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WP1-SPECIFICS: Plant traits for pest control of pulses in pesticide-free cropping systems

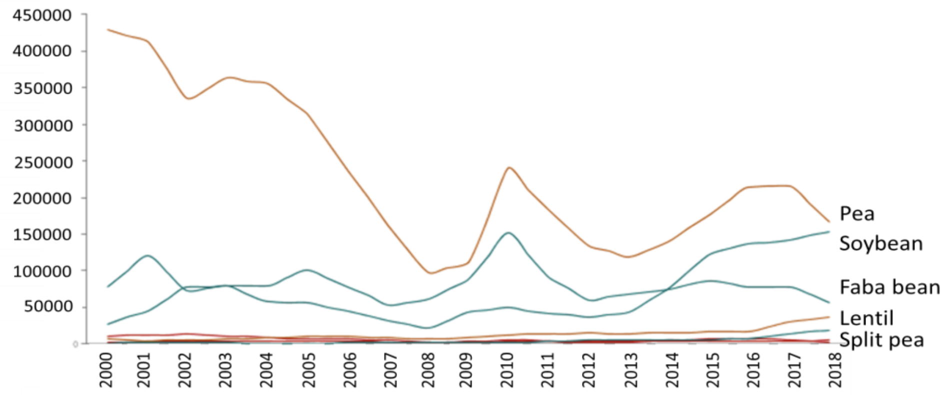
Marie-Laure PILET-NAYEL

Réunion Comité Consultatif, 06 décembre 2022

Partners: Agroécologie, IGEPP, IRHS, URGI, BioEcoAgro; Service provider: Terres Inovia :

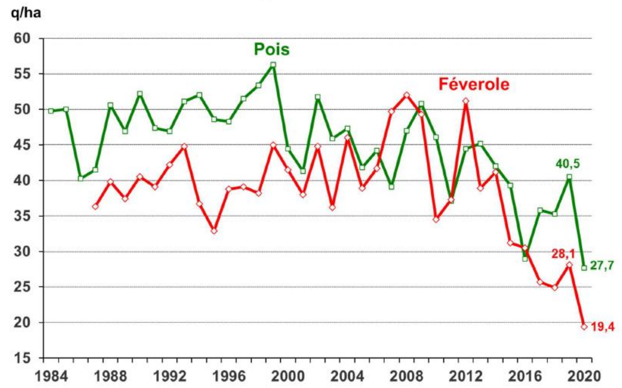
Background

Multiple diseases and pests are responsible for yield instability of major grain legume crops in France



Evolution of cultivated areas in grain legumes in France

(Source: Agreste)



Yield evolution of major pulse crops in France

(Source: Terres Univia)

Root rots

Aphanomyces euteiches

Fusarium solani

Aschochyta blight

Didymella pinodes

Chocolate spot

Botrytis fabae

Diseases

Aphid

Acyrthosiphon pisum

Bruchid

Bruchus pisorum

Sitona

Sitona lineatus

Bruchid

Bruchus rufimanus

Pests

Weeds

Limiting grain legume development; Dependant on pesticides

Compendium Pea Diseases & Pests, 3rd Ed (2021)
https://www.terresinovia.fr/

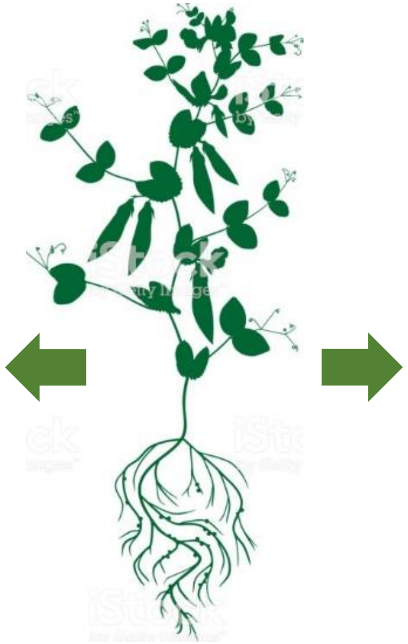
Objectives

WP1 will focus on the **lever of the plant** to enhance protection of pulses and foster their development in pesticide-free systems.

Plant resistance

Seed weevil
Aphid
Sitona

Root rots
Ascochyta blight

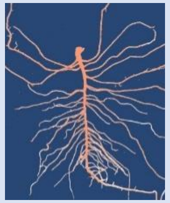


Other plant traits

Competitiveness against weeds

- Competition for soil resources
- Stand establishment

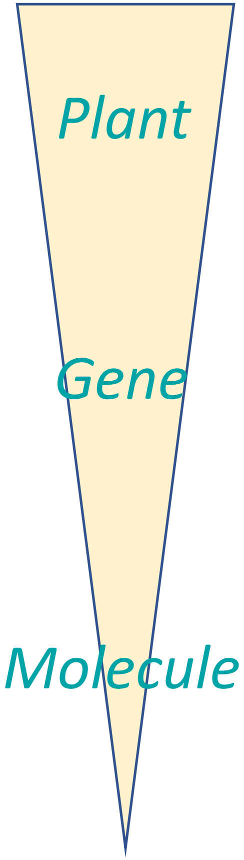
Root architecture



Objectives

WP1 will focus on the **lever of the plant** to enhance protection of pulses and foster their development in pesticide-free systems.

1. Identify novel **sources of resistance** and **protective traits** against damaging pests in pesticide-free cropping systems
2. Analyze and exploit **genomic conservation** between plant species to facilitate gene discovery, **integrate omics** knowledge of pest resistance in pulses to identify and combine multi-pest resistance loci
3. Analyze and compare **defense mechanisms** of pulses in response to pests to diversify their use in durable pest management and identify future breeding targets.



WP1 tasks

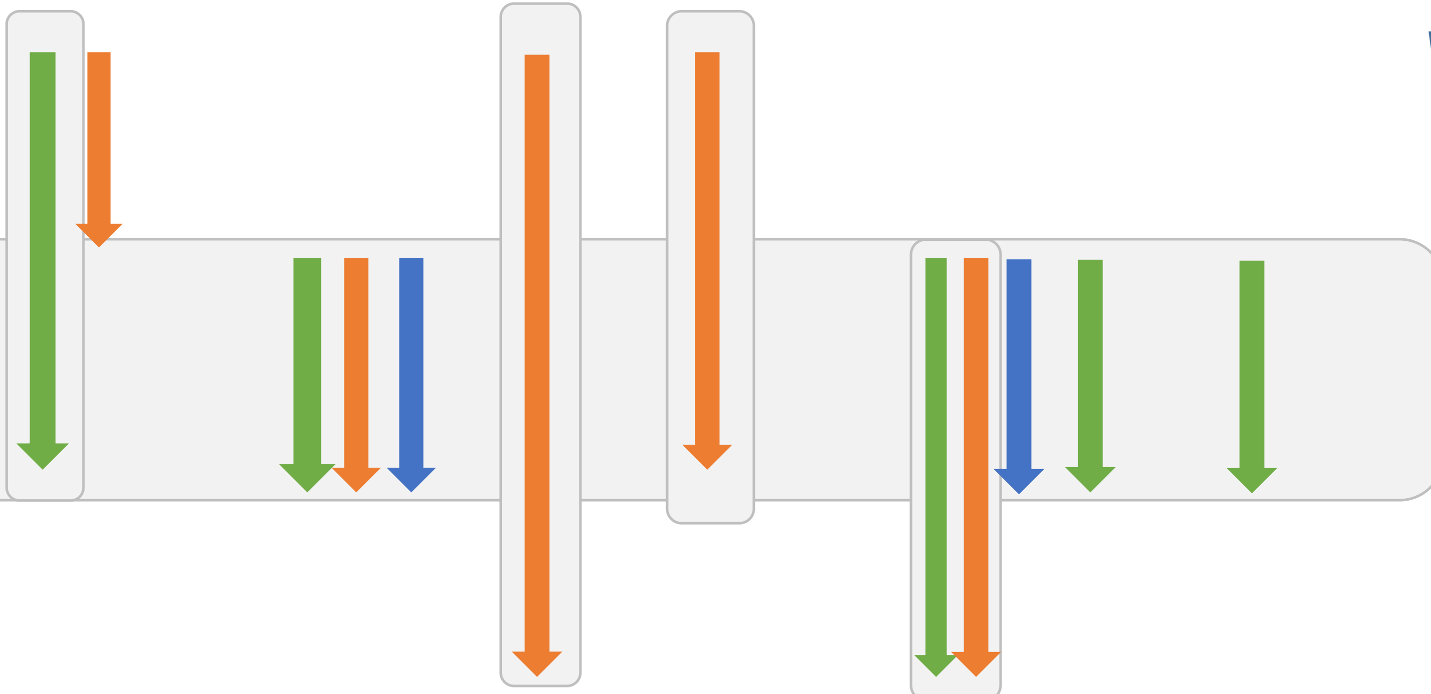
Weeds

Insects

Seed weevil *aphid* *Sitona /root archit.*

Diseases

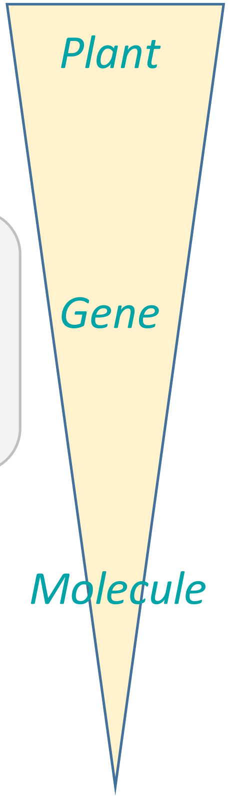
Aphanomyces *Fusarium* *Ascochyta*



Task 1.1. Novel sources of resistance and protective traits

Task 1.2. Integrative and translational genomics for multi-pest resistance

Task 1.3. Defense mechanisms in plant immunity



Related Previous/On going projects

RAID

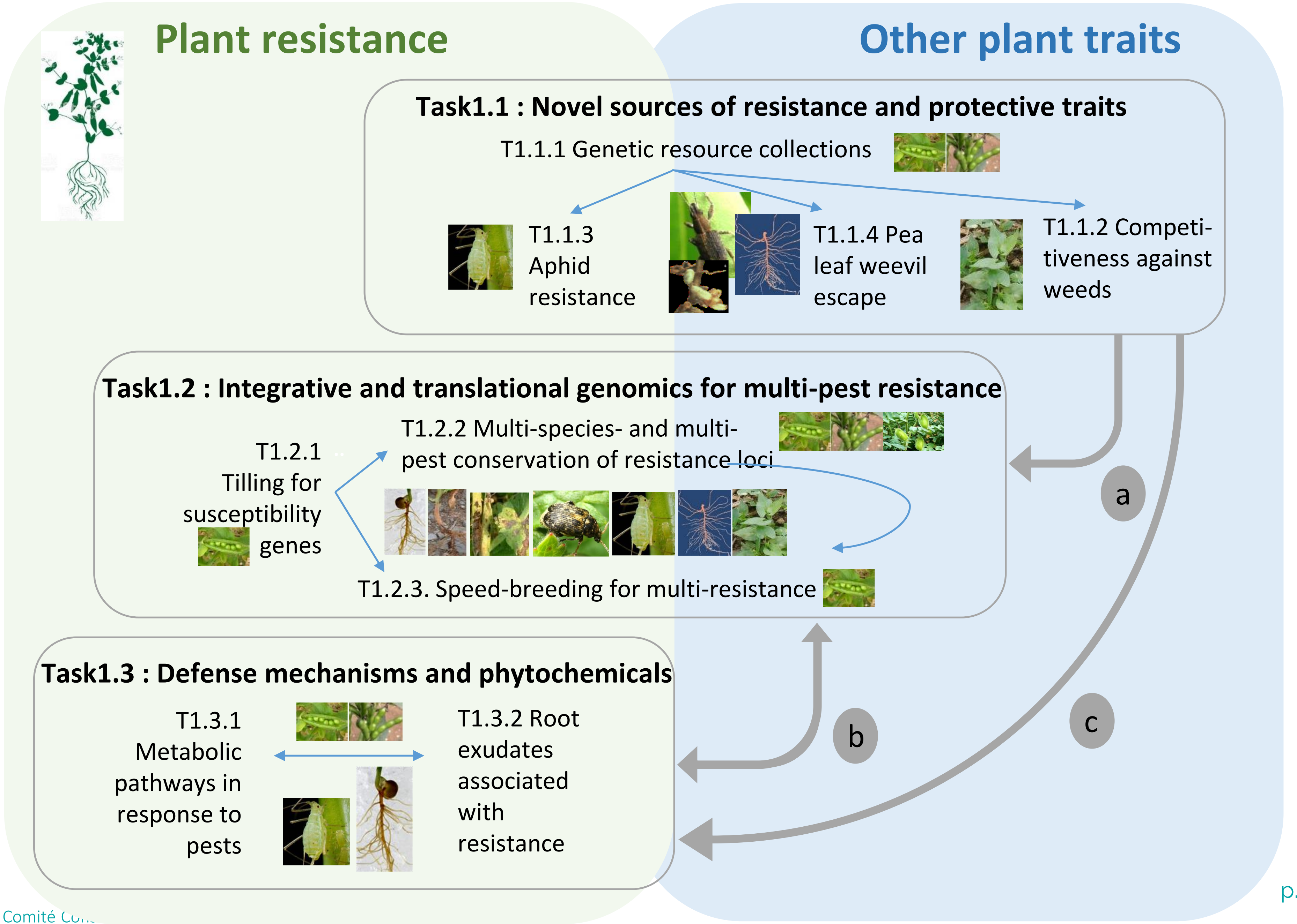
PeaMUST
Profaba
Rési*Lens*

P-Aphid
Chara-P

PeaMUST, PEAMAS
FINAPEA, DORA

WP1 tasks

- a New sources, loci and markers for T1.2
- b Integration of genetics, omics and phytochemical approaches from T1.2 and 1.3
- c Resistant and susceptible genotypes to aphids used for omics assays in T1.3



Plant resistance

Other plant traits

Task1.1 : Novel sources of resistance and protective traits

T1.1.1 Genetic resource collections

T1.1.3 Aphid resistance

T1.1.4 Pea leaf weevil escape

T1.1.2 Competitiveness against weeds

Task1.2 : Integrative and translational genomics for multi-pest resistance

T1.2.1 Tilling for susceptibility genes

T1.2.2 Multi-species- and multi-pest conservation of resistance loci

T1.2.3. Speed-breeding for multi-resistance

Task1.3 : Defense mechanisms and phytochemicals

T1.3.1 Metabolic pathways in response to pests

T1.3.2 Root exudates associated with resistance

T1.1.1. Mining genetic resources for ideotype identification (Agroécologie-ECP, URGI)

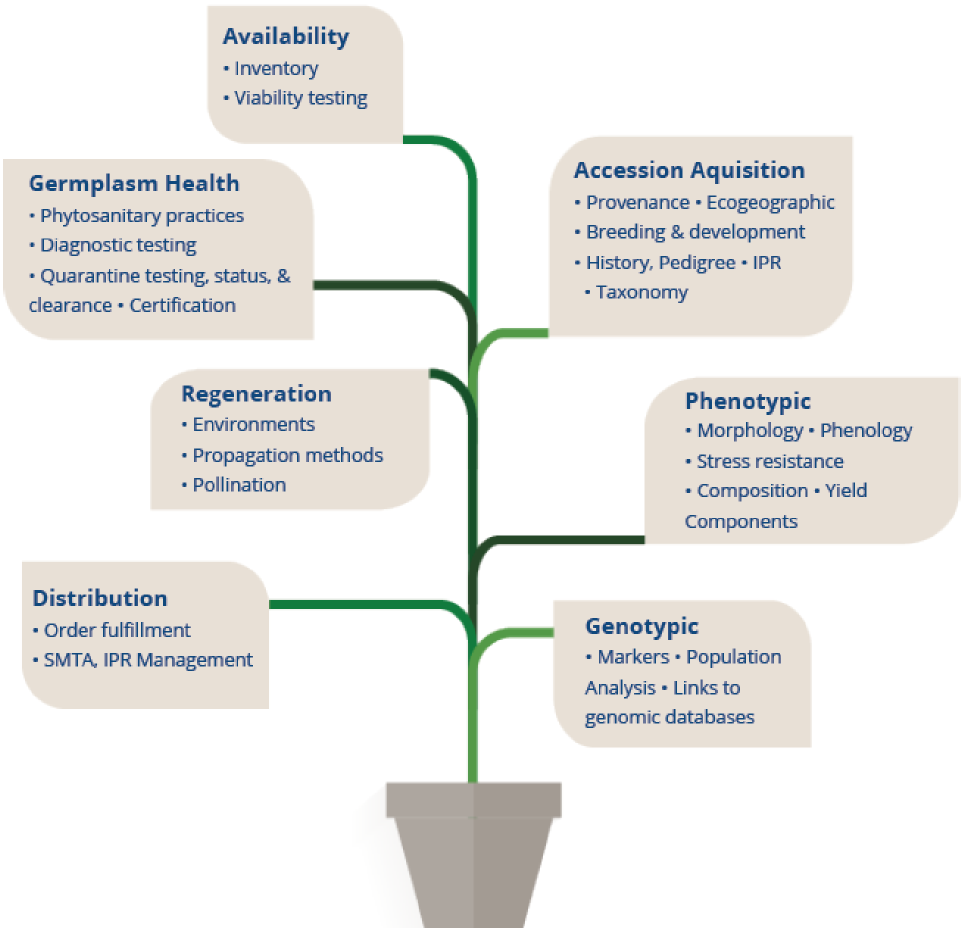
Objective

- Take advantage of unique genetic collections of pea (3800 accessions), fababean (2000 accessions) and lentil (300) maintained at INRAE of Dijon to identify new sources of resilience.

Methodology

- Each year, 10% of the collections will be grown and plant development traits measured. Seeds will be provided for the screening of sources of resistance to pests
- An instance of GRIN-Global (Germplasm Resource Information Network) will be implemented to enable efficient management and use of the genetic resources.
- The data will then be integrated into the GnpIS Information System for consultation and ideotype identification

What data is stored in GRIN-Global?



Availability
- Inventory
- Viability testing

Germplasm Health
- Phytosanitary practices
- Diagnostic testing
- Quarantine testing, status, & clearance • Certification

Accession Aquisition
- Provenance • Ecogeographic
- Breeding & development
- History, Pedigree • IPR
- Taxonomy

Regeneration
- Environments
- Propagation methods
- Pollination

Phenotypic
- Morphology • Phenology
- Stress resistance
- Composition • Yield Components

Distribution
- Order fulfillment
- SMTA, IPR Management

Genotypic
- Markers • Population Analysis • Links to genomic databases

T1.1.2 Competitiveness against weeds (Agroecologie-GESTAD-EcoLeg, IRHS-SEED)

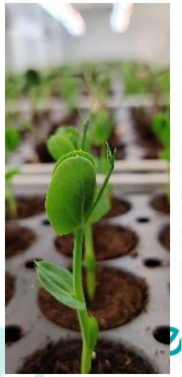
Stand establishment: uniform and rapid emergence and coverage

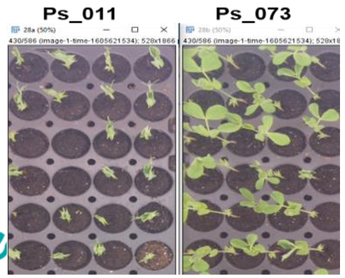
Objective 1: Identify loci controlling seedling and plant canopy emergence under conditions mimicking normal and early cold sowing conditions.

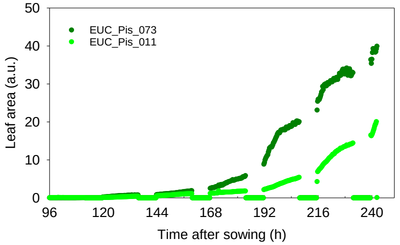
Methodology:

- Automated phenotyping on 170 pea genotypes
- Correlation between traits and GWAS

% Emergence; speed_EM, homogeneity_EM,
% coverage, Kinetics parameter_coverage







Ability to extract/use resources in conditions of resource limitation

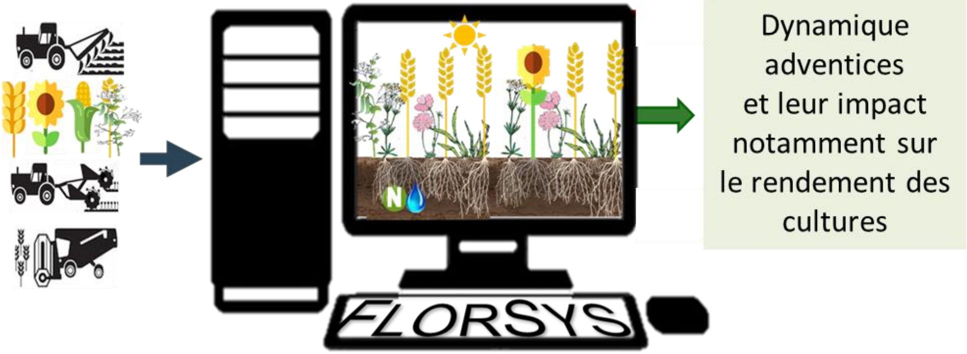
Objective 2: Phenotypic characterisation of :

- Contrasted genotypes (pea and faba bean) in response to water limitation
- Weed species in response to nitrogen and water limitations

Methodology:

- Automated phenotyping on 4PMI plat-form, analysis of previous data
- Measure of key parameters of FLORSYS model

Colbach et al. (2014; 2020)



Dynamique adventices et leur impact notamment sur le rendement des cultures

T1.1.3 Resistance to aphids (IGEPP-EGI)

Acyrthosiphon pisum
Legume specialist
transmits more than 30 viruses

https://prairiefava.com

Vicia faba Faba bean

250 exome-sequenced accessions

Aphis fabae
Generalist with preference to legumes
Infests faba bean, beet, potato, rapeseed…
transmits serious beet yellow virus (BYV)

Objective

- Screening genetic variability for resistance to aphids in faba bean
- Identify loci controlling resistance

Methodology

- Development of an efficient phenotyping method
- Phenotyping of 250 *V. faba* accessions with two aphid species
- GWAS to identify genetic loci involved in resistance

T1.1.4. Pea leaf weevil escape (Agroécologie-ECP)

Objective

- Select 20 contrasted faba bean accessions for root and nodule-related traits by screening a 250-accession reference collection
- Identify key breeding targets for plant escape to *Sitona lineatus* by characterizing the response of the selected accessions to this pest

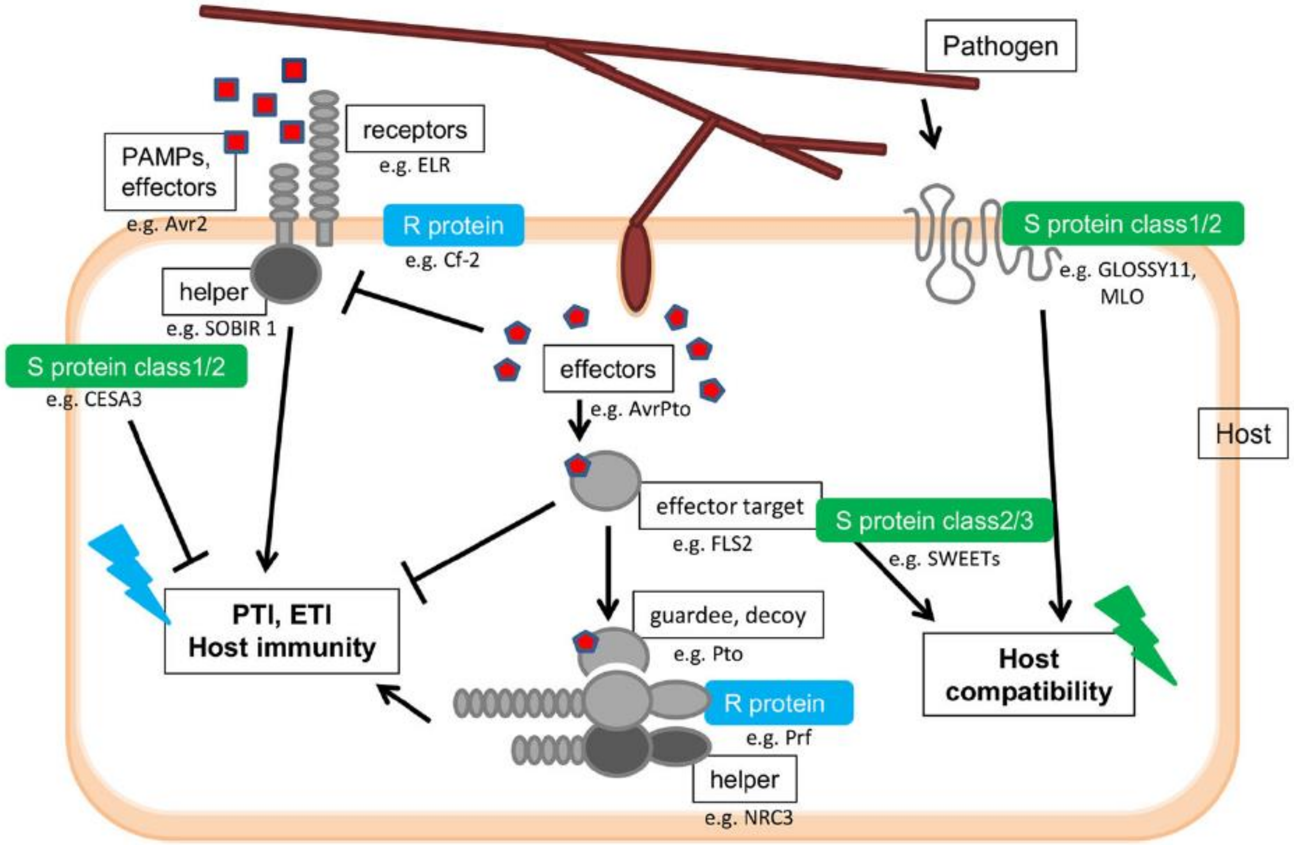
Methodology

- Run root, nodule and vegetative growth phenotyping on the 4PMI high-throughput phenotyping platform located in Dijon
- Study the response to S. lineatus under controlled conditions and use X-Ray 3D tomography to quantify nodule damage

T1.2.1. TILLING for susceptibility genes

(Agroécologie-FILEAS-ECP-IPM; Provider: Terres Inovia)

- Objective: Generate Pea lines with increased resistance to pathogens, to be used for varietal selection
- Methodology/Approach: Identification of TILLING mutants in pathogen susceptibility (S) genes



→ S-genes encode host proteins required for successful fungal infection. If mutated the host is no longer susceptible to infection.

Transgenic Res (2016) 25:731–742
DOI 10.1007/s11248-016-9964-2

T1.2.2- Multi-species- and multi-pest conservation of resistance loci
(Agroécologie-ECP, URGI, IGEPP-RD-MIR-EGI, BioEcoAgro)

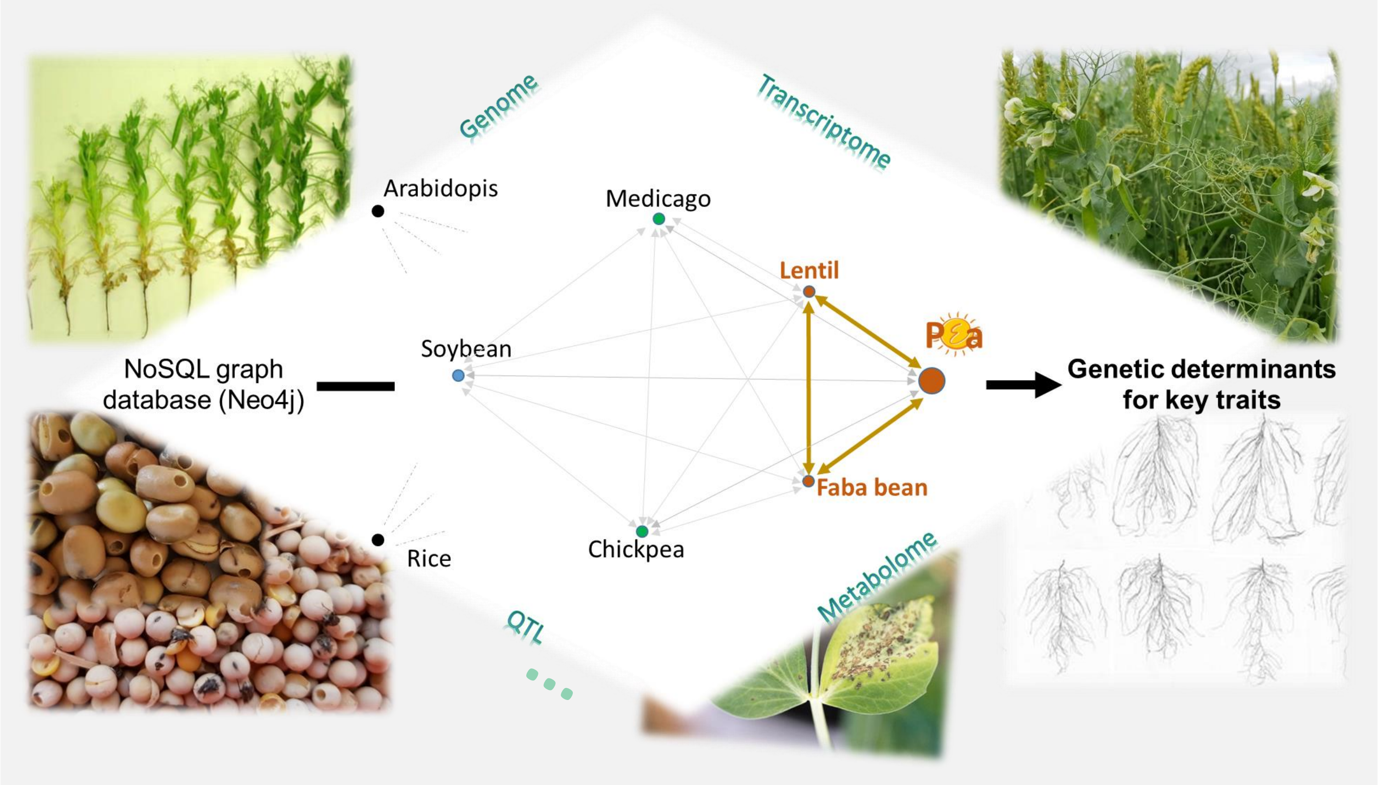
Objective

Integrate multi-scale, multi-species datasets to investigate the mechanisms of resistance to main biotic stresses in pulses and highlight:

- ✓ shared loci between traits;
- ✓ syntenic loci in target species

Methodology

- Build on available and new datasets from target crop species and model non-target species to address the scientific questions
- Use a **NoSQL** graph database to connect and explore heterogeneous, complementary datasets



Integrative pulse-specific bioinformatics tool

Non-funded partner:
UMR Transfrontalière BioEcoAgro

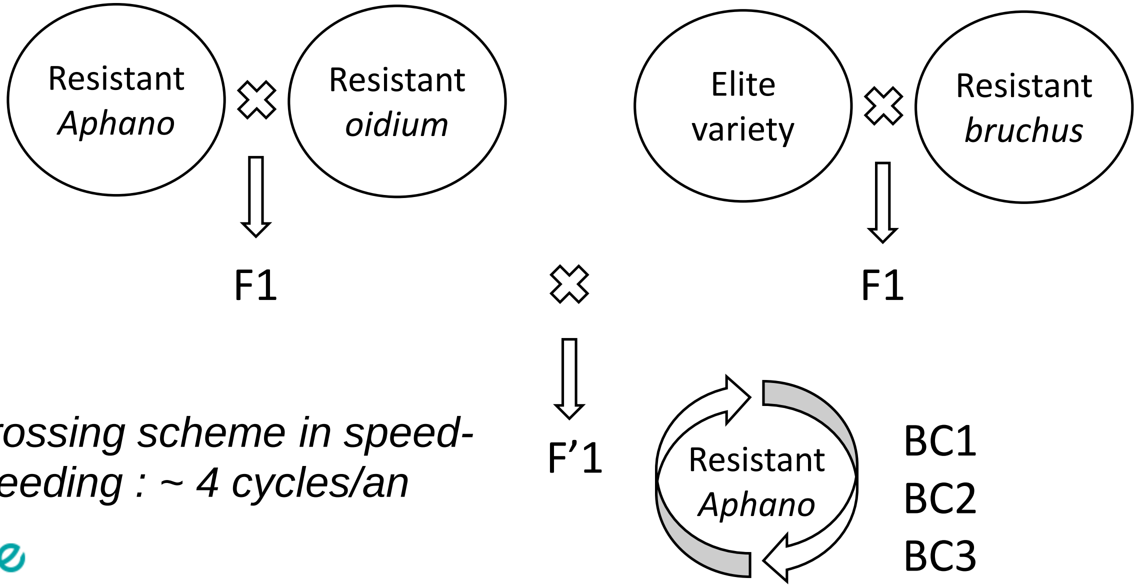
T1.2.3. Speed-breeding for multi-resistance (Agroécologie-ECP)

Objective: Speed breeding technologies will be tested as proof of concept for accelerating introgression of resistance genes and produced a pre-breeding material for pesticide-free cropping system.

Methodology:

1 – Methodological development (environmental conditions)

2 – Production and phenotyping of breeding material by back-cross (BC) marker-assisted selection



Resistant *Aphano* ✕ Resistant *oidium*

F1

Elite variety ✕ Resistant *bruchus*

F1

✕

F'1

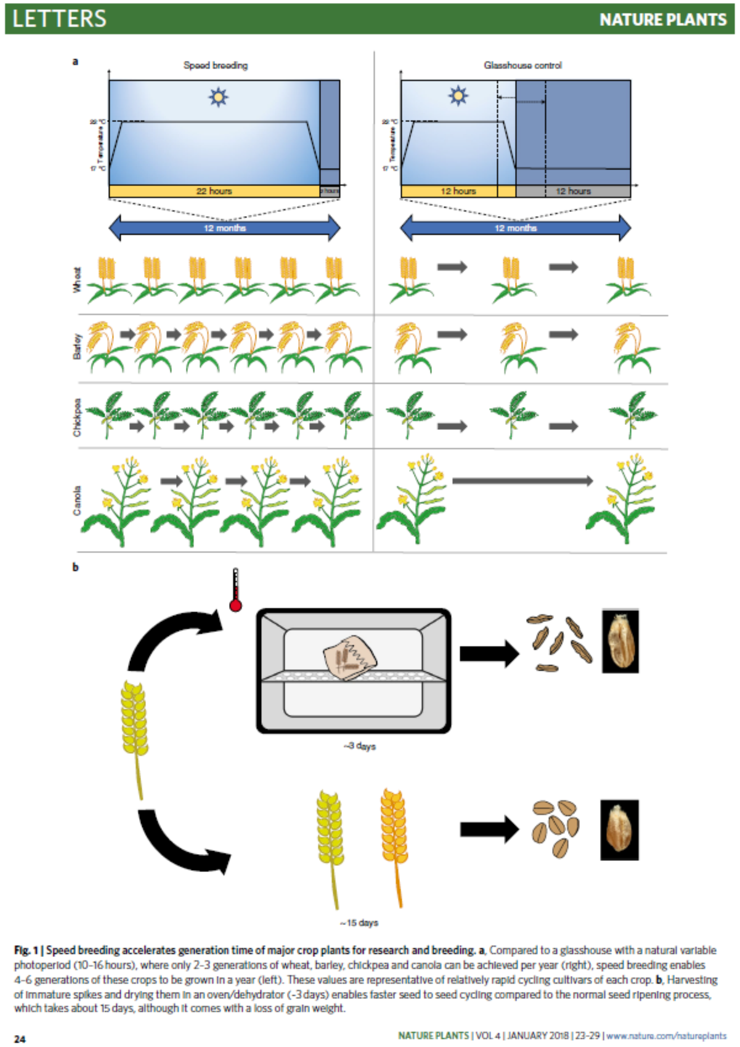
Resistant *Aphano*

BC1

BC2

BC3

Crossing scheme in speed-breeding : ~ 4 cycles/an



LETTERS NATURE PLANTS

Fig. 1 | Speed breeding accelerates generation time of major crop plants for research and breeding. a, Compared to a glasshouse with a natural variable photoperiod (10–16 hours), where only 2–3 generations of wheat, barley, chickpea and canola can be achieved per year (right), speed breeding enables 4–6 generations of these crops to be grown in a year (left). These values are representative of relatively rapid cycling cultivars of each crop. **b**, Harvesting of immature spikes and drying them in an oven/dehydrator (~3 days) enables faster seed to seed cycling compared to the normal seed ripening process, which takes about 15 days, although it comes with a loss of grain weight.

24 NATURE PLANTS | VOL 4 | JANUARY 2018 | 23–29 | www.nature.com/natureplants

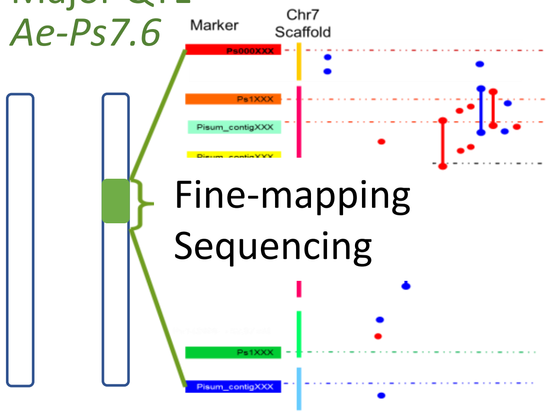
T1.3.1- Defense mechanisms against pests in pulses (IGEPP-RD-EGI)

- ✓ **Objective**: Compare **genes and metabolic pathways** recruited in pea and faba bean in response to pests, for the use of diversified defense mechanisms in durable pest management strategies;

✓ Previous results/resources

Pea/*A. euteiches*

Major QTL
Ae-Ps7.6

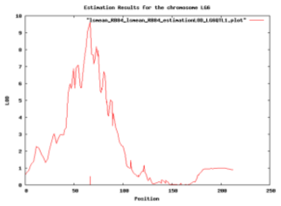


Fine-mapping
Sequencing

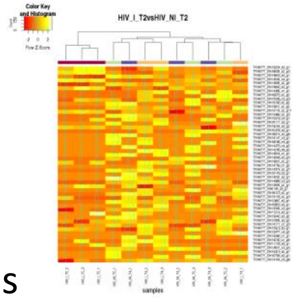
Near Isogenic Lines

PeaMUST

Faba bean/*A. euteiches*



QTL detection



RNASeq →
differentially
expressed genes

FINAPEA

✓ Works developed in SPECIFICS

from M6

Pea/*A. euteiches*

Optical mapping
RNASeq
Metabolite profiling

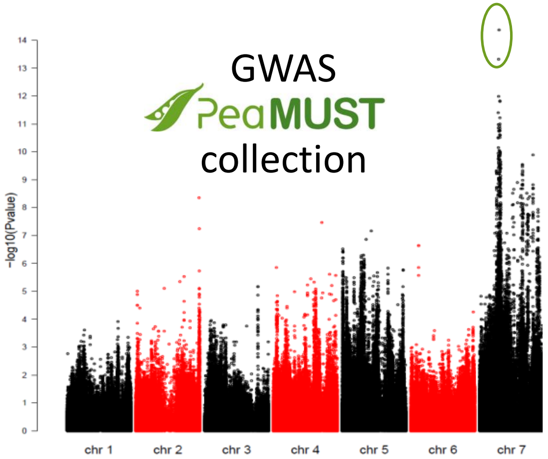
NIL pairs
differing
at QTL
Ae-Ps7.6

T1.3.1- Defense mechanisms against pests in pulses (IGEPP-RD-EGI)

- ✓ **Objective**: Compare **genes and metabolic pathways** recruited in pea and faba bean in response to pests, for the use of diversified defense mechanisms in durable pest management strategies;

✓ Previous results/resources

Pea/*A. pisum*



GWAS
PeaMUST
collection


Acyrthosiphon pisum © INRA, Bernard Chaubet

RNASeq
Targeted
metabolomics

Ollivier et al, 2022

P-Aphid

CharaP

✓ Works developed in SPECIFICS

from M42

Faba bean/*A. pisum-A.fabae*

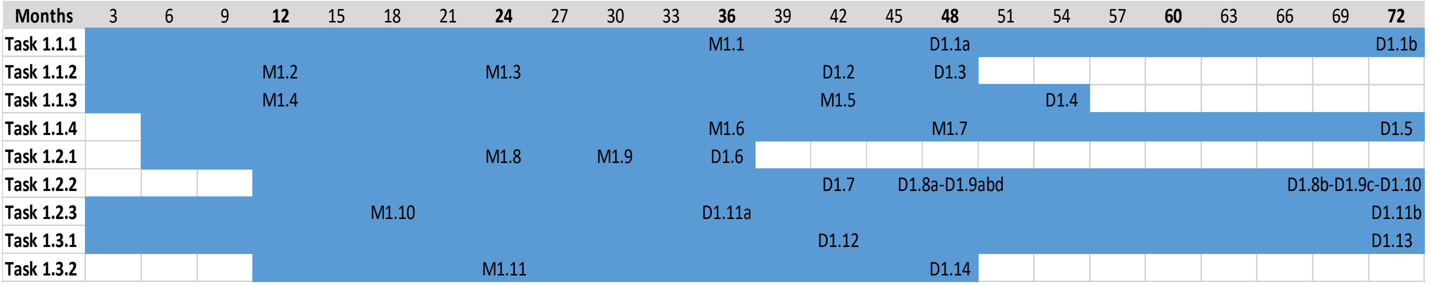
- RNAseq and targeted metabolite analysis (hormones, secondary metabolites) of selected faba bean genotypes (from T1.1.3) infested with *A. pisum* and *A. fabae*
- In depth analysis of the results by focusing on the genes identified by GWAS (from T.1.1.3)

T1.3.2. Root exudates associated with resistance (IGEPP-RD)

- ✓ **Objective**: Analyze **root exudates** effects on a soilborne pathogen and identify root exudate compounds associated with resistance
- ✓ **Approach:**
  - ✓ Effects of root exudates of resistant/susceptible pea NILs and faba bean lines on Aphanomyces pathogen life cycle (genotypes from field exp# WP2-T2.2)
  - ✓ Biochemical composition of exudates with contrasting effects on the pathogen spores; identification of compounds associated with resistance QTL
- ✓ **Expected results:** identification of key root exudate compounds impacting pathogen life cycle and associated with resistance, as new breeding targets

Expected results - Deliverables

25 Deliverables/Milestones



Months	3	6	9	**12**	15	18	21	**24**	27	30	33	**36**	39	42	45	**48**	51	54	57	**60**	63	66	69	**72**
Task 1.1.1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	M1.1	▨	▨	▨	D1.1a	▨	▨	▨	▨	▨	▨	▨	D1.1b
Task 1.1.2	▨	▨	▨	M1.2	▨	▨	▨	M1.3	▨	▨	▨	▨	▨	D1.2	▨	D1.3								
Task 1.1.3	▨	▨	▨	M1.4	▨	▨	▨	▨	▨	▨	▨	▨	▨	M1.5	▨	▨	▨	D1.4						
Task 1.1.4		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	M1.6	▨	▨	▨	M1.7	▨	▨	▨	▨	▨	▨	▨	D1.5
Task 1.2.1		▨	▨	▨	▨	▨	▨	M1.8	▨	M1.9	▨	D1.6												
Task 1.2.2				▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	D1.7	▨	D1.8a-D1.9abd	▨	▨	▨	▨	▨	▨	▨	D1.8b-D1.9c-D1.10
Task 1.2.3	▨	▨	▨	▨	▨	M1.10	▨	▨	▨	▨	▨	D1.11a	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	D1.11b
Task 1.3.1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	D1.12	▨	▨	▨	▨	▨	▨	▨	▨	▨	D1.13
Task 1.3.2				▨	▨	▨	▨	M1.11	▨	▨	▨	▨	▨	▨	▨	D1.14								

Resources

(i) Sources (accessions, mutants) of resistance or other plant traits; Breeding lines having integrated resistance alleles to multiple pests by speed breeding

(ii) Markers fully or closely linked to genomic regions and genes controlling resistance to single or multiple-pests

(iii) Database for genetic resources and bioinformatic tool for integrative and translational genomics

Knowledge

(i) Diversity, conservation and multiple effects of genomic regions/genes controlling resistance or regulation of pests

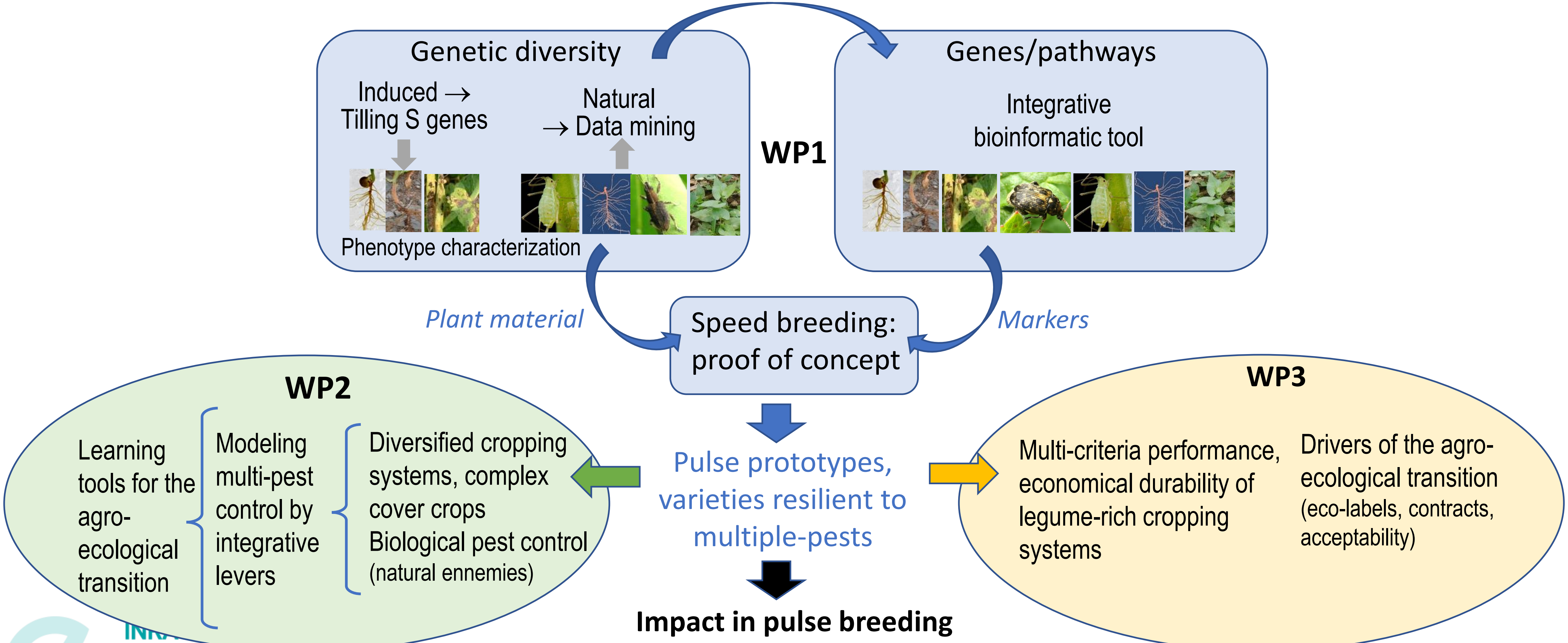
(ii) Defense pathways and phytochemical compounds associated with plant responses to pests

→ ***Optimize strategies of QTL combination in breeding for durable resistant varieties to multiple pests***

ANNEXES

Inter-WPs#1: Plant genetics for multi-pest protection of pulses

The plant lever is increasingly mobilized to control a diversity of pests of pulses without pesticides
Little knowledge is available about loci/genes/mechanisms and protective plant traits involved in resistance to multiple pests



WP1

Genetic diversity

Induced → Tilling S genes

Natural → Data mining

Phenotype characterization

Genes/pathways

Integrative bioinformatic tool

Plant material

Markers

Speed breeding: proof of concept

Pulse prototypes, varieties resilient to multiple-pests

WP2

Learning tools for the agro-ecological transition

Modeling multi-pest control by integrative levers

Diversified cropping systems, complex cover crops
Biological pest control (natural ennemies)

WP3

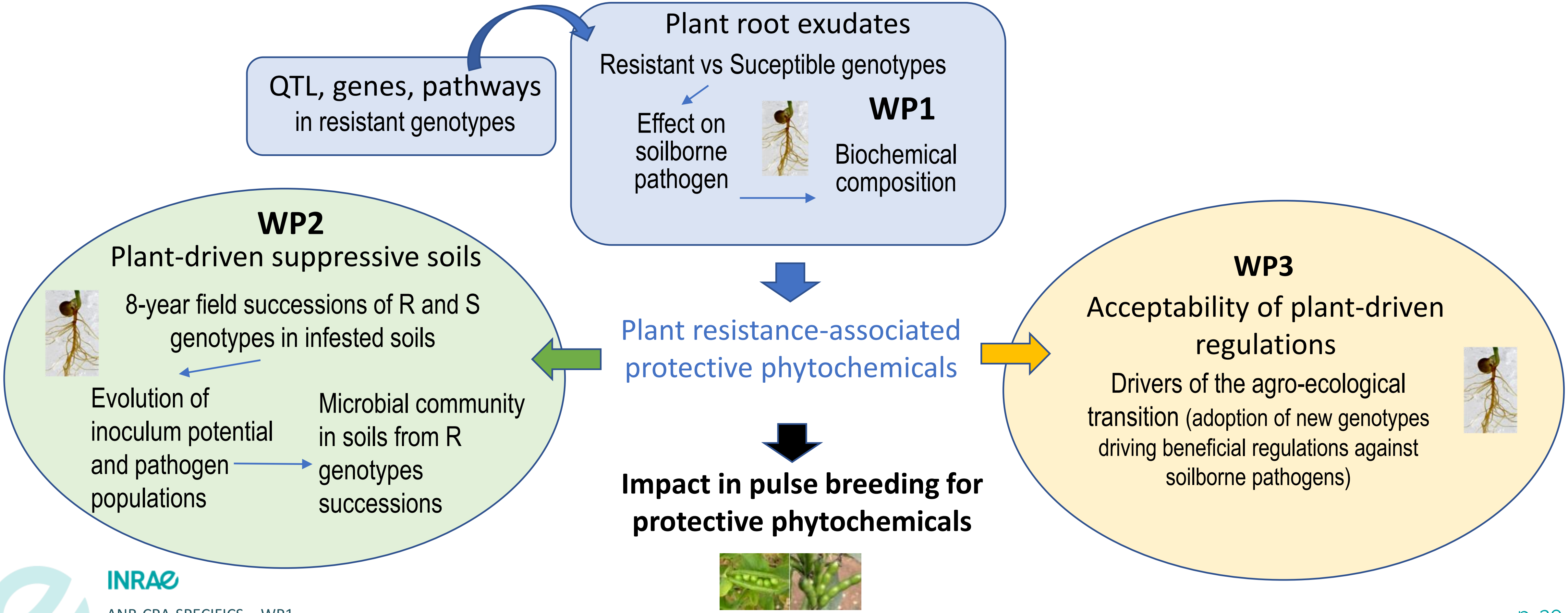
Multi-criteria performance, economical durability of legume-rich cropping systems

Drivers of the agro-ecological transition (eco-labels, contracts, acceptability)

Impact in pulse breeding

Inter-WPs#2: Plant-driven regulations for pulse protection

The plant lever as a driver of beneficial microorganisms for pulse cropping without pesticides
Little knowledge about root exudate composition from resistant plants & their effects on pathogens and beneficial organisms



QTL, genes, pathways
in resistant genotypes

Plant root exudates
Resistant vs Suceptible genotypes

Effect on soilborne pathogen

WP1

Biochemical composition

WP2
Plant-driven suppressive soils

8-year field successions of R and S genotypes in infested soils

Evolution of inoculum potential and pathogen populations

Microbial community in soils from R genotypes successions

Plant resistance-associated protective phytochemicals

WP3
Acceptability of plant-driven regulations

Drivers of the agro-ecological transition (adoption of new genotypes driving beneficial regulations against soilborne pathogens)

Impact in pulse breeding for protective phytochemicals