Epigenetic silencing of ribosomal RNA genes in allotetraploid plant *Tragopogon mirus*

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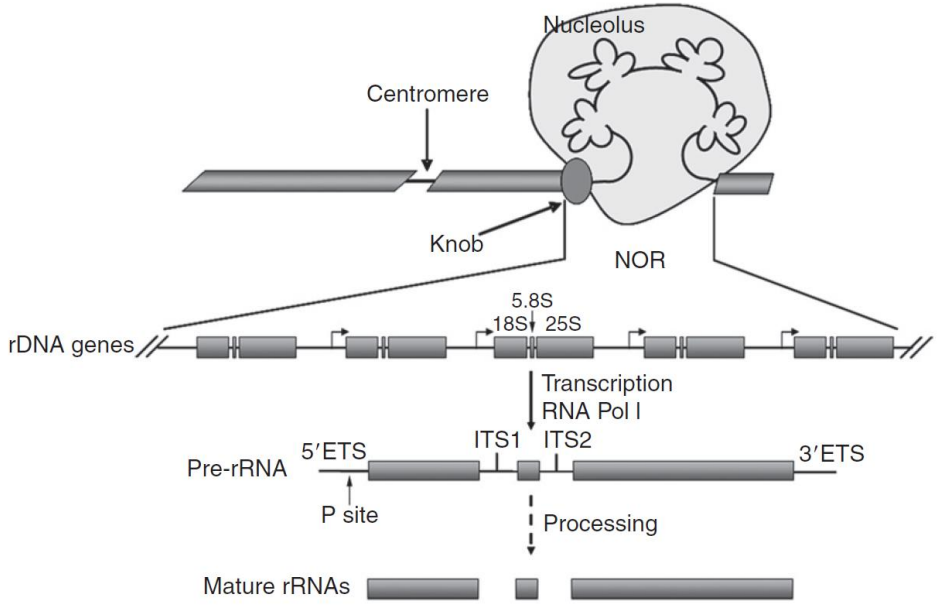
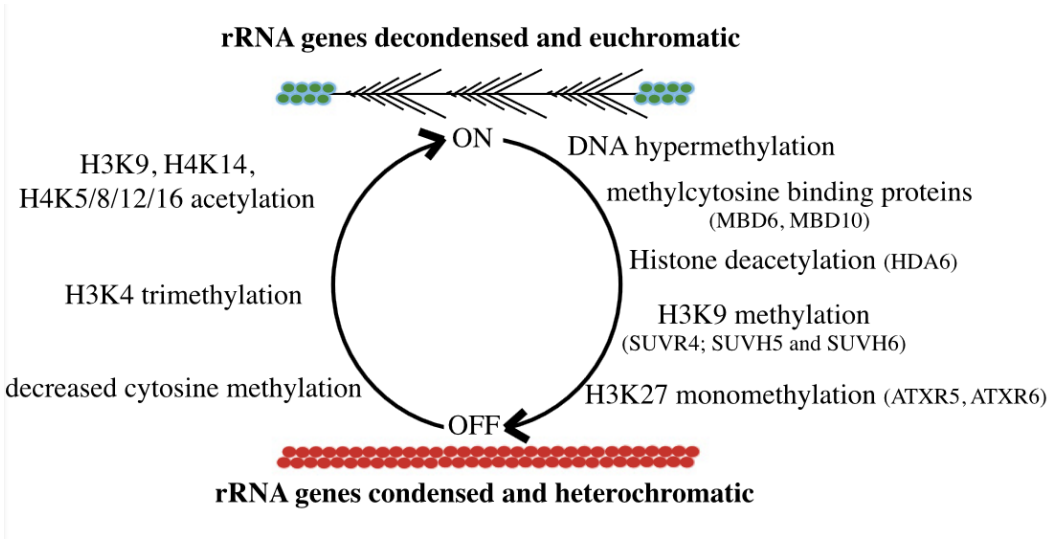
Ribosomal RNA genes

- Form head to tail tandem repeats - hundreds to thousands per haploid genome
- Polycistronic pre-rRNA transcript of 18S, 5.8S, and 25/28S rRNA - RNA Pol I
- 5S rRNA transcribed independently – Pol III

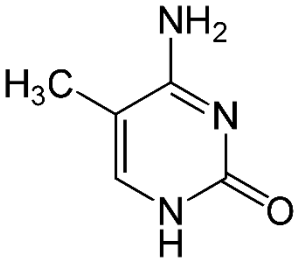


Nucleolar Dominance

- Demands for ribosomes and protein synthesis vary during development - number of active rRNA genes is subject to dosage control (*A. thaliana* ~20% of active genes)
- Interspecific hybrids: RNA genes of one progenitor are repressed - epigenetic phenomenon known as nucleolar dominance



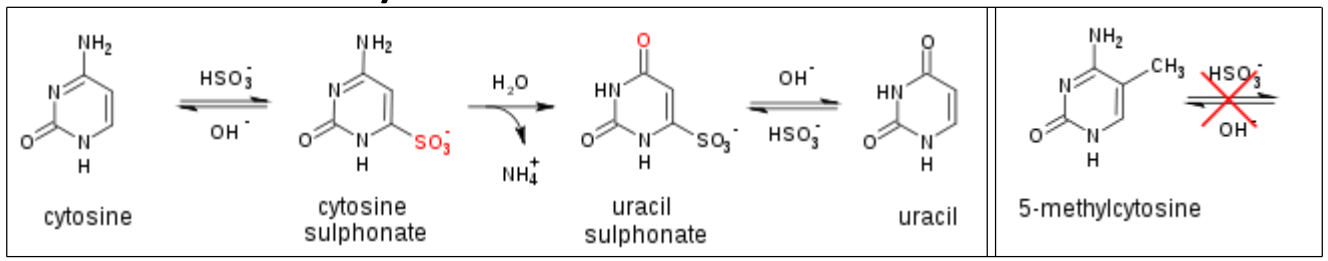
DNA methylation



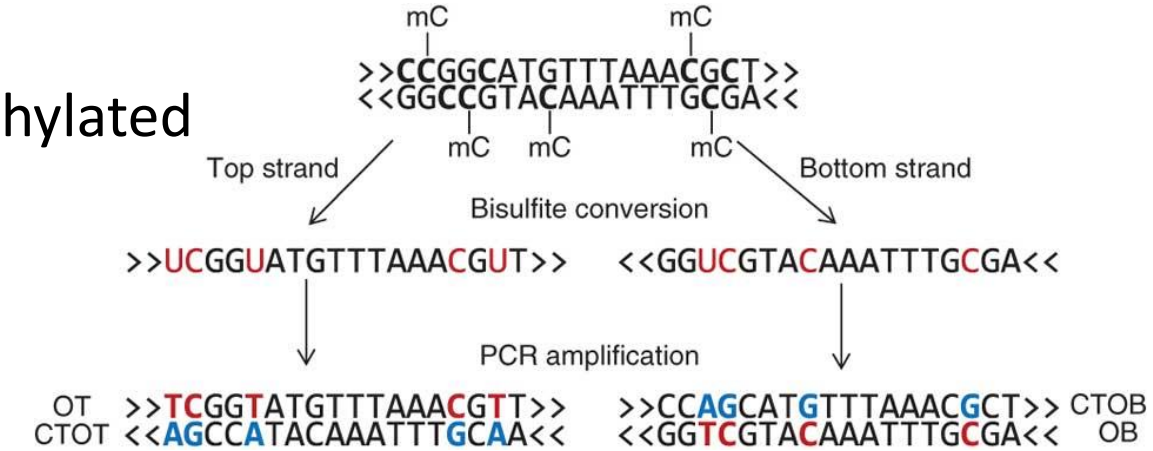
- Occurs at the C5 position of cytosines
  - in mammals: symmetric CG context
  - in plants: symmetric CG and CHG, asymmetric CHH (H = A, C or T)
- Essential for mammalian embryonic development
- X chromosome inactivation and allele-specific expression of imprinted genes
- Silencing of transposons and other repetitive sequences
- Regulating of gene expression, hypomethylation of promoters correlates with expression of rRNA genes

Bisulfite sequencing – principle of method

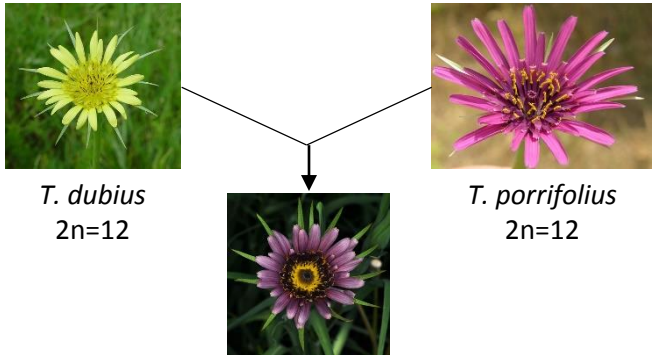
- Bisulfite treatment:
  - unmethylated cytosines: deamination -> desulfonation -> uracil
  - 5mC: not converted by bisulfite



- PCR amplification (U->T)
  - Primers not discriminating between methylated and not methylated DNA
  - Only one strand is amplified
- Cloning and sequencing
- Evaluation of sequences, CyMATE software



Tragopogon mirus



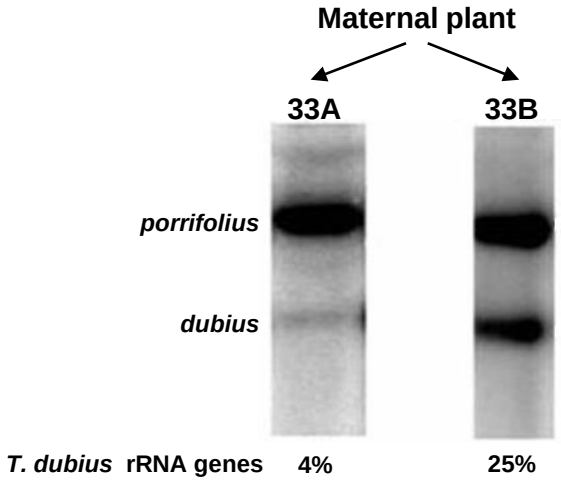
- Allotetraploid plant (2n=4x=24)
- Formed within last 80 years from diploids *T. dubius* and *T. porrifolius* in western North America
- Biennial plant => less than 40 generations
- Usually fewer 35S rRNA genes of *T. dubius* than *T. porrifolius*,

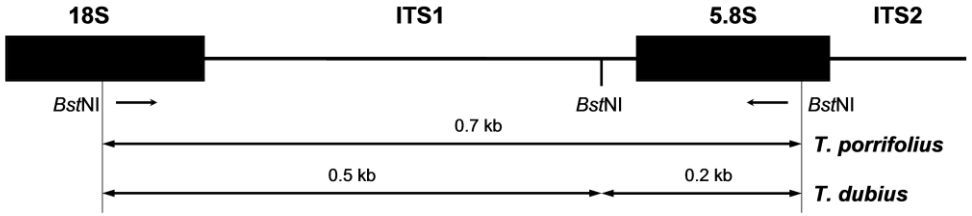
rRNA of *T. dubius* origin are transcriptionally dominant

Distribution of T. mirus plants

Tragopogon mirus

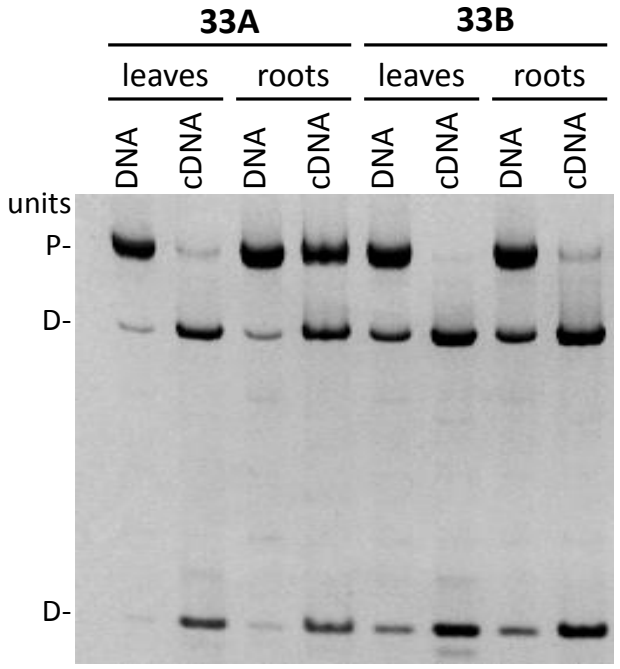
- Two sibling plants from Rosalia population with different amount of *T. dubius* rDNA
- Restriction enzyme *Bst*NI has target site in *T. dubius* origin but not *T. porrifolius* origin ITS1 sequence
- Plant 33B: 25% of *T. dubius* derived rRNA genes, 400 copies
- Plant 33A: 4% of *T. dubius* derived rRNA genes, 70 copies

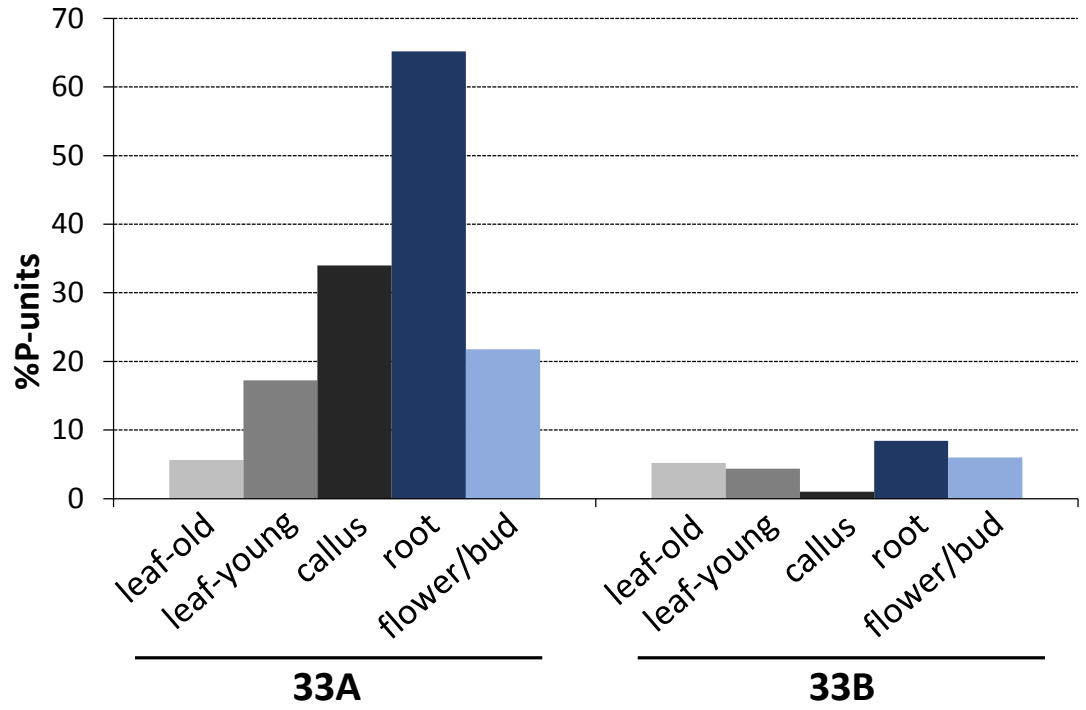




Expression of rRNA genes in different tissues

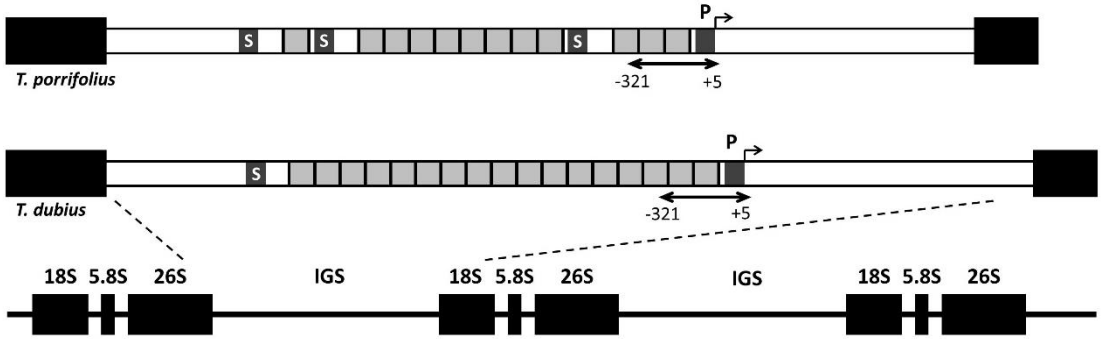
- RNA isolation -> reverse transcription -> PCR amplification -> *Bst*NI cleavage
- 33B: dominant expression of *T. dubius* derived rDNA in all tissues
- 33A: both P- and D-genome rDNA transcripts in in root, flower and callus





Bisulfite analysis

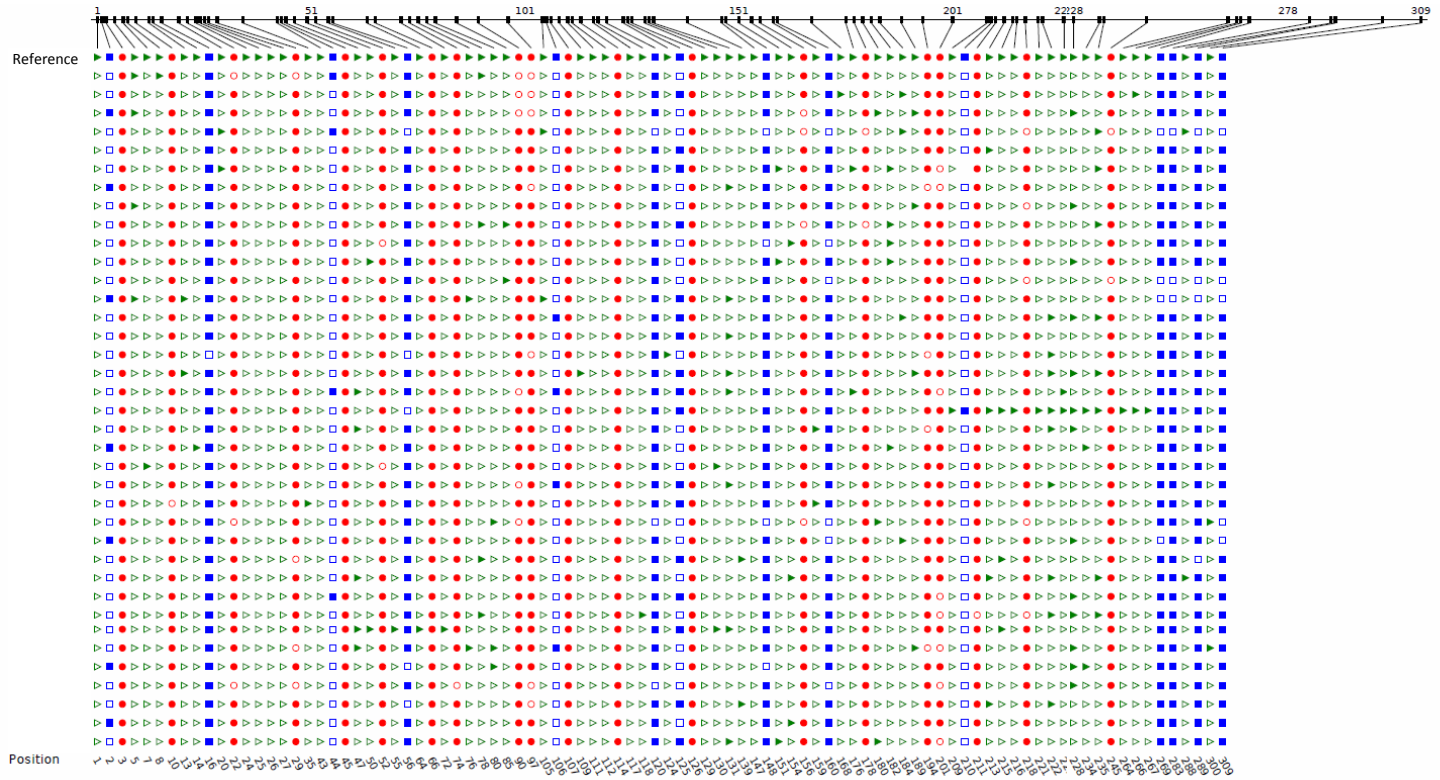
- Material: DNA from leaves and roots of 33A and 33B plants
- Analyzed region: promoter region and IGS repeats



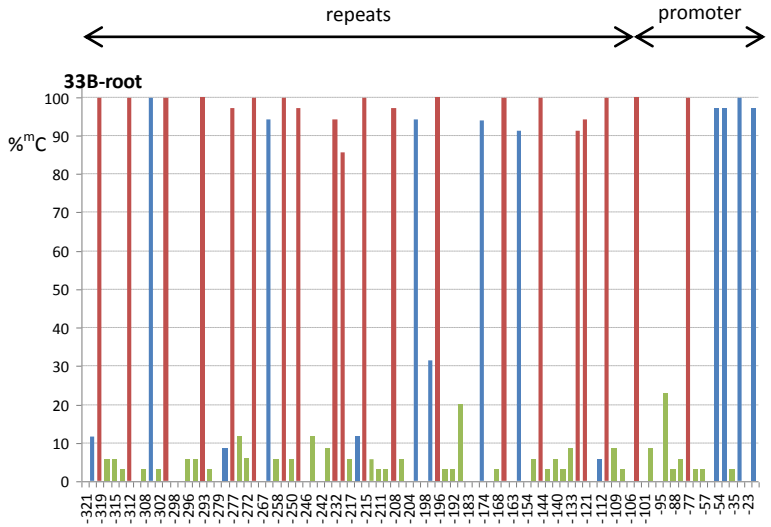
- More than 40 clones per sample were sequenced, only *T. porrifolius* derived were represented in sufficient amount and evaluated
- CyMATE software
  - Input: multiple sequence alignment (Clustal software)
  - Output: text (mC - global, per position, per sample) and graphical

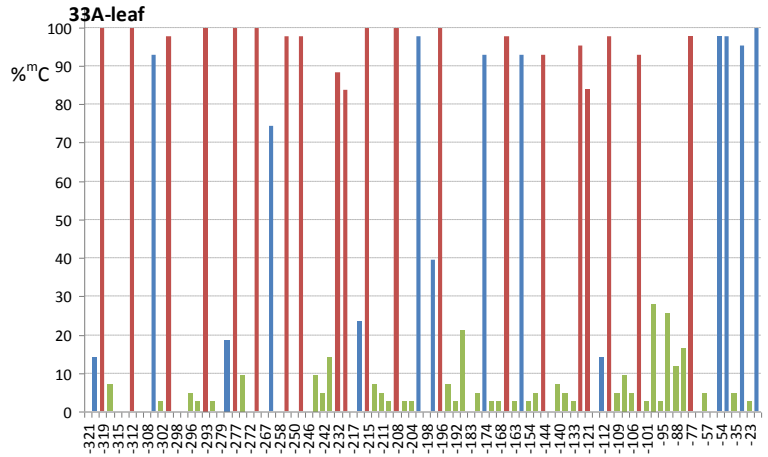
Bisulfite analysis - results

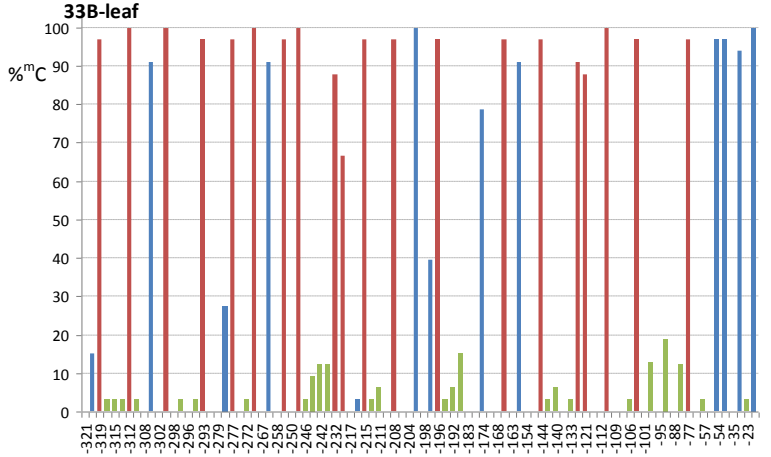
- Graphical output of CyMATE software



Bisulfite analysis - results

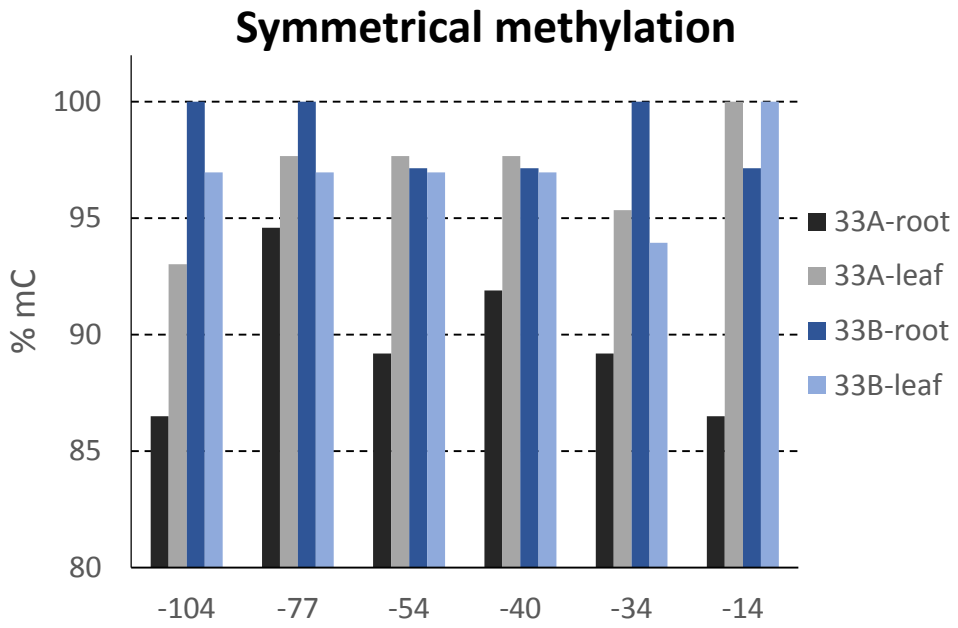


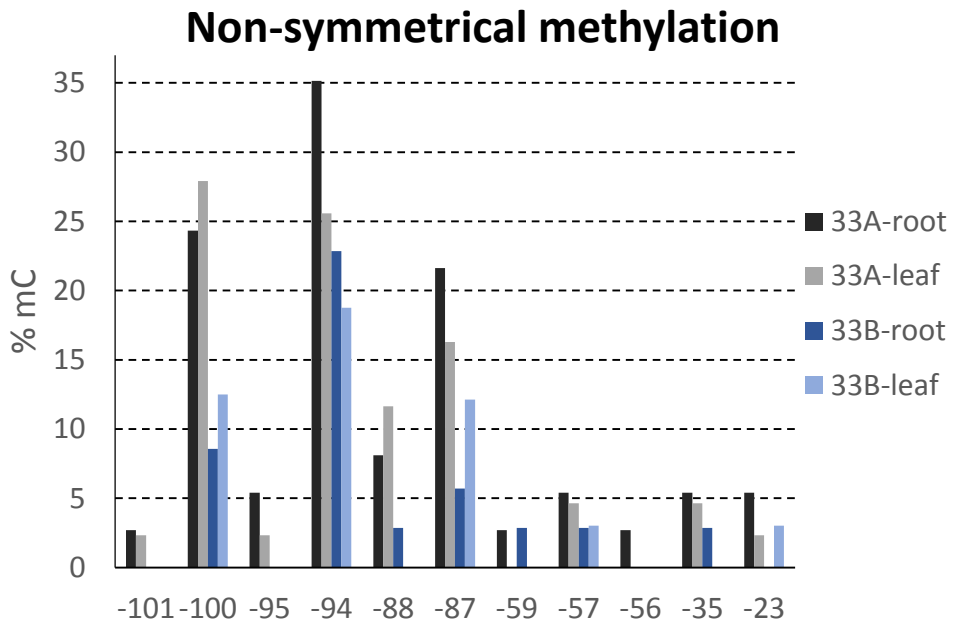




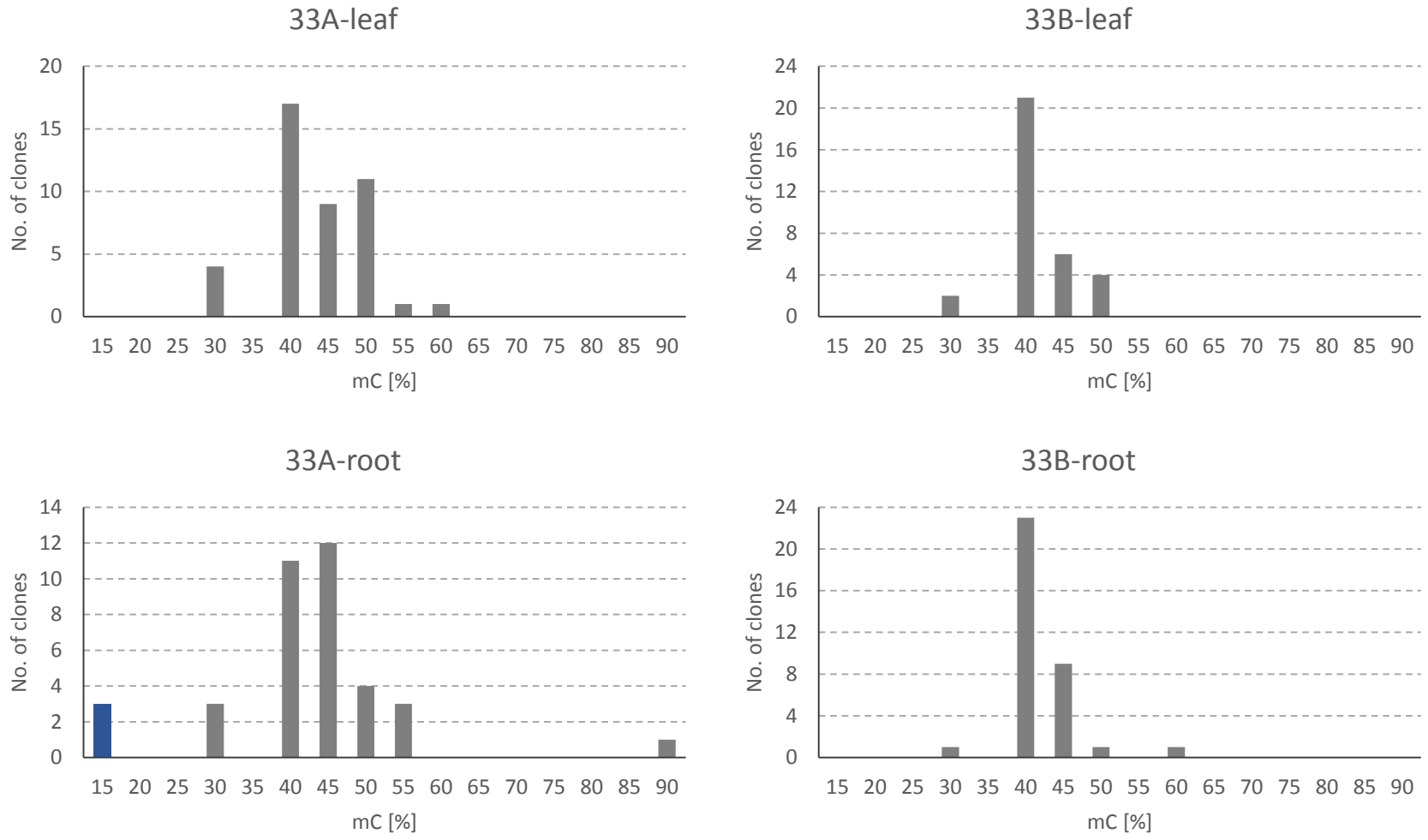
Bisulfite analysis - results

- Analysis of promoter sequence of rRNA genes derived from *T. porrifolius*
- symmetrical methylation: decreased in 33A-root
- non-symmetrical methylation: increased in 33A plant





Bisulfite analysis



- 3 demethylated clones in 33A root sample, ~10% P-genome rRNA genes are transcriptionally active

Conclusions

- Epigenetic silencing of *T. porrifolius* rDNA is disrupted in allopolyploid individuals that suffered from reduction of active gene copies of *T. dubius* origin.
- *T. porrifolius* genes were activated in tissues with high mitotic activity.
- Active rRNA genes are demethylated in their promoter sequences
- Silenced homoelog genes may be rapidly activated in polyploids after the deletion or loss of functional gene copies.