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Nicotine biosynthesis pathway: beyond tobacco

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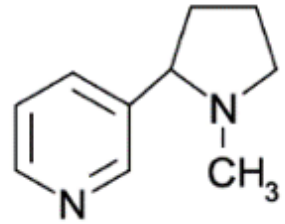
Nicotiana tabacum

- Cultivated as an economically important crop around the world
- Allotetraploid derived from the hybridization between two ancestral diploid that are closely related to current *N. sylvestris* and *N. tomentosiformis*

N. sylvestris *N. tomentosiformis* *N. tabacum*

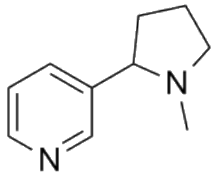
Nicotine

- Abundant predominant alkaloid in tobacco
- Consists if heterocyclic pyridine and pyrrolidine ring



- Produced in roots and accumulating in mainly in leaves
- Defense toxin produced by a jasmonate-induced response to damage by grazing herbivores

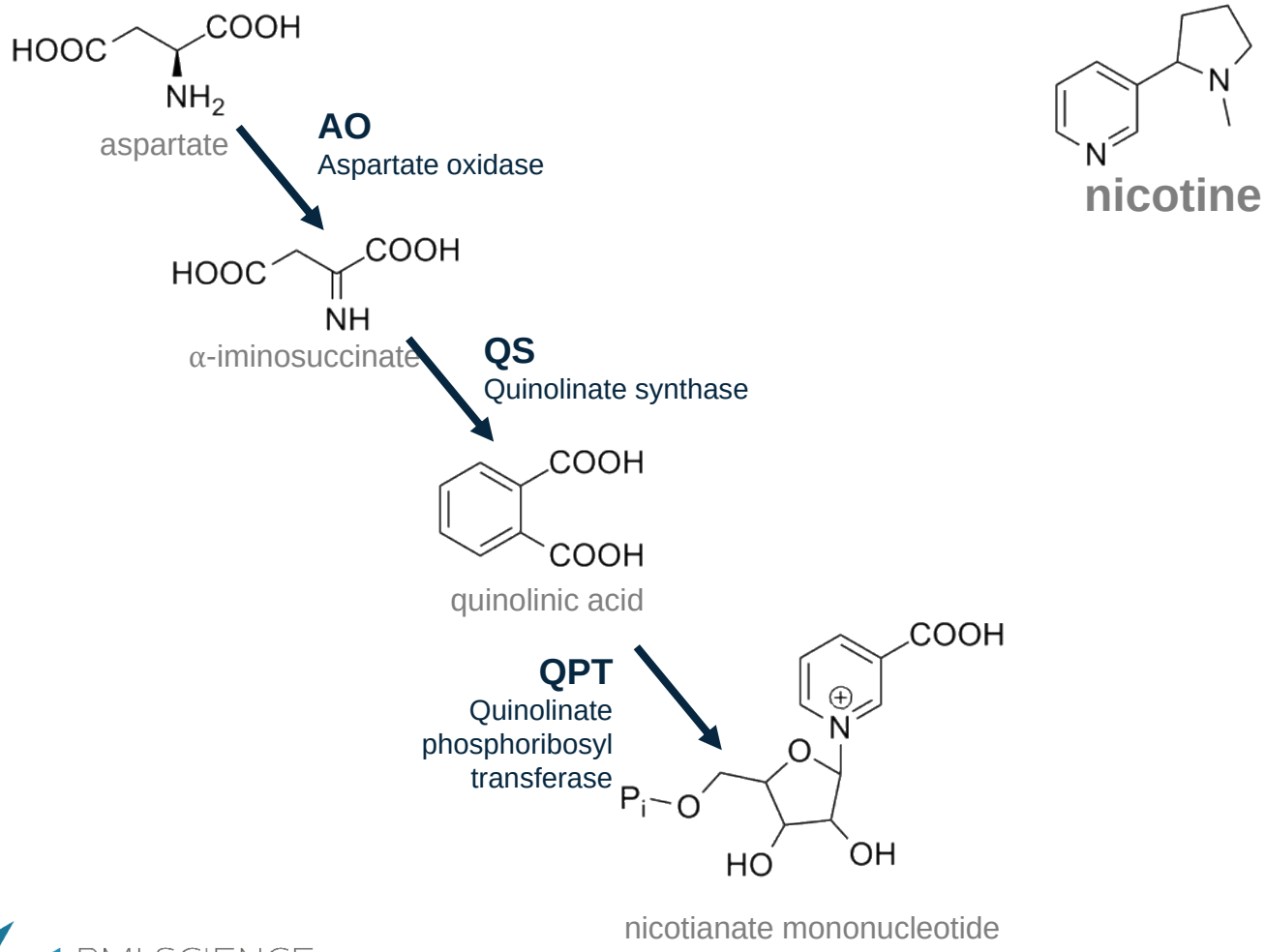
Nicotine biosynthesis



nicotine

Nicotine biosynthesis

Pyridine ring synthesis



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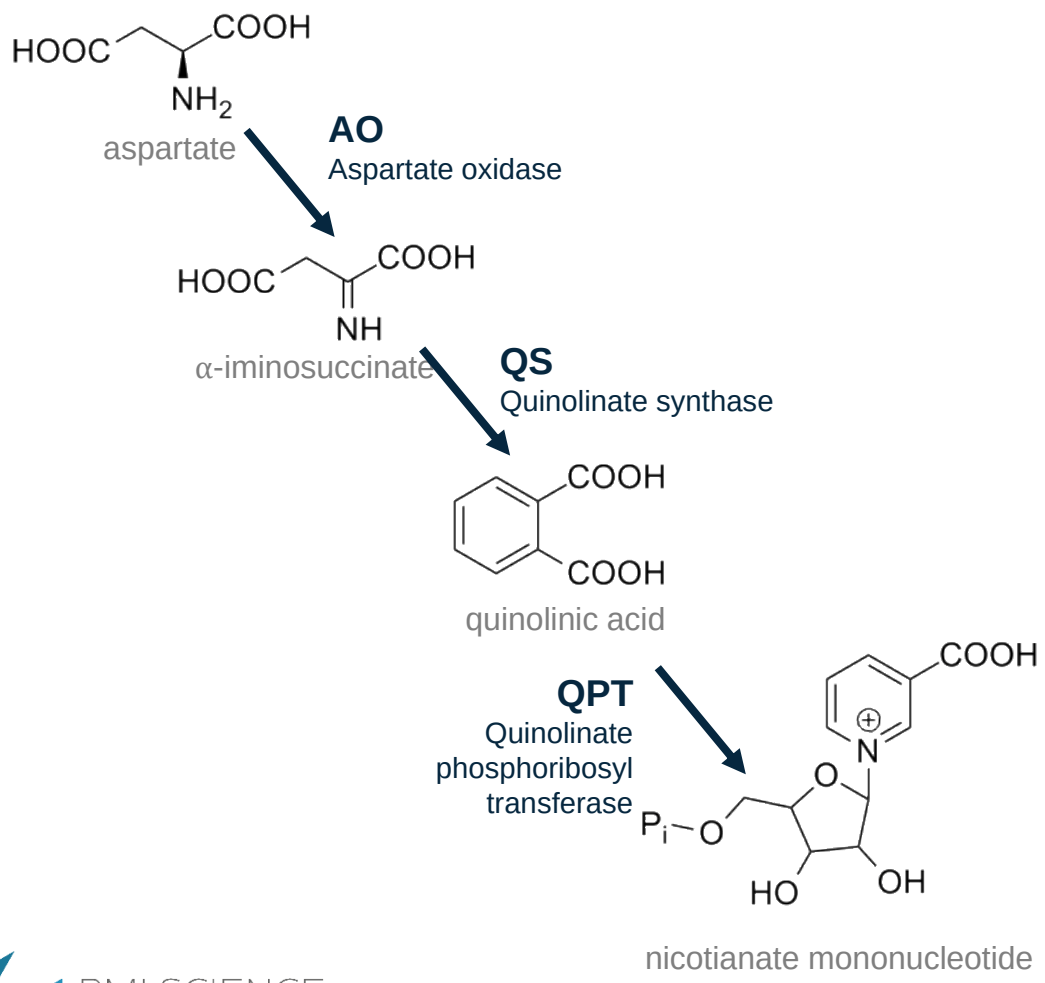
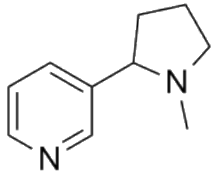
Nicotine biosynthesis

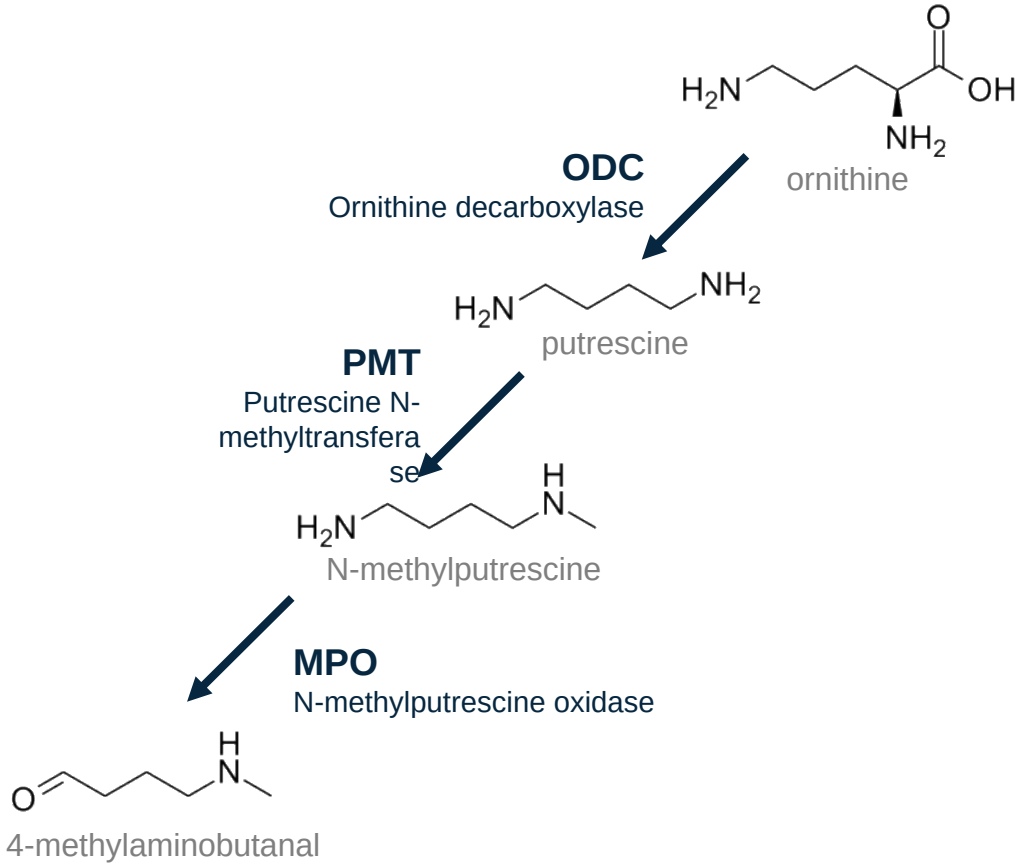
Pyridine ring synthesis



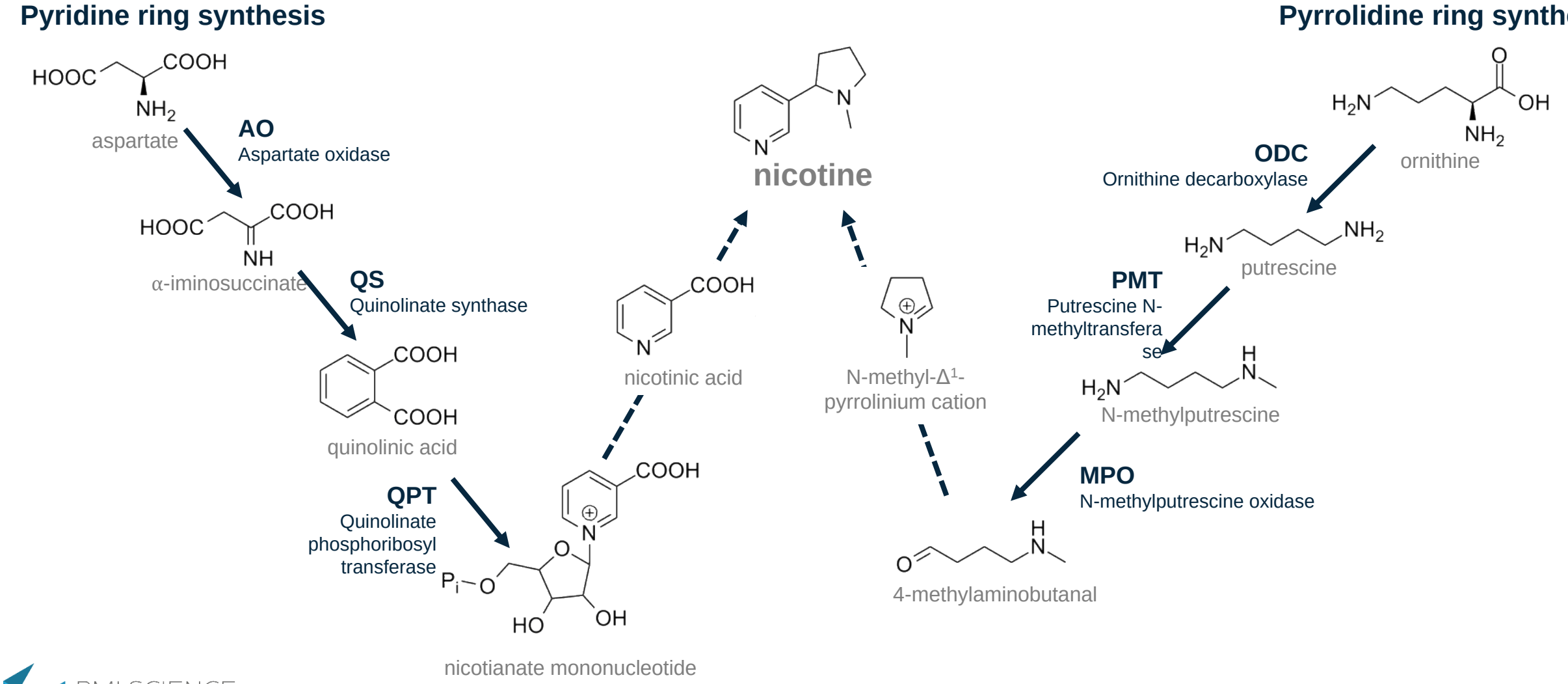


nicotine

Pyrrolidine ring synthesis



Nicotine biosynthesis



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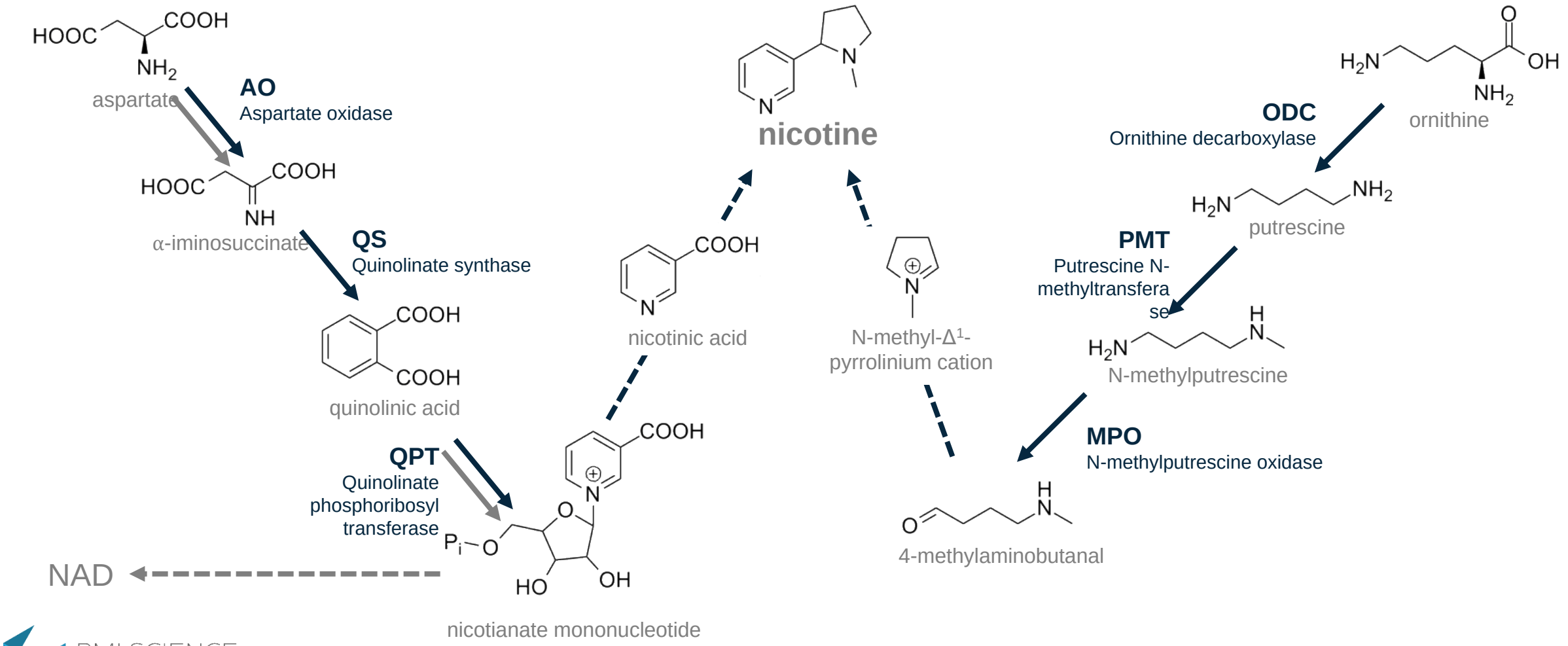
Nicotine biosynthesis



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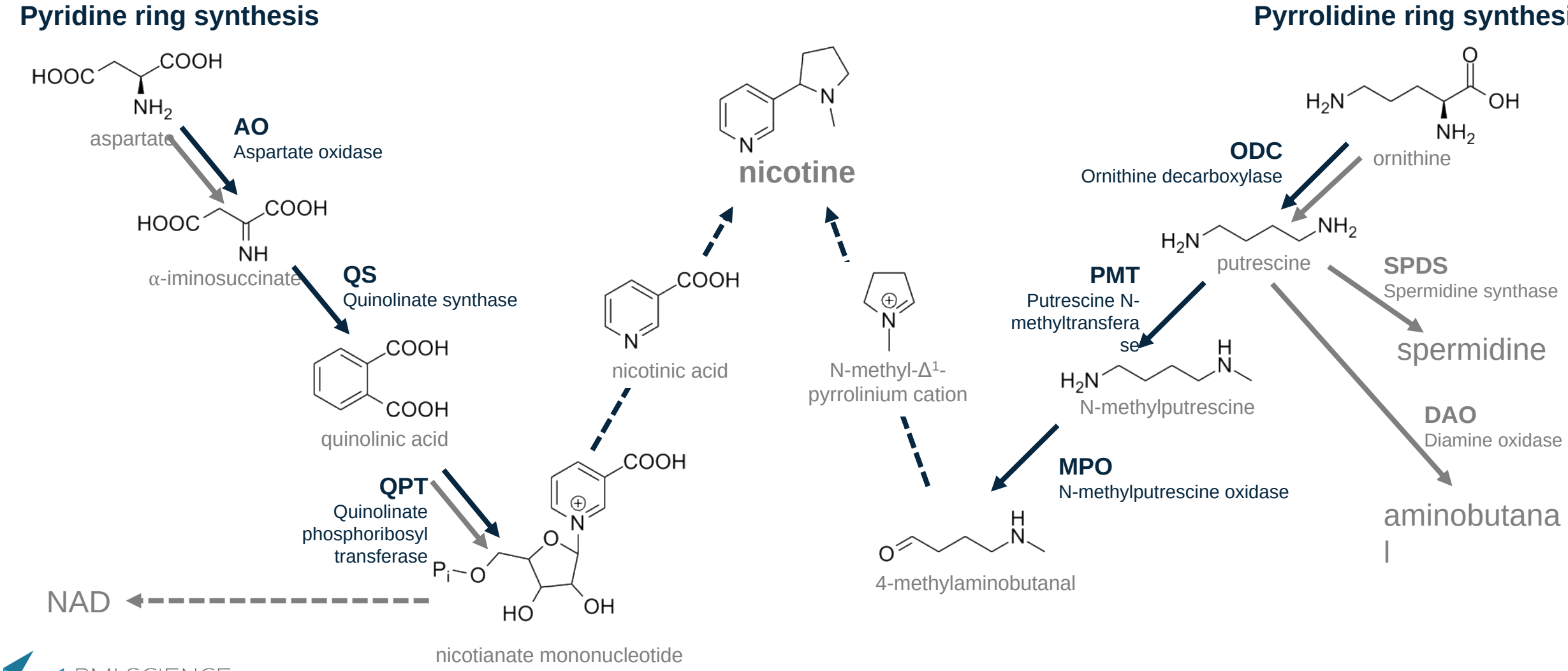
Nicotine biosynthesis



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Nicotine genetics

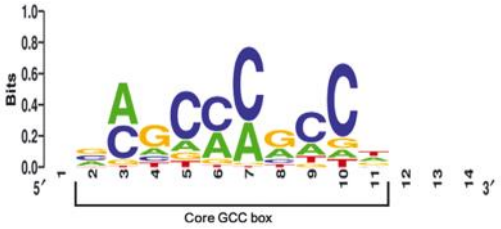
- Controlled by two distinct loci: NIC1 and NIC2
  - Their mutant alleles *nic1* and *nic2* have been used to breed low-nicotine tobacco cultivars
- At the NIC2 locus, genes encoding closely related ETHYLENE RESPONSE FACTOR (ERF) transcription factors are clustered
  - At least 7 of them are deleted in the *nic2* mutant[1]
- NIC2-locus ERF and their homologs are induced by jasmonate[1]
- Salt-stress induces expression of most ERFs but not ERF189 and its closest homolog ERF199[2]

[1]Shoji T, Kajikawa M, Hashimoto T (2010) Clustered transcription factors regulate nicotine biosynthesis in tobacco. Plant Cell 22: 3390-3409

[2]Shoji T, Hashimoto T (2015) Stress-induced expression of NICOTINE2-locus genes and their homologs encoding Ethylene Response Factor transcription factors in tobacco. Phytochemistry 113: 41-49

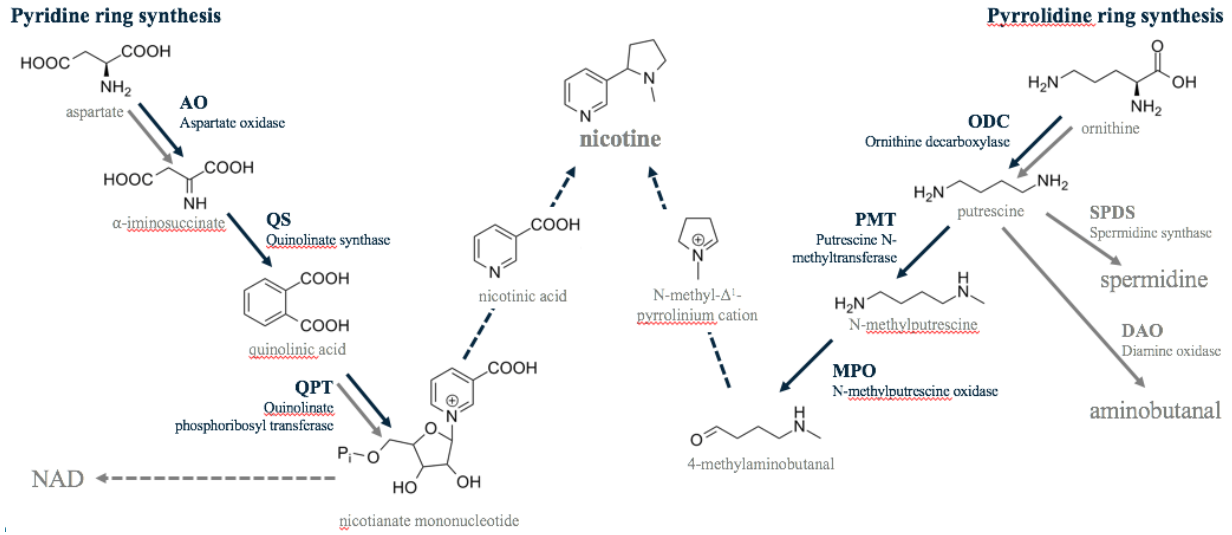
Nicotine biosynthesis pathway regulation

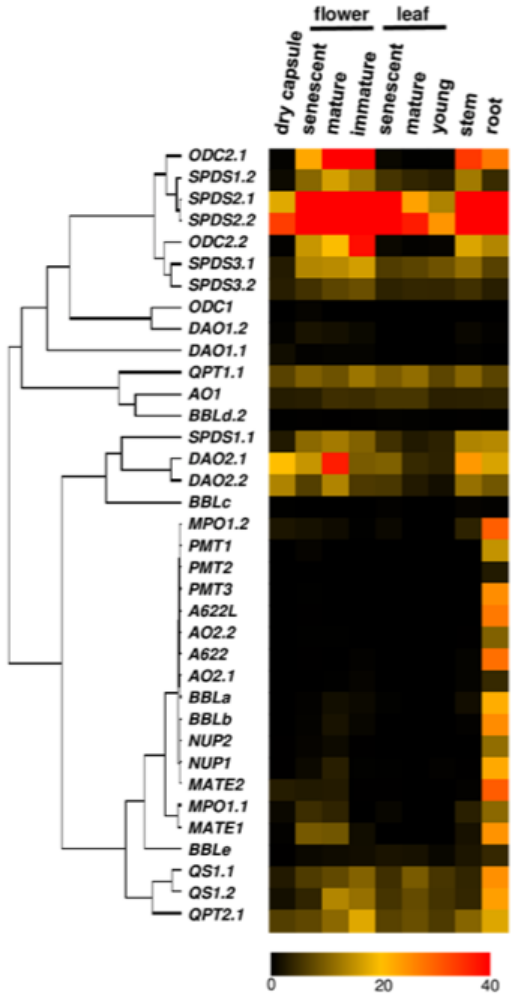
- ERF189 and ERF199
  - Master transcription factors regulating the nicotine pathw
  - Induced by jasmonate
  - Recognize GC-rich P box elements in the gene promoter

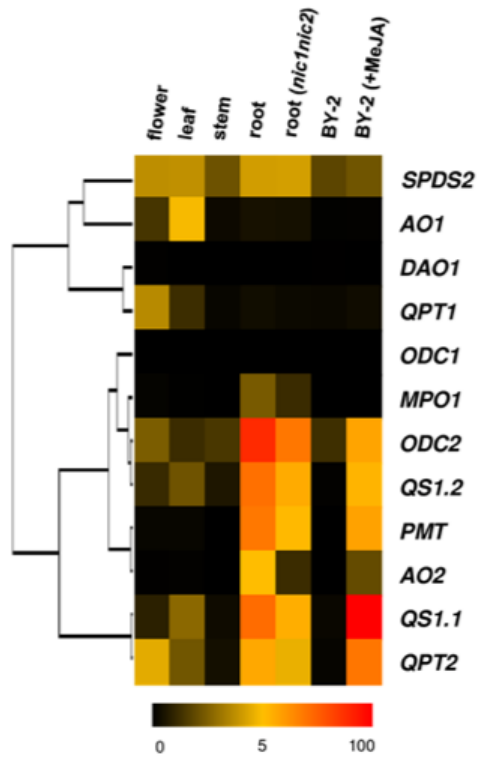


- MYC2
  - bHLH-family transcription factor
  - Key component in conserved jasmonate signaling
  - Binding to G box elements in the gene promoters

Genes of the nicotine biosynthesis pathway



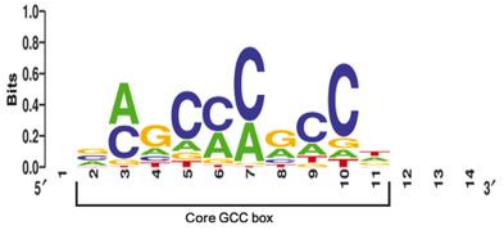




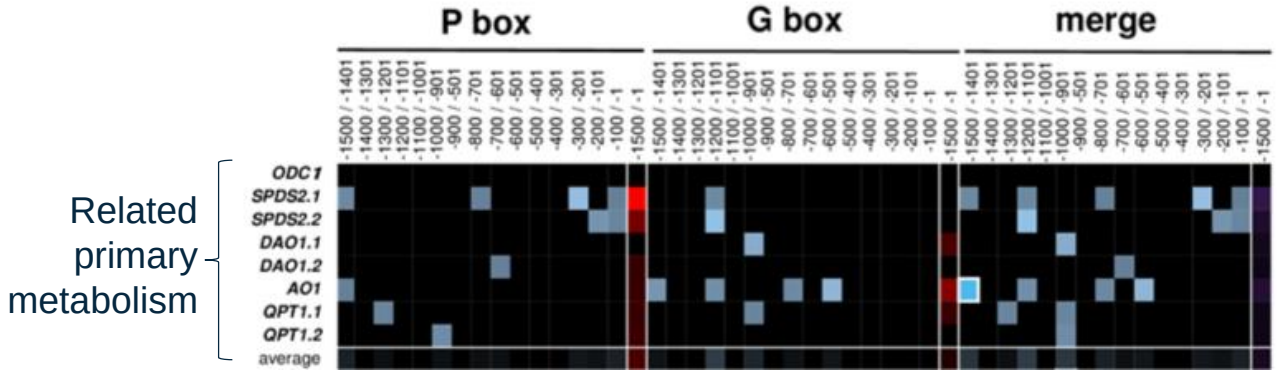
Cis-regulatory elements

P box and G box enrichment in the promoters of metabolic and transport genes involved in nicotine and related primary metabolism

P box



G box



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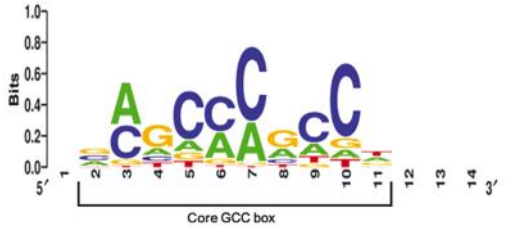
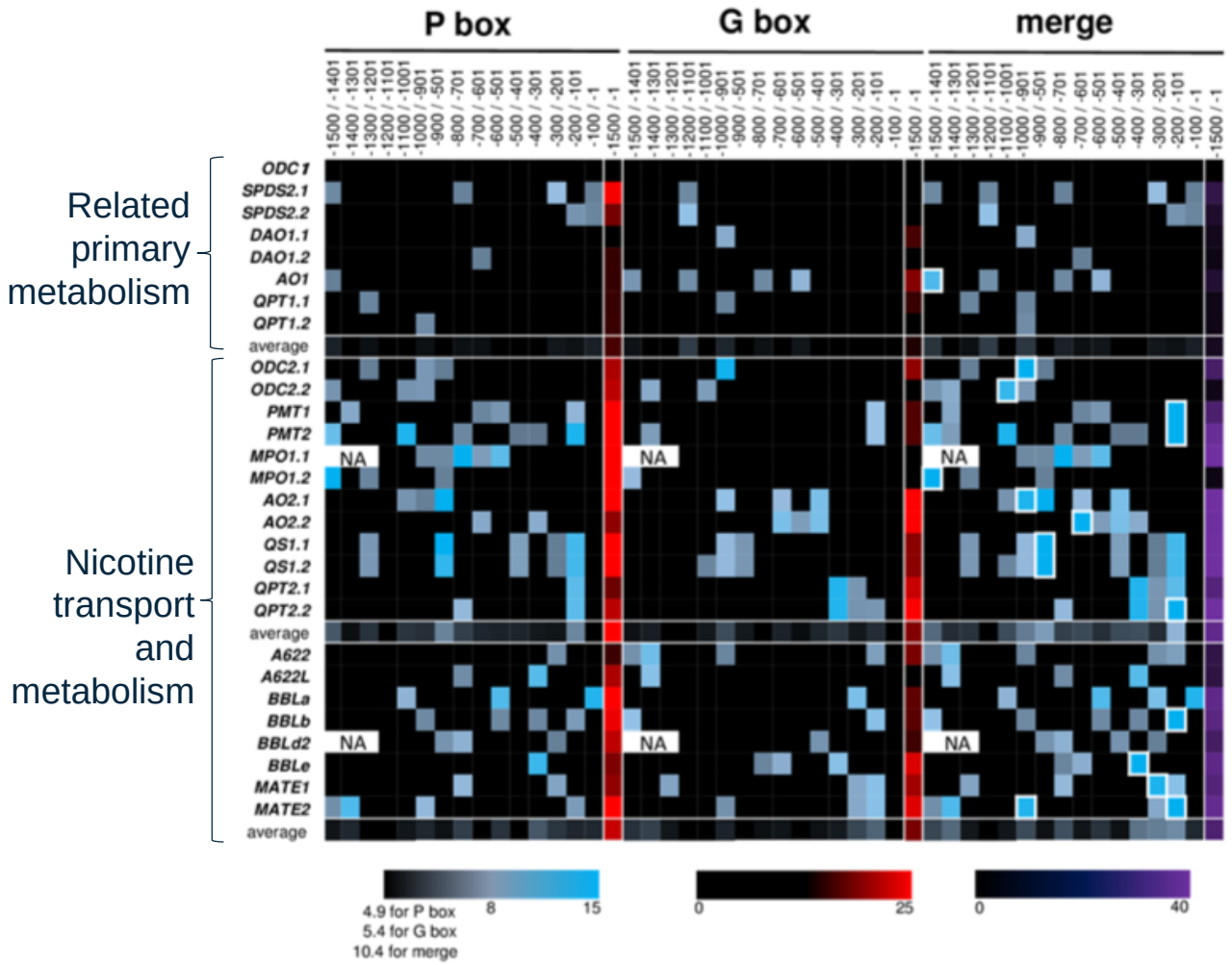


G box



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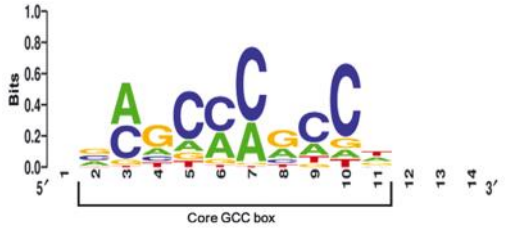
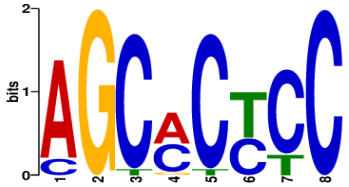
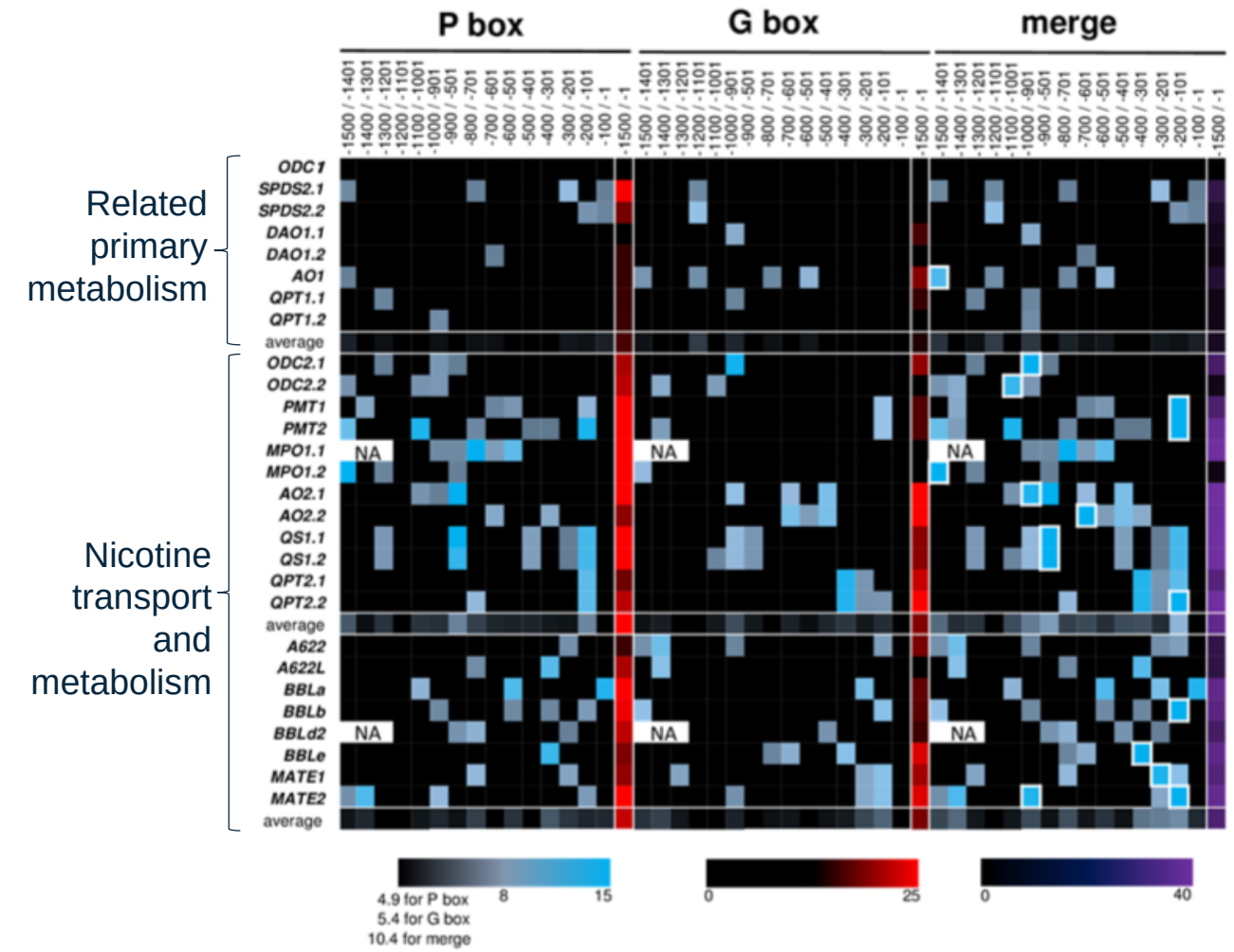


G box

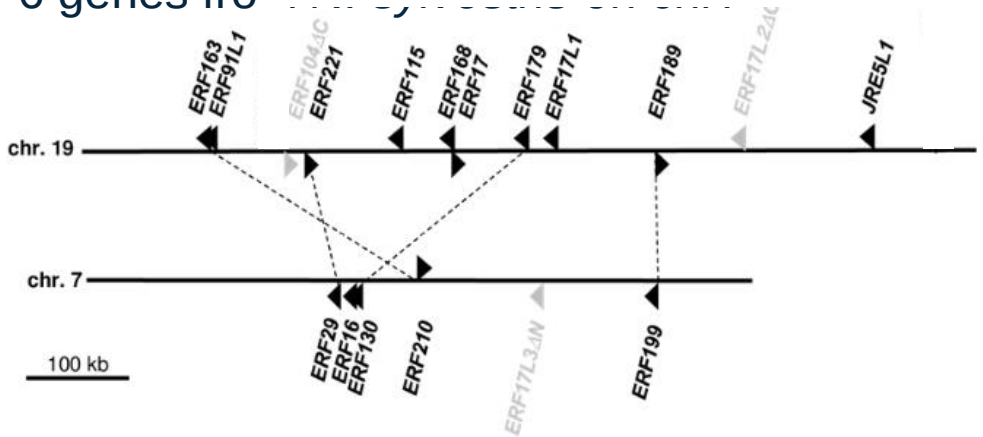
Conserved motif prediction in the promoters of metabolic and transport genes involved in nicotine and metabolism using MEME



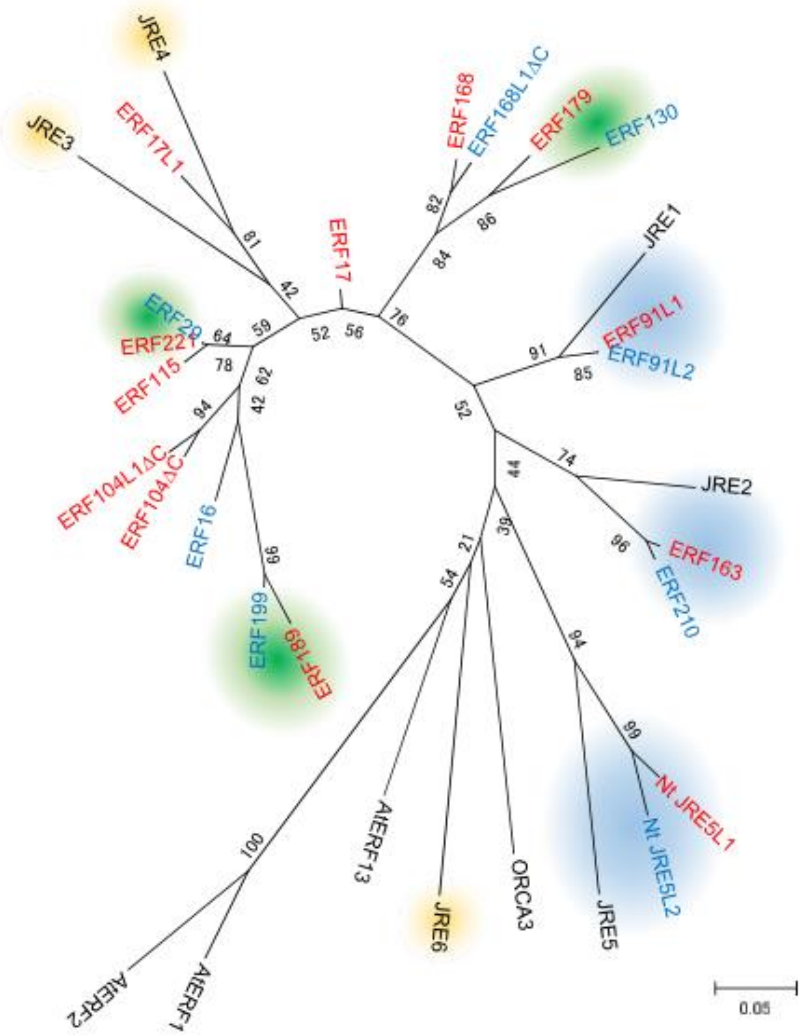


NIC2-locus ERF genes and their homologs

- 22 ERF genes retrieved from the tobacco genome
- Two clusters of multiple ERF genes
  - 12 genes from *N. tomentosiformis* on chr19
  - 6 genes fro



Dashed line indicate functional orthologs



Background

Solanaceae

Tomato

Nicotiana

Text

Tobacco S-genome

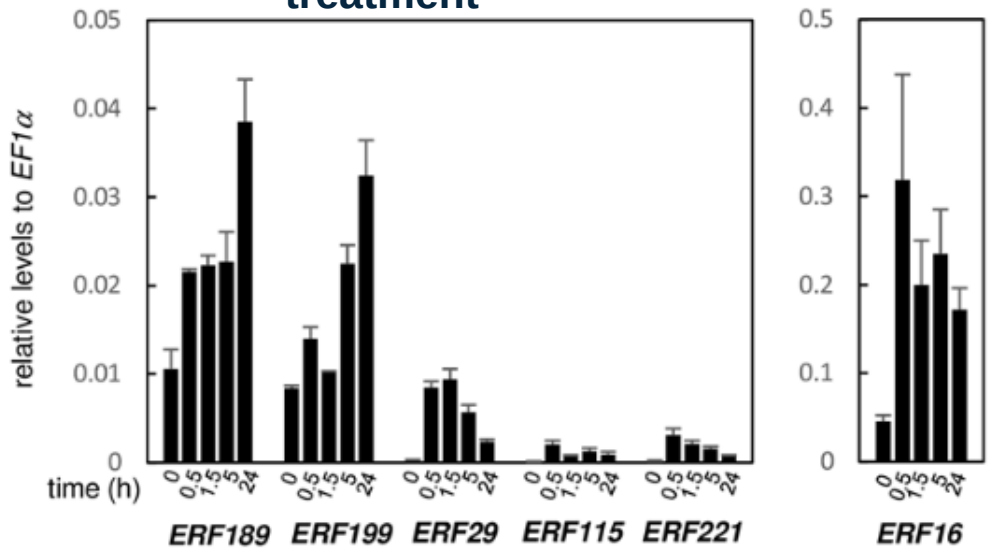
Tobacco T-genome

Tomato

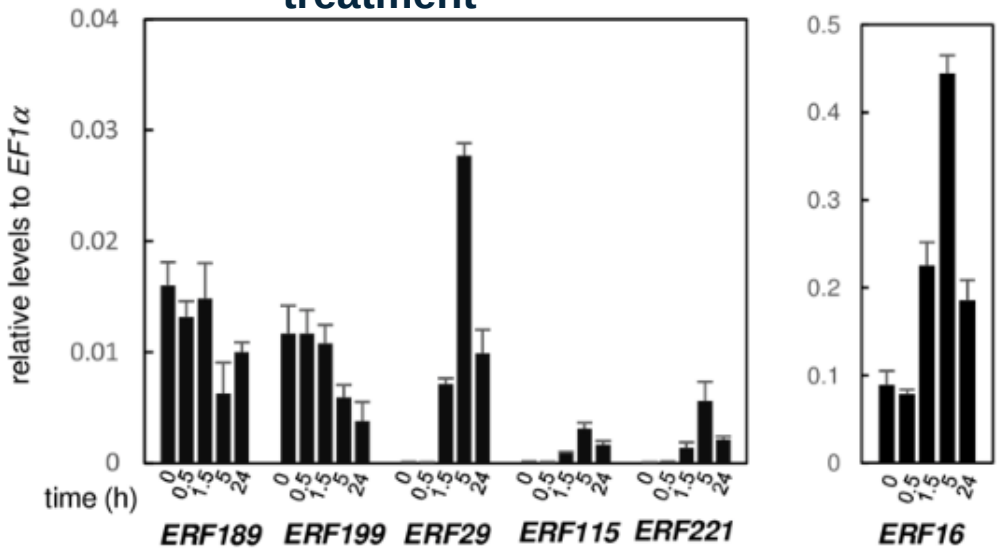
Response to jasmonate and salt stress

Jasmonate and salt stress in tobacco hairy roots was analyzed by qRT-PCR

100 µM MeJA treatment

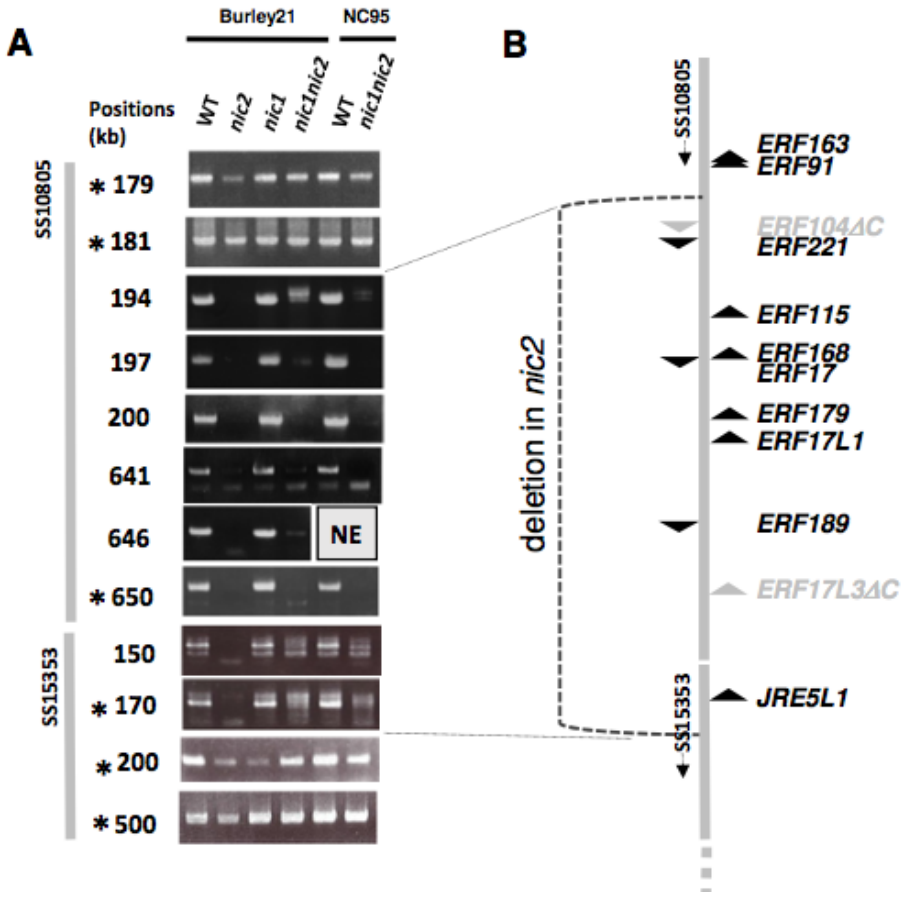


300 mM NaCl treatment



ERF16 originate from *N. sylvestris* and had no identified homolog from *N. tomentosiformis*

Deletion in *nic2* mutant

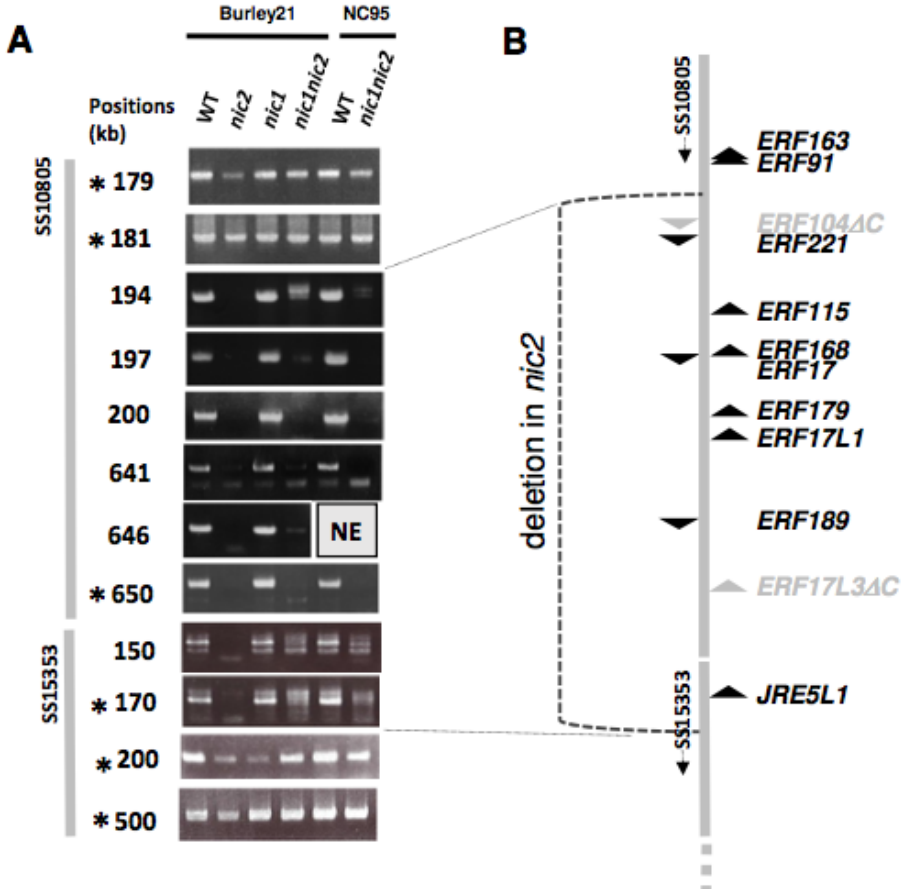


* specific amplifications confirmed by sequencing of amplified PCR fragment

Genomic PCR analysis of positions around the NIC2-locus gene cluster was performed in various *NIC* genotypes in two cultivars, Burley21 and NC95.

These PCR results are in line with gene expression results in *nic2* mutant.

Deletion in *nic2* mutant



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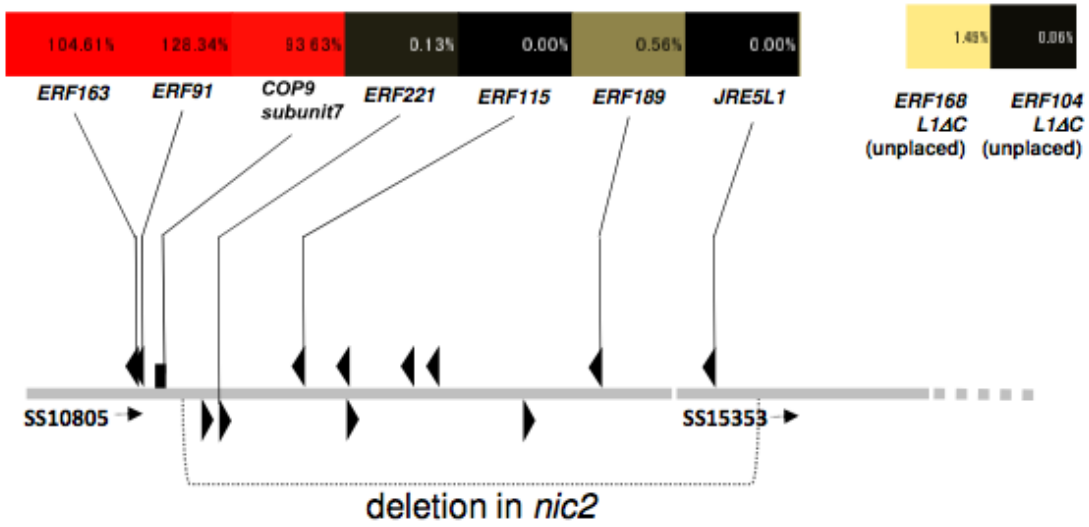

Conclusions

- Enzymes involved in nicotine biosynthesis and transport are expressed almost exclusively in roots
  - Different set of homolog enzymes for the related primary pathways
  - Promoters of their encoding genes are enriched in P box and G box elements
    - Controlled by ERF189/199 and Myc2 transcription factors
    - Not the case for their related primary pathways homologs
- 6 ERF genes of *N. sylvestris*-origin are clustered on chr7, 12 ERF genes of *N. tomentosiformis*-origin are clustered chr19
  - 4 pairs of functional orthologs
- Burley21 and NC95 *nic2* mutants have a deletion of about 650kb covering 10 out of the 12 ERF genes clustered on chr19

Acknowlegments

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  - Masataka Kajikawa
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  - James Battey
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Questions?