Revealing the past: New insights into plant evolution using a novel snoRNA marker-system

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Introduction

Despite the existence of various marker systems there are still limitations in distinguishing between closely related species, especially when information on hybridization events should be incorporated. The characterisation of plant small nucleolar RNA (snoRNA) genes and their organisation in multigene clusters provides a potential nuclear marker system which could help to discover the phylogenetic history of plants. Using three closely related *Senecio* species, we are investigating a combination of fragment and sequence variation of snoRNA-genes to assess the utility of this marker system.

What are snoRNAs?

snoRNA genes are spread over the whole genome and encode stable small RNAs (ca. 100-200nt) involved in ribosome biogenesis. Many snoRNA "guide" sequences are highly conserved across eukaryotes (from yeast to animals). In plants, snoRNA genes have a unique organisation where the genes are arranged in closely linked clusters. Analysis of snoRNAs from plant databases has identified over 250 genes in 20 different species including gymnosperms and mosses (Fig. 1a). On the basis of conserved gene order, universal primer sets have been designed to "guide" sequences (Fig. 1b).

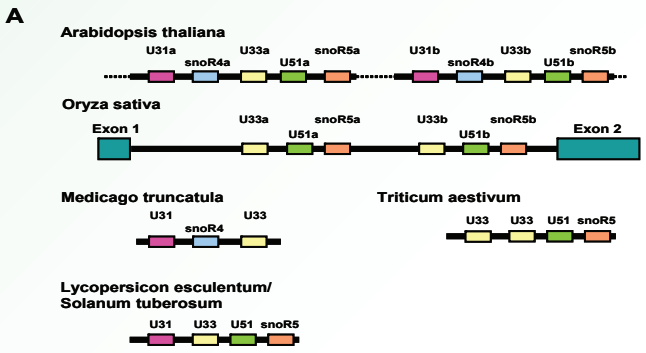

Figure 1A shows conservation of some gene order in genomic and EST sequences for one snoRNA gene cluster. In addition, there are gene and cluster duplications and different chromosomal locations of clusters.

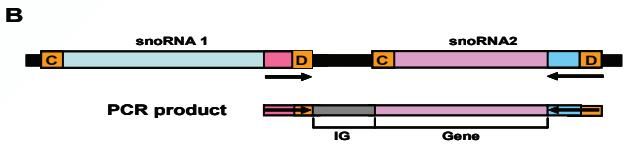

Figure 1B shows the universal primerdesign for most snoRNAs. C, D - conserved sequences; arrows - primers.

Results

Fluorescence genotyping of 23 primer sets representing 11 different gene-clusters showed similar gene order in *Senecio* species and *Arabidopsis*. Furthermore, there were more fragments than expected in most of the genotype profiles suggesting more copies of these gene clusters are present in *Senecio*. For example, in *Arabidopsis* the snoRNA29/snoRNA30 gene cluster can be found only once but appears to have at least 3 copies (6 fragments of different sizes found in one sample) in *Senecio*. Likewise, the U61/snoRNA14, a single copy gene-cluster in *Arabidopsis*, is present at least twice in *Senecio*.

Sequence data revealed three to four well bootstrap (bs)-supported clades/subclades for the snoRNA29/snoRNA30 (Fig. 2A) and two for the U61/snoRNA14 gene-cluster (Fig. 2B). Remarkable, in both cases sequences of particular sizes can be found only in one particular clade.
These clades might represent different genes which is further supported by genotyping.

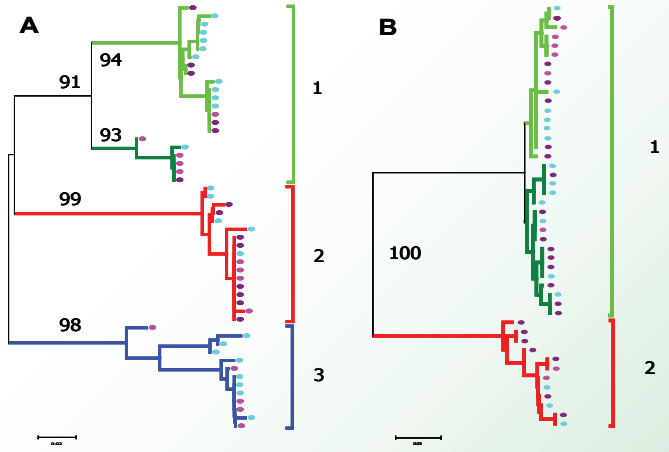

Figure 2. Neighbour joining tree of two different snoRNA gene cluster of three closely related *Senecio* species (blue, pink and violet dots) revealed four (A: snoRNA29/snoRNA30 cluster) and two (B: U61/snoRNA14 cluster) well supported (sub)clades (1 light/dark green, 2 red, 3 blue), respectively. Numbers above branches are bootstrap values.

Conclusion

snoRNA gene clusters appear to have more copies in *Senecio* than in *Arabidopsis*, which might be distinguished by a combined sequencing/genotyping approach. Thus, studying snoRNA genes/gene clusters seem to be a good marker system for studying gene evolution and may also be useful for plant evolution once single copy genes are identified.