



Breeding for high nicotine tobacco varieties

Anna Malpica, AP17, 12th October
CORESTA 2022

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1. High nicotine demand, global context
2. French local demand, project context and objective
3. Crop management guidance
4. Breeding strategy, literature background
5. Our breeding strategy
6. Some results
7. Conclusion and next steps

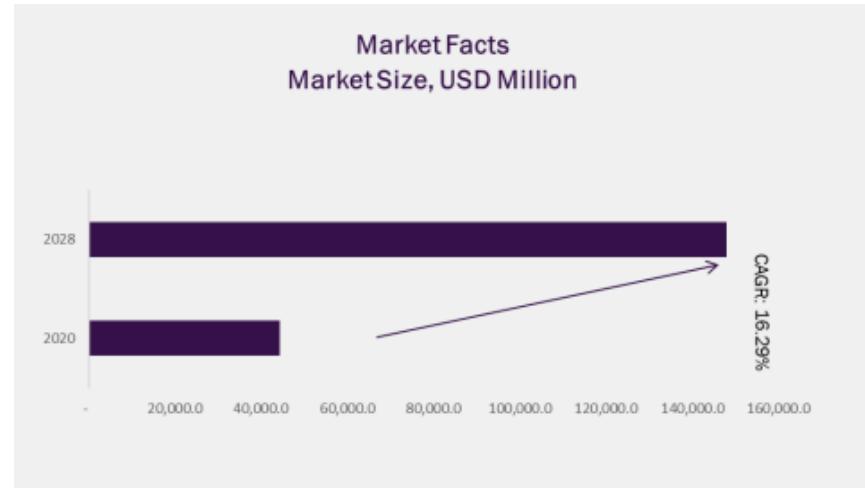
High nicotine demand, global context

-increase of the use of new tobacco products around the world with key market participants from big tobacco and big pharma industries.

→ NRT products, e-cigarettes, heat not burn devices, pouches, etc.



-market increase for nicotine production with key worldwide players (Siegfried-CNT, Porton Fine chemicals, Actarius) + development of many local players around the world



Key Market Participants

Cipla Inc.	Pfizer Inc.	Glenmark Pharmaceuticals	Fertin Pharma
Philip Morris International	British American Tobacco Plc	Japan Tobacco, Inc.	Imperial Brands
Johnson and Johnson Services, Inc.			

Source: U.S. FDA, CDC, WHO, Industry Journals, Annual Reports, Investor Presentations, Primary Interviews, Grand View Research

-high price of synthetic S nicotine (20-30 times more expensive)

French local demand, project context and objective

-Local demand for domestic and “green” production and purification

→ raw tobacco produced locally (52 growers in 2021)

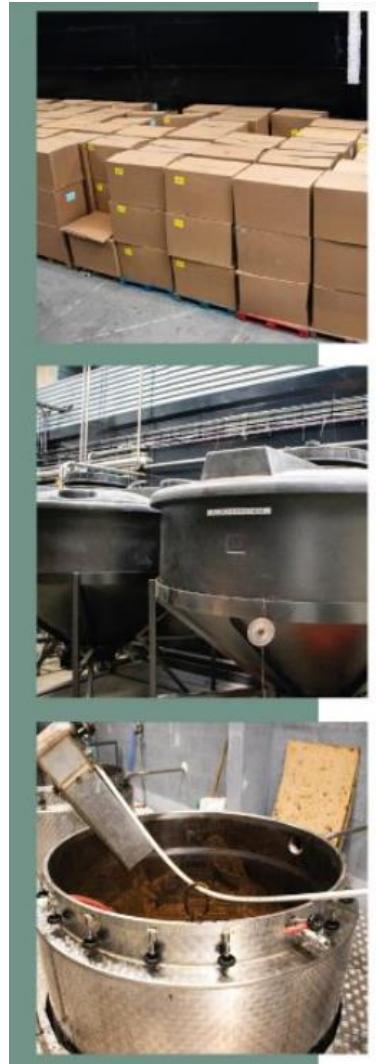
→ experimentation program for crop management improvement

→ **breeding program** for high nicotine varieties adapted to local soil, climatic and growers' conditions



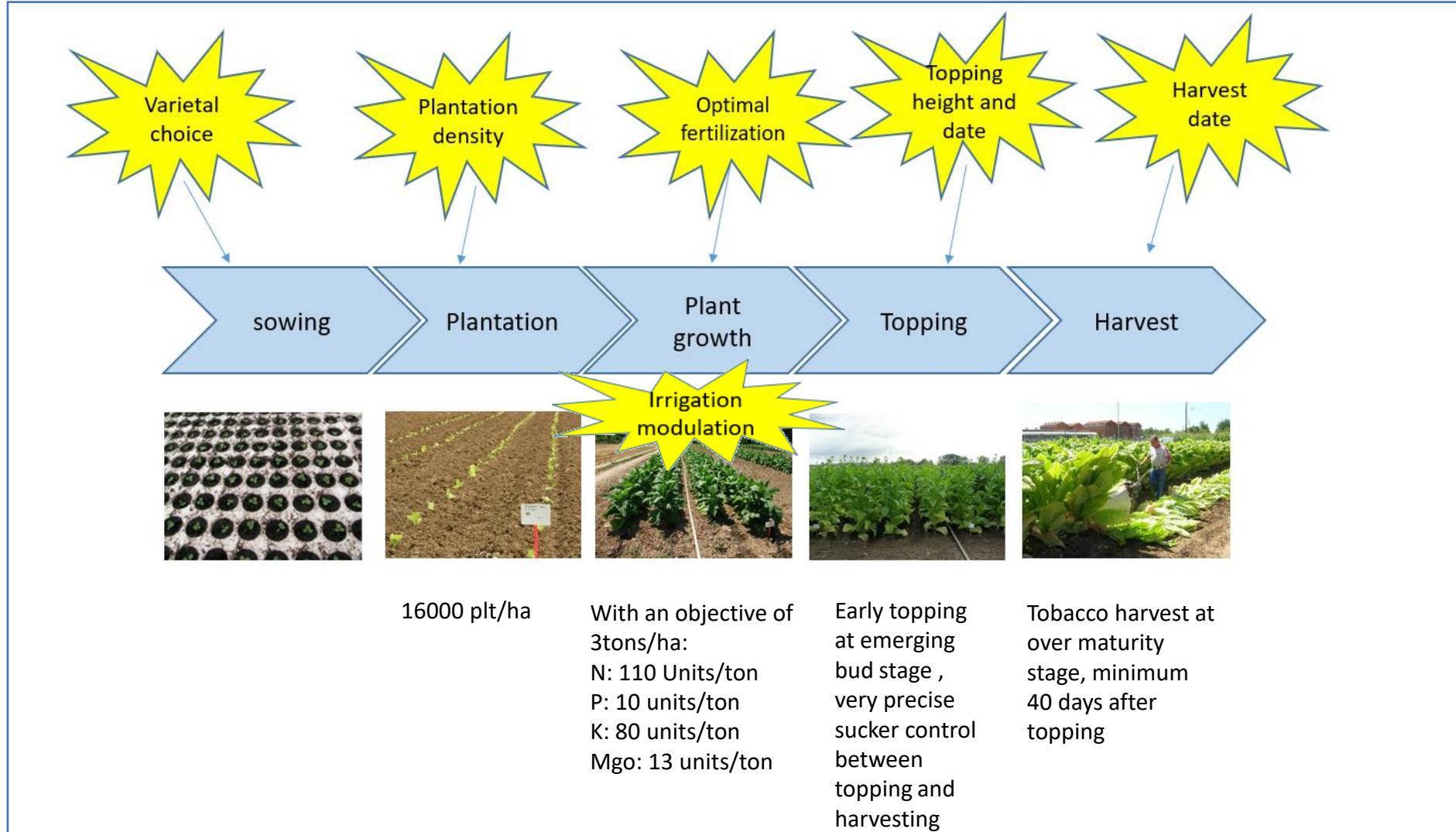
Target variety profile:

- high nicotine in average on all stalk position (mini 5% dwb)
- early variety adapted to our French short cycles
- variety with high levels of resistances to major biotic (PVY, black root rot, TMV) and abiotic (stand up ability, low sucker production) local stresses
- variety adapted to mechanical harvest



Crop management guidance

-an adapted crop management guidance has been finetuned, in parallel of the breeding program work, during 5 years of trials with growers' technical services for high nicotine screenings in SW France conditions



Breeding strategy, literature background

-lack of literature available on the topic

-breeding of Dr Chaplin from NCSU in the 70ies → Y-1 and Y-2

Used commercially by Brown & Williamson company in the 80ies, it led to a legal scandal in the 90ies in the US

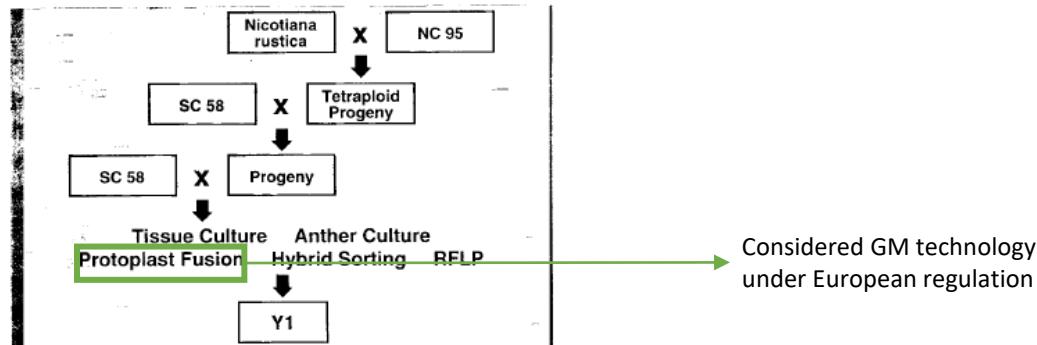


Figure 2 The breeding of Y-1. Sources: Brazilian Patent P1 9203690A and DNA Plant Technology Corporation

-In Indian production, nicotine is often extracted on tobacco scraps coming from local bidis or chewing tobacco, from *N. tabacum* and *N. rustica* landraces

Our breeding strategy

Germplasm available in Bergerac as raw matter starting point:

-High nicotine accessions in Imperial accession collection

-High nicotine lines coming from our breeding program

<i>N. tabacum</i>	2 Dark fire cured accessions 1 Dark air cured accession 2 Oriental tobaccos accessions	ITB 188, ITB GO ITB SUM
<i>N. rustica</i>	2 accessions	
		X X
		Eliminated, not adapted to local mechanical harvest tools
<i>N. tabacum</i>	1 Burley line 1 Dark air cured accession	ITB 93 ITB 164

year	Bergerac, BSB station	Growers' locations
2017	-genetic materials evaluation for nicotine production potential, agronomical key traits and resistances to key pathogens for French market (PVY, black root rot, TMV)	
2017-2018	-breeding population creation: F2, 3-way hybrids and 4-way hybrids	Evaluation of a selection of best lines and materials in growers' location, finetuning of crop management guide
2018 ↓ 2022	-traditional genealogic breeding strategy supported by selection assisted by marker for key resistance genes and intense screening for high nicotine plants at each generation	
2023	-commercial large-scale seed production	

Project breeding results

	varieties	resistances	2017			2018			2019			2020			2021		
			nicotine (% dwb)	yield (kg/ha)	nicotine yield (kg/ha)												
parental lines	ITB SUM		6,0	3131,0	186,6	6,7	1803,0	120,1	5,7	4079,4	232,5	7,0	2772,0	194,0	5,7	1993,0	113,6
	ITB 188	PVY	5,5	2817,0	154,1	6,4	1688,0	107,2	6,8	2829,3	192,4	5,9	2301,0	135,8	5,5	2105,0	115,8
	ITB 164	BRR, PVY, TMV	3,7	4448,0	162,8	6,2	1789,0	111,1	5,3	4166,7	220,8	6,8	3246,0	219,8	4,9	2717,0	133,1
	ITB 93	BRR, PVY, TMV	4,4	2399,0	104,4	4,9	2187,0	106,9	4,1	2488,9	102,0	5,4	2574,0	139,0	4,5	2800,0	126,0
	ITB GO	PVY							5,7	2804,1	159,8	6,9	3106,0	214,3	5,6	2790,0	156,2
	average		4,9	3198,8	152,0	6,0	1866,8	111,3	5,5	3273,7	181,5	6,4	2799,8	180,6	5,2	2481,0	128,9
	standard deviation		0,9	766,6	30,0	0,7	190,1	5,3	1,0	742,4	51,1	0,6	345,0	35,9	0,5	358,2	7,9
breeding populations	BSB SUM93	BRR, PVY, TMV												5,5			5,7
	BSB 164GO	BRR, PVY, TMV												5,8			6,9
	BSB 16405	BRR, PVY, TMV												5,6			4,7
	standard deviation																

	2018	2019	2020	2021
ITB SUM		7,39		
ITB 188	5,47	6,18	6,57	6,23
ITB 164		5,42	5,71	5,51
ITB 93		4,51	4,68	5,35
ITB GO			5,66	
growers	7	16	52	52
ha	5	18	62	65

-ITB SUM, ITB 188 always provided nicotine rates above 5% in experimental station and growers location

-ITB 164, ITB 93 and ITB GO are less stable and used as donors for resistances and plant architecture

Conclusion & next steps

-delivery of our new varieties in 2024 specifically for French market and VDLV project:

- secure 6% nicotine dwb
- secure a yield of 2500kg/ha, 150kg nicotine/ha
- facilitate sucker control, mechanical harvest and fast natural drying
- avoid key pathogens pressure

-surface development expected in 2025 in France

-development of high nicotine varieties adapted to other territories

