Nicotine biosynthesis pathway: beyond tobacco

Masataka Kajikawa, Nicolas Sierro, Haruhiko Kawaguchi, Nicolas Bakahe, Nikolai V Ivanov, Takashi Hashimoto, Tsubasa Shoji

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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*
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 molecule](image)
Nicotine biosynthesis

**Pyridine ring synthesis**

\[
\text{HOOC-NH}_2\rightarrow\text{COOH}\rightarrow\text{AO (Aspartate oxidase)}
\]

\[
\text{HOOC-\(\alpha\)-iminosuccinate}\rightarrow\text{QS (Quinolinate synthase)}
\]

\[
\text{quinolinic acid}\rightarrow\text{QPT (Quinolinate phosphoribosyl transferase)}
\]

\[
\text{nicotianate mononucleotide}\rightarrow\text{nicotine}
\]
Nicotine biosynthesis

**Pyridine ring synthesis**

1. Aspartate oxidase (AO)
2. Aspartate (aspartate)
3. α-iminosuccinate
4. Quinolinate synthase (QS)
5. Quinolinate (quinolinic acid)
6. Quinolinate phosphoribosyltransferase (QPT)
7. Nicotianate mononucleotide

**Pyrrolidine ring synthesis**

1. Ornithine decarboxylase (ODC)
2. Ornithine (ornithine)
3. Putrescine N-methyltransferase (PMT)
4. Putrescine N-methylputrescine oxidase (MPO)
5. 4-methylaminobutanal
Nicotine biosynthesis

**Pyridine ring synthesis**

- Aspartate oxidase (AO)
- Quinolinate synthase (QS)
- Quinolinate phosphoribosyltransferase (QPT)
- Ornithine decarboxylase (ODC)
- Putrescine N-methyltransferase (PMT)
- N-methylputrescine oxidase (MPO)

**Pyrrolidine ring synthesis**

- Aspartate
- α-Iminosuccinate
- Nicotinic acid
- Nicotianate mononucleotide
- N-methyl-Δ¹-pyrrolinium cation
- 4-Methylaminobutanal
Nicotine biosynthesis

Pyridine ring synthesis

HOOC₅H₄NO₂ → nicotine

α-iminocarboxylate → quinoline → nicotine

AO: Aspartate oxidase
QS: Quinolinate synthase
QPT: Quinolinate phosphoribosyl transferase

NAD

Pyrrolidine ring synthesis

H₂N(CH₂)₄NH₂ → putrescine

ornithine decarboxylase (ODC)
Putrescine N-methyltransferase (PMT)
N-methylputrescine oxidase (MPO)

4-methylaminobutanal

Nicotine mononucleotide
Nicotine biosynthesis

Pyridine ring synthesis

\[
\text{nicotinate} \rightarrow \text{nicotinic acid} \rightarrow \text{nicotine}
\]

\begin{align*}
\text{aspartate} &\rightarrow \alpha\text{-iminocitrate} \\
\text{AO} &\rightarrow \text{nicotinate} \\
\text{QS} &\rightarrow \text{nicotinic acid} \\
\text{QPT} &\rightarrow \text{nicotine}
\end{align*}

\[
\text{NAD} \rightarrow \text{nicotianate mononucleotide}
\]

Pyrrolidine ring synthesis

\[
\text{ornithine} \rightarrow \text{putrescine} \rightarrow \text{N-methylputrescine} \rightarrow \text{nicotine}
\]

\begin{align*}
\text{ornithine} &\rightarrow \text{putrescine} \\
\text{ODC} &\rightarrow \text{N-methylputrescine} \\
\text{PMT} &\rightarrow \text{nicotine}
\end{align*}

\[
\text{spermidine} \rightarrow \text{4-methylaminobutanal} \rightarrow \text{nicotine}
\]

\[
\text{DAO} \rightarrow \text{Diamine oxidase}
\]

\[
\text{SPDS} \rightarrow \text{Spermidine synthase}
\]

\[
\text{MPO} \rightarrow \text{N-methylputrescine oxidase}
\]

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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

• NIC2-locus ERF and their homologs are induced by jasmonate

• Salt-stress induces expression of most ERFs but not ERF189 and its closest homolog ERF199

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Nicotine biosynthesis pathway regulation

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

- 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.
Cis-regulatory elements

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

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 from *N. sylvestris* on chr7

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

ERF16 originate from *N. sylvestris* and had no identified homolog from *N. tomentosiformis*
Deletion in $nic2$ mutant

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.

* specific amplifications confirmed by sequencing of amplified PCR fragment
Deletion in \( \textit{nic2} \) mutant

Genomic PCR analysis of positions around the NIC2-locus gene cluster was performed in various \( \textit{NIC} \) genotypes in two cultivars, Burley21 and NC95.

These PCR results are in line with gene expression results in \( \textit{nic2} \) 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
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Questions?