Theor Appl Genet (1995) 91: 367–373

J. V. Monte · R. B. Flavell · J. P. Gustafson

WIS 2-1A: an ancient retrotransposon in the *Triticeae* tribe

Received: 10 December 1994 / Accepted: 3 February 1995

Abstract WIS 2-1A, the first retrotransposon found in wheat, has been recently studied and characterized. Southern hybridization experiments utilizing several species from the *Triticeae* revealed the presence of homologous sequences in all the taxa tested, showing high levels of interspecific variability and almost no intraspecific differentiation. Further experiments using in situ hybridization in several species showed that the retroposon was almost completely dispersed throughout the genomes tested. These results suggested that WIS 2-1A is an ancient element that probably was present in the unknown common ancestor of the *Triticeae* and that only under rare circumstances does it become active. DNA fragments homologous to the WIS 2-1A reverse transcriptase gene were isolated from most of the *Triticeae* species using PCR. The fragments obtained were sequenced and analyzed. Even though the sequence alignment was consistent with the phylogenetic studies made in the past, the genus *Thinopyrum* showed new evidence for a possible horizontal propagation of the retroelement.

Key words *Triticeae* · Retrotransposon · In situ hybridization · DNA sequence alignment

This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the Universidade de tras-os-Montes e Alto Douro, the University of Adelaide, the USDA, or the University of Missouri. Contribution for the U.S. Department of Agriculture, Agricultural Research Service, Plant Genetics Research Unit, and the University of Missouri Agricultural Experiment Station Journal Series No. 11992

Mention of a trade name or proprietary product does not constitute a guarantee, warranty or recommendation of the product by the U.S. Department of Agriculture or the University of Missouri and does not imply its approval to the exclusion of other products that may be suitable

All programs and services of the U.S. Department of Agriculture are offered on a non-discriminatory basis without regard to race, colour, national origin, religion, sex, age, marital status, or handicap.

Communicated by A. L. Kahler

J. V. Monte · J. P. Gustafson (✉)
USDA-Agricultural Research Service,
Plant Genetics Research Unit, and Plant Science Unit,
University of Missouri, Columbia, MO 65211, USA

R. B. Flavell
John Innes Institute.
The John Innes Centre for Plant Science Research,
Colney Lane, Norwich, Norfolk, NR4 7HU,
United Kingdom

Introduction

Harberd et al. (1987), studying a silent allele of a high-molecular-weight (HMW) storage protein gene in *Triticum aestivum* L. em Thell (hexaploid wheat) found that the allele was modified due to an exogenous 8-kb DNA insertion. This gene, *Glu-1*, is located on the long arm of the chromosome 1A. When the varieties Cheyenne and Chinese Spring were compared, the 8-kb inserted alien fragment was found disrupting the coding sequence of the *Glu-1* allele. The authors named the fragment WIS 2-1A (wheat insertion sequence isolated from within the *Glu-1A* locus). When analysed, it was found that the insertion site was flanked by a 5-bp duplication, with the two ends of WIS 2-1A containing similar sequences over 500 bp long (LTR), and its termini containing almost the same 6-bp sequence, but in opposite orientations beginning with TG and ending with CA. A further characterization of the sequence of this element (Moore et al. 1991) confirmed that the size of the long terminal repeat (LTR) sequence was 1.8 kb, with the internal domain being about 4.5 kb. They also located a priming t-RNA binding site immediately adjacent to the left-hand LTR. Sequencing of the internal fragment did not show open reading frames, due to an accumulation of mutations, and frame shifts in that domain, even though in the 5′ end a putative methionine codon for the initiation of translation was detected. However, by making the proper adjustments for frame shifting in those regions they were able to deduce sequences related to the reverse transcriptase and integrase genes (Fig. 1). All these features make it most likely that WIS 2-1A is a retrotransposon belonging to the *copia*-like and *Ty* family that has lost its capacity to autonomously transpose due to an accumulation of

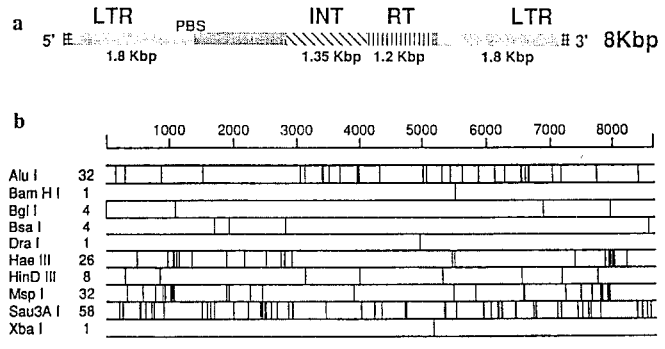


Fig. 1a WIS 2-1A physical map. Flanking the long terminal repeat sequences are two short inverse repeats. The primer-binding site is located in the 3′ end of the left LTR. The integrase and reverse transcriptase are the only genes conserved in the internal domain. **b** WIS 2-1A restriction map. The *Hin*dIII fragments 5 and 6 contain, respectively, the integrase and reverse transcriptase domains

mutations during evolution. However, the fact that it was found disrupting the *Glu-1* gene in only one variety of wheat suggests that is LTR ends are still functional, and that it can accomplish transposition mediated by trans-acting factors.

The structures of these elements are similar to those of retroviruses, which suggests a common evolutionary origin (Finnegan 1985; Hansen et al. 1988; Doolittle et al. 1989; Xiong and Eickbush 1990; Gojobori et al. 1990; Katz and Skalka 1990; McDonald 1990). Lucas et al. (1992) analysed the LTRs of WIS 2-1A, which are unusually long compared with other retrotransposons. They found that the length increase was due to the accumulation of tolerated mutations leading to an increase in DNA size. These mutations may have originated during the insertion of transposable elements and to stem from errors in DNA replication when the DNA switches RNA templates during reverse transcription. The result is an accumulation of hairpin structures that occupy almost half (44%) of the length of the LTRs. Moore et al. (1991) also detected the presence of solo LTRs in the wheat genome (transposition "footprints") which suggested a mechanism of excision through recombination in elements of the WIS family similar to one found in *Ty* retroposons. However, the presence of LTRs, or LTRs and WIS 2-1A retroelements adjacent to each other in genomic clones, indicated that either this element can amplify other than by retrotransposition or that WIS retroelements have preferred targets. The copy number of WIS 2-1A was estimated in hexaploid wheat to be about 200 copies per haploid genome. In cross-hybridization experiments, homologous sequences to WIS 2-1A subclones were detected in barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), oats (*Avena sativa* L.), and wild relatives of wheat (*Aegilops* spp.), ranging in copy number from 70 to 20 in these diploid species. Based on Southern experiments, Moore et al. (1991) observed that, for members of the WIS 2 family, in one variety of wheat there appeared to be ongoing genomic variation associated within individual plants and between specific varieties in plants regenerated from anther cultures. These observations could mean that active retrotransposition was taking place in those varieties either because sequences homologous to WIS 2-1A become active during meiosis, or because transacting factors like retroviruses were operating. However, this variation could also be accounted for by changes in the methylation pattern of the restriction enzymes' target sites, which still leaves open the question of the degree of activity of WIS 2-like elements.

Since WIS 2-1A was discovered, other retrotransposable elements have been identified in cereals. A related element called BIS 1 was recently isolated in barley (Moore et al. 1991). BIS 1-related sequences in barley constitute repetitive DNA that accounts for up to 5% of the genome. Additional WIS 2-1A-related elements found in other cereals are being analyzed at the present time. In addition to the cereals mentioned previously, *copia*-like retroposons have recently been found in a wide range of plant species (Schwarts-Sommer et al. 1987; Huijser et al. 1988; Smyth et al. 1989; Konieczny et al. 1991; Voytas et al. 1992). At the present, only wheat, tobacco (*Nicotiana tabacum* L.) and maize (*Zea mays* L.) are known to possess active retroelements (Grandbastien et al. 1989), with the tobacco element *Tnt 1* being the only autonomously active one.

The *Triticeae* tribe represents a group of plant species which have had a significant impact on the cultural and economic history of human development. The goal of the present work was to study the possible impact that WIS 2-1A could have had in the genomic diversification of the *Triticeae* since retroelements are a type of sequence capable of severely affecting the stability of their host genomes. We approached this problem in three ways. First, by studying the presence and organization of WIS 2-1A using WIS 2-1A subclones as probes in Southern hybridization experiments on genomic DNA blots of the *Triticeae* genera, species, and accessions used in this work. Second, to physically map the sequences homologous to WIS 2-1A in several *Triticeae* species by using in situ hybridization in order to study insertion targets within the chromosomes. Finally, PCR amplifications using primers based on the sequence of the WIS 2-1A reverse transcriptase were carried out on genomic DNA from the 11 *Triticeae* genomes tested. The amplified reverse transcriptase fragments were then sequenced and compared in an attempt to determine the relative importance of the vertical versus the horizontal transmission of this element during evolution (Saigo et al. 1984; Miyamoto and Cracraft 1991), and to assess the degree of conservation of this locus within the *Triticeae* (Sanderson and Doyle 1992).

Materials and methods

Plant material

Sixteen species representing 11 genomes of the *Triticeae* tribe and several accessions per species were selected (species, accessions, and their sources are listed in Table 1).

Table 1 Germplasms utilized in the present study

Species	Genome	Accessions	Geographical origin
Triticum monococcum L.	A	A1	Turkey
		A2	Iran
		A4	USSR
		A5	USSR
Triticum speltoides (Tausch) Godron	B	B1	Turkey
		B2	Israel
		B3	Iraq
		B4	Rumania
Triticum tauschii (Coss.) Schmalh.	D	D1	Afghanistan
		D2	USSR
		D3	USSR
		D5	Turkey
Secale cereale L.	R	R1	Poland
		R2	USA
		R3	Ecuador
		R4	USA
		R5	Mexico
Hordeum vulgare (L.)	I	I1	USA (Nebraska)
		I2	Canada (Central)
		I3	Netherlands
		I4	Canada (Ontario)
Critesion bogganii (Wilensky) Love	H	H1	USSR
		H2	Afghanistan
		H3	Iran
		H4	PRC
Critesion brevisubulatum (Tnn.) Love s. lat.	H	H5	Iran
		H6	Iran
		H7	USSR
		H8	USSR
Agropyron cristatum (L.) Gaetner	P	P1	USA
		P2	Germany
		P3	USSR
		P4	USSR
Agropyron desertorum (Fisher ex Link) Schultes	PP	P5	Denmark
		P6	Portugal
		P7	Turkey
		P8	USSR
Dasypyrum villosum (Cosson and Durieau) T. Durand	V	V	USSR
Psathyrostachys juncea (Fisher) Nevski	N	N1	USSR
		N2	USSR
		N3	Canada
		N4	USA
Psathyrostachys fragilis (Borss.) Nevski	N	N5	Iran
		N6	Iran
		N7	Iran
		N8	Iran
Pseudoroegneria spicata (Pursh) Love	S	S1	USA
		S2	Canada
		S3	USA
		S4	USA
Pseudoroegneria libanotica	S	S5	Iran
		S6	Iran
		S7	Iran
		S8	Iran
Thinopyrum elongatum (Host) D. R. Dewey	E	E1	France
		E2	France
Thinopyrum bessarabicum (Savul and Rayss) Love	J	J1	USSR
		J2	USSR
		J3	France
		J4	USSR
		J5	USSR

WIS 2-1A probes

Subclones in pBS (a modified pUC 18 vector) of the WIS 2-1A *Hin*dIII fragments representing the LTR, reverse transcriptase, and integrase domains (2300 bp, 1200 bp, and 1350 bp, respectively, in length) were used as probes in the Southern hybridization experiments. A clone named pECo5 containing the whole 8-kb element (originally an *Eco*RI fragment in Chinese Spring wheat) was used for the in situ hybridization assays. All these probes were obtained from the Cambridge Laboratory, John Innes Centre for Plant Science Research, Norwich, England (Moore et al. 1991).

Southern-hybridization assays

The restriction enzymes *Bam*HI, and *Hin*dIII were used to digest DNA in Southern-blot hybridization experiments (Sambrook et al. 1989) because their restriction patterns in the WIS 2-1A sequence contained fragment sizes most suitable for analysis.

Seeds from the selected species and accessions were grown in growth chambers and greenhouses on the University of Missouri-Columbia campus. Plant genomic DNA was extracted from young, green leaves by a sap extractor using a technique described in Clark et al. (1989). Genomic DNA of each plant sample was digested with the restriction enzymes *Bam*HI or *Hin*dIII separated by electrophoresis in agarose gel, transferred to nylon filters by Southern blotting, and hybridised with the three ^{32}P-labelled subclone probes using standard techniques (Sambrook et al. 1989).

In situ hybridization

Protoplasts from fixed root tips were obtained in *Triticum monococcum* (L.) Love, *T. speltoides* (Tausch) Godron, *H. vulgare* L., and *S. cereale* L., according to the technique developed by Dille' et al. (1990) (see Figs. 3 and 4). The plasmid pECo5 was biotin-labelled, and hybridised to protoplast chromosome preparations as described in Gustafson et al. (1990). The hybridizations were detected using the DAB method (Gustafson and Dille' 1992).

PCR cloning

The synthetic primers AAG AAA GGT TGT ATG TGA TA and GTC AAC AAC ATA TAC TCA TC were designed for the PCR experiments after comparing the DNA and protein sequence of the WIS 2-1A reverse transcriptase *Hin*dIII domain with the reverse transcriptase plant sequences described by Voytas et al. (1992) (Fig. 1). The PCR protocols of Innis et al. (1990) were applied to genomic DNA of the species and accessions.

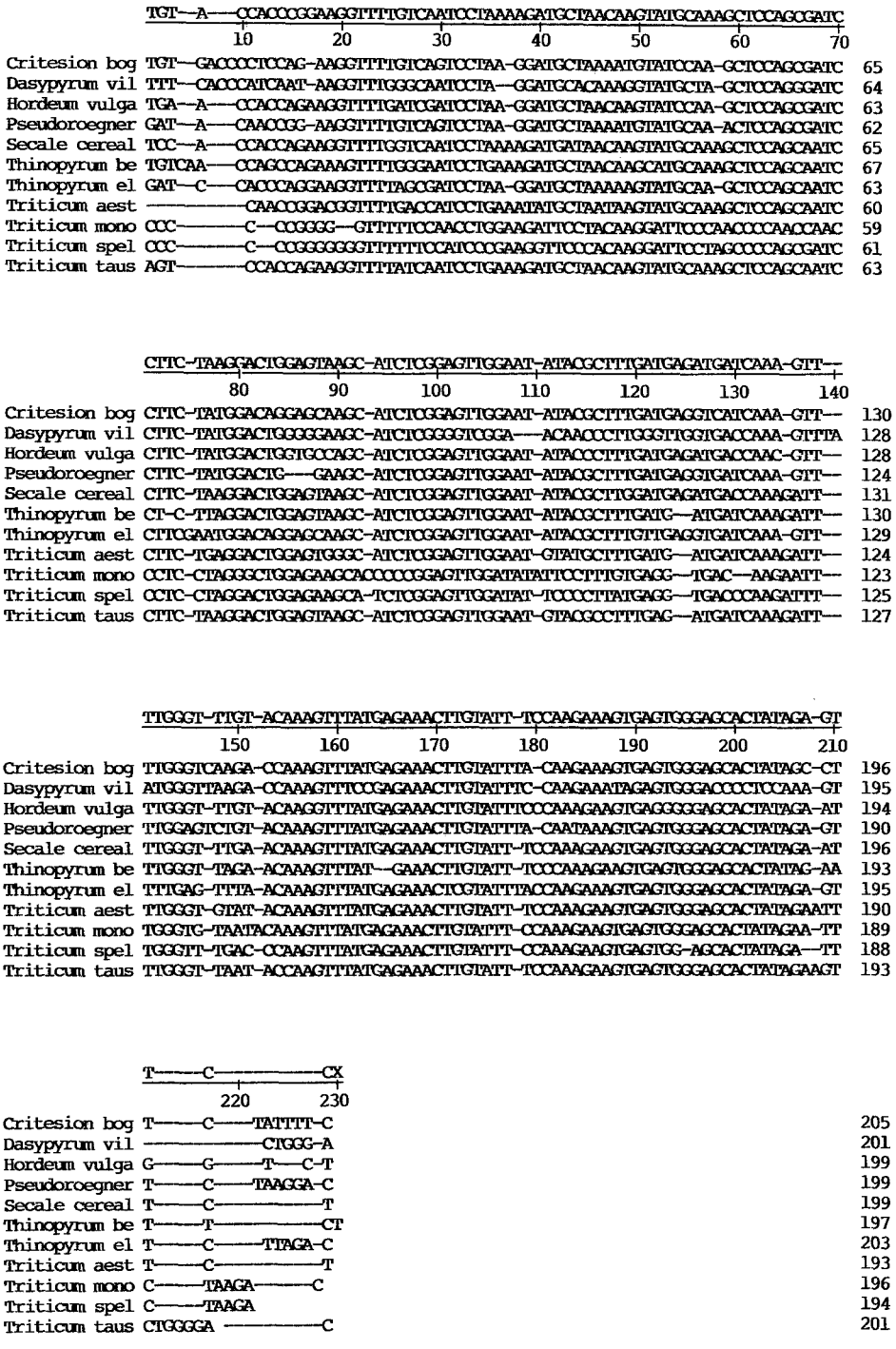
PCR products' purification

The PCR products were separated by electrophoresis in acrylamide gels and recovered as described in the *Taq* DyeDoxy™ Terminator Cycle Sequencing Kit protocol (Applied Biosystems Inc.).

DNA sequencing

The dideoxy chain-termination method described by Sanger et al. (1977) was used to sequence the PCR-amplified DNA fragments homologous to the WIS 2-1A reverse transcriptase fragments. The *Taq* Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems) was used. Direct bi-directional sequencing of the *Triticeae* PCR fragments unambiguously showed that the purified PCR bands represented single reverse transcriptase DNA copies. Thus the recomended further cloning step of the amplified PCR fragments in a vector, advisable in these types of analyses, was not needed.

Table 2 Alignment report of the *Triticeae* reverse transcriptase sequences, using the Jotun-Hein method of DNAStar with a gap penalty g_k=11+3k



DNA sequence-alignment analysis

The Jotun-Hein method present in DNASTAR version 1.3 (DNASTAR Inc. 1992) was applied using a Macintosh II ci computer, with a gap penalty g_k=11+3k, in order to generate a phylogenetic tree representing the evolution of the sequences analysed (see Table 2, Fig. 5). This method aligns each sequence pair maintaining each pair relationship in a tree which is, in a further step, re-examined for the best possible arrangement of ancestral branches using parsimony. The program assumes the sequences are somehow related and builds alignments to minimize evolutionary change (Hein 1990). When higher gap penalty values were used, the clustering patterns obtained were similar (see Fig. 5).

Results and discussion

Hybridization of the restriction-digested genomic DNA of all the species and accessions with the 3 WIS 2-1A probes, representing the LTRs, reverse transcriptase and integrase domains, showed, in all cases, high levels of interspecific variability but no intraspecific polymorphisms (Fig. 2). That the retroelement hybridized with all the taxa suggested that WIS 2-1A was an ancient element and was probably already present in the unknown diploid common ancestor of the tribe *Triticeae*. The high degree of interspe-

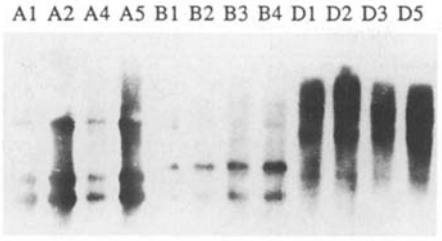


Fig. 2 Southern hybridization of the integrase domain of WIS 2-1A on genomic DNA of different populations of *T. monococcum* (*A1*, *A2*, *A4*, *A5*), *Tr. speltoides* (*B1*, *B2*, *B3*, *B4*), and *T. tauschii* (*D1*, *D2*, *D3*, *D5*)

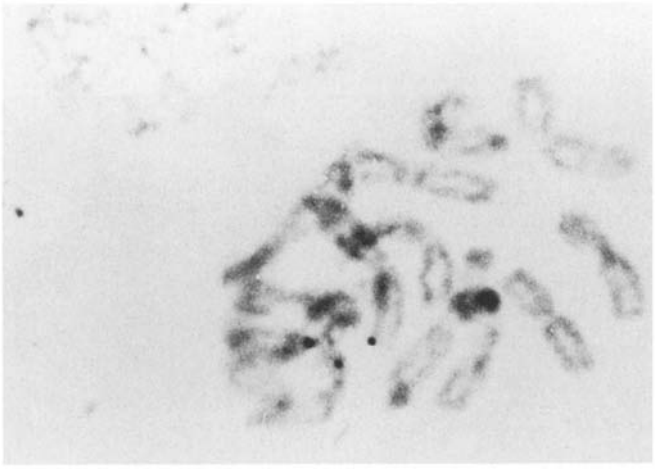


Fig. 3 In situ hybridization of WIS 2-1A on metaphase chromosomes of *Tr. monococcum*. In situ experiments showed the retroelement dispersed in the *Triticeae* genomes (see Fig. 4)

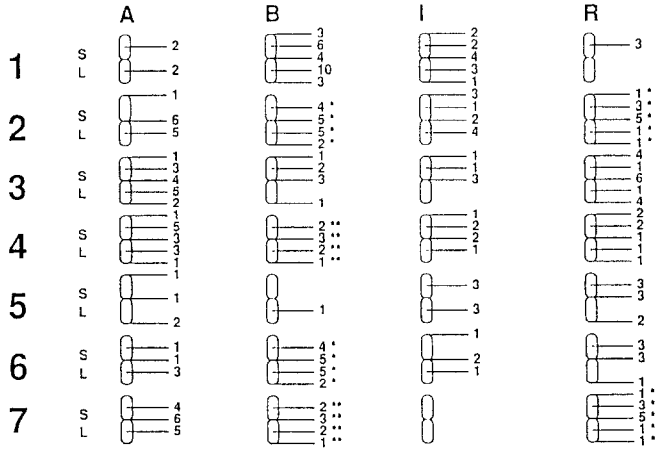


Fig. 4 In situ hybridization of WIS 2-1A on metaphase chromosomes of *T. monococcum* (*A*), *T. speltoides* (*B*), barley (*H*), and rye (*R*). The *numbers* represent the frequency of hybridization in telomeric, centromeric,and internal arm positions. The arm ratios of the B chromosomes pairs 2–6, 4–7, and the R pair 2–7; are not sufficiently discriminative to individualize these chromosomes. The ambiguities are represented with *asterisks* (*, **)

cific variation can be explained by chromosomal rearrangements, or other gross mutations, that were involved in the genome divergence and speciation of the *Triticeae* tribe. Any trans-acting factor capable of providing reverse transcriptase molecules (such as retroviruses) in the cellular cytoplasm could induce transposition of these elements, as has been previously described in the case of the *Gly* locus in Chinese Spring wheat (Moore et al. 1991). The fact that transposition events have not been detected in the *Triticeae* tribe could mean that retroviral infective processes in cereals are unusual or else are restricted to a few species or varieties. Another possible explanation could be that the accessibility of the reverse transcriptase to the retroposon mRNA could be mediated by an unknown WIS 2-1A factor involved in priming, or regulating, the different retrotransposition and insertion steps. The absence or alteration of one of those factors could lead to problems with the transposition process.

The in situ hybridization experiments of WIS 2-1A on metaphase chromosomes of *T. speltoides*, *T. monococcum*, barley and rye, clearly showed that the element was completely dispersed throughout the *Triticeae* genomes (Figs. 3, 4). In all cases, discrete, small hybridization dots were scored on almost all chromosomes. These results suggest that either this element does not have preferential insertion targets or that, if it does, the preference is not strong enough to avoid insertion elsewhere in the genome and/or the preferred site is also scattered. If the first hypothesis is correct, the tandem organization described in some WIS 2-1A clones and the hairpin structures detected in the LTRs could be due to unequal recombination and/or reverse transcription errors, respectively. In addition, the wide physical dispersion of WIS 2-1A could be a consequence of its ancient nature; large periods of time could then explain the dispersion pattern.

By scanning the sequence of the *Hin*dIII reverse transcriptase domain of WIS 2-1A (Fig. 1) it was possible to detect a fragment of about 200 bp homologous to the reverse transcriptase reading frames described in other plants and organism (Voytas et al. 1992). Two synthetic primers were chosen corresponding to two highly conserved protein motifs also present in WIS 2-1A. The primers amplified homologous fragments in all the genera tested except for *Agropyron* Gaetner and *Psathyrostachys* (Boiss.). These exceptions indicate a higher degree of evolutionary divergence in the reverse transcriptase fragments of those genomes since one or both WIS 2-1A primers apparently did not recognise complementary sequences. Southern hybridization experiments using the amplified fragments as probes against *Hin*dIII-digested WIS 2-1A DNA showed that these probes hybridized to fragments of the size expected for reverse transcriptase domains (data not shown).

Experiments using restriction enzymes recognizing 4 bp suggested sequence differences among the fragments obtained (data not shown), indicating some internal sequence divergence between species. Ten fragments corresponding to homologous WIS reverse transcriptase domains, and representing ten different *Triticeae* genomic

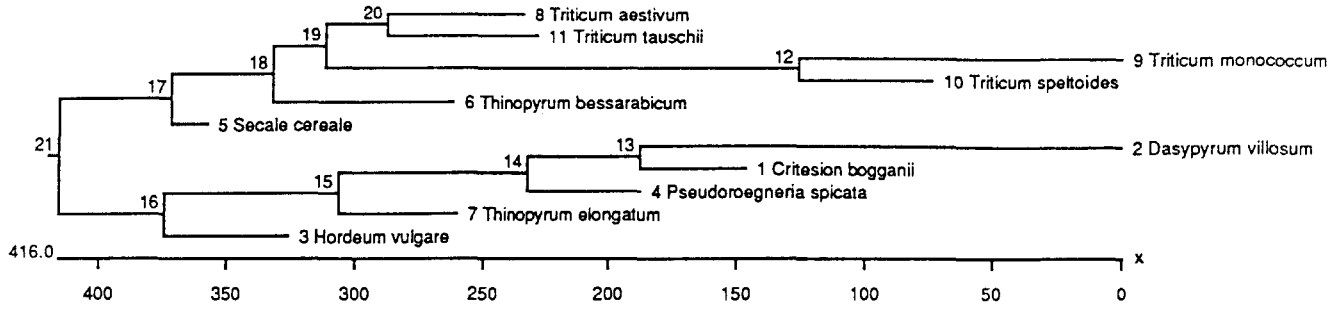


Fig. 5 Phylogenetic tree based on the alignment of the *Triticeae* reverse transcriptase sequences, using the Jotun-Hein method of DNA-Star with a gap penalty $g_k=11+3k$. The length of each pair of branches (X axis) represents the distance between sequence pairs. The sequences are numbered according to their appearance in the Meg Align work table. Nodes are assigned numbers sequentially as each branch was assembled in the tree

types in the genera *Triticum*, *Hordeum*, *Secale*, *Critesion*, *Dasypyrum*, *Thinopyrum*, and *Pseudoroegneria* Love, were sequenced and analysed. The Jotun-Hein method of DNA alignment contained in the computer program DNA-STAR was used to generate a phylogenetic tree representing the evolution of the WIS 2-1A reverse transcriptase domain in the species tested (Fig. 5). The tree clearly separated two large groups: one cluster included the A, B, D, J, and R genomes, and the other included the E, H, I, S, and V genomes. The large clustering patterns, *Triticum-Secale* and *Critesion-Hordeum-Pseudoroegneria-Dasypyrum*, are consistent with phylogenetic studies made in the past. The A and B genomes, and the ABD represented by WIS 2-1A, closely rotated, as expected, suggesting that *T. tauschii* (Coss.) Schmalh. was the donor of WIS 2-1A to hexaploid wheat. However, the most intriguing result is that the two *Thinopyrum* species representing the E and J genomes appear to be separated in the two main clusters. Since in phylogenetic studies the *Triticeae* suggest that both clusters are clearly separated, this unexpected result could be explained by a hypothetical horizontal transmission of the retrotransposon either from the *Triticum* cluster to *Thinopyrum bessarabicum* (J), or from the H-I-S-V group to *Thinopyrum elongatum* (E). Even though a certain degree of crossability occurs in the tribe between species with different genomes (Dewey 1984), the fact that the *Thinopyrum* species tested are self-fertilized suggests that a different mechanism of horizontal transfer may be involved in these species. Recent evidence for horizontal transmission of retrotransposons has been widely reported (Smyth et al. 1989; Doolittle et al. 1990; Jakubczak et al. 1990; Mizrohi and Mazo 1990; Calvi et al. 1991; Houck et al. 1991; Maruyama and Hartl 1991). How these events could take place is not clear, but passive transduction in a viral capsid is one likely possibility (Smyth 1991). Although retroviral cycles like that described for retroviruses of the HIV type in mammals have not yet been described in plants, future crop research around retroelements and reverse transcription could lead to the discovery of similar processes in plants. In addition, efforts toward understanding how WIS 2-1A is trans-activated could provide a new and powerful molecular experimental tool for cereal geneticists.

Conclusions

Wis 2-1A is a non-active retrotransposon that only under rare circumstances can be trans-activated, representing an ancient DNA element that probably was already present in the common diploid unknown ancestor of the *Triticeae* tribe. In addition, WIS 2-1A does not appear to have preferences for insertion targets, and if it does, those preferences are not strong enough to avoid insertion anywhere else in the genome. The DNA sequence alignment of the WIS reverse transcriptase domains in 10 *Triticeae* species is consistent with the taxonomic information provided by earlier morphological studies, meiotic pairing analyses, isozyme tests, RFLP assays, and DNA sequence alignment (Appels et al. 1989; Monte et al. 1993). Indirect evidence for a possible horizontal transmission was obtained in the genus *Thinopyrum*.

Acknowledgements J. V. Monte expresses gratitude to the Instituto Nacional de Investigaciones Agrarias (Spain) for the financial support that has made this work possible (Monte et al. 1993).

References

Calvi BR, Hibg TJ, Findley SD, Gelbart WM (1991) Evidence for a common evolutionary origin of inverted repeat transposons in *Drosophila* and plants: *hobo*, *activator*, and *Tam3*. Cell 66: 465–471

Clark BC, Moran LB, Appels R (1989) DNA analyses in wheat breeding. Genome 32:334–339

Dewey DR (1984) The genomic system of classification as a guide to intergeneric hybridization within the perennial *Triticeae*. Stadler Genet Symp 16:209–280

Dille' JE, Bittle DC, Ross K, Gustafson JP (1990) Preparing plant chromosomes for scanning electron microscopy. Genome 33: 333–339

Doolittle RF, Feng D-F, Johnson MS, McClure MA (1989) Origins and evolutionary relationships of retroviruses. Quart Rev Biol 64:1–30

Doolittle RF, Feng D-F, Anderson KL, Alberro MR (1990) A naturally occurring horizontal gene transfer from a eukaryote to a prokaryote. J Mol Evol 31:383–388

Finnegan DJ (1985) Transposable elements in eukaryotes. Int Rev Cytol 93:281–320

Gojobori T, Moriyama EN, Kimura M (1990) Molecular clock of viral evolution, and the neutral theory. Proc Natl Acad Sci USA 87: 10015–10018

Grandbastien MA, Spielmann A, Caboche M (1989) *Tnt 1*, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. Nature 337:376–380

Gustafson JP, Dille' JE (1992) Chromosome location of *Oryza sativa* recombination linkage groups. Proc Natl Acad Sci USA 89: 8646–8650

Gustafson JP, Butler E, McIntyre CL (1990) Physical mapping of a low-copy DNA sequence in rye (*Secale cereale* L.). Proc Natl Acad Sci USA 87:1899–1902

Hansen LJ, Chalker DL, Sandmeyer SB (1988) *Ty 3*, a yeast retrotransposon associated with tRNA genes, has homology to animal retroviruses. Mol Cell Biol 8:5245–5256

Harberd NP, Flavell RB, Thompson RD (1987) Identification of a transposon-like insertion in a *Glu-1* allele of wheat. Mol Gen Genet 209:326–332

Hein HH (1990) Unified approach to alignment and phylogenies. Methods Enzymol 183:626–645

Houck MA, Clark JB, Peterson KR, Kidwell MG (1991) Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. Science 253:1065–1180

Huijser P, Kirchhoff C, Lankenau DH, Henning W (1988) Retrotransposon-like sequences are expressed in Y chromosomal lampbrush loops of *Drosophila hydei*. J Mol Biol 203:233–246

Innis MA, Gelfand DH, Sninsky JJ White TJ (1990) PCR protocols In: White TJ (ed) Academic press Inc, San Diego London, pp 1–426

Jakubczak JL, Xiong Y, Eickbush TH (1990) Type-I (R1) and type-II (R2) ribosomal DNA insertions of *Drosophila melanogaster* are retrotransposable elements closely related to those of *Bombyx mori*. J Mol Biol 212:37–52

Katz RA, Skalka AM (1990) Generation of diversity in retroviruses. Annu Rev Genet 24:409–445

Konieczny A, Voytas DF, Cummings MP, Ausubel FM (1991) A superfamily of *Arabidopsis thaliana* retrotransposons. Genetics 127:801–809

Lucas H, Moore G, Murphy G, Flavell RB (1992) Inverted repeats in the long-terminal repeats of the wheat retrotransposon Wis 2-1A. Mol Biol Evol 9:716–728

Maruyama K, Hartl D (1991) Evidence for interspecific transfer of the transposable element *mariner* between *Drosophila* and *Zoprionus*. J Mol Evol 33:514–524

McDonald JF (1990) Macroevolution and retroviral elements. Bioscience 40:183–191

Miyamoto MM, Cracraft J (eds.) (1991) Phylogenetic analysis of DNA sequences. Oxford University Press, New York

Mizrokhi LJ, Mazo AM (1990) Evidence for horizontal transmission of the mobile element jockey between distant *Drosophila* species. Proc Natl Acad Sci USA 87:9216–9220

Monte JV, McIntyre CL, Gustafson JP (1993) Phylogenetic relationships in the *Triticeae* tribe based on RFLPs. Theor Appl Genet 86:649–655

Moore G, Lucas H, Batty N, Flavell RB (1991) A family of retrotransposons and associated genomic variation in wheat. Genomics 10:461–468

Saigo K, Kugimiya W, Matsuo Y, Inouye S, Yoshioka K, Yuki S (1984) Identification of the coding sequence for a reverse transcriptase-like enzyme in a transposable genetic element in *Drosophila melanogaster*. Nature 312:659–661

Sambrook J, Fritsch EJ, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York

Sanderson MJ, Doyle J (1992) Reconstruction of organismal and gene phylogenies from data on multigene families: concerted evolution, homoplasy, and confidence. Systematic Biol 41:4–17

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schwartz-Sommer Z, LeClerq L, Gobel E, Saedler H (1987) *Cin4*, an insert altering the structure of the A1 gene in *Zea mays*, exhibits properties of nonviral retrotransposons. EMBO J 6:3873–3880

Smyth DR (1991) Dispersed repeats in plant genomes. Chromosoma 100:355–359

Smyth DR, Kalitsis P, Joseph JL, Sentry JW (1989) Plant retrotransposons from *Lilium henryi* is related to Ty3 of yeast and the gypsy group of *Drosophila*. Proc Natl Acad Sci USA 86:5015–5019

Voytas DF, Cummings MP, Konieczy A, Ausubel lLFM, Rodermel S (1992) Copia-like retrotransposons are ubiquitous among plants. Proc Natl Acad Sci USA 89:7124–7128

Xiong Y, Eickbush TH (1990) Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J 9: 3353–3362