



Tobacco Alkaloid Genetics (TAG) 2017 Report

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Objectives

- 1. To understand the genetics that control alkaloid formation in tobacco plants.**
- 2. To understand the feasibility of conventional and non-conventional breeding techniques to modify alkaloid formation in tobacco plants.**
- 3. To understand the impact of tobacco alkaloid levels on leaf production and quality.**



❖ Before 2017 CORESTA AP meeting

- To complete the collection of various kinds of publicly available literature and data related to tobacco alkaloids.
- To cluster the alkaloid related factors into different categories including tobacco alkaloid biosynthesis genes, transporters, and regulators.
- To list the known mechanisms of tobacco alkaloid genetic control.

❖ Before 2018 CORESTA Congress

- To complete the collection of various kinds of publicly available literature related to tobacco breeding technology.
- To identify conventional and non-conventional technologies used in tobacco breeding to modify alkaloid levels.
- To make a summary of techniques used in modification of tobacco alkaloids.

❖ Before 2019 CORESTA AP meeting

- To complete the collection of various kinds of publicly available literature related to the evaluation of tobacco quality and production from tobacco plant containing different alkaloid levels.
- To draft a final report of the TAG TF.

❖ **Work had finished**

- **Collected available literature and data related to tobacco alkaloids**
- **Alkaloid biosynthesis**
- **Alkaloid transporters**
- **Alkaloid regulation**





Alkaloid in tobacco

❖ Commercial tobacco cultivars: Alkaloid 2%-4% of total dry weight

- Nicotine ----- 90% of the total alkaloid content
 - Nornicotine
 - Anatabine
 - Anabasine
- } Nearly 10%



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- The diagram illustrates the metabolic pathway of nicotine synthesis in tobacco plants, showing the conversion of various precursors into the final product, Nicotine.
- Left Pathway (Anabasine and Anatabine):**
- Lysine** is converted to **Cadaverine**, then to **5-aminopentanal**, and finally to **Δ^1 -piperidine**.
 - Δ^1 -piperidine** is converted to **Anabasine**.
 - Anabasine** is converted to **Anatabine** via a step labeled **BBL?**.
- Central Pathway (Nicotinic Acid and Nicotine):**
- Aspartic acid** is converted to **α -iminosuccinic acid**.
 - Gly-3-P** and **α -iminosuccinic acid** are converted to **Quinolinic acid**.
 - Quinolinic acid** is converted to **NAMN** via a step labeled **QPT** (highlighted with a red circle).
 - NAMN** is converted to **Nicotinic Acid** via a step labeled **NAD**.
 - Nicotinic Acid** is converted to **3,6 dihydronicotinic acid** via a step labeled **A622?**.
 - 3,6 dihydronicotinic acid** is converted to **Nicotine** via a step labeled **BBL?**.
 - 3,6 dihydronicotinic acid** is also converted to **2,5 dihydropyridine** via a step labeled **BBL?**.
 - 2,5 dihydropyridine** is converted to **Anatabine** via a step labeled **BBL?**.
- Right Pathway (Putrescine and Nicotine):**
- Arginine** is converted to **Agmatine** via a step labeled **ADC**.
 - Agmatine** is converted to **N-Carbamoyl putrescine**.
 - N-Carbamoyl putrescine** is converted to **Putrescine**.
 - Putrescine** is converted to **N-methylputrescine** via a step labeled **PMT** (highlighted with a red circle).
 - N-methylputrescine** is converted to **4-methylaminobutanol** via a step labeled **MPO**.
 - 4-methylaminobutanol** is converted to **N-methyl- Δ^1 -pyrrolinium**.
 - N-methyl- Δ^1 -pyrrolinium** is converted to **Nicotine** via a step labeled **NND**.
- Chemical Structures:**
- Anabasine**: C1CCN1c2cccnc2
 - Anatabine**: C1CCN1c2cccnc2
 - Nicotinic Acid**: N#Cc1cccnc1C(=O)O
 - 3,6 dihydronicotinic acid**: N#Cc1cccnc1C(=O)O
 - 2,5 dihydropyridine**: C1=CC=CC=C1
 - Nicotine**: CN1CCCC1c2cccnc2
 - Nornicotine**: C1CCN1c2cccnc2

Fig. 1. Schematic diagram of alkaloid biosynthesis in *N. tabacum*.

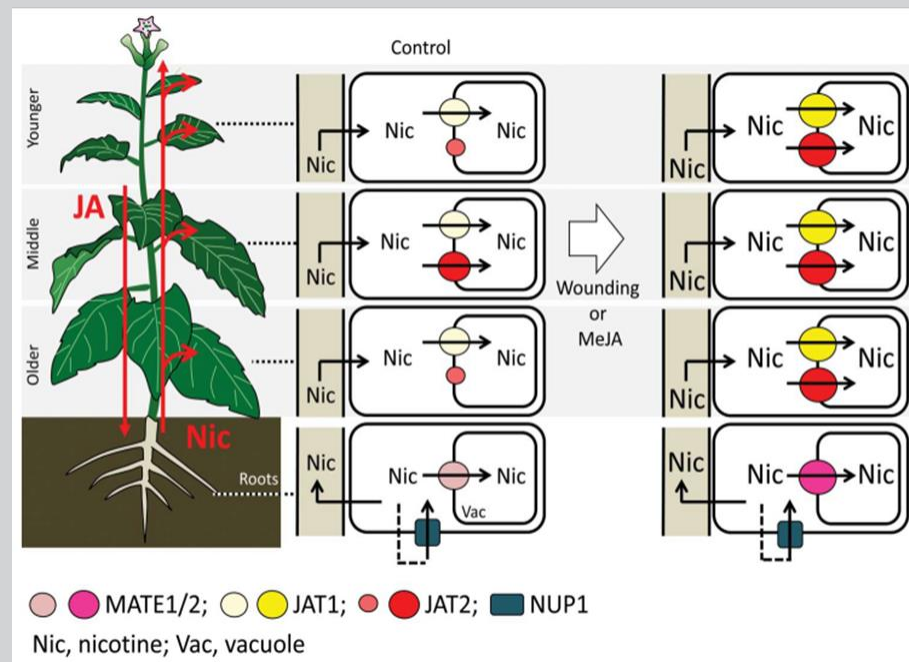
Alkaloid transporters

❖ Root tip: NUP1

❖ Root: JAT1, MATE1/2

❖ Leave: JAT2

- MATE1/2: Multidrug and toxic compound extrusion 1/2
- JAT1/2: Jasmonate-inducible alkaloid transporter 1/2
- NUP1: Nicotine uptake permease1

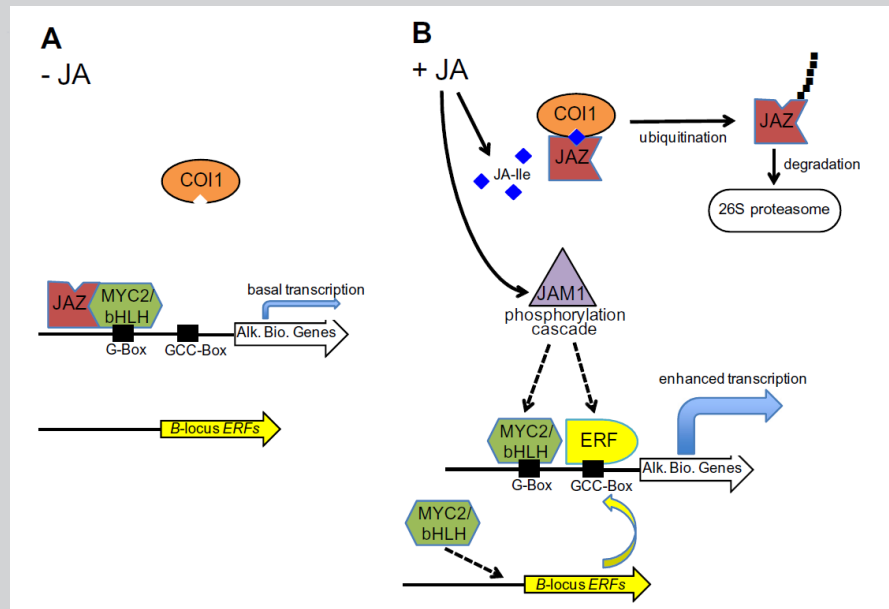


Shitan.et al. (2015)

Fig. 2. Hypothetical model of nicotine transporter functions in *N. tabacum*.

Alkaloid Regulators

- ❖ ERF:189, 221
- ❖ MYC2/bHLH
- ❖ COI1,JAZ
- ❖ JAM1

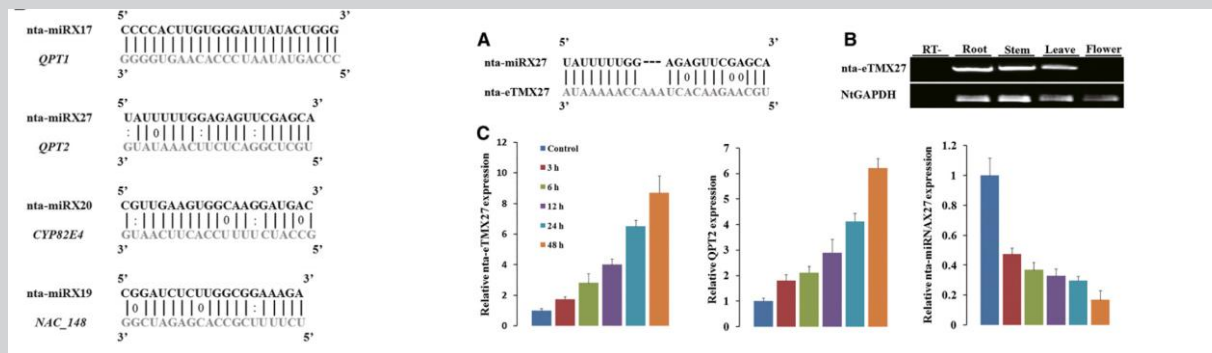


Dewey and Xie (2013)

Fig. 3. Model depicting our current understanding of JA-stimulated induction of alkaloid biosynthetic genes in tobacco

Transcriptional regulation of Alkaloid

- ❖ Nicotine Biosynthesis-Related miRNAs(miRX17,27)
- ❖ Novel tobacco miRNA (nta)-eTMX27 in regulation of nicotine biosynthesis by acting as a decoy of nta-miRX27 to sequester and degrade nta-miRX27 that targets *QPT2*.



Fangfang Li et al. (2015)

Fig. 4. Topping-induced expression of endogenous nta-eTMX27 in tobacco root



Group meeting-Brazil 2017

❖ Meeting Sunday 22 Oct

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THANK YOU