BERGERAC SEED & BREEDING

PROGRESS IN DEVELOPMENT OF A HIGH-THROUGHPUT PHENOTYPING TEST AS BREEDING TOOL FOR BROOMRAPE RESISTANCE IN TOBACCO

CORESTA Santa Cruz do Sul, October 2017, Brazil, AP42

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-Our breeding strategy and associated phenotyping tools

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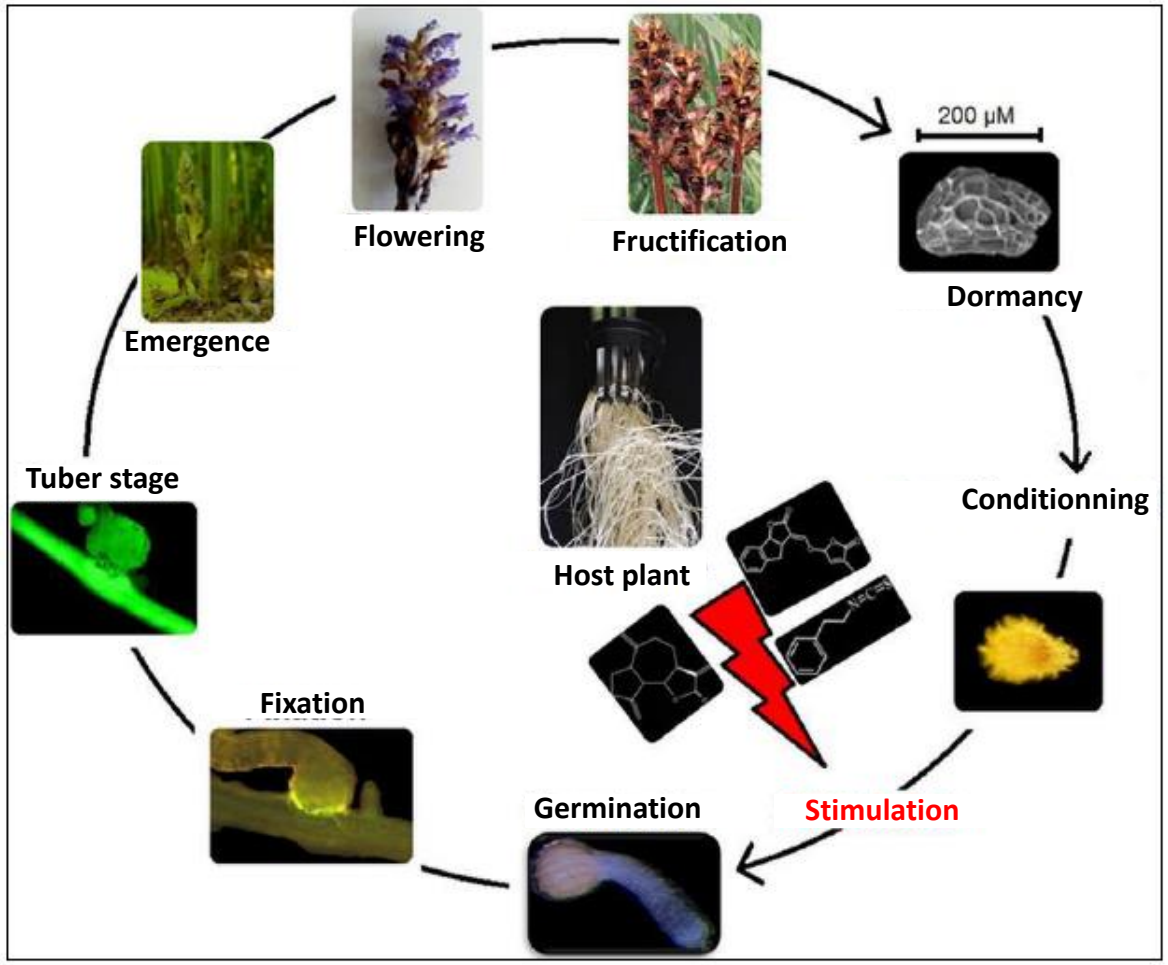
Tobacco broomrapes

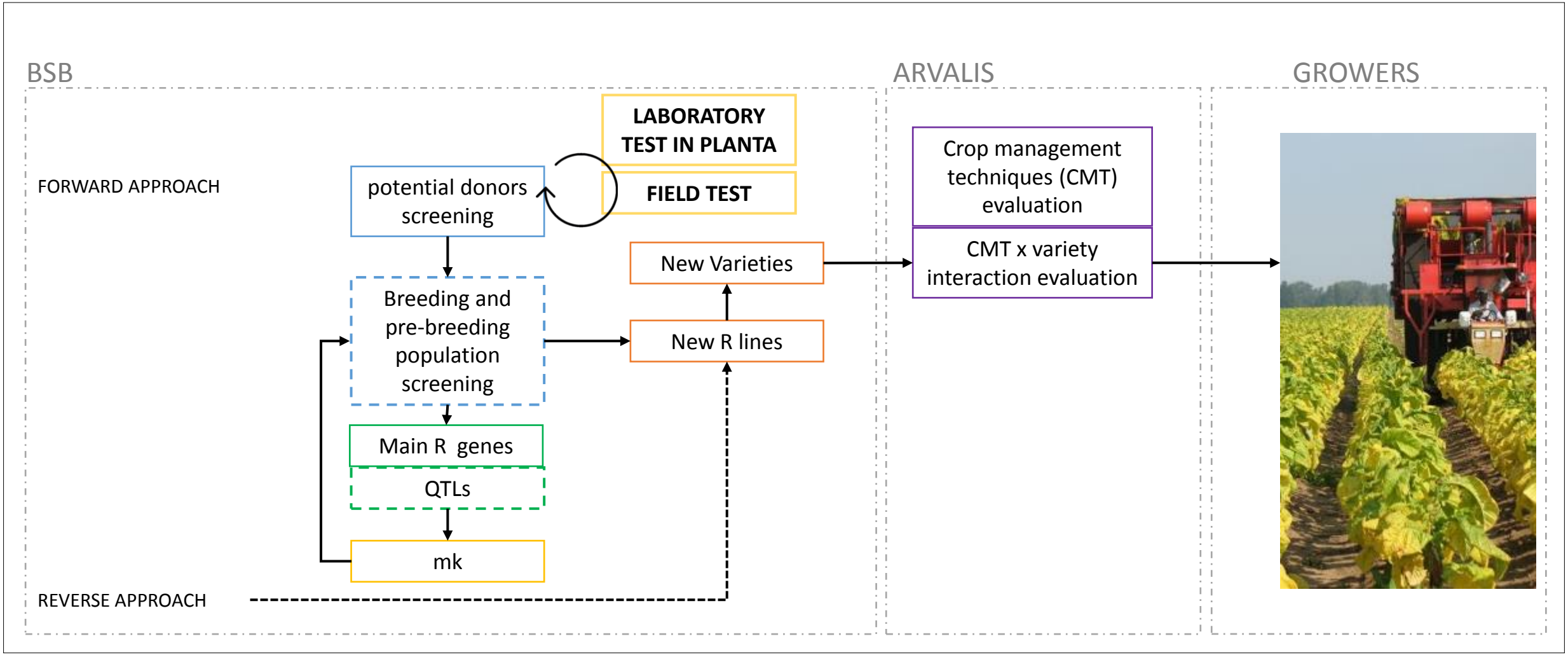


Biological cycle of *Phelipanche ramosa* (Lechat, 2004)

- Holoparasitic plants, main species affecting tobacco *P. ramosa*, *P. aegyptiaca*, *O. cernua*
- Present in Europe, Middle east, Asia and some African countries
- Large range of hosts: Solanaceae, Fabaceae, Brassicas, Sunflower, and many other crops

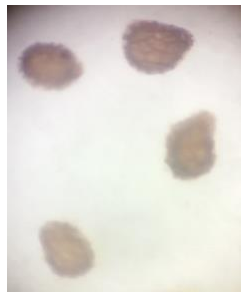
→ Economically destructive weeds causing large crop losses (from 5 to 100%) leading to the interruption of tobacco cultivation in very impacted areas.

Our breeding to grower strategy



Our phenotyping tools

LABORATORY TEST IN PLANTA



+ Straightforward and robust test
+ Clear differences observed

- Tedious and time consuming test
- Many false positive detected

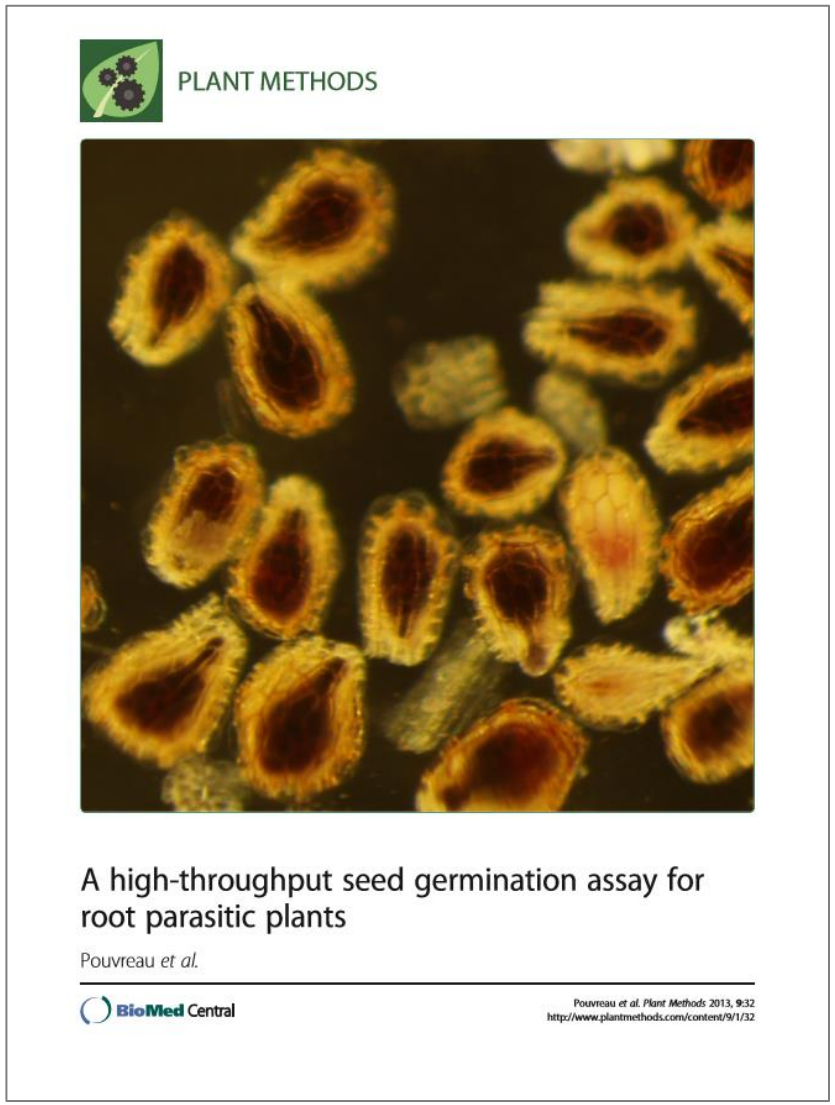
FIELD TEST

+ Tolerant genotype validation in a grower environement
+ Evaluation of plant vigor and contamination intensity

- One cycle a year test
- Space and time consuming

→ **Is a quantitative, efficient and easy to read test available?**

New phenotyping tool



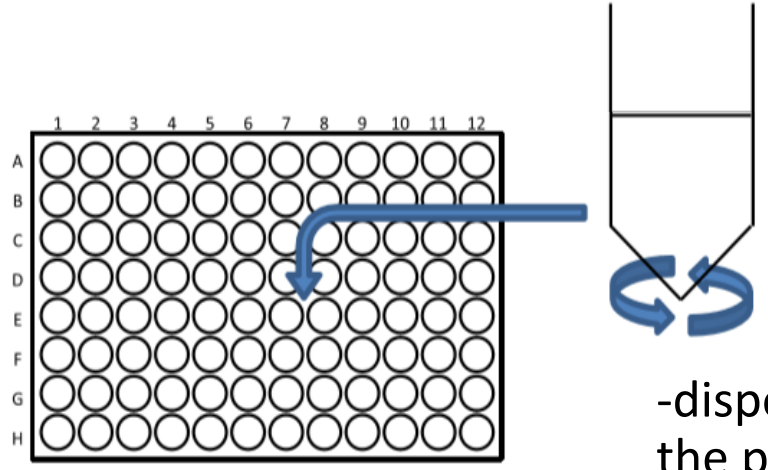
Nantes University team
Jean-Bernard Pouvraud, Philippe Simier

→development of an easy and fast method for broomrape germination rate determination on rapeseed model

→adaptation to *tobacco-Phelipanche ramosa* model
Fatma Ben Jamaa

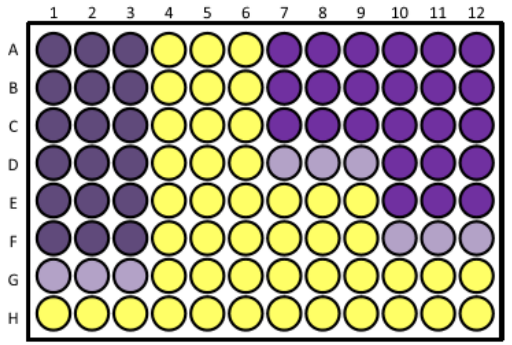
Test protocol

Plantlets cultivation on inert substrate (glass), weekly collection of exudates



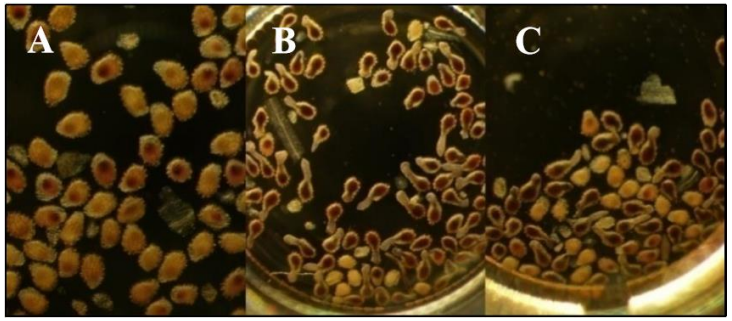
-disposal of broomrape seeds preconditioned in 96 wells

-disposal of collected exudates on the plate



-MTT (methylthiazolyldiphenyl-tetrazolium bromide) application

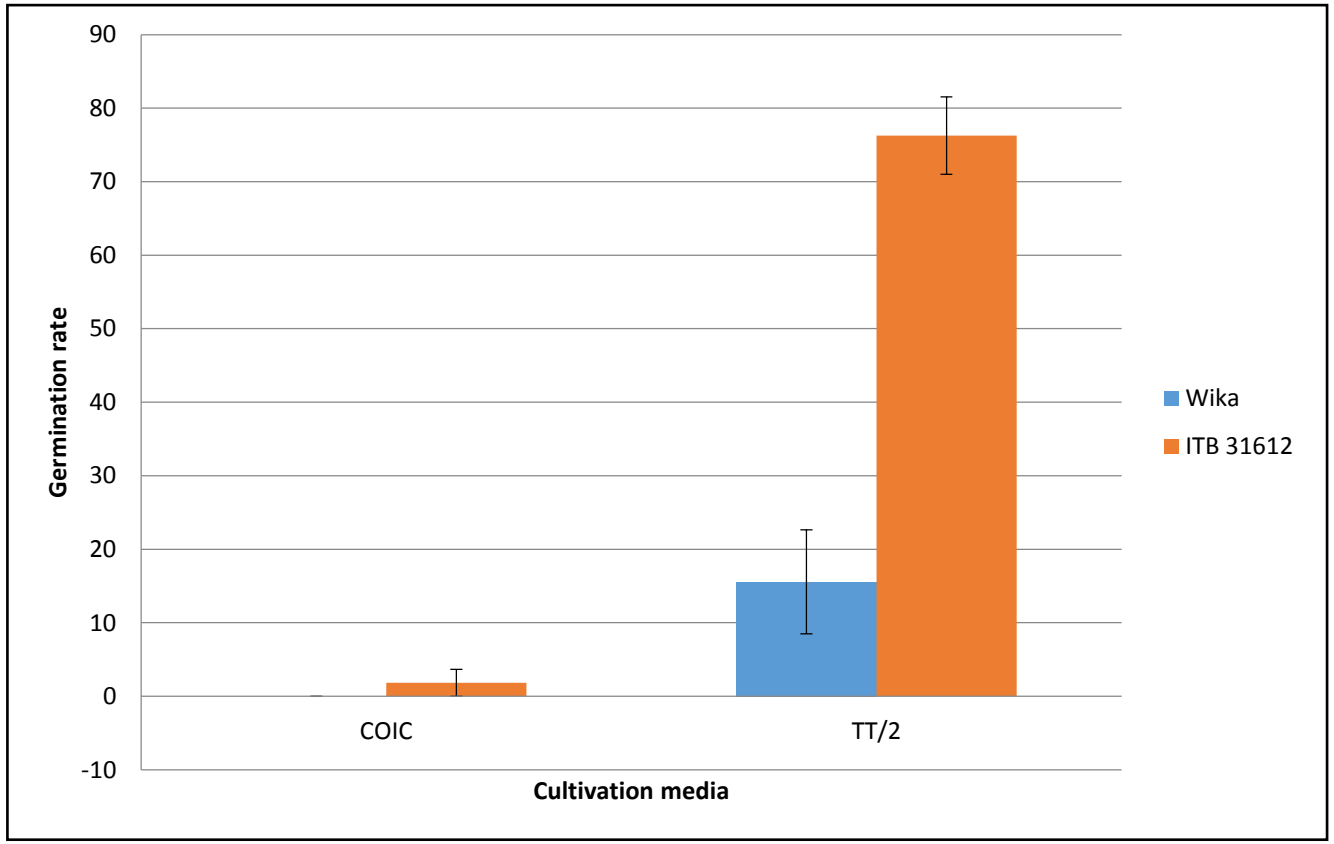
-Solubilization of formazan salts and absorbance reading



A. Negative control (water), B. positive control (GR24), C. orobanche seeds under exudates stimulation (BenJemaa, 2017)

-calibration curves are calculated based on broomrape seeds germinated with GR24

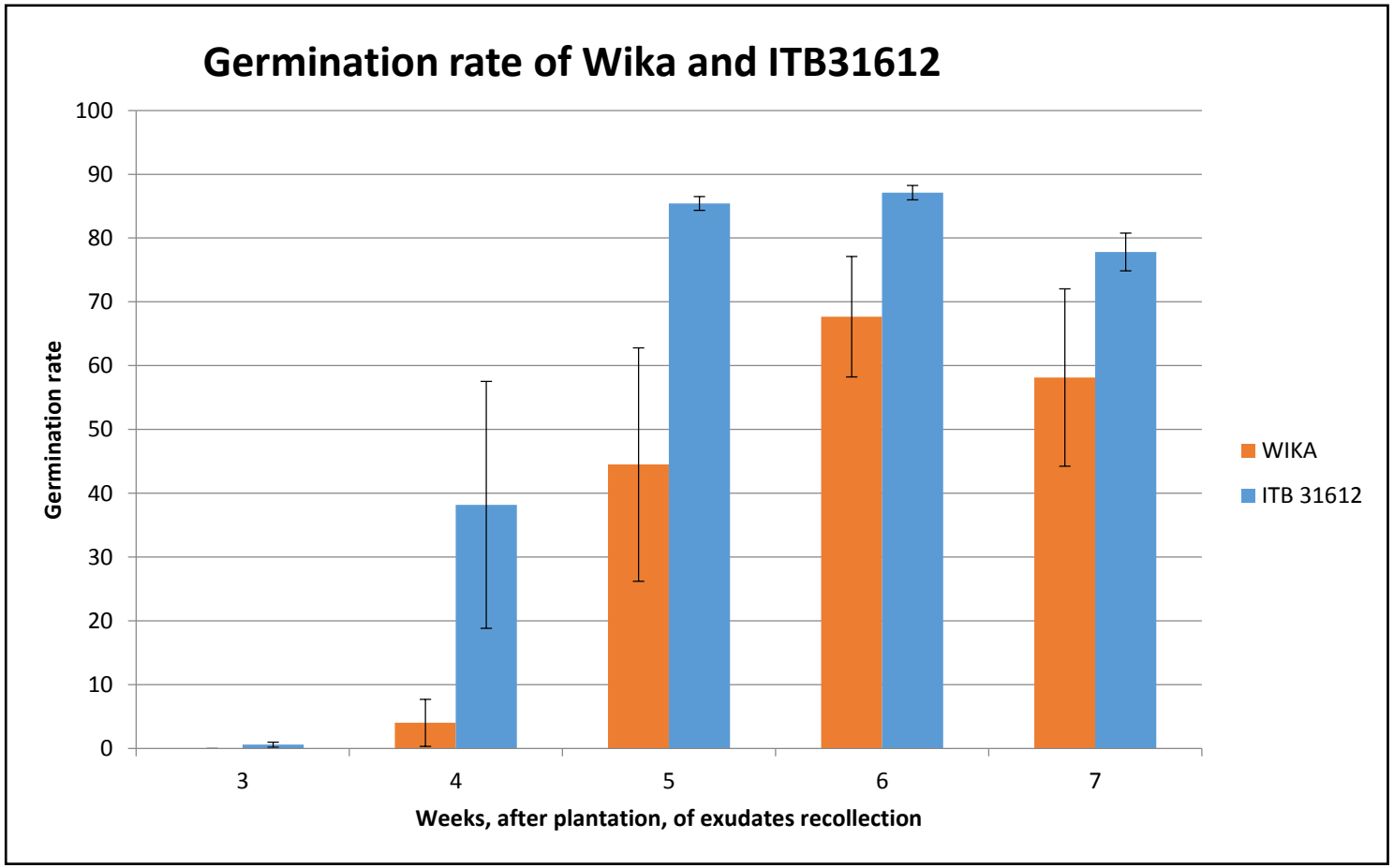
Results



Total	Coïc	Coïc 1/2	TT	TT 1/2
N	14,2	7,1	7,72	3,86
P	1,1	0,55	0,32	0,16
K	5,2	2,6	4	2
S	0,75	0,375	4	2
Mg	0,75	0,375	2	1
Na	0,4	0,2	2,32	1,16

→ Cultivation media validation: TT/2 media generates more roots exudates

Results



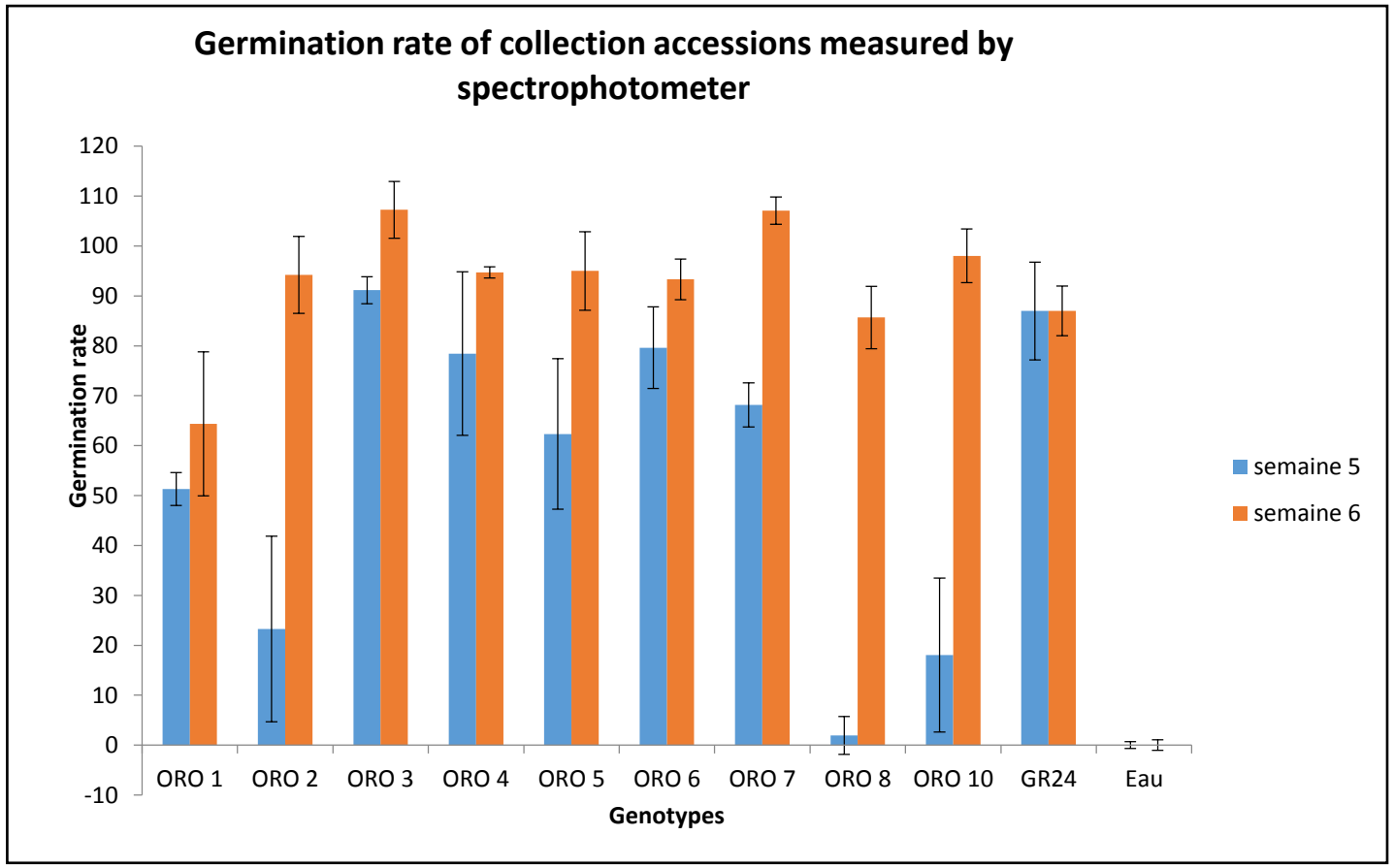
- Significant difference between germination rates of Wika (tolerant genotype) front to ITB31612 (susceptible genotype)

→Validation of the test accuracy in tobacco resistance screening

- Most significant difference is obtained 4 to 5 weeks after plantation

→determination of the best screening window

Results



- Large screening of new potential sources of resistance.
- Significant difference between genotypes

→ validation of candidates

Conclusion and discussion

An efficient test: easier and faster measurements of germination rates
→enables to work with larger populations
→enables a precise quantitative screening

→enables screening of germination inhibitors

Limitation
Detection of tolerance mechanisms linked to exudation and broomrape germination stimulation only

- Implementation of this phenotyping test in our breeding routine
- Validation of tolerance levels of accessions screened
- Screening of larger breeding and pre-breeding populations

Thanks for your attention