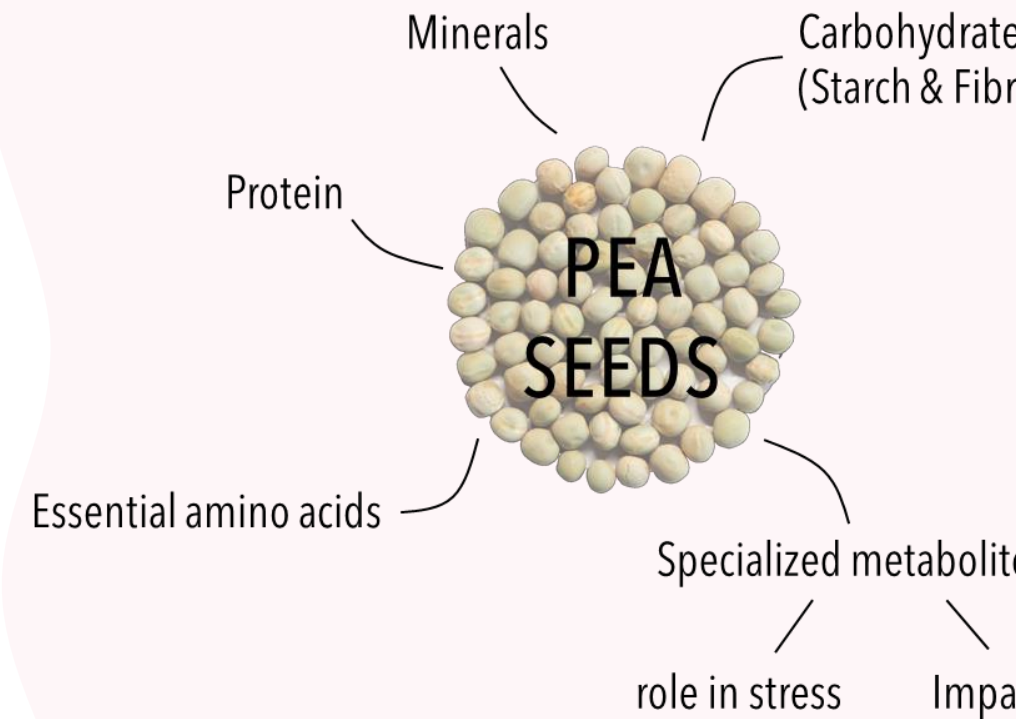


INTRODUCTION



Legumes species, including pea (*Pisum sativum*), have a key role in the crop rotation, in part thanks to their ability to fix atmospheric nitrogen (Palmero et al., 2022). Pea (*P. sativum*) seeds have a rich and varied composition. Seeds are characterized by high protein content (around 25%), depending on accessions (Nutrition Handouts : Protein In Common Foods, 2020).

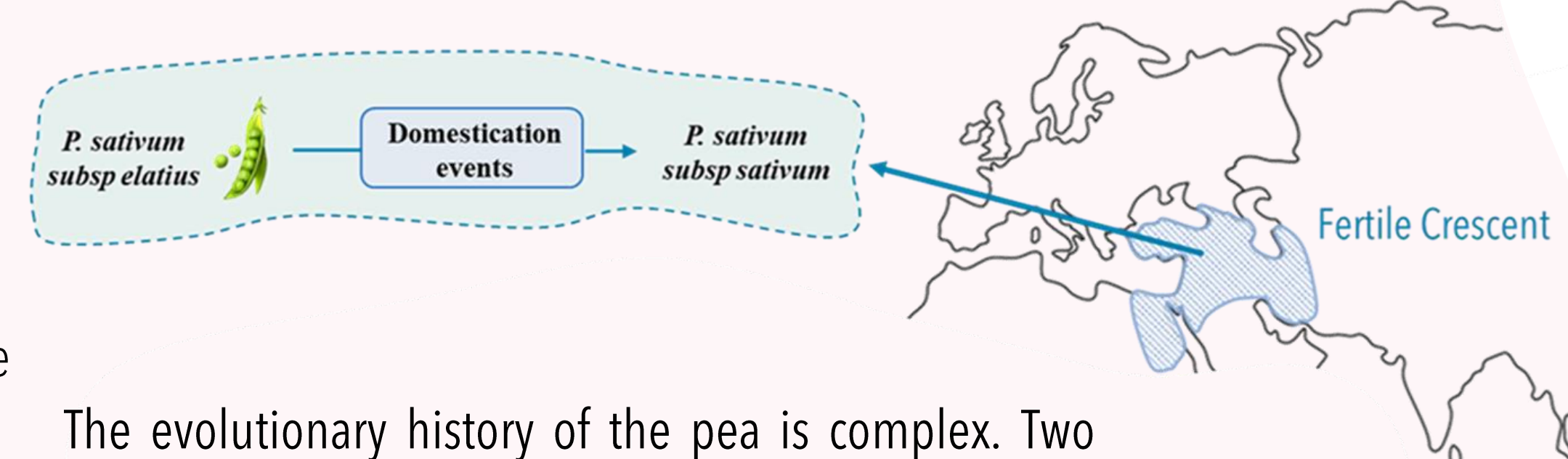
Domestication and evolution led to a drastic modification of the metabolic composition (Ku et al., 2020 ; Klčová et al., 2024). In the case of peas, most of the studies focus on the seed coat and on specific metabolic classes (such as flavonoids).

The evolutionary history of the pea is complex. Two independent domestication events in time and space are thought to have taken place (Kreplak et al., 2019).

The LetsProSeed project:
"Pushing back the limits to the use of legume proteins in human nutrition by improving seed quality and processing without compromising stress resistance"

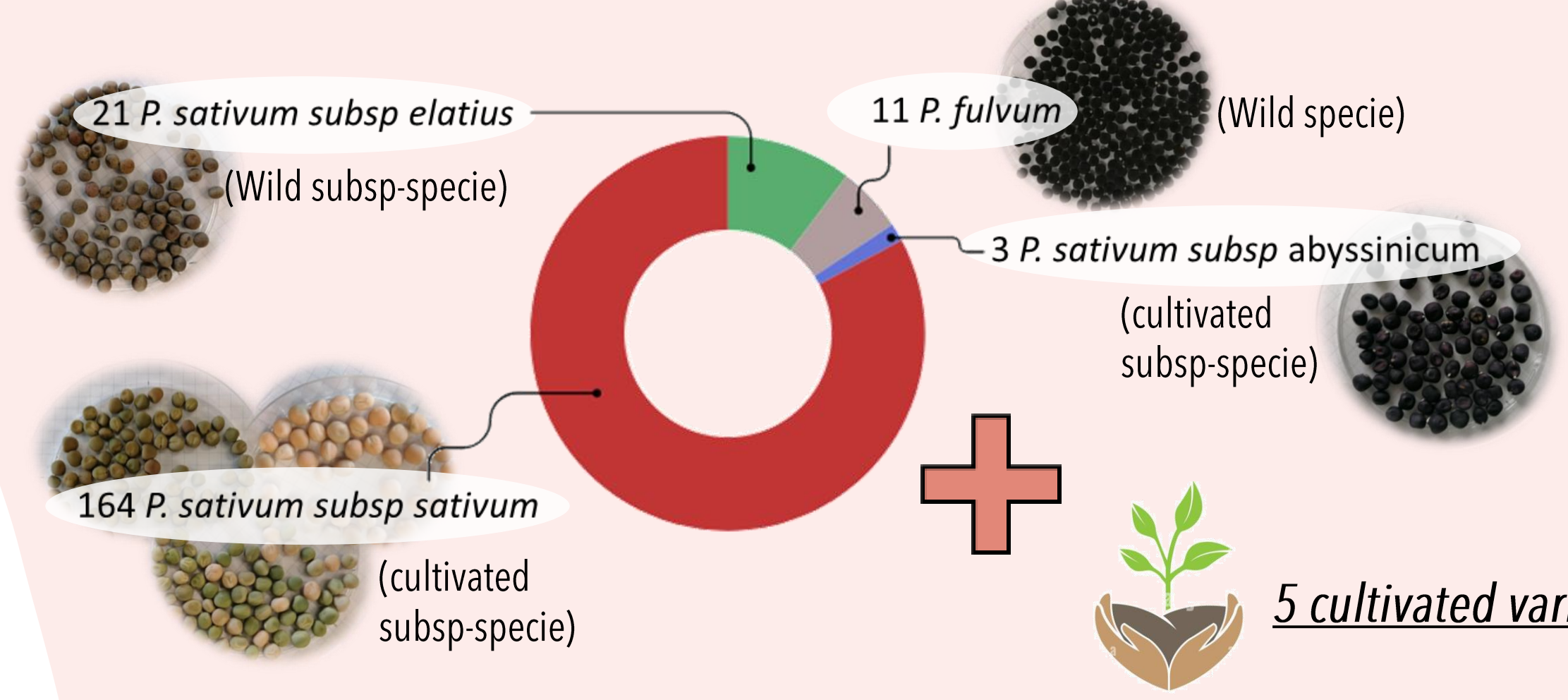
Objectives :

- What is the extent of specialized metabolites (SM) natural diversity , evolution and domestication in pea seeds ? And what are the effects of the environment on seed SM profiles and content ?
- What are the genetic determinants of SM accumulation ?



MATERIAL AND METHODS

A large genetic diversity will be characterized during the project:
199 pea accessions of four species/subspecies from the genus *Pisum*



Cultivation in controlled greenhouse conditions (Dijon , feb-July 2023)
Cotyledons have been separated from seed coats
Untargeted metabolomic analyses (LC – MS/MS) of seed coats and cotyledons
Data processing with MzMine3
Metabolite annotation and visualization with MetGem, Cytoscape and SIRIUS
Exploring the metabolomics diversity through statistical analyses to identify specialized metabolites impacted by the domestication and/or evolution

Genotype sequencing by exome capture (> 500,000 genome-wide SNPs to study the genetic basis of traits of interest)
Metabolic-GWAS (mGWAS) to identify the genetic determinants involved in metabolite accumulation (ongoing)

RESULTS

Cotyledons (embryos) dataset

1 800 ions detected in positive mode
115 metabolic cluster have been constructed

23 % has annotation proposals

Seed coats dataset

735 ions detected in positive mode
55 metabolic cluster have been constructed

28 % has annotation proposals

LEGEND

Species and subspecies

- P. fulvum*
- P. sativum subsp. elatius*
- P. sativum subsp. sativum*

Metabolic class

- Alkaloids and nitrogen derivatives & Phenylpropanoids
- Carbohydrates & Alkaloids and nitrogen derivatives
- Alkaloids and nitrogen derivatives
- Lipids and derivatives
- Carbohydrates
- Terpenoids
- Not significant
- Phenylpropanoids
- Cyclic esters
- Unknown

Metabolic clusters

- Ions under-accumulated
- Ions over-accumulated

Pairwise analysis between *P. sativum subsp. elatius* and *P. sativum subsp. sativum*

Enrichment analysis :
Higher in *P. sativum subsp. sativum*
5 multi-charged clusters
11 unknown clusters

Higher in *P. sativum subsp. elatius*
7 multi-charged clusters
12 unknown clusters

Pairwise analysis between *P. fulvum* and *P. sativum subsp. sativum*

Enrichment analysis :
Higher in *P. sativum subsp. sativum*
3 multi-charged clusters
7 unknown clusters
1 cyclic esters clusters
1 alkaloids and derivatives clusters

Higher in *P. fulvum*
1 lipids and derivatives clusters
4 unknown clusters
4 multi-charged clusters
1 alkaloids and derivatives clusters

Higher in *P. sativum subsp. sativum*
1 chromones & coumarins clusters
2 unknown clusters
1 alkaloids and derivatives clusters

Higher in *P. sativum subsp. elatius*
1 phenolics acids and derivatives clusters
4 unknown clusters

Higher in *P. fulvum*
1 lipids and derivatives clusters
4 unknown clusters

PERSPECTIVES

- Large metabolic diversity in pea seed coat and embryo
- A majority of ions are clustered
- Pea species showed specific metabolite signature in seed-coat and embryo
- Domestication/evolution seems to have had more impact on seed coat metabolic landscapes
- Improve annotation (exploring the spectra)
- Exploring the structuration of *P. sativum subsp. sativum*
- Metabolomic genome-wide study association (mGWAS)
- Heterologous expression to validate enzymes associated with specialized metabolites

BIBLIOGRAPHY

Klčová et al (2024) *Plant Journal* 10.1111/tpj.16734

Ku et al (2020) *Frontiers in Genetics* 11: 581357

Kreplak et al (2019) *Nature genetics* 51, 1411–1422

Palmero et al (2022) *European Journal of Agronomy* 136:126514

Valadon Anne-Solenn

Institut Jean-Pierre Bourgin & Agroécologie
INRAE-Versailles & INRAE-Dijon

E-mail: anne-solenn.valadon@inrae.fr