Tobacco Alkaloid Genetics (TAG) 2019 Report

Coordinator: Yongfeng Guo, TRI-CAAS, China

Victoria Falls, Zimbabwe

17 October 2019

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.

Progress

Project No.	Activity	Status	Time
140	Initiation	Completed	March 2017
	Soliciting participants	Completed	January 2018
	Breaking down the report into 9 subtitles	Completed	February 2018
	Assignment of subtitles to participants (9/12)	Completed	April 2018
	Collecting writings	Completed	October 2018
	First draft	Completed	April 2019
	Final report	On-going	December 2019

List of participants

- **Ernie Hiatt** (RJ Reynolds, USA), **Tijs Gilles** (BAT, UK), **Irving J. Berger** (BAT, Brazil), Alkaloid biosynthesis
- **Christelle Bonnet** (JTI, Switzerland), Regulatory mechanisms of alkaloid accumulation
- **Chengalrayan Kudithipudi** (Altria Client Service, USA), Regulatory mechanisms of alkaloid accumulation
- **Ramsey S. Lewis** (NS State University, USA), Traditional breeding for low alkaloid tobacco
- **Hongzhi Shi**, **Mengyue Zhang** (Henan Agricultural University, China), **Marcos Lusso** (Altria Client Service, USA), Leaf production and quality affected by low nicotine tobacco production
- **Barunava Patra**, **Shengming Yang** (University of Kentucky, USA), Transportation of alkaloids
- **Xue Zhao**, **Hongbo Zhang** (Tobacco Research Institute, CAAS, China), Transportation of alkaloids
- **François Dorlhac de Borne** (Imperial Tobacco, France), Introduction
- **Yongfeng Guo**, **Liuying Wen** (Tobacco Research Institute, CAAS, China), **Dongmei Xu** (Altria Client Service, USA), Organization

Structure of the report

- Title: Molecular regulation and genetic manipulation of alkaloid accumulation in tobacco plants
- Abstract
- Introduction
- Alkaloid biosynthesis in tobacco
- Transportation of alkaloids between cells and within the plant
- Regulatory mechanisms of alkaloid accumulation in tobacco
- Development and use of low alkaloid tobacco
- Future prospects for ultra-low alkaloid tobacco

Alkaloid biosynthesis

1. **Formation of nicotinic acid (the pyridine ring);**
2. **Formation of N-methyl- Δ1-pyrrolinium cation (the pyrrolidine ring);**
3. **Condensation of a pyridine ring and a pyrrolidine ring (A662 and BBLs);**
4. **Nornicotine biosynthesis;**
5. **Anabasine biosynthesis;**
6. **Anatabine biosynthesis;**

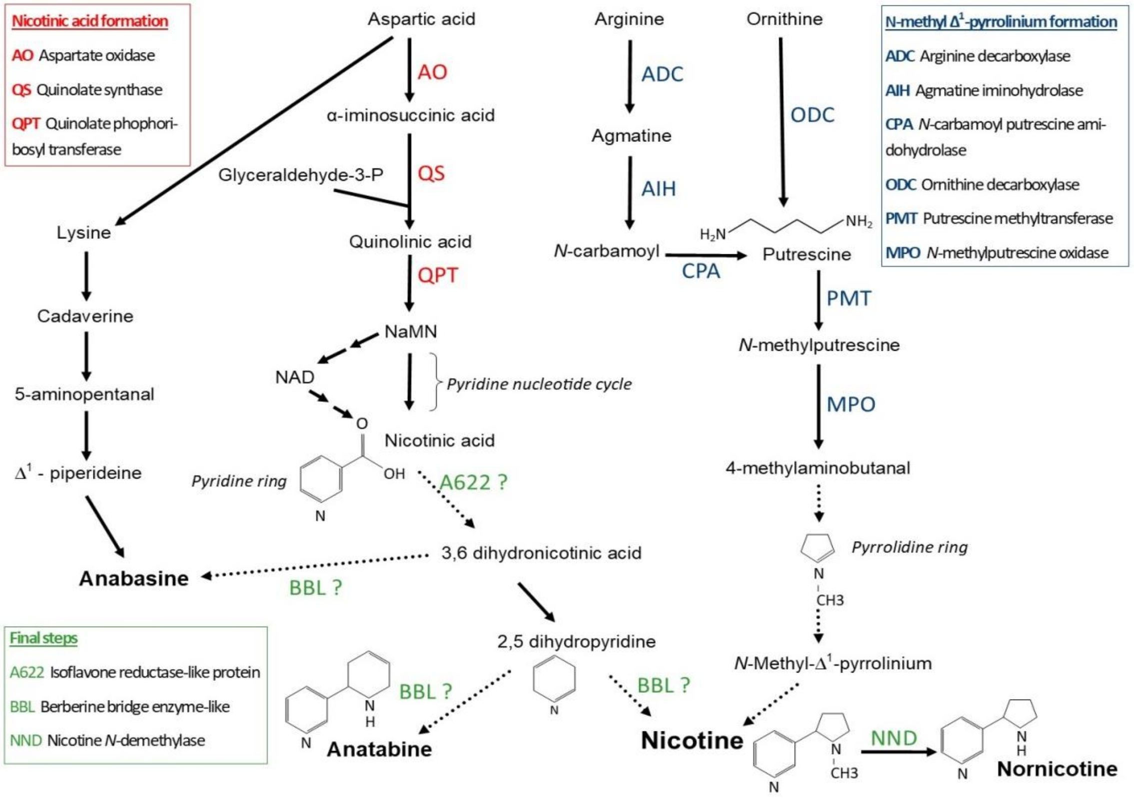


Figure by Christelle Bonnet

Alkaloid transportation

1. **Multidrug and toxic compound extrusion proteins NtMATE 1 and NtMATE2;**
2. **Jasmonate inducible alkaloid transporters JAT1 and JAT2;**
3. **Purine uptake permease-like transporter NUP1;**

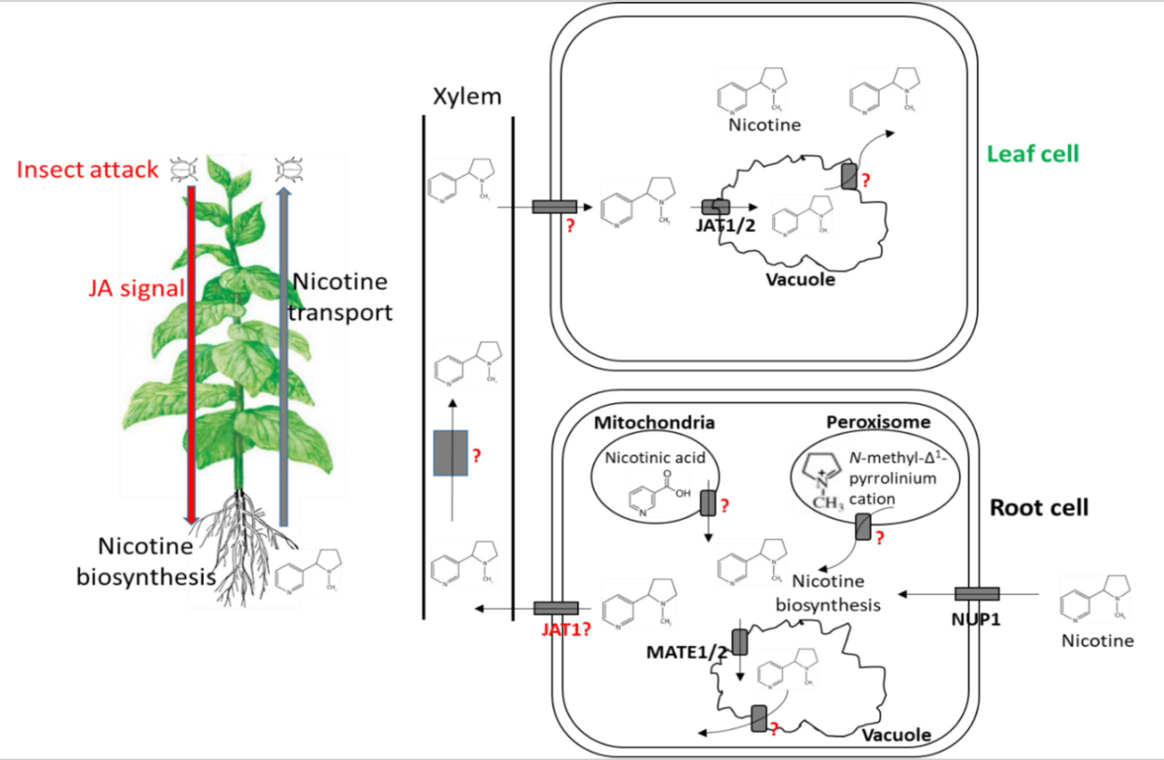


Figure by Shengming Yang

Regulatory mechanisms

1. **The Nic loci and ERF transcription factors;**
2. **The jasmonate pathway and MYC2-like bHLH transcription factors;**
3. **Induction of JA-mediated nicotine accumulation by senescence and abiotic stresses;**
4. **Inhibitory effects of ethylene and auxin on nicotine biosynthesis;**
5. **Small and long non-protein-coding RNAs in nicotine biosynthesis;**

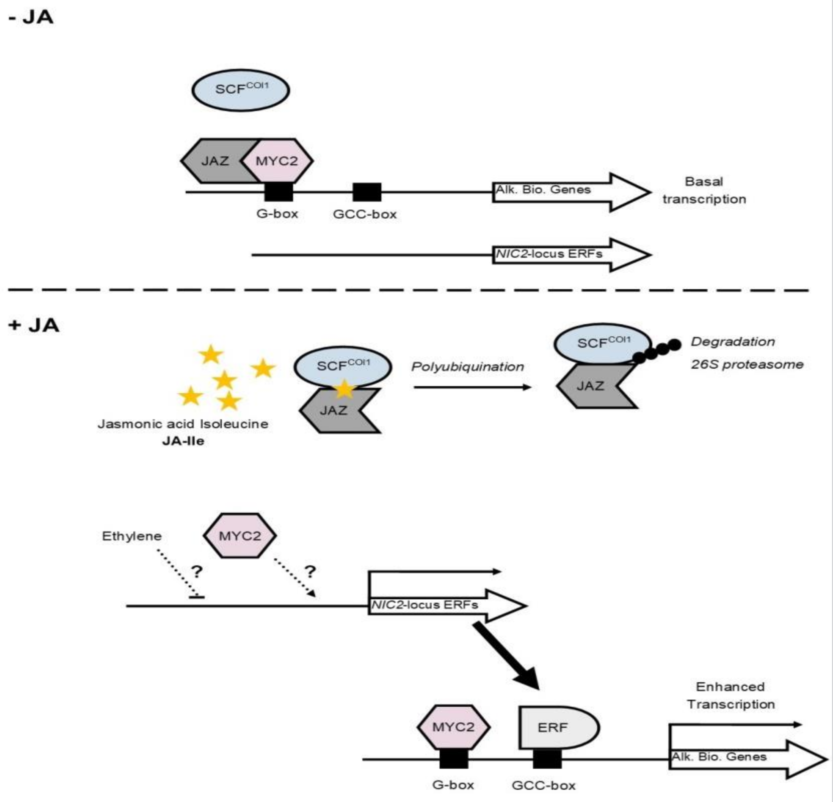


Figure by Christelle Bonnet

Low alkaloid tobacco

1. Low alkaloid tobacco developed by traditional breeding;
2. Low alkaloid tobacco developed by genetic engineering;
3. Reduction of alkaloid accumulation through agronomic practices.

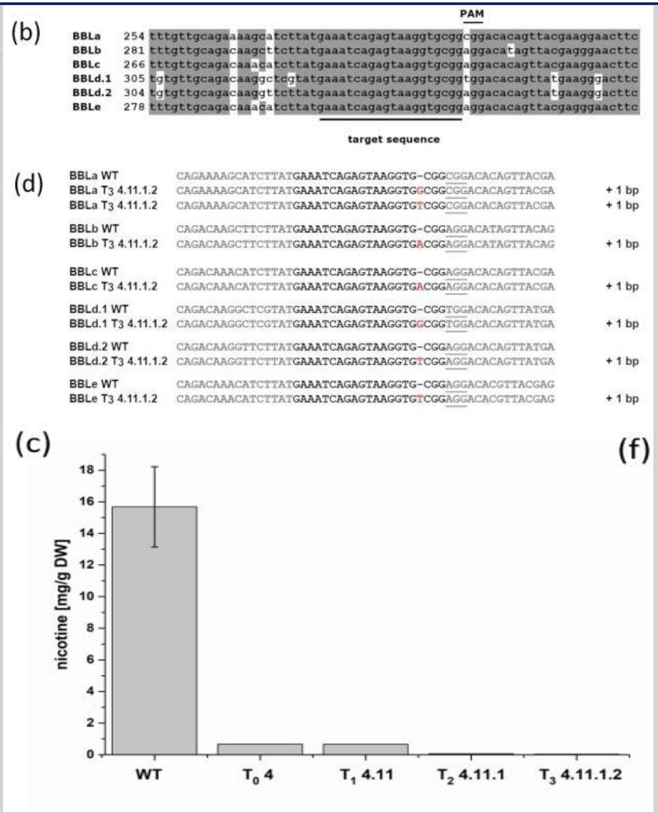
Variability Type	Mechanism	Nicotine (mg/g)	Sample Type	Reference
Wild type	Nic1Nic2	15 – 45	Composite cured leaf sample	Lewis 2018 [121]
Naturally-Occurring	nic1/nic2 (also known as a/b)	2.0 – 2.5[b]	Composite cured leaf sample [c]	Legg and Collins 1971 [118]
		2.99	Composite cured leaf sample	Lewis et al. 2015 [41]
		4.52	Composite cured leaf sample	Lewis 2016 (unpublished data)
Naturally-Occurring	CYP82E4 (nicotine demethylase)	6.45 – 8.33	Fourth leaf from the top	Lewis et al. 2008 [51]
Transgenic	NtQPT1 Antisense	1.44	Composite cured leaf sample	Vector Tobacco Ltd. 2001 [22]
Transgenic	NtPMT Family RNA Interference	0.60	Composite cured leaf sample	Lewis 2014 (unpublished data)
Transgenic	NtPMT Family Co-Suppression	2.20	Composite cured leaf sample	Lewis 2014 (unpublished data)
Transgenic	NtBBL Family RNA Interference	4.14	Composite cured leaf sample	Lewis et al. 2015 [41]
Knockout Mutation	NtBBL Family Inactivation	4.43	Composite cured leaf sample	Lewis 2016 (unpublished data)

Table by Ramsey Lewis

Recent progress

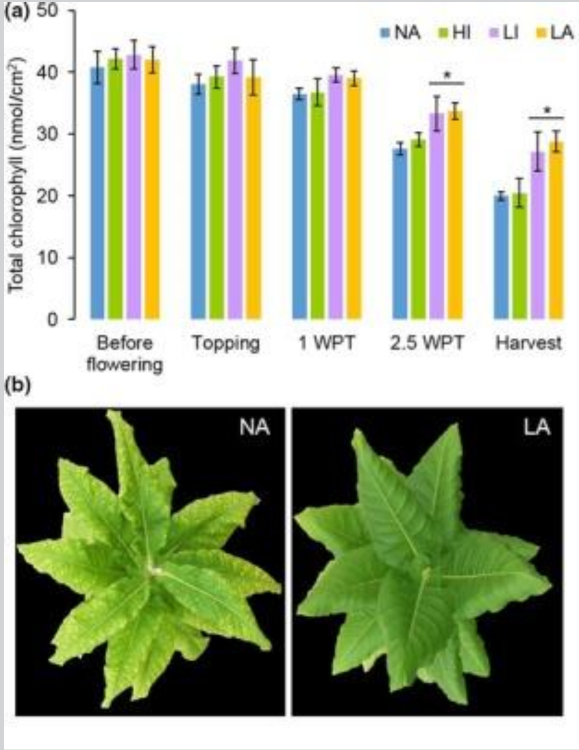
1. Targeted 6 BBL genes
2. 0.04 mg g per DW
3. 99.7% reduction
4. Normal growth

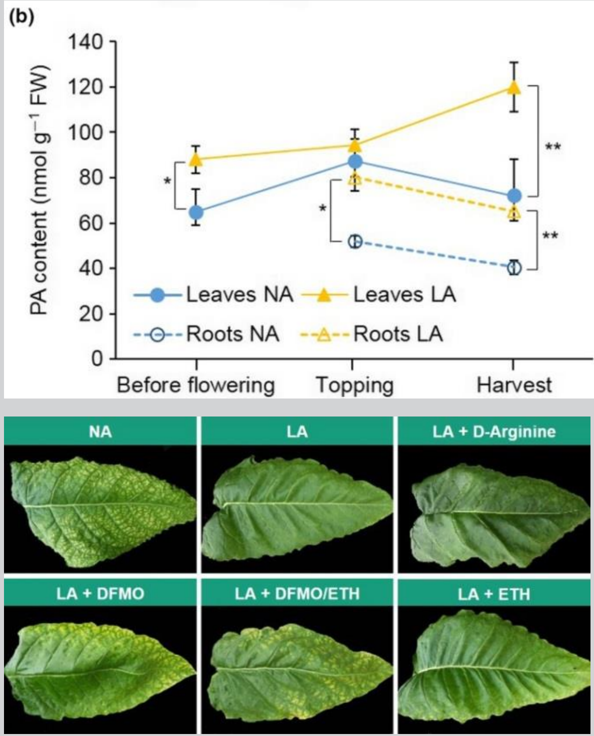
J. Schachtsiek, F. Stehle, Nicotine-free, nontransgenic tobacco (Nicotiana tabacum l.) edited by CRISPR-Cas9. Plant Biotechnol. J. (2019), pp. 1-3, 10.1111/pbi.13193



Recent progress

1. **Slower chlorophyll degradation in LA plants**
2. **LA plants accumulate higher levels of polyamines**
3. **Inhibition of polyamine biosynthesis in LA leaves achieved partial amelioration**





Nölke G, Volke D, Chudobová I, et al. Polyamines delay leaf maturation in low-alkaloid tobacco varieties. Plant Direct. 2018;2:1–12. 10.1002/pld3.77

Group meeting - Zimbabwe 2019

- Meeting Sunday 12 Oct

Issues discussed:

Publication in a peer-reviewed journal

What’s next for the TF

Acknowledgements:

We thank Paolo Donini of JTI, Toru Hiyoshi of JT-LTRC, and Peter Edde of ALCS for their help in reviewing the manuscript. We also thank Eeva Marignac, Colin Fisher, Henri Papenfus, Paul Harp, Masahiro Miyoshi, Bernhard Eitzinger, Xavier Cahours, Anne Fisher, and Mauri Winegardner of CORESTA, and the 2018-2020 Scientific Commission and Board members of CORESTA for critical reading of the manuscript.

THANK YOU