

Agrobacterium T-DNA in *N. tomentosiformis* and other *Nicotiana* species

Ke CHEN¹, François DORLHAC DE BORNE², Ernő SZEGEDI³ and Léon OTTEN¹

Abstract

Agrobacterium rhizogenes induces growth of hairy roots with bacterial T-DNA, T-DNAs gene expression leads to root growth and synthesis of opines, used by the agrobacterium (Otten *et al.*, 2008). Normal tobacco contains T-DNA and is therefore derived from an ancient *Agrobacterium* infection event. We deep-sequenced the DNA of the tobacco ancestor *Nicotiana tomentosiformis*, and found 4 T-DNAs: TA, TB, TC and TD, derived from four transformation events. Each T-DNA consists of partially repeated sequences. Their divergence is proportional to time and allowed us to reconstruct the order of T-DNA introduction. In tobacco, TC was lost. Most of the T-DNA genes lost their function, but TB-*mas2'* encodes desoxyfructosylglutamine synthesis. Some tobacco cultivars show high *mas2'* expression, suggesting that *mas2'* plays a role in the physiology of these cultivars.

Results

- . *N. tomentosiformis* TA, TB, TC and TD structures (Fig. 1) were derived by blasting genomic contigs to known T-DNAs and repeat polymorphisms were determined by PCR .
- . Repeat divergence was used to estimate insertion times, and PCR was used to test T-DNA presence in other *Nicotiana* species, yielding the evolutionary tree shown in Fig. 2.
- . An additional T-DNA, TE, was found in sequences from Sierró *et al.* 2014, TE has not yet been assembled (Fig. 3).
- . The *mas2'* gene was artificially expressed in *N. benthamiana* and found to be active (Fig. 4)
- . The present results will soon be published in The Plant Journal (Ke *et al.*, 2014).

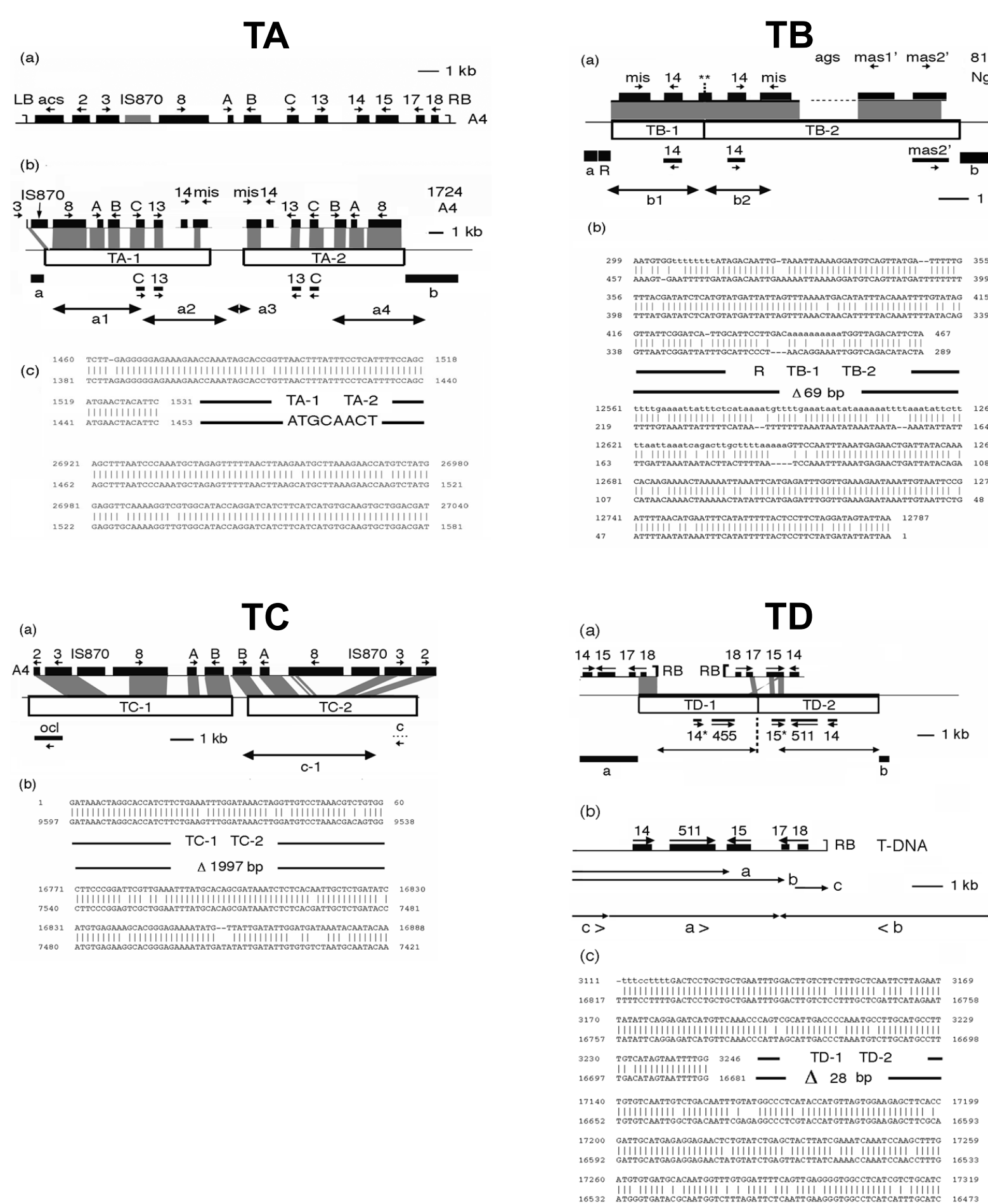


Figure 1: *N. tomentosiformis* TA, TB, TC and TD structures.

Top: maps, bottom: T-DNA insertion sites.

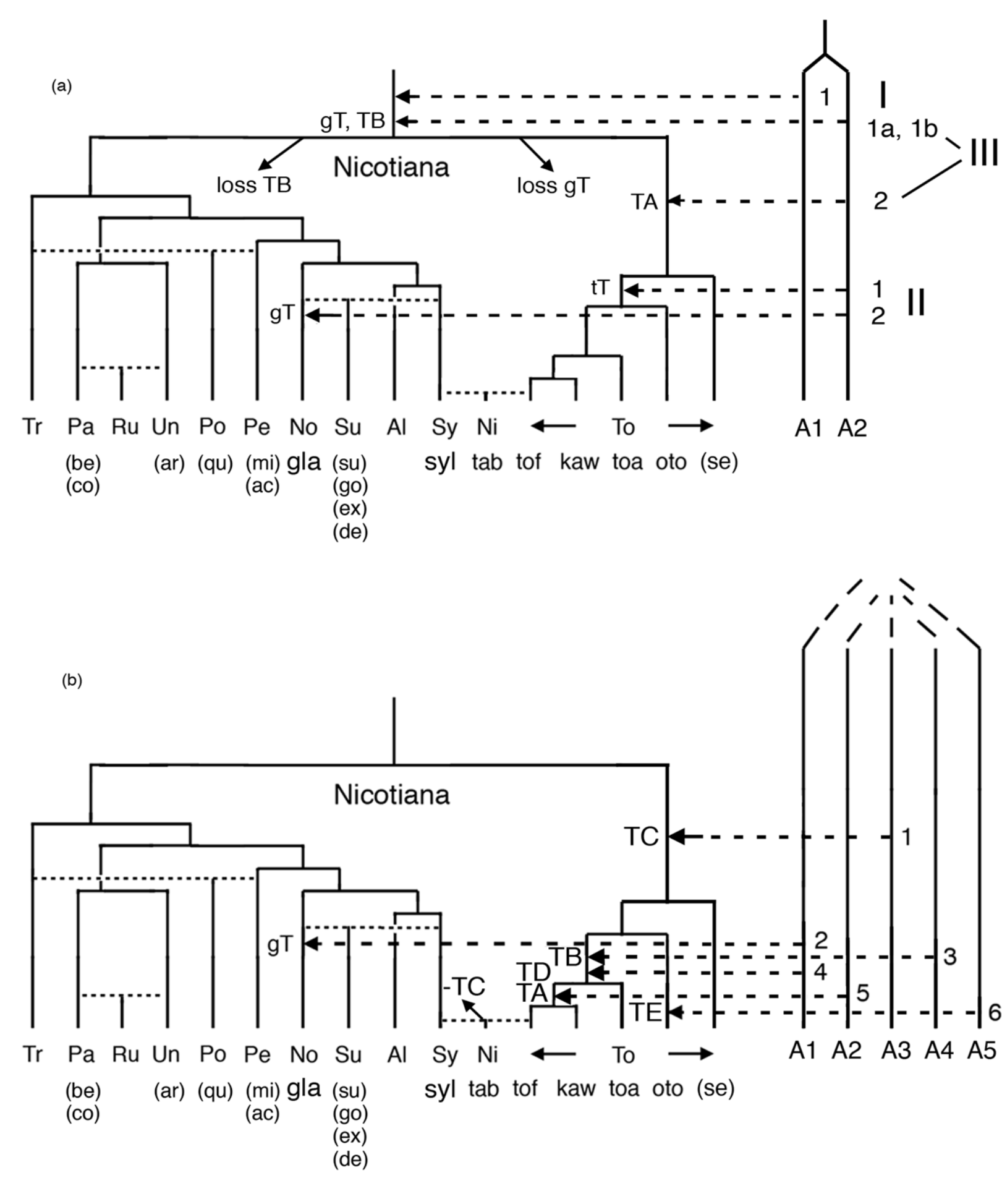


Figure 2: Model of Nicotiana T-DNA evolution.
a. Earlier model.
b. Present model.
1-6: different insertions.
Abbreviations of Nicotiana species: Ke *et al.* 2014.

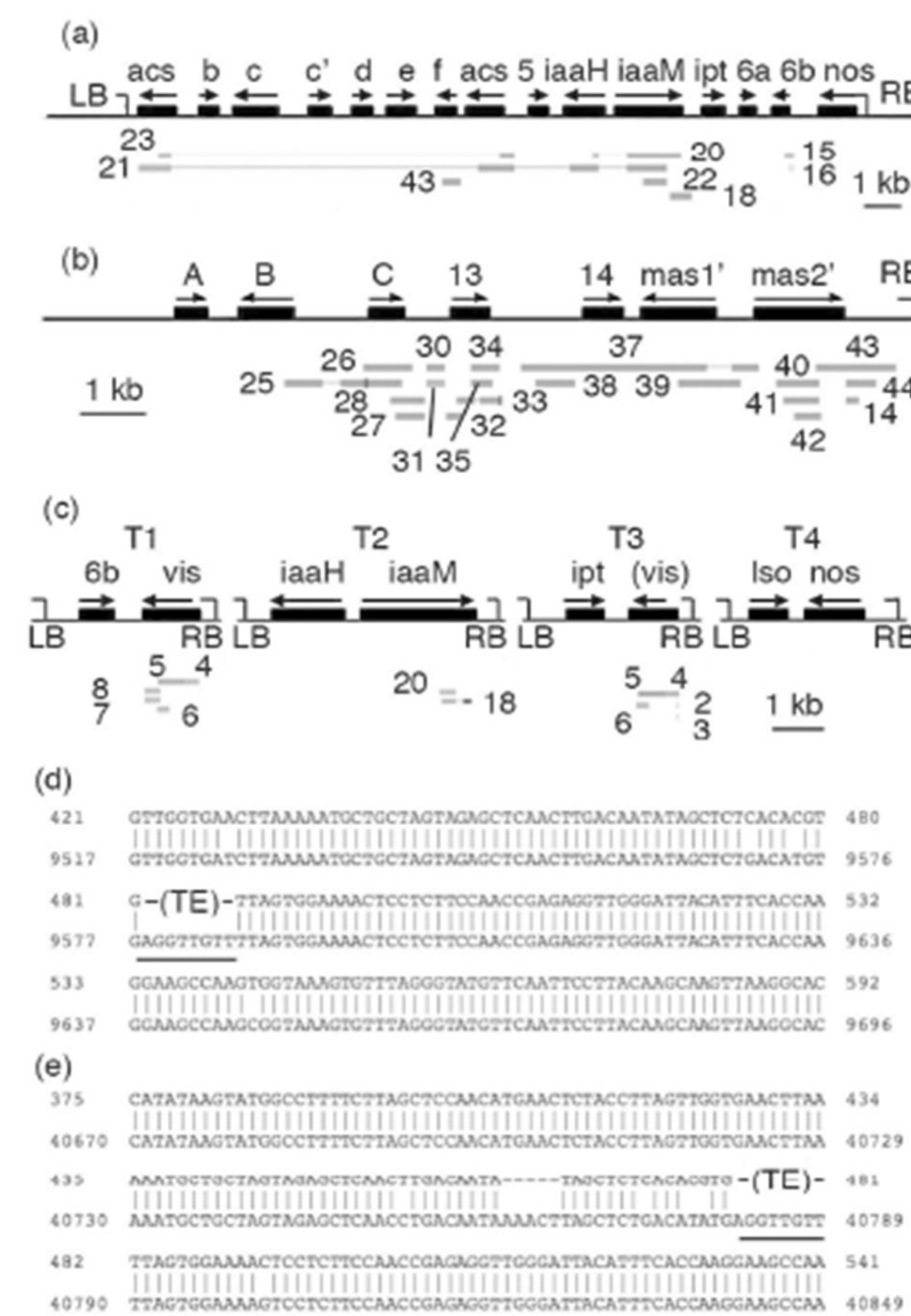


Figure 3: TE from *N. otophthora* (Sierro *et al.* 2014).

This region remains to be assembled. Comparison with T-DNAs from strains C58, 8196 and S4 (a to c).

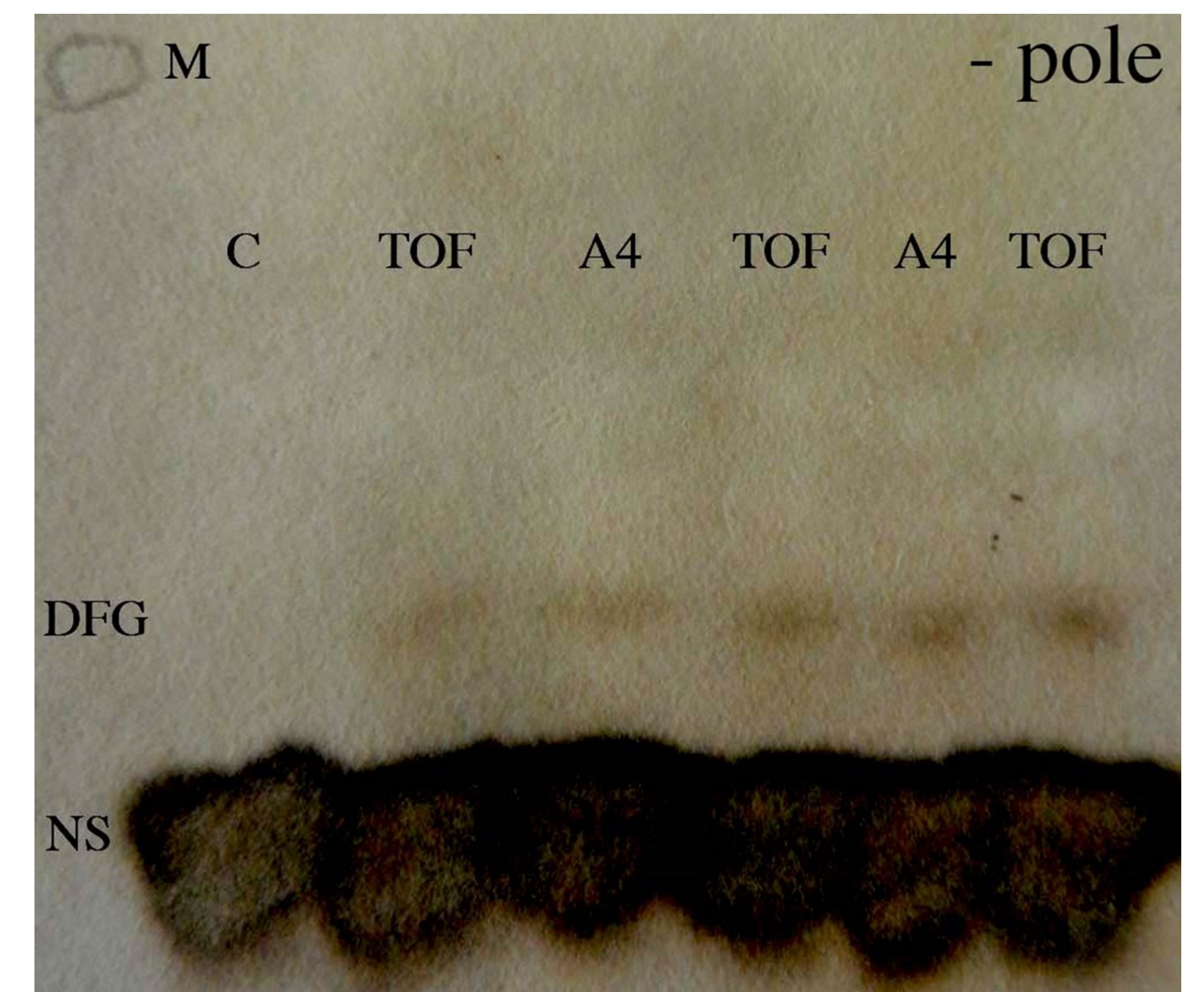


Figure 4: TB-*mas2'* gene codes for an active enzyme producing DFG.

Paper electrophoresis analysis of *N. benthamiana* extracts after introduction of A4 or *N. tom. mas2'* gene.
NS: neutral sugars, M: marker.

Perspectives

- . Assemble TE and TC region from *N. otophora*
- . Test possible importance of DFG production
- . Test other Nicotiana species for cT-DNA
- . Test expression of cT-DNA open reading frames Test functions open reading frames.

References

- Ke, C., Dorlhac de Borne, F., Szegedi, E., Otten, L. (2014) The Plant Journal, in press.
- Sierro, *et al.* (2014) Nature Commun. 5:3833, DOI:10/1038.
- Otten, L., Burr, T. and Szegedi, E. (2008) In:: Agrobacterium: from biology to biotechnology. Eds. Tzfira and V. Citovsky, Springer Press.