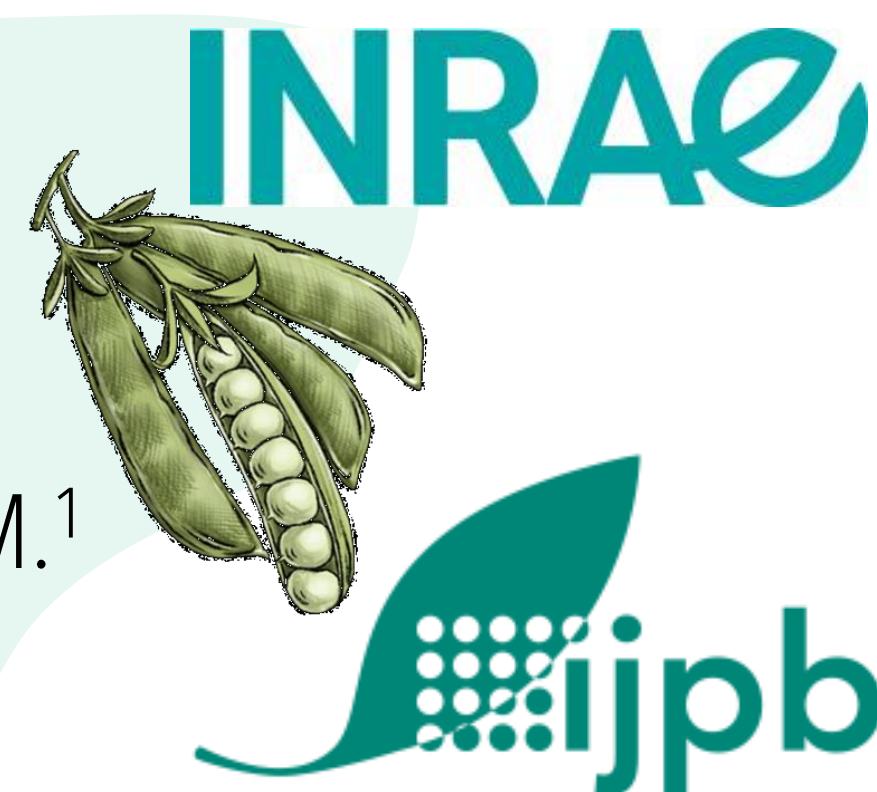




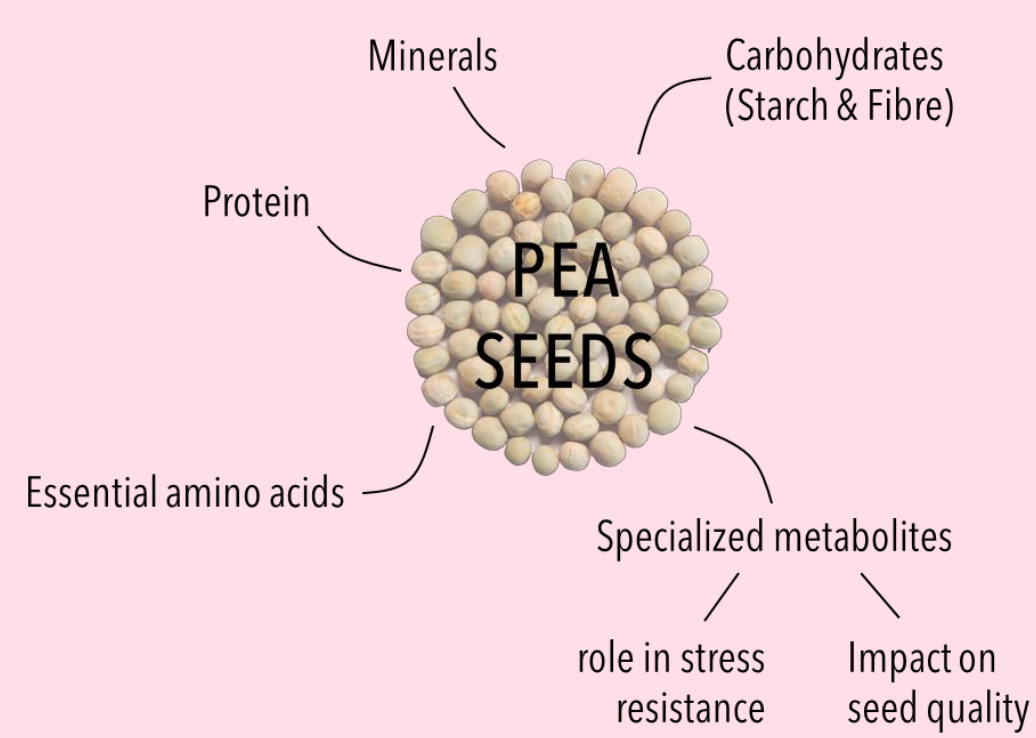
Diversity, Plasticity and Genetic Determinism of the Specialized Metabolome of Pea Seeds (Cotyledons and Seed coats)

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INTRODUCTION



Legumes species, including pea (*Pisum sativum* L.), play a key role in crop rotation, because of their ability to fix atmospheric nitrogen. Pea produces protein-rich seeds (**about 25%**) which are increasingly used as a source of proteins for food applications.

However, the presence of some specialized metabolites (SM) in mature pea seeds can alter the organoleptic properties and the digestibility of the protein fractions, while they can also protect the seeds against stress.

These include saponins which contribute to bitterness (Heng et al., J. Sci. Food Agric., 2006) or phenolic compounds which are associated with astringency (Troszyńska A et al., Food Qual., 2006).

→ This project aims to balance beneficial and antinutritional SMs in peas to improve seed quality and resilience in the context of climate change and agroecology.

Previous work suggested that domestication led to a drastic modification of the metabolism in legumes (Ku et al., Front. in Genetics, 2020), but how the pea seed metabolome has been shaped during crop evolution still need to be investigated.

Objectives :

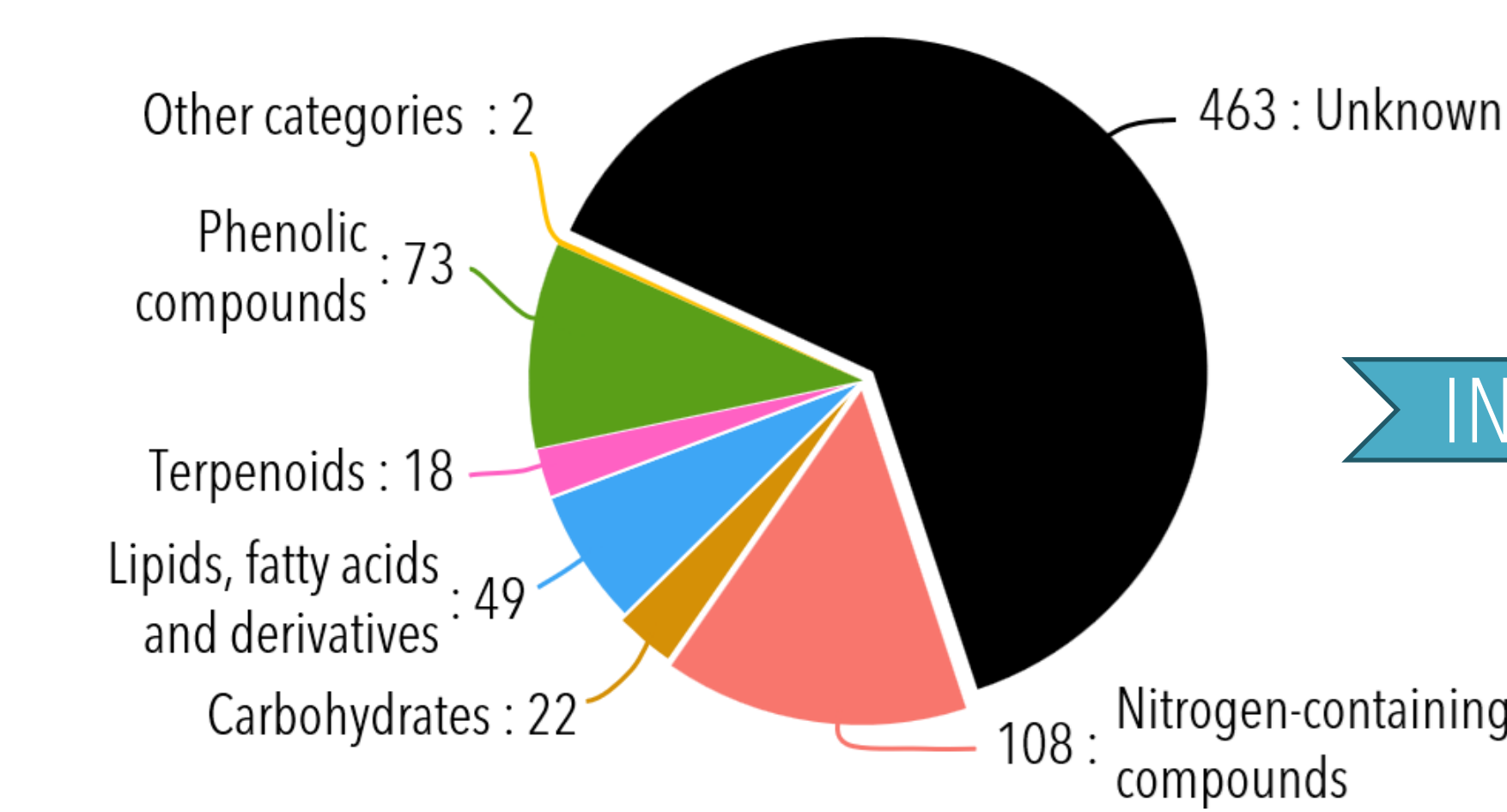
1. To gain new knowledge on SM diversity in pea seeds and to study how crop evolution impacted the seed specialized metabolome
2. To investigate to which extent SM influence seed quality, in particular organoleptic properties
3. To reveal key genes controlling SM accumulation in seeds
4. To study the impact of the environment on SM profile and content and reveal environmental plasticity in pea seeds

This thesis is part of the ANR-France 2030 LETSPROSEED project (ANR-22-PLG-0002), whose main objective is to provide knowledge and tools to improve the quality (nutritional and organoleptic) of legume seeds (mainly pea and faba bean) without compromising plant resistance to stress.



Thesis co-supervised between IJPB – Versailles, M. Corso (SEDEV Team) and Agroecology – Dijon, V. Vernoud (FILEAS Team), started at IJPB in December 2023

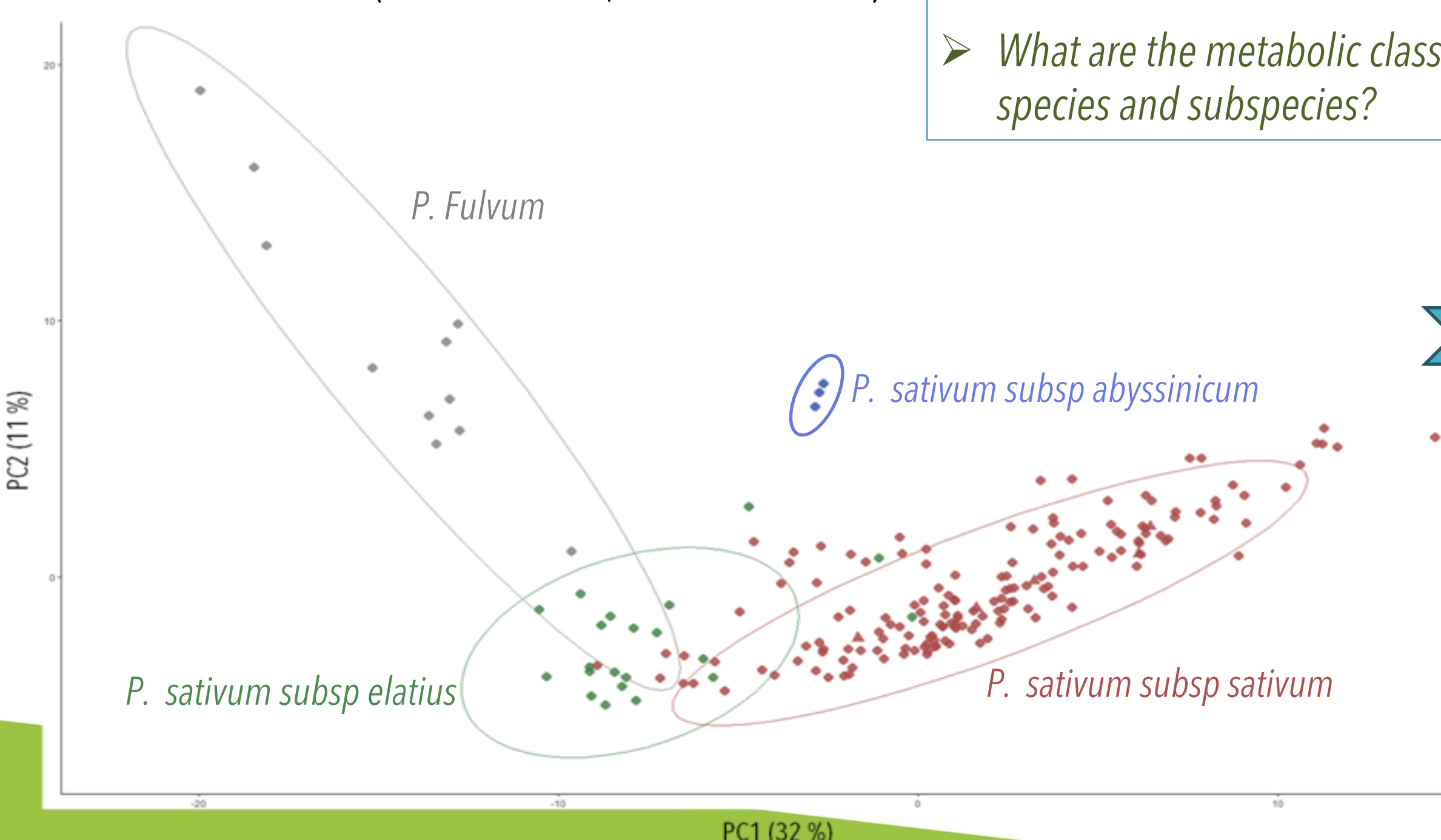
FIRST RESULTS FOR SEED COATS (Obj. 1)



- 735 ions detected in **seed coats** in positive mode
- 28% of ions are annotated

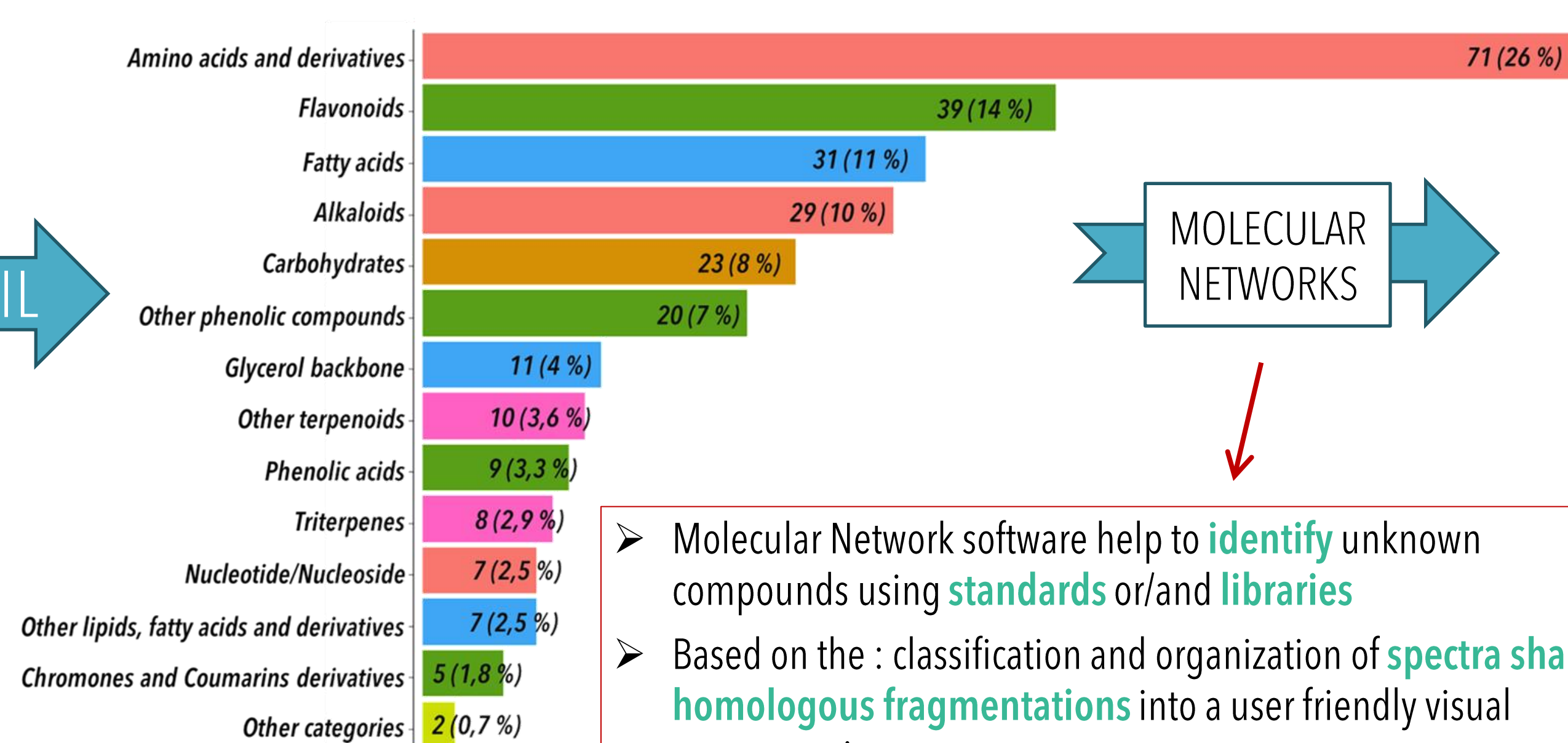
METABOLOME SPECIFICITY BETWEEN SPECIES AND SUBSPECIES

PLS-DA – seed coat (VIP-score > 1, 18% of dataset)



- A distinction between species and subspecies is observed, suggesting specific metabolic signatures in wild and cultivated accessions
- *What are the metabolic classes that differentiate the species and subspecies?*

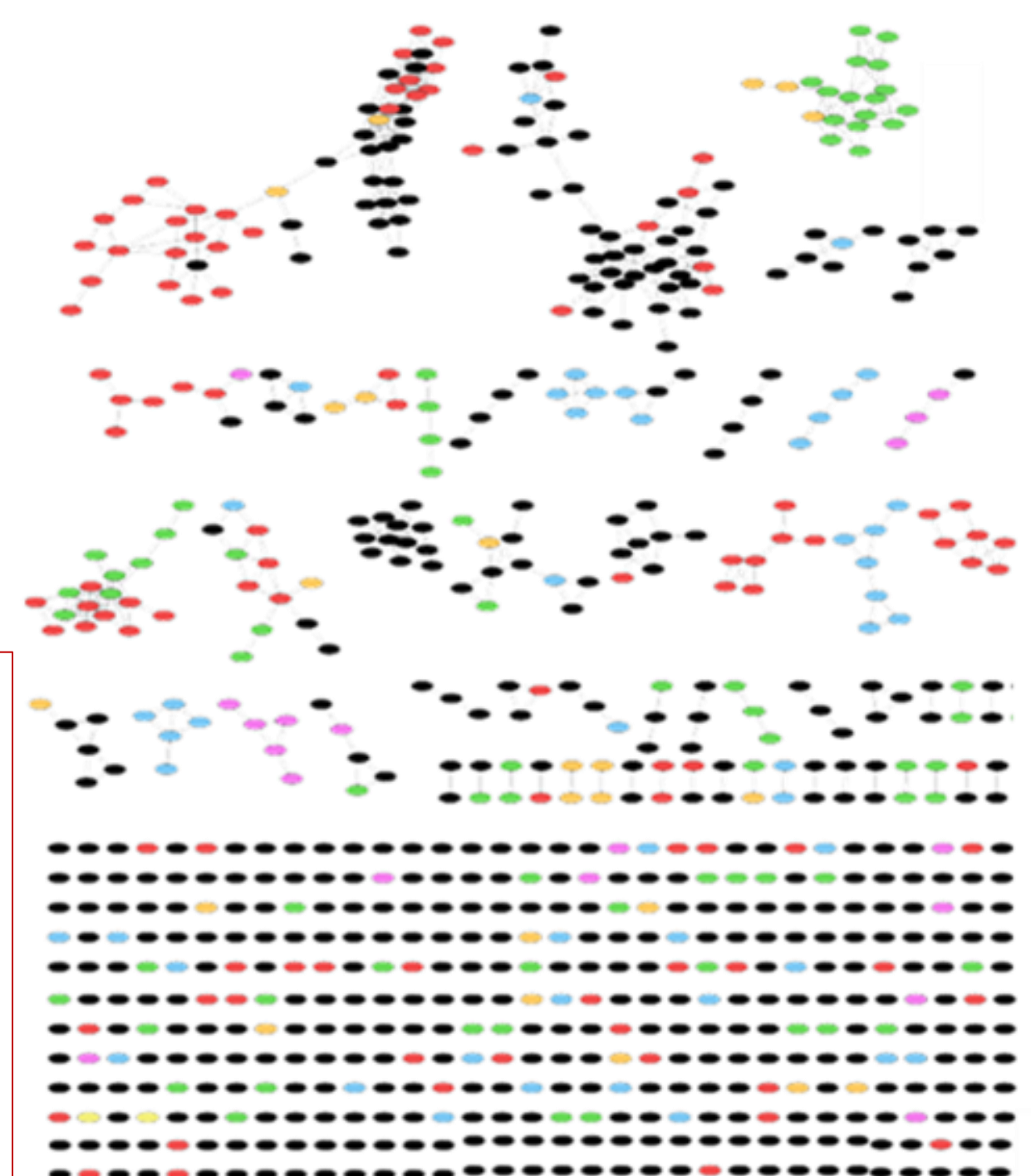
PAIRWISE COMPARISON ANALYSIS



MOLECULAR NETWORKS

- Molecular Network software help to **identify** unknown compounds using **standards** or/and **libraries**
- Based on the : classification and organization of **spectra sharing homologous fragmentations** into a user friendly visual representation
- *Postulate: Compounds with similar chemical functions will have similar fragmentation profiles in mass spectrometry*

Metabolic clusters



<i>P. fulvum</i> VS <i>P. sativum</i> subsp <i>sativum</i>	Enrichment analyses: - Sphingolipids - Fatty acids - Lipids - Esters cycliques	173	Enrichment analyses: - Chromones & coumarins - Terpenoids	138
<i>P. fulvum</i> VS <i>P. sativum</i> subsp <i>elatius</i>	Enrichment analyses: - Other categories - Lipids - Fatty acids	209	Enrichment analyses: - Carbohydrates - Phenolic acids - Amino acids and derivatives - Diterpenoids - Terpenoids (other)	81
<i>P. sativum</i> subsp <i>elatius</i> VS <i>P. sativum</i> subsp <i>sativum</i>	Enrichment analyses: - Sphingolipids - Phenolic acids	205	Enrichment analyses: - Chromones & coumarins	128

▲ Up-regulated ▼ Down-regulated

➔ The same analyses were carried out on cotyledons

➔ Statistical analyses highlighted ions with contrasting accumulation profiles between species and subspecies

PERSPECTIVES

Obj. 1 :

- Statistical analyses to characterize the diversity present within the *P. sativum* subsp *sativum* group (158 genotypes).
- Consolidate the metabolomic data: select 15 genotypes to be analyzed by LC/MS-MS for the 3 replicates for both tissues (cotyledons and seed coats)

Obj. 2 :

- Characterization of the astringency of seed extracts from the 199 accessions and the 5 varieties using a biosensor developed by the CSGA (INRAE, Dijon).
- Correlations between metabolomic data and astringency data will be performed to reveal SM or SM pathways implicated in this off-flavour.

Obj. 3 :

- Metabolomic genome-wide association (mGWAS) will be performed to identify genomic regions involved in the control of key SM accumulation and astringency intensity of the pea extracts.

Obj. 4 :

- The 5 commercial field pea varieties cultivated in greenhouse will also be grown in field trials over several years and sites (Terres Inovia field networks, Coll. V. Biarnes)
- Meteorological and phenological data are planned to be recorded.
- Untargeted metabolomic analyses will be carried out on the seeds coming from these field trials to study the effect of the environment on the seed specialized metabolome.