CORESTA: Scientific Cooperation in Tobacco Leaf Research and Production

Dr François DORLHAC

CORESTA Scientific Commission / Phytopathology & Genetics

INVESTA

9-13 December 2019

CORESTA

Cooperation Centre for Scientific Research Relative to Tobacco

A non-profit organisation created in 1956 governed by French law

Purpose

To promote cooperation in scientific research relative to tobacco and its derived products

Agronomy and Phytopathology

Agronomy and Leaf Integrity

- Production of all tobacco leaf types
  - Crop management practices and environmental factors that influence crop production
  - Good Agricultural Practices for efficient, sustainable tobacco production and leaf supply
- Guidance on pest & sanitation of stored tobaccos.

Phytopathology and Genetics

- Study of tobacco pests and diseases and plant breeding
- Integrated Pest Management
- Genetic mapping, molecular markers and genetic diversity.

5-Year Roadmap 2018-2022

Three priorities in tobacco leaf and production:

1. **Protecting raw material integrity from issues that originate in the pre-production sectors: Regulations and risk issues of substances in product**

2. **Compliance with International Regulations on Biotechnologies**

3. **Product standard for low nicotine.**

1. Protecting raw material integrity from issues that originate in the pre-production sectors

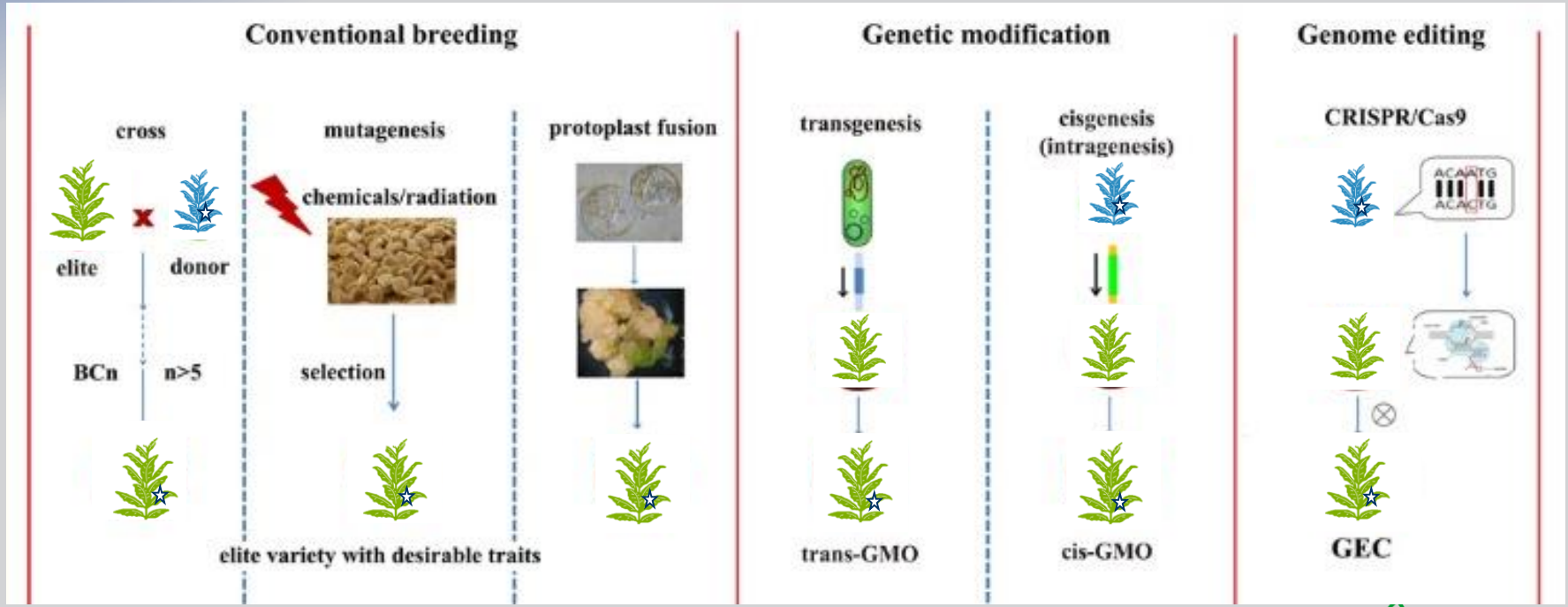
- **CORESTA is developing a range of actions to help the supply chain to limit the risks in the production:**
  - Development of a **tool to identify diseases** at an early stage or in the field
  - Elaboration of a **document** for agronomists & farmers to **promote Integrated Pest Management**
  - Preparation and maintenance of a **list of agrochemicals necessary to sustain successful leaf production** and for which GRLs have to be set or reviewed
  - Assessment of efficacy of **biological and eco-friendly Crop Protection Agents (CPAs)**
  - Studies on specific diseases like **Black shank and viruses**
  - Investigations on new technologies and issues related to **infestation control in post-harvested tobacco**.

1. Protecting raw material integrity from issues that originate in the pre-production sectors

- **Development of an innovative tool to identify diseases and geo-localize**
  - **Di@gnoplant** developed in collaboration with D. Blancard (INRA)
  - Identification of diseases with a **smartphone** app (iOS or Android).

2. Compliance with International Regulations on Biotechnologies

❖ Gene Editing: a revolution in plant breeding



2. Compliance with International Regulations on Biotechnologies

❖ Gene Editing:

- ➢ Pros:
  - Fast to generate a new trait
  - Very precise and no other changes expected in the genome.
- ➢ Cons:
  - New technology, no real feedback available
  - **Patent on Crispr/Cas9: tobacco excluded in U.S. in commercial products**
  - **New regulations in some countries could slow down the development of the gene editing**.

2. Compliance with International Regulations on Biotechnologies

- **Development worldwide of regulations on Genetically Modified Organisms (GMOs)**
  - **The supply chain and all the tobacco stakeholders must be compliant to these international regulations**
    - Many countries have adopted regulations on cultivation, transportation, imports, marketing, labelling
    - No GM tobaccos are allowed, except one cultivar Vector 21-41 in US
    - Detection methods are needed to check raw tobaccos and products
    - Proficiency testing on detection of transgenic tobaccos organized by the CORESTA.

2. Compliance with International Regulations on Biotechnologies

❖ CORESTA database on GMO regulations (GMO dossier)

➢ International databases

- United Nations (Environmental Programme (UNEP))
- Codex Alimentarius Commission(WHO/FAO)
- UNEP-GEF Biosafety Project
- USDA, NGOs and regional communities (USA, Europe, Africa).

➢ Countries

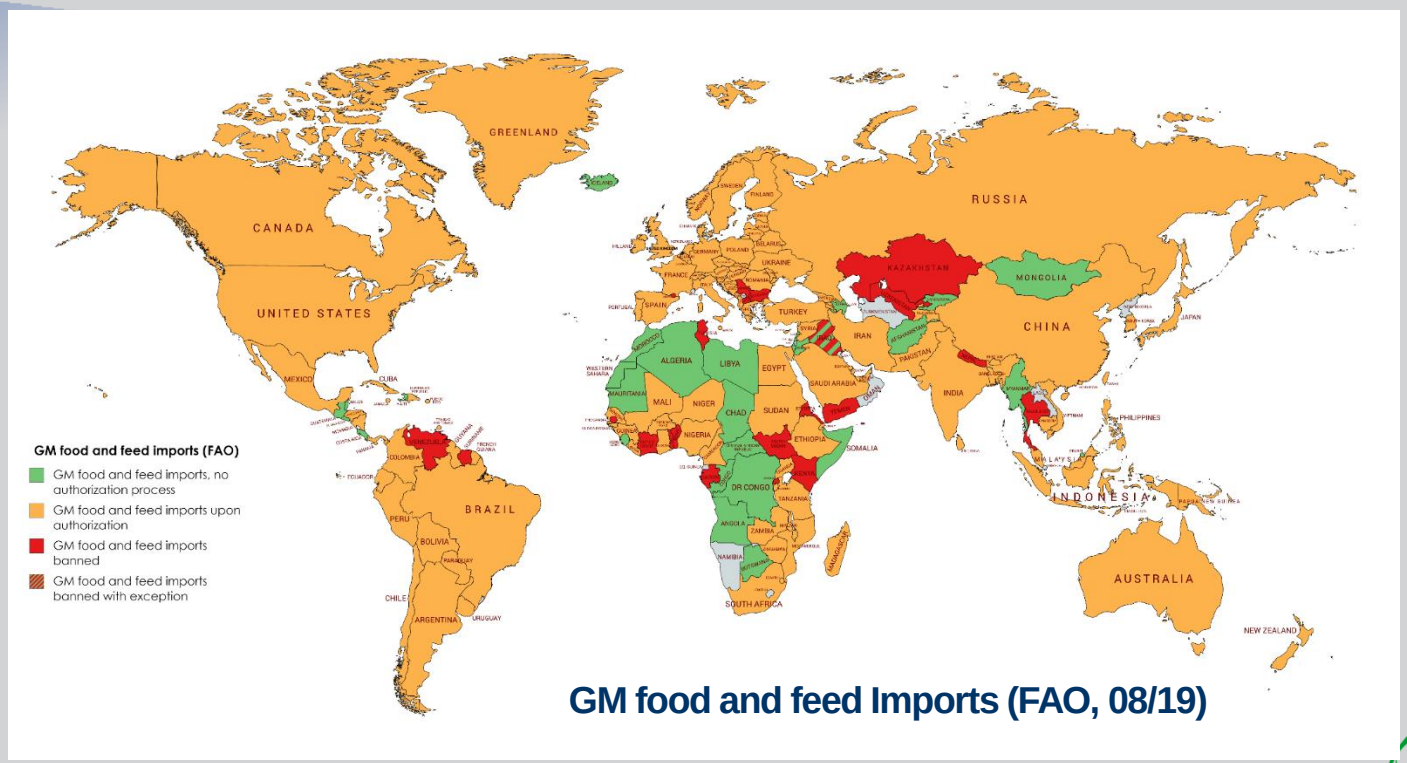
- 199 entries

➢ Information

- Information on Regulations, Approved GMOs, Labelling and Social Acceptance.
- Links of website/document (> 1300)

➢ GMO dossier available online to CORESTA members.

2. Compliance with International Regulations on Biotechnologies



GM food and feed Imports (FAO, 08/19)

Genome editing: Regulated or not regulated around the world

ROTHAMSTED RESEARCH

	No transgene / foreign DNA					Foreign DNA
	Targeted small deletions	Targeted small additions /edits	Targeted allelic replacements	Targeted insertions	Null segregant	Targeted insertion
Argentina	No	No	No	Yes?	No	Yes
Brazil	No	No	No	Yes	No	Yes
Canada	No	No	No	Yes	No	Yes
Chile	No	No	No	Yes?	No	Yes
Colombia	No	No	No	Yes	No	Yes
USA	No unless plant pest	No unless plant pest	No unless plant pest	No unless plant pest	No	No unless plant pest
Australia	No	Yes	Yes	Yes	No	Yes
New Zealand	Yes	Yes	Yes	Yes	?	Yes
Israel	No	No?	No?	?	?	Yes
China	?	?	?	Yes	?	Yes
Japan	No	No	No?	?	No	Yes
EU	Yes	Yes?	Yes?	Yes?	?	Yes

Some of these policies are still in development (the EU still has no formal policy)

2. Compliance with International Regulations on Biotechnologies

- **New Breeding Techniques (NBTs) and Gene Editing status**
  - **No database available on this topic**
  - **European Union could update its laws (2001/18 directive particularly)**
    - The European Court of Justice (ECJ) has classified the NBTs, Gene Editing included, as GMOs
    - but the ECJ has confirmed the exclusion of irradiated and chemical mutants from the GMO regulation.

3. Product standard for low nicotine

❖ WHO and FDA positions

- WHO Study Group on Tobacco Product Regulation, October 2015: *"**The maximum nicotine content should be as low as is technically feasible**. At present, that level would appear to be 0.4mg nicotine per gram of cigarette tobacco filler".* **That means 0.04% in raw tobacco**.
- *"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"* said Scott Gottlieb, the FDA commissioner, July 2017.
- "*FDA is particularly interested in comments about the merits of nicotine levels like 0.3, 0.4, and 0.5mg nicotine/g of tobacco filler, as well as other levels of nicotine"*, March 2018. **That means 0.03 to 0.05% in raw tobacco**.

3. Product standard for low nicotine

- **CORESTA is working to have a better knowledge on alkaloid control**

- **Tobacco Alkaloid Genetics Task Force**
  - To understand the genetics that control alkaloid formation in tobacco plants
  - To understand the feasibility of conventional and non-conventional breeding techniques to modify alkaloid formation in tobacco plants
  - To understand the impact of tobacco alkaloid levels on leaf production and quality.

3. Product standard for low nicotine

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.

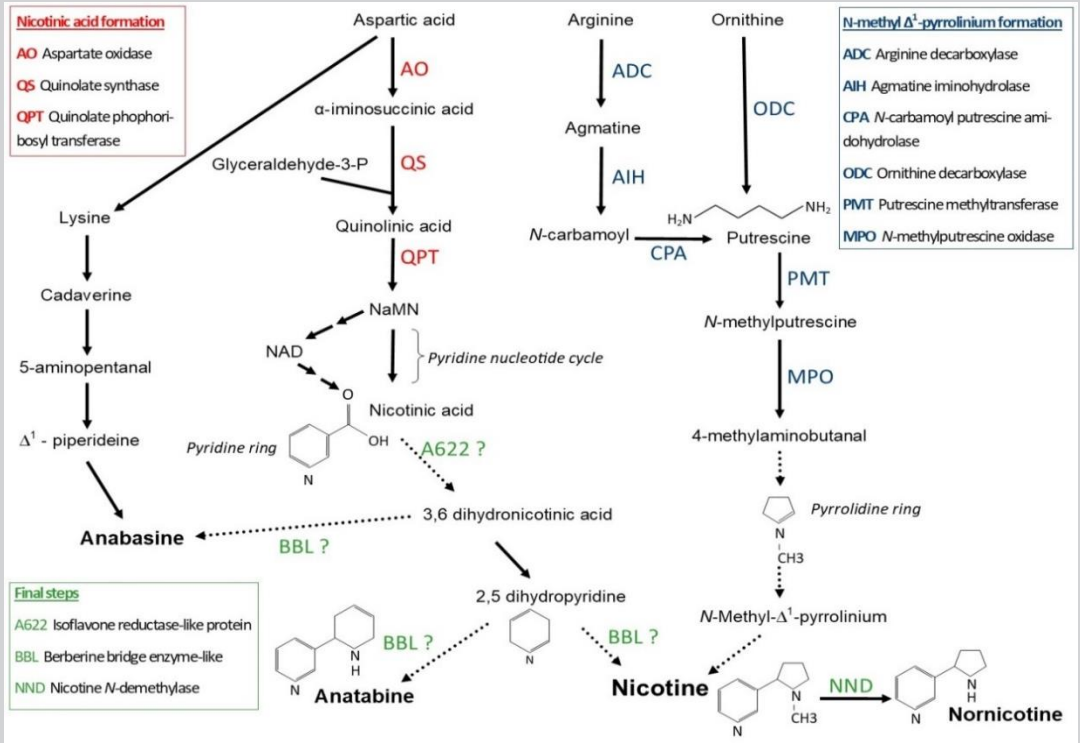


Figure by Christelle Bonnet (JTI)

3. Product standard for low nicotine

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.

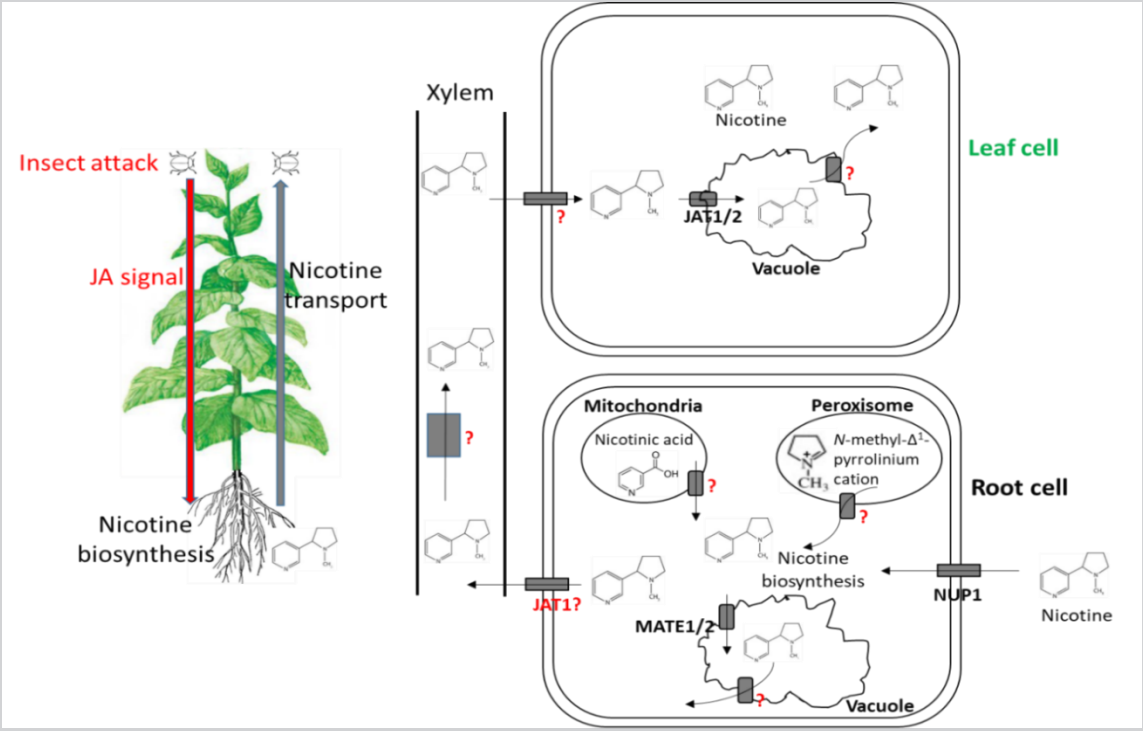


Figure by Shengming Yang (University of Kentucky)

3. Product standard for low nicotine

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.

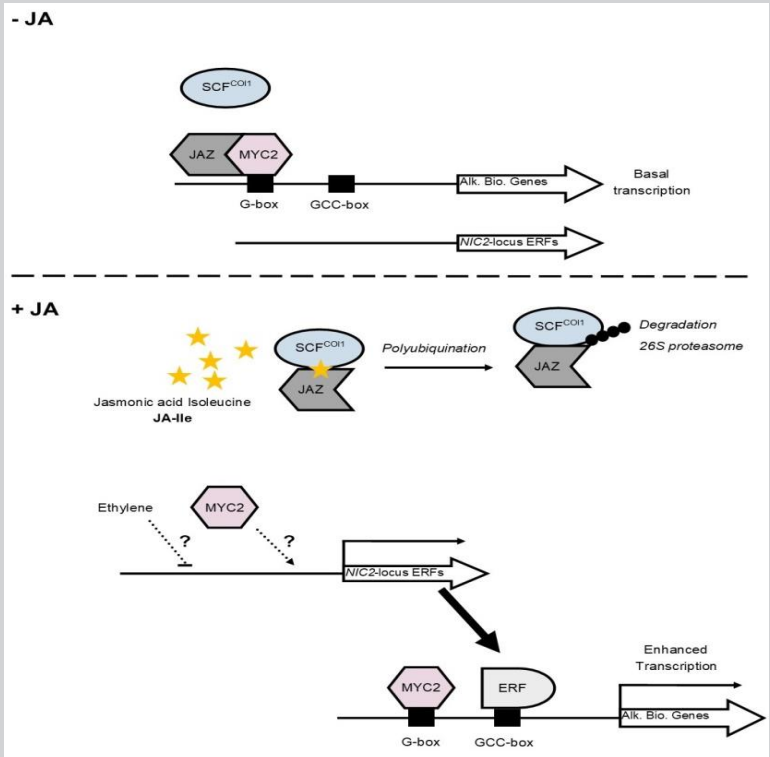


Figure by Christelle Bonnet (JTI)

3. Product standard for low nicotine

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.**

Table by Ramsey Lewis (NCSU)

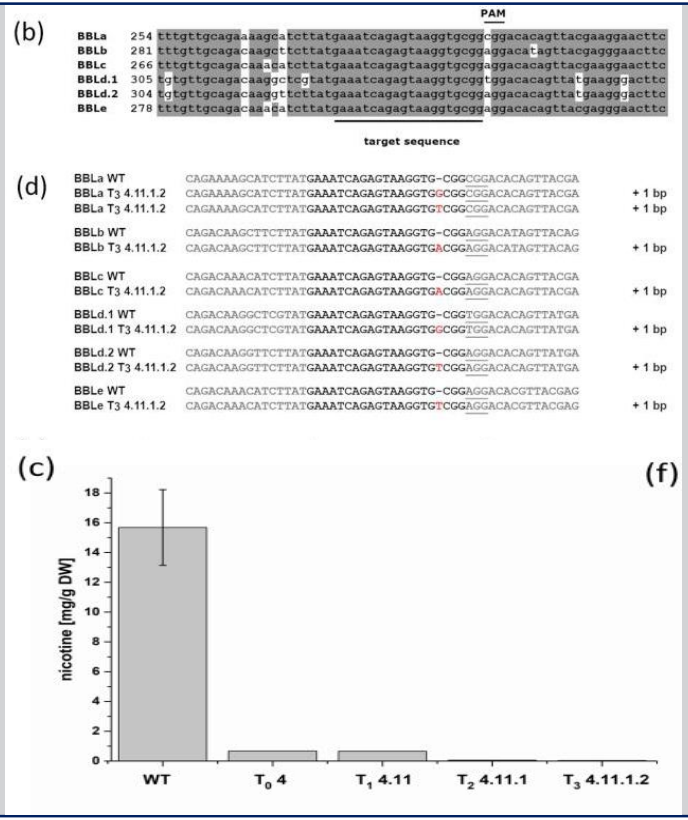
Variability Type	Mechanism	Nicotine (mg/g)	Sample Type	Reference
Wild type	Nic1Nic2	15 – 45	Composite cured leaf sample	Lewis 2018 [121]
Naturally-Occurring	nic1/nic2 (also known as a/b)	2.0 – 2.5[b]	Composite cured leaf sample [c]	Legg and Collins 1971 [118]
		2.99	Composite cured leaf sample	Lewis 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)

3. Product standard for low nicotine

Recent progress

1. Targeted 6 BBL genes by Crispr-Cas9
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



3. Product standard for low nicotine

- **Nicotine control with GMOs is difficult to implement due to regulatory and intellectual property limitations.**

- CORESTA is launching now a collaborative study on tobacco varieties obtained by conventional breeding cultivated in different geographic areas to define the limit of feasibility by this technique.

Conclusion

- **CORESTA is open to all the tobacco stakeholders and all of you are welcome to participate to the different sub-groups and taskforces**.

- Our next congress will be in Vienna (Austria), October 11-15, 2020.

Acknowledgements

- **Ana Yansy Cuéllar Gonzalez** (Instituto de Investigaciones del Tabaco).
- **Amaury Borges Miranda** (Instituto de Investigaciones del Tabaco).
- **Lea Scott** (Universal Tobacco Leaf), **Dongmei Xu** (Altria) and **Anthony Jackson** (Premium Tobacco).
- **Pierre-Marie Guitton, Eeva Marignac, Natacha de Tervarent** and **Stéphane Colard** (CORESTA).