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SEED QUALITY MANAGEMENT  
AS A COMPONENT OF  
TOBACCO IDENTITY PRESERVATION

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# Tobacco seed quality

## ■ Seed integrity

→ Identity to the variety

→ « Purity »

- Off types
- Adventitious *GMO*
- Converter plants

} Absent or lower than threshold

## ■ Biological properties

→ Seed viability

→ Seed vigor

→ Seed health

# Seed integrity

## ■ Methods to assess seed integrity

- Variety description
  - UPOV guidelines,
  - Disease resistance tests,
  - Other characters
- GMO detection
- Converter plants detection

## ■ Seed production & control scheme

# Identity to the variety

Variety : UPOV definition (simplified)

## Plant grouping

1. Defined by the expression of characters resulting from a genotype or combination of genotypes,
2. Distinct from any other plant grouping,
3. Suitable for being propagated unchanged.

<http://www.upov.int/fr/publications/conventions/1991/act1991.htm>

# Identity to the variety

1. Reference phenotypic description of the variety,
  2. From a reference seed lot.
- A prerequisite : uniformity
- F1 male-sterile hybrids
3. Pollinator inbred line + fertile maintainer:  $\geq F7$
  4. Male-sterile line:  $\geq BC6$  to fertile maintainer
    - Genetic and phenotypic variation within lines may still exist
      - Mean number of heterozygous chromosome fragments following 6 selfing generations: 2 for a genome size of 1000 cM. (Hanson, 1959)
  5. Checking phenotypic uniformity before using lines.
    - Future : molecular markers to estimate the number and length of heterozygous chromosome fragments within candidate lines.

# UPOV guidelines for tobacco

- for describing varieties in view of the DUS examination,
- issued in 2002.
- 35 traits

	Qualitative	Measured	Total
Plant	3	2	5
Leaf	14	3	17
Flower	6	2	8
Inflorescence	3	1	4
Fruit	1		1

- 2 independent growing cycles
- 2 replicates, total at least 40 plants.

- « Level of expression » : **relative to standard cultivars**

# UPOV guidelines

Ex. : Leaf traits

« After the beginning of flowering on the largest fully-developed leaf in the middle third of the main stem, excluding the inflorescence »

## 7: Leaf angle

3 levels of expression for this trait

35 traits : 2 to 9 levels of expression depending on trait

Add. 7 : Feuille : angle d'insertion



1. Very acute

**K149**

2. Moderately acute

3. Right angle

**K326**



# UPOV guidelines, part 3.

## Example provided by Brazil

Example varieties provided by le Brazil / Variétés exemples proposées par le Brésil / Von Brasilien vorgeschlagene Beispielsorten /  
Variedades ejemplo propuestas por Brasil

Example varieties Beispielssorten Exemples Variedades ejemplo	Characteristic number Merkmalsnummer Numéro de caractère Número del carácter																																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Banket A1		7																			2															
BY21							1		5														3											3	3	
BY37		3		3																																
BY64 #			2	7			1	7		7													7											7	1	
C176 #								7	7	3						3	5			7								5								
C371 G #																												3	4							7
K149 #							1		3	7			1								3															
K326 #		5	3	5	3	1	3	5	5			5	2	2	7	1	3			5	4	3	5	5	5		5	7	3	2	1		2	5	3	
K346 #																				7																
KY10												3				3																				
KY14													1							7																
KY8959		7			5				7						5	1	3											5					1			
KY907									7	3				3																						
MN373 #	1	3	2		5		1					3								3		2	3											3		
NC2326 #				3											9																					
NC27 NF #		7		7				3															7													
NC82 #				3																																
SPG70 #														3	5																					
TN86	2		1									5			3					7	3	1										1				
TN90		5		5	3	1	3	3	3			2			2				5	5	4	2	5	5	5		5	7	3	2	2		2	5	5	
VA509	1							5							7																					
VA510														2																						
VA528																													1							

States of expression/Niveaux d'expression  
Ausprägungsstufe/Niveaus de expression

States of expression/Niveaux d'expression  
Ausprägungsstufe/Niveles de expression

States of expression

[http://www.upov.int/en/publications/tg-rom/tg195/tg\\_195\\_1.pdf](http://www.upov.int/en/publications/tg-rom/tg195/tg_195_1.pdf)



# Disease resistance tests

Disease	Pathogen	Expression of resistance			Test			
		Factor	Host reaction	Dev. stage	# plants	Days to reading	R	S
<b>Mosaic</b>	TMV	1 N	HR	All	16	30-35	N. glutinosa, PBD6	P48, TN86
<b>Black root-rot</b>	<i>Thielaviopsis basicola</i>	1	Immunity	All	100	18-22	Ky17, ITB32	VD, PBD6
<b>Vein necrosis</b>	PVY <sup>N</sup>	1 va	No necrotic symptom	All	12	40-50	TN86, ITB32, PBD6	Ky17, ITB30
<b>Powdery mildew</b>	<i>Erysiphe cichoracearum</i>	1	No spots on leaves	All	18	40-50	TB22	ITB32

TMV res: from *N. glutinosa* to *N. tabacum* Samsun H, HOLMES 1938.

*Th. b* res: from *N. Debneyi* to *N. tabacum* Br-RMW, CLAYTON, 1969.

PVY res: VAM, VD (Virginia SCR), Havana 307, etc. ANO, 1995.

*E. cich.* res: from *N. tomentosiformis* to *N. tabacum* TB22, SMEETON B.

All reading  
on individual plants



Outcome :

- Fixed for resistance
- Segregating
- Fixed for susceptibility

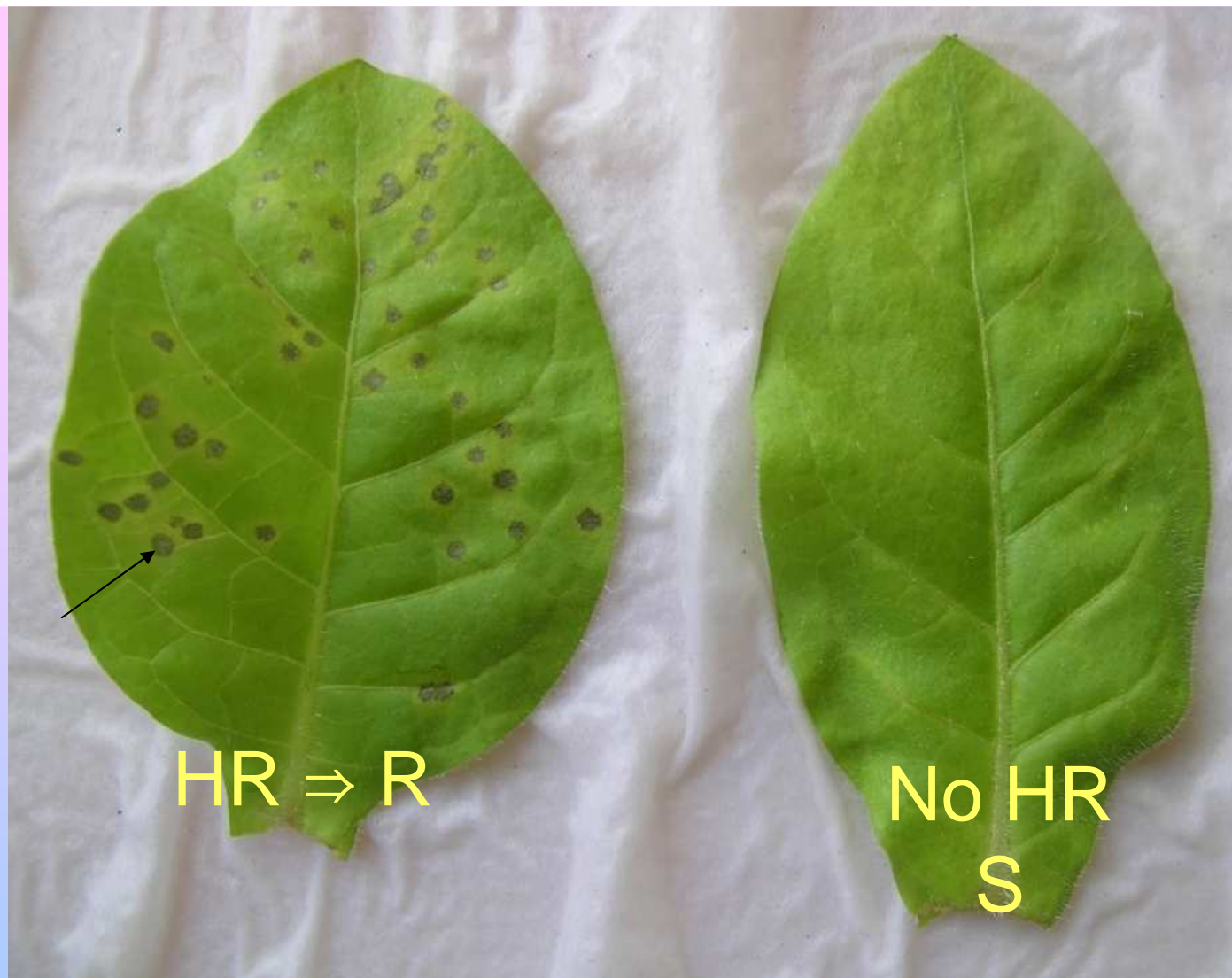
# Test for N gene

## Layout

- Detached leaves,
- 1 leaf/ plant,
- Wet paper,
- Covered tray

## Inoculum

- Sap
- **TMV** infected
  - P48 plant,
  - *Nicot.*
- Filtered
- Gently spread
- Paintbrush



**Hypersensitive Reaction (HR) development :**  
3 days, room temperature.

# Test for *N. debneyi* BRR res.

## Layout

- Petri dishes (Ø 50mm)
- Hole for water feeding

## Inoculum

- Substrate
- 60 ml  $\frac{1}{2}$  Knop solution
- $10^6$  conidia /ml
- 100 g pure silica sand

## Disease development

- Day/Night 10/14h
- Temp. 23/16°C

## Reading

- Binocular lense (x20)
- presence/absence
- chlamydopsors





# Test for the va gene

## Layout

- Floating trays

## Inoculum

- Sap, VaVa plant
- **PVYN** strain
- from indexation with vava / VaVa lines (VAM, VD, Ky17...)
- carborundum

## Disease development

- Day/Night 10/14h
- Temp. 23/18°C

## Reading

- Presence/absence
- Necrotic veins



# TB22 res. to powdery mildew

## Layout

- Floating trays

## Inoculum

- **E. cich.** spots
- Susceptible plant
- Blown above tray
- Air pump
- No air movement



## Disease development

- Day/Night 10/14h
- No liquid water on leaves
- Temp. 23/18°C
- High relative moisture

## Reading

- Presence/absence
- Spots



# Flue-cured

Levels of expression relative to the standard K326

Cultivars			MN 944	K 394	K 326	K 399	NC55	VD	ITB 31612	ITB 3304	ITB 30808	ITB 33024	ITB 620	ITB 623	ITB 609	ITB 30804	ITB 667	ITB 333	ITB 683
Disease resistance genes	<i>Thielaviopsis</i>		0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
	PVY		0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1
	<i>E. cich.</i>		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Average leaf harvest date		7 days	5	5	5	4	6	4	3	5	4	3	3	3	3	3	2	4	3
UPOV traits	30 Stamens		1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
	2 Plant height	10 cm	4	4	5	2	3	6	8	7	7	6	6	7	7	7	6	8	7
	10 Leaf ratio L / W	0,2	5	4	5	3	5	1	2	2	2	1	2	2	2	2	2	2	2
	4 Leaf number	1	-	4	5	4	5	3	6	4	6	5	5	6	5	5	4	-	5
	23 50% flowered date	2 days	-	5	5	4	7	4	3	4	5	3	3	4	3	3	3	5	4

Difference between neighbour levels of expression

Average leaf harvest date: variance analysis from 71 RCB trials, 10 years, the most stable and discriminant trait for flue-cured var.

Stamens: 0=male-sterile, 1= male-fertile

Leaf ratio:5=narrow, 1=round.

# Burley

Levels of expression relative to the standard TN90

Cultivars				BB 16	B 217	ITB 2204	KY 17	ITB 221	ITB 2604	ITB 501	ITB 218	ITB 503	ITB 502	ITB 219	TN 90	ITB 573	ITB 529	ITB 574
Disease resistances	<i>Thielaviopsis</i>			0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	PVY <sup>N</sup>			0	1	0	0	1	1	1	1	1	1	1	1	1	1	1
	TMV			1	0	1	1	0	0	0	0	0	0	0	1	1	1	1
	<i>E. cich.</i>			0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
UPOV traits	30	Stamens		1	1	0	1	0	0	0	0	0	0	0	1	0	0	0
	2	Height	10 cm	5	6	5	5	6	6	5	5	5	4	4	5	4	6	4
	4	Nb. leaves	1,5	3	3	5	4	4	4	4	3	2	4	4	5	4	4	6
	10	Leaf ratio L / W	0,2	5	3	6	5	6	5	5	6	3	5	5	5	5	4	5
	23	50% flowered	3 days	2	2	5	4	4	4	4	4	4	5	5	5	4	2	4

Difference between neighbour levels of expression

Stamens: 0=male-sterile, 1= male-fertile

Leaf ratio: 5=narrow, 1=round.



# Adventitious *GMO* seed detection: biological test

## ■ Preparation :

- Petri dishes 90 mm (4 holes for water circulation)
- Pure silica sand + Knop nutrient solution
- 250 seeds/ dish, 8 dish (2000 seed)/ seed lot
- Day/night 14 / 10 h temp. 26 / 23°C

## ■ Detection of kanamycine resistant *GMO*:

- Kanamycine: 300 mg/l
- In the nutrient solution
- Mixed to the substrate before seeding

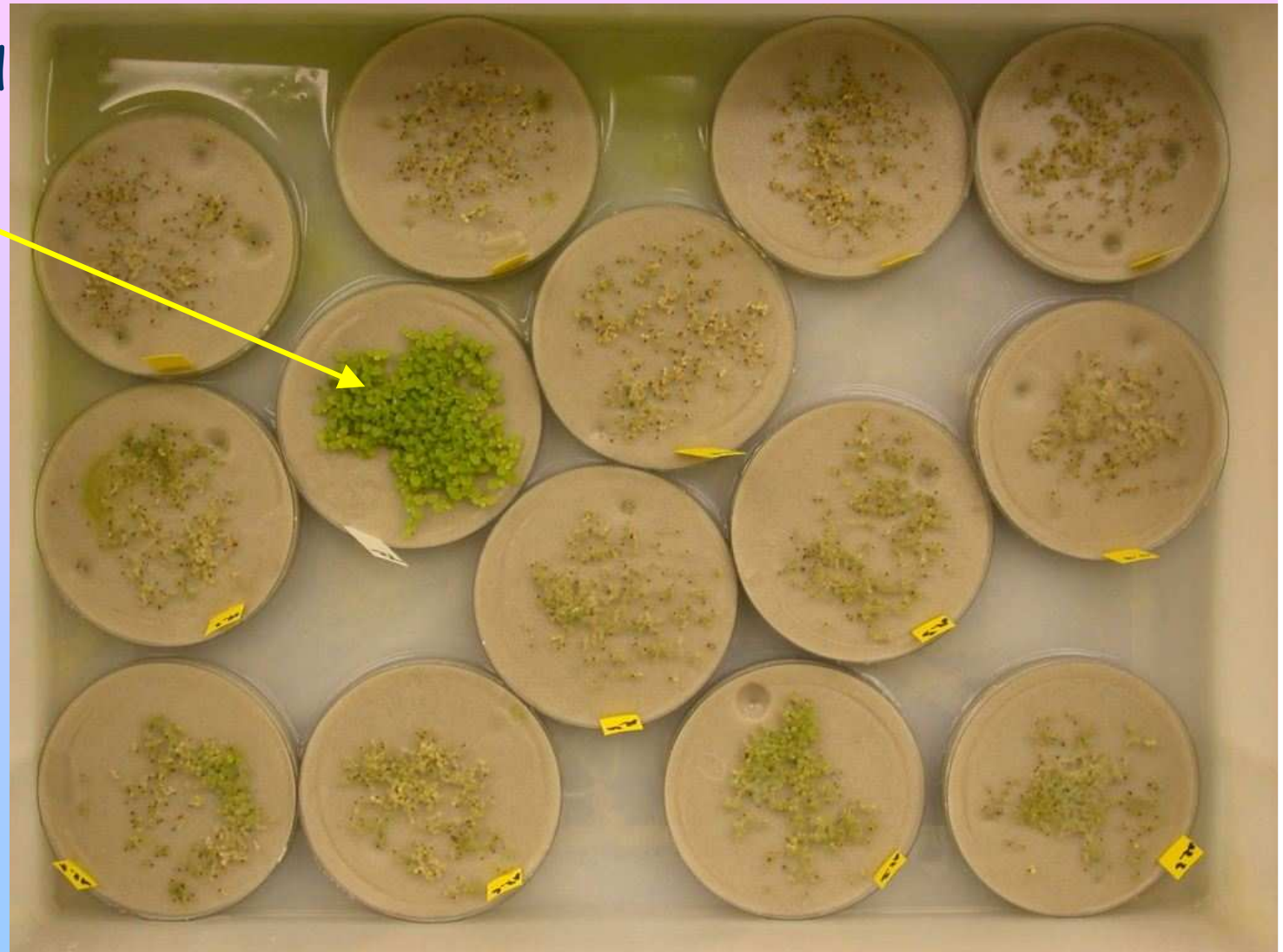
## ■ Detection of bromoxynil resistant *GMO*:

- Sprayed on test
  - 45 mg/l, bromoxynil octanoate
- 8 days after seeding :1.2 ml/dish
- Then 13 days after seeding: 1.4 ml/dish



# Kanamycine resistance test

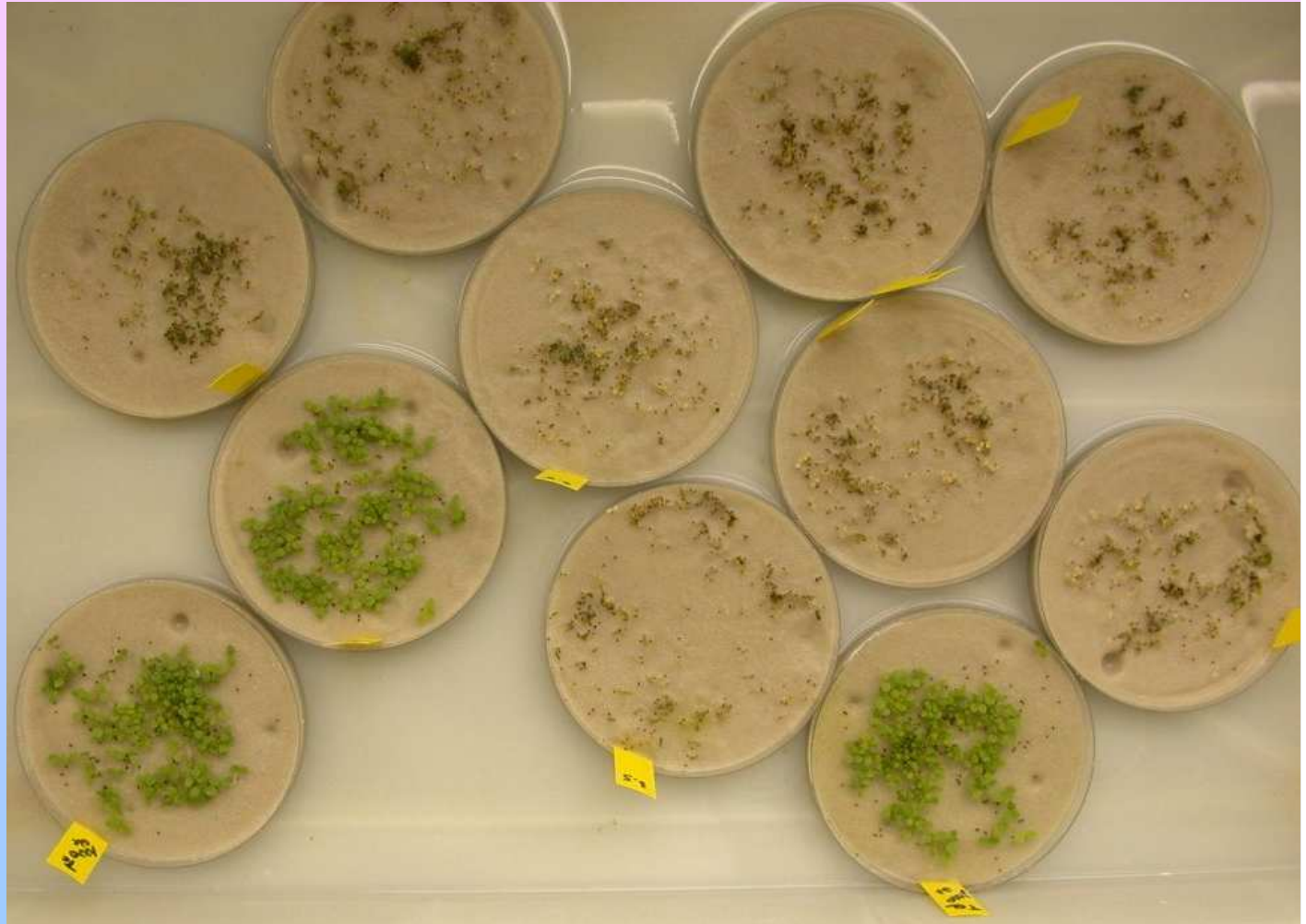
Res. Standard  
K8-5-1  
GMO



# Bromoxynil resistance test

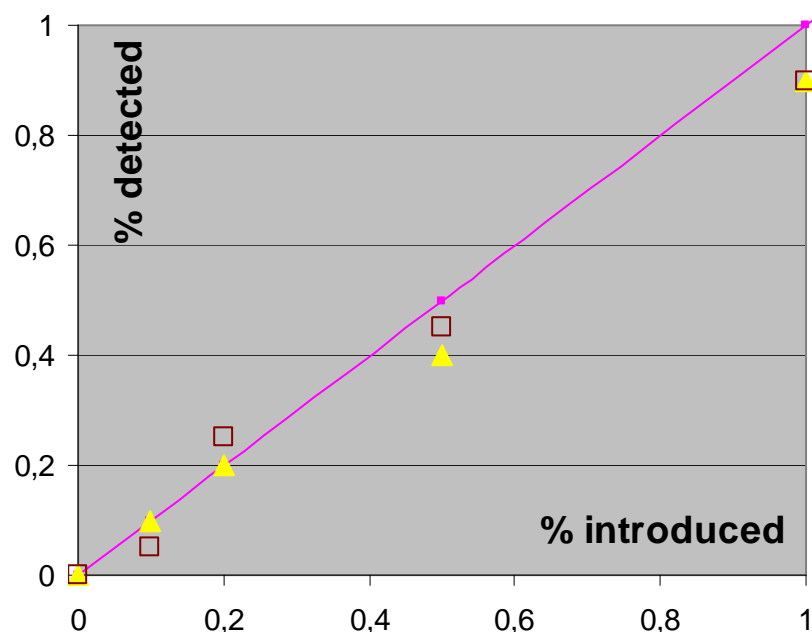
ITB 1000 O×

Res. standard

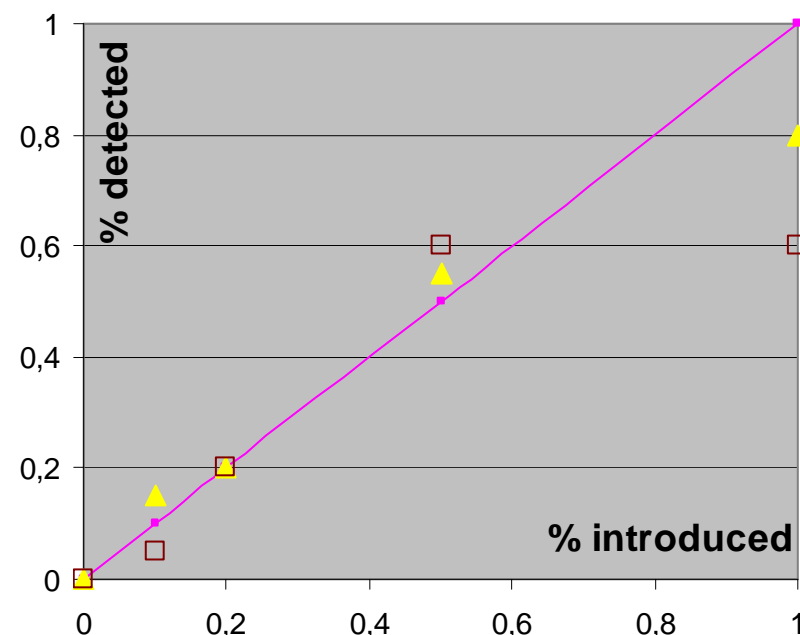


# Assessment of biological test for *GMO* detection

**Bromoxynil res. *GMO***



**Kanamycine res. *GMO***



Resistant plants saved and grown to extract DNA and PCR test  
35S (Kanamycine res. Plants) : 93% confirmed  
Bromoxynil res. Gene : 80% confirmed

# Detection of converter plants

## ■ Early detection: prior to flowering

- Knee high
- 2 middle leaves / plant
- Dipped into 1% NaH CO<sub>3</sub> solution,
- Yellowing : 4 days, 36°C and 85% HR, then drying (60°C).

## ■ Detection on adult plants

- 2 middle leavaes/plant
- Air-cured with high enough moisture for complete yellowing,
- Drying (60°C)

## ■ Chemical analysis

- Alkaloids : CORESTA method n°35 (continuous flow)
- Nornicotine : colorimetric test (same extract than for alkaloids)

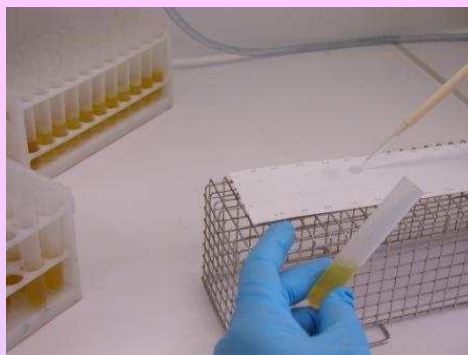
% nornicotine standard concentration array									
0,02	0,04	0,08	0,1	0,2	0,4	0,8	1	1,5	2



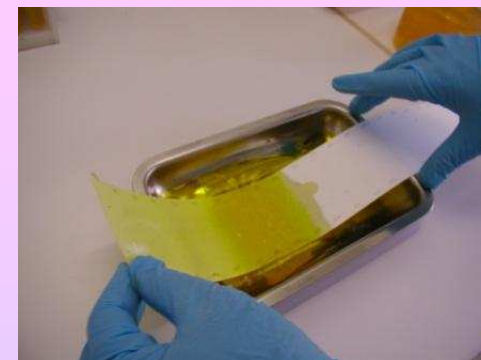
# Nornicotine colorimetric test



2,5 ml CH<sub>3</sub>COOH extract  
(CORESTA n°35)  
+ 0,5 ml CHCl<sub>3</sub>  
+ 1 ml de NaOH 10 N  
Shake strongly (no vortex)



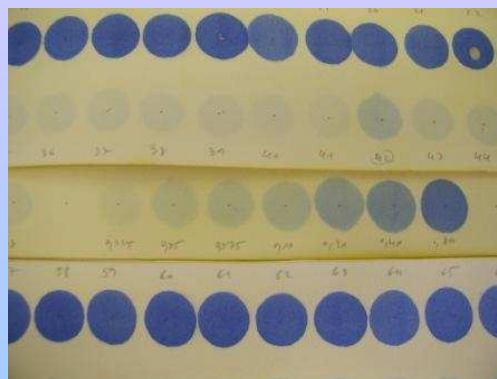
40 µl on watman paper  
Dried at room temp. (air flux)



Dipping in Isatin solution



Oven 100°C for 5 minutes

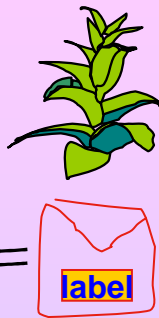
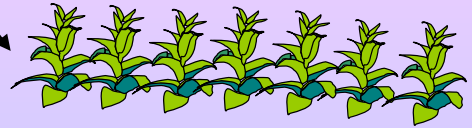
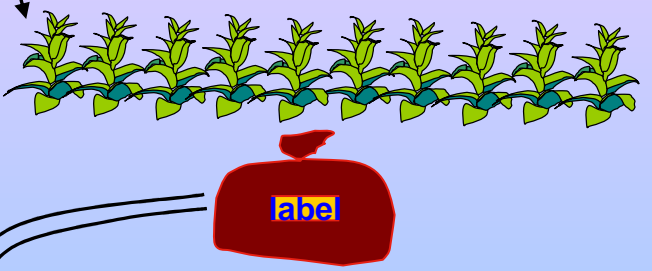
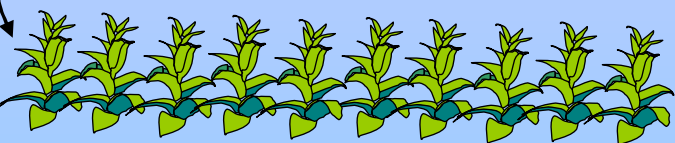
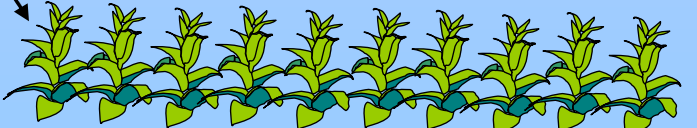


The blue color appears  
when total evaporation



Reading with Spectropen  
Computerised calibration curve ->  
estimates of concentrations

# Tobacco seed production & control

Year	Step	Tests
n	Pollinator & MS lines  Foundation seed 	<u>Individual plants</u>  Low conversion Early + adult
n+1	Checking foundation seed 	Low conversion (10-20 plants) Disease resistances Uniformity GMO detection
n+2	Commercial seed production  Commercial seed 	<u>Population</u>
n+3	Checking commercial seed 	Low conversion (20 plants) GMO detection Identity & uniformity Disease resistance
n+4	Seed sale & Leaf production 	



# The team



J. Jubely



J.-C. Bardon



B. Cailleteau



J.-L. Verrier

Thank you  
for  
your attention !