

Projet WEAB

WEAB

Wheat Effector Assisted Breeding for Resistance to Fungal Pathogens

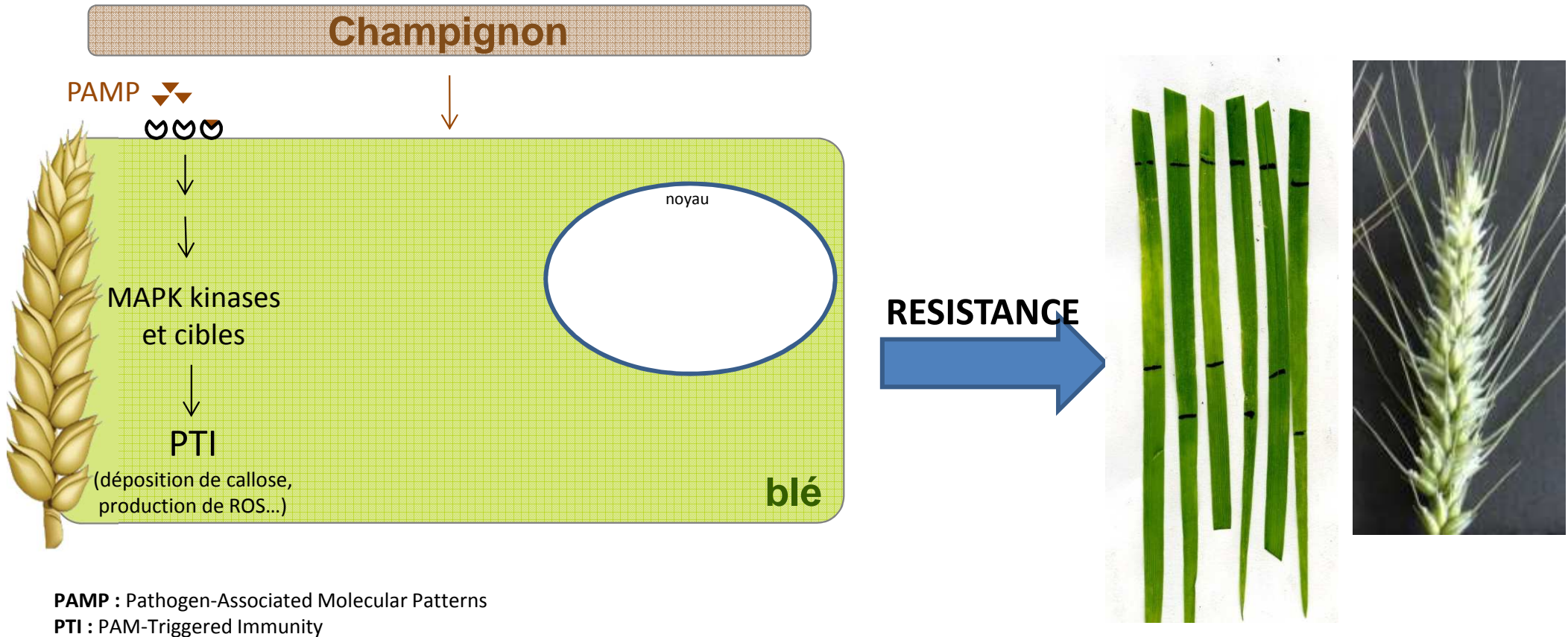
Coordinateur : Marc-Henri LEBRUN

Marc-Henri LEBRUN, INRA BIOGER, Thiverval-Grignon, Thierry LANGIN, INRA GDEC, Clermont-Ferrand ; Thomas KROJ, INRA BGPI, Montpellier ; James COCKRAM, NIAB, Grande Bretagne ; Richard OLIVER, ACNFP, Australie ; Gert KEMA, PRI, Hollande ; Romain VALADE, Arvalis, Thiverval-Grignon ; Sébastien PRAUD, BIOGEMMA, Clermont-Ferrand ; Valérie LAURENT, Florimond-Desprez, Cappelle en Pévèle ; Laure Duchalais, RAGT, Louville la Chenard; Volker LEIN, CETAC, Estrées-Saint-Denis



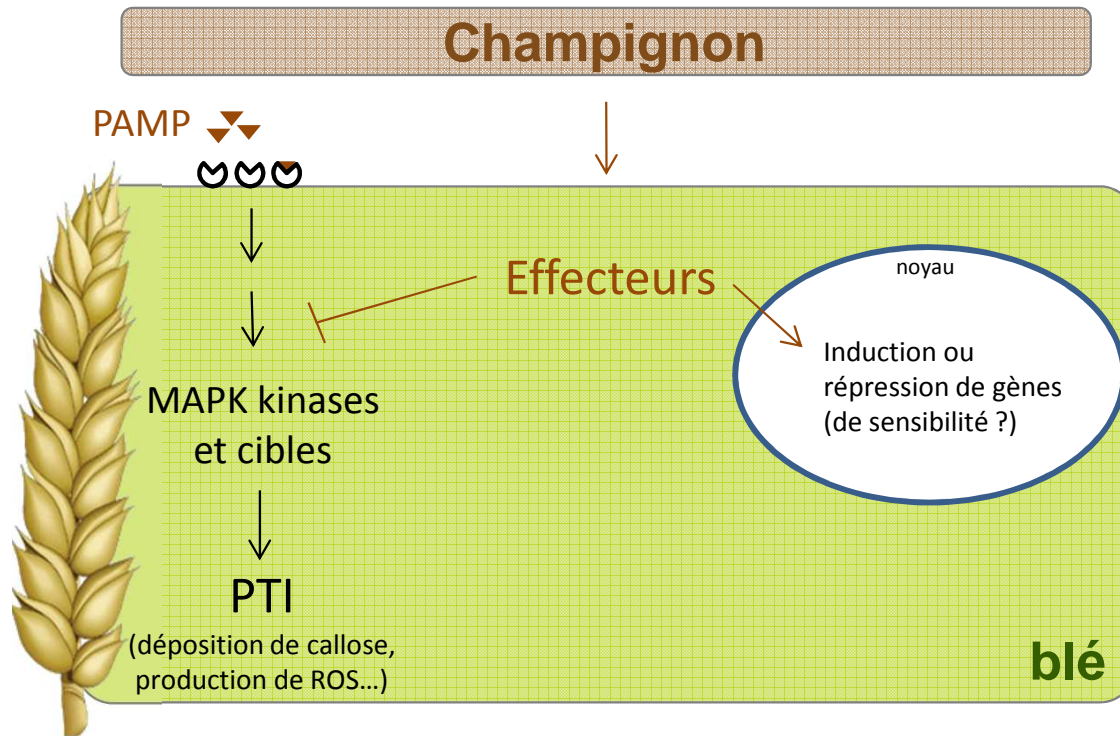
Comprendre les interactions blé/pathogènes

Première voie de défense chez la plante (PTI)



Comprendre les interactions blé/pathogènes

Adaptation du pathogène aux premières voies de défenses



PAMP : Pathogen-Associated Molecular Patterns

PTI : PAM-Triggered Immunity

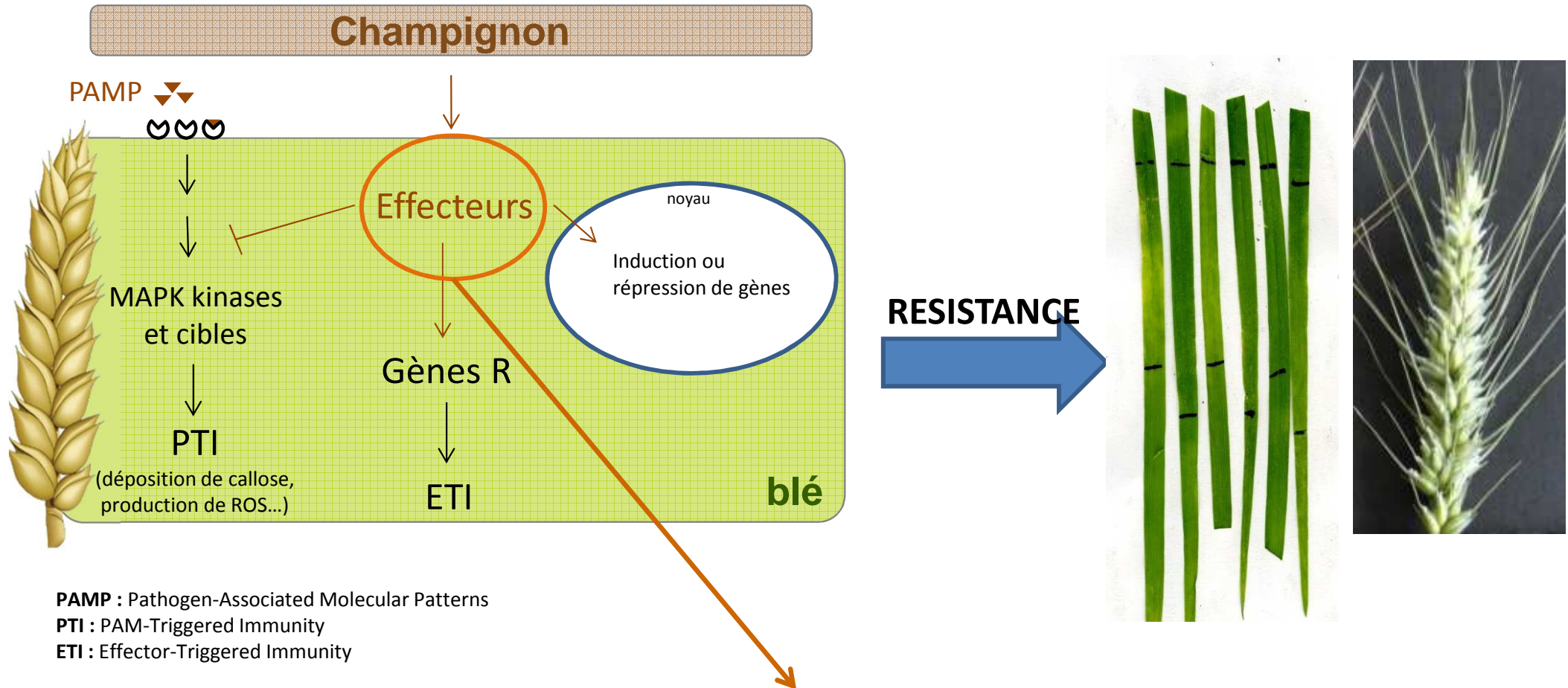
SENSIBILITE



Effecteurs : cocktail de molécules de petites tailles, sécrétées, sans fonction connue

Comprendre les interactions blé/pathogènes

Deuxième voie de défense chez la plante (ETI)



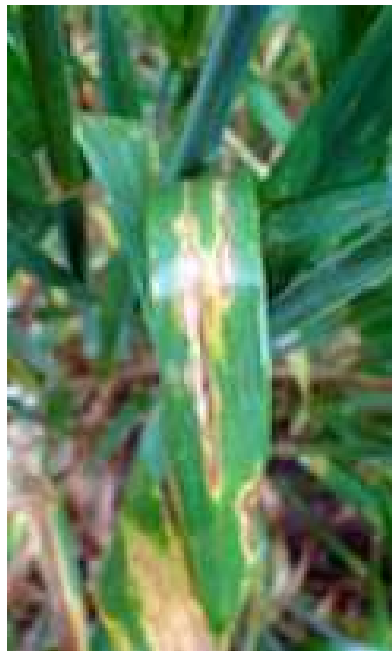
Projet WEAB : Utilisation des effecteurs inducteurs de nécroses (transition biotrophie / nécrotrophie) pour identifier des nouvelles résistances

Focus on fungal pathogens of wheat
Fusarium graminearum
Mycosphaerella graminicola
Stagonospora nodorum

M. graminicola



S. nodorum

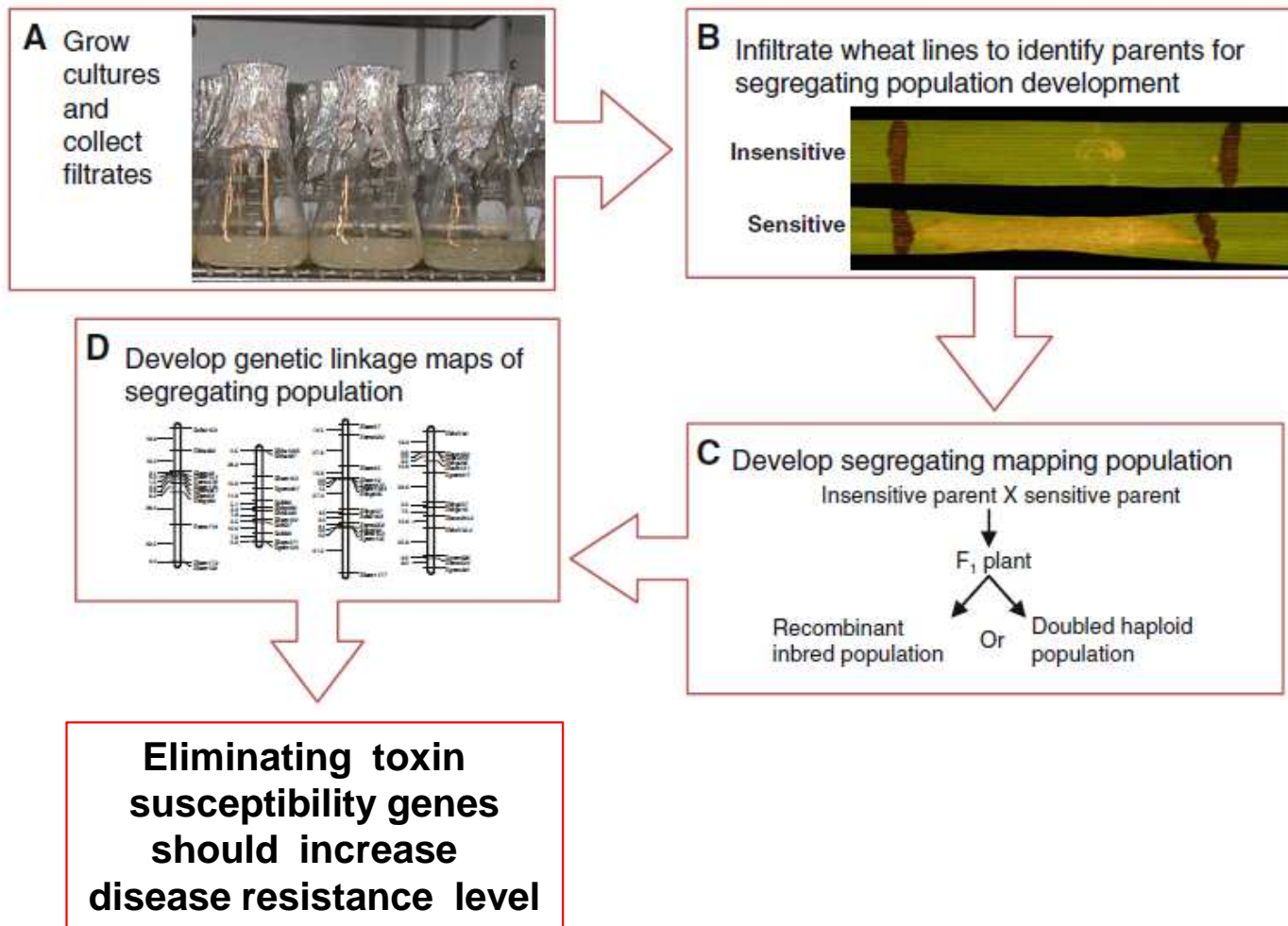


F. graminearum



Strategy:

Using fungal toxic protein effectors to screen for resistant/insensitive wheat cultivars: example *S. nodorum*

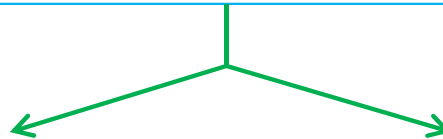


WP1 Effectors from *F. graminearum* (*Fg*), *M. graminicola*, (*Mg*), *S. nodorum* (*Sn*) genomes : Expression during infection, toxic domains
Deliverables: priority list for testing on wheat

WP2 Effector evolution in *F. graminearum* and *M. graminicola*
Deliverables: refined priority list for testing on wheat



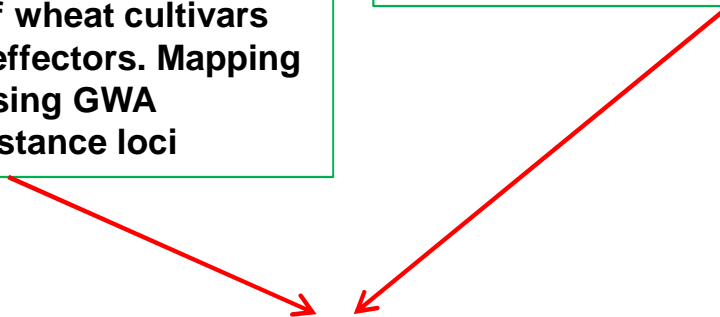
XX effectors from *Fg*, *Mg*, *Sn*



WP3 Identification of toxic effectors
Production and testing by infiltration in wheat leaves
Deliverables: toxic effectors

WP4 Screening of wheat cultivars for resistance to effectors. Mapping resistance loci using GWA
Deliverables: resistance loci

WP5 Wheat effector targets (WET)
Identification of effector targets in wheat and their mapping
Deliverables: WET genes linked to resistance QTLs

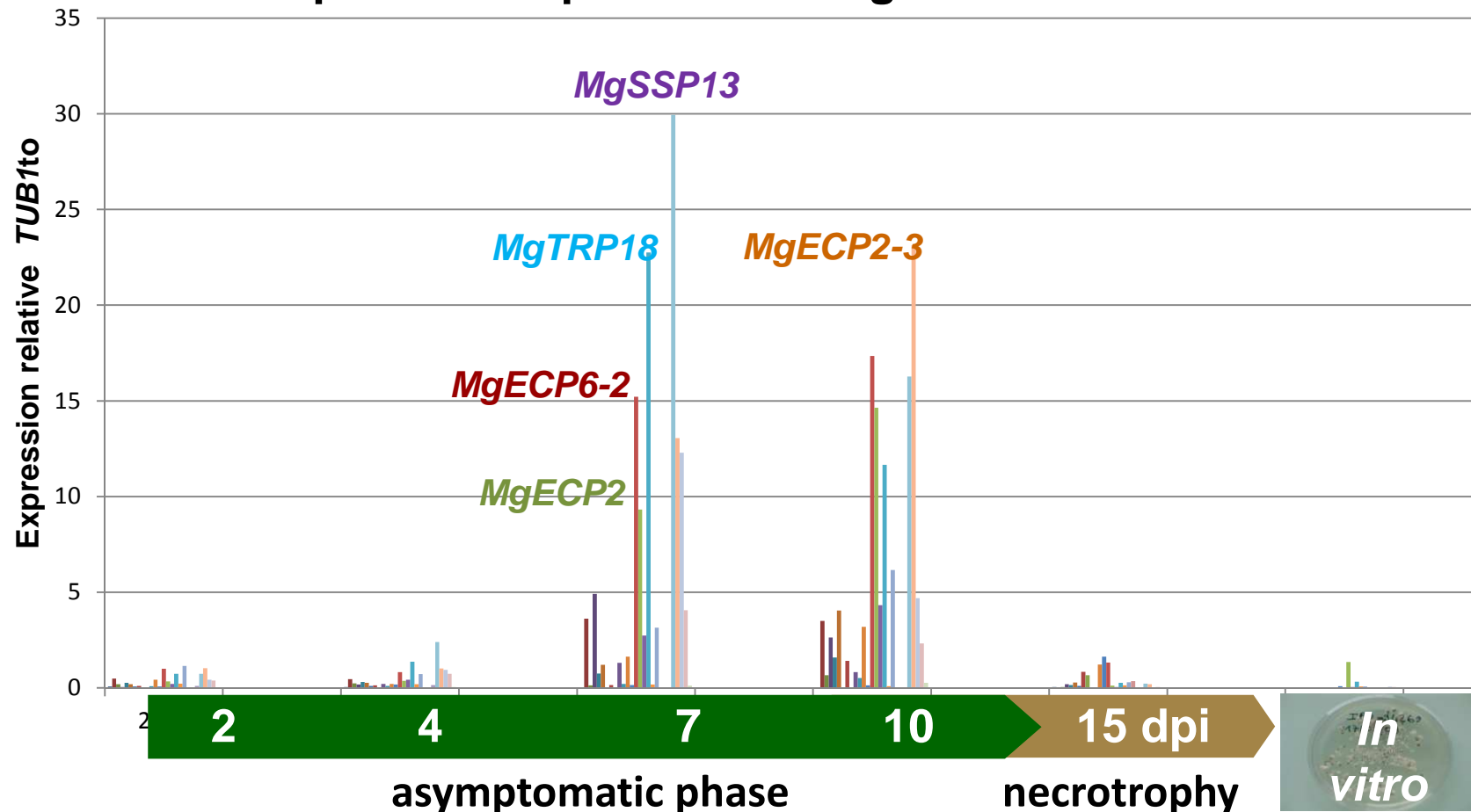


novel resistance genes / QTLs to fungal pathogens

WP1/2 Selection of effectors from *F. graminearum*, *M. graminicola* and *S. nodorum*

M. graminicola : No toxic effector found yet (may be some ?)

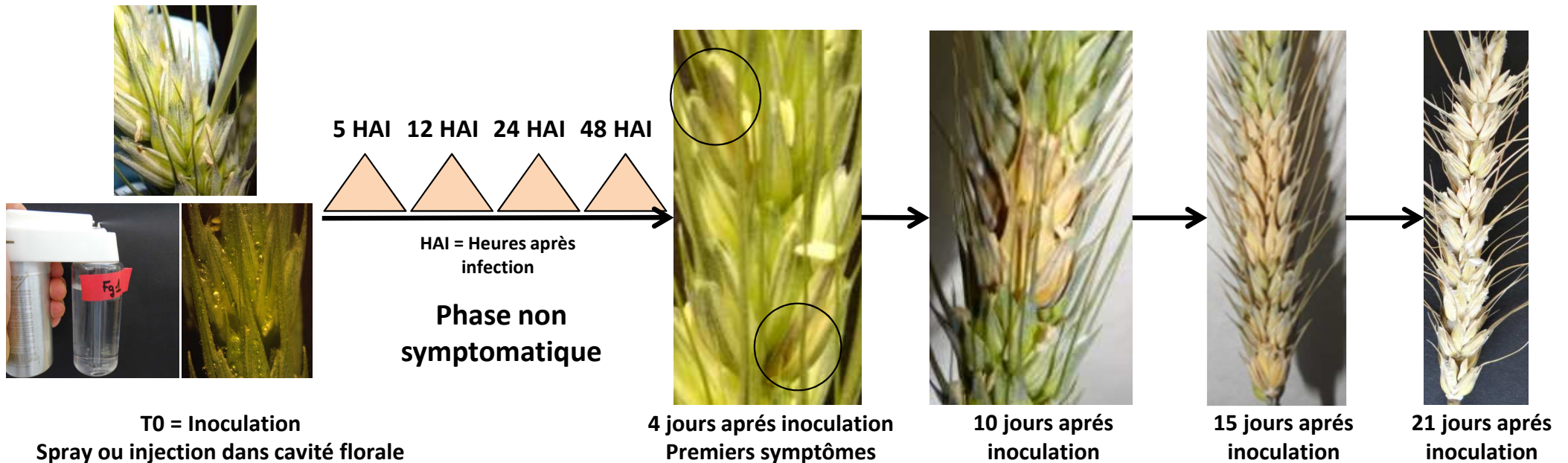
secreted proteins expressed during wheat leaves infection



WP1/2 Selection of effectors from *F. graminearum*, *M. graminicola* and *S. nodorum*

Suivi de l'expression d'effecteurs potentiels au cours d'une cinétique d'infection *in planta*

Extraction des ARNm de grains infectés au cours d'une cinétique



	Expression of the selected candidates in early stages of infection in wheat spikes									Expression of selected candidates non plant medium			<i>in silico</i> data	
	0 hour after infection	5 hours after infection	12 hours after infection	24 hours after infection	48 hours after infection	4 days post infection	10 days post infection	15 days post infection	21 days post infection	PDA solid medium	PDB liquid medium	Germinating spores	Predicted PSS	NLS
Candidat-1	-	-	+	+	+	+	+	+	+	-	-	-	+	-
Candidat-2	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Candidat-3	-	-	-	+	+	+	+	+	+	-	-	-	+	-
Candidat-4	-	+	+	+	+	+	+	+	+	-	-	+	+	+
Candidat-5	-	-	+	+	+	+	+	+	+	-	-	-	+	+
Candidat-6	-	-	-	+	+	+	+	+	+	-	-	+	+	+
Candidat-7	-	-	-	-	-	-	-	+	+	-	-	-	+	-
Candidat-8	-	-	-	+	+	+	+	+	+	-	-	-	+	-

WP1/2 Selection of effectors from *F. graminearum*, *M. graminicola* and *S. nodorum*

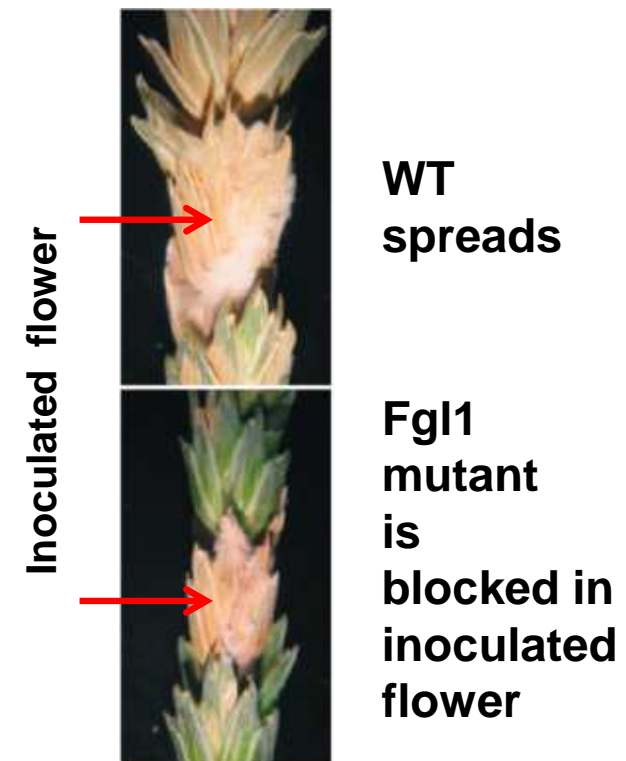
***F. graminearum* toxic effectors :**
proteins secreted in infected leaves

Lipase Fgl1

- Secreted in infected wheat tissues
- Required for full infection
- Inhibits callose synthase
- Induces necrosis of wheat flowers



Pathogenicity defect of Fgl1 mutant



No information on resistance / insensitivity of wheat cultivars to Fgl1

WP3 Identification of toxic effectors

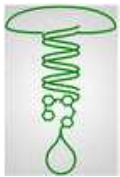
Production and testing by infiltration in wheat leaves

Candidate effectors

**Gene synthesized
and cloned in Pichia
expression vector**

Production in Pichia

Purification



**BBF
Marseille**

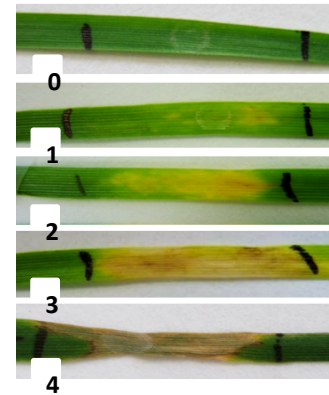


**Injection into
wheat leaves**

Symptoms ?

**Test on
differential
cultivars and
French
commercial
panel**

GWA mapping



Example SnToxA



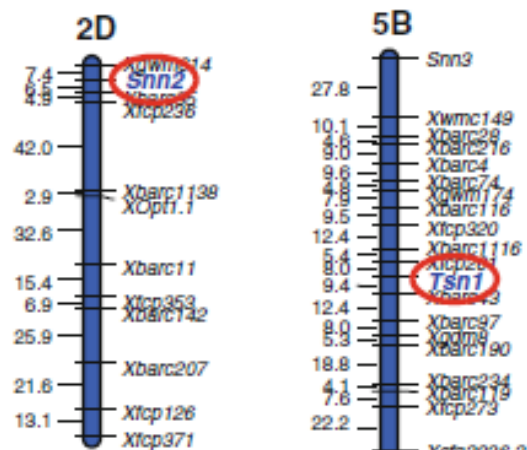
Curtin University



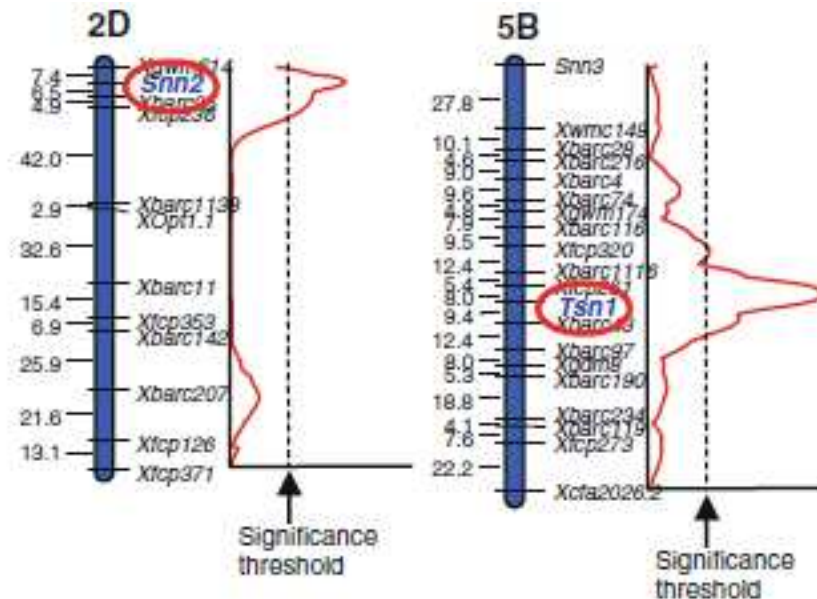
WP4 Screening of wheat cultivars for resistance to effectors Mapping resistance loci using GWA

Mapping Sn, Mg, Fg resistance QTLs of French wheat cultivars
relative to toxin resistance / insensitivity loci

Loci conferring insensitivity
to toxins (*S nodorum*)



Co-localization with QTLs conferring
Resistance to the disease (*S nodorum*)



WP5 Wheat effector targets (WET)

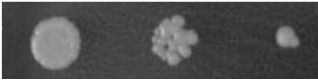

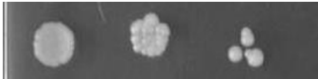







Identification of effector targets in wheat and their mapping

For few selected toxic effectors :
Screening for interactants in a wheat Y2H library

Successful example of such a screening in rice

Rice RGA5 = CC-NBC-LRR-**c term domain**
interacts with Pia CC-NBS-LRR

Y2H
M. oryzae
Effectors

BD	AD	Mating control (DDO)	Selection (TDO)
AVR-CO39	RGA5_L		
AVR-Pia	RGA5_L		
Empty	RGA5_L		
AVR-CO39	Empty		
AVR-Pia			

Perspectives de WEAB

Identification d'effecteurs toxiques de Fg / Zt
Essais sur feuille pour tester l'effet toxique des effecteurs

Identification de cultivars tolérants et/ou résistants à ces effecteurs toxiques

Identification des protéines de blé ciblées par ces effecteurs toxiques
Cartographie des loci conférant la résistance à ces effecteurs toxiques

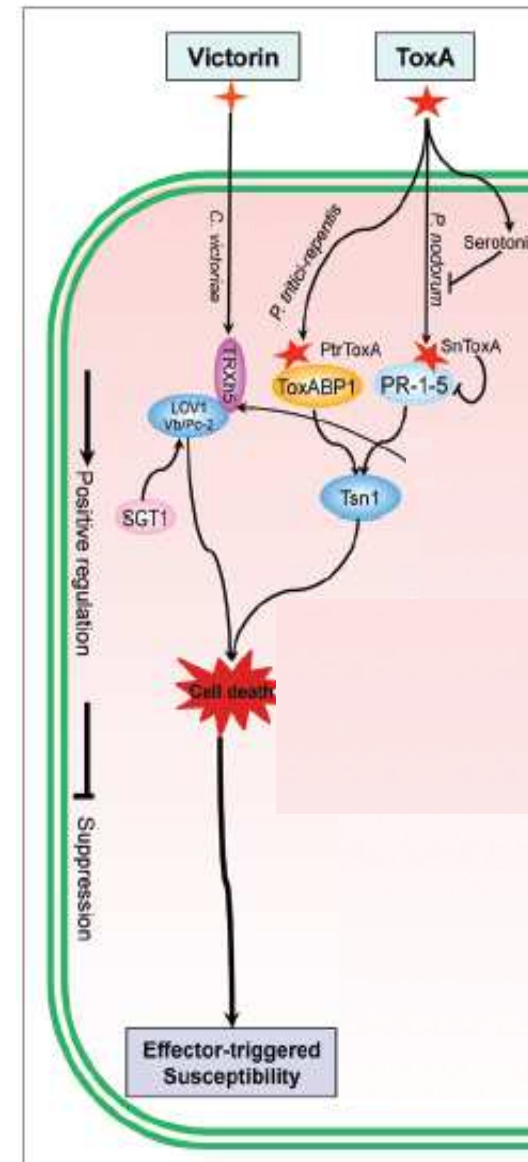
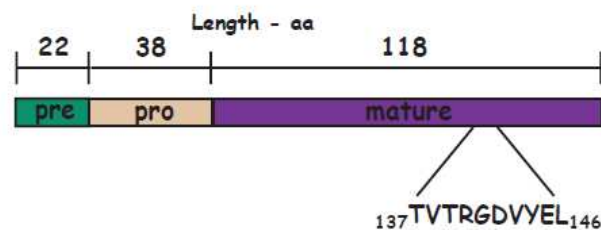
Evaluation des cultivars français pour leur résistance à Sn

**Merci de votre
attention**

WP1/2 Selection of effectors from *F. graminearum*, *M. graminicola* and *S. nodorum*

***S. nodorum* toxic effectors:**
proteins secreted in infected leaves

ToxA



WP1/2 Selection of effectors from *F. graminearum*,
M. graminicola and *S. nodorum*

***S. nodorum* toxic effectors:**
proteins secreted in infected leaves

	cell target	target proteins	susceptibility gene
SnToxA	Chloroplasts	ToxABP1, PR1	Tsn1 (NBS-LRR)
SnTox1	Chloroplasts		Snn1 (WAK)
SnTox3	Unknown		Snn3
SnTox4	Chloroplasts		Snn4
SnTox5	Unknown		Snn5

WP4 Screening of wheat cultivars for resistance to *S. nodorum*

Snod durum wheat SE13001
On Halberd Euclid



Snod bread wheat SE13004
On Halberd Euclid



Snod tritcale SE14005
On Halberd Euclid

