XV INTERNATIONAL LUPIN CONFERENCE 2019

Cochabamba - Bolivia

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19/03/019

Session 4: Agronomy, Farming

17:15 – 17:30

EVALUATION OF TARWI (*LUPINUS MUTABILIS* SWEET) ADAPTABILITY TO CULTIVATION UNDER MEDITERRANEAN CLIMATE CONDITIONS

Norberto Guilengue, Sofia Alves, Pedro Talhinhas, João Neves Martins

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Evaluation of tarwi (*Lupinus mutabilis* Sweet) adaptability under mediterranean conditions

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Lupin Bioeconomy Development | BIO-BASED INDUSTRIES Public-Private Partnership | Horizon 2020 European Union Funding for Research & Innovation | Bio·based Industries Consortium | FCT Fundação para a Ciência e a Tecnologia

Horizon 20-20: Proj. 720726 **L**upinus mutabilis for **I**ncreased **B**iomass from marginal lands and value for **BIO**refineries

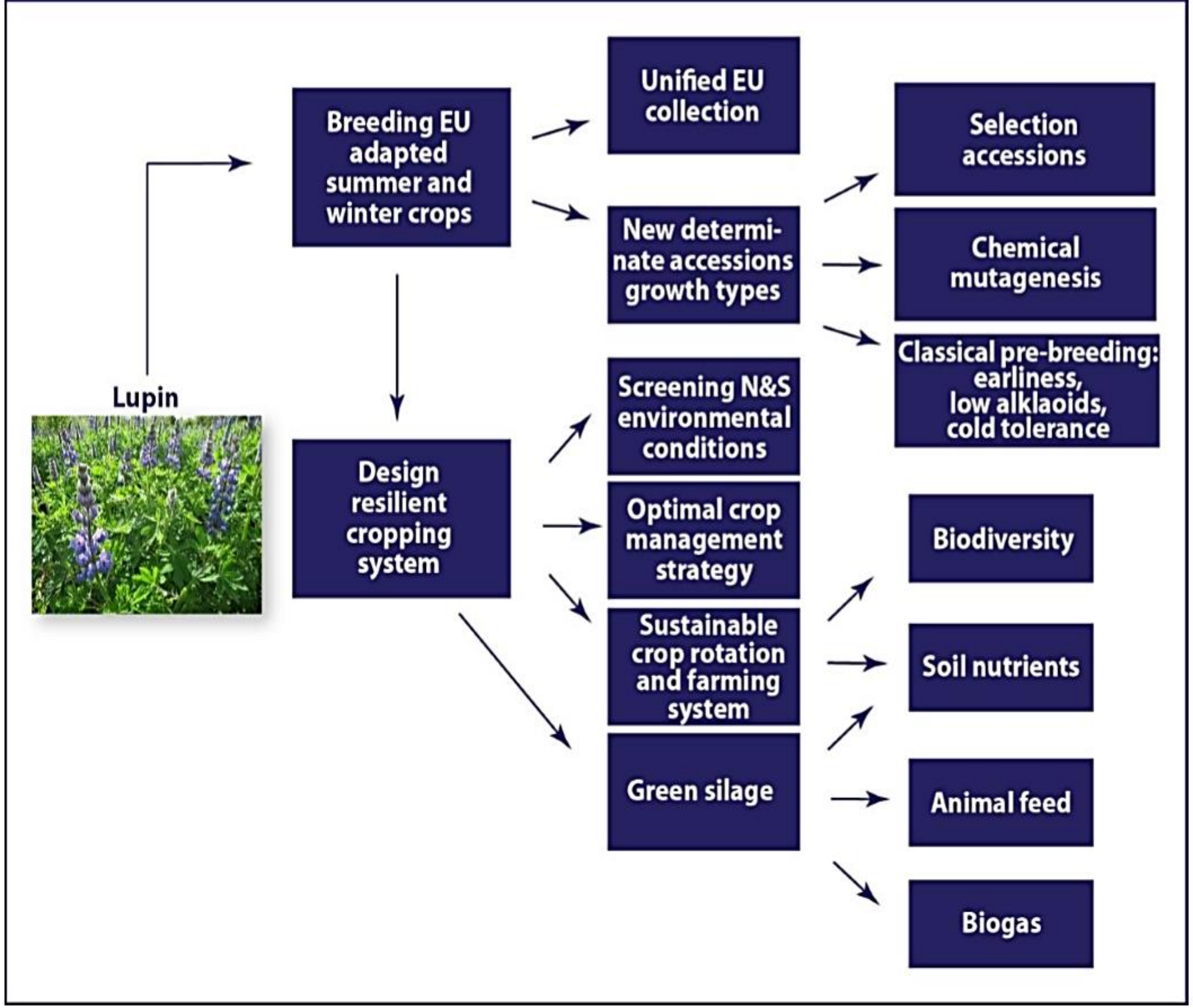
LIBBIO Tasks

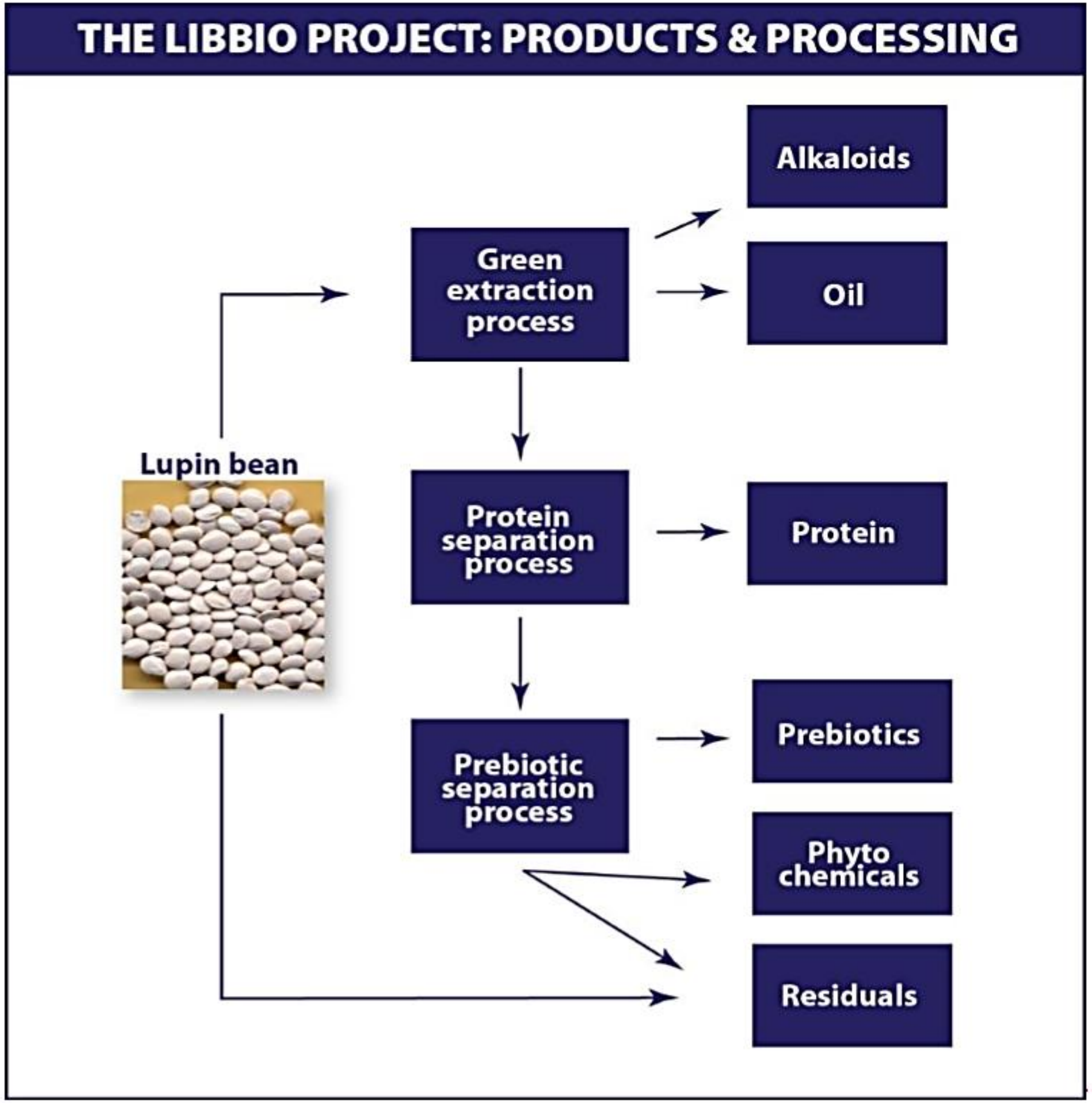
de Lisboa para o mundo

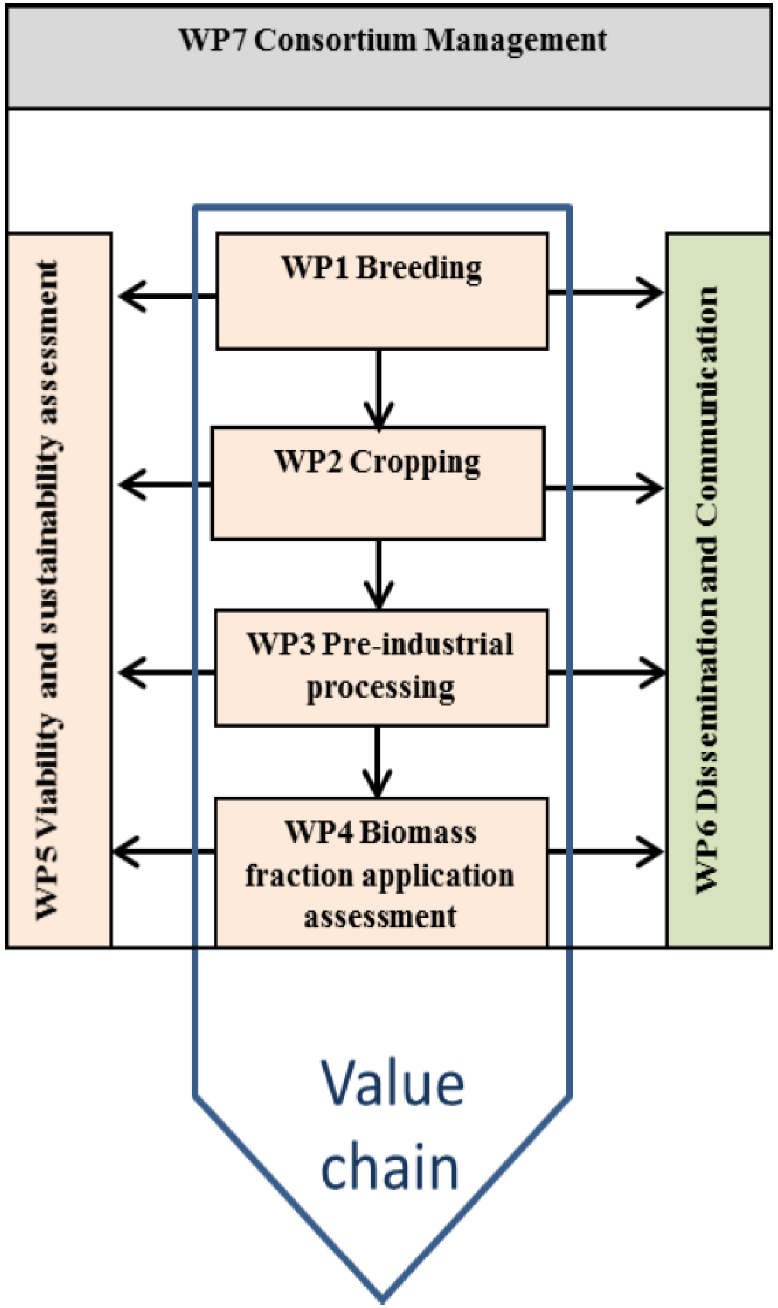
Norberto Guilengue, Sofia Alves, Pedro Talhinhas, Catarina Sousa, Mª do Carmo Alvim Joana Mota, Ana Lima, Ricardo Boavida Ferreira & João Neves-Martins

Horizon 2020
European Union Funding
for Research & Innovation

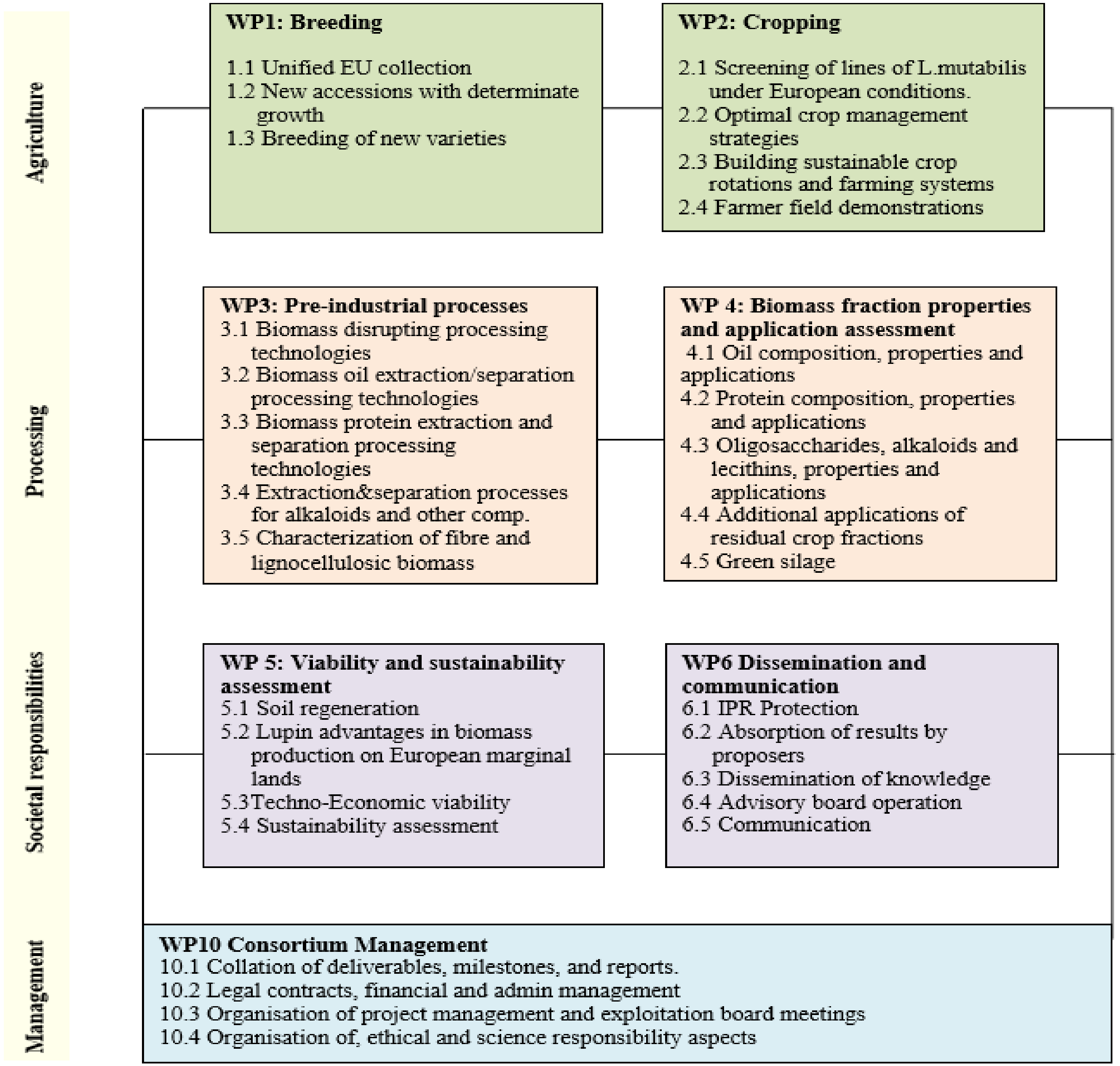
THE LIBBIO PROJECT: BREEDING & AGRONOMY




THE LIBBIO PROJECT: PRODUCTS & PROCESSING
Lupin bean
Green extraction process
Protein separation process
Prebiotic separation process
Alkaloids
Oil
Protein
Prebiotics
Phyto chemicals
Residuals


WP7 Consortium Management
WP5 Viability and sustainability assessment
WP1 Breeding
WP2 Cropping
WP3 Pre-industrial processing
WP4 Biomass fraction application assessment
WP6 Dissemination and Communication
Value chain

Pert chart illustrating the WPs' inter-dependency.



Tasks

Task 1.1 Unified EU collection
Task 1.2 New accessions with determinate growth
Task 1.3 Breeding of new varieties

Task 2.1

2.1.1 Variety trials; 2.1.2 Soil types; 2.1.3 Calcium tolerance; 2.1.4 Salinity; 2.1.5 Drought; 2.1.6 Frost tolerance; 2.1.7 Susceptibilty to diseases; 2.1.8 Susceptibilty to pests.

Task 2.2

2.2.1 Herbicide screening; 2.2.2 In field experience with resistance to harrowing; 2.2.3 Effect of row spacing on yield; 2.2.4 Effect of sowing date on crop development; 2.2.5 Effect of sowing density on yield; 2.2.6 Optimal irrigation timing

Task 2.3

2.3.1 Nitrogen fixation 2.3.2 Phosphorous mobilisation and phosphorous transfer 2.3.3 Effect of L. mutabilis on soil health

2.3.4 Effect of L. mutabilis on above ground biodiversity

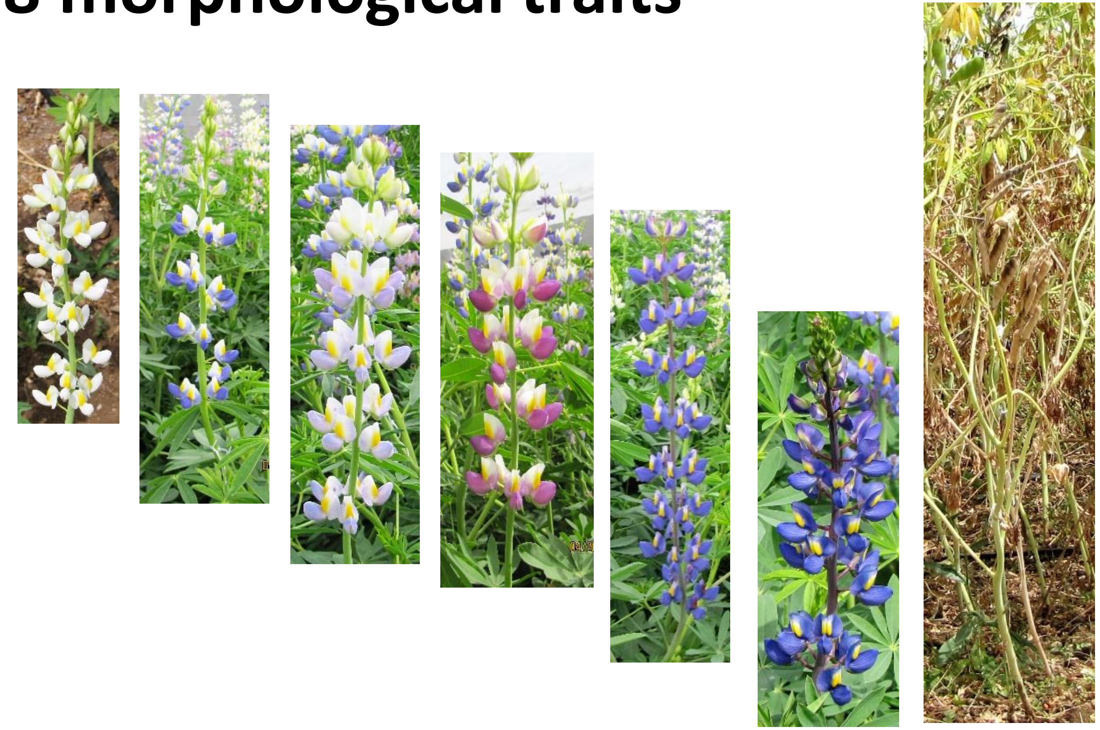
Summary

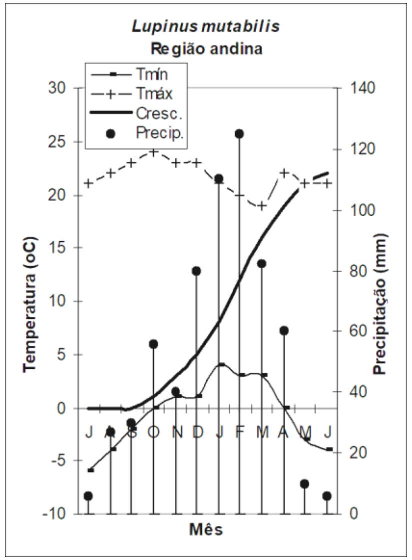
- Tarwi accessions morphological types
- Their genome size (GS)
- Assessing antracnose (R & S)
- Molecular characterization with ISSRs
- Tarwi deflamin evaluation

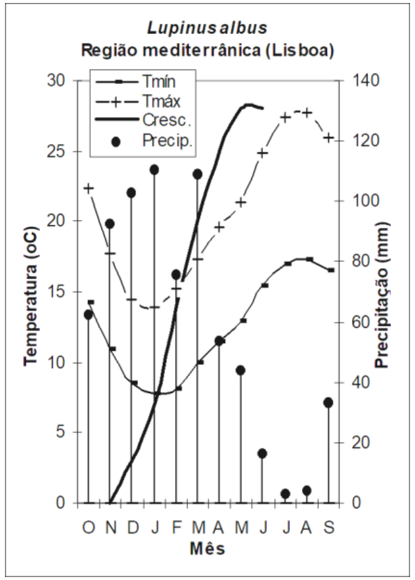
Material and Methods

- **26 accessions:**
- **Evaluated with 28 morphological traits**

CM-157	P-20993
I-82	Potosi-Alem
INTI	Potosi-ISA
LM-13	PRT79
LM-18	SBP
LM-231	XM1-39
LM-268	XM-5
LM-27	JKL-210
LM-32	JKL-295
LM-34	JKL-309
LM-81	JKL-377
MUTAL	Blanco
	MISAK (*L. albus*)







225 new accessions from INIAP, Equator

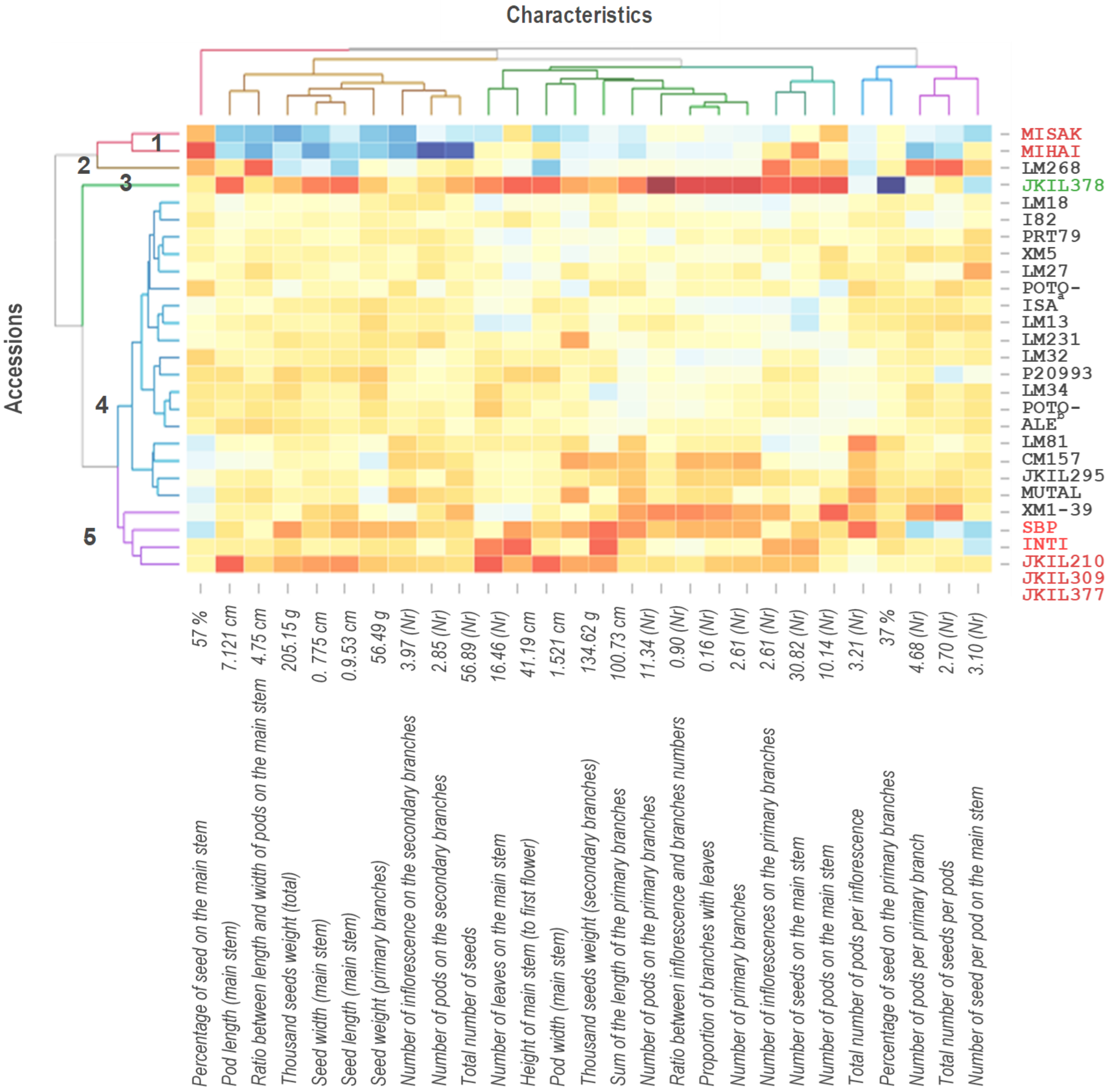
Evaluation & Characterization

Taging & marking

CM-157
I-82
INTI
LM-13
LM-18
LM231
LM-268
LM-27
LM-32
LM-34
LM-81
MUTAL
P-20993
Potosi-Alem
Potosi-ISA
PRT79
SBP
XM1-39
XM-5
JKL 210
JKL 295
JKL 309
JKL 377
Blanco

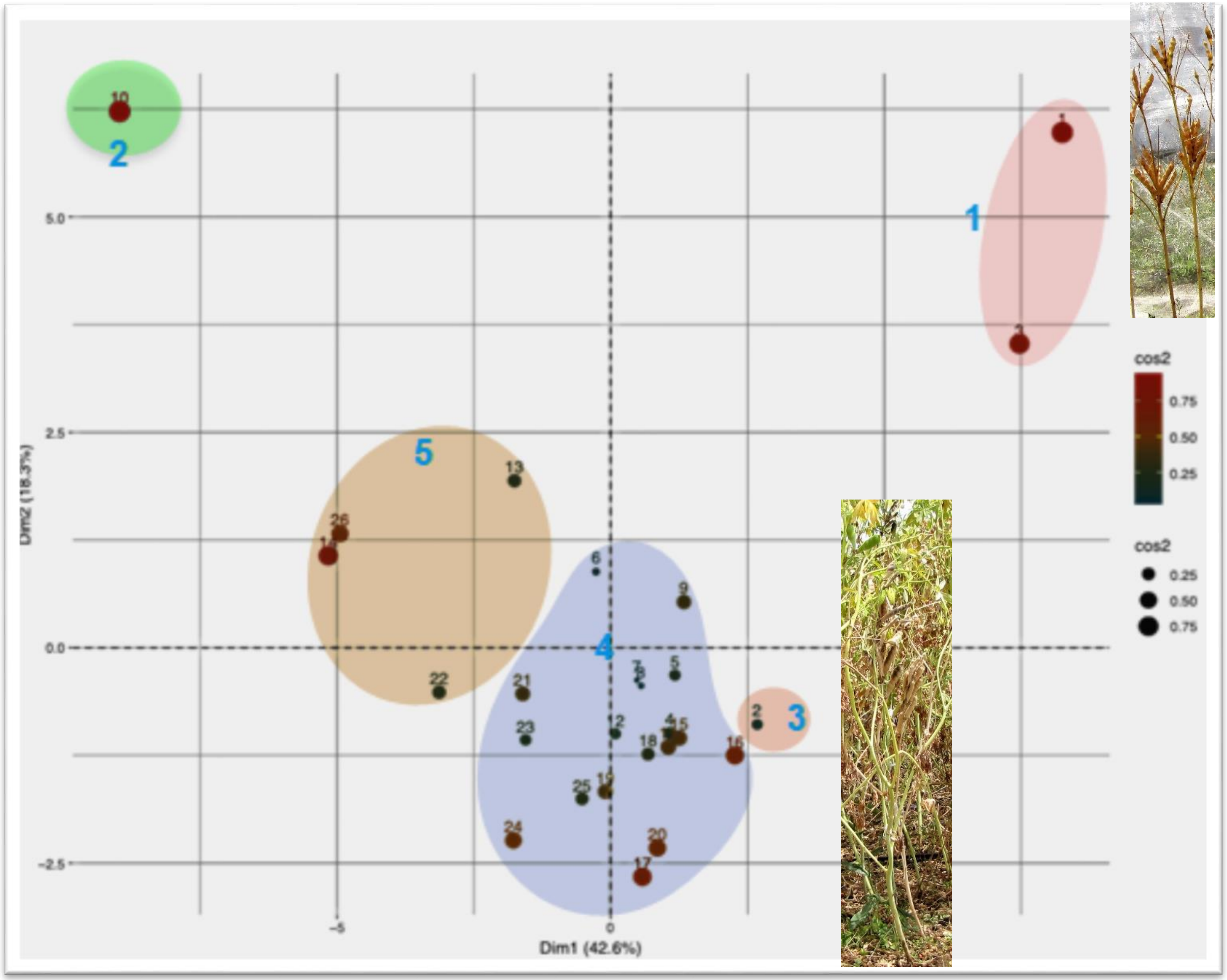
Heat map of the 26 accessions evaluated by 28 morphological traits

1. Two accessions (Vars ´MISAK´ and ´MIHAI´ of *Lupinus albus* show high values (bands in blue) for most of characters, confirming the high adaptability of this species to this Mediterranean region.
2. Accession (LM 268), is characterized by high values for total thousand seeds weight, width and length of seeds on the main stem, pod width of the main stem and percentage of seed on the primary branches;
3. Next 18 accessions characterized mostly by the absence of characters expressing extreme values;
4. Accession (JKI L378), evaluated by the lowest values in the heat map for many characteristic (bands in red): number of inflorescence/branches ratio, branches proportions with leaves, numbers for primary branches, inflorescence on the primary branches, seeds and pods on the main stem, and total number of pods per inflorescence;
5. Bottom 4 accessions; characterized by low values for pods (length and width), number of main stem leaves, main stem height, total length of the primary branches and seed percentage on the primary branches, since are early flowering types, selected on Northern Europe.

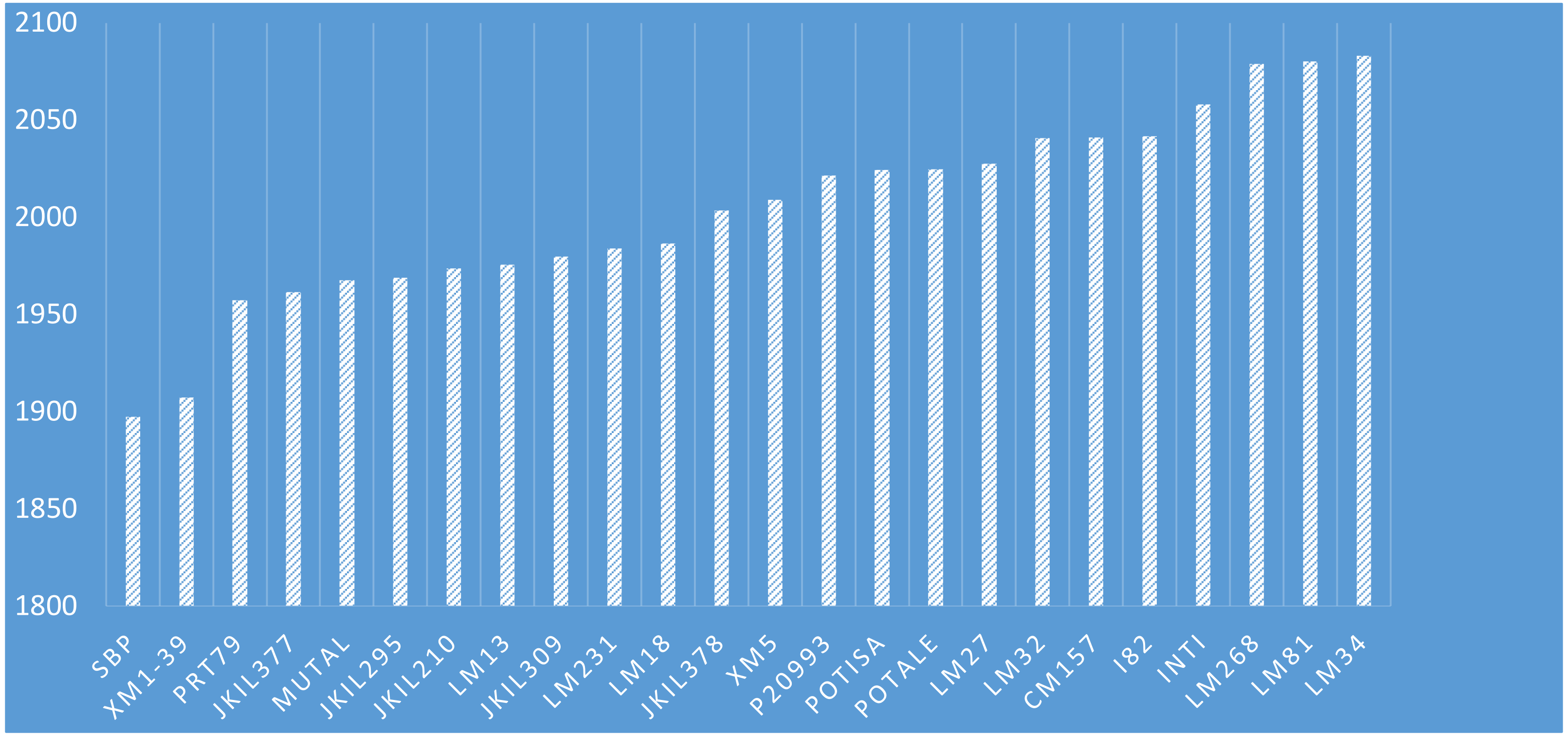


PCA

The two dimensions (PCA 1 & 2 explain 66.9%) of original normalized data for morphological evaluation regarding characterization of the 26 accessions. The first three principal components explain 78.2% of the total variance.



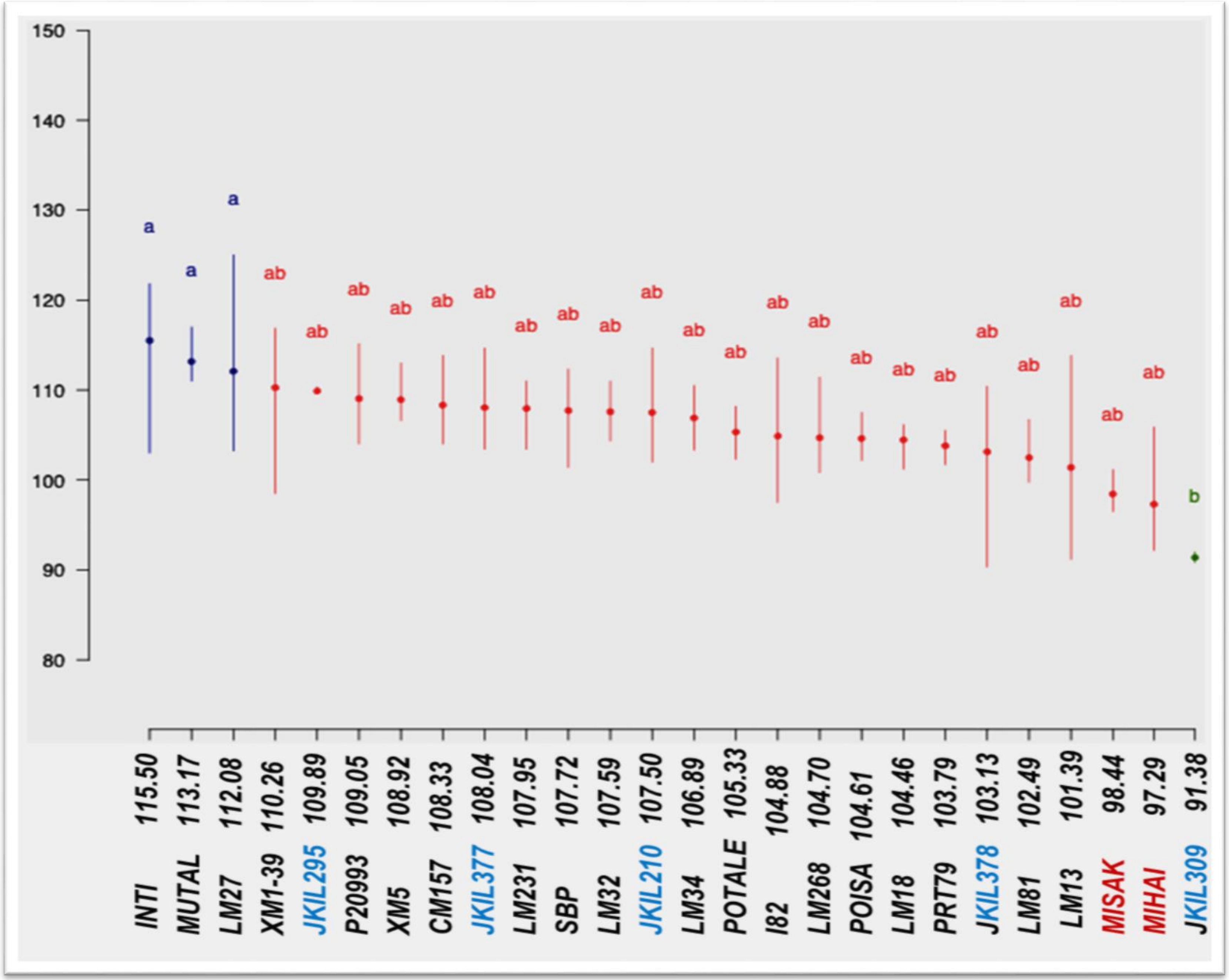
Genome size (GS)



Genome Size estimates (Flow Cytometry) for each of the 26 accessions data is not correlate with the morphological data

MISAK MIHAI	Means Genome size (Mbp)
LM268	2078.89 a
JKIL378	2003.63 efg
LM18	1986.36 cde
I82	2041.63 bc
PRT79	1957.40 hi
XM5	2009.06 def
LM27	2027.66 cde
POTO-ISA[a]	2024.33 cde
LM13	1975.70 ab
LM231	1984.06 ab
LM32	2040.86 bc
P20993	2021.50 cde
LM34	2083.17 a
POTO-ALE[b]	2024.76 cde
LM81	2080.22 a
CM157	2040.90 bcd
JKIL295	1968.97 ghi
MUTAL	1967.55 ab
XM1-39	1907.34 i
SBP	1897.26 i
INTI	2058.08 ab
JKIL210	1973.60 fgh
JKIL309	1979.66 fgh
JKIL377	1961.38 ghi

Number of days from sowing to 50% flowering plants by accession. Means comparison using Kruskal-Wallis with 95% confidence.



Molecular Characterization by six ISSR primers

- The electrophoresis show the **six ISSR primers** used that resulted in the production of **1571 bands** (262 bands per primer) (Table)

- The **24 accessions** were divided in seven major groups, in part relatable with flower colour (e.g., cluster 2 is formed only by accessions with blue flowers) (Figure). Unlike cluster 2, cluster 1, 3 and 6 are composed by accessions that exhibit distinct phenotypic patterns in flower colours. Cluster 4 and 5 are formed by an accession each with blue and purple colour, respectively.

Molecular Characterization by six ISSR primers

Table 1: List of ISSR primers used in this study, their total numbers of band per primer, polymorphic and monomorphic band and polymorphism percentage per primer.

Nr.	Primer	Band	Polymorphic	Monomorphic	Polymorphism (%)
1	HVH(TG)7	471	151	320	32.1
2	GA8YT	173	93	80	53.8
3	AG8YT	125	125	0	100.0
4	GT8YC	140	140	0	100.0
5	AG8YC	204	204	0	100.0
6	AG8YG	458	221	237	48.3

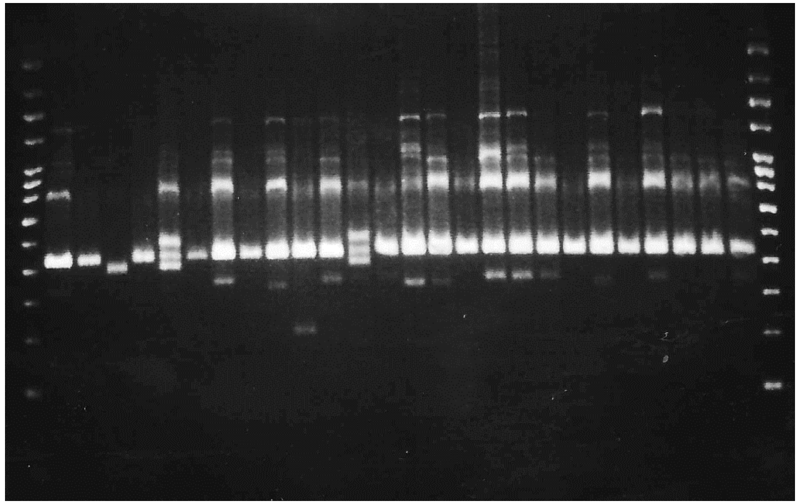
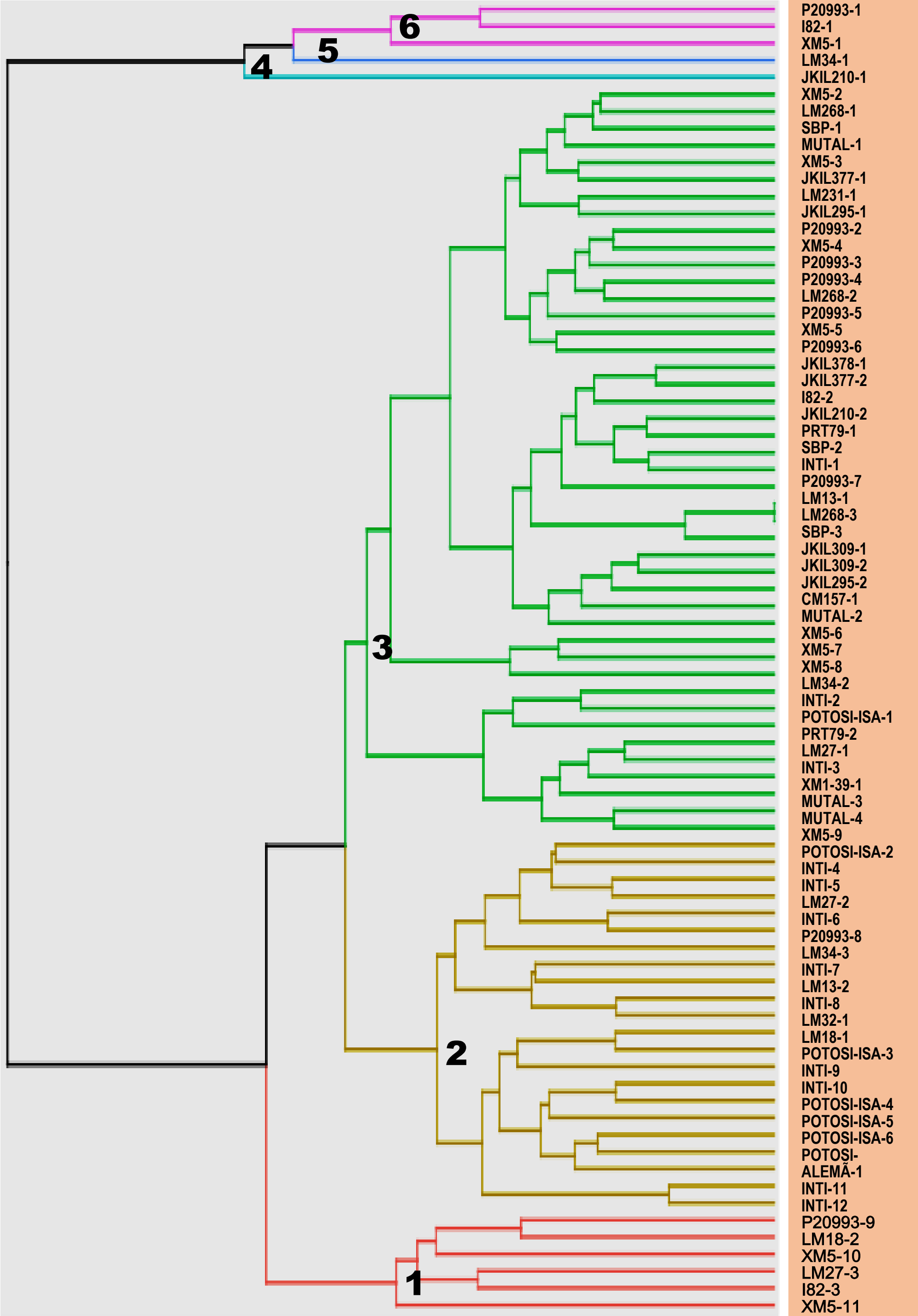


Fig. 4: Example of amplification profiles for 24 accessions of *Lupinus mutabilis* using ISSR primers and separated on agarose gel at 2% of concentration

Figure. Dendogram obtained by the UPGMA method from the coefficients of similarity (DICE) between the accessions of *Lupinus mutabilis* from six ISSR markers. r =0.8631271



LM34-1, JKIL378-1, I82-2,

JKIL377-1, MUTAL-2, XM5-7, XM5-8, P20993-9, XM5-

P20993-1, XM5-1, XM5-5, P20993-7, LM268-3, JKIL295-2, LM27-1, MUTAL-4, XM5-11

I82-1, JKIL210-1, XM5-2, LM268-1, SBP-1, MUTAL-1, XM5-3, LM231-1, JKIL295-1, P20993-2, XM5-4, P20993-3, P20993-4, LM268-2, P20993-5, P20993-6, JKIL377-2, JKIL210-2

PRT79-1, SBP-2, INTI-1, LM13-1, SBP-3, JKIL309-2, CM157-1, XM5-6, LM34-2, INTI-2, POTOSI-ISA-1,

PRT79-2, INTI-3, XM1-39-1, MUTAL-3, XM5-9, POTOSI-ISA-2, INTI-4, INTI-5

Tarwi deflamin inhibits colon cancer cell invasion

- Our results have shown that deflamin might be a common protein within the Lupin genus with not only similar molecular weight but also with anti-cancer invasion activity.
- *Lupinus mutabilis* seems to be a good source of bioactive deflamin, adding more economic value to this species.
- Further steps include sequencing *Lupinus mutabilis* deflamin from different varieties and produce it as recombinant proteins for cancer treatment

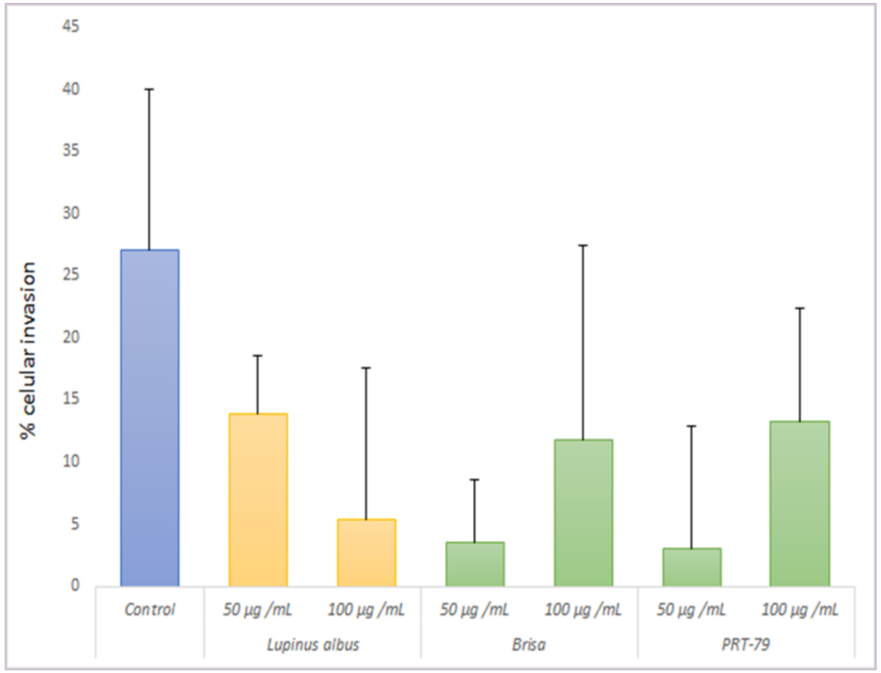
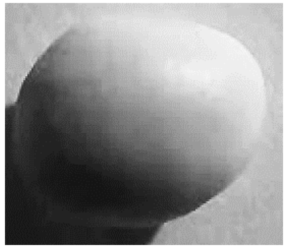


Figure 3 – Percentage of HT29 celular invasion after 48h of exposure to *L.mutabilis* varieties Brisa and PRT-79 and *L.albus* with 50 µg and 100 µg of each proteic inhibitor. The bars represent the average of 3 diferent replicates ± SD.

Conclusions



1. The agronomic performance of the Andean species in Portuguese conditions was very good. Our results highlight the LM268 line with larger seeds and a total thousand weight similar of *Lupinus albus*. LM18 was the accession with highest yield producing 1584 Kg/ha, grouping with the accessions Potosi-Isa, I82, and LM 268 being above 1500 kg/ha. These accessions are a starting point for enhancement and improvement of our collection.

2. The JKI Lines (210, 295, 309, 377 and 378) had low production of biomass and exhibits determined growth with short arquitecture structure. The JKI L309 stood out as the most precocious needing only 91 days to flower. This line could be a good option for regional drought, low precipitation and soils with low capacity for water retention. The only limitation of this accession is its low yield.

3. The third branches of all accessions of *Lupinus mutabilis* were not productive enough because short cycles and environment limitations to water supply. These early types are used in breeding.

4. The ISSR results indicate existence of few genetic variability between accessions. The Portuguese collection is composed by accessions with different genome size that we are keen to relate with phenotypic selecting traits seen in north european lines that were used.

Tarwi flower types

Seed Colours…

Thanks your attention

Muchas Gracias