

# Nicotine biosynthesis pathway: beyond tobacco

Masataka Kajikawa, Nicolas Sierro, Haruhiko Kawaguchi, Nicolas Bakaher, <u>Nikolai V Ivanov</u>, Takashi Hashimoto, Tsubasa Shoji

#### 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. tabacum





- 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







#### Pyridine ring synthesis





nicotianate mononucleotide

nicotine









nicotianate mononucleotide





nicotianate mononucleotide





nicotianate mononucleotide

#### **Pyridine ring synthesis** Pyrrolidine ring synthesis COOH HOOC $H_2N$ OH $NH_2$ AO $NH_2$ aspartate ODC Aspartate oxidase ornithine nicotine Ornithine decarboxylase COOH HOOC $NH_2$ $H_2N$ NH putrescine **SPDS** QS **PMT** α-iminosuccinate COOH Spermidine synthase Quinolinate synthase Putrescine N- $\oplus$ N methyltransfera COOH spermidine Se N-methyl- $\Delta^1$ nicotinic acid $H_2N$ pyrrolinium cation N-methylputrescine COOH DAO quinolinic acid Diamine oxidase COOH **MPO QPT** N-methylputrescine oxidase $\oplus$ Quinolinate aminobutana phosphoribosyl transferase Pi~0 $\bigcap$ 4-methylaminobutanal NAD OH



nicotianate mononucleotide

HO

9

#### **Nicotine genetics**

- Controlled by two distinct loci: NIC1 and NIC2
  - Their mutant alleles *nic1* and *nic2* have been used to breed lownicotine 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<sup>1</sup>
- NIC2-locus ERF and their homologs are induced by jasmonate<sup>1</sup>
- Salt-stress induces expression of most ERFs but not ERF189 and its closest homolog ERF199<sup>2</sup>

<sup>1</sup>Shoji T, Kajikawa M, Hashimoto T (2010) Clustered transcription factors regulate nicotine biosynthesis in tobacco. Plant Cell 22: 3390-3409 <sup>2</sup>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





www.pmiscience.com

#### **Cis-regulatory elements**

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



Related primary metabolism





#### **Cis-regulatory elements**

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



ODC 1 SPDS2.1 Related SPDS2.2 DA01.1 primary -DA01.2 A01 metabolism **QPT1.1** QPT1.2 average ODC2.1 ODC2.2 PMT PMT: MPO1.1 NA MPO1.2 AO2.1 A02.2 QS1.1 Nicotine QS1.2 QPT2.1 transport QPT2.2 average and A622 A622L metabolism BBLa BBLb BBLd2 BBLe MATE MATE<sub>2</sub> averac 4.9 for P box 5.4 for G box 10.4 for merge

G box

merge

P box



### **Cis-regulatory elements**

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



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





G box P box merge ODC SPDS2.1 Related SPDS2.2 DA01.1 primary -DA01.2 AO1 metabolism QPT1.1 QPT1.2 average ODC2.1 ODC2.2 MPO1. **MPO1.2** AO2. A02.2 QS1.1 Nicotine QS1.2 QPT2.1 transport QPT2.2 average and A622 A622L metabolism **BBL**a BBLL BBLd2 BBL MATE MATE2 4.9 for P box 5.4 for G box 10.4 for merge

## 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



Dashed line indicate functional





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



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

PMI SCIENCE PHILIP MORRIS INTERNATIONAL Genomic PCR analysis of positions around the NIC2locus 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



\* specific amplifications confirmed by sequencing of amplified PCR fragment

Genomic PCR analysis of positions around the NIC2locus 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.





#### 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 orthology
  - 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

#### NAIST

- Masataka Kajikawa
- Haruhiko Kawaguchi
- Takashi Hashimoto
- Tsubasa Shoji

#### • PMI

- Nicolas Bakaher
- Sonia Ouadi
- Jerome Thomas
- James Battey
- Nicolas Sierro
- Manuel C. Peitsch



# Questions?



www.pmiscience.com