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Frequencies of Ty1-*copia* and Ty3-*gypsy* retroelements within the *Triticeae* EST databases

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Abstract The frequency of Ty1-*copia*-type and Ty3-*gypsy*-type retrotransposons in the International *Triticeae* EST Consortium (ITEC) database (61,942 sequences: 82% wheat, 10% barley, 8% rye) and the DuPont EST database (86,628 wheat sequences) was estimated using BLASTN searches. These ESTs were obtained from 94 cDNA libraries from different tissues (leaves, roots, spikes, flowers and seeds) and different growing conditions, excluding subtracted and normalized cDNA libraries. *Triticeae* EST databases were screened using four different Ty1-*copia*-type, 12 reverse transcriptase sequences, and three Ty3-*gypsy*-type *Triticeae* retrotransposon sequences. Using a selection threshold of BLASTN scores higher than 100 or *E* values smaller than e^{-20}, 0.145% of the ESTs were found to be significantly similar to at least one of the retrotransposons used in the search (0.064% Ty1-*copia*, 0.081% Ty3-*gypsy*). This percentage increased to 0.176% when the BLASTN threshold was changed to $E<e^{-10}$. The percentage of ESTs similar to retrotransposons was significantly higher ($P < 0.05$) in cDNA libraries from leaf tissues than in cDNA libraries from roots, anthers, or spikes. In addition, the percentage of ESTs similar to retrotransposons in cDNA libraries from plants under stress conditions (0.25% at $E<e^{-20}$, and 0.30% at $E<e^{-10}$) was three to four folds higher ($P < 0.0001$) than in cDNA libraries from plants grown under normal conditions (0.07% at $E<e^{-20}$, and 0.09% at $E<e^{-10}$). Identification of retrotransposons within the *Triticeae* EST databases provides an indirect estimation of the patterns of transcriptional activity of these repetitive elements and is important to improve the annotation of genomic sequences used to search these EST databases.

Keywords EST databases · *Triticeae* · Retrotransposons · *Gypsy* · *Copia*

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Introduction

Expressed **S**equence **T**ags (ESTs) are produced by single sequencing reactions of random cDNAs, generating short DNA sequences (300–400 bp) to be used as tags for a particular cDNA. EST projects in wheat, barley and rye are starting to provide valuable functional information for numerous genes from the *Triticeae* family. Most of the EST sequences are from cDNA libraries (http://wheat.pw.usda.gov/NSF/libraries.html) that had not been normalized or subtracted and, therefore, provide a unique opportunity to perform "electronic Northerns" on different tissues, developmental stages, and environmental conditions. In these electronic Northerns, the relative levels of expression of a particular class of genes can be estimated by counting the number of significant hits found when that gene is compared with all the sequences present in the database using BLASTN searches (Altschul et al. 1997).

The EST databases are also useful tools to identify genes within genomic sequences and to validate the results of gene-prediction programs. Several Bacterial Artificial Chromosome (BAC) libraries are now available for *Triticeae* species (Lijavetzky et al. 1999; Moullet et al. 1999; Liu et al. 2000; Yu et al. 2000; Cenci et al. 2001) and several BAC clones have been sequenced (Panstruga et al. 1998; Shirasu et al. 2000; Dubcovsky et

al. 2001; Lagudah et al. 2001). BLASTN searches of the *Triticeae* EST databases using these sequences detected ESTs with significant similarities to genes present in each of these BACs (Dubcovsky et al. 2001). However, several ESTs were also detected with significant similarity to transposable retroelements present within the BAC sequences.

Retrotransposons are a major class of eukaryotic mobile elements that transpose via an RNA transcript that is reversed transcribed to DNA by a reverse transcriptase coded by the retrotransposon (for a recent review see Kumar and Bennetzen 1999). Ty1-*copia*-type and Ty3-*gypsy*-type retroelements are two abundant classes of retrotransposons in the *Triticeae* genomes (Suoniemi et al. 1996a; Gribbon et al. 1999; Vicient et al. 1999), comprising approximately 35% of the rye genome (Pearce et al. 1997) and a minimum of 20% of the wheat genome (Lagudah et al. 2001).

Most copies of these retroelements are inactive and their representation in the mRNA population is smaller than in the genomic DNA. In maize, for example, the retrotransposons make up 50–80% of the genomic DNA but contribute less than 10% of the mRNA in most tissues (Kumar and Bennetzen 1999). This percentage is similar to those observed in *Drosophila* and yeast. Transcripts homologous to some Ty1-*copia*-type retrotransposons amount to between 0.5 to 3% of the total RNA of *Drosophila* and 5 to 10% of the polyadenylated RNA of yeast (Singer and Berg 1991). Previous studies have shown that some of these retroelements are also transcribed in different *Triticeae* species (Pearce et al. 1997; Suoniemi et al. 1996b) though no quantification of their abundance is available. Translation products of Ty1-*copia*-type retrotransposons have been recently detected immunologically in barley (Jääskeläinen et al. 1999).

RNA transcripts from Ty1-*copia*-type and Ty3-*gypsy*-type retroelements have a polyA tail and, therefore, have the potential to be included in cDNA libraries constructed from these mRNA populations. The proportion of these elements included in the cDNA libraries is expected to be proportional to the abundance of these elements in the RNA population. The accumulation of approximately 150,000 *Triticeae* ESTs from almost 100 different cDNA libraries from different tissues, developmental stages, and environmental conditions provides a unique opportunity to estimate the relative proportion of these retroelements in these cDNA libraries. The aim of this work was to study the representation of different classes of Ty1-*copia*-type and Ty3-*gypsy*-type retrotransposons in these different cDNA libraries as an indirect estimate of their patterns of transcriptional activity. In addition, the annotation of the ESTs with significant similarity to repetitive elements will contribute to the annotation of genomic sequences used to search the *Triticeae* EST databases.

Materials and methods

Database searches were carried out with the BLASTN search tool (Altschul et al. 1997) as implemented on the International Triticeae EST Consortium (ITEC; http://wheat.pw.usda.gov/wEST/blast/) and DuPont proprietary EST database. A BLASTN score of 100 or a E-value of $E<^{-20}$ was used as the first selection threshold (*P*-values and E-value are nearly identical when $E<0.01$). This stringent selection threshold eliminated some ESTs showing certain similarity to retrotransposons, but minimized the number of putative false positives. A second search was performed using a threshold of $E<e^{-10}$ to determine the effect of the reduction of the selection stringency on the proportion of detected retrotransposons.

Ninety four cDNA libraries including 148,570 ESTs from different tissues (leaves, shoot apexes, roots, spikes and spikelets, anthers and pistils, and seeds) and different growing conditions (including biotic and abiotic stresses) were included in this study.

Ty1-*copia*-type ESTs were screened using complete retrotransposon sequences from *Hordeum vulgare* (BARE1, Z17327.1), *Triticum aestivum* (WIS-2-1A, X63184.1) *Triticum monococcum* (WIS-2-1A, AF339051), *Secale cereale* (R1713, X64100.1), and 12 different reverse transcriptase sequences from wheat and barley (D90620.1, D90619, D90628, AJ241105, D90649, D90675, D90662, AJ241106.1, AJ241101, AJ241114, AJ241113, AJ241112; Gribbon et al. 1999). Ty3-*gypsy*-type ESTs were screened using retrotransposon sequences from *H. vulgare* (CEREBA, AF078801.1; and BAGY-1, Y14573) and *T. aestivum* (WHRE1, AB014747.1).

Comparisons of the frequencies of retrotransposons in different tissues and stress conditions were performed using 2×2 contingency tables and the Fisher Exact Test (SAS Institute 1994). This test was preferred over the χ^2 test because of the presence of cells with frequencies equal to zero.

Results and discussion

Since the available *T. aestivum* WIS-2-1A retrotransposon sequence (GenBank accession No. X63184.1) did not include the Long Terminal Repeats (LTRs), a complete WIS-2-1A retrotransposon from *T. monococcum* BAC 60B2 (Lijavetzky et al. 1999) was sequenced, annotated and deposited in GenBank AF339051 (Fig. 1). The genomic sequence includes a complete 8.7-kb WIS-2-1A retrotransposon flanked by two 5 bp (GTTTT) direct repeats from the host DNA (Fig. 1). This retrotransposon includes two 1.8-kb LTRs that are 99% identical, suggesting a recent insertion event. This sequence was added to the other retroelements listed in Materials and methods for the screening of the EST databases.

The ITEC *Triticeae* EST database included 61,942 sequences from 54 non-normalized cDNA libraries when this study was performed (Table 1). Eighty two percent of these sequences were from *Triticum*, 9.5% from *Hordeum*, and 8.5% from *Secale*. ESTs from 23 of these libraries showed significant similarity with at least one of the retrotransposons used in this database screening. When BLASTN searches were performed at the higher stringency level ($E<e^{-20}$), 24 ESTs (0.04%) showed significant similarities with Ty1-*copia*-type and 30 (0.05%) with Ty3-*gypsy*-type retrotransposons, representing in total 0.09% of the ESTs present in the ITEC database (Table 1). This percentage increased to 0.11%

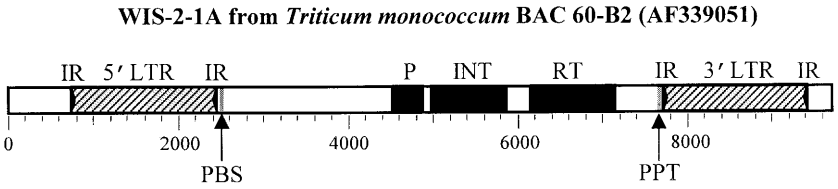

Fig. 1 Genomic sequence from *T. monococcum* BAC 60-B2 including a complete WIS-2-1A Ty1-*copia*-type retrotransposon (AF339051). *LTR* long terminal repeat, *IR* inverted repeats, *P* protease region, *INT* integrase region, *RT* reverse transcriptase region, *PBS* minus-strand cDNA priming site, and *PPT* plus-strand priming site

Table 1 Frequency of Ty1-*copia* and Ty3-*gypsy*-type retrotransposons in the ITEC and DuPont *Triticeae* EST databases.

Item	cDNA libraries from stressed plants				No stress
	Biotic	Abiotic	Biotic+Abiotic	Total	Total
No. of cDNA libraries					
ITEC	4	20	0	24	30
DuPont	7	6	3	16	24
Total	11	26	3	40	54
No. of ESTs					
ITEC	4,607	14,818	0	19,425	42,517
DuPont	27,104	8,336	6,725	42,165	44,463
Total	31,711	23,154	6,725	61,590	86,980
No. of significant hits to Ty1-*copia* at $E<e^{-20}$ ($E<e^{-10}$ values are between brackets)					
ITEC	0 (0)	9 (11)	–	9 (11)	15 (20)
DuPont	36 (47)	16 (19)	7 (7)	59 (73)	12 (18)
Total	36 (47)	25 (33)	7 (7)	68 (84)	27 (38)
No. of significant hits to Ty3-*gypsy*- at $E<e^{-20}$ ($E<e^{-10}$ values are between brackets)					
ITEC	4 (6)	16 (17)	–	20 (23)	10 (14)
DuPont	44 (50)	11 (11)	14 (14)	69 (75)	22 (28)
Total	48 (56)	27 (29)	14 (14)	89 (98)	32 (42)
Percentage of ESTs significantly similar to any retroelement at $E<e^{-20}$ ($E<e^{-10}$)					
ITEC	0.09 (0.13)	0.17 (0.19)	–	0.15 (0.16)	0.06 (0.08)
DuPont	0.29 (0.36)	0.32 (0.36)	0.31 (0.31)	0.30 (0.35)	0.08 (0.11)
Total	0.26 (0.32)	0.22 (0.27)	0.31 (0.31)	0.25 (0.30)	0.07 (0.09)

when the BLASTN selection threshold was changed to $E<e^{-10}$.

The DuPont EST database included 86,628 sequences from 40 non-normalized wheat cDNA libraries (Table 1). At the $E<e^{-20}$ threshold, 162 (0.19%) ESTs from 15 cDNA libraries showed significant similarity with the retrotransposons used in the database screening. Seventy-one of these sequences (0.08%) showed significant similarities to Ty1-*copia*-type retrotransposons and 91 (0.11%) with Ty3-*gypsy*-type retrotransposons (Table 1). The total percentage in the DuPont EST database increased from 0.19% to 0.22% when the BLASTN selection threshold was changed from $E<e^{-20}$ to $E<^{-10}$.

Results from both databases suggest that a minimum of 0.15% of the approximately 150,000 ESTs present in the *Triticeae* databases is represented by retrotransposons from these two groups (Table 1). This percentage increased to 0.18% when the selection threshold was changed from $E<e^{-20}$ to $E<e^{-10}$.

These two groups of retroelements are represented by highly heterogeneous populations of mRNAs (Gribbon et al. 1999) and some of them may not show significant similarities to the retrotransposons used for the database screening at the selected thresholds. Therefore, the 0.18% estimate of Ty1-*copia*-type and Ty3-*gypsy*-type retrotransposons in the EST databases should be considered as a minimum estimate of the actual proportion of these retrotransposons within the mRNA population.

These ESTs showed similarities to different regions of the retrotransposon sequences and most of them showed significant alignment with most of the good quality EST sequence (80 to 100%). Two ESTs from the ITEC database and four from the DuPont database showed significant similarity with the reverse transcriptase (RT) portion of the Ty1-*copia*-type retrotransposons ($E<e^{-10}$) allowing a more precise classification of the selected ESTs. Comparison of this sequence with the different families of RTs (Gribbon et al. 1999) showed that these

Table 2 Comparison of the total frequency of retrotransposons in cDNA libraries from different tissues and between the relative frequencies of Ty1-*copia* and Ty3-*gypsy*-type retrotransposons in the same tissues (BLASTN threshold = $E<e^{-20}$). Tissues with different letters at the Fisher Exact Test are significantly different with $P < 0.05$. NS = Non significant.

cDNA library tissue	Number of ESTs	Ty1-*copia*	Ty3-*gypsy*	Total %	Fisher Exact Test	
					Ty1 vs Ty3	Between tissues
Leaves	8,175	10	8	0.22%	$P = 0.81$ NS	A
Shoot apex	1,777	1	0	0.06%	$P = 1.00$ NS	ABC
Roots	29,136	8	12	0.07%	$P = 0.50$ NS	B
Grain tissues	26,680	7	11	0.07%	$P = 0.48$ NS	B
Spike and spikelet	9,381	1	1	0.02%	$P = 1.00$ NS	BC
Anthers and pistils	6,465	0	0	0.00%	$P = 1.00$ NS	C

ESTs were most-closely related to the BARE-1 subgroup of the Ty1-*copia*-type retrotransposons.

Many of the plant retrotransposons studied to-date have shown different levels of transcription under stress conditions. Therefore, the cDNA libraries included in this study were divided into those developed from plants under biotic stress (virus or fungal infection), abiotic stress (cold, heat, drought, tissue culture, wounding and chemical agents), simultaneous biotic and abiotic stress, or no stress (including libraries from etiolated seedlings) (Table 1). In both databases, the proportion of ESTs similar to the selected retrotransposons was significantly higher (Fisher Exact Test $P < 0.001$) in cDNA libraries from stressed plants (0.25% at $E<e^{-20}$, and 0.30% at $E<e^{-10}$) than in cDNA libraries from normal plants (0.07% at $E<e^{-20}$, and 0.09% at $E<^{-10}$). These results suggest that stress conditions may produce approximately a 3–4-fold increase in the proportion of retrotransposons in the RNA population.

The cDNA libraries from non-stressed plants from both databases were further divided according to the different tissues used to generate the libraries (81,614 ESTs, Table 2). ESTs from cDNA libraries constructed from complete plants (5,366 ESTs) were not included in this study. ESTs significantly similar ($E<e^{-20}$) to the selected retroelements were detected in cDNA libraries from leaves (0.22%), roots (0.07%), different grain tissues (0.07%), shoot apical region (0.06%), spikes and spikelets (0.02%), and were not detected in cDNA libraries from anthers and pistils (Table 2). The frequency of retrotransposons in the cDNA libraries from leaves was significantly higher than the frequency of these elements in the cDNA libraries from all other tissues (Fisher Exact Test $P < 0.001$) with the exception of the one from the shoot apex region (Fisher Exact Test $P = 0.12$). The frequency of retrotransposons in the cDNA libraries from anthers and pistils was significantly lower than the frequency of these elements in the cDNA libraries from leaves, roots, and grain tissues (Table 2). Lack of significant differences between the shoot apex region and all other tissues may be related to the small number of sequences available from the shoot apex cDNA library at the time of the screening (1,777 ESTs).

These results suggest a tissue-specific regulation of the transcription of Ty1-*copia* and Ty3-*gypsy*-type retrotransposons in the *Triticeae*. In all tissues analyzed, the frequency of Ty1-*copia* retrotransposons was not significantly different from the frequency of Ty3-*gypsy*-type retrotransposons suggesting a similar pattern of activation (Table 2). The large proportion of ESTs similar to Ty1-*copia*-type in the leaves is consistent with experimental evidence on the presence of BARE-1 transcripts in leaves using RNase protection assays (Suoniemi et al. 1996b).

The different proportions of Ty1-*copia* and Ty3-*gypsy*-type retrotransposons in different tissues and at different stress conditions are responsible for the higher percentage of ESTs with significant similarities to retrotransposons observed in the DuPont database (0.19%) relative to the ITEC database (0.09%). Fifty percent of the ESTs from the DuPont database are from cDNA libraries from stressed plants, whereas only 31% of the ITEC ESTs are from stressed plants. Moreover, 33% of the ESTs from the non-stressed plants in the ITEC database are from libraries with low proportions of retrotransposons (spikes, spikelets, anthers, pistils, and shoot apexes), whereas only 8% of the ESTs from non-stressed plants in the DuPont database are from cDNA libraries from these tissues.

Determination of the level of transcription of different retrotransposons in different tissues and environmental conditions is important because transposition of these retroelements seems to be regulated mainly at the transcriptional level. A correlation between transcription and transposition has been demonstrated for the tobacco *Tto1* and rice *Tos17* Ty1-*copia*-type retrotransposons (Hirochika 1993; Hirochika et al. 1996). Therefore, control mechanisms of the transcription may be crucial to minimize deleterious effects of retrotransposon transposition on the host.

Identification of ESTs similar to retrotransposons in cDNA libraries from specific tissues or under specific stress conditions may provide a strategy to identify new regulatory sequences in these retrotransposons. Transcription of plant retrotransposons is controlled by *cis*-acting regulatory elements located within the U3 region of the 5′LTR (for a review see Kumar and Bennetzen 1999). Comparative sequence analysis of selected ESTs from different *Triticeae* species activated under similar conditions could be used to identify the conserved regulatory elements. For example, the cDNA library from peeled leaf epidermis (barley cultivar Franka, developed

by K. Feuillet and B. Keller, ITEC HV Franka, SFR) showed an unusually high proportion of ESTs with significant similarity to the barley CEREBA retrotransposon (0.6%). This result suggests that these elements may carry some regulatory sequence to rapidly induce transcription after wounding as reported for other retrotransposons (Takeda et al. 1999).

It should be pointed out that the presence of a particular retrotransposon in the EST database does not necessarily demonstrate that it is transcriptionally active. Retrotransposons initiated in the promoters of adjacent genes, and genomic DNA contamination during the construction of the cDNA libraries, can result in the inclusion of inactive retrotransposons within the EST database. To test this possibility, we compared the orientation of the ESTs from directional cDNA libraries with the annotated BARE-I and WIS-2-A1 retrotransposons. If the ESTs were all products of transcription, a large proportion of them should be in the sense orientation (a small percentage of the clones of directional libraries are still cloned in reverted orientation), and if they were the product of genomic contamination, both orientations should be equally frequent. From the ten ESTs from directional cDNA libraries showing significant similarity to the Ty1-*copia*-type, seven were in the sense-orientation and three in the antisense-orientation. One of the ESTs in antisense-orientation matched perfectly the 3′ LTR and started close to the polyadenylation signal suggesting that it was cloned in reverted orientation. The second one was also similar to the 3′ LTR but far from the polyadenylation signal, and the last one was similar to the polyprotein region. Therefore, 80% of these ESTs are putative products of translation.

In addition, the correlation between the EST frequencies observed in the screening of the Ty1-*copia*-type and Ty3-*gypsy*-type retrotransposons (Table 2) suggests that these frequencies reflect particular transcriptional profiles of the retrotransposons screened in this study. The increased frequency of ESTs similar to retrotransposons in cDNA libraries from stressed plants also parallels similar results based on experimental evidence (Kumar and Bennetzen 1999; Vicient et al. 1999) and suggests that the observed frequencies are the result of a particular transcription pattern rather than an artifact produced by genomic contamination of the cDNA libraries.

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