Global Journal of Science Frontier Research: C
Biological Science
Volume 17 Issue 3 Version 1.0 Year 2017
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Characterization of a Classical W14 Albino Mutation and a Putative New Robertson's Mutator-Induced Allele in Maize (Zea Mays)

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GJSFR-C Classification: *FOR Code: 069999*

Strictly as per the compliance and regulations of :

Characterization of a Classical W14 Albino Mutation and a Putative New Robertson's Mutator-Induced Allele in Maize (Zea Mays)

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I. Introduction

Albino mutations are well known in plant and animal species. These mutations are caused by various recessive alleles which, when homozygous, lead directly or indirectly to a reduction in normal pigmentation in the organism. Genes that cause albinism in maize are identified by a number of different designations (Vancetovic et al., 2010): w1, w2, w3 represent three white-albino loci; wd1 the white deficiency locus; lw1, lw2, lw3, lw4 four lemon white loci; vp2, vp5, vp9 three viviparous loci; and y10 the yellow endosperm locus (Neuffer et al., 1997).

A common feature of these genetic defects is that among their phenotypic effects is a depletion of carotenoid pigmentation in some or all tissues. The concomitant loss of chlorophyll and other biomolecules in affected tissues is due to the loss of photo- protective carotenoids. The first comprehensive review of albino maize mutants was by Robertson (1971). Since then, studies of these mutants have focused on developmental and metabolic features. Most studies have only described the mutations phenotypically, there are no molecular assays for the different genes that produce albino mutants in maize (Vančetović, et al., 2004; Vančetović, et al., 2010).

Robertson divided albino mutations into two classes. Class I albino mutations produce white-endosperm and white seedlings, whereas class II albino mutants produce both white and yellow endosperm and off-white seedlings (Robertson, 1975). In the albino mutant that is the focus of this research, designated w*-5200, trace amounts of chlorophyll are formed under dim light conditions. Since these albino seedlings grow from both white kernels and yellow kernels, they bear a Class II albino mutation, most likely in the w14 locus (Stinard, 2013, and personal communication). Based on chromosome mapping of previously characterized w14 alleles (chromosome 6L), and putative gene identification within the B73 draft genomic sequence, the likely identity of the w14 locus is Dxs1, which encodes 1-deoxy-D-xylulose-5-phosphate synthase.

The newly identified mutation was derived from a population carrying Robertson's Mutator transposable elements. To determine whether the new albino mutation was due to a lesion at the w14 locus, it was compared with another genetically confirmed w14 allele: 616B w14-N335 (generated by EMS mutagensis). Since most studies on albinism in maize have only described these mutations phenotypically, there was no comprehensive molecular assay for the potential genes that produce these albino mutants or to identify the mutation causing the new w*-5200 phenotype. The DNA sequences of w*-5200 and w14 allele 616B w14-N335 were compared following amplification of gene segments by Polymerase Chain Reaction (PCR). We hypothesized that the new albino phenotype was caused by failure of the mutant to produce normal levels of carotenoids due to a mutation that affects the w14 gene product. Specifically, we pursued the hypothesis that the defect was due to the insertion of a Roberton's Mutator transposable element into the w14 locus.

Typically, allelism of a new lethal mutation is confirmed by field crosses between plants heterozygous for the new mutation and plants heterozygous for a mutation in the target locus. However, at the outset of this project municipal water restrictions, which continued for three successive summers, prohibited field work, which would have included allelism testing. Given the availability of a draft genome sequence, confirmed alleles of the putative target gene, and the facilities to perform the required molecular operations, the sequences of the new mutation and a known w14 allele were determined and compared to identify the lesions resulting in their mutant phenotypes.

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II. Literature Review

a) *Maize as a Model Organism*

Maize has historically been an important model organism for classical genetic research. However, it has a long reproductive cycle (four-months) and tall stature that are not easily accommodated in a greenhouse setting, as well as a large (2.3 x 109 bp) haploid genome. By comparison, Arabidopsis thaliana, which has become the preferred flowering plant model, boasts diminutive size, a short (~6-week) reproductive cycle, and a small (1.35 x 108 bp) haploid genome size (Johnston et al., 2007). Although Arabidopsis is superior for many studies, maize remains an attractive model organism for the study of mutations that result in seedling lethal phenotypes. Arabidopsis seeds provide too little stored energy to support the growth of homozygous photosynthesis- defective seedlings to a size useful for biochemical analysis, even when grown on sucrose-supplemented medium (Koornneef and Meinke, 2010). In contrast, typical maize kernels store adequate starch to produce 3- or 4-leaf seedlings, which provides sufficient tissue for many biochemical analyses. Furthermore, the mechanics of classical genetic manipulation of maize remain as straightforward as ever, including simple and reliable self- and outcrosses.

b) *Transposable Elements in Maize*

In maize, transposable elements (TE) make a big contribution to the genome (about 85% of the genomic material). Although most of the transposable elements are silenced most of the time to maintain genome stability, TEs still play an important role in plant evolution and environmental adaptation (Tenaillon et al., 2010). Robertson's Mutator (Mu) has been frequently utilized for mutant induction (Vollbercht et al., 2010). The Mutator family of elements includes both an autonomous (master) element, Mu9/MuDR, and nonautonomous components, Mu1-Mu8 (Chomet, 1994). The elements routinely insert to unlinked sites in high numbers, making the family very mutagenic (McCarty et al., 2013). There are six classes within the Mu family, all of which share a comparable ~200 bp terminal inverted repeats (Bennetzen et al., 1993).

Miniature inverted-repeat transposable elements (MITE) are class II transposable elements. MITEs are short (80-500 bp) non-autonomous DNA transposons that are present near or within plant genes. Most MITEs are AT rich and produce target-site duplications of between 2 and 9 bp (Charrier et al., 1999; Patel et al., 2004).

c) *Effect of Albino Mutations on Leaves*

Chloroplasts degenerate in sunlight if they lack photo-protective carotenoids. Besides their photo-synthetic role, chloroplasts perform a variety of other essential biochemical functions, such as biosynthesis of amino acids, vitamins, and storage proteins. In the absence of carotenoids, the variety of compounds that are synthesized by chloroplasts, are reduced or absent. Plants bearing such mutations are only able to grow to maturity as heterozygotes or with sugar in their growth media (Walles, 1963). In particular, carotenoids play an important role in protection of chlorophyll from photochemical degradation. Without carotenoids, chlorophyll is destroyed under normal light conditions resulting in a white (albino) leaf phenotype. Different albino mutations produce phenotypes that differ developmentally depending on the step in carotenoid biosynthesis affected and on the expression pattern of the gene that is mutated.

The w*-5200 mutation produces unpigmented white albino seedlings in ambient sunlight. In very dim light, mutant seedlings produce very low levels of chlorophyll. Furthermore, there is no linkage between the white seedling phenotype and white endosperm as is the case for certain carotenoid-deficient mutations. Based on this phenotype, w*-5200 is hypothesized to encode DXS (1-deoxy-D-xylulose-5- phosphate synthase), which catalyzes the first step in the 2-C-methyl-D-erythritol-4-P (MEP), isoprenoid biosynthetic pathway (Fig. 1). In maize, DXS is encoded by three different genes: Dxs1 on chromosome 6, Dxs2 on chromosome 7, and Dxs3 on chromosome 9 (Cordoba et al., 2011). Dxs1 is expressed primarily in young leaves and at lower levels in husks, tassel and mature leaves. Dxs2 is expressed primarily in mature leaves and at lower levels in yellow kernels and roots. Dxs3 is expressed in all vegetative and reproductive tissues but at the highest level in mature leaves. In addition to tissue-specific differences in expression, the three genes differ in the magnitude of their responses to light.

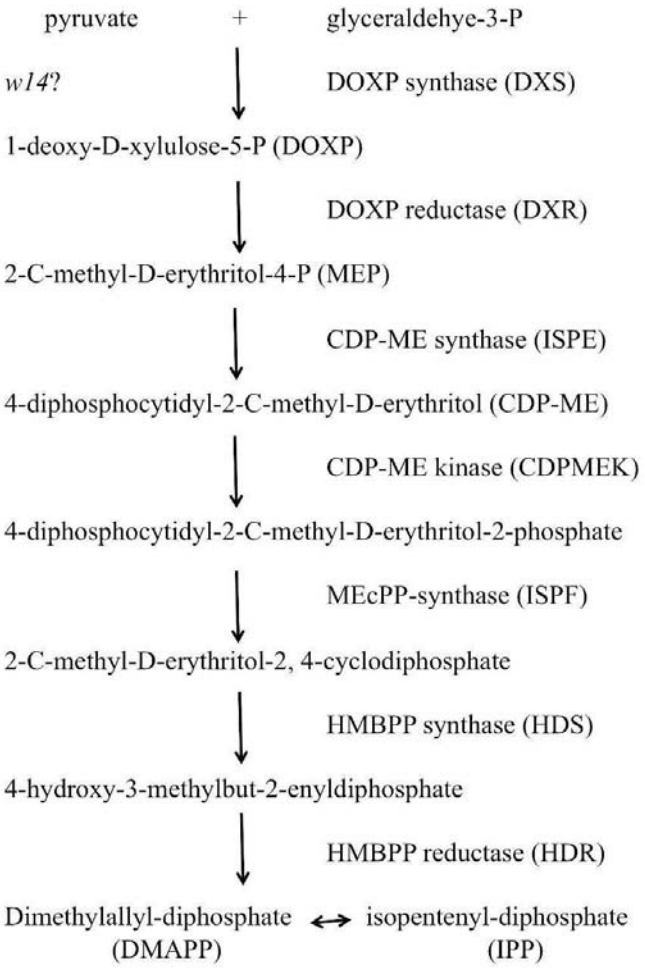

Figure 1: Chloroplast MEP Isoprenoid Biosynthetic Pathway (adapted from Stinard, 2013)

III. Materials and Methods

a) Genetic Seed Sources

The maize (Zea mays) seed stock bearing w*-5200 arose from a self-pollination in a family that segregated another photosynthetic mutation independent of the w14 locus. The immediate source of experimental seed was ear 5200-a5 from the 2012 field season, which was the last field season before water use restrictions interrupted field production. The 616B w14-N335 EMS-induced stock was obtained from the Maize Genetics Cooperation Stock Center at the University of Illinois.

b) Growth Conditions

Zea mays kernels were grown in potting soil in the greenhouse at 21 °C - 25 °C (70 °F - 77 °F) under ambient light (360-650 μmol photons m^{-2} s^{-1}) For phenotypic verification of low light chlorophyll production, seedlings were germinated at 22 °C - 23°C at a light intensity of approximately 6 μmol photons m-2 s-1

c) Mutant Screening

Mutant seedlings were screened visually and recognized by their paper-white leaf color under ambient natural light in the greenhouse. Sprouting w*-5200 mutant seedlings were screened for the w14 albino phenotype using cool-yellow fluorescent lamps (approximately 6 μmol photons m^{-2} s^{-1} 100-fold lower photon flux compared to the midday green house) in the dark. The use of a low photon flux permitted the light dependent conversion of barely detectable amounts of protochlorphyllide to chlorophyll without its subsequent destruction by photo-bleaching. The weakly pigmented leaves subsequently turned to paper-white under greenhouse lighting. To distinguish between homozygous and heterozygous phenotypically normal 616B seedlings with or without a MITE insertion in intron 3, genomic DNA was amplified using PCR primers A5F and A5R to produce products of one size in homozygotes (~800 bp) and products of two different sizes (~650 bp and ~800 bp) in heterozygotes (Table 1).

d) DNA Extraction

For Polymerase Chain Reaction (PCR) screens, genomic DNA was extracted from albino mutant seedlings and normal green seedlings (modified from Doyle and Doyle, 1987). Fresh, young leaves of albino and normal plants were collected. The tips (3 cm2 of fresh leaves were cut into small pieces. Leaf tissue was ground thoroughly with a plastic pestle in 1.5 ml microfuge tubes containing 250 μl of extraction buffer (2% cetyl trimethylammonium bromide [CTAB], 20 mM EDTA (pH 8), 280 mM Tris-HCl (pH 8), 1.4 M NaCl, 8.2 mM 2-mercaptoethanol and 10 μg ml-1 RNAse A). An additional 750 μl extraction buffer were added to the test tube and the mixture was heated at 55 °C in a shaking water bath for 10 min then left at room temperature (25 °C) for 5 min to cool. To the cooled homogenate, 400 μl chloroform/isoamyl alcohol (24:1) were added and shaken gently to form an emulsion. Samples were centrifuged at 13,000g for 10 min at 5°C. The aqueous phase was transferred to a new microfuge tube and 2/3 volume of isopropanol (-20 °C) was added. Samples were placed at -20 °C for 10 min then centrifuged (13,000g) for 10 min to precipitate the DNA. The supernatant was removed and the pellet was washed with 76% EtOH and centrifuged (13,000g) for 10 min at 4 °C. The supernatant was removed and the pellet was air dried at room temperature. The DNA pellet was resuspended in 100 μl deionized H2O (dH2O).

e) Polymerase Chain Reaction (PCR) Amplification

The quality of the DNA samples was verified by PCR amplification using a primer pair unrelated to the w14 locus. Screening PCR was performed using Phusion Master Mix with HF Buffer (NEB). The PCR reactions of 20 μl contained 50 ng template DNA, 1X

Phusion High-Fidelity buffer mix and forward and reverse primers, each at 500nM. PCR amplification was carried out in an Applied Biosystems thermal cycler (v.2.09) with the following profile: initial denaturation for 1 min at 98 °C, followed by 35 cycles each of 10 s at 98 °C (denaturation), 30 s at 66 °C (annealing), 2.0-3.0 min depending on product size at 72°C (extension), followed by a final extension of 5 min at 72 °C. Screening PCR was also performed using Stratagene 1X master mix. The thermal profile for using Stratagene master mix was 5 min at 94°C for initial denaturation, followed by 35 cycles each of 1 min at 94 °C (denaturation), 1 min at 60 °C (annealing), 3.0-4.0 min depending on product size at 72 °C (extension), followed by a final extension of 5 min at 72 °C.

f) PCR Product Analyses

The amplification products were electrophoresed in 1% agarose gels in 1× TAE and stained with ethidium bromide (0.5 μg ml^{-1} for detection. PCR product sizes were determined by comparison to Hi-Lo DNA size markers (Minnesota Molecular). Selected PCR products were cloned in vector pHSG299 by blunt-end ligation in reactions containing 0.1 μg vector and 3 μl PCR product in 1x T4 DNA ligase reaction mix (New England Biolabs) incubated overnight in water at 10 °C and allowed to warm to room temperature through the night. Three microliters of the ligation reaction were added to NEB 5-alpha competent E. coli cells, mixed by flicking, and incubated on ice for 30 min. Cells were heat shocked at 42 °C for exactly 30 s, then incubated on ice for 5 min before addition of 950 μl of room temperature SOC. The mixture was incubated in a shaking incubator (260 rpm) at 37 °C for 60 min. The transformation was diluted 10-fold in SOC and 100 μl were plated on LB-Kanamycin plates supplemented with 50 μl X-gal (40 μg ml^{-1}) and 50μl IPTG(100 μg ml-1) and dried just before plating. Colonies with recombinant plasmids were selected by blue white screening, checked for insert size by PCR, and sequenced by Eurofins Genomics. Alternatively, some PCR products were sequenced directly by the same commercial lab. Sequences were analyzed manually using SerialCloner® software (SerialBasics) by comparison to the Dxs1 region of maize inbred line B73 chromosome 6, RefGen_v4, whole genome shotgun sequence (NCBI accession number: NC_024464) to identify potentially mutagenic lesions. Putative mutagenic regions were sequenced with 3X or better coverage.

Table 1: Tm for selected primer pairs at 500 nM in 1X Master Mix

1F4	GCACACTCTCTCCCCGGC	Tm (°C)	
Primer	Primer Sequence	Phu	Stra
1F4	GCACACTCTCTCCCCGGC		
1R4	CCACCGCCATCCCGA	66	59
2F4	GAGTACGACAGCTTCGGCACG		
2R4	GAATGGGCCGGTCAAAACCTAG	69	60
3F4	GGATCTCAGGTCGCAGCAAGTT		
3R4	ACGACGTCGATCTGCAGAAGCTA	69	60
4F4	GGTCCTCGACTGACGCCG		
4R4	CGGTAACTGTTGTTCCGGCG	67	60
A5F	ATCCTCAACGACAACAAGCA		
A5R	AGAGTCAACTTGCTGCGACC	67	60
6BF	ACGTCGGGATCGCGGAGCAG		
6BR	CAACGGGACGCCAACGCCGT	69	60
1Falig	CCAACATGGTCGTCATG		
1Ralig	AAGTTCAGACACTCTAG	69	60
2Falig	GCACACTCTCTCCCCGG		
2Ralig	GGTGGTTAATTAGCTAG	65	60
3Falig	ATGGCTCTGGGTAACGT		
3Ralig	ACAGTCTGGAAATTTGA	69	60
UpSF	CATGGGGCTTTAGGAGCATAGGTCT		

UpSR	TGCGAGCAATGGGTGTCCTACCAAT	69	60
1021UpSR	GTCAGCGGTGGCAAAGTGAAGATTA		
UpSR	TGCGAGCAATGGGTGTCCTACCAAT	68	60
DSF	GCCAAACGCGTAGAACTTGTGCTGA		
DSR	TTCCCAGAAATGGAGAAATTGGATCT	69	60

g) Allelism Test

Three years into the project municipal outdoor watering restrictions were lifted permitting a return to field propagation and the opportunity for a genetic test of allelism between w*-5200 and 616B in the summer of 2016. Reciprocal crosses were performed between individuals heterozygous for the w*-5200 allele and one individual heterozygous for 616B. When present on cross participants, a second viable ear shoot was self-pollinated in order to distinguish between heterozygous and homozygous-normal individuals. Progeny of allelism crosses were screened for segregation of the albino phenotype indicating heterozygous parentage.

IV. Results

a) Seeding Screening

Albino mutants were initially identified by their white leaf phenotypes. In subsequent screens albino seedlings were examined for low-light greening using 15W cool-yellow fluorescent lamps (approximately 6 μmol photons m^{-2} s^{-1} in a darkened room before transfer to the greenhouse for photo-bleaching (Fig. 2).

Figure 2: Pale green pigmentation in w14 mutant leaves. A Chlorophyll (black arrow), following low-light treatment; B Chlorophyll (white arrow) on day 4 of the greenhouse growth before sunrise; C No trace of chlorophyll following growth in full sunlight

b) Allelism Test

Allelism crosses confirmed that w*-5200 is allelic to 616B w14-N335 (Table 2). After a nearly complete crop failure, one individual of the 616B stock survived. This individual served as pollen donor for one cross to w*-5200 heterozygotes and as pollen recipient in addition to supporting a self-pollination to verify heterozygosity.

Table 2: Allelism test w*-5200 × 616B w14-N335

Cross	Phenotype		Ratio	Result
	Albino	Normal		
w-5200⊗*	82	247	1:3.01	heterozygous
w-5200-sib × w*-5200-sib*	37	102	1:2.76	heterozygous
616B⊗	17	53	1:3.12	heterozygous
w-5200 × 616B*	15	37	1:2.47	allelic

⊗indicates self-pollination

c) Sequence Analysis of DXS from w-5200 and 616B*

To identify the mutations causing the w*-5200 and 616B phenotype, their normal and mutant Dxs1 alleles were sequenced. The sequences of the two mutants were compared to each other, their normal siblings, and to the reference genome sequence of B73.

Figures 3 and 4 illustrate typical results from the amplification of the Dxs1 locus. Primers pairs 1F-2R, and 3F-4R gave similar results in that both amplified products of approximately the same sizes in mutant and normal samples.

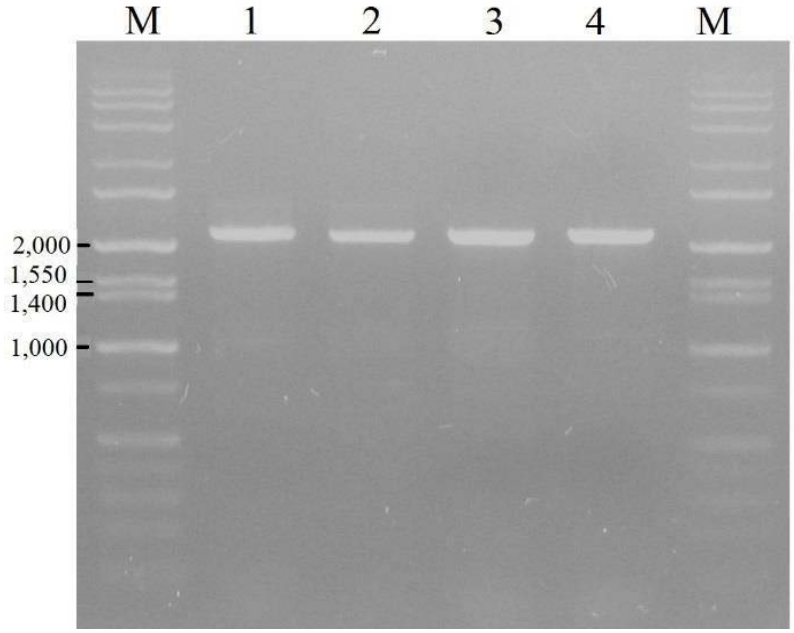

Figure 3: Agarose gel electrophoresis (1 %) of PCR products amplified with primers 1F and 2R. Lane 1: w*-5200 albino; lane 2: w*-5200 normal; lane 3: 616B albino; lane 4: 616B normal. M: Hi-Lo DNA markers (bp).

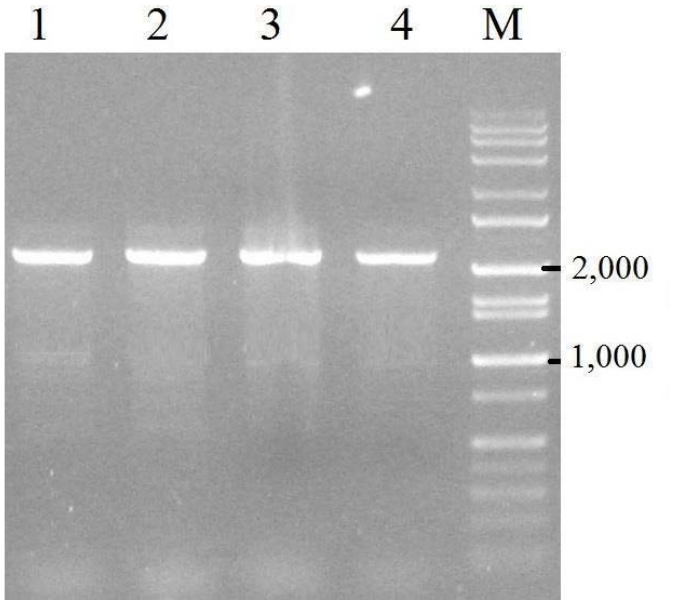

Figure 4: Agarose gel electrophoresis (1 %) of PCR products amplified with primers 3F and 4R. Lane 1: w*-5200 albino; lane 2: w*-5200 normal; lane 3: 616B albino; lane 4: 616B normal. M: Hi-Lo DNA markers (bp).

17 The sequence alignment of the DNA from the four w14 alleles is shown in Figure 5. The alignment identifies no consequential difference between w*-5200 and 616B w14.

There are a few difference between the Dxs1 reference sequence and w*-5200 and 616B w14 (Table 3). Most of sequence differences occur in non-coding regions: in 5′ or 3′ untranslated regions (UTR) (G15T and C50T) or within the introns (A1291C, C1322A, T1338A, A1396G) in locations unlikely to affect splicing. Among mutations within exons, most were silent or consevative mutations (G1183T).

Table 3: Consequential differences between B73 and 616B albino, w*-5200 albino, w*- 5200 normal, 616B normal and effects on protein sequence

Difference Relative to B73	Effect on Amino Acid Sequence	*616B* Albino	*w*-5200* Albino	*w*5200* Normal	*616B* Normal
A294C	N31H	√	√	√	
G2769A	S436N	√	√	√	√
G3063A	D484N	√		√	
G3314A	G568D	√	√	√	
C3452T	A586V	√		√	

The sequences of the Dxs1 locus from the two mutants were more than 90% identical. In comparision to the Dxs1 sequence from B73, w*-5200 albino, w*-5200 normal and 616B normal, the 616B albino sequence includes a 140-bp deletion from intron 3 (Fig. 5). Where present, the 140-bp sequence is flanked by 3-bp direct repeats and contains 15-bp inverted repeats, each a signature of a minature inverted-repeat transposable element (MITE) of the PIF/Harbinger class (Wessler et al., 1995).

Figure 5: Map of maize Dxs1 transcribed region. Grey: 5′, 3′ UTR or intron; white: exon; black: MITE insert when present. Segment lengths drawn to scale. Scale bar= ~200 bp.

As the MITE was inserted into intron 3 of the 616B normal allele and in both w*- 5200 albino and normal alleles, the possibility that the deletion of the element from 616B was the cause of the albino phenotype was evaluated. The region surrounding the site of the MITE insertion was amplified in order to determine the presence or absence of the element in 616B and three other genetically confirmed w14 alleles: 612A, 612N, and 612M using A5F and A5R (Table 1). To determine whether the MITE elements, when present, were inserted in the same location in all cases, the amplification products were sequenced.

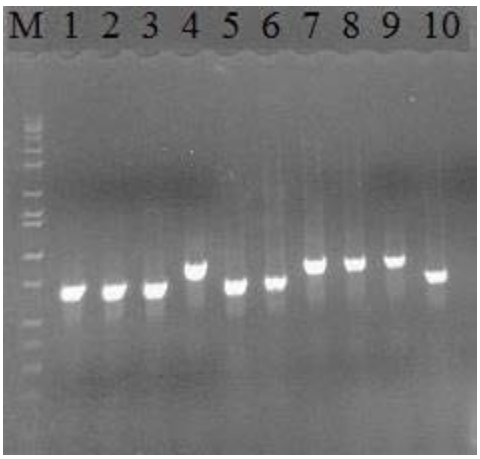

Figure 6: Agarose gel electrophoresis (1 %) of PCR products amplified with primers A5F and A5R. Lane 1: 612A normal; lane 2: 612A albino; lane 3: 612M normal; lane 4: 612M normal; lane 5: 612N normal; lane 6: 612N albino; lane 7: w*-5200 normal, lane 8: w*-5200 albino; lane 9: 616B normal; lane 10: 616B albino. M: Hi-Lo DNA markers (bp).

The results in both PCR products (Fig. 6) and sequences (appendix A3) provide evidence that the MITE is present in either or both normal and mutant DNAs among the five different allele-bearing stocks and that in each case where it is present, the MITE insertion is in the same location.

An additional mutation was considered as the possible cause of one or more of the albino phenotypes. A mutation that affects a critical upstream and downstream regulatory region could prevent expression of the gene and result in the observed phenotypes. To evaluate this possibility, the 2,000 bp regions immediately upstream and downstream of the coding regions were amplified and the products compared to detect any large-scale insertions, deletions or other rearrangement that might affect gene expression (Fig. 7, 8).

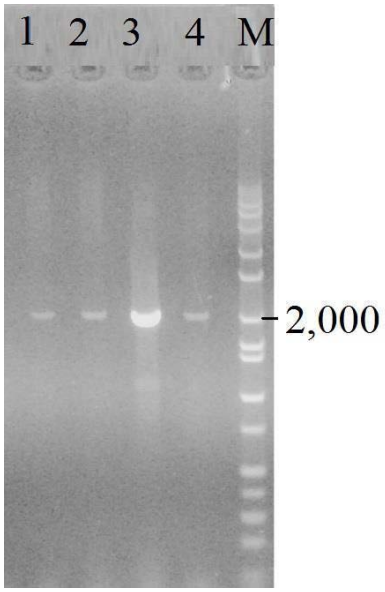

Figure 7: Agarose gel electrophoresis (1 %) of PCR products amplified downstream of Dxs1 with primers DSF and DSR. Lane 1: w*-5200 normal, lane 2: w*-5200 albino; lane 3: 616B normal; lane 4: 616B albino. M: Hi-Lo DNA markers (bp).

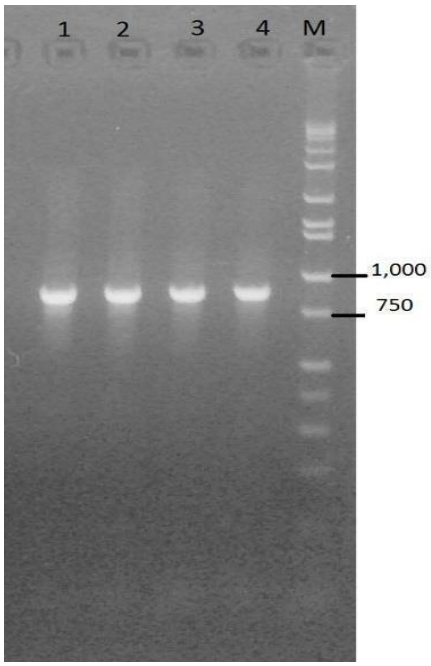

Figure 8: Agarose gel electrophoresis (1 %) of PCR products amplified upstream of Dxs1 with primers 1021UpSF and UpSR. Lane 1: w*-5200 normal, lane 2: w*- 5200 albino; lane 3: 616B normal; lane 4: 616B albino. M: Hi-Lo DNA markers (bp).

V. Discussion and Conclusion

The allelism test confirmed that the newly isolated albino mutation affects the w14 locus. The mutations in w*-5200 and 616B were determined to affect the same locus because the segregation ratio among progeny of the allelism crosses approximated the 1 (albino): 3 (normal) expected for a recessive trait (Table 1).

Although several sequence differences were identified among the samples that were examined, none was likely to inactivate DXS (Table A1). The MITE insert that is present in w*-5200 albino, w*-5200 normal, and 616B normal samples is absent from the 616B albino sequence (Fig. 6), which suggests that the excision may be the cause of the albino mutation in 616B. However, the MITE element was absent from both 612A samples, both 612N samples, and from 612M normal, but present in the 612M albino sequence. The presence or absence of the MITE element is independent of the albino phenotype and not its cause.

The w*-5200 Dxs1 sequence included neither a large-scale disruption, such as the hypothesized Robertson's Mutator transposable element insertion, nor any smaller variations that would obviously result in failure to produce a functional gene product.

The unexpected absence of obvious molecular evidence for the cause of the mutation in either w*-5200 or 616B may be due to one of two possible explanations. The first is that the albino mutation that affects the w14 gene product is not present within the transcribed sequence of the locus. Gene expression may be affected by 5′ or 3′ sequence features outside the coding region that was examined. Although the MITE element that was detected in this study was present in the transcribed region of the locus, it raises the question of whether another MITE element may be inserted outside the transcribed region in a location able to affect expression. Reduced expression of ZmRAP2.7, a flowering time repressor gene, is associated with increased methylation in a regulatory element (Vgt1) that bears a highly methylated MITE element although a causative relationship between the two phenomena has not been demonstrated (Castelletti et al., 2014; Salvi et al., 2007). An ancient transposable element insertion ~60 kb upstream of tb1 locus contributes to the enhanced expression of the gene and the resulting apical dominance seen in modern maize (Studer et al., 2011). A MITE insertion in the 3'UTR of TaHSP16.9 (wheat) affects gene expression by stabilizing transcripts after exposure to heat stress (Li et al., 2014).

Amplification of the 5′ and 3′ UTRs identified no large-scale insertions or deletions that might prevent expression of Dsx1 (Figs. 7 and 8). Although no large-scale changes were detected within 2,000 bp of the gene, smaller scale changes may be present that would have to be detected by more focused sequence analysis as was done for the transcription unit in this study. Modifications to more distant elements that may affect transcription of the Dxs1 would be detected only by more elaborate genomic analyses. While disruption of a regulatory region is a plausible explanation for altered gene expression in individual cases, the likelihood that both mutation events (w*-5200 and

616B), each generated in a mutagenesis project of known mechanism, would result from such disruptions seems small.

The second possible explanation for the lack of evidence of mutations in this locus is that Dxs1 is not the locus affected in w14 mutations. Although the circumstantial evidence on which the hypothesis of w14 involvement was based seems solid, to date it has not been confirmed by molecular means.

While it has been impossible to identify a sequence change that would obviously affect the expression of the w14 gene, it may be that a change eluded detection by sequencing that can be detected by other means. In future studies isolation and comparison of mRNA from mutant and normal sibling individuals should be performed. Northern analysis would provide information about the presence or absence of the transcripts from w14 and their sizes. In the case that mRNA is detected by Northern analysis, Real Time PCR should be employed to more precisely determine the relative levels of transcript accumulation between mutant and normal siblings.

While it seems unlikely that two independent mutations affecting the same locus would leave no molecular evidence in the transcribed sequence, it is possible. Fortunately, due to the long and active history of mutational analysis of maize, several other independent mutations affecting the w14 locus exist. Three of these will be propagated during the 2017 crop season to prepare DNA for sequence analysis. While this study did not yield a satisfying answer regarding the identity of the w14 locus or the nature of the mutations affecting it, data from additional w14 mutations and results from further experiments described above may shed light on the question.

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Appendix

Table A1: List of all sequence differences between B73 and 616B albino, w*-5200 albino, w*-5200 normal, 616B normal and effects on protein synthesis

Difference Relative to B73	Effect on Amino Acid Sequence	*616B* Albino	*w*-5200* Albino	*w*-5200* Normal	*616B* Normal
G15T	5'UTR	√	√		
C50T	5'UTR	√	√		
A236G	silent	√	√		
C293T	silent	√	√		
A294C	N31H	√	√	√	
A1396G	intron		√	√	
C2674A	silent		√	_	
G2769A	S436N	√	√	√	√
T2904C	intron	√	√	√	
A2951C	intron	√	√	√	
C2952G	intron	√	√	√	
T2953A	intron	√	√	√	
C2954T	intron	√	√	√	
G2955C	intron	√	√	√	
A2956G	intron	√	√	√	
ins 2956TTT	intron	√	√	√	
G3063A	D484N	√	_	√	
G3083A	silent	√	√	√	
C3084A	silent	√	√	√	
T3140G	silent	√	√	√	
C3239A	silent	√	√	√	
G3314A	G568D	√	√	√	
C3333T	intron	√	√	√	
C3335A	intron	√	√	√	
C3452T	A586V	√	_	√	
T3492C	silent	√	√	√	
C3633A	silent	√	√	√	
C3680T	silent	√	√	√	
C3707A	intron	√	√	√	
del 3753GA	intron	√	√	√	
A3761C	intron	√	√	√	
del 3787AA	intron	√	√	√	
A3975T	silent	√	√	√	
A4015G	3'UTR	√	√	√	

```
w5200          gcacactctctcccctgccacttcccaaatccgcccgccattcatgcactcttctgtgca
616B           gcacactctctcccctgccacttcccaaatccgcccgccattcatgcactcttctgtgca
w5200norma     gcacactctctccccggccacttcccaaatccgcccgccattcatgcacccttctgtgca
Dxs1           gcacactctctccccggccacttcccaaatccgcccgccattcatgcacccttctgtgca
616Bnormal
```

```
w5200          ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
616B           ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
w5200normal    ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
Dxs1           ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
616Bnormal
```

```
w5200          gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
616B           gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
w5200normal    gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
Dxs1           gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
616Bnormal
```

```
w5200          tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGTTC
616B           tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGTTC
w5200normal    tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGATTC
Dxs1           tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGATTC
616Bnormal
```

```
w5200          CTCGGCGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTTCACAAG
616B           CTCGGCGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTTCACAAG
w5200normal    CTCGGTGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTCAACAAG
Dxs1           CTCGGTGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTCAACAAG
616Bnormal
```

```
w5200          CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
616B           CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
w5200normal     CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
Dxs1           CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
616Bnormal
```

```
w5200          taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
616B           taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
w5200normal    taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
Dxs1           taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
616Bnormal
```

```
w5200          GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
616B           GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
w5200normal    GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
Dxs1           GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
616Bnormal     ------------------------------------------------------------
```

```
w5200          GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
616B           GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
w5200normal    GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
 Dxs1          GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
616Bnormal     ------------------------------------------------------------
```

```
w5200          TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
616B           TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
w5200normal    TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
 Dxs1          TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
616Bnormal     ------------------------------------------------------------
```

```
w5200                CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
616B                 CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
w5200normal          CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
Dxs1                 CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
616Bnormal           ------------------------------------------------------------

w5200                CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
616B                 CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
w5200normal          CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
 Dxs1                CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
616Bnormal           ------------------------------------------------------------


w5200                atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
616B                 atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
w5200normal          atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
Dxs1                 atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
616Bnormal           ------------------------------------------------------------


w5200                ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
616B                 ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
W5200normal          ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
Dxs1                 ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
616Bnormal           ------------------------------------------------------------


w5200                gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
616B                 gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
w5200normal          gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
Dxs1                 gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
616Bnormal           ------------------------------------------------------------


w5200                CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
616B                 CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
w5200normal          CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
Dxs1                 CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
616Bnormal


w5200                ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
616B                 ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
w5200normal          ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
Dxs1                 ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
616Bnormal


w5200                GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCGTGA
616B                 GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCATGA
w5200normal          GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCATGA
Dxs1                 GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCATGA
616Bnormal


w5200                   CGCCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
616B                    CGGCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
w5200normal             CGGCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
Dxs1                    CGGCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
616Bnormal
```

```
w5200                TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACTCTCGACGGGCCGGTGC
616B                 TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC
w5200normal          TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC
Dxs1                 TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC
616Bnormal           ACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC

w5200                CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
616B                 CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
w5200normal          CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
Dxs1                 CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
616Bnormal          CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG

w5200                AGCTCAGGGAGGTGGCCAAGGtacgtccgcaaaaaccttggatgggacgctacatcaccc
616B                 AGCTCAGGGAGGTGGCCAAGGtacgtccgcacaaaccttggatgggacgctacatcaccc
w5200normal          AGCTCAGGGAGGTGGCCAAGGtacgtccgcacaaaccttggatgggacgctacatcaccc
Dxs1                 AGCTCAGGGAGGTGGCCAAGGtacgtccgcacaaaccttggatgggacgctacatcaccc
616Bnormal           AGCTCAGGGAGGTGGCCAAGGtacgtccgcacaaaccttggatgggacgctacatcaccc

w5200                tattgacagcccggccggataggcaagcgccacgtaagggcttgttcggttattcccaat
616B                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
W5200normal          tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
Dxs1                 tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
616Bnormal          tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat

w5200                acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
616B                 ------------------------------------------------------------
w5200normal          acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
Dxs1                 acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca
616Bnormal           acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca

w5200                aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616B                 ------------------------------------------------------------cac
w5200normal          aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
Dxs1                 aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616Bnormal           aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac

w5200                gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616B                 gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
W5200normal          gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
Dxs1                 gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616Bnormal           gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat

w5200                tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
616B                 tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
W5200normal          tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
Dxs1                 tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
616Bnormal           tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa

w5200                ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca
616B                 ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca
w5200normal          ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca
Dxs1                 ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca
616Bnormal           ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca

w5200                tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
616B                 tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
W5200normal          tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
Dxs1                 tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
616Bnormal           tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
```

```
w5200              tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
616B               tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
w5200normal        tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
Dxs1               tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
616Bnormal         tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct

w5200              gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
616B               gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
w5200normal        gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
Dxs1               gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
616Bnormal         gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtata

w5200              gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
616B               gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
W5200normal        gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
Dxs1               gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
616Bnormal         gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt

w5200              gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
616B               gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
W5200normal        gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
Dxs1               gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
616Bnormal         gtataaacaaagctactcaaagcatggatgatggatct----------------------

w5200              agaaatagtttatcatgctactcgagctgtatcca-gtttgactgacattggttcatctt
616B               agaaatagtttatcatgctactcgagctgtatccaagtttgactgacattggttcatctt
W5200normal        agaaatagtttatcatgctactcgagctgtatccaagtttgactgacattggttcatctt
Dxs1               agaaatagtttatcatgctactcgagctgtatccaagtttgactgacattggttcatctt
616Bnormal         ------------------------------------------------------------

w5200              ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
616B               ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
W5200normal        ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
Dxs1               ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
616Bnormal         ------------------------------------------------------------

w5200              cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
616B               cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
W5200normal        cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
Dxs1               cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
616Bnormal         ------------------------------------------------------------

w5200              cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
616B               cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
w5200normal        cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
Dxs1               cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
616Bnormal         ------------------------------------------------------------

w5200              ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
616B               ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
w5200normal        ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
Dxs1               ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
616Bnormal         ------------------------------------------------------------
```

```
w5200                CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
616B                 CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
w5200normal          CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
Dxs1                 CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
616Bnormal           ------------------------------------------------------------

w5200                ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
616B                 ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
w5200normal          ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
Dxs1                 ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
616Bnormal           ............................................................

w5200                ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
616B                 ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
w5200normal          ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
Dxs1                 ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
616Bnormal           ............................................................

w5200                ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
616B                 ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
w5200normal          ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
Dxs1                 ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
616Bnormal           ............................................................

w5200                gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
616B                 gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
W5200normal           gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
Dxs1                 gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
616Bnormal           ............................................................

w5200                gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
616B                 gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
w5200normal           gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
Dxs1                 gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
616Bnormal           ............................................................

                     2641

w5200                CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTAATCGCCGAGGCGGAGCAGGACAGCA
616B                 CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTCATCGCCGAGGCGGAGCAGGACAGCA
w5200normal          CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTCATCGCCGAGGCGGAGCAGGACAGCA
Dxs1                 CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTCATCGCCGAGGCGGAGCAGGACAGCA
616Bnormal           ............................................................

w5200                AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
616B                 AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
w5200normal          AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
Dxs1                 AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
616Bnormal           ............................................................

w5200                GCTTCCCGAACCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
616B                 GCTTCCCGAACCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
w5200normal          GCTTCCCGAACCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
Dxs1                 GCTTCCCGAGCCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
616Bnormal           ............................................................
```

```
w5200              CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
616B               CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
w5200normal      CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
Dxs1               CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
616Bnormal

w5200                GCGGCTACGACCAGGTgcgcacgccgccgtgtgcccgccggccgggccgttcttcgcatt
616B                 GCGGCTACGACCAGGTgcgcacgccgccgtgtgcccgccggccgggccgttcttcgcatt
w5200normal          GCGGCTACGACCAGGTgcgcacgccgccgtgtgcccgccggccgggccgttcttcgcatt
Dxs1                 GCGGCTACGACCAGGTgcgcacgctgccgtgtgcccgccggccgggccgttcttcgcatt
616Bnormal

w5200                tgcttgctgctcgatcgtttcgttttcttcttttgtgcgggcgcggtcctcgactgacgc
616B                 tgcttgctgctcgatcgtttcgttttcttcttttgtgcgggcgcggtcctcgactaacgc
W5200normal          tgcttgctgctcgatcgtttcgttttcttcttttgtgcgggcgcggtcctcgactgacgc
Dxs1                 tgcttgctgctactcgcgttttcttcttttgtgcgggcgcggtcctcgactgacgc---
616Bnormal

W5200                cgtacgcacgtcgccgatgggccggtgtgggtggtggcgcaggtcgTGCACG
616B                 cgtacgcacgtcgccgatgggccggtgtgggtggtggcgcaggtcgTGCACG
W5200normal          cgtacgcacgtcgccgatgggccggtgtgggtggtggcgcaggtcgTGCACG
Dxs1                 cgtacgcacgtcgccgatgggccggtgtggccgtgtgggtggtggcgcaggtcgTGCACG
616Bnormal

w5200                 ACGTCAATCTGCAGAAGCTACCGGTAAGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
616B                  ACGTCAATCTGCAGAAGCTACCGGTAAGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
w5200normal           ACGTCAATCTGCAGAAGCTACCGGTAAGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
Dxs1                  ACGTCGATCTGCAGAAGCTACCGGTGCGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
616Bnormal

w5200                CGGACGGGCCGACCCACTGCGGGGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
616B                 CGGACGGGCCGACCCACTGCGGTGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
w5200normal          CGGACGGGCCGACCCACTGCGGGGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
Dxs1                 CGGACGGGCCGACCCACTGCGGGGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
616Bnormal

w5200                TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACAGCCGCGG
616B                 TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACAGCCGCGG
w5200normal TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACAGCCGCGG
Dxs1                 TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACCGCCGCGG
616Bnormal

w5200                CAATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
616B                 CAATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
w5200normal          CAATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
Dxs1                 CCATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
616Bnormal

   w5200             TGCCGCCCAACTACAAAGACACTCCCCTCGAGGTATGTATACATCGATCGATGGTGTATT
   616B              TGCCGCCCAACTACAAAGACACTCCCCTCGAGGTATGTATACATCGATCGATGGTGTATT
   w5200normal       TGCCGCCCAACTACAAAGACACTCCCCTCGAGGTATGTATACATCGATCGATGGTGTATT
   Dxs1              TGCCGCCCAACTACAAAGGCACTCCCCTCGAGGTACGTATACATCGATCGATGGTGTCTT
   616Bnormal
```

```
w5200          TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
616B           TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
w5200normal    TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
Dxs1           TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
616Bnormal     ..............................................................

w5200          AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGCGCTGCTGGGGTACGGGTCGGCAGTG
616B           AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGTGCTGCTGGGGTACGGGTCGGCAGTG
w5200normal    AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGTGCTGCTGGGGTACGGGTCGGCAGTG
Dxs1           AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGCGCTGCTGGGGTACGGGTCGGCAGTG
616Bnormal     ..............................................................

w5200          CAGTACTGCCTGACCGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
616B           CAGTACTGCCTGACCGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
w5200normal    CAGTACTGCCTGACCGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
Dxs1           CAGTACTGCCTGACTGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
616Bnormal     ..............................................................

w5200          GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
616B           GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
w5200normal    GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
Dxs1           GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
616Bnormal     ..............................................................

w5200          GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGAGGGTTCGGCTCGCACGTCGCCCAG
616B           GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGAGGGTTCGGCTCGCACGTCGCCCAG
w5200normal    GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGAGGGTTCGGCTCGCACGTCGCCCAG
Dxs1           GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGCGGGTTCGGCTCGCACGTCGCCCAG
616Bnormal     ..............................................................

w5200          TTCATGGCCCTGGACGGCCTTCTTGACGGCAAACTCAAGgcaagtctcacactagctagc
616B           TTCATGGCCCTGGACGGCCTTCTTGACGGCAAACTCAAGgcaagtctcacactagctagc
w5200normal    TTCATGGCCCTGGACGGCCTTCTTGACGGCAAACTCAAGgcaagtctcacactagctagc
Dxs1           TTCATGGCCCTGGACGGCCTTCTCGACGGCAAACTCAAGgcaagtctcaccctagctagc
616Bnormal     ..............................................................

w5200          tgctcggtcgccctaatgataacgagagagagagaaaaaaactccgaactccatcttt
616B           tgctcggtcgccctaatgataacgagagagagagaaaaaaactccgaactccatcttt
w5200normal    tgctcggtcgccctaatgataacgagagagagagaaaaaaactccgaactccatcttt
Dxs1           tgctcggtcgccctaatgataacgagagagagagaaaaaaaatccgaactccatcttt
616Bnormal     ..............................................................

w5200              agctgacaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGGTG
616B               agctgacaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGGTG
w5200normal        agctgacaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGG
Dxs1                agctgacaaaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGGTG
616Bnormal          ------------------------------------------------------------

w5200               CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
616B                CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
w5200normal        CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
Dxs1                CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
616Bnormal          ..............................................

w5200          ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGAACAGGGAGGCTCTT
616B           ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGA----   GGAGGCTCTT
w5200normal    ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGAACAGGGAGGCTCTT
Dxs1           ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGAACAGGGAGGCTCTT
616Bnormal     ------------------------------------------------------------
```

```
w5200                GCCATCATGGCAGTGCCTAACGCGTAGaacttgtgctgatcttggcctatagagatggtt
616B                 GCCATCATGGCAGTGCCTAACGCGTAGaacttgtgctgatctgggcctatagagatggtt
w5200normal         GCCATCATGGCAGTGCCTAACGCGTAGaacttgtgctgatcttggcctatagagatggtt
Dxs1                 GCCATCATGGCAGTGCCAAACGCGTAGaacttgtgctgatctgggcctatagagatgatt
616Bnormal

w5200                gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
616B                 gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
W5200normal         gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
Dxs1                 gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
616Bnormal

W5200                gtgtcgccggaacaacagttaccg
616B                 gtgtcgccggaacaacagttaccg
W5200normal         gtgtcgccggaacaacagttaccg
Dxs1                 gtgtcgccggaacaacagttaccg
616Bnormal
```

Figure A1: Multiple sequence alignment of Dxs1 alleles from normal and mutant maize seedlings. Dxs1: B73 sequence. Intron sequences are in small cap and exon sequences are in all caps. Grey shading highlights the differences between sequences. Dashes (-) indicate gaps inserted to optimized the alignment

```
w5200                tattgacagcccggccggataggcaagcgccacgtaagggcttgttcggttattcccaat
616B                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
W5200normal          tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
Dxs1                 tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
616Bnormal           tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
612A                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612Anormal           tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612M                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612Mnormal           tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
612N                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612Nnormal           tcttgacagcccggccggttaggcaagcgccacgtaa----------------------

w5200                acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
616B                 ------------------------------------------------------------
w5200normal          acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
Dxs1                 acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca
616Bnormal           acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca
612A                 ------------------------------------------------------------
612Anormal           ------------------------------------------------------------
612M                 ------------------------------------------------------------
612Mnormal           acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
612N                 ------------------------------------------------------------
612Nnormal           ------------------------------------------------------------

w5200                aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616B                 ------------------------------------------------------------cac
w5200normal          aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
Dxs1                 aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616Bnormal           aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
612A                 ------------------------------------------------------------cac
612Anormal           ------------------------------------------------------------cac
612M                 ------------------------------------------------------------cac
612Mnormal           aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
612N                 ------------------------------------------------------------cac
612Nnormal           ------------------------------------------------------------cac
```

```
w5200              gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616B               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
W5200normal        gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
Dxs1               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616Bnormal         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612A               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612Anormal         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612M               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612Mnormal         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612N               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612Nnoraml         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
```

Figure A2: Multiple sequence alignment of MITE region in Dxs1 alleles from normal and mutant maize seedlings. Dxs1: B73 sequence. Intron sequences are lower case and exon sequences are upper case. Dashes (-) indicate the deletion of MITE transposable element sequences leaving a TAA footprint.