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# Genetic mapping of the *Isaac*-CACTA transposon in maize

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**Abstract** We constructed a genetic linkage map with *Isaac*-TD, SSR, and SNAP markers in a RIL population which had been derived from a cross of waxy corn (KW7) and dent corn (Mo17). A total of 368 markers, including 241 *Isaac*-TD, 121 SSR, and 6 SNAP markers, were assigned to 10 linkage groups, encompassing 1687.0 cM, with an average genetic distance of 4.6 cM between markers. SSR markers were utilized as chromosome anchors, in order to assign the *Isaac*-TD markers to the chromosomes, and the number of markers in each of the linkage groups ranged between 22 and 49. The majority of the *Isaac*-TD markers were determined to have been distributed throughout the ten maize chromosomes. In linkage analysis of the *Isaac*-TD markers with genes of agronomic interest, six genes related with maize kernel starch biosynthesis, *ae1*, *bt2*, *sh1*, *sh2*, *su1*, and *wx1*, were analyzed and shown that they were closely linked with either the *Isaac*-TD or SSR markers on chromosomes of 3, 4, 5, and 9. We observed and mapped segregation-distorted markers on chromosomes 1, 5, 6, 7, 8, and 10, where these markers were clustered. The *Isaac*-TD or SSR markers which were closely linked with starch synthesis genes may prove useful in marker-assisted breeding programs.

## Introduction

Genetic mapping using DNA probes and PCR-based molecular markers has resulted in the identification of many loci in the maize genome (Davis et al. 1999). PCR-based techniques, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR) have been utilized in the construction of linkage maps (Giese et al. 1994; Senior et al. 1996; Castiglioni et al. 1999; Sharopova et al. 2002). Recently, transposons have also been employed as genetic markers via transposon display (TD) techniques (Casa et al. 2000; Kwon et al. 2005; Lee et al. 2005). Transposons constitute a sizeable fraction of the plant genome, and are of fundamental importance with regard to the formation of the current genome organization, and also constitute a primary source of allelic and subsequent genetic diversity in plant species (Casa et al. 2000; Wessler et al. 2001; Lee et al. 2005). CACTA is a family of Class 2 transposons, which is found exclusively in plants with high copy numbers (Kunze and Weil 2002). The CACTA family is comprised of several elements, and each of the elements exhibits consensus nucleotide sequences at its terminal invert repeats (TIRs), as well as some internal motifs, which can be utilized for PCR amplification in TD analyses. The association of transposons with protein-coding genes has been thought to be a possible advantage of the TD technique, especially considering the anonymity of other molecular markers (Bureau and Wessler 1994a, b). In the CACTA superfamily, several subfamilies, including *Casper, Mandrake, Isaac, Baldwin, Jorge, Enac*, and *TAT-1*, have been identified in the *Gramineae* species, and the sequence homology between each of the subfamilies was reportedly limited, with the exception of the terminal 20–30 bp region, which contains the CACTA motif in each of the variants (Wicker et al. 2003). Recently, Lee et al. (2005) reported the *Isaac*-CACTA transposons for genetic markers in maize and sorghum, using the conserved sequence motif in the *Isaac* transposon for TD analysis. In an attempt to check genetic linkage of the *Isaac*-CACTA transposon markers with these genes of agronomic interest, we carried out single nucleotide amplified polymorphism (SNAP) analysis in starch synthesis genes, amylase extender1 (*ae1*), brittle endosperm2

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(*bt2*), shrunken1 (*sh1*), *sh2*, *sugary1* (*su1*), and *waxy1* (*wx1*), as the protocols of Drenkard et al. (2000).

This study describes the chromosomal distribution of the *Isaac*-TD markers, determined using recombinant inbred lines (RILs) derived from a cross between Mo17 and KW7. The genes related to starch synthesis were also mapped in association with the *Isaac*-TD markers. Mo17 is one of the primary known inbred lines, and is used extensively in corn breeding programs in the USA during 1960s–1980s (Lamkey 1992). KW7 is an inbred parental line and is being used for developing waxy corns in Korea.

## Materials and methods

### Plant material and DNA isolation

The RIL population used in this study was developed as follows: $F_1$ seeds were generated by a cross between Mo17 (U.S Corn Belt maize inbred) and KW7 (an inbred derived from Korean waxy corn landrace). A total of 200 $F_2$ plants were self-pollinated and allowed to advance to the $F_7$ generation, via the single-seed descent method. Due to sterility problems, the final population of the $F_7$ generation consisted of 80 lines, each of which originated from different $F_2$ individuals. The DNA from the parents and RIL mapping populations was isolated from young leaves, via the method described by Dellaporta et al. (1983), with some minor modifications.

### CACTA-TD, SSR and SNAP analyses

The CACTA-*Isaac* primer and adaptors were designed according to the consensus sequences acquired from the GenBank database, using the basic information provided by the study of Wicker et al. (2003). The sequences of the employed primers are shown in Table 1. The conditions for PCR amplification were as reported (Lee et al. 2005).

Simple sequence repeats amplifications were conducted in a total volume of 30 µl, and consisted of 20 ng of genomic DNA, 1× PCR buffer, 0.3 µM of forward and reverse primers, 0.2 mM dNTPs, and 1 unit of Taq Polymerase (Biotools). The PCR profile consisted of an 5-min initial denaturation period at 94°C, followed by two 1-min denaturation cycles at 94°C, a 1-min annealing cycle at 65°C, and a 2-min extension at 72°C. After the second cycle, the annealing temperature was decreased by 1°C increments at every second cycle, to a final temperature of 55°C. The last cycle was then repeated 20 times. Upon the completion of the cycles, the extension cycle was extended for 10 min at 72°C.

The SNAP primers were designed on the basis of the genetic structures determined for the six starch synthesis candidate genes (*ae1, bt2, sh1, sh2, su1*, and *wx1*) reported by Wilson et al. (2004). The nucleotide sequences of the SNAP primers in each of the loci are shown in Table 2.

**Table 1** Primer sequences of the *Isaac*-TD analysis

| Primer name | Sequence |
|---|---|
| Adaptors | |
| KRMA-1 | GACGATGAGTCCTGAG |
| KRMA-2 | TACTCAGGACTCAT |
| *Mse*I anchors | |
| KRMP-0 | GACGATGAGTCCTGAGTAA |
| KRMP-CAC | GACGATGAGTCCTGAGTAACAC |
| KRMP-CAT | GACGATGAGTCCTGAGTAACAT |
| KRMP-CTA | GACGATGAGTCCTGAGTAACTA |
| KRMP-CTC | GACGATGAGTCCTGAGTAACTC |
| KRMP-GAA | GACGATGAGTCCTGAGTAAGAA |
| KRMP-GAC | GACGATGAGTCCTGAGTAAGAC |
| KRMP-GAG | GACGATGAGTCCTGAGTAAGAG |
| KRMP-GAT | GACGATGAGTCCTGAGTAAGAT |
| KRMP-GTA | GACGATGAGTCCTGAGTAAGTA |
| KRMP-GTC | GACGATGAGTCCTGAGTAAGTC |
| KRMP-GTG | GACGATGAGTCCTGAGTAAGTG |
| KRMP-GTT | GACGATGAGTCCTGAGTAAGTT |
| *Isaac* | |
| Isaac MAP | ATAGGGTGCGATTCCGGTAGTG |

The SNAP amplifications were conducted in a total volume of 20 µl, and consisted of 10 ng of genomic DNA, 1× PCR buffer, 0.5 µM of forward and reverse primers, 0.2 mM dNTPs, and 1 unit of Taq Polymerase (Biotools). The PCR profile consisted of a 5-min initial denaturation cycle at 94°C, followed by 30 10-s denaturation cycles at 94°C, a 20-s annealing cycle at 65°C, and a 20-s extension at 72°C. This step was followed by a 3-min final extension at 72°C.

### Detection of CACTA-TD, SSR and SNAP bands

The CACTA-TD and SSR reaction products were added to an equal volume of stop solution (98% deionized formamide, 2 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) and heated for 5 min at 95°C. A 3 µl aliquot of each of the reaction mixtures was then analyzed via 6% denaturing polyacrylamide gel electrophoresis, and stained with silver. The SNAP reaction products were resolved via electrophoresis on 1.5% agarose gel, and stained with ethidium bromide. The identities of the SNAP amplicons were verified by checking expected molecular sizes and sequencing before linkage analysis.

### Data analysis and linkage map construction

Chi-square analysis was conducted at a significance threshold of 5%, in order to detect any deviations from the expected Mendelian segregation ratio of 1:1. The SSR markers were employed as anchors, and were selected on the basis of the coverage of maize chromosomes according to the published mapping information contained in the maize genetics and genomics database (http://www.maizeGDB.org). Linkage analysis was conducted using the Mapmaker program, version 3.0 (Lincoln et al. 1992). A framework of SSR markers was chosen for each of the mapping populations, in order to

**Table 2** SNAP primers and their PCR conditions

| Gene | SNAP primer | Primer sequence | Length (bp)[a] | Annealing temperature (°C) |
|---|---|---|---|---|
| *ae1* | Ae1-19605-F | CTATTTGTACCAGGTGGTCT | 233 | 60 |
| | Ae1-19605-RA | TTGGTGTATAAACCGAGAAT | | |
| | Ae1-19605-RG | TTGGTGTATAAACCGAGAAC | | |
| *bt2* | Bt2-817-F | CGTTAAGGAATGGTATTACACT | 288 | 60 |
| | Bt2-817-RA | GATTGATCTTACGAATTAAGTTT | | |
| | Bt2-817-RG | GATTGATCTTACGAATTAAGTTC | | |
| *sh1* | Sh1-4424-FA | TGAGTAAATACTGAAGCCATA | 260 | 60 |
| | Sh1-4424-FG | TGAGTAAATACTGAAGCCATG | | |
| | Sh1-4424-R | GCAAGTATGTACAAAACCAT | | |
| *sh2* | Sh2-7025-F | GAATCTTGTGTGCTCTATC | 222 | 60 |
| | Sh2-7025-RG | TTCTTGTTTTGTAGACGCAC | | |
| | Sh2-7025-RT | TTCTTGTTTTGTAGACGCAA | | |
| *su1* | Su1-8210-F | AGTGTAGCGCACTTTAGAGTTT | 241 | 60 |
| | Su1-8210-RG | ATGATGCATATTCCAGCATAC | | |
| | Su1-8210-RT | ATGATGCATATTCCAGCATAT | | |
| *wx1* | Wx1-1425-FT | ATGTGTTTCCTCCTGGCTT | 283 | 60 |
| | Wx1-1425-FC | ATGTGTTTCCTCCTGGCTC | | |
| | Wx1-1425-R | GTAGCAGTGGAAGAACCTGAC | | |

[a] Amplicon length of the PCR product

facilitate chromosome assignment and map comparisons. The linkage groups were created with LOD scores of 5.0, and recombination fractions of 0.4. All map distances were calculated according to the Kosambi mapping function (Kosambi 1944).

## Results and discussion

### Screening and scoring of polymorphic markers in CACTA-TD, SSR and SNAP analyses

In order to construct a linkage map, the DNA polymorphisms occurring between the two parents, Mo17 and KW7, were determined using 12 *Isaac*-TD primer combinations, 250 SSR primer pairs, and 6 SNAP primer combinations. In *Isaac*-TD, the overall amplification profiles were determined to be rather similar to those observed with conventional AFLP. The numbers of amplified fragments, as well as the polymorphic fragments detected in each of the primer combinations are shown in Table 3. A total of 814 fragments (100–800 bp) from 12 primer combinations were unambiguously scored in the parental lines. Among all of the primer combinations, the number of amplified fragments ranged between 39 (-GAT) and 84 (-GAA), with a mean 68 fragments. Among the 814 *Isaac*-TD fragments, 319 (39.2%) were determined to be polymorphic between the parents, Mo17 and KW7. While the-GTC primer combination exhibited the most abundant quantity of polymorphic markers, with a polymorphism level of 46.8%, the-GAC primer combination exhibited the least abundant quantity of polymorphic markers, with a polymorphism level of 32.4% (Table 3). Although this level of polymorphism was lower than that observed for the MITE transposon markers in maize (Casa et al. 2000), it was determined to be higher than that observed for AFLP markers in a previously analyzed maize mapping population (Castiglioni et al. 1999).

In the SSR analysis, 250 SSR markers, which had been selected in an attempt to encompass the 10

**Table 3** Comparison of *Isaac*-TD primer between the parents Mo17 and KW7 and within RIL mapping population

| Primer pair | Number of resolvable bands | Number of polymorphic bands | Polymorphism (%) | Number of polymorphism scored in RIL population |
|---|---|---|---|---|
| -CAC | 59 | 23 | 39.0 | 19 |
| -CAT | 65 | 30 | 46.2 | 29 |
| -CTA | 74 | 31 | 41.9 | 19 |
| -CTC | 77 | 27 | 35.1 | 21 |
| -GAA | 84 | 36 | 42.9 | 33 |
| -GAC | 68 | 22 | 32.4 | 12 |
| -GAG | 73 | 25 | 34.2 | 20 |
| -GAT | 39 | 14 | 35.9 | 11 |
| -GTA | 59 | 26 | 44.1 | 21 |
| -GTC | 62 | 29 | 46.8 | 16 |
| -GTG | 75 | 26 | 34.7 | 17 |
| -GTT | 79 | 30 | 38.0 | 23 |
| Total | 814 | 319 | 39.2 | 241 |

**Table 4** The number of marker loci per chromosome and the chromosome length (cM) in the RIL mapping population

| Molecular marker | Number of loci in each chromosome | | | | | | | | | | Total loci |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| *Isaac*-TD | 31 | 37 | 26 | 34 | 23 | 22 | 14 | 19 | 16 | 19 | 241 |
| SSR | 11 | 12 | 15 | 11 | 13 | 11 | 8 | 12 | 12 | 16 | 121 |
| SNAP | | | 1 | 2 | 1 | | | | 2 | | 6 |
| Total | 42 | 49 | 42 | 47 | 37 | 33 | 22 | 31 | 30 | 35 | 368 |
| Length (cM) | 200.4 | 207.8 | 205.5 | 193.2 | 186.3 | 130.3 | 110.5 | 173.1 | 135.3 | 144.6 | 1,687.0 |
| Average loci interval | 4.8 | 4.2 | 4.9 | 4.1 | 5.0 | 4.0 | 5.0 | 5.6 | 4.5 | 4.1 | 4.6 |

maize chromosomes from the maize database (http://www.maizeGDB.org), were evaluated for polymorphisms in the parental lines. Among these, 152 (60.8 %) of the primers exhibited polymorphisms between the parents, which were then used as anchors in the construction of a linkage map. The six SNAP primer combinations, one each of the *ae1, bt2, sh1, sh2, su1*, and *wx1* genes, generated polymorphisms via the presence or absence of the amplicons.

## Linkage map construction

In order to construct a linkage map, a total of 477 markers, consisting of 319 *Isaac*-TD, 152 SSR, and six SNAP markers, were utilized along with 80 $F_7$ mapping lines. Two-point linkage at LOD 3 analyses revealed that the 477 markers were grouped into two big linkage groups, 4 small linkage groups and a group with 7 unlinked markers. When the LOD value was increased to 5, the two big linkage groups were divided into 22 linkage groups to represent 10 maize chromosomes with 457 of the 477 markers (95.8%), including 305 *Isaac*-TD, 146 SSR, and six SNAP. Because the remaining 20 markers (4.2%) of the *Isaac*-TD and SSR markers were not linked to any of the groups at a LOD value of 5.0, they were excluded from further mapping analysis.

In order to construct the framework map, the 457 above-listed informative markers were confirmed via three-point linkage analyses, at a LOD value of 5.0. Of these, a total of 368 (80.5%) loci, including 241 *Isaac*-TD, 121 SSR, and 6 SNAP loci, were located within the 10 linkage groups (Fig. 1, Table 4). 78 *Isaac*-TD markers (24.4%) and 31 SSR markers (20.3%) were not located on the framework maps, as they were found to exist at a distance exceeding 50 cM from the main clusters of each chromosome. The elimination of high number of markers in the linkage group may be due to the low number of RILs (80 lines) which have been derived from 200 $F_2$ lines by lost of many RILs in early generation. As a result, the framework map encompassed a length of 1687.0 cM, representing all 10 linkage groups. The average genetic distance between each pair of loci among all of the linkage groups was 4.6 cM. The number of loci per linkage group ranged between 22 and 49. Chromosome 2 exhibited the highest number of loci with the longest genetic distance and chromosome 7 manifested the lowest genetic loci, and the shortest genetic length.

Following the report by Casa et al. (2000) regarding the chromosomal distribution of the MITE transposon, this report is the second to address the chromosomal mapping of transposons via TD analysis in maize. In the report of Casa et al. (2000), the *Heartbreaker* MITE loci were determined to be distributed throughout the all ten linkage groups. Similarly, most of the *Isaac*-TD loci were also distributed throughout the ten maize chromosomes in our analysis. However, the *Rim2*/*Hipa* CACTA transposon was located primarily in the regions proximal to the centromeres in the chromosomes of rice (Kwon et al. 2005). Therefore, the chromosomal distributions of these transposons may differ between species, as well as between the transposon families or subfamilies. The distribution of markers within the genome bears important implications with regard to the general applicability and utility of the marker class (Waugh et al. 1997). As transposons have been shown to constitute the major driving force in the creation of genomic diversity and allelic variations (Kumar and Bennetzen 1999; Fedoroff 2000; Zhang et al. 2000; Casa et al. 2000), the high polymorphism observed among accessions in crop species may suggest that the TD markers are suitable for use in genomic analyses. Also, the association of the transposons with protein coding genes is another recommendation for the TD markers with regard to genomic analysis, especially when compared to the anonymity associated with conventional AFLP markers. Therefore, TD analysis may provide direct information regarding genetic mapping and markers tagged for specific traits, and may also constitute an efficient method for polymorphic detection within a mapping population. In our analysis, the genetic distances of the *Isaac*-TD loci to the genes associated with kernel starch synthesis ranged between 0.6 cM (*bt2*-IsCAT30) and 8.0 cM (*wx1*-IsGAA20). However, more tight linkage may be detected upon the performance of future TD analyses using other transposons.

## Segregation and chromosomal distribution of *Isaac*-TD, SSR and SNAP loci

Among the 241 *Isaac*-TD loci located in the linkage map, 113 were determined to be Mo17-specific alleles, and 128 were KW7-specific. Of the 241 *Isaac*-TD loci, 214 (88.7%) exhibited a segregation ratio of 1:1, and the other 27 (11.2%) loci deviated from the expected ratio of

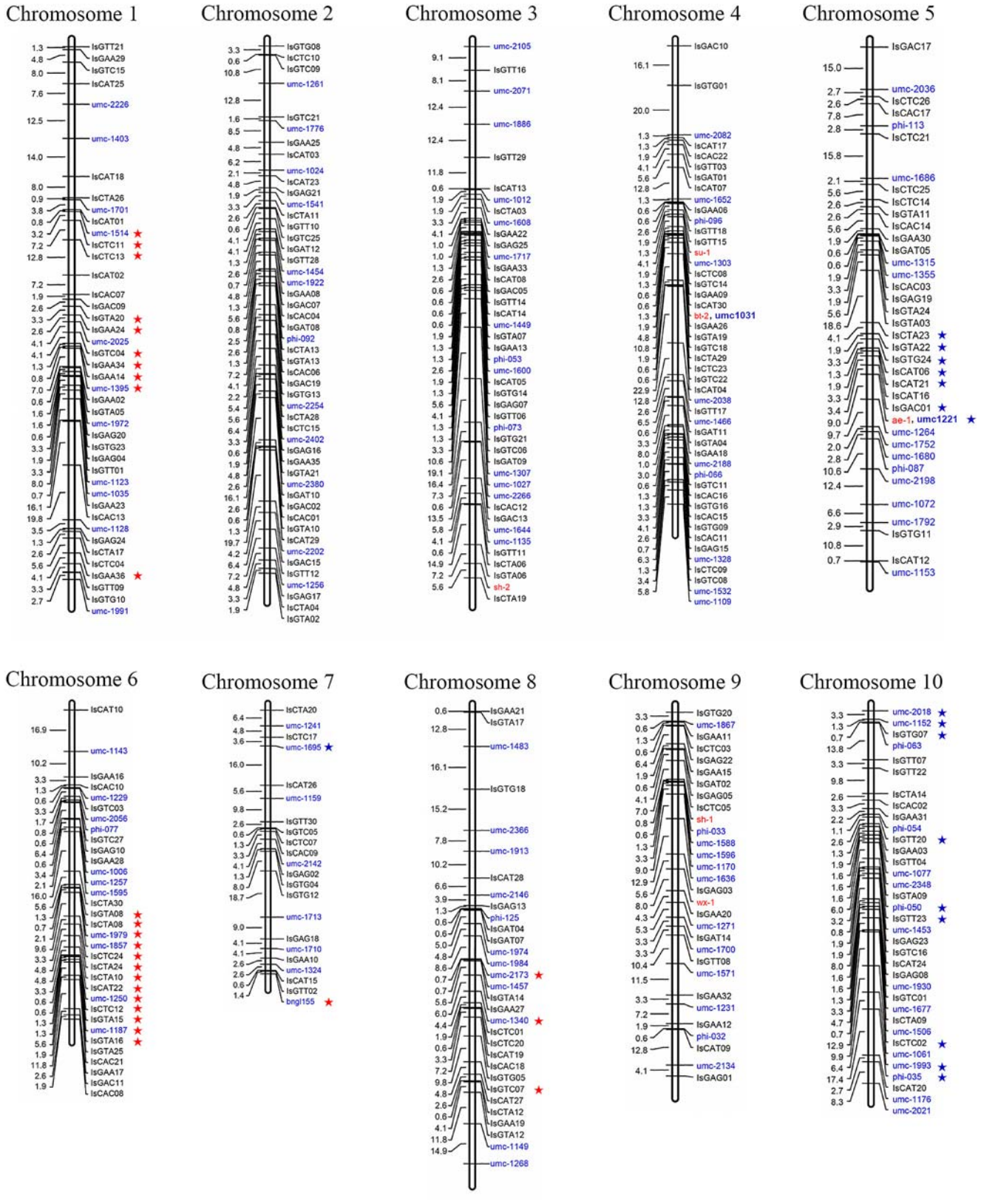


**Fig. 1** The genetic map based on the *Isaac*-TD, SSR and kernel starch synthesis related SNAP markers. Map distances (*on the left*) are expressed in cM (Kosambi mapping function). *Isaac*-TD, SSR and SNAP markers are shown in *black*, *blue*, and *red letters*, respectively (*on the right*). The segregation distorted markers are highlighted by *asterisks*. *Red* and *blue asterisks* denote dent (Mo17) and waxy (KW7) preferentially distorted markers, respectively

1:1, at a 5% significance threshold. With regard to the SSR loci, 106 (87.6%) out of 121 SSR loci exhibited a segregation ratio of 1:1 for the 80 $F_7$ mapping population, and the remaining 15 (12.3%) SSR loci were shown to deviate from the expected 1:1 ratio, at a significance level of 5%. Among the six mapped SNP loci, only the *ae1* SNAP locus was shown to deviate from the expected 1:1 ratio, at a 5% level of significance. Among the 43 segregation-distorted loci, 26 (17 *Isaac*-TD and 9 SSR loci) were skewed towards Mo17, and these were clustered in chromosomes 1, 6, and 8. The remaining 17 (10 *Isaac*-TD, 6 SSR and 1 SNAP loci) loci skewed towards KW7, and were clustered in chromosomes 5 and 10. These distorted loci are highlighted by asterisks in Fig. 1. In addition, we also observed non-parental *Isaac*-TD markers among the $F_7$ segregants. The non-parental *Isaac*-TD markers occurred in all 12 primer combinations in a range of 1–3 with a mean of 1.75 per primer combination. Among the 814 resolvable *Isaac*-TD bands, 21 bands (2.58%) showed non-parental inheritance. Since they do not follow the parental inheritance, we did not include them in linkage analysis.

Deviation from the expected Mendelian segregation ratios has been reported previously in a variety of mapping populations (Gardiner et al. 1993; Xu et al. 1997; Cho et al. 1998; Haanstra et al. 1999; Casa et al. 2000; Lu et al. 2002; Pradhan et al. 2003). In general, segregation distortion may be induced as the result of chromosomal rearrangements, including translocations and residual heterozygosity (Burr et al. 1988; Gardiner et al. 1993; Casa et al. 2000; Lu et al. 2002). According to our results, the majority of the *Isaac*-TD (88.7%) and SSR (87.6%) loci conformed to the expected Mendelian segregation ratios in the RIL population. The RILs lost in early generations might contribute the observed segregation distortion. The percentiles of the distorted loci between the *Isaac*-TD and SSR were fairly similar, implying that the TD loci do not differ significantly from other types of markers with regard to segregation.

The appearance of the non-parental segregating TD markers was also noted by others in maize (Casa et al. 2000) and in rice (Kwon et al. 2005). In our study, the frequency of non-parental *Isaac*-TD fragments ranged from 1.2 to 5.0% with an average of 2.6%, depending on the primer combination assayed. These levels of frequency of non-parental fragment were similar or higher than those of *Heartbreaker* MITE-TD by the previous report by Casa et al. (2000), who speculated that these non-parental fragments might derive from the loss of some degree of parental variation over subsequent generations of inbreeding. Pollen contamination, genomic rearrangement, mutation on the restriction sites, or even transposon transposition may not be ruled out for the appearance of non-parental inheritance. Another possible explanation can be put forward that the CACTA transposon might be active in recombinant inbred lines after cross of the two parents, because residual heterozygosity is commonly observed in inbred lines of maize (Burr et al. 1988).

Among the six mapped SNAP loci, *sh2* was located on chromosome 3, linked to two flanking *Isaac*-TD loci, IsGTA06 and IsCTA19, respectively. *su1* and *bt2* were identified on chromosome 4, where *su1* was linked by two flanking loci, the *Isaac*-TD IsGTT15 and the SSR locus umc1303, respectively. *bt2* was determined to be linked completely with an SSR locus, umc1031, which was also closely linked with two flanking *Isaac*-TD loci, the IsCAT30 and IsGAA26, respectively. On chromosome 5, *ae1* was linked completely with the SSR locus, umc1221, which was linked to two flanking loci, and the *Isaac*-TD IsGAC01 and the SSR locus umc1264, respectively. *sh1* and *wx1* were located on chromosome 9. *sh1* was linked to two flanking loci, the *Isaac*-TD locus IsCTC05, and the SSR locus phi033, respectively. *wx1* was linked to two flanking *Isaac*-TD loci, IsGAG03 and IsGAA20, respectively.

## Concluding remarks

The framework map constructed in this study is the first genetic map which uses a maize RIL population derived from a cross between waxy corn and dent corn. As the current reports also include the chromosomal locations of the starch synthesis genes in relation to the *Issac*-TD markers, a detailed physical map in the regions of the starch synthesis genes which are closely linked with the TD loci may provide us with a modicum of insight into the processes inherent to the regulation of genes by transposons. The *Isaac*-TD markers which are closely linked with these kernel starch synthesis genes may be converted to SCAR markers, for use in future breeding programs. The current genetic map may also be useful in the dissection of quantitative traits and the identification of superior QTLs from waxy hybrid corn.

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