



Conventional breeding using low nicotine germplasm materials

Conventional breeding

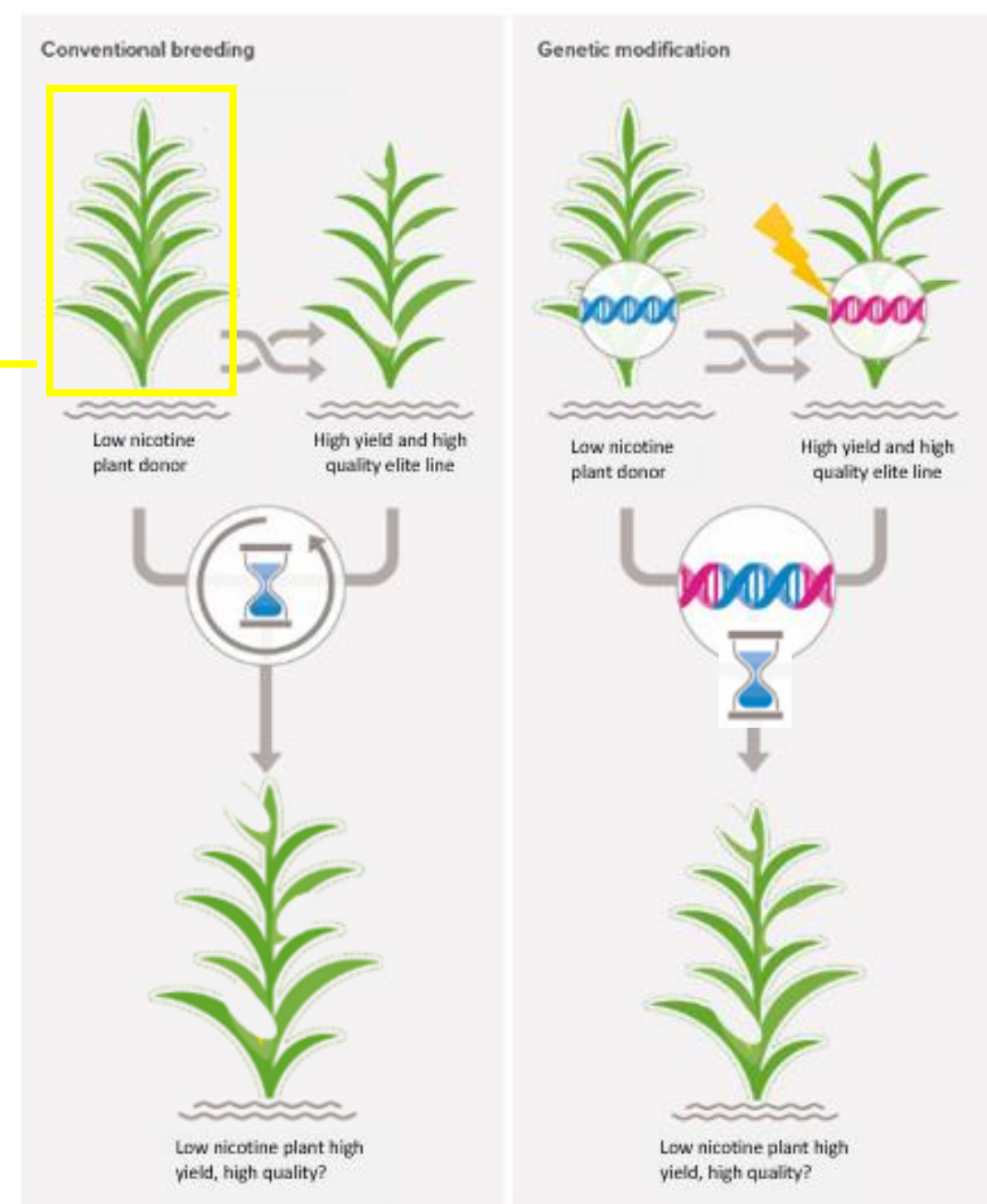
- Natural variation: Germplasm collection
- Natural variation: Spontaneous mutations
- Induced variation: Induced non targeted mutations (EMS)

▪ Induced variation: gene editing technologies

▪ Induced variation: GM technologies

→ Breeder target:
reduced nicotine levels (within an industrial variety)

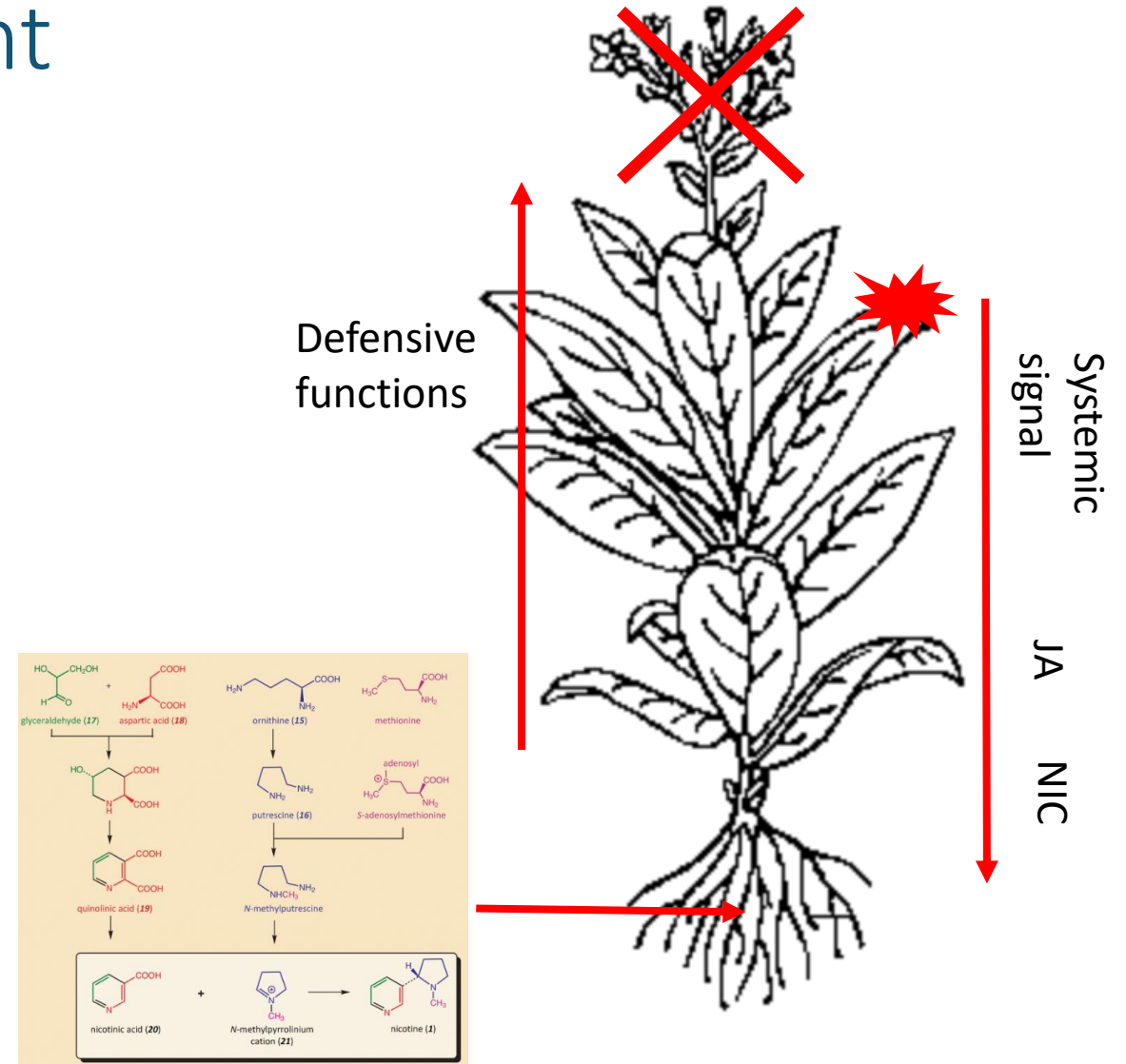
Nicotine level = **Genetics** + Environment + Interaction GE



Trait of interest:

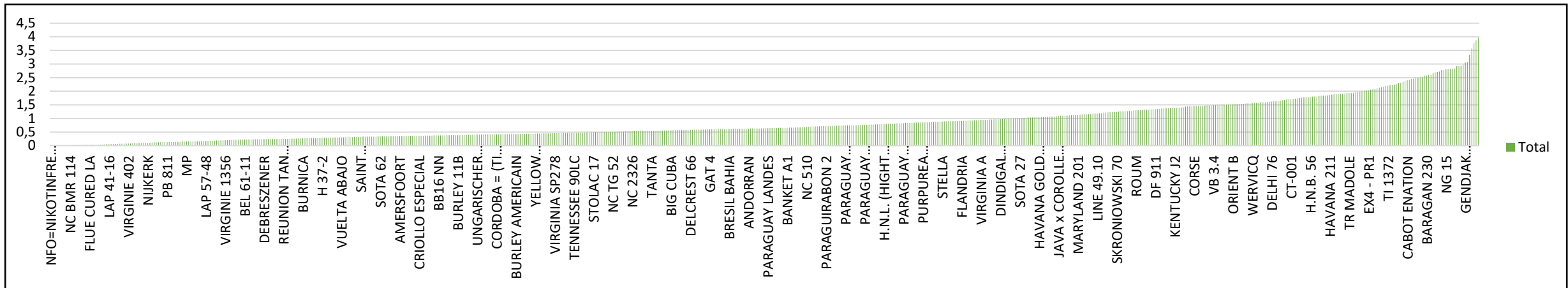
Nicotine level in tobacco plant

- Nicotine is the predominant alkaloid in tobacco (90-95%)
- It is synthesized exclusively in tobacco roots, transported to leaves and stored in leaf cell vacuole
- Nicotine pathway is well known and described
- Genome sequence of tobacco provides an almost complete inventory of structural and regulatory genes involved in this pathway



Low nicotine conventional breeding: the example of Bergerac program

- Basic donors of low nicotine trait in our breeding programs:
 - Imperial tobacco germplasm collection, 1211 accessions including wild tobaccos, levels from 0,01 to 3,97. Nicotine measured in seed production conditions.



- Short cycle, fast ripening breeding lines (North Europe origins)
 - the shorter the plant cycle is the less nicotine accumulation is observed in the plant
- nic1 nic2 breeding lines (initial donors LAFC, VBLA)
 - Stable low nicotine behaviour in different climatic conditions
- EMS mutant lines

Low nicotine conventional breeding: the example of Bergerac program

- Flue cured varieties, lowest alkaloid contents, filler varieties, filler crop management
 - Database extract from Bergerac trials from 1997 to 2017
 - Nicotine, 'Nic' expressed in % of dry weight, measured in mid stalk position (whole leaf).

VAR	lqb	Dvp	rdtb	Nic	flowering	height	l nb	red Sug
MS33319	34	112		0,3				16,2
MS33320	44	115	3250	0,5	78	142,7	20,3	19,8
MS32N16	68	103	3521	0,6	76	139,0	19,2	24,4
MS35613	58	110	3401	0,6	78	144,4	21,6	17,7
MS33322	64	113	3591	0,7	78	139,5	19,4	20,5
MS35614	59	117	4181	0,7	78	140,4	21,9	18,1
MS35417	60	114	3961	0,8	78	143,9	19,7	21,3
MS39207	69	101	3690	0,8	68	120,3	18,0	17,9
MS35420	60	110	3271	1,0	78	134,5	18,9	18,8
MS37901	55	110	3496	1,0	73	134,7	22,0	17,2
ITB697	67	106	3643	1,1	73		19,6	19,8
ITB6184	81	104	3873	1,1	78	137,5	20,0	20,4
MS32N10	74	103	3738	1,1	75	137,7	19,4	
MS35418	51	112	3701	1,1	76	130,7	18,7	19,6
MS35409	65	112	3614	1,2				17,5
MS31653	52	118	3383	1,2	73		20,6	21,2
ITB683	78	101	3639	1,3	74	134,2	20,7	18,4

Low nicotine conventional breeding: the example of Bergerac program

- Burley varieties, lowest alkaloid contents
 - Database extract from Bergerac trials from 1997 to 2017
 - Nicotine, 'Nic' expressed in % of dry weight, measured in mid stalk position (whole leaf)

VAR	yield	lqn	mat	datflo	l nb	Nic
ITB5101	3557	45	1,1	66,2		0,1
ITB259	3125	42	0,6	64,4		0,2
MS25905		30	1,3	65,2		0,3
MS25904		33	1,3	64,2		0,4
ITB270	2964	22	2,0	62,2	21,4	0,6
BURLEY21LA	3148	32	2,0	68,1	21,0	0,8
MS21210	3254	46	3,7	74,3	24,7	2,8
MS22602		59	2,3	69,3		2,9
MS27903	3365	55	2,1	80,0	26,3	2,9
ITB550	3203	58	2,6	70,2	20,4	3,1
BSB5120	3361	78	2,4	65,7	18,7	3,2
ITB2604	3369	72	2,8	68,7	19,9	3,9
ITB583	3331	76	2,9	68,4	21,0	4,0

nic1 nic2
materials

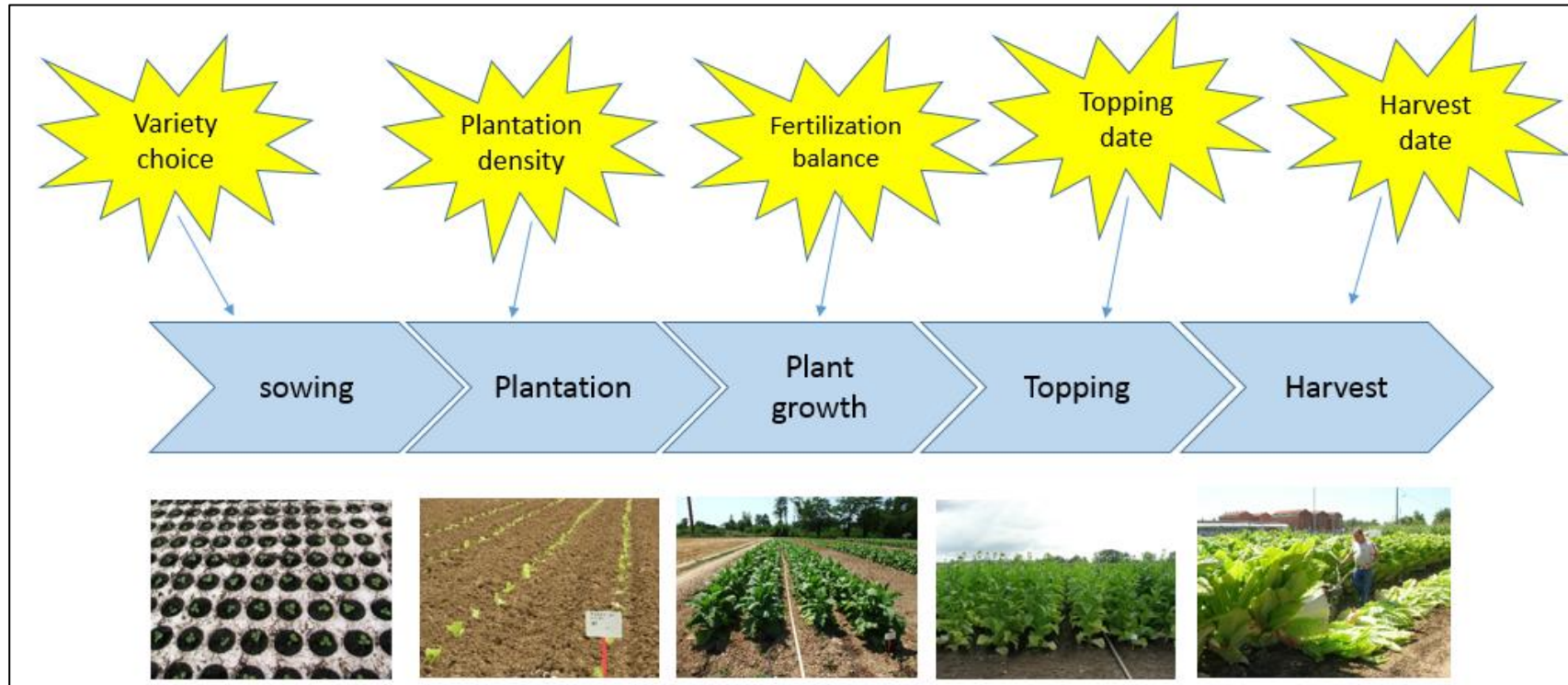
Environment impact on nicotine level trait

$$\text{Nicotine level} = \text{Genetics} + \text{Environment} + \text{Interaction GE}$$

Nicotine decrease ↑

Stability of decrease ↑

climate + soil + crop management ↓



Conclusions, at conventional breeding level:

- **Low** nicotine levels are feasible and existing in varieties obtained with conventional breeding
- **Very low** nicotine contents are reachable cumulating low nicotine genetics + adapted agronomical practices
- **Ultra low** nicotine contents seem difficult to reach with conventional breeding, the use of GM or gene editing techniques are necessary



Breeding for variations in nicotine contents is closely linked to yield, leaf quality, and taste challenges.

Decrease of nicotine level → increase of insect control needs → increase of CPA risks

Time of variety development + time of practical implementation

Thanks for your attention

