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

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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 Sierro 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).

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


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. otophora (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.