

GENETIC AND GENOMIC DIVERSITY IN A TARWI (*Lupinus mutabilis* SWEET) GERmplasm COLLECTION AND PHENOTYPIC VARIABILITY UNDER MEDITERRANEAN CONDITIONS

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INTRODUCTION

Tarwi (*Lupinus mutabilis* Sweet) exhibits key traits of domestication, including indehiscent pods and seeds with permeable tegument. As for other domesticated crops with little availability of wild specimens, the genetic diversity of tarwi is low, but concealing important morphological variability, relatable with the diverse agroecological conditions found across its native range. In an attempt to introduce the cultivation of tarwi into Mediterranean-climate regions, a germplasm collection was created focused on promising accessions and studied for its genetic and morphological diversity.

MATERIALS AND METHODS

In this work we report the characterization of the genetic and genomic diversity of 24 accessions of the *Lupinus mutabilis* specie, using ISSR markers and flow cytometry genome size estimates, respectively, paralleled by the characterization of the phenotypic variability, including morphological/architectural traits. These accessions were selected from the Andean material to study their adaptability to European conditions and in particular to Portugal. In this study were used two accessions of *Lupinus albus* as reference.

RESULTS

Morphologic characterization

The cluster (Fig.1) and principal component analysis (Fig.2) identified 5 groups between accessions:

- 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.
- 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;
- Next 18 accessions characterized mostly by the absence of characters expressing extreme values;
- 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;
- 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.

Molecular Characterization

The electrophoresis show the six ISSR primers used that resulted in the production of 1571 bands (262 bands per primer) (Table 1). The percentage of polymorphic bands obtained in this study correspond to 59.4%, suggesting the existence of considerable genetic variability in the accessions under study. Similar results was found by Chirinos-Arias *et al.* (2015) in their study about genetic diversity of 30 accessions of *L. mutabilis* using 8 primers ISSR. In this work we verify that the genetic variability do not correlate with phenotypic and genomic variability, indicating the need for incorporation of more molecular markers. Galek *et al.* (2017) also did not find congruence between the genetic and morphological variability in accessions of *L. mutabilis*. The 24 accessions was divided in seven major groups, in part relatable with flower color (e.g., cluster 2 is formed only by accessions with blue flowers) (Fig. 5). Unlike cluster 2, cluster 1, 3 and 6 are composed by accessions that exhibit distinct phenotypic patterns in flower colors. Cluster 4 and 5 are formed by an accession each with blue and purple color, respectively. The existence of several distinct groups that aggregate different colors of flowers probably reflect few differences on genetic constitutions, which makes sense being the same species. On the other hand the distinct group can reflect into distinct morphological characters and variations between them.

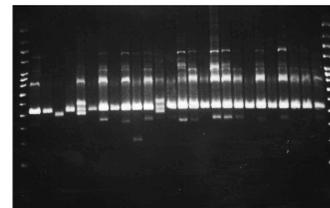


Fig. 4: Example of amplification profiles for 24 accessions of *Lupinus mutabilis* using ISSR primers and separated on agarose gel at 2% of concentration.

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

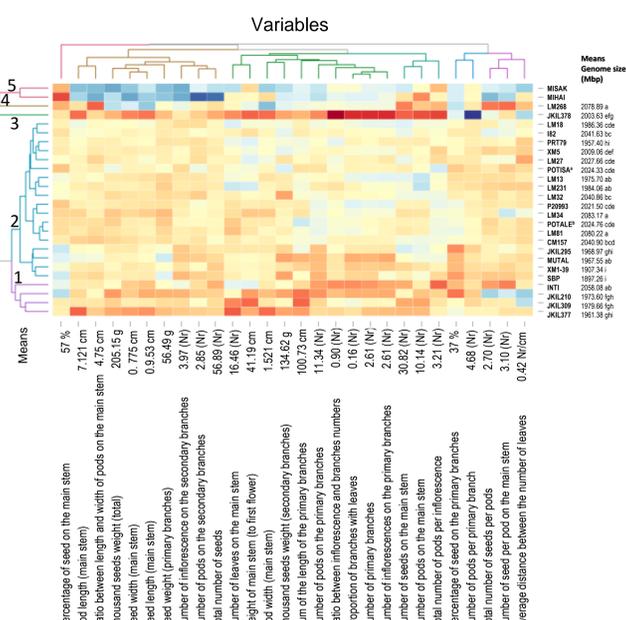


Fig. 1: Heat map of the 26 accessions obtained from morphological characterization data for the 28 traits. Relation between accessions and genome size measured by the technical cytometry flow. Accessions with one or more letter in common are not different statistically for 95% of significance. *POTISA-Potosi-ISA; *POTALE-Potosi Alemão.

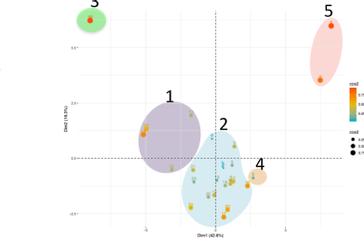


Fig. 2: Representation in two dimensions (first and second principal component explain 66.9%) of normalized original data of morphological characterization of the 26 accessions in a space defined by the vectors and own values. The first three principal components together explain 78.2% of the total variance.

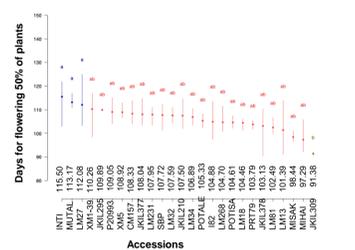


Fig. 3: Number of days to flowering 50% of plants in each accession counted since the first day sowing. Means comparison using Kruskal-Wallis with 95% confidence. Averages with the same letter are not significantly different.

Genome size (GS) data is not correlate with the morphological data that we got. Similar results of the present study were founded in various species. Gallagher *et al.* (2011) does not found correlation between genome size and morphological traits on the invasive and noninvasive acacias species in Australian. Realini *et al.* (2016) reported low association between GS and morphological traits in maize. Very recently Basak *et al.* (2019) assessing the variation of morphological traits with the genome size in turnip found no correlation between genome size and morphological traits. Beaulieu *et al.* (2007) found similar results like ours suggesting that morphological traits are controlled by other factors.

Studying five environments (including Portugal) in Europe Hardy *et al.* (1998) reported that flowering dates vary widely between environments (42-128 days). According to these authors, *Lupinus mutabilis* requires low temperatures to enter vernalization. The flowering date ranged 91-115 days after sowing (Fig.3). This is probably cause of the late onset of flowering in our conditions because the temperatures in Portugal are warmer than in Europe. On the other hand our climatic conditions allow that from May the plants enter in accelerated maturation interrupting the vegetative growth and diminishing the cycle of the plant.

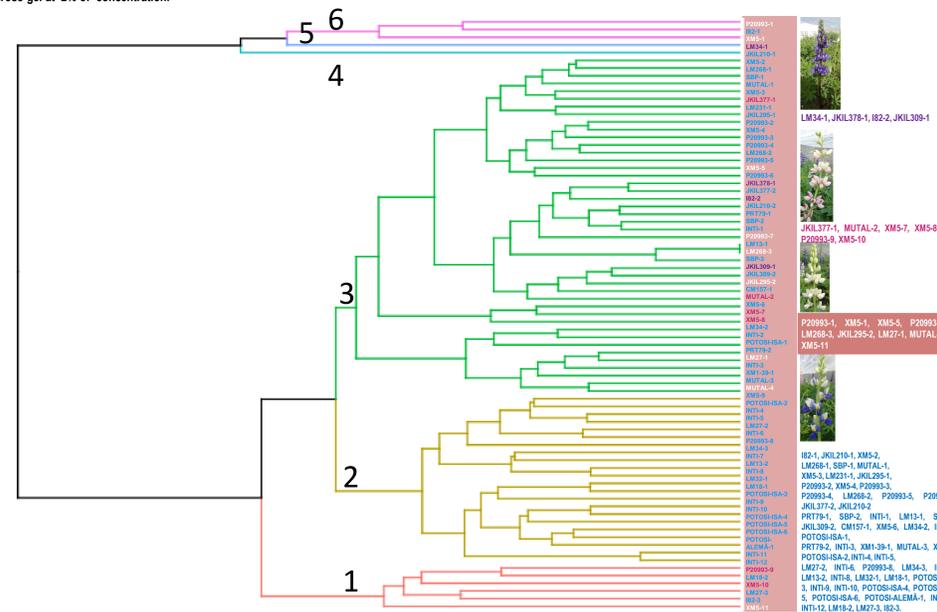


Fig. 5: Dendrogram obtained by the UPGMA method from the coefficients of similarity (DICE) between the accessions of *Lupinus mutabilis* from six ISSR markers. $r = 0.8631271$

CONCLUSIONS

- 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.
- The JKI Lines (210, 295, 309, 377 and 378) had low production of biomass and exhibits determined growth with short architecture 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.
- 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.
- 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 used.

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ACKNOWLEDGMENTS

This research was supported by the Fundação para a Ciência e a Tecnologia (FCT), Portugal, concerning financial support to the research unit LEAF (UID/AGR/04129/2013).

Lupin Bioeconomy Development

Horizon 2020 European Union Funding for Research & Innovation

Bio-based Industries Consortium