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Research Article

Evolutionary history of the Tip100 transposon in the genus *Ipomoea*

Ana-Paula Christoff[1], Elgion L.S. Loreto[2] and Lenira M.N. Sepel[2]

[1]*Curso de Ciências Biológicas, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.*
[2]*Departamento de Biologia, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.*

Abstract

Tip100 is an Ac-like transposable element that belongs to the *hAT* superfamily. First discovered in *Ipomoea purpurea* (common morning glory), it was classified as an autonomous element capable of movement within the genome. As *Tip100* data were already available in databases, the sequences of related elements in ten additional species of *Ipomoea* and five commercial varieties were isolated and analyzed. Evolutionary analysis based on sequence diversity in nuclear ribosomal Internal Transcribed Spacers (ITS), was also applied to compare the evolution of these elements with that of *Tip100* in the *Ipomoea* genus. *Tip100* sequences were found in *I. purpurea, I. nil, I. indica* and *I. alba*, all of which showed high levels of similarity. The results of phylogenetic analysis of transposon sequences were congruent with the phylogenetic topology obtained for ITS sequences, thereby demonstrating that *Tip100* is restricted to a particular group of species within *Ipomoea*. We hypothesize that *Tip100* was probably acquired from a common ancestor and has been transmitted vertically within this genus.

Key words: hAT, transposable elements, Ac-Ds, *Ipomoea*, genome evolution, ITS.

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Introduction

Transposable elements (TE), which are also referred to as "jumping genes", due to their ability to move around inside the genome, are important sources of genetic variability that have contributed to genome evolution (Biémont and Vieira, 2006; Slotkin and Martienssen, 2007; Naito *et al.*, 2009; Blumenstiel, 2010). Through their being extremely variable in sequence, molecular organization and replication mechanisms, these characteristics have been used to classify TEs in a hierarchical manner (Wicker *et al.*, 2007). Some transposable elements can also be domesticated by their host genomes, thereby contributing to important processes in the organism (Knon *el al.,* 2009).

The transposable element *Tip100* was initially identified in *Ipomoea purpurea* by Habu *et al.* (1998). It is a class II transposable element that moves through a DNA intermediary, and is classified in the order TIR and the superfamily *hAT* (Wicker *et al.*, 2007). It possesses 11 bp-long TIRs (terminal inverted repeats), produces 8 bp target site duplications (TSDs) as co-products of mobilization, and has a conserved *hATC* (hAT family dimerization domain) protein domain in the transposase, all these being characteristic features of the *hAT* superfamily (Kempken and Windhofer, 2001; Rubin *et al.*, 2001; Arensburger *et al.*, 2011).

Tip100 is an autonomous, freely moving element in *I. purpurea* (Ishikawa *et al.*, 2002), to which has been attributed the color variegation patterns observed in flowers of some strains. Habu *et al.* (1998) demonstrated that this TE is inserted into either the 5' regulatory region, or the intron of the *Chalcone Synthase D* gene (*CHS-D*). Its presence in this gene, which encodes the enzyme responsible for the first step of anthocyanin production, can induce modification of colors in flowers. Recurrent somatic excision of *Tip100* in the *CHS-D* gene can generate the variegated patterns observed in some *I. purpurea* plants (Iida *et al.*, 2004). Likewise, many other transposable elements are capable of affecting the genes of the anthocyanin pathway (Park *et al.*, 2004).

The genus *Ipomoea* is a member of the Convolvulaceae, one of the large families of Solanales. It includes numerous species that are mainly distributed in the Americas (Austin and Huáman, 1996; Austin and Bianchini, 1998). Some are simply weeds, whereas others are economically important, viz., sweet potatoes and ornamental plants, such as the morning glories *I. purpurea* and *I. nil.* Plants of this genus are appropriate biological models for research, through presenting exceptional morphological

Send correspondence to Lenira M.N. Sepel. Departamento de Biologia, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Caixa Postal 5050, Agência Campus, Camobi, 97105-900 Santa Maria, RS, Brazil. E-mail: lenirasepel@gmail.com.

and habitat-use diversity, whereby their extensive experimental versatility (Stefanovi *et al.*, 2003; Clegg and Durbin, 2003).

In plants, nuclear ribosomal internal transcribed spacers (ITS) comprise one of the most useful sequences for phylogenetic studies at the species level (Feliner and Rosselló, 2007). Results from previous studies on *Ipomoea* using ITS sequences (Miller *et al.*, 1999, 2004; Manos *et al.*, 2001), are congruent with those based on morphological characteristics (McDonald and Mabry, 1992; Austin and Huáman, 1996; Austin and Bianchini, 1998).

In the present study, *Tip100*-related elements in ten *Ipomoea* species and five commercial cultivars were investigated. These species are representative of the three subgenera of *Ipomoea*, namely *Eriospermum, Ipomoea* and *Quamoclit* (Austin, 1975; Austin and Huáman, 1996; Austin and Bianchini, 1998). Our aim was to shed light on how the *Tip100* transposable element is distributed among *Ipomoea* species, and how it may have evolved, by comparing the phylogenetic relationships of TE sequences with the host-species phylogeny.

Materials and Methods

DNA extraction

DNA was extracted from 0.1 g of germinated plant-leaf tissue, according to the protocol described by Oliveira *et al.* (2009). The species examined in this study and their origins are shown in Table 1.

PCR cloning and sequencing of *Tip100* sequences

Primers, designed with Oligo 4.1 software (Rychlik, 1992) were based on the *Tip100* sequence from *I. purpurea* (Habu *et al.*, 1998). The forward primer (5'-CGTTCTCC TTTTGTTGGTGT-3') anneals in the putative regulatory region of the element at positions 621-640 and the reverse primer (5'-GCTTCTCAATGGGGCACTTC-3') does so in the first region of the transposase ORF at positions 1526-1545. A non-coding sequence region was chosen, as this part is expected to be more variable, and so, phylogenetically more informative. PCR assays were performed in 10 μL volumes with 20 ng of genomic DNA, 0.2 U Taq DNA polymerase (Invitrogen), 1X Reaction Buffer, 1.5 mM of $MgCl_2$ and 200 pmol of each primer. The following thermocycler amplification process was used: 94 °C for 5 min, 30 cycles at 94 °C for 45 s, 55 °C for 30 s and 72 °C for 60 s, followed by a final extension cycle at 72 °C for 7 min. The amplified fragments were cloned using the TA Cloning Kit pCR 2.1 Vector (Invitrogen). Plasmid DNA was isolated by miniprep alkaline lysis (Sambrook and Russel, 2001), and then precipitated with 13% PEG and 1.6 M NaCl. 35 plasmids from all the species and varieties were selected, for direct sequencing of the two strands in a MegaBACE 500 automatic sequencer. The dideoxy chain-termination reaction was implemented with the DYEnamic ET kit (GE Healthcare). To obtain sequences for each clone, reads, were assembled using Gap4 software from the Staden Package (Staden, 1996), with assembly continuing until a confidence value higher than 30 was obtained. The *Tip100* sequence described by Habu *et al.* (1998) (GenBank AB004906) was also included in the analysis. All the new sequences obtained in this study were deposited in GenBank (Accession No: HM014415-HM014422).

Table 1 - *Ipomoea* species and localities, as well as PCR results for the transposon *Tip100* and Internal Transcribed Spacers (ITS).

Species	Origin of samples	Amplification	
		Tip100	ITS
Ipomoea alba	Lat 29° 42' S Long 53° 42' W	+	+
Ipomoea batatas	Lat 29° 42' S Long 53° 42' W	-	+
Ipomoea cairica	Lat 29° 42' S Long 53° 42' W	-	+
Ipomoea carnea	Lat 29° 42' S Long 53° 42' W	-	+
Ipomoea indica	Lat 29° 42' S Long 53° 42' W	+	+
Ipomoea coccinea	Lat 29° 42' S Long 53° 42' W	-	+
Ipomoea nil	Lat 29° 42' S Long 53° 42' W	+	+
Ipomoea purpurea	Lat 29° 42' S Long 53° 42' W	+	+
Ipomoea triloba	Lat 29° 42' S Long 53° 42' W	-	+
Ipomoea quamoclit	Lat 29° 42' S Long 53° 42' W	-	+
Varieties			
Ipomoea nil 'Candy Pink'	Seeds of Thompson & Morgan (Group) Ltd	+	+
Ipomoea purpurea 'Kniolas Black Knight'	Seeds of Thompson & Morgan (Group) Ltd	+	+
Ipomoea purpurea 'Light Blue Star'	Seeds of Thompson & Morgan (Group) Ltd	+	+
Ipomoea purpurea 'Split Personality'	Seeds of Thompson & Morgan (Group) Ltd	+	+
Ipomoea X Slotari	Seeds of Thompson & Morgan (Group) Ltd	-	+

(+) positive amplification, (-) negative amplification.

Analysis of transposon sequences

The identity of the cloned sequences was determined by Blast searches (Altschul *et al.*, 1990) in the NCBI and RepBase databases. Nucleotide sequences were aligned using Clustal W (Thompson *et al.*, 1994), with default parameters. Cons software (Rice *et al.*, 2000) was used to obtain consensus sequences of clones that presented divergences of less than 8.5%, and belonged to the same species or variety. Mega 4 software (Tamura *et al.*, 2007) was used to obtain divergences for sequences with Tamura 3 parameters.

Phylogenetic analysis using Bayesian criteria was done in MrBayes 3.1.2 software (Huelsenbeck and Ronquist, 2001). The HKY evolutionary model was chosen in the MrModelTest 2.2 software (Nylander, 2004) implemented in PAUP 4.0b10 (Swofford 2003), and using the Akaike (AIC) criterion (Akaike 1974). Two independent runs of four heated Monte Carlo Markov chains (MCMC)

were carried out, each for 1,000,000 generations. Results were saved every 100 generations.

PCR and sequencing of internal transcribed spacers (ITS)

The primers used to amplify ITS sequences, viz., ITS92 (5'-AAGGTTTCCGTAGGTGAAC-3') and ITS75 (5'-TATGCTTAAACTCAGCGGG-3'), had already been described by Baldwin (1992). The amplified region corresponded to the two internal spacers (ITS1 and ITS2), as well as the complete 5.8S ribosomal gene region between these. PCR conditions were similar to those used for the *Tip100* PCR runs, except for the temperature cycles which were as follows: 94 °C for 5 min, 35 cycles at 94 °C for 40 s, 55 °C for 30 s and 72 °C for 80 s, followed by a final cycle of 72 °C for 7 min. The resultant PCR fragments were purified with 13% PEG and 1.6 M NaCl, and directly sequenced in a MegaBACE 500 automatic sequencer. The dideoxy chain-termination reaction was carried out with a DYEnamic ET kit (GE Healthcare). An ITS sequence for *Merremia tuberosa* (AF110909), obtained from GenBank, was used as outgroup during analysis. The newly obtained sequences were deposited in GenBank (Accession No: HM14423-HM14437).

Analysis of ITS sequences

ITS sequence-processing was the same as that for *Tip100* sequences, except that sequence-distance calculations were performed using a Tamura Nei model in Mega 4 (Tamura *et al.*, 2007), and Bayesian analysis with a GTR+G model.

Results

The *Tip100* transposon

The molecular investigation of *Tip100* homologous sequences in ten different *Ipomoea* species and five *Ipomoea* commercial varieties, lead to identification of the transposon in four species and four varieties, through positive PCR amplification of the expected 900 bp fragment (Table 1).

Sequence analysis showed the different cloned elements to be very similar, with levels of divergence varying from 0.0% to 2.8% (Table 2). The only exception was the *Tip100* sequence in *I. alba*, which was more divergent (14.9%) from that in other species. The second highest divergence was 2.8% between *I. nil* 'Candy Pink' and the *Tip100* sequence described by Habu *et al.* (1998) for *I. purpurea* (*Tip100*-AB004906). The lowest levels of divergence were found between *I. nil* and *I. nil* 'Candy Pink' (0.1%), among *I. purpurea* 'Kniolas Black Knight', *I. purpurea* and *I. purpurea* 'Split Personality' (0.1%), and between *I. purpurea* and *I. purpurea* 'Split Personality' (0.0%).

The complete *Tip100* transposase CDS contains 2,426 bp that encode 808 amino acids. The region analyzed in this study covers the first 268 bp of the 5' end of the transposase CDS, corresponding to 73 amino acids. In this region, amino acid sequences are well conserved among the different *Ipomoea*. Although some nucleotide changes were found, amino acid sequences and physiochemical properties remained conserved in the analyzed region. The only exception was nucleotide loss at position 30 of the transposase ORF in *I. nil* and *I. nil* 'Candy Pink sequences', thereby causing amino acid deletion (Figure S1, Supplementary Material).

Table 2 - Nucleotide divergence analysis of *Tip100* transposon.

	I alba	I nil	I CP	I ind	I LBS	I KBK	I SP	I purp
I alba								
I nil	0.147							
I CP	0.149	0.001						
I ind	0.152	0.023	0.024					
I LBS	0.151	0.024	0.026	0.02				
I KBK	0.147	0.019	0.02	0.015	0.008			
I SP	0.146	0.018	0.019	0.013	0.007	0.001		
I purp	0.146	0.018	0.019	0.013	0.007	0.001	0	
Tip100	0.157	0.027	0.028	0.023	0.016	0.011	0.009	0.009

I. alba = *Ipomoea alba*, Inil = *I. nil*, I CP = *I. nil* Candy Pink, I. ind = *I. indica*, I LBS = *I. purpurea* Light Blue Star, I KBK = *I. purpurea* Kniolas Black Knight, I SP = *I. purpurea* Split Personality, I. purp = *I. purpurea*, Tip100 = Transposon *Tip100* described by Habu *et al.*, (1998) for *I. purpurea* (AB004906).

Bayesian analysis indicated three clusters in an unrooted tree. As expected, the most divergent clade was formed by *I. alba Tip100* (Figure 1). The second clade included the two transposons in *I. nil* and *I. nil* 'Candy Pink'. Posterior probability (1.00) conferred strong support for this clade. The third clade, also well supported (0.98), was formed by the *Tip100* sequences in *I. indica* and *I. purpurea*, *Tip100*-AB004906 and *I. purpurea* commercial varieties.

ITS variability

PCR amplification of ITS sequences was uniform and positive for all the species and varieties tested (Table 1). The obtained PCR fragments matched the expected fragment size of 550 bp for the ITS1 and ITS2 spacers, and the 5.8S sequence.

Comparison among sequences indicated the largest divergence to be between *Merremia tuberosa* and *I. quamoclit* (36.2%). No sequence difference was observed between *I. purpurea* and the *I. purpurea* varieties (*I. purpurea* 'Kniolas Black Knight', 'Light Blue Star' and 'Split Personality') (Table 3).

ITS sequences appeared to be good markers for reconstructing the phylogenetic history of *Ipomoea*, since all the clades received highly satisfactory statistical support (Figure 2). Miller *et al.* (2004) proposed that *Ipomoea* is formed by two principal clades. Our results are in partial

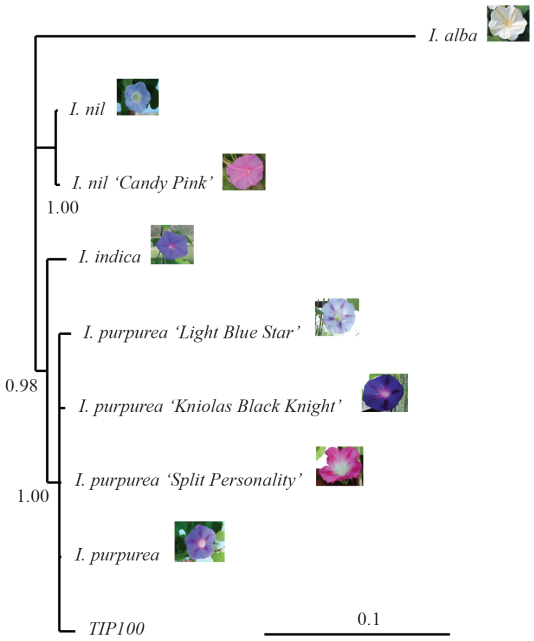

Figure 1 - Unrooted phylogenetic tree of *Tip100* transposons, with pictures of the respective flowers, as defined through Bayesian analysis. The evolutionary model used was HKY, as determined by the Akaike criterion. The numbers on the branches correspond to posterior probabilities estimated from the Bayesian analysis.

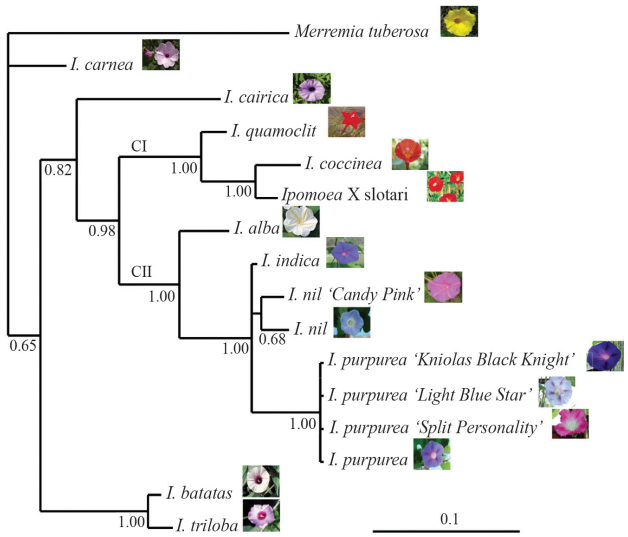

Figure 2 - Phylogenetic tree of the ITS region, as defined through Bayesian analysis. The evolutionary model used was GTR+G. The numbers on the branch correspond to posterior probabilities estimated from the Bayesian analysis. *M. tuberosa* was used as outgroup. Clades I and II (indicated by CI and CII) are as described by Miller *et al.* (2004).

agreement, since Clade I was identified as including *I. quamoclit, I. coccinea* and '*Ipomoea* X Slotari' with strong statistical support, and Clade II as containing the remaining *Ipomoea* taxa, with *I. alba* as the basal member of the group. Within Clade II, three clusters were formed, one for *I. indica,* a second for *I. nil* and *I. nil* 'Candy Pink', and a third for *I. purpurea* and its varieties. All the branches are strongly supported, except for that joining *I. nil* and *I. nil* 'Candy Pink'. *I. cairica* is basal to these clades, and Miller *et al.* (2004) used this very species as outgroup of the genus. The other species that were studied here, but were not included in the phylogenetic analysis done by Miller *et al.* (2004), are *I. batatas, I. triloba* and *I. carnea*. These three species appear to be basal to Clades I and II. The basal clade of *I. batatas* and *I. triloba* is strongly supported.

Table 3 - Nucleotide divergence analysis of ITS.

	M. tub	I cair	I carn	I bat	I tril	I cocc	I quam	I SLT	I alba	I CP	I nil	I ind	I KBK	I LBS	I SP
I cair	0.299														
I carn	0.277	0.162													
I bat	0.267	0.175	0.134												
I tril	0.244	0.189	0.139	0.017											
I cocc	0.347	0.214	0.17	0.257	0.293										
I quam	0.362	0.164	0.128	0.133	0.147	0.078									
I SLT	0.153	0.119	0.107	0.215	0.212	0.032	0.06								
I alba	0.251	0.152	0.159	0.183	0.193	0.17	0.107	0.152							
I CP	0.261	0.204	0.236	0.225	0.226	0.2	0.159	0.211	0.098						
I nil	0.236	0.176	0.202	0.241	0.241	0.188	0.149	0.16	0.091	0.028					
I ind	0.214	0.163	0.189	0.185	0.186	0.168	0.138	0.136	0.074	0.017	0.024				
I KBK	0.295	0.236	0.259	0.35	0.331	0.235	0.172	0.161	0.122	0.063	0.066	0.044			
I LBS	0.295	0.236	0.259	0.35	0.331	0.235	0.172	0.161	0.122	0.063	0.066	0.044	0		
I SP	0.295	0.236	0.259	0.35	0.331	0.235	0.172	0.161	0.122	0.063	0.066	0.044	0	0	
I purp	0.295	0.236	0.259	0.35	0.331	0.235	0.172	0.161	0.122	0.063	0.066	0.044	0	0	0

M.tub = *Merremia tuberosa*, I cair = *Ipomoea cairica*, I carn = *I. carnea*, I bat = *I. batatas*, I tril = *I. triloba*, I cocc = *I. coccinea,* I quam = *I. quamoclit*, I SLT = Ipomoea X Slotari, I alba = *I. alba*, I CP = *I. nil* Candy Pink, I nil = *I. nil*, I indica = *I. indica,* I KBK = *I. purpurea* Kniolas Black Knight, I LBS = *I. purpurea* Light Blue Star, I SP = *Ipomoea purpurea* Split Personality.

Discussion

Numerous transposable elements known to be involved in the process of variegation in *Ipomoea*, thereby leading to wide diversification in flower pigmentation, also represent an important evolutionary process. One of these elements is *Tip100*, which is inserted in the CHS-D gene. After extensive searches in the NCBI database with Blastn, Blastx and tBlastx, no similarities between the *Tip100* sequence and other transposable elements came to light. Habu *et al.* (1998) classified *Tip100* as a member of the Ac/Ds family (Kunze *et al.*,1997). However, according to more recent criteria for TE classification (*e.g.*, Wicker *et al.*, 2007), "two elements belong to the same family if they share at least 80% of sequence identity in their coding domain, or within their terminal repeat regions, or in both". Hence, *Tip100* would not belong to the Ac/Ds family, since no close similarity was found between *Tip100* and the other transposons of this family. Nevertheless, *Tip100* sequences and structural characteristics clearly place this element in the *hAT* superfamily (Kempken and Windhofer, 2001; Rubin *et al.*, 2001). Therefore, we propose that *Tip100* belongs to a new TE family, which, to date, has only been observed in the genus *Ipomoea*.

Recently, Arensburger *et al.* (2011) undertook a rigorous phylogenetic analysis of the *hAT* superfamily. They discovered that this superfamily is formed by two large families, namely Buster and AC, and even indicated the existence of a third clade, maybe a new family, which currently contains only three members, viz., *Tip100* of Ipomoea and two *Tip100*-related sequences, one from a hydra (*H. magnipapillata*) and the other from zebrafish (*Danio rerio*). These findings give to understand that this possibly new family may be widely distributed.

The species included in this study are representatives of three *Ipomoea* subgenera. The well-supported, mutual phylogenetic relationships established by ITS analysis are congruent with other studies based on morphological and molecular data (McDonald and Mabry, 1992; Austin and Huáman, 1996; Austin and Bianchini, 1998; Miller *et al.*, 1999, 2002, 2004; Stefanovi et *al*., 2003), whereby, *I. batatas, I. triloba* and *I. carnea* were identified as members of the subgenus *Eriospermum, I. purpurea, I. nil* and *I. indica* as members of the subgenus *Ipomoea*, and *I. cairica*, *I. coccinea*, *I. quamoclit* and *I. alba* as part of the subgenus *Quamoclit*. In the present analysis, we found that *I. alba* is more closely related to species of the subgenus *Ipomoea*, rather than to *Quamoclit,* as previously proposed by Miller *et al.* (1999; 2004). Furthermore, *I. cairica,* formally in the subgenus *Ipomoea*, appears as outgroup to the clade formed by the subgenera *Quamoclit* and *Ipomoea*, although the statistical support for this branch (0.82) is less than for the other branches.

There is significant consistency between the phylogeny built with species with *Tip100* sequences and the one constructed with ITS data, the latter including more species representing the host phylogeny. As *Tip100* was found only in a restricted clade in the ITS phylogeny (Figure 2, Clade II), we propose that this element was present in an ancestor of these related species, thereby implying that *Tip100* was vertically transferred during evolution of the genus. Thus, it was more effectively maintained in the subgenus *Ipomoea*, were it apparently remains more conserved. Although, *Tip100* was more divergent in *I. alba*, this is consistent with its basal position in relation to the subgenus *Ipomoea*, possibly through more available time to diverge from other species of *Ipomoea* subgenus. Nevertheless, why *Tip100* is restricted to only one cluster in *Ipomoea* is unknown A possible explanation for the emergence of this element in this species could be horizontal transfer of *Tip100* from an unknown donor to an ancestor of *I. alba, I. indica* and *I. nil*, and the *I. purpurea* cluster (Clade II, Figure 2). Horizontal transfer of TEs has been recognized as an important evolutionary force in eukaryotes (Keeling and Palmer, 2008), although few examples have been encountered in plants (Diao *et al.*, 2006; Roulin *et al.*, 2009). As an alternative explanation, the element was present in all the other clusters of the genus *Ipomoea*, but could have been stochastically lost.

A more plausible explanation for this peculiar TE occurrence could be the presence of *Tip100* sequences in other species of the genus that have diverged throughout the evolution and expansion of these plants, since the evolutionary history of the genus *Ipomoea* is relatively recent, *i.e.*,approximately 35 to 40-million-years, as calculated by molecular clock inference (Clegg and Durbin, 2003). Hence, additional studies are required to determine whether TE arrival in this genus was through horizontal transfer, or whether it is an ancient genome component.

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Supplementary Material

The following online material is available for this article:

- Figure S1 - Nucleotide sequence alignment of the 5' end of the *Tip100* transposase CDS.

This material is available as part of the online article from http://www.scielo.br/gmb.

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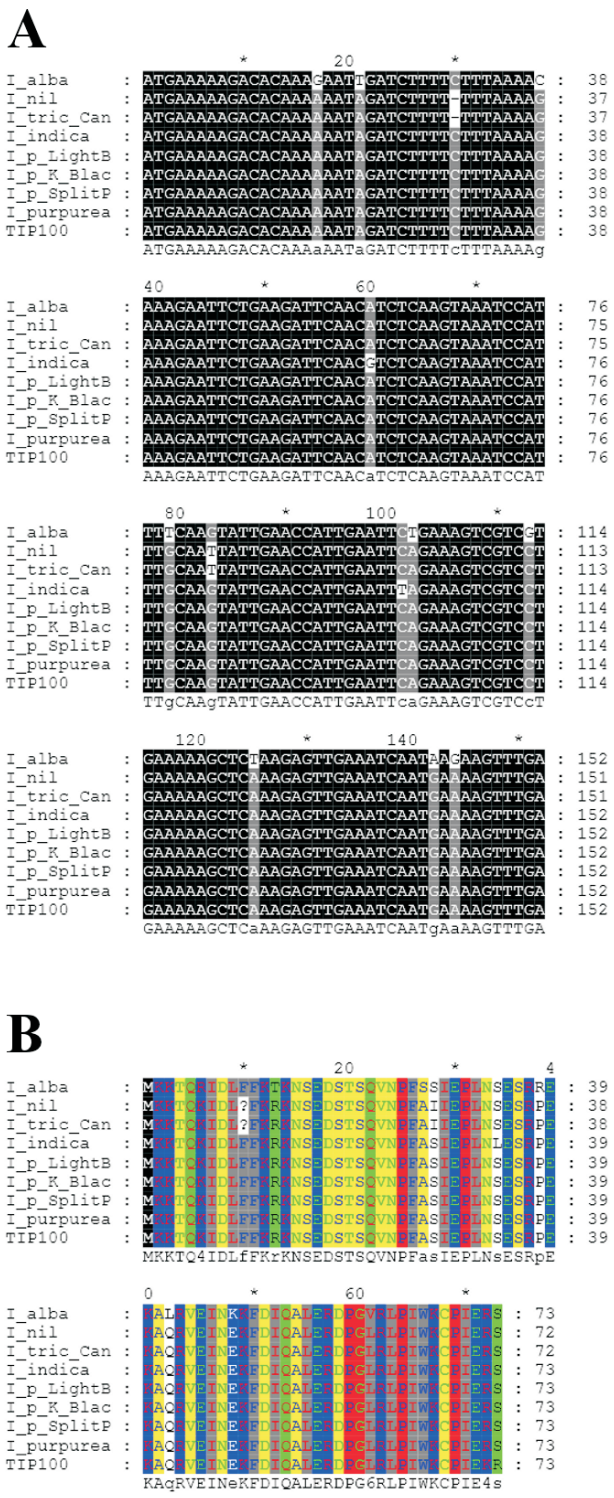

Figure S1 - Multiple alignment of the 5' end of the *Tip100* transposase CDS. (A) Nucleotide sequences in the different species and varieties analyzed in this study. (B) Protein sequences of the first 73 residues of the *Tip100* transposase coding sequence. Colors show the physiochemical properties of the amino acids.