AP14 - RNA-SEQ ANALYSIS OF OROBANCHE RESISTANCE IN TOBACCO: DEVELOPMENT OF MOLECULAR MARKERS FOR BREEDING RECESSIVE RESISTANCE FROM WIKA TOBACCO VARIETY.

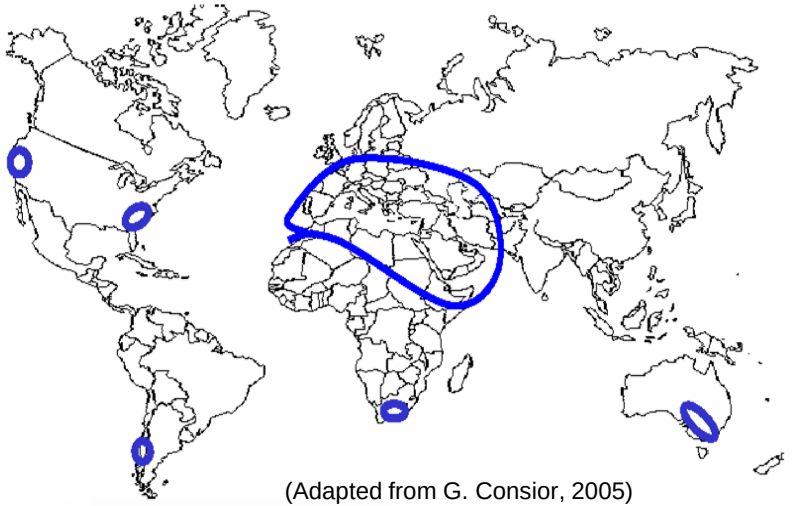
Julio, E. (1) ; Malpica, A. (2) ; Cotucheau, J. (1) ; Bachet, S. (2); Volpatti, R. (1); Decorps, C. (1); Dorlhac de Borne, F. (1).

(1) Imperial Brands, Leaf research, La Tour, 24100 Bergerac, France

(2) Bergerac Seed and Breeding, La Tour, 24100 Bergerac, France

BROOMRAPE/OROBANCHE RAMOSA

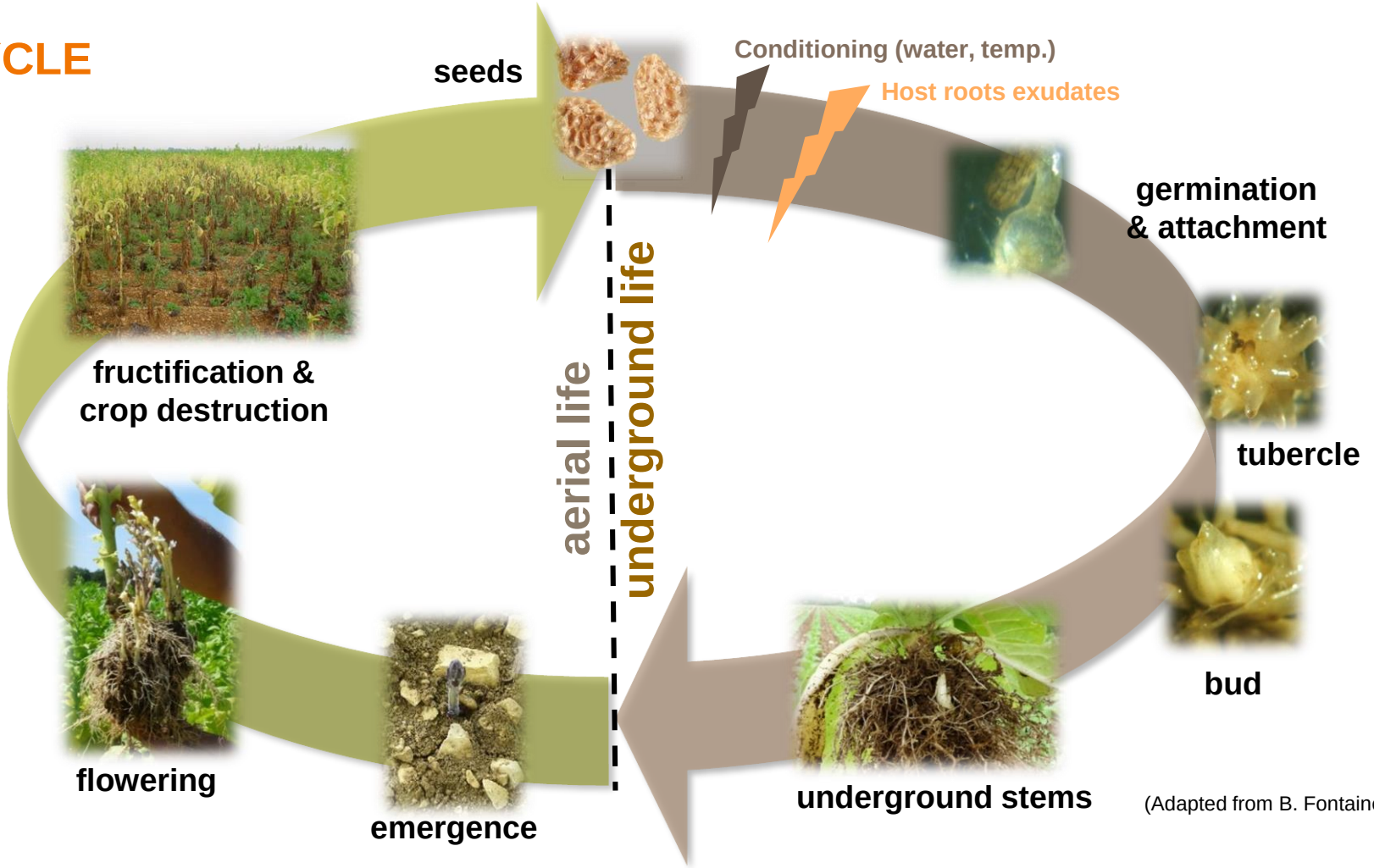
- Broomrape are dicotyledonous parasitic flowering plants that cause heavy economic losses in many crops worldwide.
- Economically destructive (from 5 to 100%) leading to the interruption of tobacco cultivation in very impacted areas.
  - 2018: south of France, up to 20% and more !
- Broomrape control is challenging:
  - Use of agrochemicals is difficult because of host-parasite connection
  - Prolific seed production, seed longevity…



(Adapted from G. Consior, 2005)

Need for resistant varieties
Assist selection with markers

CYCLE



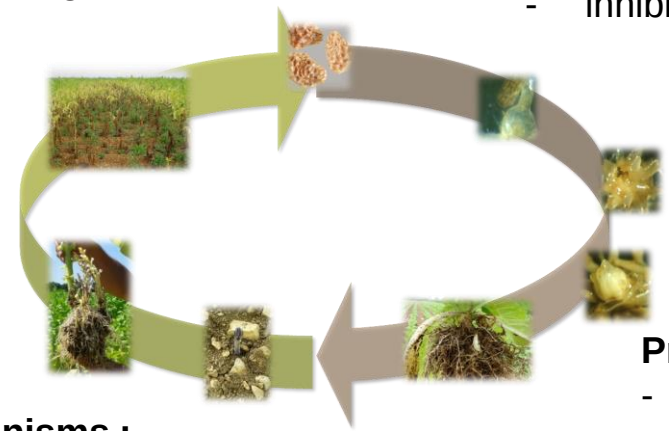
(Adapted from B. Fontaine, 2013)

RESISTANCE MECHANISMS

CAN BE QUANTITATIVE, MONOGENIC, RACE SPECIFIC, COMBINED MECHANISMS…

Preventing germination of seeds:
- low germination stimulant production
- exudation of parasitic-seed germination inhibitor

Preventing the penetration of the parasite:
- low haustorium stimulant production
- inhibition of haustorium formation



Preventing establishment :
- formation of a physical barrier (deposition of callose , suberin)
- host necrosis at attachment point

Postestablishment mechanisms :
- occlusion of vessels with mucilage
- production by the host and delivery into the parasite of toxic metabolites

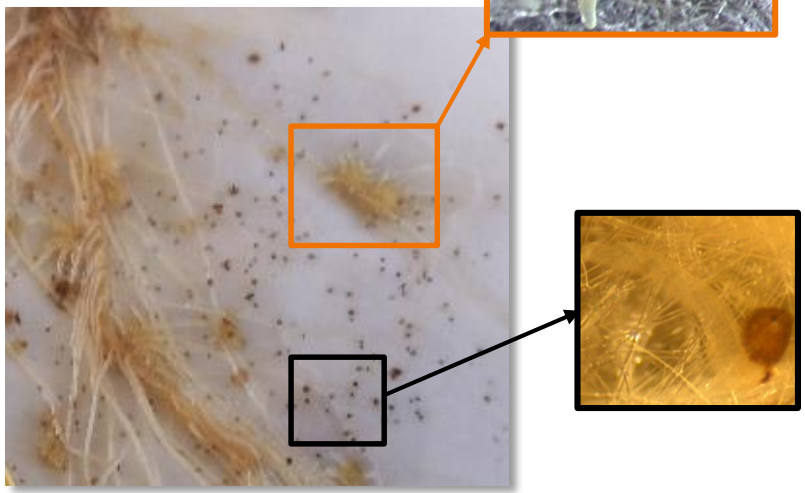
GENETIC MATERIAL USED

- Wika shows later/lower stimulation of *Orobanche* seed germination (IT germplasm collection screening : 68 entries Nicotiana species + various tobacco types, field trials from 2003 to 2006).
- Compared to a susceptible variety, no biological activity from Wika roots exudates have been found (Brault- Hernandez, PhD, 2006).
- This tolerance is recessively inherited (Cailleteau *et al*., CORESTA 2006)
- Breeding lines were developed by conventionnal breeding from Wika tolerance.

ITB 31612

Wika

THE PHENOTYPING TOOL



- Plantlets in plastic Petri dishes inoculated with 500-1000 Orobanche seeds.
- Scoring of germination near roots:
  - % of seeds germination
  - Number of seed germinated at star level.
- Warning:
  - Elimination of plantlets with small root system to avoid false negative.
  - Additionnal scoring for plants with 2<% germination<10

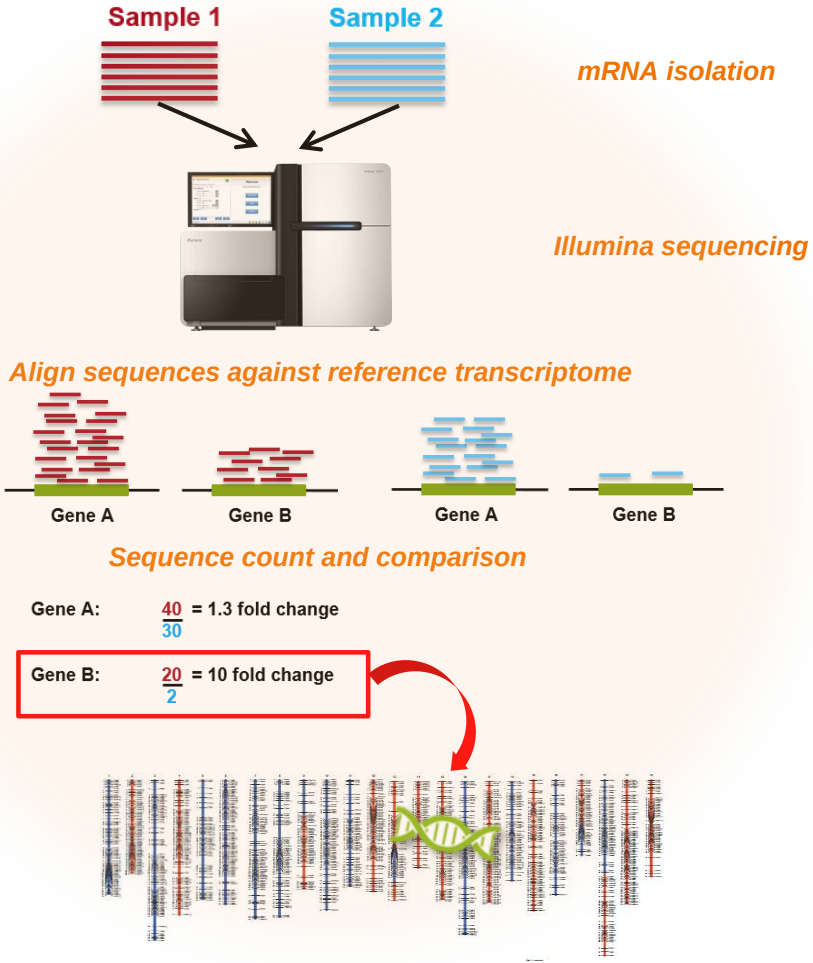
STRATEGY

I. RNA-Seq analysis of BSB breeding lines:
   - R control Wika + 7 resistant lines.
   - S control VD + 5 susceptible lines

II. Identification of candidate markers.

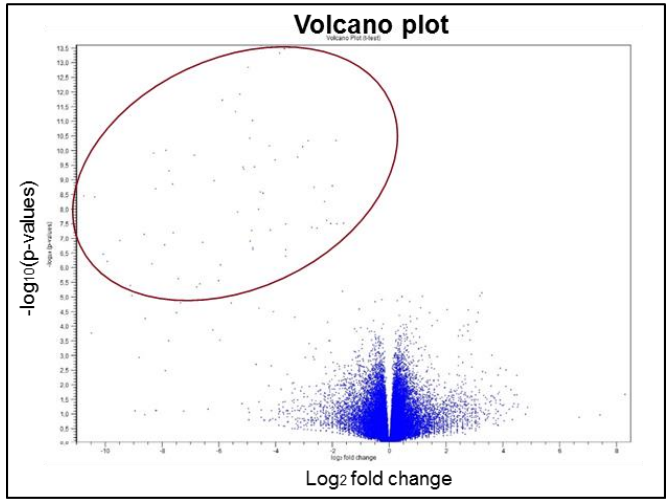
III. Validation and mapping on F2 segregating population characterized for tolerance.

IV. Screening of the Nicotiana collection

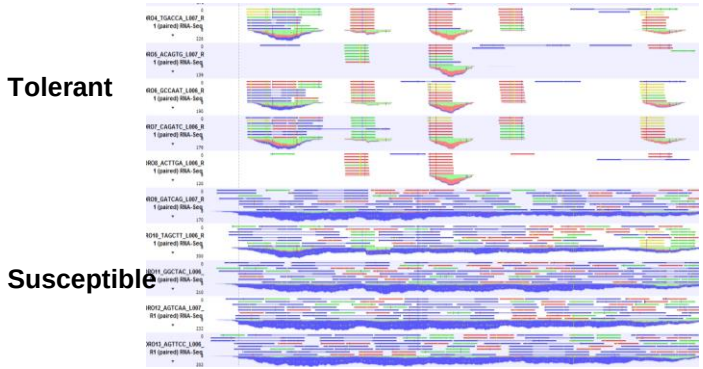


RNA-SEQ RESULTS

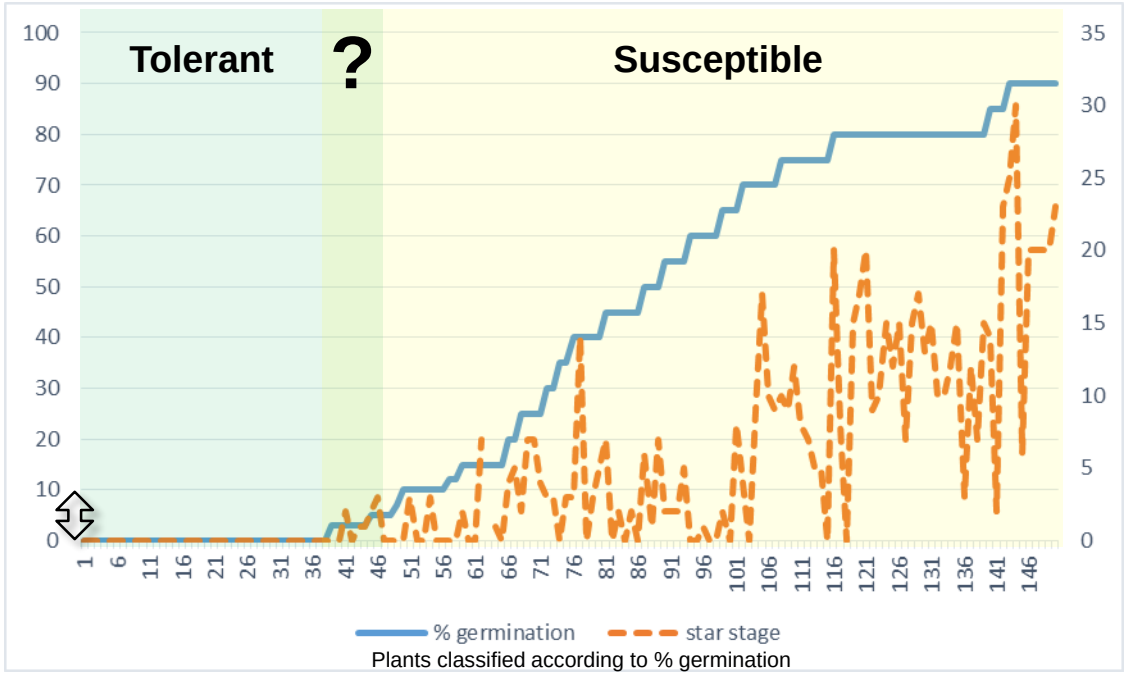
- Reference transcriptome : 62395 contigs.
- T-test, tolerant vs susceptible lines
  - More than 90 genes with P-value < 10^{-6}.
  - On these 90 genes, 95% are preferentially expressed in susceptible lines.
  - 94% are from *N. tomentosiformis* origin.



Deletion on a chromosome from *N. tomentosiformis* origin ?



TESTING ON TWO F2 SEGREGATING POPULATION
BIOLOGICAL TEST RESULTS



Plants classified according to % germination

	F2-1	F2-2
R parent	Wika	Wika
S parent	V4K	KYR
Number of ind.	83	134
R:S	16:67	35:99
P value (1:3)	0,229	0,765

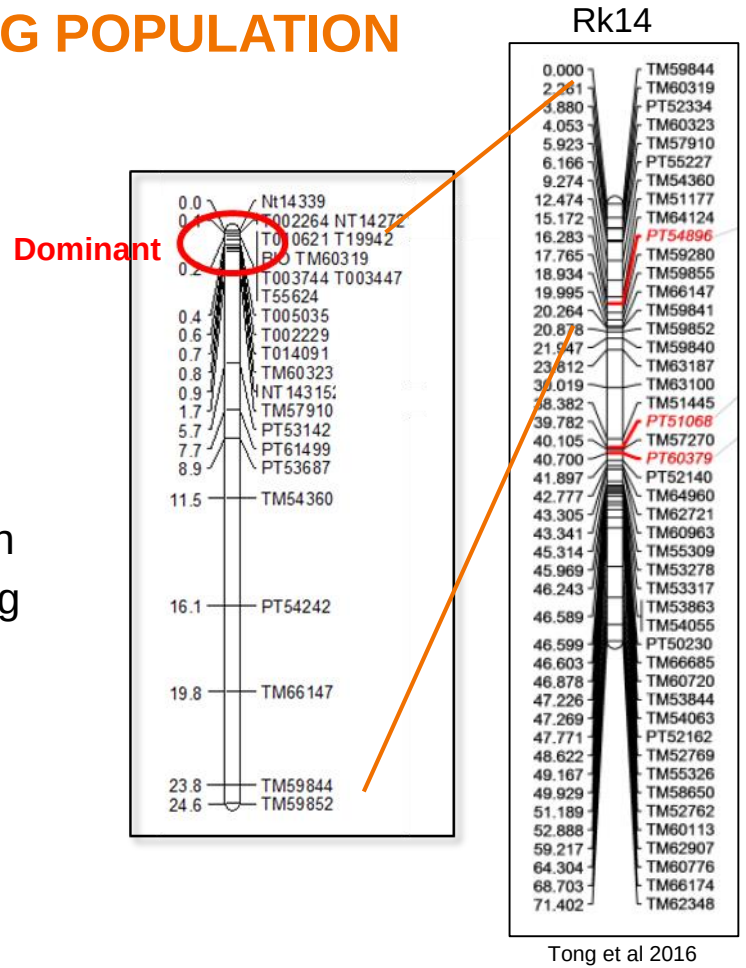
Recessive resistance confirmed

TESTING ON TWO F2 SEGREGATING POPULATION
LINKAGE MAPPING

- Alignement of differentially expressed genes on *N. tabacum* genome (Edwards *et al* 2017): all genes are mapping on the extremity of Nt.14.

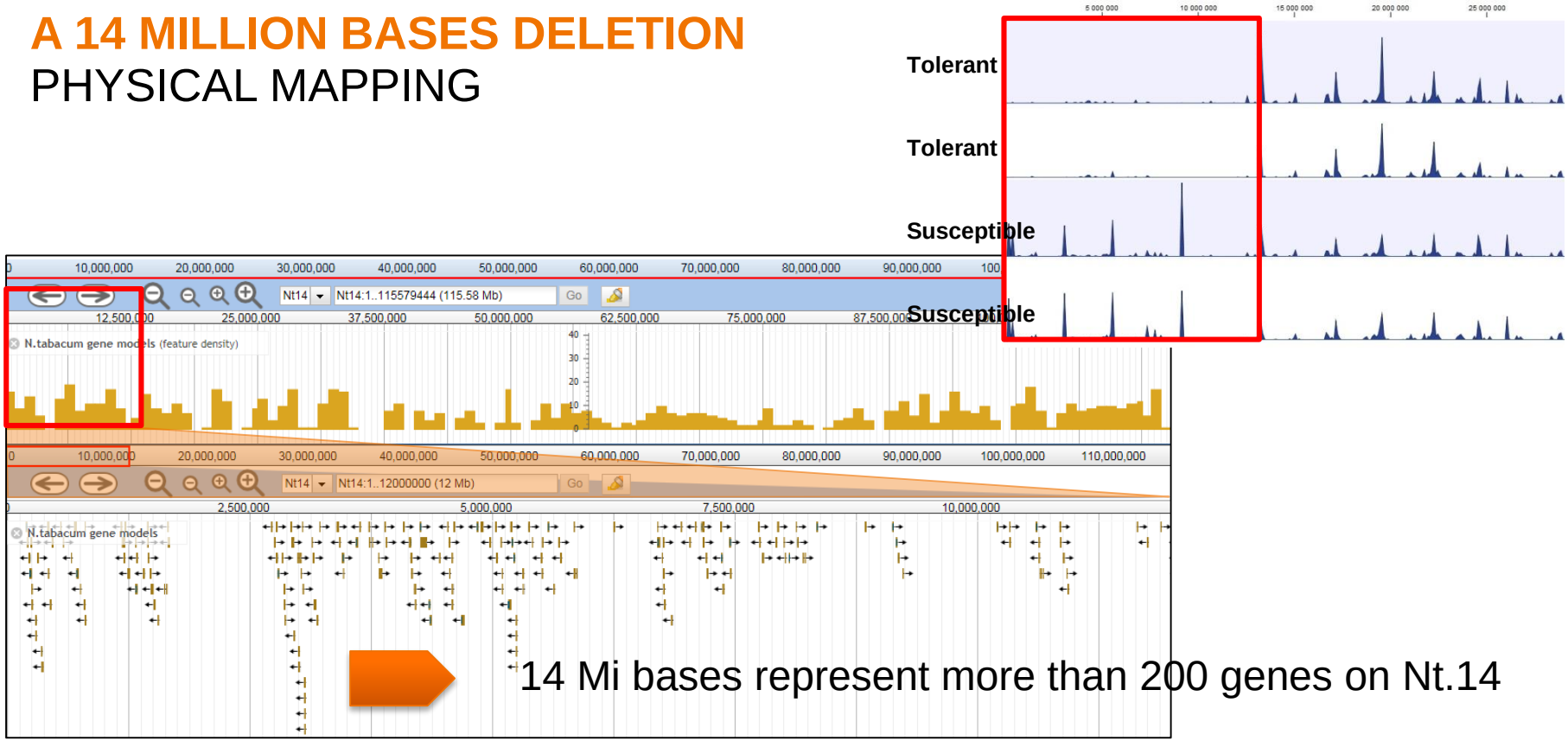
- Linkage mapping of some candidates along with SSR markers from Bindler *et al* (2011) and Tong *et al* (2016): confirmation of Nt.14.

Deletion on Nt14 confirmed



Tong et al 2016

A 14 MILLION BASES DELETION
PHYSICAL MAPPING



14 Mi bases represent more than 200 genes on Nt.14

SCREENING OF THE COLLECTION

DNA AND SSR MARKERS

Designed on DNA (Edwards et al 2017)

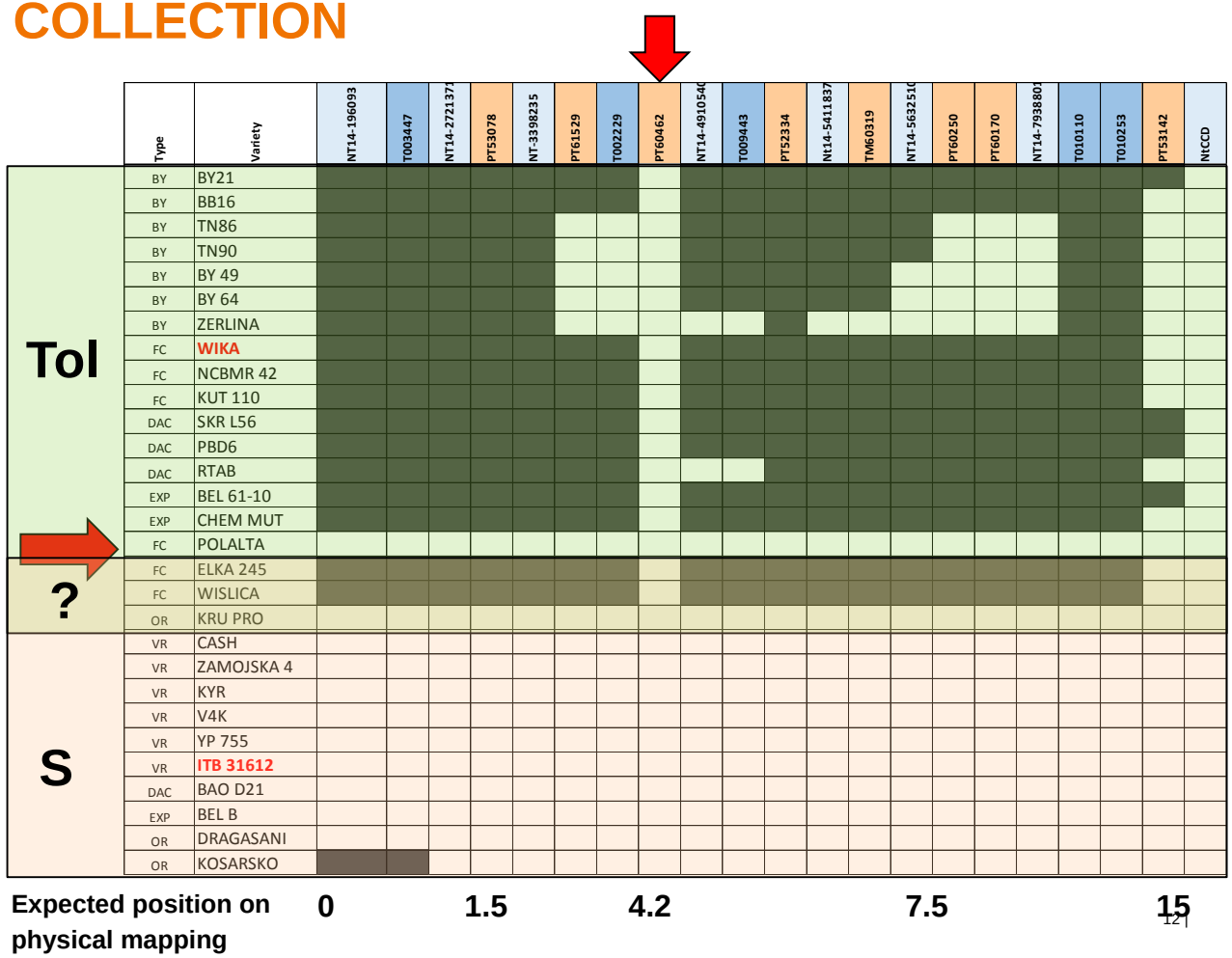
Designed on diff. expr. contigs

SSR (Bindler et al. 2011+Tong et al. 2016)

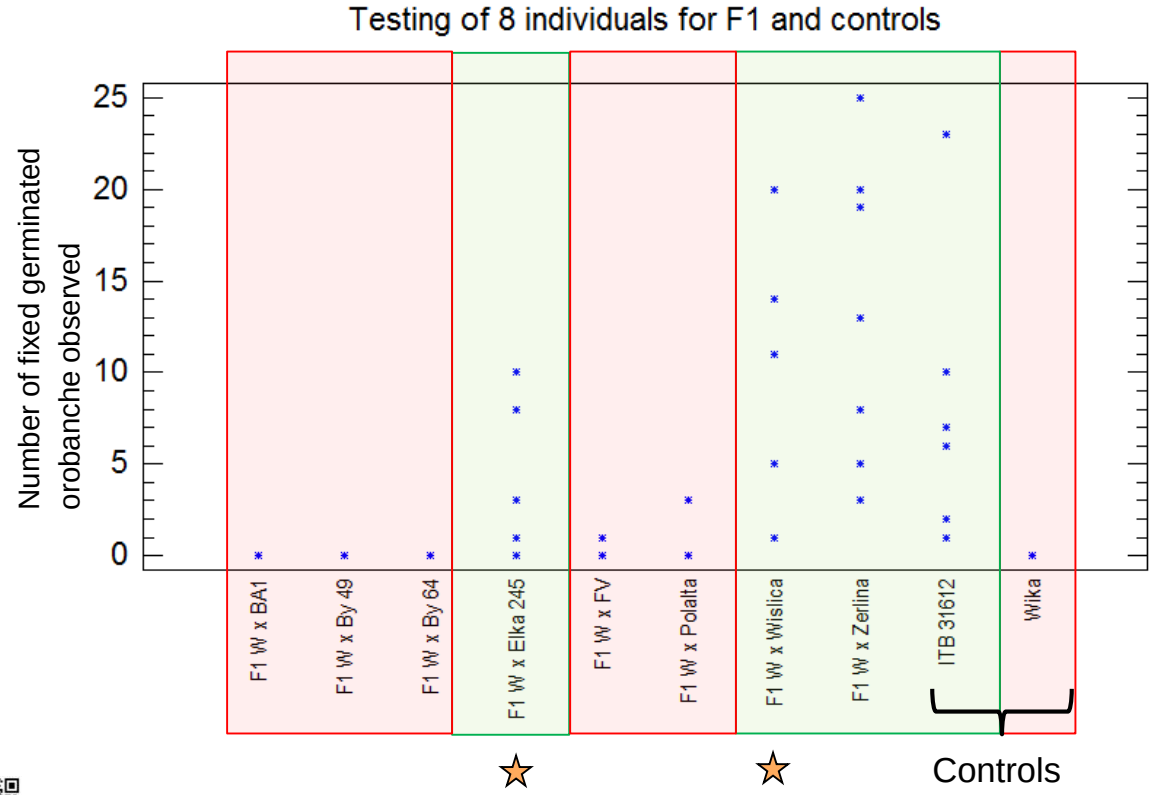
PCR +

PCR -

Different sizes and patterns of deletion



F1 ALLELISM TESTS WITH WIKA

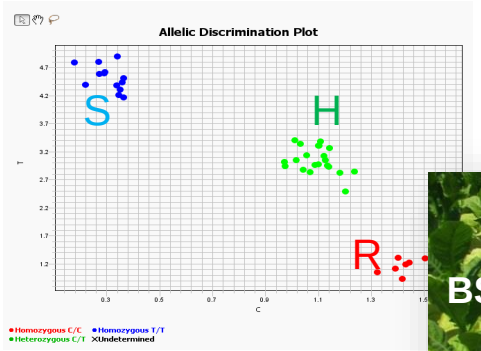


- Some varieties are allelic to Wika.
- Varieties with intermediate resistance ☆ not allelic to Wika
- Zerlina have a deletion on Nt14 but is not allelic
- Polalta looks like allelic to Wika but without a deletion on Nt14

MARKERS VALIDATED ON THE COLLECTION OF VARIETIES AND ON BSB COMMERCIAL LINES

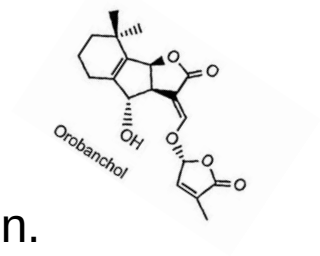
N°	MS/F/H	Origin	Commercial name	R/S	T002229	T002264	T003447
1	Variety	Susceptible control	VD	S			
2	Fertile Line	Susceptible control		S			
3	**Hybrid F1**	Susceptible control	ITB31612	S			
4	MS line	Susceptible line		S			
5	MS line	Susceptible line		S			
6	MS line	Susceptible line		S			
7	**Hybrid F1**	One parent tolerant only	BSB 6199	S (het)			
8	**Hybrid F1**	Both parent susceptible	ITB188	S			
9	***Variety***	***Resistant control***	***Wika***	*R*			
10	Fertile Line	Tolerant line		R			
11	Fertile Line	Tolerant line		R			
12	MS line	Tolerant line		R			
13	MS line	Tolerant line		R			
14	MS line	Tolerant line		R			
15	MS line	Tolerant line		R			
16	MS line	Tolerant line		R			
17	**Hybrid F1**	Both parents tolerant		R			
18	**Hybrid F1**	Both parents tolerant		R			
19	**Hybrid F1**	Both parents tolerant		R			
20	***Hybrid F1***	***Both parents tolerant***	***BSB 6190***	*R*			
21	***Hybrid F1***	***Both parents tolerant***	***BSB 6191***	*R*			

PCR markers and KASP markers available



http://www.bergeracsb.com

CONCLUSION



WIKA resistance

- Wika tolerance is related to low broomrape germination.
- This tolerance is under the control of a recessive gene on Nt.14.

New sources identfied

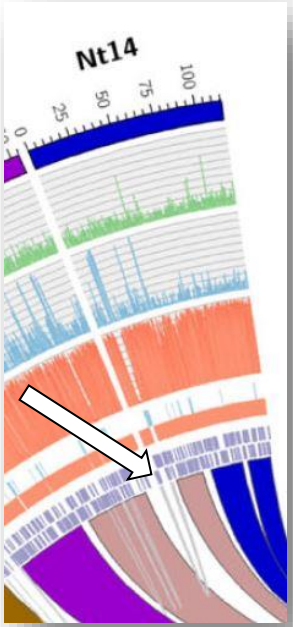
- Others sources of tolerance have been identified in the collection of varieties, allelic or with a different mechanism.

Solutions available

- User-friendly molecular markers are available to pilot the transfer of Wika tolerance in breeding lines.
- BSB tolerant lines are available.

PERSPECTIVES

- Local structure of the genome still unclear: multiple deletion with local rearrangement, differences according to the genotype.
- Candidate genes have been identified in this area, and are under evaluation.
- Need for segregation tests to assess the new sources of tolerance found in the Imperial Tobacco collection, in order to improve breeding.
- Tolerance to others species of Orobanche will be tested.



Thank you.

Emilie Julio
Julien Cotucheau
Roxane Volpatti
Christophe Decorps
François Dorlhac de Borne

Anna Malpica
Sonia Bachet
Julie L’Humeau