Theor Appl Genet (2005) 110: 276–282
DOI 10.1007/s00122-004-1830-6

ORIGINAL PAPER

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Two point mutations identified in emmer wheat generate null *Wx-A1* alleles

Received: 6 August 2004 / Accepted: 27 September 2004 / Published online: 8 December 2004

Abstract In this report, the *Wx-A1* mutations carried by a *Triticum dicoccoides* line from Israel and a *Triticum dicoccum* line from Yugoslavia are characterized. A single nucleotide insertion in the *T. dicoccoides* null allele and a single nucleotide deletion in the *T. dicoccum* null allele each cause frameshift mutations that induce premature termination codons more than 55 nucleotides upstream of the last exon-exon junction. In both mutants, *Wx-A1* transcripts were detectable in 10 day post-anthesis endosperm by relative RT-PCR. However, transcript levels of the *T. dicoccoides* and *T. dicoccum* null alleles were reduced to approximately 6.5 and 1.5% of wild-type, respectively. Therefore, the lack of Wx-A1 protein in the mutants appears to be largely due to nonsense-mediated mRNA decay. The two mutations described here arose independently, and are not related to either of the *Wx-A1* mutations identified in common wheat.

Introduction

The *waxy* gene encodes the waxy (Wx) protein, or granule bound starch synthase I (GBSSI), which is responsible for amylose biosynthesis in storage tissues. *Waxy* mutations may affect amylose levels, and *waxy* mutants with little or no amylose have been described in a number of plant species (Eriksson 1970). The ease of identifying amylose-free mutants using iodine staining has facilitated the use of *waxy* mutants in genetic studies of diploid plants such as maize, rice and barley (Eriksson 1970). More recently, *waxy* mutations have been analyzed in detail at the molecular level. In maize, many spontaneous *waxy* mutations are caused by transposable elements (Fedoroff et al. 1983; Wessler and Varagona 1985; Wessler et al. 1987), whereas a single nucleotide substitution leads to aberrant splicing and reduces both *waxy* mRNA level and amylose content in rice (Cai et al. 1998; Hirano et al. 1998; Isshiki et al. 1998). In barley, a deletion in the region of the promoter and first intron results in lowered amylose levels, while a single nucleotide substitution in the coding region produces amylose-free cultivars (Patron et al. 2002; Domon et al. 2002).

The development of a method for the separation of the Wx proteins in common or emmer wheat (Nakamura et al. 1993a, 1993b) was followed by the identification of several null alleles for Wx protein (Yamamori et al. 1994, 1995; Demeke et al. 1997; Graybosch et al. 1998; Zhao et al. 1998; Nieto-Taladriz et al. 2000; Urbano et al. 2002). Combining null alleles from each genome allowed the production of *waxy* mutants in common and tetraploid wheat (Nakamura et al. 1995). A molecular analysis of the three null alleles for the *Wx-A1*, *Wx-B1* and *Wx-D1* genes of the hexaploid mutant identified a deletion in the null *Wx-A1* gene (Vrinten et al. 1999), and allowed the development of PCR markers that were used to analyze *waxy* mutations in common wheat germplasm (Nakamura et al. 2002; Saito et al. 2004). In consequence, a new type of mutation induced by an insertion was identified in the *Wx-A1* gene of Turkish cultivars (Saito et al. 2004). It appeared that the deletion and the insertion mutations occurred separately and while the deletion spread to several areas of the world, the insertion was conserved only in Turkish cultivars.

Although fewer lines of emmer than common wheat have been analyzed for *waxy* mutations, several accessions that carry null alleles for *Wx-A1* have been identified in the emmer wheat types *Triticum dicoccoides* Körn., *Triticum dicoccum* Schübl. and *Triticum durum* Desf. (Yamamori et al. 1995; Urbano et al. 2002). From

Communicated by C. Möllers

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an evolutionary point of view, it would be interesting to deduce the relationship of mutations in emmer versus common wheat, or among emmer wheat lines. The *Wx-A1* mutation in Turkish durum wheat may be due to an insertion since amplification of a region of the null *Wx-A1* allele produced a larger fragment than did the wild-type allele (Urbano et al. 2002). However, besides this PCR data, there are no molecular-based analyses to indicate the origin of the *Wx-A1* mutations reported in emmer wheat. In this study, we characterize the *Wx-A1* mutations occurring in *T. dicoccoides* and *T. dicoccum* and discuss the effects of these lesions on *waxy* gene expression.

Materials and methods

Plant materials

Seed accessions of *T. dicoccoides*, *T. dicoccum* and *T. durum* were obtained from the Plant Germplasm Institute, Faculty of Agriculture, Kyoto University and the gene bank of the National Institute of Agrobiological Sciences (NIAS) (Tsukuba, Japan). The tetraploid waxy wheat used in this study was selected from the BC5F3 generation of a cross between durum wheat (*T. durum* Desf. cv. Plenty) with our original waxy tetraploid wheat (Nakamura et al. 1995).

SDS–PAGE

Preparation of starch granules and separation of Wx proteins by low-bis acrylamide SDS-PAGE was performed as described by Nakamura et al. (1993a, 1993b).

DNA extraction and amplification of *Wx-A1* gene

Plant DNA was extracted from young leaf tissue using the Nucleon Phytopure Plant DNA Extraction kit (Amersham Biosciences, Little Chalfont, UK) according to the manufacturer's instructions. The primers and PCR conditions used to amplify null *Wx-A1* alleles in hexaploid wheat were the same as those used by Nakamura et al. (2002). To obtain genomic sequence information from emmer wheat, the coding region of the *Wx-A1* gene was amplified using KOD-Plus polymerase (Toyobo, Osaka) with the primers 5′-ATGGCGGCTCTGGTCACGTC-3′ and 5′-TCAGGGAGCGGCGACGTTC-3′, which were designed based on the *Wx-A1* sequence of common wheat.

Cloning of PCR products and sequencing analysis

PCR products were cloned using the Zero Blunt TOPO PCR Cloning Kit for sequencing or the TOPO TA Cloning Kit for sequencing (Invitrogen, Carlsbad, Calif.). Inserts were sequenced using a BigDye Terminator v1.1 Cycle Sequencing Kit and an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, Calif.).

RNA extraction and RT-PCR

Total RNA was extracted from developing seeds at 10 days post-anthesis (10 DPA) using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. RNA was treated with DNase from a DNA-free kit (Ambion, Austin, Tex.), and 5 μg of total RNA was used for reverse transcription reactions. First-strand cDNA was synthesized at 50°C from random hexamers using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). After the reaction was terminated at 85°C and cooled at room temperature, the remaining RNA was digested with RNase H. The synthesized cDNA was used for a comparative PCR between *Wx-A1* and 18S rRNA, which was employed as an endogenous internal standard. The *Wx-A1*-specific primers 5′-TCCGAGATCAAGGTCGTTGACA-3′ and 5′-CCGGTCTTGCCTTCCACGATA-3′ were used for the detection of *Wx-A1* transcript, and the Quantum RNA 18S internal standards primer set (Ambion) was used for 18S rRNA amplification. Each 50 μl reaction contained 1 μl of the RT reaction, 1× KOD Dash buffer, 0.2 mM dNTP, 0.2 μM of each *Wx-A1* primer, 0.12 μM 18S primer mix, 0.28 μM 18S competimer and 1 U KOD Dash polymerase (Toyobo). The PCR cycle consisted of an initial 1 min denaturation at 94°C, followed by 25 or 30 cycles of 98°C for 10 s, 62°C for 2 s and 74°C for 30 s. PCR products were separated on a 3% (w/v) agarose gel and quantified with an Agilent 2100 Bioanalyzer using the DNA 7500 LabChip kit (Agilent Technologies, Palo Alto, Calif., USA).

Results

PCR analysis of *Wx-A1*

Previous studies of emmer wheat germplasm collected throughout the world identified several lines lacking the Wx-A1 protein (Yamamori et al. 1995, 1998; our unpublished data), including a *T. dicoccoides* accession from Israel (KU13454) and seven *T. dicoccum* accessions (Table 1), one from Ethiopia (KU9004) and six from

Table 1 Number of null *waxy* alleles in emmer wheat

Triticum species	Total number analyzed	Number of null alleles	
		Wx-A1	*Wx-B1*
T. dicoccoides	68	1	0
T. dicoccum	114	7	0
T. durum	226	0	0

Yugoslavia (KU14293, 14294, 14298, 14299, 14302, 14303). None of the accessions analyzed lacked the Wx-B1 protein. Protein from the null *Wx-A1* lines was re-analyzed to confirm that these lines lacked the Wx-A1 protein (Fig. 1).

Two different null mutations within the *Wx-A1* gene of common wheat have been characterized; one is a 19-bp deletion (Vrinten et al. 1999) which is found in varieties from throughout the world and the second is a 173-bp transposon-like insertion found only in Turkish germplasm (Saito et al. 2004). A primer set capable of distinguishing both types of mutation (Nakamura et al. 2002) was used to analyze DNA from null (KU13454) and wild-type (KU13453) *T. dicoccoides* lines, and from null (KU14294) and wild-type (KU14306) *T. dicoccum* lines (Fig. 2). All lines produced a fragment of approximately 389 bp, which is the size expected based on the wild-type hexaploid *Wx-A1* genomic sequence. Neither the 19-bp deletion nor the 173-bp insertion were observed, indicating the null *Wx-A1* alleles identified in *T. dicoccoides* and *T. dicoccum* have a different origin than those found in *T. aestivum* germplasm.

Sequence analysis of mutant *Wx-A1* gene

To obtain sequences of the coding regions of the null *Wx-A1* alleles, we designed primers that map to sequences that are conserved among the three *T. aestivum waxy* genes (see section Materials and methods). A product with the expected size of approximately 2.8 kb was amplified from all four accessions. The amplified fragments were expected to contain *waxy* sequences derived from both *Wx-A1* and *Wx-B1* genes. Sequences of several clones from each accession were compared with those of *T. aestivum*, allowing us to clearly classify clones as *Wx-A1* or *Wx-B1* genes. The positions of exons in the *Wx-A1* genomic sequences were predicted based on *T. aestivum waxy* cDNA and genomic sequences from publicly available databases. The nucleotide sequence from the wild-type alleles of KU13453 and KU14306 showed several differences from *T. aestivum* cultivar Chinese Spring (data not shown), but these nucleotide changes resulted in only two amino acid substitutions; positions 60 and 61 are phenylalanine and aspartic acid in *T. aestivum* but glycine and asparagine in both *T. dicoccoides* and *T. dicoccum* (Fig. 3). Amino acid sequences of the two emmer accessions were identical, indicating a high level of conservation of Wx-A1 protein sequences.

The alignment also revealed several nucleotide differences between the null and wild-type *Wx-A1* genes (Fig. 4). In KU13454, one missense substitution, two translationally silent substitutions and a single nucleotide insertion were found (Fig. 4a). The insertion led to a frameshift mutation and a premature termination codon was generated at amino acid position 523 (Figs. 3 and 4b).

In KU14294, a single nucleotide deletion at position 23 of exon 4 also caused a frameshift mutation (Fig. 4a, c). A premature termination codon was generated at amino acid position 144 (Figs. 3, 4c). Although mRNA is transcribed from both genes, it is unlikely that functional Wx protein would be produced from either transcript.

Expression of *Wx-A1* gene in emmer wheat

In waxy common wheat, the amount of *waxy* transcript is greatly reduced in comparison to non-waxy wheat (Vrinten et al. 1999). However, the amylose-free barley cultivar Yon M Kei, which also carries a premature termination codon in its *waxy* gene, shows the same level of *waxy* transcript as wild-type barley (Patron et al. 2002). Therefore, relative RT-PCR experiments were performed to determine if *Wx-A1* transcript levels were affected by the mutations described here. The coding region of the *Wx-A1* gene was amplified from 10 DPA endosperm total RNA using *Wx-A1* specific primers, and 18S rRNA was co-amplified as an internal standard. The amount of 18S rRNA was almost identical in all RNA samples examined. However, critical differences

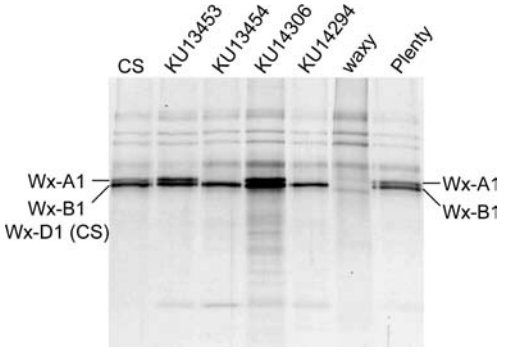

Fig. 1 SDS-PAGE analysis of Wx protein bound to starch granules. *CS*: Chinese Spring, *KU13453*: *Triticum dicoccoides*, *KU13454*: *T. dicoccoides*, *KU14306*: *T. dicoccum*, *KU14294*: *T. dicoccum*, *waxy* tetraploid waxy wheat, *Plenty T. durum*

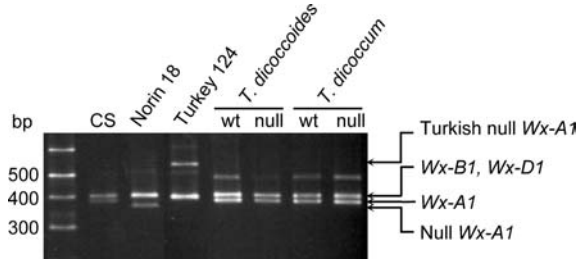

Fig. 2 PCR assay of *Wx-A1* genes in emmer wheat accessions. The primer set used here distinguishes common wheat lines carrying null *Wx-A1* alleles due to a 19-bp deletion (*Norin 18*) or a 173-bp insertion (*Turkey 124*). However, no size differences were detectable between amplification products of wild-type and null *Wx-A1* alleles of emmer wheat. *Wt* (wild-type) *T. dicoccoides*: KU13453, *null T. dicoccoides*: KU13454, *wt T. dicoccum*: KU14306, *null T. dicoccum*: KU14294

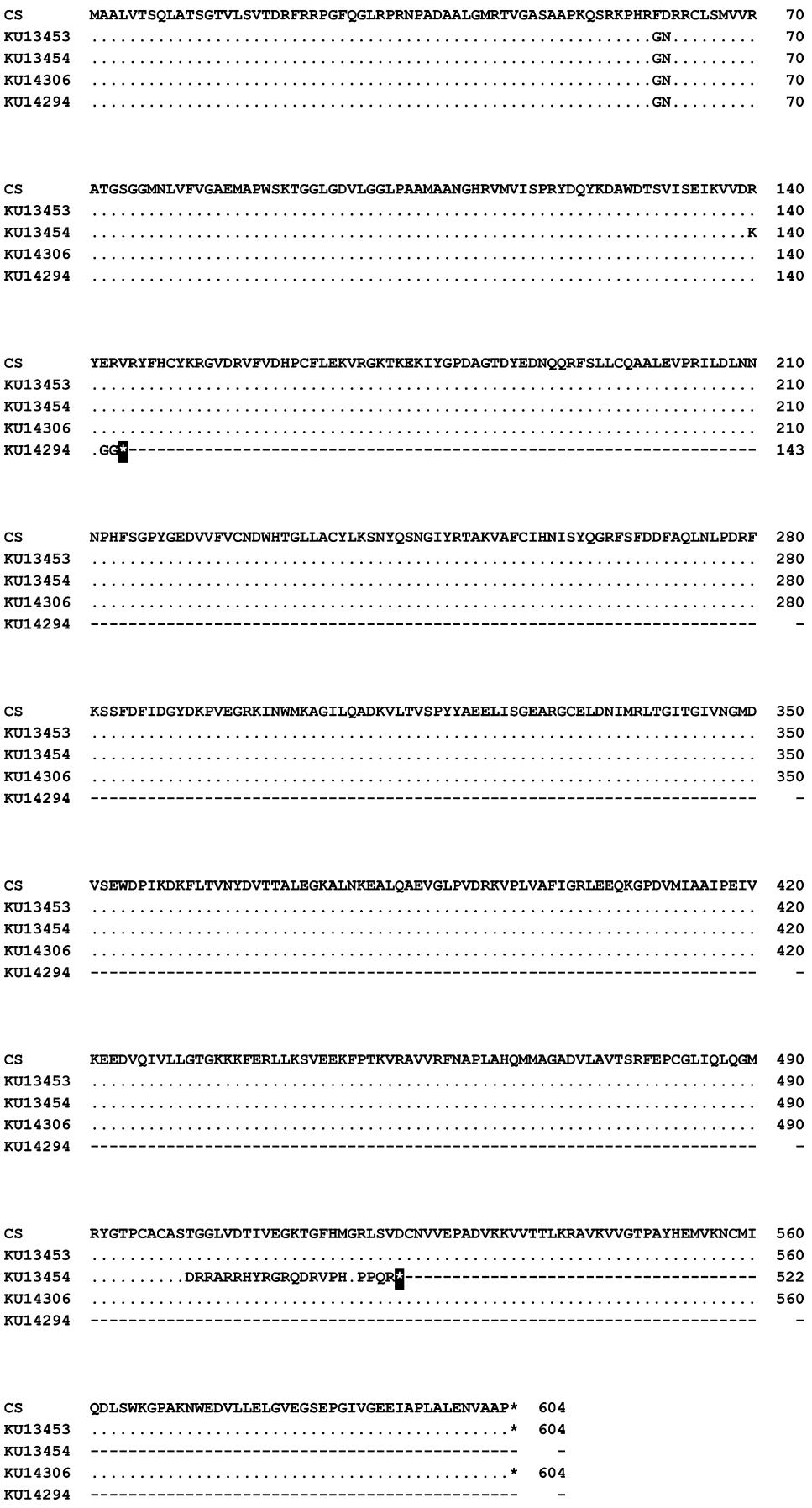

Fig. 3 Alignment of deduced amino acid sequences of *Wx-A1* genes. Identical residues are represented by *dots*. Positions of premature termination codons and normal stop codons are indicated by *outlined asterisks* and *black asterisks*, respectively

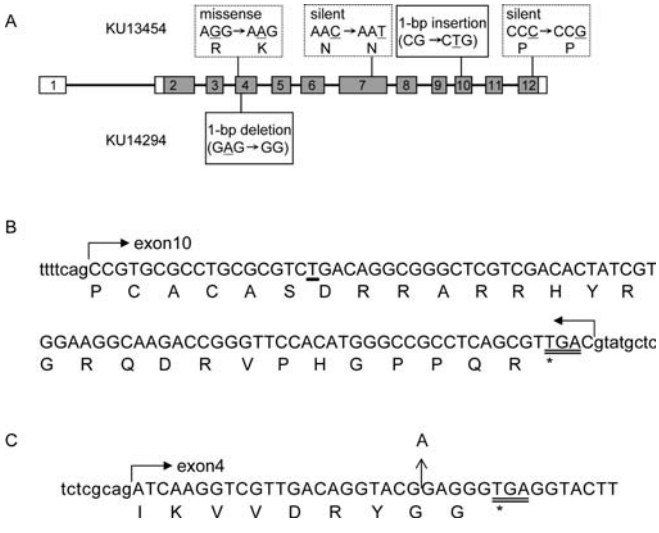

Fig. 4 Sequence comparison between wild-type and null *Wx-A1* alleles of emmer accessions. **a** Schematic illustration of *Wx-A1* gene and locations of nucleotide sequence differences observed in mutated *Wx-A1* genes from KU13454 and KU14294. *Boxes* indicate exons, and *gray shading* represents coding regions. Insertions and deletions, as well as missense and translationally silent changes, are indicated above (KU13454) or below (KU14294) the exon boxes. **b** Frameshift mutation in the null *Wx-A1* allele of KU13454. A 1-bp insertion in exon 10 changes the reading frame, and creates a premature termination codon at the 3′ end of exon 10. **c** Frameshift mutation in the null *Wx-A1* allele of KU14294. A 1-bp deletion at nucleotide 23 of exon 4 creates a premature termination codon

were observed in the amount of *Wx-A1* transcript found in wild-type versus mutant plants (Fig. 5a); gel electrophoresis followed by ethidium bromide staining detected a small amount of amplification product in KU13454 reactions, while no product was visible in KU14294 samples. However, product could be detected even in KU14294 when samples were analyzed using an Agilent Bioanalyzer. The ratio of *Wx-A1* to 18S rRNA molarity was taken as the relative amount of *Wx-A1* transcript (Fig. 6). From this, the levels of *Wx-A1* transcripts in KU13454 and KU14294 were calculated as approxi-

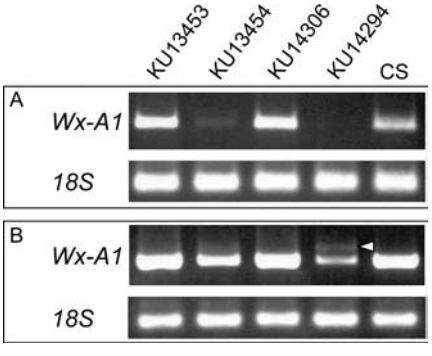

Fig. 5 Expression of *Wx-A1* genes in KU13454 and KU14294. The amounts of *Wx-A1* transcripts and 18S rRNA were analyzed by multiplex RT-PCR. PCR was performed with 25 (**a**) or 30 reaction cycles (**b**). The *arrowhead* indicates PCR product amplified from *Wx-A1* transcripts with retained introns

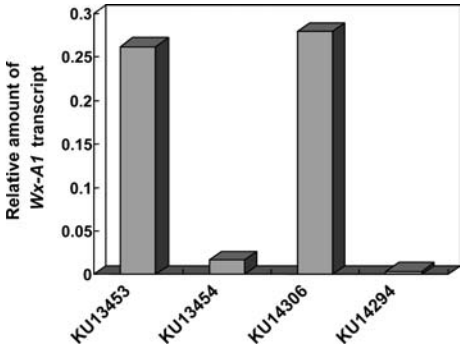

Fig. 6 Quantitative comparison of mature *Wx-A1* transcripts. Comparative RT-PCR (25 PCR cycles) was conducted with *Wx-A1* and 18S rRNA-specific primer sets and amplified products were analyzed using an Agilent 2100 Bioanalyzer. The amounts of *Wx-A1* and 18S rRNA PCR products were calculated in molarity (nmol/l) and the *Wx-A1* product amount was normalized to the rRNA product amount

mately 6.5% of KU 13453 and 1.4% of KU14306, respectively.

When the number of PCR cycles was increased to 30, an amplification product with a higher molecular weight than the 1,145-bp fully spliced *Wx-A1* transcript was detected in KU14294 (Fig. 5b, arrowhead). Sequencing revealed this product consisted of at least three types of sequence carrying one or two unspliced introns (Fig. 7). A similar-sized product was also found in KU13454 when the number of cycles and the amount of product loaded were increased (Fig. 7). This fragment included two different amplification products containing intron sequences as well as a product that resulted from the use of a variant splicing site.

Discussion

The wild emmer wheat *T. dicoccoides* is thought to have primarily originated in the western arc of the Fertile Crescent from the upper Jordan Valley to southeastern

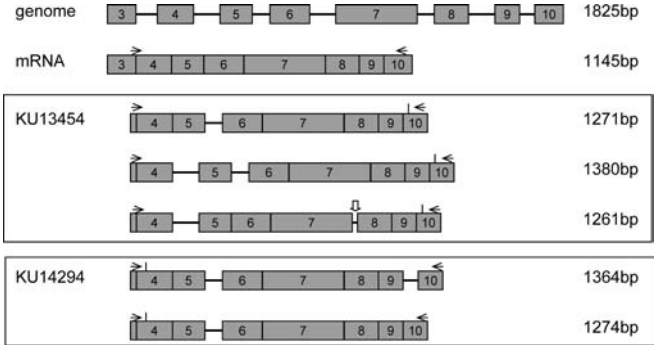

Fig. 7 Schematic representation of *Wx-A1* fragments amplified from total RNA of KU13454 and KU14294 using RT-PCR. *Wx-A1* specific primers, indicated by *horizontal arrows*, were used. *Boxes* indicate exons, and *vertical lines* indicate the positions of premature termination codons. One or two introns were retained in transcripts (*lines*), and the *outlined arrow* indicates an intron that originated from an aberrant splicing site

Anatolia (Johnson 1975). *T. dicoccoides* is the progenitor of the domesticated emmer wheat types *T. dicoccum* and *T. durum*. Archaeological evidence indicates that emmer wheat has been cultivated since the early Neolithic era, and was the most important crop until the early Bronze Age in the Near East (Bell 1987). Although tetraploid wheat evolution has been studied using *waxy* gene sequences (Yan and Bhave 2000, 2001), there are few reports relating to *waxy* mutations in tetraploid wheat. In this report, we were able to elucidate the causes of the mutations of the *Wx-A1* genes in *T. dicoccoides* and *T. dicoccum*. In both cases, the mutation originated from a frameshift mutation caused by a single nucleotide insertion or deletion (Fig. 4). However, the *Wx-A1* mutation in *T. dicoccum* was not related to that in its progenitor, *T. dicoccoides*, and the two mutations clearly arose independently.

The frameshift mutations described here generated premature termination codons in the RNA sequences (Fig. 4), which would reduce the length of the translation products by 82 and 461 amino acids in KU13454 and KU14294, respectively (Fig. 3). Both mutated genes were transcribed, but dramatic reductions in transcript levels were observed, suggesting that mRNA was recognized and degraded by a process referred to as nonsense-mediated mRNA decay (NMD). NMD or RNA surveillance is a transcript quality control system (reviewed in Wilusz et al. 2001; Schell et al. 2002; Wagner and Lykke-Andersen 2002; Maquat 2004) that prevents the accumulation of high levels of truncated proteins translated from mRNAs containing a premature termination codon. Such truncated proteins may exert a dominant-negative effect (Maquat 2004), and NMD prevents their accumulation by selectively eliminating aberrant transcripts. In mammals, the position of the premature termination codon and the presence of introns are important in eliciting NMD. Only those premature termination codons positioned more than 50–55 nucleotides upstream of the final exon-exon junction result in NMD, and premature termination codons occurring in the last exon or in intronless genes cannot elicit NMD (Maquat 2004).

The mechanisms involved in NMD in plants are still under investigation. Evidence that premature termination codons can reduce mRNA level was initially presented in studies of the Kunitz trypsin inhibitor (*KTi*) gene in soybean (Jofuku et al. 1989), the phytohemagglutinin (*PHA*) gene in common bean (Voelker et al. 1990), and the ferredoxin-1 (*Fed-1*) gene in *Pisum* (Dickey et al. 1994). These are all intronless genes, therefore the rules governing NMD in plants may differ from those in mammals. Reduction in mRNA abundance also occurs in intron-containing genes such as the phytochrome A (Dehesh et al. 1993) and *AUX1* (Marchant and Bennett 1998) genes of *Arabidopsis*, and the *waxy* gene of rice (Isshiki et al. 2001). In these cases, the premature termination codons are located more than 50–55 nucleotides upstream of the final exon-exon junction, consistent with the mammalian NMD rules. The premature termination codons identified in the mutated *Wx-A1* genes of KU13454 and KU14294 also appear to fit these conditions, and drastic reductions in transcript levels were observed in both cases. Therefore, the deficiency of Wx-A1 protein observed here is likely to be largely due to rapid mRNA degradation because of NMD. The amount of transcript detected was very small, but if some translation still occurred, the resulting protein would not likely be functional since it would lack the C-terminal region. The active form of GBSSI is tightly bound to the starch matrix, and the C-terminal region is thought to be involved in this attachment (van de Wal et al. 1998).

Wx-A1 fragments retaining introns were amplified from KU13454 and KU14294 but not from the wild-type alleles (Figs. 5b, 7). In addition, KU13454 carried a 1,261-bp fragment (Fig. 7) in which a different splicing site replaced the primary site between exons seven and eight (Fig. 7). Point mutations in the coding region may affect not only amino acid sequence but also pre-mRNA splicing (Cartegni et al. 2002; Faustino and Cooper 2003). In particular, frameshift and nonsense mutations can elicit nonsense-associated altered splicing (NAS) in mammalian cells, including exon skipping, alternative splicing site choice and intron retention (Cartegni et al. 2002; Maquat 2002). NAS may be involved in the production of the aberrantly spliced *Wx-A1* transcripts in KU13454 and KU14294, although we cannot rule out the possibility that the relatively low levels of correctly processed transcripts in these genotypes led to selective amplification of incompletely or improperly processed transcripts.

Although *Wx-A1* mutations were identified in eight accessions of emmer wheat (Table 1), only two mutants were subjected to molecular analysis in this study. The six *T. dicoccum* accessions from Yugoslavia were all collected from the same region, and it is likely that they all carry the same point mutation. Unfortunately, due to a limited number of seed and a lack of seed germination, we were unable to analyze the *T. dicoccum* accession from Ethiopia, and the molecular details of the mutation carried by this line remain unknown. The cause of a *Wx-A1* mutation carried by a durum wheat line (Urbano et al. 2002) is also unknown. Further analysis of these mutants will reveal whether the lesions they carry are related to the mutations described here.

Acknowledgements We thank Drs. Shoji Ohta and Makoto Yamamori, and NIAS for providing emmer wheat seeds. We also thank Dr. Patricia Vrinten for helpful discussions and useful editorial comments on the manuscript. This study was supported by a grant from the Japan Science and Technology Corporation.

References

Bell GDH (1987) The history of wheat cultivation. In: Lupton FGH (ed) Wheat breeding. Chapman and Hall, New York, pp 31–49

Cai XL, Wang ZY, Xing YY, Zhang JL, Hong MM (1998) Aberrant splicing of intron 1 leads to the heterogeneous 5′ UTR and decreased expression of *waxy* gene in rice cultivars of intermediate amylose content. Plant J 14:459–465

Cartegni L, Chew SL, Krainer AR (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat Rev Genet 3:285–298

Dehesh K, Franci C, Parks BM, Seeley KA, Short TW, Tepperman JM, Quail PH (1993) *Arabidopsis HY8* locus encodes phytochrome A. Plant Cell 5:1081–1088

Demeke T, Hucl P, Nair RB, Nakamura T, Chibbar RN (1997) Evaluation of Canadian and other wheats for waxy proteins. Cereal Chem 74:442–444

Dickey LF, Nguyen TT, Allen GC, Thompson WF (1994) Light modulation of ferredoxin mRNA abundance requires an open reading frame. Plant Cell 6:1171–1176

Domon E, Fujita M, Ishikawa N (2002) The insertion/deletion polymorphisms in the *waxy* gene of barley genetic resources from East Asia. Theor Appl Genet 104:132–138

Eriksson G (1970) The waxy character. Hereditas 63:180–204

Faustino NA, Cooper TA (2003) Pre-mRNA splicing and human disease. Genes Dev 17:419–437

Fedoroff N, Wessler S, Shure M (1983) Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell 35:235–242

Graybosch RA, Peterson CJ, Hansen LE, Rahman S, Hill A, Skerritt JH (1998) Identification and characterization of U.S. wheats carrying null alleles at the *wx* loci. Cereal Chem 75:162–165

Hirano HY, Eiguchi M, Sano Y (1998) A single base change altered the regulation of the *waxy* gene at the posttranscriptional level during the domestication of rice. Mol Biol Evol 15:978–987

Isshiki M, Morino K, Nakajima M, Okagaki RJ, Wessler SR, Izawa T, Shimamoto K (1998) A naturally occurring functional allele of the rice *waxy* locus has a GT to TT mutation at the 5′ splice site of the first intron. Plant J 15:133–138

Isshiki M, Yamamoto Y, Satoh H, Shimamoto K (2001) Nonsense-mediated decay of mutant *waxy* mRNA in rice. Plant Physiol 125:1388–1395

Jofuku KD, Schipper RD, Goldberg RB (1989) A frameshift mutation prevents Kunitz trypsin inhibitor mRNA accumulation in soybean embryos. Plant Cell 1:427–435

Johnson BL (1975) Identification of the apparent B-genome donor of wheat. Can J Genet Cytol 17:21–39

Maquat LE (2002) NASty effects on fibrillin pre-mRNA splicing: another case of ESE does it, but proposals for translation-dependent splice site choice live on. Genes Dev 16:1743–1753

Maquat LE (2004) Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat Rev Mol Cell Biol 5:89–99

Marchant A, Bennett MJ (1998) The *Arabidopsis AUX1* gene: a model system to study mRNA processing in plants. Plant Mol Biol 36:463–471

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993a) Decrease of waxy (Wx) protein in two common wheat cultivars with low amylose content. Plant Breed 111:99–105

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993b) Identification of three Wx proteins in wheat (*Tritium aestivum* L.). Biochem Genet 31:75–86

Nakamura T, Yamamori M, Hirano H, Hidaka S, Nagamine T (1995) Production of waxy (amylose-free) wheats. Mol Gen Genet 248:253–259

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

Nieto-Taradriz MT, Rodriguez-Quijano M, Carrillo JM (2000) Polymorphism of waxy proteins in Spanish durum wheats. Plant Breed 119:277–279

Patron NJ, Smith AM, Fahy BF, Hylton CM, Naldrett MJ, Rossnagel BG, Denyer K (2002) The altered pattern of amylose accumulation in the endosperm of low-amylose barley cultivars is attributable to a single mutant allele of granule-bound starch synthase I with a deletion in the 5′-non-coding region. Plant Physiol 130:190–198

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

Schell T, Kulozik AE, Hentze MW (2002) Integration of splicing, transport and translation to achieve mRNA quality control by the nonsense-mediated decay pathway. Genome Biol 3:10061–10066

Urbano M, Margiotta B, Colaprico G, Lafiandra D (2002) Waxy proteins in diploid, tetraploid and hexaploid wheats. Plant Breed 121:465–469

Voelker TA, Moreno J, Chrispeels MJ (1990) Expression analysis of a pseudogene in transgenic tobacco: a frameshift mutation prevents mRNA accumulation. Plant Cell 2:255–261

Vrinten P, Nakamura T, Yamamori M (1999) Molecular characterization of *waxy* mutations in wheat. Mol Gen Genet 261:463–471

Wagner E, Lykke-Andersen J (2002) mRNA surveillance: the perfect persist. J Cell Sci 115:3033–3038

van de Wal M, D'Hulst C, Vincken JP, Buléon A, Visser R, Ball S (1998) Amylose is synthesized in vitro by extension of and cleavage from amylopectin. J Biol Chem 273:22232–22240

Wessler SR, Varagona MJ (1985) Molecular basis of mutations at the *waxy* locus of maize: correlation with the fine structure genetic map. Proc Natl Acad Sci USA 82:4177–4181

Wessler SR, Baran G, Varagona M (1987) The maize transposable element *Ds* is spliced from RNA. Science 237:916–918

Wilusz CJ, Wang W, Peltz SW (2001) Curbing the nonsense: the activation and regulation of mRNA surveillance. Genes Dev 15:2781–2785

Yamamori M, Nakamura T, Endo TR, Nagamine T (1994) Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theor Appl Genet 89:179–184

Yamamori M, Nakamura T, Nagamine T (1995) Polymorphism of two waxy proteins in the emmer group of tetraploid wheat, *Triticum dicoccoides*, *T. dicoccum*, and *T. durum*. Plant Breed 114:215–218

Yamamori M, Nakamura T, Kiribuchi-Otobe C (1998) Waxy protein alleles in common and emmer wheat germplasm. Misc Publ Natl Inst Agrobiol Resour 12:57–104

Yan L, Bhave M (2000) Sequences of the *waxy* loci of wheat: utility in analysis of waxy proteins and developing molecular markers. Biochem Genet 38:391–411

Yan L, Bhave M (2001) Characterization of waxy proteins and *waxy* genes of *Triticum timopheevii* and *T. Zhukovskyi* and implications for evolution of wheat. Genome 44:582–588

Zhao XC, Batey IL, Sharp PJ, Crosbie G, Barclay I, Wilson R, Morell MK, Appels R (1998) A single genetic locus associated with starch granule properties and noodle quality in wheat. J Cereal Sci 27:7–13