Delve into the potential of wild populations of Lupinus hispanicus

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Delve into the potential of wild populations of *Lupinus hispanicus*

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Abstract

Lupinus hispanicus Boiss. et Reut. is a Mediterranean wild species of lupin or lupine that grows naturally in the Iberian Peninsula. This species can be an interesting alternative leguminous crop adapted to marginal areas and is suitable for low-input cultivation. Thirty-one populations were collected in northwestern Spain from a diverse range of altitudes (from 25 to 1063 m). To know their potential to produce green mass for forage and/or grain for animal feed, populations were evaluated under winter Mediterranean conditions in northwestern Spain. Twenty-one traits related to growing cycle, biomass and seed yield and domestication were measured during two years at two different locations. Based on morphological characteristics we classified all the populations into the sub-species *L. hispanicus* subsp. *bicolor* (synonymous with *L. gredensis* Gand.). This result helps clarifying the distribution of this sub-species in the Iberian Peninsula. Performance of genotypes across environments was stable, since the interaction of the genotypes with the environment was non- significant for most part of traits. Populations showed phenotypical variability in their growing cycle and seed traits. Some accessions may have potential for grain (LUP-0048 and LUP-0052) or forage (LUP-0048 and LUP-0188) production and became an interesting alternative to other more input-dependent fodder crops in areas with poor soils and low supplies. Improving crucial traits as alkaloid content and seed permeability would be required before their utilization. Trials in each planting zone should be performed in order to establish adequate crop density, planting date and ideal plant type. Useful characteristics from *Lupinus hispanicus* accessions (i.e. yield stability, waterlogging and poor soil tolerance and frost resistance) could be also introduced into yellow lupin cultivars.

Keywords: germplasm collection, genetic resources, wild populations, lupins, forage, plant yield.

Introduction

Grain legumes are an important crop within the frame of sustainable agriculture. They provide food and also are important for livestock feeding. They do not require the employment of inorganic nitrogen fertilizers, as they can fix atmospheric nitrogen thanks to their symbiotic association with *Rhizobium* bacteria (Abraham et al. 2019). Lupins are native European legumes. Among their nutritional properties, they have high protein content with good quality, with potential benefits to human health and acceptability to consumers (Lucas et al. 2015). Because of that, their cultivation is growing in Europe. Besides, lupin species could be a sustainable alternative source of protein for animal feeding (Abraham et al. 2019). Cultivation of lupins as a source of plant proteins for human consumption and livestock production can be an alternative to soybean and in this way, the risk associated to the global trade of soybean and the dependence of the European Union on the imported soybean could be reduced (Abraham et al. 2019). They could be grown for direct grazing or as winter forage crop for silage, intercropped with maize, in areas with high rainfall and elevation and cool-temperate conditions (Lema and Lindner 2010). Moreover, cultivation of lupins can help in improving soil fertility and break disease cycles in crop rotation under rainy Mediterranean climatic conditions and poor acid soils (Lopez-Bellido and Fuentes 1986).

Lupinus genus accounts for approximately 300 species. However, only four of them: *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis* are employed as crops (Abraham et al. 2019). Breeding efforts in lupin species are focused in improving yield, resistance to biotic and abiotic stresses and seed quality. The genetic pool available for lupin breeders consist in landraces, and wild species, which can provide resistance to abiotic and biotic stresses and adaptation to low-input agricultural systems. Wild species of *Lupinus* are naturally distributed in North and South America, the Mediterranean region and northern Africa. However, they have been profoundly influenced by the activities of humans (Gladstones 1998), which may have threatened their existence, and enhance the spread of other wild or semi domesticated lupin species.

Lupinus hispanicus Boiss. et Reut. is a wild species that grows naturally in the Iberian Peninsula. There are no historical records of cultivation of *L. hispanicus*, although it has been cultivated experimentally (Arrieta et al. 1993). However, its high productivity and protein content (between 35.4 and 42.1%, unpublished data) makes it a promising and interesting alternative leguminous crop for low-input cultivation, and thereby able to contribute to solve the local problems of forage scarcity during the summer by its use as a self-seeding pasture (Arrieta et al. 1994). *L. hispanicus* could also be used as a source of desirable characteristics to

be introduced into other species of lupins (Hondelmann 1984). In fact, introgressions of *L. hispanicus* subsp. *bicolor* into yellow lupin (*L. luteus*) can be found naturally in Spain (Gladstones 1974). The production of interspecific hybrids is feasible (Święcicki et al. 1985, 1999) and potentially, *L. hispanicus* could be a donor of waterlogging tolerance and frost resistance (López-Bellido and Fuentes 1986). *L. hispanicus* is structured in two different subspecies: *L. hispanicus* subsp. *hispanicus* and *L. hispanicus* subsp. *bicolor* (Merino) Gladst. (synonymous with *L. gredensis* Gand.). They occupy different habitats. *L. hispanicus* subsp. *hispanicus* grows at low altitudes 0-1500 masl in central and west parts of the Iberian Peninsula. *L. hispanicus* subsp. *bicolor* grows at intermediate to high altitudes (600-1600 masl) in middle west and central Iberian Peninsula. *L. hispanicus* subsp. *bicolor* substitutes to *L. hispanicus* subsp. *hispanicu*s in occidental latitudes, especially at lower altitude (Castroviejo and Pascual 1999). Natural hybridization between both species occur in central Spain (Arrieta et al. 1993). But there is lack of information of their distribution in other parts of the Iberian Peninsula.

Our objectives were to describe the collection of populations of the wild lupin species *L. hispanicus* in northwestern Spain, to determine the taxonomical status and the relationships among accessions and to study of their morphological and agronomic characteristics with the aim of knowing their potential to be grown as a crop and to be employed in breeding programs.

Materials and methods

Plant material and experimental design

Thirty-one wild populations of *L. hispanicus* were collected in northwestern Spain (Table 1) and they were kept at the Gene Bank placed at Misión Biológica de Galicia (CSIC, Spain). Sampling was planned to cover the widest geographic distribution in northwestern Spain, including different habitats and geographical conditions (Table 1). This material was characterized and evaluated in two growing seasons (2000/2001 and 2001/2002) and at two locations, Pontevedra (42º 25' N, 8º 38' W, 20 masl) and Lalín (42º 39' N, 8º 06' W, 552 masl). Each combination of growing season and location was considered as different environments in the analyses. Pontevedra, is a location near to the coast with an annual rainfall of 1650 mm and mean temperature of 14 ºC. Lalín is an inner location, with an annual rainfall of 1100 mm and mean temperature of 11 ºC. The growing season extended from October to July. Soils in both locations were granitic with low pH (<5.5), high matter content (>7%) and low effective

exchange capacity [<6 cmol (+) kg^{-1}]. No irrigation was utilized in the trials. Fertilization was applied before sowing following the soil analysis recommendations (150 kg ha^{-1} of N, 150 kg ha^{-1} of P, 150 kg ha^{-1} of K).

A randomized complete block design with two replications was employed to evaluate the accessions. Before sowing, seed was scarified to facilitate the emergence. Each experimental plot consisted of a single 3.8 m-length row with an inter-row spacing of 0.4 m and inter-plant spacing of 0.2 m, corresponding with a density of 125,000 plants ha^{-1}. Plots were over planted and thinned to 20 plants after emergence.

Observations of seed and corolla colour were recorded for all the populations. Twenty one morphological, phenological and agronomic traits (Table 2) were measured in all plots, according to the IBPGR Lupin Descriptors (IBPGR 1981). Traits include some related to the cycle of the plant, biomass production, seed yield and other traits of particular relevance to the domestication and cultivation of this species.

Statistical analyses

Combined analysis of variance over environments was performed for each quantitative trait. Replications were considered as random factors and environments and populations as fixed factors. The Proc Mixed of SAS® 9.4 software, specifically designed to fit mixed effect models, was used for the analysis of variance. The random factors were not included in the model and their variance was obtained following the REML procedure of SAS. Mean comparisons were performed using Fisher's protected Least Significant Difference (LSD) at the 0.05 level of probability (Steel et al. 1997). Correlations between traits were determined using the mean values for each character. A principal component analysis (PCA) and a cluster analysis (CA) were performed with the standardized mean values for each quantitative trait to display the taxonomical relationships among populations. Principal components were standardized to produce the Mahalanobi's distances and a cluster analysis was made using UPGMA (Unweighted Pair Group Method using Arithmetic Averages) (Dunn and Everitt 1982; Romesburg 1984). PCA and CA were carried out with the NTSYS 2.1 software.

Results and discussion

Description of the collection

Wild *L. hispanicus* populations were collected from a diverse range of altitudes (ranging from 25 to 1063 m) and soil types. They were collected on shoulders of roads and along margin fields as reflected in Table 1. In all the populations, flowers were cream coloured in the upper whorls, while they were lilac in the lower ones (**Fig. 1**). Therefore, all the populations belong to *L. hispanicus* subsp. *bicolour*, characterized by the change in the colour of flowers with the development of the whorls. On the contrary, flowers of *L. hispanicus* subsp. *hispanicus* do not turn their colour and they are all beige. After the sampling and characterization of wild populations, we conclude that *L. hispanicus* subsp. *bicolor* is the only subspecies of *L. hispanicus* present in northwestern Spain, while natural populations in the west central regions of Spain belong to *L. hispanicus* subsp. *bicolor* and *L. hispanicus* subsp. *hispanicus*. Therefore, this study helps to clarify the natural distribution of the species in the Iberian Peninsula. Morphologically, all the populations form rosettes in the early stages of growth, and in the warmer part of the spring. Later on, the main stem and several lateral branches elongate. Seeds were beige with darker brown markings (**Fig. 1**).

In order to have a collection which represents the natural variability of this species, it is necessary to sample accessions in as many geographical locations as possible and to sample adequately the variable populations at each location. In spite of collecting samples from different locations we did not find significant differences among environments or among replications within environments for any character (data not shown). The population × environment interaction was significant only for pod length, seed weight, seeds per pod, pods per main inflorescence and proportion of yield on main inflorescence. The lack of significant population × environment interaction for most traits, including seed yield and dry mass, indicates a consistency of population's response to environmental variation. There was variation for morphological, phenological and agronomic traits among the *L. hispanicus* populations, but a strong influence of geographical environment on morphology and agronomic traits was not observed. Therefore, it seems that only a few environments are needed to initially evaluate seed yield and phenological and agronomic characteristics of *L. hispanicus* accessions in northwestern Spain. Our results in agreement with (Arrieta et al. 1993) suggest that a unique geographical and climate feature of the northwestern Iberian Peninsula region has generated a considerable degree of genetic variation in natural *L. hispanicus*.

Due to the lack of population × environment interaction in most traits, combined means across environments. will be employed to describe the accessions (Table 2). To get an idea on how this wild populations could be employed as crop or can be utilized in breeding programs we measured traits related to phenology (flowering, maturity), plant biomass (plant height,

green and dry biomass), seed productivity (pod and seeds dimensions, seed yield, etc.) and domestication (permeability of seed coat, pod shattering) (Table 2). Regarding phenological traits, *L. hispanicus* populations tended to flower late with a narrow range of variation, ranging from 158 to 179 days. Approximately, three months later, populations ripened. In agreement with this, Buirchell and Cowling (1998) find an average of 143 days to first flower in this species. Arrieta et al. (1993) found an average of 211 days to first flower in *L. hispanicus* populations from west central of Spain and Cowling et al. (1998) did not find early flowering types in this species. The growing cycle of *L. hispanicus* is similar to that of yellow lupin (*L. luteus*) collected in the same region and characterized by Lema and Lindner (2010). High vernalization requirements and small and impermeable seeds maybe the cause of late flowering and maturity of the accessions. Although there is no strong influence of the environment in the analysed traits, populations from warmer locations trend to be early flowering and are more vigorous. Late flowering populations showed long and numerous lower branches and short upper branches. No significant variation for emergence was found because the seed was scarified before planting.

L. hispanicus populations have a height average of 73.9 cms and they produce 159 g of green biomass and 14.2 g of dry biomass (Table 2). These parameters are lower than that found in a collection of yellow lupin (*L. luteus*) population of northwestern Spain (Lema and Lindner 2010), with an average of 91.6 cm and 194 and 19.1 g of green and dry biomass, respectively. Traits related to plant biomass are of special importance to consider this species for green manure and for forage. Seed weight in *L. hispanicus* wild populations ranged from 4.7 to 6.6 g/100 seeds, which is lower than that of *L. luteus.* Productivity is of special importance if we consider that seeds can be employed for human or animal consumption. Although parameters for green biomass and seed yield are lower than that of a cultivated yellow lupin collected in the same area, *L. hispanicus* population have the advantage of being more rustic, requiring less supplies. Moreover, this specie is very interesting because of its yield stability (Clements and Cowling, 1991), and adaptation to poor soils (Jambrina, 1996). In Poland, this species has been employed to introduce resistance to cold and excess moisture in the soil in *L. luteus*, since it supports clay, poorly drained soils (Swiecicki, 1985).

The average of water permeable seed coat in *L. hispanicus* was 36%, with a minimum of 13 and a maximum of 58%. The average of the populations is higher than that observed by Arrieta et al. (1994) in the subspecies *hispanicus* with an average of 4% and higher than the average of *L. luteus* with an average of 33 % (Lema and Lindner 2010). This can be considered an essential trait for domestication, which could be easily improved by selection, since there is

significant variability for this trait in the collection and the trait is controlled by a single gene (Arrieta et al. 1994). Populations have a high pod shattering values, with an average of 6.8 in a scale from 0 (non-shattering) to 9 (complete shattering). If we want to cultivate the populations to obtain seed, this value should also be improved by selection.

Variability among accessions

Variability among accessions was assessed in order to known their possibilities to be selected for different uses. According to the ANOVA (data not shown), the accessions differed significantly for first flowering, plant height and lodging, pod length and width, seed length and width, seed weight, permeability of seed coat and number of seeds per pod. Therefore, we could find variation mainly for traits related to seed yield or biomass production. We did not find any correlation among first flowering and seed coat permeability and the other traits (Table 3). Lodging was significantly correlated with plant height (0.45) at $P \leq 0.05$. Seed weight was significantly correlated ($P \leq 0.01$) with the dimensions of the pod (length: 0.42 and width: 0.65) and of the seed (length: 0.79, width: 0.73). Accordingly, Talhinhas (2002) found positive and significant correlations among seed yield and pod length in a collection of yellow lupins from Portugal. Therefore, the dimension of the pod may be employed as a first proxy of seed yield if a high number of accessions is going to be tested. Seed yield on the main inflorescence was positively correlated with number of pods on the main inflorescence, and negatively with time to flowering. Therefore, late populations trend to have lower seed yield.

To have a clearer idea on the classification and ordination of populations, we carried out a multivariate analysis. Multivariate analysis are useful when evaluating a large number of agronomic traits in a germplasm collection (Peeters and Martinelli 1989). This type of techniques have been successfully employed in other grain legumes collections, such as that of soybean (Broich and Palmer 1980) and lentil (Erskine et al. 1989). Moreover, PCA was employed to investigate patterns of variation in a *L. albus* collection from Spain (Simpson 1986), in *L. angustifolius* from the Aegean region (Clements and Cowling 1994) and Spain (Lema et al. 2005), in *L. pilosus* from Australia (Clements et al. 1996) and in *L. luteus* from Spain (Lema and Lindner 2010). In the PCA, 59% of the total variation was explained by the first two vectors, which accounted for 39 and 20% of the observed variation, respectively. Considering the eigenvector coefficients with higher absolute value, discrimination along the first principal component was done by pod width and seed traits and in the second principal component by plant height, first flowering and lodging (Table 4). Populations with the longest

seed, and widest pods were distributed to the left side of the graph because of the negative relation of pod width and seed traits with the first principal component, while those with taller plants and late flowering grouped toward the upper-right quadrant (**Fig. 2**).

Two main groups of populations were identified by CA (**Fig. 3**) which are also shown in the graph from the PCA (**Fig. 2**). Generally speaking, group 1 included populations with late and tall plants, with low seed size and high lodging percentage and group 2 comprised earlier populations with higher seed size (**Fig. 3**, Table 5). Group 1 consisted of three subgroups. Subgroup 1a contained 5 populations with tall plants, large pods, and small impermeable seeds. Subgroup 1b consisted in populations with narrow pods and small permeable seeds. Populations of subgroup 1c are very late, tall and show high lodging percentage, and permeable seeds. Group 2 consisted of two subgroups. Populations from subgroup 2a have short plants with large pods, and permeable and large-sized seeds. Subgroup 2b contained three early flowering populations with plants showing very low percentage of lodging. No association between groups and geographical origin was found (**Fig. 3**, Table 5).

As a first conclusion of multivariate analysis, we have found that there is no relationship among phenotypic variation and geographical origin of the populations. Based on the results from PCA and CA we can choose populations according to their overall performance for traits that demonstrated significant variability among accessions. We can select larger-seeded genotypes based in the first principal component and belonging to group 2 from cluster analysis. Subsequently larger seed size populations could be employed to introgress useful traits (i.e. adaptation) to yellow lupin cultivars for grain production for animal feed. Introgression of new useful traits is especially important in this species, because it shows reduced diversity of physiological and agronomic characteristics compared to white and narrow-leafed lupins (López-Bellido and Fuentes, 1986). Long cycle genotypes could be employed for biomass production to obtain green manure or to be used as pasture, allowing more time for dry matter accumulation (López-Bellido and Fuentes 1986). Agreeing with this, Kurlovich (2002) highlighted the potential of materials from northwestern Spain (Galician ecotype) to be used as highly productive forage cultivars. Among the group of larger-seeded accessions, late genotypes are ideal for the autumn-sown conditions in northwestern Spain. Earlier genotypes would have the advantage of reducing the incidence of some pests and diseases and allow rotation with other crops.

Potential for production and breeding

The main objectives of lupin breeding are focussed in reducing the alkaloid content in the seed, increasing yield and its components (Abraham et al. 2019), improving plant type and adaptation to climatic conditions and soil types (Brennan et al. 2001). According to Lema and Lindner (2010), in the autumn season of the northwestern Spain, the ideal yellow lupin type of plant for grain production are high yielding genotypes with rapid emergence and relatively tall plants and earliness in flowering and ripening. Those characteristics are crucial to avoid weed competition, to reduce the incidence of some pests and diseases (mainly Fusarium wilt and anthracnose), and to allow rotation with other crops. Similar ideotype is to be expected for cultivated *L. hispanicus*.

In this study, all accessions seem to show the same potential to be cultivated for their seed or grown as green manure or pasture, because there were no significant differences in seed yield and dry and fresh matter among accessions. Nevertheless, other important characteristics must be considered for production and breeding. Accessions LUP-0047, LUP-0048, LUP-0052, and LUP-0188, which belongs to group 2a from cluster analysis, are the most promising accessions for breeding purposes. They showed high seed weight and low percentage of lodging, being close to 0 in LUP-0047 and LUP-0052 (Table 6). LUP-0052 was the earliest population in this group (163.8 days to flowering) and LUP-0188 the latest one (182.7 days to flowering). LUP-0047 showed high seed coat permeability (50%) and accordingly better germination, and its value was close to the maximum found in the collection (58%). LUP-0048 and LUP-0052 could be the most suitable for grain production. They had similar seed yield per plant (27.8 and 29.5 g, respectively) than the high yielding Galician yellow lupin populations (ranged from 25.7 and 36.6 g) found by Lema and Lindner (2010) and the largest seeds in this study (6.3 and 6.1 g per 100 seeds, respectively), differing significantly from most accessions. In many species, including legumes, plants with large seeds have a competitive advantage over those which produce short seeds, since they produce bigger seedlings (Fuller 2007). In addition to being a desirable characteristic for seed production, higher seed size offers other advantages related to deeper burial and weed competition.

In addition to seed production, this species could be used for forage production, either for silage or direct grazing, or as a green manure crop to improve soil characteristics. Among the early maturing accessions, LUP-0048 was the best accession for forage production because of its high green (233.4 g) and dry (20.2 g) mass yields. Besides, the late maturing LUP-0188 shows high green (174.4 g) and dry (14.3 g) mass yields, besides to pods with lower dehiscence (4.3 on a scale from 0 (non-shattering) to 9 (complete shattering)). As suggested by Lema and Lindner (2010), the most adequate use for *L. luteus*, and probably also for *L. hispanicus*, in

304 northwestern Spain would be to employ it as a winter forage crop for silage, although further
305 research will be necessary to confirm this assumption and to determine appropriate sowing
306 dates and plant densities. Combination of early and late maturing genotypes could be desirable
307 to avoid forage scarcity periods. The accessions LUP-0048 and LUP-0188 could be employed
308 in breeding programs to obtain forage crops adapted to cold-temperate environments. Besides,
309 *L. hispanicus* can be used as a self-seeding pasture, and in this way, help to solve the local
310 problems of forage scarcity in the central-west Iberian Peninsula during the end of the summer
311 and the winter (Arrieta et al. 1993). In this case, to maintain partial shattering may be interesting
312 to ensure adequate reseeding.

313 We have already pointed out that *Lupinus hispanicus* ssp. *bicolor* plants are adapted to
314 survive in marginal conditions and could be grown in different agro-climatic zones in Europe
315 under low winter temperatures (to -15ºC) (Wolko et al. 2011) and low-input farming.
316 Moreover, their partial compatibility with *L. luteus* will allow the introgression of genes
317 between both genomes and help to improve some characteristics of the yellow lupin, such as
318 adaptation (Swiecicki 1985). Mentioned accessions from group 2a could be also useful to
319 introgress adaptation traits in yellow lupin (i.e. waterlogging and poor soil tolerance and frost
320 resistance), to develop new cultivars and to increase the supply of protein derived from plants
321 in inland Spain and in other zones in Europe.

322 As conclusion, a representative set of 31 wild populations of *L. hispanicus* has been
323 collected and characterized in northwestern Spain to assess their potential for high green mass
324 and grain production. Populations showed environmental stability for most of the analyzed
325 traits, including biomass and seed yield, but there was phenotypical variability in their growing
326 cycle and seed traits. Several outstanding accessions can be used to introgress adaptation traits
327 to yellow lupin or to be grown as crops for grain or forage production and became an alternative
328 to other cultivated lupins in colder areas with poor soils and low supplies. Early efforts should
329 focus on reducing its alkaloid content and increasing seed permeability, to obtain finally novel
330 low-alkaloid and productive varieties adapted to the environmental conditions of the region.

Statements & Declarations

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Competing interests

Authors declare no competing interest.

Authors contribution

M. Lema executed the experirmental work and the statistical analysis. M. Lema and P. Soengas wrotte the paper.

Data availability

Data is available upon request to the authors.

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441 Table 1. Geographical origin and collection details of 31 *Lupinus hispanicus* accessions from
442 northwestern Spain.

Code	Origin	Location	Latitude	Longitude	Altitude (masl)
LUP-0046	Roadside	Verín, Tamaguelos	41.863971	-7.439028	368
LUP-0047	Grassland	O Rosal, Calvario	41.936111	-8.841389	25
LUP-0048	Grassland	Castrelo de Miño, Vide	42.321327	-8.051330	102
LUP-0049	Roadside	Boborás, Nogueira	42.466778	-8.167807	469
LUP-0050	Roadside	Boborás, Xuvencos	42.434208	-8.135119	400
LUP-0051	Roadside	Coles, A Barra	42.429463	-7.820165	303
LUP-0052	Land	Porqueira, Sabucedo	42.024167	-7.820278	654
LUP-0053	Roadside	Xinzo de Limia, Boado	42.060556	-7.685833	624
LUP-0054	Roadside	Trasmirás, Vila de Rei	42.013335	-7.582255	708
LUP-0055	Roadside	Cualedro, As Estibadas	41.986889	-7.531083	704
LUP-0056	Grassland	Crecente, Angudes	42.183575	-8.188212	256
LUP-0057	Scrubland	A Arnoia, A Reza	42.265966	-8.151221	119
LUP-0058	Roadside	Ourense	42.335000	-7.847778	286
LUP-0059	Roadside	Baltar	41.950797	-7.707865	812
LUP-0060	Roadside	Baltar, A Quinta	41.914698	-7.788452	885
LUP-0061	Roadside	Os Blancos, Covas	41.959930	-7.774421	1063
LUP-0062	Roadside	Riós, Fumaces	41.955761	-7.333662	868
LUP-0064	Scrubland	Monforte de Lemos, Seoane	42.539933	-7.554722	310
LUP-0112	Roadside	O Irixo, Alto do Paraño	42.492500	-8.220278	797
LUP-0113	Woodland	Arbo, Consistorio	42.104402	-8.326792	74
LUP-0117	Scrubland	Manzaneda	42.297335	-7.229272	780
LUP-0146	Roadside	Ribadavia	42.281633	-8.142038	75
LUP-0150	Roadside	Ourense, Barrio	42.342778	-7.938333	135
LUP-0155	Roadside	Sandiás	42.117778	-7.756667	632
LUP-0158	Grassland	Xinzo de Limia	42.054444	-7.716944	622
LUP-0160	Roadside	Xinzo de Limia	42.036355	-7.717880	626
LUP-0161	Grassland	Xinzo de Limia, Parada de Ribeira	42.020000	-7.722778	713
LUP-0164	Roadside	Os Blancos, Covelas	42.003333	-7.716667	782
LUP-0166	Roadside	Os Blancos	42.000278	-7.746389	798
LUP-0188	Roadside	Coles, Sta. María Meliás	42.391667	-7.792500	182
LUP-0191	Roadside	Pereiro de Aguiar	42.356678	-7.795654	385

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Table 2. Morphological, phenological and agronomic traits taken in 31 wild accessions of *Lupinus hispanicus* from northwestern Spain.

Trait	**Description**	**Average (range)**
Phenological		
Emergence (days)	Days from sowing until 50% of plants emerged	20.3 (17.2-25.8)
First flowering (days)	Days from emergence until 50% of plants had the first flower	168.0 (157.5-178.7)
First ripe pod (days)	Days from emergence until 50% of plants had the first ripe pod	248.1 (230.7-254.0)
Total ripening (days)	Days from emergence until 50% of plants were fully ripped	264.7 (256.0-272.5)
Pod shattering (0-9)	Visual subjective scale per plot. From 0 (non-shattering) to 9 (complete shattering)	6.8 (4.3-9.0)
Morphological and agronomic		
Lodging (%)	Proportion of truncate plants per plot	8.9 (0.0-25.4)
Plant height (cm)	Height of plants measured at end of flowering; average of five plants per plot	73.9 (63.7-84.0)
Green mass (g)	Fresh weight of the plants measured before flowering; average of three plants per plot	159.0 (54.0-241.0)
Dry mass (g)	Dry weight of the plants, measured after 24 hours at 80ºC; average of three plants per plot	14.2 (5.0-21.8)
Pods per main inflorescence (no.)	Number of pods on main stem; average of five plants per plot	21.6 (10.7-28.3)
Pods per plant (no.)	Number of pods per plant; average of five plants per plot	65.0 (22.0-125.0)
Pod length (mm)	Distance from the lowest insertion point on the main inflorescence to the end of the pod; average of five pods from the main inflorescence of five different plants per plot	55.9 (48.4-58.9)
Pod width (mm)	Distance from the dorsal to ventral pod suture; measured on the same pods that the previous trait	7.7 (6.6-8.3)
Seeds per pod (no.)	Number of viable seeds per pod; taken on the same pods that the previous trait	5.8 (4.9-6.8)
Seed weight (g)	Weight of 100 viable seeds per plot	5.6 (4.7-6.6)
Seed yield per plant (g)	Weight of seeds by plant; average of five plants per plot	15.9 (8.7-29.5)
Seed yield per main inflorescence (g)	Weight of seeds on main stem; average of five plants per plot	5.8 (2.2-8.0)
Proportion of yield on main inflorescence (%)	Proportion of seed yield on main stem; average of five plants per plot	41.0 (21.0-73.0)

Seed length (mm)	Maximum distance of the seed; average of 25 healthy seeds per plot	5.0 (4.6-5.4)
Seed width (mm)	Distance at widest point; measured on the same seeds that the previous trait	4.7 (4.5-5.2)
Permeability of seed coat (%)	Proportion of germinated seeds; measured after 14 days growing on moisture filter paper in Petri dishes; average of 25 healthy seeds per plot	36.0 (13.0-58.0)

Table 3. Phenotypic correlations (n = 31) between nine significant traits of *Lupinus hispanicus* accessions evaluated in four environments.

	First flowering (days)	Lodging (%)	Plant height (cm)	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	Seed weight (g)
First flowering	1.00							
Lodging	0.32	1.00						
Plant height	0.29	0.45*	1.00					
Pod length	0.25	-0.09	0.04	1.00				
Pod width	-0.03	-0.08	0.01	0.63**	1.00			
Seed length	-0.12	-0.15	-0.15	0.47**	0.78**	1.00		
Seed width	-0.22	0.04	-0.20	0.25	0.47**	0.79**	1.00	
Seed weight	-0.07	-0.08	-0.15	0.42*	0.65**	0.79**	0.73**	1.00
Seed coat permeability	-0.18	0.16	-0.32	-0.09	-0.21	-0.31	-0.08	-0.05

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 4. Eigenvectors of the significant traits in the first four principal components in 31 *Lupinus hispanicus* accessions studied in four environments in northwestern Spain.

Trait	PC1 (39%)	PC2 (20%)	PC3 (13%)	PC4 (11%)
First flowering	0.0638	**0.5393**	0.0138	**0.4093**
Plant height	0.0919	**0.5849**	0.0054	-0.3338
Lodging	0.0976	**0.4228**	**-0.6645**	-0.1880
Pod length	-0.3254	0.2462	0.1032	**0.5901**
Pod width	**-0.4493**	0.1368	0.0585	0.1359
Seed length	**-0.5076**	0.0033	0.0250	-0.1521
Seed width	**-0.4257**	-0.0982	-0.2921	-0.3095
Seed weight	**-0.4651**	-0.0224	-0.1954	-0.0268
Seed coat permeability	0.1222	-0.3144	**-0.6481**	**0.4465**

Absolute values > 0.40 for each principal component are shown in bold.

Table 5. Means of significant traits of groups and subgroups of the cluster analysis made with *Lupinus hispanicus* populations studied in four environments in northwestern Spain.

Groups	No. of accessions	First flowering	Lodging	Plant height	Pod length	Pod width	Seed length	Seed width	Seed weight	Seed coat permeability
1	18	174.81a	11.54a	76.12a	55.49a	7.56b	4.93b	4.65b	5.38b	35.96a
1a	5	174.84ab	5.54bc	77.16a	57.05a	7.82ab	5.00c	4.58b	5.27c	21.10b
1b	8	173.48bc	8.42b	74.29ab	54.45b	7.25c	4.78d	4.60b	5.18c	44.39a
1c	5	176.78a	21.39a	77.86a	55.56ab	7.76ab	5.09bc	4.79a	5.74b	36.11a
2	13	170.12b	4.90b	70.71b	56.62a	7.88a	5.19a	4.86a	5.98a	35.23a
2a	10	171.41c	6.24bc	70.12c	57.29a	7.95a	5.20a	4.87a	6.05a	39.51a
2b	3	166.09d	0.58c	72.62bc	54.52b	7.64b	5.17ab	4.86a	5.74b	20.58b
Average	31	172.87	8.88	73.95	55.94	7.68	5.04	4.74	5.62	35.66
LSD[1]		1.88	4.36	2.34	1.21	0.16	0.07	0.06	0.16	7.42
LSD[2]		3.13	6.93	3.93	1.89	0.25	0.11	0.09	0.25	11.94

[1]Least significant difference to compare among groups and [2]among subgroups. Groups and subgroups with the same letter do not significantly differ at $p \leq 0.05$.

Table 6. Means of significant traits of the most outstanding accessions of *Lupinus hispanicus* evaluated in four environments in northwestern Spain.

		First flowering (days)	Lodging (%)	Plant height (cm)	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	Seed weight (g)	Seed coat permeability (%)
Accessions										
LUP-0047		165.4bc	2a	63.7b	57.9a	7.78a	5.31a	4.92b	5.80c	50.0a
LUP-0048		170.0b	11.3a	84.0a	57.4a	8.13a	5.36a	5.14a	6.29ab	15.2b
LUP-0052		164.7c	1.8a	68.9b	58.6a	7.87a	5.19a	4.82b	6.06bc	25.7b
LUP-0188		182.7a	6.2a	67.8b	58.9a	8.03a	5.36a	5.19a	6.60a	22.0b
Range	Minimum	163.8	0	63.7	48.4	6.60	4.62	4.48	4.75	13.0
	Maximum	183.0	25.4	84.0	58.9	8.28	5.36	5.19	6.60	58.0
LSD[1]		5.0	14.9	7.3	3.2	0.41	0.19	0.16	0.45	22.1

[1]Least significant difference to compare among populations. Populations with the same letter do not significantly differ at $p \leq 0.05$.

Figure legends

Fig. 1. Morphological features of *Lupinus hispanicus* collected in northwestern Spain. A) Wild population found in the edge of a road. B) Inflorescence. C) Leaf. D) Pods. E) Seeds.

Fig. 2. Plot generated with the two first principal components grouping morphological, phenological and agronomic traits in 31 *Lupinus hispanicus* wild populations from northwestern Spain. PC1: first principal component; PC2: second principal component.

Fig. 3. Dendrogram of 31 *Lupinus hispanicus* wild populations from northwestern Spain based on Mahalanobi's distance of morphological, phenological and agronomic traits after evaluation in four environments.

Figures

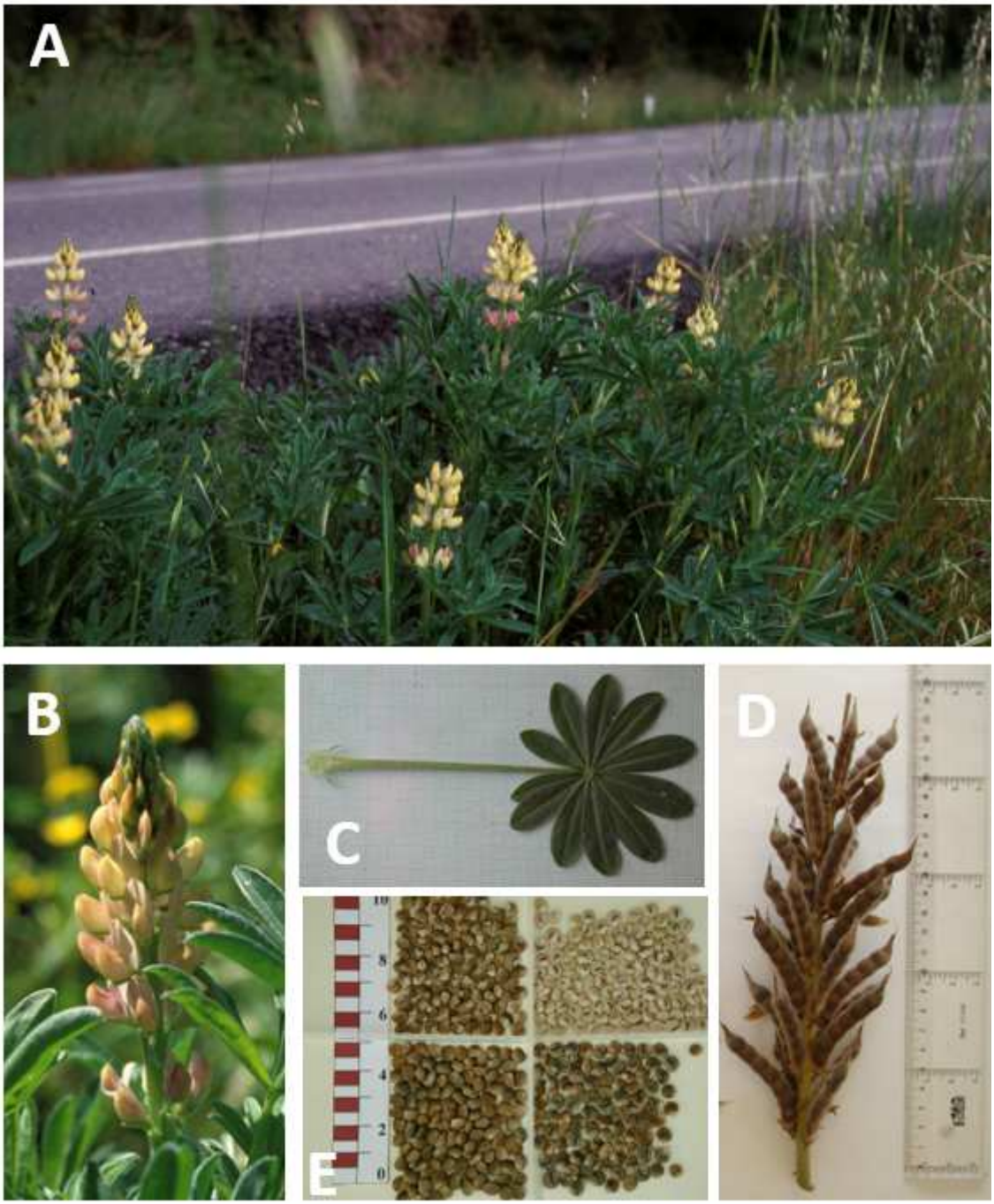

Figure 1

Morphological features of Lupinus hispanicus collected in northwestern Spain. A) Wild population found in the edge of a road. B) Inflorescence. C) Leaf. D) Pods. E) Seeds.

Figure 2

Plot generated with the two first principal components grouping morphological, phenological and agronomic traits in 31 Lupinus hispanicus wild populations from northwestern Spain. PC1: first principal component; PC2: second principal component.

Figure 3

Dendrogram of 31 Lupinus hispanicus wild populations from northwestern Spain based on Mahalanobi's distance of morphological, phenological and agronomic traits after evaluation in four environments.