Genetics and Molecular Biology, 28, 3 (suppl), 634-639 (2005)
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Short Communication

Identification and frequency of transposable elements in *Eucalyptus*

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Abstract

Transposable elements (TE) are major components of eukaryotic genomes and involved in cell regulation and organism evolution. We have analyzed 123,889 expressed sequence tags of the *Eucalyptus* Genome Project database and found 124 sequences representing 76 TE in 9 groups, of which *copia*, *MuDR* and *FAR1* groups were the most abundant. The low amount of sequences of TE may reflect the high efficiency of repression of these elements, a process that is called TE silencing. Frequency of groups of TE in *Eucalyptus* libraries which were prepared with different tissues or physiologic conditions from seedlings or adult plants indicated that developing plants experience the expression of a much wider spectrum of TE groups than that seen in adult plants. These are preliminary results that identify the most relevant TE groups involved with *Eucalyptus* development, which is important for industrial wood production.

Key words: Eucalyptus, transposable element.

Received: May 28, 2004; Accepted: May 30, 2005.

Transposable elements (TE) are present in most of the eukaryotic cells. They represent up to 40% of the human genome size (Yoder *et al.,* 1997; Smit, 1999) and 50-90% of the genome size of important agricultural plants, such as maize, wheat and barley (Flavel, 1986; SanMiguel *et al.*, 1996; Shirasu *et al.*, 2000). They are tightly related to chromosome structure and evolution and thus, ultimately, to organism evolution (McDonald, 1995; Britten, 1996; Kidwell and Lisch, 1997; Fedoroff, 2000).

Based on the mode by which TE can move from one location to another in the genome, they are divided into two classes. Class I TE are transcribed to RNA intermediates, reverse-transcribed and integrated in a new genome site. Because of this activity, members of this class are also called retroelements, which are further subdivided into retrotransposons (*e.g. copia*-like and *gypsy*-like) that have long terminal repeats (LTRs), as well as the so-called non-LTR retroelements (*e.g.* long (*LINE*) and short (*SINE*) interspersed nuclear elements). Class II TE are those presenting terminal inverted repeats (TIR) and capable of moving from one site to another in the genome through a 'copy-and-paste' process that involves the action of transposases, which interact with TIR (Berg and Howe, 1989). Class II is further subdivided accordingly to TE structure and sequence or features of the target duplication site generated upon insertion (Capy *et al.*, 1996). Examples of groups from Class II are *CACTA*, which is flanked by inverted repeats that terminate in a conserved CACTA motif (Wicker *et al.*, 2003); *MuDR*, which codes for a transposase (Benito and Walbot, 1997) or other genes (Lisch *et al.*, 1999) whose function remains unknown and control the expression of TE in the *MU* system, which is the most active and mutagen transposable element described in plants (Rossi *et al.*, 2004); *hAT*, which is widespread among fungi, plants and animals (Rubin *et al.*, 2001); and *IS* (insertion sequence), which is commonly found in bacteria (Schnetz and Rak, 1995).

Knowledge of TE has advanced significantly with the sequencing of many genomes, which has led to the accumulation of a large number of sequences and resulted in the

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discovery of new TE such as those in the *Jittery* group, which are homologous to *FAR1* and *FHY3* genes (*FAR1* family) and present regulatory functions (Hudson *et al.*, 2003). Regulatory function of genes in the *FAR1* family is proposed to involve a mechanism which is similar to that occurring in the linkage of transposase to the TIR of a Class II TE in the *MuDR* group (Hudson *et al.*, 2003). Therefore, many of the genes which are currently classified in distinct groups of TE are likely to be involved in cell regulatory processes, so that by following TE expression patterns in different physiologic conditions one could identify putative regulatory genes.

In the present investigation, we have searched for TE in different tissues and physiological conditions in *Eucalyptus*, which is an important source of wood for industrial purposes. To accomplish that, we identified TE in libraries of the *Eucalyptus* Transcriptome Project (FORESTS, Table 1) through the utilization of a keyword search in the FORESTS database, which was carried out with keywords 'transposon,' 'transposase' or names of each group of TE. Only the retrieved EST (EST = expressed sequence tags) showing e-values ≤ 10^{-5} were considered. An additional search for sequences of TE in the FORESTS database was done through a blastn (Basic Local Alignment Search Tool, nucleotide-nucleotide) utilizing the query sequences from the GenBank, representing every group of TE. Then, sequences of TE that were retrieved from the FORESTS databank were classified based on their similarity to previously described sequences in the GenBank. To achieve that, each *Eucalyptus* transposable element sequence was utilized as a query in a blastx (translated queries against the GenBank protein database) analysis and protein sequences showing e-values ≤ 10^{-5} and scores ≥ 80 were considered.

This procedure for identification and classification of TE resulted in 124 EST in 16 libraries of the FORESTS database, and these EST were grouped in 76 clusters (Table 2). Many of these clusters (57) were found as singletons, which may correspond to rarely expressed genes, although it is likely that some of these singletons represented different regions of a single gene, considering the high gene size of TE (up to 9 kb (Capy *et al.*, 1996)) compared to sizes of EST that were generated in the FORESTS project (~ 800 bp). The remaining 19 identified clusters were composed of 2 to 9 EST each (Table 2), and 16 of these clusters were composed of EST from more than one library, which indicates that some of the identified TE are expressed in more than a single plant tissue.

Most of the identified TE were in Class I (retroelements) and belonged to the *FAR1* (29.8%) or *copia* (22.6%) groups while other retroelements, such as *LINE* and *gypsy*, were poorly represented (4.0 and 2.4%, respectively). Within the Class II TE, *MuDR* (16.9%) and *hAT* (12.1%) groups prevailed, followed by *CACTA* (4.8%), non-classified LTR (4.0%) and *IS* (3.2%) groups (Table 2). These results are in agreement with the high amount of expressed *MuDR* found in sugarcane (Rossi *et al.*, 2001).

Table 1 - EST libraries from *Eucalyptus*.

Library	Source	Species	EST*
BK1	Bark, heartwood, softwood and medulla of an eight-year old tree	*E. grandis*	1,052
CL1	Calli formed in the dark	*E. grandis*	9,998
CL2	Calli formed in the presence of light	*E. grandis*	2,535
FB1	Inflorescence and fruit	*E. grandis*	12,275
LV1	Leaf from seedlings	*E. grandis*	2,048
LV2	Leaf from disease-sensitive, phosphate and borate deficient tree	*E. grandis*	7,532
LV3	Leaf colonized by *Thyrinteina*	*E. grandis*	4,341
RT3	Root of seedlings	*E. grandis*	13,252
RT6	Root of frost-resistant or susceptible trees	*E. grandis*	6,877
SL1	Seedlings cultivated in the dark and exposed to light	*E. grandis*	6,182
SL4	Seedlings cultivated in the dark	*E. globulus*	6,718
SL5	Seedlings cultivated in the dark	*E. saligna*	7,165
SL6	Seedlings cultivated in the dark	*E. urophylla*	1,217
SL7	Seedlings cultivated in the dark	*E. grandis*	4,120
SL8	Seedlings cultivated in the dark	*E. calmadulensis*	2,035
ST2	Stalk of seedlings susceptible to water deficit (0.6-2.0 kb insert)	*E. grandis*	11,032
ST6	Stalk of seedlings susceptible to water deficit (0.8-3.0 kb insert)	*E. grandis*	12,558
ST7	Stalk of frost-resistant or susceptible tress	*E. grandis*	2,728
WD2	Wood	*E. grandis*	10,224

*Number of sequenced EST in each library.

Table 2 - Number of TE EST in different libraries of *Eucalyptus* and their corresponding clusters.

TE and cluster*		Library / Class I																Total
		BK1	CL1	CL2	FB1	LV2	LV3	RT3	RT6	SL1	SL4	SL5	SL7	SL8	ST2	ST6	WD2	
copia	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2
	3	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
	4	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
	5	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	9	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	10	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	2
	11	-	-	-	3	3	-	1	-	-	-	-	2	-	-	-	-	9
	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
	13	-	-	-	2	-	-	-	-	-	-	-	-	2	-		1	5
Subtotal		0	0	1	6	3	0	3	1	2	0	0	3	2	3	1	3	28
gypsy	14	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	15	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	16	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Subtotal		0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	3
LINE	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	19	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	21	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Subtotal		0	0	0	0	0	0	1	0	0	0	0	0	0	2	1	1	5
nc^{+}	22	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
	23	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	24	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	26	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Subtotal		0	0	0	0	0	0	1	0	0	0	2	1	0	0	1	0	5
MuDR	27	-	-	-	-	-	-	1	-	-	-	1	-	-	1	1	-	4
	28	-	4	-	-	-	-	-	-	-	2	-	-	-	-	-	1	7
	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	31	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	33	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
	34	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
	35	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	36	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	2
	37	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Subtotal		0	5	0	1	0	0	2	1	0	4	1	0	0	1	4	2	21
FAR1	38	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	3
	39	-	-	-	-	-	-	3	-	-	-	-	-	-	-	1	-	4
	40	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1

Table 2 (cont.)

TE and cluster*		Library / Class I																Total
		BK1	CL1	CL2	FB1	LV2	LV3	RT3	RT6	SL1	SL4	SL5	SL7	SL8	ST2	ST6	WD2	
	41	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	2
	42	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	43	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	44	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
	45	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	47	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
	48	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	49	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	51	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	52	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	53	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
	54	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	55	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	56	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	57	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	58	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	59	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
	60	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2
	61	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	2
	62	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	3
	63	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	2
Subtotal		2	1	0	3	5	0	11	1	2	1	2	2	1	3	3	0	37
hAT	64	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	66	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
	67	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	3
	68	-	4	-	-	-	-	1	-	-	-	-	-	-	-	-	-	5
	69	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	70	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
	71	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
	72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Subtotal		0	6	0	0	0	1	2	0	0	3	0	1	0	0	1	1	15
IS	73	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	1	3
	74	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Subtotal		0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	1	4
CACTA	75	-	1	-	-	2	-	-	-	-	-	-	-	1	-	-	1	5
	76	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Subtotal		0	1	0	1	2	0	0	0	0	0	0	0	1	0	0	1	6
Total		2	13	1	12	12	1	22	3	4	8	5	8	4	9	11	9	124

*Clusters are divided in groups of TE. Cluster numbers are correlated to FORESTS codes as described in http://omega.rc.unesp.br/transposable/tabela.php.

$^+$non-classified LTR

However, to our knowledge, the presence of TE in the *IS* group has never been reported in plants. Elements of this group are quite common in bacteria, where they act as enhancers (*e.g.* Schnetz and Rak, 1995). It remains to be seen whether elements in the *IS* group, which were found in libraries from roots and leaves, have a regulatory function in *Eucalyptus*.

In order to better compare the relative amounts of distinct groups of TE in *Eucalyptus*, we have calculated the TE frequency F by the equation F = (n / N) 10^3, where n is the number of EST in a given group of TE in a given library (values in Table 2) and N is the number of sequenced EST in this library (*i.e.* each of the 19 values in Table 1). The frequency FG was calculated for groups of TE, according to the equation FG = ΣF_G, where F_G represents each of the F values within a given group of TE. FG values indicated *FAR1* as the most frequent group, followed by *copia*, *MuDr*, *hAT* and *CACTA* groups, non-classified LTR and *gypsy*, *LINE* and *IS* groups (Figure 1).

To compare the relative amounts of TE in different libraries, we have calculated FL, using FL = ΣF_L, F_L representing each of the F values within a given library. Our results are shown in Figure 2, which also shows the relative contribution of each of the groups of TE to FL values. These values vary from zero to 1.96, with a mean value of 0.92 (calculated for the FL values represented in Figure 2 plus the zero FL value of LV1, SL6 and ST7 libraries). This finding indicates that FORESTS libraries contained, on average, close to 1 transposable element per 1,000 EST, suggesting that a comparable expression rate may occur in *Eucalyptus*. This average value is very low, considering that TE usually represent 50-90% of plant genomes (Flavel, 1986; SanMiguel *et al.*, 1996). This finding indicates that only a small fraction of TE that can be expressed in *Eucalyptus* is efficiently transcribed. Inhibition of expression of TE has been called silencing (Okamoto and Hirochika, 2001) and found to be widespread within many organisms such as maize (Fedoroff and Chandler, 1994; Rudenko *et al.*, 2003), *Arabdopsis* (Hirochika *et al.*, 2000; Steimer *et al.*, 2000) or *Drosophila melanogaster* (Jensen *et al.*, 1999; Malinsky *et al.*, 2000).

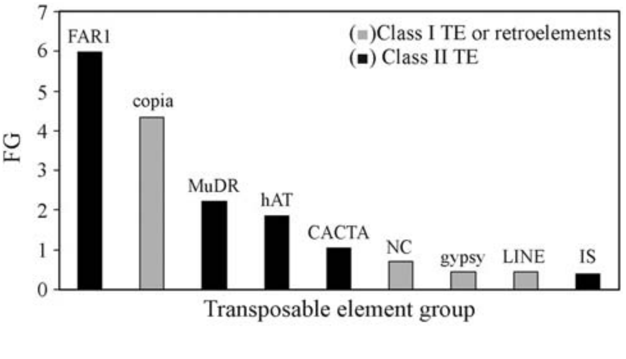

Figure 1 - Frequency of different groups of TE in *Eucalyptus* (FG). Values in Table 2 were converted to frequency values within each of the groups of TE as described in the text. nc: non-classified LTR.

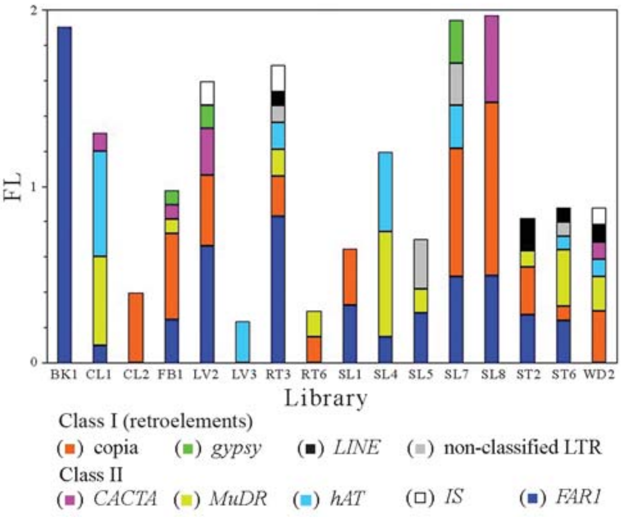

Figure 2 - Frequency of TE in different *Eucalyptus* libraries (FL). Values in Table 2 were converted to frequency values within each of the libraries as described in the text. Libraries LV1, SL6 and ST7 did not contain TE and therefore were not represented. Individual contribution of each of the groups of TE for the FL values are represented by different colors.

It is unclear whether the calculated frequencies are related to the expression levels of TE; however, the FL values that we found are indicative of a highly variable expression pattern in distinct *Eucalyptus* tissues or even within single tissues submitted to distinct physiological conditions. For instance, CL1 and CL2 libraries, which were obtained from *E. grandis* calli, in the dark or in the presence of light, respectively, presented a dramatic difference in FL. CL1 presented a high FL value and contained four different groups of TE (especially *MuDR* and *hAT*, yet small amounts of *FAR1* and *CACTA*) and CL2 showed a low FL value and contained TE only in the *copia* group.

In addition, libraries from seedlings (SL1, SL4, SL5, SL7 and SL8) contained almost all groups of *Eucalyptus* TE identified in our investigation, except members of *LINE* and *IS* groups. This pattern strongly contrasts that found in the BK1 library, which was obtained from eight-year-old trees and contained only *FAR1* representatives. Similarly, we found a greater variety and frequency of groups of TE in the RT3 library (which was made from seedlings and contained almost all groups of TE that were identified in *Eucalyptus*, except *CACTA* and *gypsy)* than those found in the RT6 library (which was made from trees and contained only small amounts of *copia* and *MuDr* representatives). Finally, libraries from seedling stalk (ST2 and ST6) also had a great variety of groups of TE, although no transposable element was detected in any library from adult tree stalk (ST7). Taken together, these findings suggest that developing plants experience the expression of a much wider spectrum of TE than that seen in adult plants.

Our current mining efforts have identified the clusters and by consequence the FORESTS clones which contain genes of TE that may be differentially expressed in distinct tissues or physiological conditions in *Eucalyptus*. Starting from this preliminary information, further studies on the expression of these genes can be carried out in order to identify the most relevant TE involved in plant development, which is important for wood production on *Eucalyptus* plantations.

Acknowledgements

We would like to thank Centro Virtual Omega / PROPE-UNESP for financing the computers utilized in this annotation work. We would also like to thank the FORESTS Consortium, which provided us with access to its database.

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Associate Editor: Marie Anne Van Sluys