Tobacco Alkaloid Genetics (TAG) Task Force 2018 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.

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	On-going	Early 2019
	Final report	On-going	October 2019

Outline of the TAG TF report and list of participants

- Introduction (the background of this Task Force, the potential mandated cigarette nicotine reduction) (François Dorlhac De Borne, Imperial Tobacco)
- Traditional breeding using low nicotine germplasm materials; (Ramsey Lewis, NC Sate Uni)
- Alkaloids biosynthesis; (Ernie Hiatt, Irving Berger & Tijs Gilles, BAT/RJ Reynolds)
- Transportation of alkaloids between cells and within the plant; (Hongbo Zhang, Tobacco Research Institute, CAAS)
- Regulatory mechanisms of biosynthesis and transportation of tobacco alkaloids; (Christelle Bonnet, JT International)
- Genetic engineering of alkaloid levels based on biosynthetic enzymes; (Dongmei Xu, Altria Client Services)
- Genetic engineering of alkaloid levels based on transporters; (Shengming Yang, Uni Kentucky)
- Genetic engineering of alkaloid levels based on regulatory genes; (Chengalrayan Kudithipudi, Altria Client Services)
- Impact of low alkaloid levels on tobacco leaf production and quality.(Hongzhi Shi, Henan Agricultural Uni; Marcos Lusso, Altria Client Services)

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% (Nornicotine, Anatabine, Anabasine)

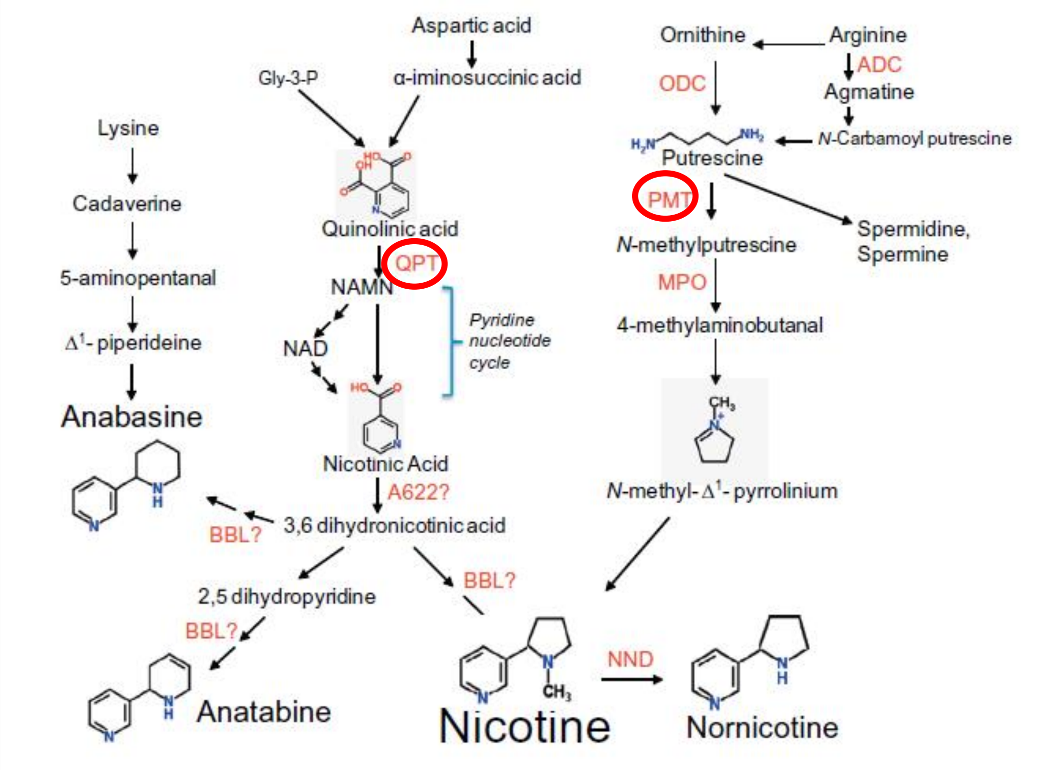
Recent Development in Nicotine Regulation

- In 2015, The WHO Study Group on Tobacco Product Regulation (TobReg) proposed to work on the addictive effect of nicotine. An advisory note was released recommending a lowering of cigarette nicotine levels to below concentrations designated as ‘sub-threshold levels of addiction’.
- In 2017, the FDA commissioner Scott Gottlieb declared “Addressing the addictive levels of nicotine in combustible cigarettes must be part of the FDA’s strategy for addressing the devastating, addiction crisis that is threatening American families”.
- More recently, in March 2018, the FDA announced in an advanced notice of proposed rulemaking to be “particularly interested in comments about the merits of nicotine levels 0.3, 0.4 and 0.5mg nicotine/g of tobacco filler, as well as others levels of nicotine”.

Alkaloid biosynthesis genes

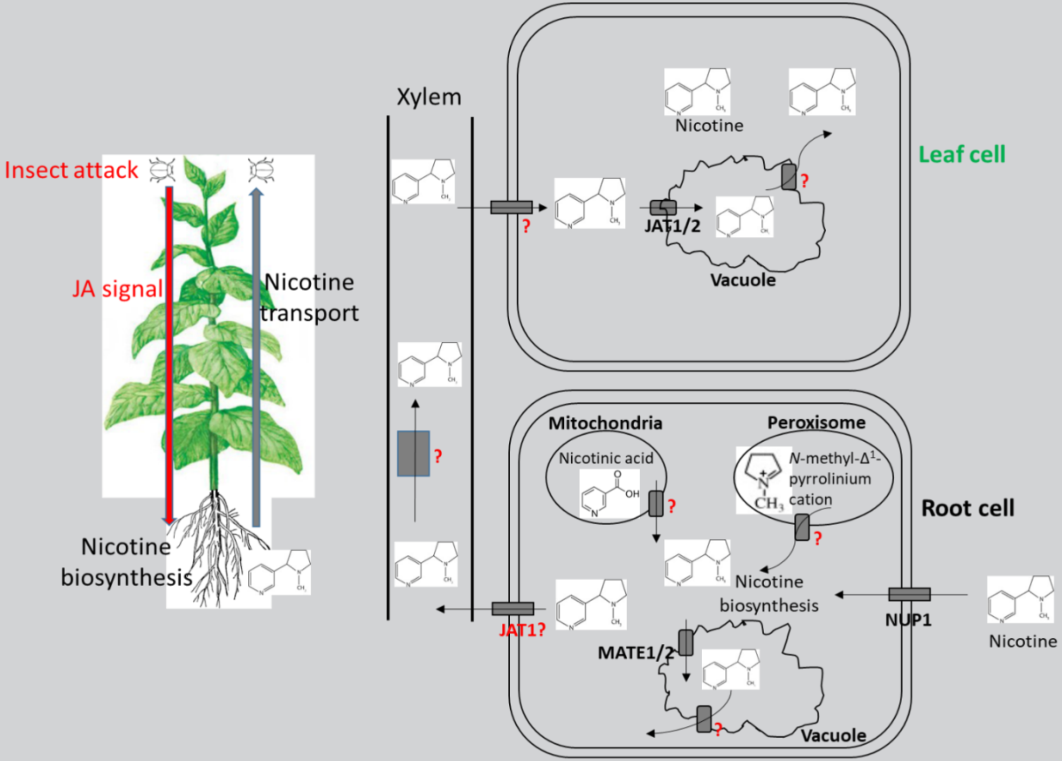
- ❖ Key rate-limiting enzyme
- ❖ PMT: putrescine methyltransferase;
- ❖ QPT: quinolinate phosphoribosyltransferase.
- ❖ ODC: ornithine decarboxylase;
- ❖ ADC: arginine decarboxylase;
- ❖ MPO: N-methylputrescine oxidase;
- ❖ BBL: berberine bridge enzyme-like;
- ❖ A622: isoflavone reductase-like protein;
- ❖ NND: nicotine N-demethylase;

Dewey and Xie (2013)



Alkaloid transporters

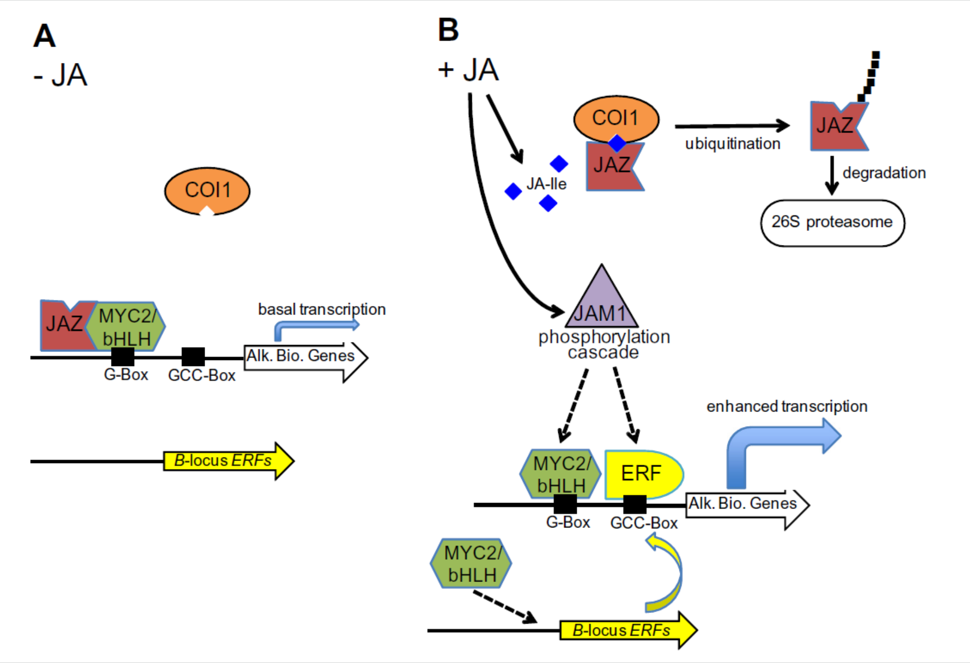
- Root tip: NUP1
- Root: JAT1,MATE1/2
- Leave: JAT2



Shitan et al. (2015)

Alkaloid Regulators

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



Dewey and Xie (2013)

Nicotine Reduction Through Genetic Approaches

Table 1. Nicotine reduction in field-grown[a] plants containing naturally-occurring or induced genetic variation affecting alkaloid accumulation.

Variability Type	Mechanism	Nicotine (mg/g)	Sample Type	Reference
Naturally-Occurring	*nic1*/*nic2* (also known as *a*/*b*)	2.0 – 2.5[b]	Composite cured leaf sample[c]	Legg and Collins 1971
		2.99	Composite cured leaf sample	Lewis et al. 2015
		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
Transgenic	*NtQPT1* Antisense	1.44	Composite cured leaf sample	Vector Tobacco Ltd. 2001
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
Knockout Mutation	*NtBBL* Family Inactivation	4.43	Composite cured leaf sample	Lewis 2016 (unpublished data)

Lewis (2018)

Impact of Low alkaloid levels on tobacco growth and leaf quality

- Reduced yield, delayed leaf maturation and chlorophyll degradation, inferior leaf quality and increased susceptibility to insect damage were observed for the naturally-occurring low alkaloid lines (Chaplin and Weeks, 1976; Chaplin and Burk, 1984).
- Less alteration in agronomic traits was observed in genetically engineered low alkaloid lines. Accumulation of a novel metabolite identified as dihydrometanicotine (DMN) was observed in the roots of transgenic BBL suppressed tobacco plants (Kajikawa et al., 2011; Lewis et al., 2015).
- Low nicotine (0.8%) tobacco leaves produced by grafting on eggplant. The content of chlorophyll was significantly higher and the leaves matured slower. The contents of amino acids increased significantly in grafted tobacco, and the higher levels of protein, starch, total nitrogen and lower levels of sugar contents were also observed (Shi et al. 2018).

Group meeting-Kunming 2018

- Group meeting on Sunday 22 Oct

Discussed:

1) Editing & finalizing the report;

2) Publication of the report;

3) What's next for the Task Force?

Acknowledgement: Dongmei Xu

THANK YOU