D in CS.

As expected, one PCR fragment of SSR *Xgwm261* was detected in *Ae. tauschii* line AS60 (Fig. 1) with D genome. It is interesting that the same fragment of *Ae. tauschii* AS60 appeared in tetraploid wheat LDN (AABB genomes). However, the fragment size was smaller than that in common wheat CS. This transferable PCR fragment in LDN was not detected in LDN 2D(2B) in which a pair of chromosome 2B were replaced by a pair of chromosome 2D of common wheat CS. A 192 bp allele was observed in the 2D of CS (Fig. 1). This result indicated that the PCR fragment in LDN was located on chromosome 2B.

In some cases, the fragment can be amplified through template-primer mismatch and/or modified PCR conditions, resulting in nonspecific amplification. To verify amplification specificity, each five clones from *Xgwm261*

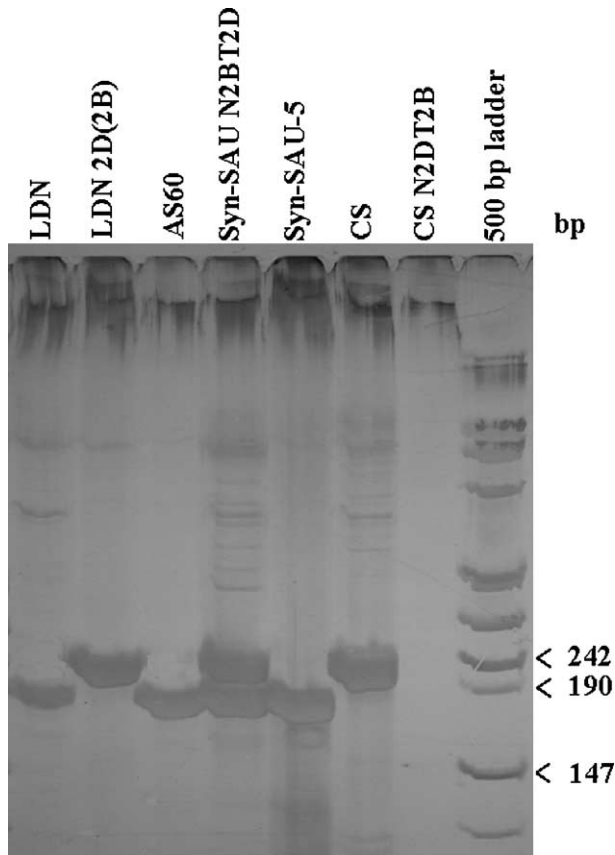


Fig. 1. The amplified products for SSR *Xgwm261* from durum LDN, LDN 2D(2B), *Ae. tauschii* AS60 and their synthetic hexaploid wheats Syn-SAU-5, Syn-SAU N2BT2D and common wheat CS and CS N2BT2D, revealed by 6% denaturing polyacrylamide gels.

amplified LDN and *Ae. tauschii* AS60 product were sequenced. Sequence alignment revealed that all the clones had the same sequences with a length of 176 bp (Fig. 2). Comparison between the 176 bp sequence and 192 bp sequence (provided by Liu et al. 2005) indicated that the 176 bp alleles is composed of [(CT)₁₁AG] repeat motif, and the 192 bp alleles is composed of [(CT)₂₁] repeat motif (Fig. 2).

The synthetic nullisomic 2B-tetrasomic 2D, Syn-SAU N2BT2D showed two distinct bands, a 192 bp from chromosome 2D of common wheat CS, a 176 bp from 2D of *Ae. tauschii*. The synthetic hexaploid wheat Syn-SAU-5 between LDN and *Ae. tauschii* displayed the 176 bp band, which probably contains two different loci, one for chromosomes 2B of LDN and the other for 2D of *Ae. tauschii* (Fig. 1). However, since the fragment size of 2D in *Ae. tauschii* and 2B in LDN is same, they can not be distinguished in synthetic wheat Syn-SAU-5 background.

SSR Analysis on F₂ Segregation Population Between Syn-SAU-5 and CS

SSR analysis on F₂ segregation population verified that SSR *Xgwm261* in Syn-SAU-5 contained two different loci. CS and Syn-SAU-5 produced two polymorphic bands, with a length of 192 bp and 176 bp in *Xgwm261* loci, respectively. All the F₁ hybrids between CS and Syn-SAU-5 showed both bands. Their F₂ generation exhibited three phenotypes, i.e., one band with 176 bp, two bands with 176 and 192 bp, and one band with 192 bp. Theoretically, if SSR *Xgwm261* with 176 bp is a single locus in Syn-SAU-5, its F₂ hybrids with CS would show a ratio of 1 (176 bp): 2 (176 bp and 192 bp): 1 (192 bp). However, if the *Xgwm261* contains two loci in different chromosomes, 2B and 2D, F₂ hybrids would result in a ratio of 4 (176 bp): 11 (176 bp and 192 bp): 1 (192 bp) (Fig. 3). Among the 185 F₂ plants from populations between Syn-SAU-5 and CS, 46, 125 and 14 plants showed the 176 bp, 176 bp+192 bp and 192 bp bands, respectively. Chi-square test revealed that *Xgwm261* was segregated at a ratio of 4 (176 bp): 11 (176 bp and 192 bp): 1 (192 bp), which was in agreement with the expected segregation of two loci, one co-dominant locus on 2D and one dominant locus on 2B, rather than single locus at 0.01 significant level (Table 1).

DISCUSSION

Identification of markers linked to Mendelian and quantitative trait loci (QTL) of agronomic importance has been achieved using co-segregation and QTL analyses, and linked markers are being used in marker-assisted selection programs. Reliable genetic maps are essential for these purposes. However, segregation distortion, deviations from expected Mendelian segregation ratios, which is a common biological phenomenon and occurs in a number of species, can affect the accuracy of genetic linkage maps (Lyttle 1991; Lorieux et al. 1995a,b; Foisset and Delourme 1996; Cloutier et al. 1997; Zhu et al. 2007). Extremely distorted segregation could result in a wrong linkage group. Low-magnitude segregation distortion may not affect the detection of proper linkage. However, it can affect the estimation of map distances and the order of markers.

There are many possible causes for segregation distortion (Lyttle 1991; Foisset and Delourme 1996). One of them may result from a selection process during gametogenesis, fertilization or germination caused by segregation-distortion loci (SDL). If segregation distortion is caused by SDL, all markers in the vicinity of the SDL will be affected and result in a cluster with distorted ratios. Errors in marker genotyping may also cause systematic deviations from expected segregation ratio. However, this could lead to mis-scoring of only

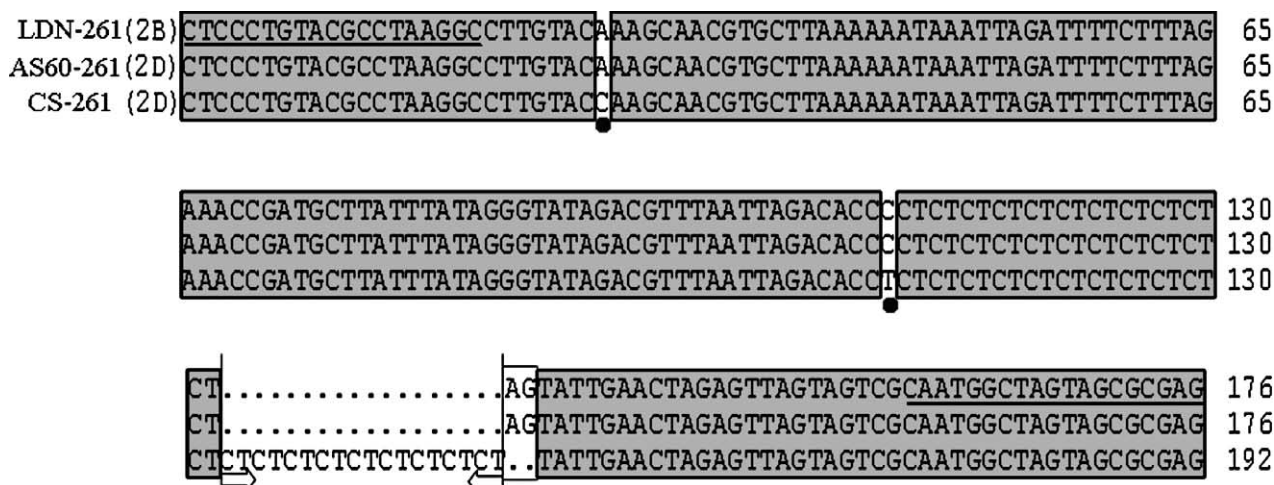


Fig. 2. Sequence comparison of SSR *Xgwm261* among the alleles from chromosomes 2D in *Ae. tauschii* AS60 and common wheat CS and 2B in durum wheat LDN. Primer sequences were underlined; bold circles represent the nucleotide replacements; arrow heads showed the dinucleotide (CT) motif insertions/deletions; The AG insertion/deletion were boxed.

a single marker. SSR bands could have been mis-scored and given the wrong allelic designation. The discriminative ability of gel-separation technology can affect accuracy and reproducibility of band scoring. “Stutter” bands, which are especially prone to occur in di-repeat SSRs, can also cause incorrect genetic scoring of bands (Smith et al. 1997).

The present study demonstrated that wrong locus determination may be an alternative cause for segregation distortion. According to the data of the chromosomal location and DNA sequence, two *Xgwm261* loci, each

existed in chromosomes 2B and 2D of Syn-SAU-5, respectively. In the F₂ segregation population of Syn-SAU-5 × CS, *Xgwm261* bands were segregated into a 4:11:1 ratio in agreement with the segregation of two loci, one co-dominant locus on 2D and one dominant locus on 2B. However, since the two loci in Syn-SAU-5 have the same size, they could not be distinguished from each other in the SSR analyses. According to classic analysis procedure on SSR, *Xgwm261* would be mis-scored as single locus with the 1:2:1 ratio in genetic analyses. As a consequence, the segregation ratio for

Parental generation: 2B¹⁷⁶2B¹⁷⁶2D¹⁷⁶2D¹⁷⁶ X 2B^{null}2B^{null}2D¹⁹²2D¹⁹²
 (Synthetic wheat Syn-SAU-5) ↓ (Chinese Spring)

F₁ generation: 2B¹⁷⁶2B^{null}2D¹⁷⁶2D¹⁹²

F₂ generation:

	Female gametes			
Male gametes	2B ¹⁷⁶ 2D ¹⁷⁶	2B ¹⁷⁶ 2D ¹⁹²	2B ^{null} 2D ¹⁷⁶	2B ^{null} 2D ¹⁹²
2B ¹⁷⁶ 2D ¹⁷⁶	2B ¹⁷⁶ 2B ¹⁷⁶ 2D ¹⁷⁶ 2D ¹⁷⁶ 1/16 (176 bp)	2B ¹⁷⁶ 2B ¹⁷⁶ 2D ¹⁷⁶ 2D ¹⁹² 1/16 (176 + 192 bp)	2B ¹⁷⁶ 2B ^{null} 2D ¹⁷⁶ 2D ¹⁷⁶ 1/16 (176 bp)	2B ¹⁷⁶ 2B ^{null} 2D ¹⁷⁶ 2D ¹⁹² 1/16 (176 + 192 bp)
2B ¹⁷⁶ 2D ¹⁹²	2B ¹⁷⁶ 2B ¹⁷⁶ 2D ¹⁹² 2D ¹⁷⁶ 1/16 (176 + 192 bp)	2B ¹⁷⁶ 2B ¹⁷⁶ 2D ¹⁹² 2D ¹⁹² 1/16 (176 + 192 bp)	2B ¹⁷⁶ 2B ^{null} 2D ¹⁹² 2D ¹⁷⁶ 1/16 (176 + 192 bp)	2B ¹⁷⁶ 2B ^{null} 2D ¹⁹² 2D ¹⁹² 1/16 (176 + 192 bp)
2B ^{null} 2D ¹⁷⁶	2B ^{null} 2B ¹⁷⁶ 2D ¹⁷⁶ 2D ¹⁷⁶ 1/16 (176 bp)	2B ^{null} 2B ¹⁷⁶ 2D ¹⁷⁶ 2D ¹⁹² 1/16 (176 + 192 bp)	2B ^{null} 2B ^{null} 2D ¹⁷⁶ 2D ¹⁷⁶ 1/16 (176 bp)	2B ^{null} 2B ^{null} 2D ¹⁷⁶ 2D ¹⁹² 1/16 (176 + 192 bp)
2B ^{null} 2D ¹⁹²	2B ^{null} 2B ¹⁷⁶ 2D ¹⁹² 2D ¹⁷⁶ 1/16 (176 + 192 bp)	2B ^{null} 2B ¹⁷⁶ 2D ¹⁹² 2D ¹⁹² 1/16 (176 + 192 bp)	2B ^{null} 2B ^{null} 2D ¹⁹² 2D ¹⁷⁶ 1/16 (176 + 192 bp)	2B ^{null} 2B ^{null} 2D ¹⁹² 2D ¹⁹² 1/16 (192 bp)

Fig. 3. The Punnett square of the SSR genotypes and phenotypes in the hybrid of Syn-SAU-5 × Chinese Spring.

Table 1. Chi-square analysis for F₂ segregation population of Syn-SAU-5 × Chinese Spring

Genotypes	No. of observed plants	No. of expected plants for two loci (4:11:1)	No. of expected plants for single locus (1:2:1)
176 bp	46	46.25	46.25
176 bp+192 bp	125	127.19	92.50
192 bp	14	11.56	46.25
		$\chi^2 = 0.554$	$\chi^2 = 33.91^{**}$

$\chi^2_{0.01, 2} = 9.21$.

Xgwm261 would be distorted with a higher frequency for heterozygous (176 bp+192 bp) and lower for CS (192 bp). Although only F₂ segregation populations were analyzed in the present study, distorted segregations would be produced in other populations, such as DH populations and recombined inbred lines if we considered two loci as single locus.

This study only demonstrated an example for two loci by using well-studied SSR *Xgwm261*. Previous studies have indicated that many SSRs are transferable from common wheat to its donor species, *T. turgidum* and *Ae. tauschii*, and their SHWs (Pestova et al. 2000; Sourdille et al. 2001; Guyomarc'h et al. 2002a,b; Sharma et al. 2002; Zhang et al. 2004, 2005, 2007a), which suggested the possibility that some SSRs, specific for a single locus in common wheat, could have two, three or more loci in SHWs.

In summary, our data suggest that caution should be used in analyzing the genetic population between common wheat and SHW. As example of *Xgwm261*, in genetic mapping, those transferable SSRs could be mis-scored as single locus, and would yield inaccurate information. To avoid the problems from this type of SSR markers, an effective method is to use the corresponding parents of SHWs, *T. turgidum* and *Ae. tauschii*, as controls in polymorphism analysis of SSR. The SSRs that failed to reveal the differences between D-genome of *Ae. tauschii* and A/B genomes of *T. turgidum* should not be selected in further analysis.

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