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Agro-Morphological Evaluation of *Lupinus mutabilis* in Two Locations in Greece and Association with Insect Pollinators

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Abstract: *Lupinus mutabilis* Sweet is an Andean protein crop with agro-economic potential. However, it is characterized by low yields and phenotypic plasticity related to environmental conditions when cultivated in different locations in Europe. Current research objective was to evaluate *L. mutabilis* agro-morphological performance in two locations in Greece and to record its pollinators, since these can contribute to optimization of crop performance. For this purpose, eight Andean lupin accessions, one white and one blue lupin commercial varieties were evaluated for 71 agro-morphological traits in a Randomized Complete Block design with three replications. Combined Analysis over Location presented a significant accession-location interaction for traits of economic interest such as seed crude protein and 100 seed weight. Seed crude protein was higher in *L. mutabilis* accessions (up to 43.8 g 100 g^{-1} seed) than white and blue lupins. Andean lupin yielded up to 327 kg ha^{-1} (LIB214) in Kalamata, while its yield was lower than the white lupin in Athens. Using principal component analysis, three groups of accessions were formed, one by each lupin species and three within Andean lupin accessions. Floral traits such as color and size are related to pollinators diversity and preference. Insect pollinators and floral traits interplay among different lupin species was examined. Pollinators visited *L. mutabilis* flowers belong to the genera *Anthophora*, *Bombus*, *Megachile* and *Xylocopa*. The results of the present study, indicate that *L. mutabilis* has agronomic potential and enhances above ground biodiversity, however breeding for higher yields is required.

Keywords: *Lupinus mutabilis*; Mediterranean; South Europe; pollinators; agro-morphological; crude protein; yield; Andean lupin

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1. Introduction

The Andean lupin, *Lupinus mutabilis* Sweet (tauri, tarwi, tarhui, chocho or kirku), is an important crop originated in the central Andes [1–3] with high seed crude protein (32.0–52.6 g 100 g^{-1} seed), and oil (13.0–24.6 g 100 g^{-1} seed) content [4,5]. Its domestication started in the Cajamarca region in Peru [6]. It has been cultivated for over 2500 years in the plains of South America, from Colombia to northern Argentina since the pre-Inca times [3,7]. In North and South America, *L. mutabilis* is the only agronomically cultivated lupin species [8,9]. Andean lupin was an important component of crop rotation in the Andean mountainous cultivating regions, contributing to soil fertility through nitrogen fixation and phosphorus mobilization [10,11]. However, since the Incan era its cultivation was declined, while interest for this crop was recently renewed [2,12–14].

Since 1930s studies on *L. mutabilis* adaptation initiated in Europe resulting in a low alkaloid Andean lupin line, "Inti" in 1977 [15]. Agri-food, pharmaceutical, and cosmetics industries have shown interest for this crop due to its high seed crude protein and high oil content and the development of low alkaloid lines [6,16–18]. In this context the interest of European Union in cultivating *L. mutabilis* in Europe emerged [1,8,19,20]. Studies regarding the adaptation of Andean lupin continued in many European countries such as Germany, the United Kingdom [15,21,22], France, Poland and Portugal [23–25], Spain [2,26,27] while recently also in Austria, The Netherlands and Greece [6,19,20,28]. Yields of Andean lupin in Europe are low and unstable [4,6,14,26,27,29], and the main reasons are the differences in climatic conditions during cultivation period [4,27] between the Andean region and Europe. Andean lupin genetic material, affected both by genotype and diverse climatic conditions, expresses plasticity with various morphological types [2,30]. Depending on the geographical European region that Andean lupin is cultivated (northern or southern Europe), diversity in the expression of growth type characters such as height and branching has been observed [4]. One of the most important breeding aims to increase seed yield in northern Europe was to develop determinate Andean lupin genotypes [31], because the indeterminate ones expressed continual growth with high biomass but low seed yield [6]. On the contrary, the indeterminate types produce higher seed yield than the determinate ones in South Europe, as they perform better under interannual climate variations due to the partitioning of yield in successive order branches [4]. Under Mediterranean climate, due to summer drought, plants usually ripen after seed setting maximum on the second order of inflorescence [20]. Another inhibitory factor is the frost (−3 to 6 °C) occurring in early winter (for autumn sown lupins) or late spring (for spring sown lupins) at early growth stages [32] that can be detrimental for plant growth and development [6,16,27,32]. Contrary to frost stress, temperatures above 27 °C and heat stress during the flowering stage are responsible for flower abscission [6,33–35], resulting in lower yields [35]. Another reason for flower abscission is the competition for nutrients between flowers and lateral branches [4,36,37] which is mostly expressed in the plants of the indeterminate morphotype [14,31].

Under interannual and over location climate variations in southern Europe the seed yield of *L. mutabilis* ranges greatly and seems to be higher in the frost-free regions [4,38] than in locations with frost [2,27]. Greece is characterized by a high variation of climatic conditions [39] and a highly fragmented landscape with more than 15,000 km of coastline and four fifths of mountainous mainland [40] resulting in various microclimates [41]. Wild forms of Old-World lupins can be found in many places in Greece [42] and landraces of these species have been cultivated and consumed since antiquity [7,43,44] even though the presence of alkaloids and the necessary debittering process of the seeds have reduced their cultivation [45] that continues only for local culinary habits. In the last decade commercial cultivars of white and blue lupin have been introduced and cultivated mainly for their seed crude protein content that can lead to the replacement of other crops. *Lupinus mutabilis* was introduced in Greece to our knowledge, with LIBBIO project (*Lupinus mutabilis* for Increased Biomass from marginal lands and value for BIOrefineries) with the aim to evaluate the agronomic potential and the morphological variability of several accessions with semi-determinate and indeterminate growth under different cropping practices and locations. A publication regarding phenology and planting dates showed that an early planting in November was preferable [20]. Moreover, the accessions with indeterminate growth exhibited the highest yields [20], as also observed in other studies in southern Europe [4,20]. However, the morphology and performance of *L. mutabilis'* accessions in different Greek locations have not been previously addressed.

Furthermore, the adaptation of Andean lupin in a new location includes its interaction with the local fauna [6]. Recent studies highlight the role of plant-pollinator interplay to enhance ecosystem biodiversity and production services particularly for resilient and sustainable agriculture [6,46–51]. Even though lupins are capable of self-pollinating [52] they present various pollination modes [6] with outcrossing up to 58.8% [14]. Pollinators can act as cross-pollination agents but also induce self-pollination [52] increasing seed yields of lupins [53–55]. Previous studies [6,53] on the insect-pollinators that could pollinate *L. mutabilis* crop, highlight that a study on the potential factors affecting the floral preferences in lupin species is important. Moreover, pollen transfer efficiency is also of a great interest. Andean lupin cultivation in Europe has been reviewed reporting also some preliminary data regarding pollinator insects that we observed in Greece [6,56]. In the present paper we describe how this research was conducted, the identification of the species visiting *L. mutabilis* and their preference compared to the other cultivated lupins examined.

Given the limited published work on the effect of location to the agro-morphological performance of Andean lupin under winter cultivation in south Europe, we used two locations with different microclimate and soil conditions hypothesizing that agro-morphological differences that affect its adaptation will be observed between locations. Additionally, we hypothesized that different pollinators will be present in the two different locations.

This work aims to evaluate the agro-morphological performance of selected *L. mutabilis* accessions. Furthermore, we recorded their insect pollinators in comparison to commercial Old-World lupin (*L. albus* and *L. angustifolius*) varieties ('Multitalia' and 'Polo'), in two locations in Greece, characterized by different microclimate.

2. Materials and Methods

2.1. Plant Material

The plant material was comprised of eight *L. mutabilis* accessions, namely LIB209, LIB212, LIB214, LIB219, LIB220, LIB221, LIB222 and LIB223. Among the accessions, LIB209, LIB214 and LIB223 were characterized by a prolonged growth habit, one has been characterized as a semi-determinate genotype (LIB220) [38], one as semi-indeterminate (LIB212) while the other two (LIB221 and LIB222) were characterized by a restricted indeterminate growth habit [20]. Two commercial varieties *L. albus* 'Multitalia' and *L. angustifolius* 'Polo' were also used. Due to limited availability, seeds were put for germination in Petri dishes which were incubated at 25 °C after disinfection with hypochlorite solution (20%). The seeds were inoculated with *Bradyrhizobium lupini* (HiStick® Lupin, BASF Nederland B.V., Arnhem, The Netherlands) and transplanted to the greenhouse with substrate peat: perlite (2:1) and after two weeks in their final position in the field. Transplantation took place in December 2016 and harvest in June 2017.

2.2. Experimental Design

The experiment was carried out at the experimental fields of the University of the Peloponnese, Greece (Kalamata location) (coordinates: 37°03′39.4′′ N, 22°03′49.3′′ E) and the Agricultural University of Athens, Greece (Athens location) (coordinates: 37°59′03.5′′ N, 23°42′10.0′′ E) (Figure 1).

Figure 1. Map of Greece with the experimental locations.

The meteorological data were collected on monthly basis from the meteorological stations of the National Observatory of Athens sourced from meteo.gr (Figure 2).

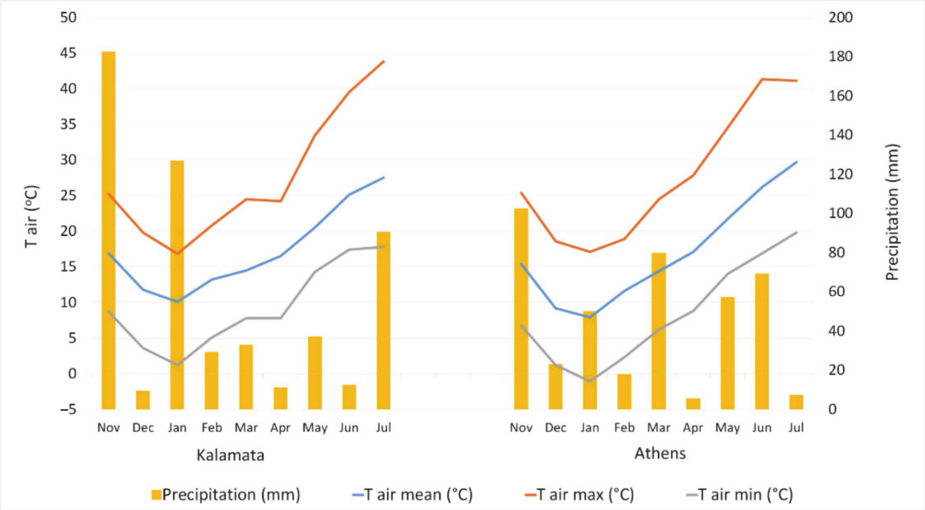

Figure 2. Mean, max and min monthly air temperature (°C) and precipitation (mm) for Kalamata (left) and Athens (right) during the growing period (November 2016 to July 2017).

The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates. Plants were spaced at 30 cm between rows and 25 cm apart within the row forming 2.25 m^2 experimental plots. Plants were cultivated in a loam soil, with a pH value of 7.6 and a $CaCO_3$ content of 15.47% in Kalamata field. On the other hand, in Athens field plants were cultivated in a silt clay loam soil, with corresponding values for pH and $CaCO_3$, 7.9 and 17.47% (Table 1).

Table 1. Soil traits of the experimental fields in Athens and Kalamata locations.

Trait	Units	Analysis	
Location		Kalamata	Athens
Soil texture		Loam	Sandy Clay Loam
Clay	%	23.63	31.83
Silt	%	48.93	22.40
Sand	%	27.43	45.77
Organic Mater	%	1.08	3.22
$CaCO_3$	%	15.47	17.47
pH		7.62	7.87
Available P	ppm	41.37	43.07
Available K	ppm	197.33	881.33
Total N	%	0.12	0.20
NO_3^-	ppm	10.03	6.35

2.3. Plant Measurements

Measurements were taken on 51 morphological and agronomic traits, based on lupin descriptor list (International Board for Plant Genetic Resources descriptor list, IBPGR 1983) and 20 additional agro-morphological traits all presented in Tables S1–S4.

2.4. Determination of Seed Crude Protein Content

Fifty seeds of each accession were used. Each sample was powdered using a ball mill (Bosch MKM6000 Type: KM13) and three replications were formed. Kjeldhal-N method was used to determine the nitrogen content of lupin accessions in dry seed samples (Kjeltec™ 8400 Analyzer unit and FOSS Analytical LabtecTM Digestor Basic, Hillerød, Denmark). Crude protein content was calculated by multiplying N by the factor 6.25 [57].

2.5. Determination of Soil Type and Quality

The soil analysis was conducted at the Laboratory of Soil Science and Agricultural Chemistry of the Agricultural University of Athens. Soil particle size was measured with a hydrometer (Bouyoucos method [58]) and soil texture by Davis and Bennett [59]. $CaCO_3$ was measured by the Bernard calcimetry Method and pH with the use of electrodes. Total nitrogen was measured by Kjeldahl method (Kjeltec™ 8400 Foss Analyzer unit and FOSS Analytical LabtecTM Digestor Basic, Hillerød, Denmark). Organic matter was measured by the Walkley-Black method [60]. Potassium was quantified by flame photometer (Sherwood Scientific Model 410, Cambridge, UK), and phosphorus with Murphy Riley method [61]. Nitrate and ammonium nitrogen were measured by Copperized Cadmium Reduction Method and Indophenol Blue method as mentioned in Karavidas et al. [62] in soil extracted by Mehlich 3 method [63].

2.6. Pollinator Recording

Insect pollinators actively visiting lupin flowers were recorded by visual observation in both experimental fields [56]. The observations took place during the flowering period between 8 am and 11 am (4 min/plot, plot area 2.25 m^2, 3 replications/observation, 3 different dates for Kalamata 20/4/2017, 25/4/2017, 12/5/2017 and 4 different days for Athens 10/4/2017, 15/4/2017, 28/4/2017, 5/5/2017) [56,64]. Observation time was determined based on preliminary recordings on the highest mobility of the pollinators during the day. Specimens of the different species that were observed to pollinate lupin plants were trapped with the traditional net collection method. They were kept in entomological jars at −18 °C and subsequently taxonomized using the Keys by Collins [65].

2.7. Data Analysis

Relative frequency (percentage %) of the values of qualitative (discontinued) traits of the accessions examined was calculated using Microsoft Excel 2007.

Analysis of Variance (ANOVA) with regard to accession was applied for the quantitative traits. Means were separated by the Tukey-Kramer Honest Significant Difference (HSD) test at the 0.05 significance level. Furthermore, in some cases small deviation from normality was observed. Although ANOVA is robust to small deviations from normality [66], the non-parametric Kruskal-Wallis test was also carried out giving the same results.

The Fmax criterion for each trait tested, shown that there is a statistically significant heterogeneity in the experimental error between the two experimental fields. In addition, there is a different set of observations between the two experimental fields as a result of plant loss during the cultivation period. Thus, Mixed Model Analysis, defining the effects of the replications (blocks) as random effects, was used in order to apply combined analysis over location. The following model was adapted (Equation (1)):

$$y_{ijk} = \mu + b_{jk} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}, \tag{1}$$

where y_{ijk} ($i = 1, 2, \ldots 9$; $j = 1, 2$; $k = 1, 2, 3$) is the yield of genotype i, in location j, block k; μ is the overall mean; b_{jk} is the effect of block k within location j, α_i is the main effect of genotype i; β_j is the main effect of location j; $(\alpha\beta)_{ij}$ is the effect of the interaction of genotype i with location j and e_{ijk} is the residual error term associated with observation y_{ijk}. The model is described in detail by Hu et al. [67]. In order to estimate the variance components, the Restricted Maximum Likelihood (REML) method was used. Traits studied are those for which the method can be applied and those considered most important for the purpose of this study.

Moreover, Pearson Correlation Coefficients were used to investigate correlations among traits. The correlation coefficients are presented with two decimals within a parenthesis followed by a number of "*" that indicate statistical significance level (p-value < 0.001 as ***, p-value < 0.01 as ** and p-value < 0.05 as *). In addition, study of the correlations contributed to the number reduction of the traits to be used in Principal Components Analysis (PCA) resulting in 31 traits.

Principal Component Analysis was performed to examine the contribution of each trait in the generation of new variables (axes) from which a small number explains a large part of the total variance. Also, PCA was applied to study the contribution of each trait in the classification of lupin accessions. A second PCA, using 19 agronomic traits, was conducted concerning Andean lupin data, to study their contribution in the differentiation of the accessions in each location.

All the statistical analyses except from the Mixed Model Analysis by REML method were performed using JMP-10 statistical packages (SAS Institute Inc., Cary, NC, USA, 2012). Mixed model analysis was performed using the SAS Edition 9.1.3 statistical package. Pearson correlation analysis was performed using STATGRAPHICS Centurion XVII (Version 17.2.00).

3. Results

3.1. Agronomic and Yield Traits

The majority of the agronomic traits measured, presented statistically significant differences among the accessions studied (Tables 2–5). Specifically, plant height (PH) was found to differ statistically significantly among accessions in Kalamata location with LIB223 presenting the statistically significantly highest mean (52 cm) (Table 2). In Athens statistically significant differences were found mostly among species with 'Polo' presenting the lowest height (26 cm), and 'Multitalia' (55 cm) along with a *L. mutabilis* LIB223 (49 cm) accession presenting the highest means among the accessions tested (Table 2). In addition, plant height to the main inflorescence (MainPH) was found to be statistically significantly highest in LIB223 accession in both locations (Table 2). Days to first flowering (DFF) presented statistically significant differences among accessions with 'Multitalia' and LIB223 constituting the latest flowering accessions, whereas 'Polo' the earliest one in both locations together with LIB221 for Kalamata location (Table 2).

Table 2. Means of PH, MainPH and DFF among *L. mutabilis* accessions in comparison to white ('Multitalia') and narrow-leafed lupin ('Polo') in Kalamata and Athens locations.

Trait	**PH**		**MainPH**		**DFF**	
Location	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens
Unit	cm	cm	cm	cm	nr	nr
Accession						
'Multitalia'	36b	55a	25b	30b	132a	138a
'Polo'	27de	26c	19d	19e	106d	111d
LIB209	35bc	38b	24bc	24cd	112c	125b
LIB212	34bcd	39b	23bc	26cd	113c	126b
LIB214	40b	40b	24bc	27bc	116c	126b
LIB219	25e	36b	21cd	25cd	123b	126b
LIB220	37bc	39b	26b	27bc	121b	127b
LIB221	29cde	n.a.	20cd	n.a.	104d	n.a.
LIB222	28de	37b	19d	23d	125b	120c
LIB223	52a	49a	37a	36a	135a	139a
p-value [a]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
p-value [b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.4373

Different letters in the same column indicate statistically significant differences among accessions with Tukey HSD intervals at 95% confidence interval. [a] p-value of ANOVA; [b] p-value of non-parametric Kruskal-Wallis. nr = number, PH = plant height, MainPH = plant height to the initiation of the main inflorescence, DFF = days to first flowering, n.a. indicate that this accession was not available in the specific location.

Table 3. Mean number of branches in first, second order and the respective number of flowers and leaves in the first order, among *L. mutabilis* accessions in comparison to white ('Multitalia') and narrow-leafed lupin ('Polo') in Kalamata and Athens locations.

Trait	**NBr1st**		**NBr2nd**		**NF1st**		**NL1st**	
Location	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens
Unit	nr	nr	nr	nr	nr	nr	nr	nr
Accession								
'Multitalia'	2.2ab	3.2a	0.0b	7.0a	7.3cde	13.5a	9.3bc	8.2a
'Polo'	2.3a	2.0b	0.6ab	1.5b	2.7e	2.2c	10.3a	7.2ab
LIB209	1.5bc	1.8b	0.3b	2.7b	9.4bc	9.7ab	8.0de	6.6b
LIB212	1.2cd	2.4b	0.1b	1.0b	8.3bcde	7.4b	8.0cd	7.4ab
LIB214	1.1cd	1.9b	1.0a	4.0ab	8.7bcd	7.2bc	7.4ef	7.4ab
LIB219	0.2e	1.6b	0.0b	0.0	5.5cde	5.0bc	9.1abcde	7.3ab
LIB220	0.8cde	1.6b	0.0b	0.0	10.4ab	7.6bc	8.7abc	6.9b
LIB221	1.1cd	n.a.	0.6ab	n.a.	5.3de	n.a.	6.6f	n.a.
LIB222	0.7de	1.7b	0.3b	1.5b	13.6a	7. 7b	8.8abc	6.9b
LIB223	2.8a	2.1b	0.5ab	1.7b	8.7bc	10.4ab	8.9ab	8.4a
p-value [a]	<0.0001	<0.0001	<0.0001	0.0145	0.4455	<0.0001	0.1212	<0.0001
p-value [b]	<0.0001	<0.0001	<0.0001	0.1494	<0.0001	<0.0001	<0.0001	<0.0001

Different letters in the same column indicate statistically significant differences among accessions with Tukey HSD intervals at 95% confidence interval. [a] *p*-value of ANOVA; [b] *p*-value of non-parametric Kruskal-Wallis. nr = number, NBr1st = Number of branches in first order, NBr2nd = Number of branches in second order, NF1st = Number of flowers in first order inflorescence, NL1st = Number of leaves in first order of branch, n.a. indicate that this accession was not available in the specific location.

Table 4. Pod traits means among *L. mutabilis* accessions in comparison to white ('Multitalia') and narrow-leafed lupin ('Polo') in Kalamata and Athens locations.

Trait	**NPPMain**		**NPP1st**		**NPP2nd**		**NSPP**	
Location	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens
Unit	nr	nr	nr	nr	nr	nr	nr	nr
'Multitalia'	2.2bc	5.0b	0.1c	3.4a	0.0b	0.1ab	3.7a	5.0a
'Polo'	1.5c	1.5c	1.3bc	0.7b	0.0b	0.0ab	2.1d	1.8d
LIB209	3.8a	6.1ab	4.5ab	2.7ab	0.2b	0.3a	3.2ab	3.2c
LIB212	2.9abc	6.8ab	2.7abc	1.0b	0.0b	0.0b	2.9bcd	3.4bc
LIB214	2.5abc	6.2ab	4.9ab	1.6ab	2.8a	0.1ab	3.1abc	3.1c
LIB219	2.6abc	5.7ab	0.4c	1.5ab	0.0b	0.0b	2.6bcd	3.4bc
LIB220	4.0a	7.9a	1.3bc	1.4b	0.0b	0.0b	2.7bcd	2.3d
LIB221	2.7abc	n.a.	3.2abc	n.a.	0.6ab	n.a.	2.9abcd	n.a.
LIB222	3.5ab	7.9a	5.0ab	2.4ab	1.5ab	0.0b	3.3ab	4.1b
LIB223	3.3ab	2.6c	2.6abc	2.1ab	0.6ab	0.1ab	2.3cd	2.1d
p-value [a]	<0.0001	<0.0001	0.0012	<0.0001	0.0028	0.0001	<0.0001	<0.0001
p-value [b]	<0.0001	<0.0001	0.0002	<0.0001	0.0148	0.0094	<0.0001	<0.0001

Different letters in the same column indicate statistically significant differences among accessions with Tukey HSD intervals at 95% confidence interval. [a] *p*-value of ANOVA; [b] *p*-value of non-parametric Kruskal-Wallis. nr = number, NPPMain = number of pods in main inflorescence, NPP1st = number of pod in first order of inflorescence, NPP2nd = number of pods in 2nd order of inflorescence, NSPP number of seeds per pod, n.a. indicate that this accession was not available in the specific location.

The number of branches in first order of inflorescence (NBr1st) of LIB223 was statistically significant highest among *L. mutabilis* accessions in Kalamata. On the contrary, in Athens location there were no statistically significant differences among the accessions. The number of branches in the second order of inflorescence (NBr2nd) observed in the Andean lupin accessions in Athens was much higher than the one observed in Kalamata (Table 3).

The number of flowers in the first order of inflorescence (NF1st) was quite diverse among Andean lupin accessions in both fields. According to the ANOVA there were no statistically significant differences, but Kruskal-Wallis test showed statistically significant differences among the accessions' medians, even though non-parametric tests are less powerful than parametric (Table 3).

Table 5. Yield traits among *L. mutabilis* accessions and between locations in comparison to white ('Multitalia') and narrow-leafed lupin ('Polo').

Trait	**AGDW**		**SPPL**		**SYPPL**		**SY**	
Location	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens	Kalamata	Athens
Unit	g plant^{-1}	g plant^{-1}	nr	nr	g plant^{-1}	g plant^{-1}	kg ha^{-1}	g plant^{-1}
'Multitalia'	2.0b	7.2a	7.1de	34.6a	1.8bc	8.9a	153. 7	791.3a
'Polo'	0.8b	0.7d	5.5de	4.1f	0.6c	0.2b	52.0	21.3b
LIB209	2.6b	2.4cd	19.2abc	17.6cd	2.0b	1.6b	179.3	138.3b
LIB212	1.8b	2.1cd	11.8cde	19.0c	1.3bc	1.6b	113.0	137.3b
LIB214	6.8a	3.3bc	26.1a	18.7c	5.3a	2.0b	327.3	173.0b
LIB219	0.1b	1.9cd	3.3e	20.2c	0.5c	1.2b	35.3	103.7b
LIB220	1.4b	2.5cd	8.1de	12.8de	1.4bc	1.2b	94.0	109.3b
LIB221	1.7b	n.a.	14.9bcd	n.a.	2.2b	n.a.	121.0	n.a.
LIB222	2.6b	2.3cd	26.1ab	26.6b	2.2b	1.5b	142.7	133.3b
LIB223	6.7a	4.4b	11.6cde	8.5ef	2.1b	1.2b	167.3	103.7b
p-value [a]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6396	<0.0001
p-value [b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0469	0.2374	0.0457

Different letters in the same column indicate statistically significant differences among accessions with Tukey HSD intervals at 95% confidence interval. [a] *p*-value of ANOVA; [b] *p*-value of non-parametric Kruskal-Wallis. nr = number, AGDW = above ground dry weight, SPP = number of seeds per plant, SY = seed yield per ha, n.a. indicate that this accession was not available in the specific location.

The number of leaves in the first order branches (NL1st) presented statistically significant differences using Kruskal-Wallis test in Kalamata. The NL1st was observed to be slightly higher in Kalamata compared to Athens location (Table 3).

The trait number of pods in main inflorescence (NPPMain) did not differ statistically significantly among *L. mutabilis* accessions in Kalamata. On the contrary, in Athens *L. mutabilis* accessions means differed statistically significantly, with LIB223 accession yielding the lowest NPPMain (2.6). Moreover, 'Multitalia' in Athens yielded twice the NPPMain compared to its performance in Kalamata (5.0 and 2.2 respectively). In general, higher values were observed in Athens than in Kalamata (Table 4). The mean number of pods in the first and second order of inflorescence was higher in Kalamata compared to Athens location in the Andean lupin accessions, while they presented statistically significant different number of pods in both the first and second order of inflorescence (Table 4).

The number of pods in the third order of inflorescence (NPP3rd) was zero in 'Multitalia' and 'Polo' in both locations. However, some *L. mutabilis* accessions presented a small NPP3rd mean, more specifically LIB214 presented 0.6 pods and LIB222, 0.1 pods in Kalamata. In Athens, LIB219 and LIB223 presented 0.1 and less than 0.1 NPP3rd.

Statistically significant differences were also observed among *L. mutabilis* accessions regarding number of seeds per pod (NSPP). In Kalamata location LIB209, and LIB222 presented more seeds per pod than LIB223 did, while in Athens LIB222 presented statistically significant more seeds per pod than LIB209, LIB214, LIB220 and LIB223 but did not exceed the NSPP of 'Multitalia' (Table 3).

The accessions LIB223 and LIB214 presented statistically significantly highest means of above ground biomass without pods (AGDW) among the Andean lupin tested in Kalamata location, while in Athens LIB223 yielded the highest AGDW among Andean lupin accessions. Similar results were observed also for total above ground fresh weight (TAGFW) in both locations (Table S5). In Kalamata LIB223 presented statistically significantly higher root dry weight (RDW) than most of the accessions, while in Athens there were no statistically significant differences among Andean lupin accessions (Table S5).

Seed yield (SY) presented statistically significant differences only in Athens with 'Multitalia' showing the statistically significant highest seed yield (Table 5). Seed yield per plant (SYPPL) on the other hand was observed to vary among Andean lupin accessions in Kalamata with LIB214 presenting the highest yield followed by LIB209, LIB221, LIB222 and LIB223 that presented statistically significant higher SYPPL than 'Polo' and LIB209 (Table 5).

The number of seeds per plant (SPPL) means were statistically significant different among the accessions in both locations and the differences among accessions were higher in Kalamata location compared to Athens (Table 4). Regarding the number of seeds per main, first and second order of inflorescence, similar results to those of pods were observed (Table S5). The means of a hundred seed weight (100SW) of *L. mutabilis* accessions ranged from 6.8 to 16.1 g with LIB223 presenting the highest *L. mutabilis* 100SW in both locations (Table 6).

Table 6. Seed weight and quality traits among *L. mutabilis* accessions and between locations in comparison to white ('Multitalia') and narrow-leafed lupin ('Polo').

Trait	**100SW**		**CP**	
Location	Kalamata	Athens	Kalamata	Athens
units	g	g	g $100g^{-1}$	g $100g^{-1}$
'Multitalia'	21.7a	28.3a	35.6f	32.3d
'Polo'	9.9def	15.7bc	32.0g	34.5cd
LIB209	10.2def	9.1cd	43.8a	42.1a
LIB212	11.4cdef	12.2bcd	43.6ab	42.7a
LIB214	15.2bc	13.1bcd	43.7a	41.9ab
LIB219	7.7ef	7.6d	39.6d	37.3bc
LIB220	14.1bcd	13.9bcd	43.0b	42.2a
LIB221	10.3cdef	n.a.	41.7c	n.a.
LIB222	6.8f	7.9cd	37.6e	38.9abc
LIB223	16.1b	16.0b	43.5ab	42.5a
p-value [a]	<0.0001	<0.0001	<0.0001	<0.0001
p-value [b]	<0.0001	<0.0001	0.0297	0.0056

Different letters in the same column indicate statistically significant differences among accessions with Tukey HSD intervals at 95% confidence interval. [a] *p*-value of ANOVA; [b] *p*-value of non-parametric Kruskal-Wallis. 100SW = 100 seeds weight, CP = seed crude protein content, n.a. indicate that this accession was not available in the specific location.

Seed crude protein content (CP) differed statistically significantly among accessions with *L. mutabilis* presenting the highest CP reaching 43.8 g $100g^{-1}$ (LIB209) in the field of Kalamata and 42.7 g $100g^{-1}$ (LIB212) in Athens. 'Multitalia' presented statistically significant lower CP compared to the Andean lupin accessions in both locations reaching 35.6 g $100g^{-1}$ seed CP in Kalamata. 'Polo' accession reached 34.5 g $100g^{-1}$ CP in Athens and 32.0 g $100g^{-1}$ in Kalamata (Table 6).

3.2. Plant and Seed Morphological Traits

Statistically significant differences among the *L. mutabilis* accessions were presented in most of the morphological quantitative traits studied (CL, SHL, 1stPH, LeafD, PetL, StpL, IL, PW, PL, SWidth, SLength), Table S5, Table S6), but two of the traits (ST, H1PB) were not statistically different among *L. mutabilis* accessions in Athens location (Table S6). Moreover, variability in several morphological qualitative traits studied was observed among the accessions tested.

3.2.1. Quantitative Morphological Traits

Cotyledon length (CL) of the *L. mutabilis* accessions ranged from 0.3 to 2.7 cm and accessions LIB220, LIB223, had statistically significant higher means compared to LIB209, LIB212, LIB219, LIB221 and LIB222 (Table S6).

Among *L. mutabilis* accessions in Kalamata location, LIB223 gave the highest mean stem thickness (ST) (Table S6). Regarding stipule length (StpL), LIB209, LIB212 (Kalamata and Athens), LIB214 (only in Athens) showed statistically significant highest means among *L. mutabilis* accessions. Means of leaf diameter (LeafD) of LIB223 was the statistically significantly highest in Kalamata and of LIB212 in Athens compared to the other Andean lupin tested. A similar pattern was observed for petiole length (PetL) as well (Table S6).

The height of the lowest primary branch (HlPB) was statistically significantly higher in LIB223 and 'Multitalia' compared to the other accessions tested in the field of Kalamata (Table S6). Mean height of first pod (1stPH) of LIB223 was the statistically significantly highest in both fields (Table S6). Main inflorescence length means (IL) in Kalamata were the highest in LIB220 while in Athens in LIB209 (Table S6).

Pod width (PW) and length (PL) means of *L. mutabilis* plants ranged from 1.1 to 1.5 cm and 4.8 to 6.6 cm respectively. Statistically significant differences were found among accessions regarding PW with LIB223 presenting the highest width in Kalamata and LIB214 in Athens (Table S5).

The white lupin 'Multitalia' presented the highest seed width and length (SWidth, SLength) compared to the accessions tested. Among the Andean lupin accessions LIB220 presented the highest mean of SLength while the mean of Swidth was the highest in LIB223 (Table S6).

3.2.2. Qualitative Morphological Traits

During the vegetative growth stage (seedling stage) most of the morphological traits such as intensity of hypocotyl color (IHC) (medium), cotyledon color (SCC) (green) did not differ among accessions. However, in the case of hypocotyl color (SHC) all the *L. mutabilis* accessions presented green color with red discoloration except from LIB220 which does not present any discoloration. In addition, intensity of cotyledon color (ICC) presented differences among accessions with *L. albus* ('Multitalia') and *L. mutabilis* accessions LIB209, LIB212, LIB214 and LIB223 presenting dark intensity, whereas *L. angustifolius* ('Polo') and *L. mutabilis* accessions LIB219, LIB220, LIB221, LIB222 presenting medium ICC. Regarding stem pubescence (SP), the *L. mutabilis* accessions were glabrous contrary to 'Multitalia' and 'Polo' which were pubescent. Leaflet shape (LS) did not differ among locations and was elliptical for all the accessions tested except from 'Multitalia' and LIB214 in which was widest towards the extreme.

The central leaflet tip of the accessions 'Multitalia', 'Polo', LIB214 was not acuminate, whereas in the rest of the accessions was acuminate. Regarding pubescence of leaflet upper surface (PLeafUp), it was absent in all the accessions tested, while pubescence of leaflet lower surface (PLeafLow) was present only in 'Multitalia' and 'Polo'. Leaf color (LC) was green in all the accessions tested, while the intensity of leaf color (ILC) varied among the accessions with 'Multitalia' presenting dark intensity, LIB209, LIB212 and LIB214 medium intensity and the rest of the accessions, light intensity. All the *L. mutabilis* accessions presented stem waxiness (SW) while 'Multitalia' and 'Polo' did not.

'Multitalia', 'Polo' LIB219, LIB220, LIB221 and LIB223 had green stem color (StC) and light intensity of stem color (IStC). However, different frequencies of green, red, red and green, green and grey stem coloring were observed in LIB209, LIB212, LIB214 and LIB222 which also differed between locations. More specifically LIB209 presented green, red, red and green frequencies of 24.4%, 57.8%, 17.8% respectively in Kalamata and 39.7%, 56.9%, 3.5% in Athens, LIB212 presented green (57.9%), red (14.0%), red and green (28.1%) StC in Kalamata and green (85.2%), red (13.0%), red and green (1.9%) StC in Athens. LIB214 presented green (77.2%), red (5.3%), red and green (17.5%) StC in Kalamata and green (88.8%), red (7.4%), red and green (3.8%) StC in Athens. Finally, LIB222 presented green (91.0%), red and green (9%) StC in Kalamata and green (63.2%), red (24.6%), red and green (7.1%), green and grey (5.3%) StC in Athens. Regarding the intensity of stem color, LIB209 presented light (33.3%), medium (13.4%), dark (53.3%) IStC in Kalamata and light (13.7%), medium (22.5%), dark (63.8%) in Athens. LIB212 presented light (65.9%), medium (24.3%), dark (9.8%) IStC in Kalamata and light (87.0%), medium (1.9%), dark (11.1%) in Athens. LIB214 presented mostly light stem color intensity in both locations with light (96.5%), medium (3.5%) in Kalamata and light (92.6%), medium (5.6%) and dark (1.9%) in Athens. Finally, LIB222 presented light (78.6%), medium (19.6%) and dark (1.8%) IStC in Kalamata and light (71.4%), medium (28.6%) in Athens. Stipule color (StpC) and its intensity (IStpC) differed mostly among species, with *L. mutabilis* accessions presenting yellow color and medium intensity, except from LIB221, LIB222 which showed low intensity. Petiole color (PetC) was 100% green in 'Polo', LIB219, LIB220, LIB221 and LIB223, whereas LIB209, LIB212, LIB214, LIB222 presented green, red, green and red PetC. LIB209 presented green (26.3%) and red (73.7%) PetC in Kalamata, red (66.6%) and red and green (33.4%) in Athens, LIB212 presented green (60%), red (13.3%), red and green (26.7%) PetC frequencies in Kalamata as well as green (83.6%) and red (16.4%) in Athens. LIB214 PetC frequencies varied presenting green (53.6%), red (10.7%), red and green (35.7%) and LIB222 showed green (90.9%) and red (9.1%) PetC in Kalamata. Both LIB214 and LIB222 presented 100% green PetC in Athens. The intensity of petiole color (IPetC) was 100% light in 'Multitalia', 'Polo' and LIB219 in both locations, LIB221 in Kalamata and LIB220, LIB222 in Athens. Medium IPetC at 100% frequency was observed in LIB223 in Kalamata and LIB209, LIB214 and LIB223 in Athens location. LIB209 presented light (24.4%) and dark (75.6%) intensity in Kalamata and 100% medium IPetC in Athens. LIB212 presented light (86.7%), medium (1.7%) and dark (11.6%) and light (94.5%), medium (5.5%) IPetC respectively in Kalamata and Athens. LIB214 presented light (5.0%) and medium (95.0%) IPetC in Kalamata and medium (100%) in Athens. LIB220 and LIB222 presented 100% light IPetC in Athens, while in Kalamata they also showed 1.7% medium IPetC and 28.6% medium IPetC respectively.

Green pod pubescence (GPP) was medium in 'Multitalia', 'Polo', LIB220, LIB214 and heavy in LIB222 in both locations. On the other hand, LIB223, LIB219, LIB221 presented light GPP and LIB209, LIB212 GPP varied between locations. Specifically, LIB209 presented low (1.7%), medium (67.2%) and heavy (31.1%) GPP in Kalamata, while in Athens medium (68.3%) and heavy (31.7%). LIB212 showed medium (68.3%) and heavy (31.7%) GPP in Kalamata, whereas heavy (89.1) GPP overcame medium (10.9) in Athens.

Seed morphological traits varied greatly among the lupin species and within *L. mutabilis* accessions. Seed shape (SSh) was spherical in LIB223, flattened spherical in LIB214, LIB219 and LIB221, reniform in 'Polo', flattened oval in LIB212, LIB220 and LIB222, flattened cuboid in 'Multitalia'. LIB209 presented two types of seed shape spherical and flattened oval at 24.0% and 76.0% frequencies respectively. Seed lustre was shiny in all accessions except from 'Polo', while seed primary color was cream in all accessions except from 'Multitalia' and LIB223 that have orange and white colored seeds respectively. Intensity of seed primary color (ISPC) was light in all accessions except from 'Polo' seeds that presented dark ISPC. Seed secondary color (SSeC) was medium brown for 'Polo' and dark brown for LIB222. LIB209 seeds are characterized by 20.0% absence of SSecC and 80.0% dark brown, while LIB212 seeds are characterized by 84.0% absence of SSeC and 16.0% dark brown. The accessions 'Multitalia', LIB223, LIB219, LIB220, LIB221, LIB214 do not present SSeC. Seed secondary color distribution (SSeCD) was characterized as marbled in LIB222, and moustache with line next to hilum in 'Polo', while variable SSeCD were observed in LIB209 and LIB212. Specifically, LIB209 presented eyebrow (24.0%), marbled crescent (28.0%), marbled eyebrow (28.0%) and absent SSeCD (20.0%), whereas LIB212 presented marbled crescent (16.0%) and absent SSeCD (84.0%).

3.3. Principal Component Analysis

The first two axes of principal component analysis (PCA) explained the 50.3% of the total variation. In the respective Biplot (Figure 3) different groups of accessions can be defined. In the first quartile a group of *L. mutabilis* populations or selections from populations, LIB209, LIB212, LIB214 and LIB223, was created and the trait Slu was shown to describe better the accessions of this group. Two subgroups were also formed in the first main group. In particular, the first subgroup consists of LIB223 and LIB214 which are described mainly by the contribution of the traits inflorescence length(IL), days to first flowering (DFF), seeds per plant (SPPL) and height of first pod (1stPH), whereas the variables seed crude protein content (CP) and length of flower (FL) contributed more to the description of LIB209 and LIB212 which consisted the second subgroup. In the second quartile 'Multitalia' formed the second group which was described better by the trait stipule color (StpC). The third group was seen in the third quartile where the variable seed secondary color distribution (SSecCD) contributed more than the other variables in the description of 'Polo' accession. In the fourth quartile the breeding lines, LIB219, LIB220, LIB221, LIB222 were grouped together and described better by the contribution of the traits seed primary color (SPC) and mature pod pubescence (MPP). Overall, the traits stem waxiness (SW) and color of flower (FC) are indicative both to the first and forth group of accessions, which means to *L. mutabilis* accessions, since it is shown between the two groups.

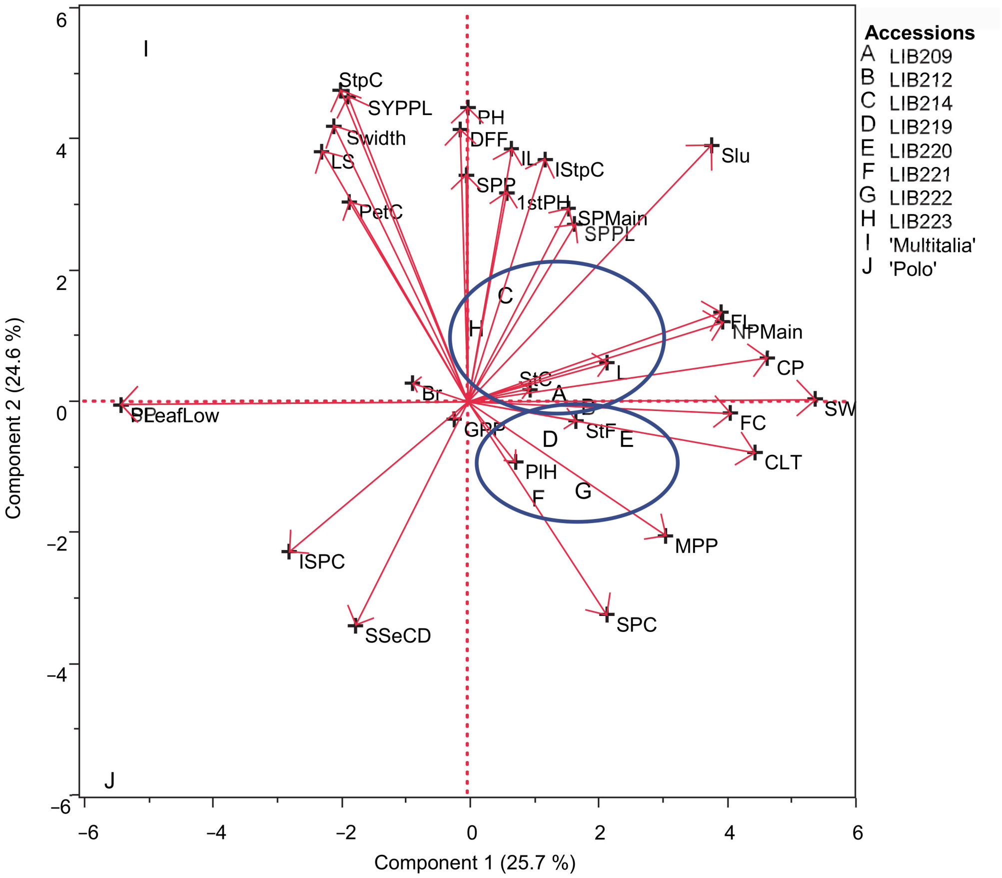

Figure 3. Two dimensional biplot of the two first components of the principal component analysis for eight *L. mutabilis*, one *L. albus* ('Multitalia') and one *L. angustifolius* ('Polo') accession. PLeafLow (pubescence of leaflet lower surface), ISPC (intensity of seed primary color), SSeCD (seed secondary color distribution), GPP (green pod pubescence), PlH (plant habit), SPC (seed primary color), MPP (mature pod pubescence), StF (stem formation), CLT (central leaflet tip), FC (color of flower), SW (stem waxiness), StC (stipule color), CP (seed crude protein content), NPPMain (number of pods in main inflorescence), FL (flower length), L (lodging), Slu (seed lustre), SPPL (seeds per plant), SPMain (seeds in main inflorescence), 1stPH (height of first pod), IStpC (intensity of stipule color), IL (inflorescence length), SPP (seeds per pod), DFF (days to first flowering), PH (plant height), PetC (petiole color), LS (leaf shape), Swidth (seed width), SYPPL (seed yield per plant), StpC (stipule color).

A second PCA using only agronomical traits and Andean lupin accessions, resulted in a differentiation between locations for the accession LIB214, in which the traits seed yield per plant (SYPPL) and total above ground fresh weight (TAGFW) contributed the most. Number of pods and seeds in main inflorescence (NPPMain, SPMain) and number of pods and seeds in second order of inflorescence (NPP1st and NSP1st) contributed to the differentiation of LIB209 and LIB222 among locations. Also, LIB223 differentiation among locations was described by the traits days to first flowering (DFF) and total above ground fresh weight (TAGFW). In Athens location the agronomical traits among Andean lupin accessions were more stable, which depicted in accessions distribution on the second and third quartile, while in Kalamata the accessions were scattered among all quartiles (Figure S1).

3.4. Over-Location Analysis of Agro-Morphological Traits

Combined analysis over location applying the Mixed Model Analysis by the REML, was used to make the comparison between the experimental fields. The *p*-values regarding location × accession revealed an interaction between factors, in all the traits studied, except from PH (Table 7). The traits seed crude protein, number of pods and seeds in main inflorescence, pods and seeds per plant, and 100 seeds weight were found to differ significantly among locations.

Table 7. Over Location analysis for particular agronomic and morphological traits regarding *L. mutabilis* accessions white ('Multitalia') and narrow-leafed lupin ('Polo') accessions.

Trait	Location	Accession	Location × Accession
	p-value	*p*-value	*p*-value
Seed crude protein content (CP)	0.0014	<0.0001	0.0245
Plant height (PH)	0.6777	0.2619	0.304
Total above ground fresh weight (TAGFW)	0.4586	<0.0001	<0.0001
Number of pods in main inflorescence (NPPMain)	0.0003	<0.0001	<0.0001
Pods per plant (PPPL)	0.0262	<0.0001	<0.0001
Number of seeds in main inflorescence (SPMain)	0.0004	<0.0001	<0.0001
Seeds per plant (SPPL)	0.0305	<0.0001	<0.0001
100 seeds weight (100SW)	<0.0001	<0.0001	<0.0001
Stem thickness (ST)	0.7821	<0.0001	<0.0001
Petiole length (PetL)	0.1882	<0.0001	<0.0001
Length of flower (FL)	0.7762	<0.0001	<0.0001

3.5. Correlations among Traits

Important tools such as correlations can be used to measure the strength of the association between traits, enhancing the breeding process. In this study Pearson correlation coefficients were calculated for each location separately (Table S7, Table S8).

In Kalamata the number of seeds and pods per plant were statistically significantly correlated to seed yield per plant (0.77 **), while the correlation coefficient of seed yield per plant with number of seeds (0.88 ***) and pods (0.89 ***) in the second order of inflorescence was higher than that of seed yield per plant with the number of seeds (0.70 *) and pods (0.69 *) in the first order of inflorescence. Seed yield per plant was also correlated (0.95 ***) with total above ground fresh weight. Regarding seed crude protein content, it was statistically significantly correlated to pod width (0.66 *) and number of pods in main inflorescence (0.65 *).

In the field of Athens seed yield per plant was statistically significantly correlated with number of seeds per plant (0.78 *) and number of pods (0.70 *) and seeds (0.96 ***) in first order of inflorescence, number of seeds per pod (0.76 *), and total above ground fresh weight (0.98 ***).

Another important and statistically significant correlation was found between stem thickness and plant height in both Athens (0.94 ***) and Kalamata (0.86 **). Total above ground fresh biomass was correlated with plant height (0.70 * and 0.85 ** for Kalamata and Athens fields respectively). Inflorescence length was correlated with pod length in both locations positively 0.78 ** (Kalamata) and 0.74 * (Athens).

3.6. Insect Pollinators Preferences on Lupinus spp.

The genera of pollinating insects visiting the flowers of the examined *Lupinus* spp., varied with the experimental location and lupin species. The insect pollinator recorded to forage on flowers of *L. mutabilis* are *Anthophora* sp., *Bombus* sp., *Megachile* sp. and

Xylocopa spp., while flowers of *L. albus* were actively visited by *Apis mellifera* and *Megachile* sp. Pollinators were not observed on flowers of *L. angustifolius* (Figure 4).

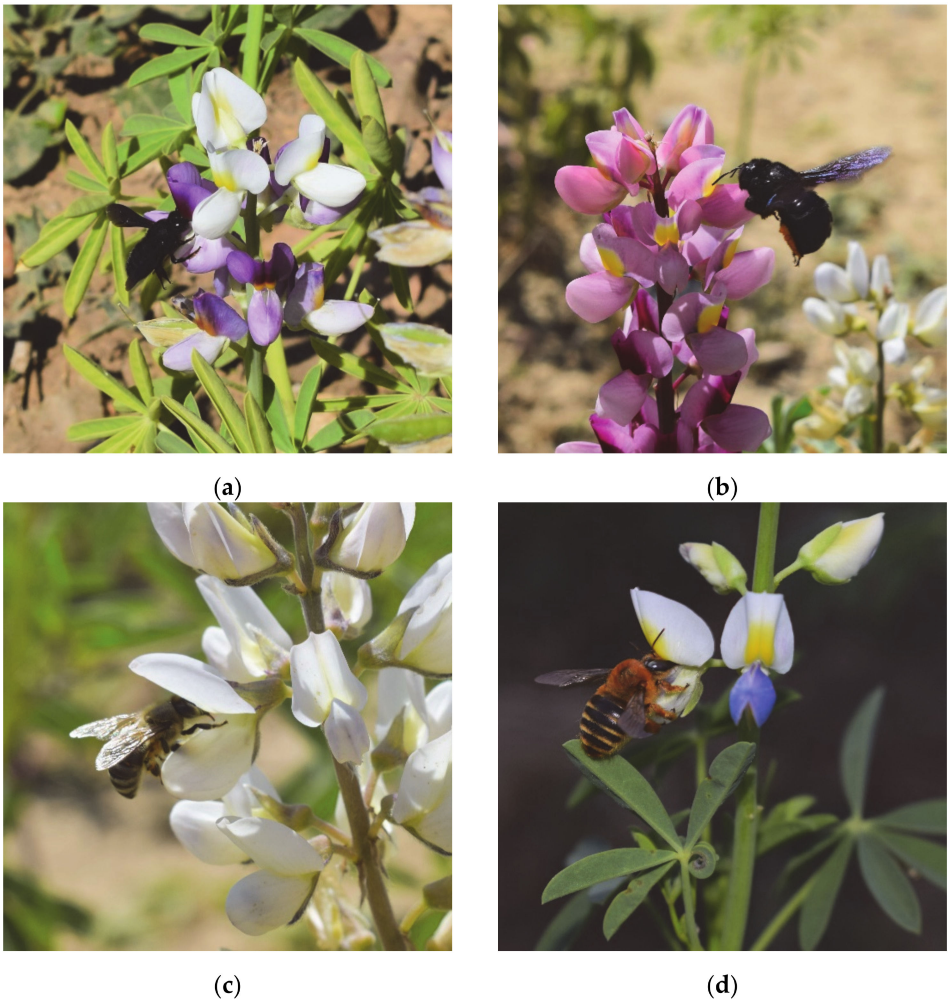
(**a**) (**b**) (**c**) (**d**)

Figure 4. (**a**) *Megachile* sp. bee foraging on a *L. mutabilis* flower; (**b**) *Xylocopa* sp. bee foraging on a *L. mutabilis* flower; (**c**) *Apis melifera*. bee foraging on a *L. albus* flower; (**d**) *Anthophora* sp. bee foraging on a *L. mutabilis* flower.

Lupinus mutabilis accessions with larger (length size) and multicolored flowers were foraged by more genera of larger pollinating species compared to those of *L. albus* and *L. angustifolius*, which have white and smaller flowers. It is noted that *A. mellifera* was not recorded on the larger flowers of *L. mutabilis* and *L. angustifolius* did not have any bee or other pollinating insect visitors. *Xylocopa* spp. were recorded steadily visiting *L. mutabilis* flowers and *A. mellifera* visiting *L. albus* flowers on Kalamata and Athens but great differences were observed between the two locations regarding the other insect pollinators detected. *Megachile* sp. was observed in one observation day (10/4/2017) and *Bombus* sp. in two observation days (10/4/2017, 15/4/2017) only in Athens whereas *Anthophora* sp. was recorded once (12/5/2017) only in Kalamata (Table S9).

In addition, great differences in flower traits such as flower length, length of main inflorescence (IL) and number of flowers in first order inflorescence (NF1st) were observed between the lupin species as well as between locations (e.g., LIB209 and LIB219 accessions, which are both selections from populations, showed 1cm longer flowers in Athens compared to Kalamata) (Tables 3, S6 and S9).

4. Discussion

4.1. Andean Lupin Agronomic Performance in Two Greek Locations

Andean lupin accessions performance, using transplantation because of limited seed, was evaluated for the first time in two Greek locations, showing that they are promising as plant material for genetic improvement. Most of the traits studied presented great variation between the accessions tested. Plant architecture (PH, MainPH, N1stBr e.t.c.) and yield (PPMain, NPP1st, NPP2nd, SPMain, SP1st, SP2nd e.t.c) traits of *L. mutabilis* has been reported to be highly dependent upon the environmental conditions [4,29,33], as also exhibited in the present study. All the agronomic traits studied in the over location analysis differed between locations except from plant height and above ground biomass.

Plant height is one of the most important traits for breeders as it is correlated with early maturity [1], and it has been reported to vary within *L. mutabilis* genetic material from 23 to 225 cm [29]. Lower plants are generally observed in south Europe than in north Europe [21,22,38], especially when plants are sown late [20] such as in the present study. Terminal drought due to low precipitation and high temperature is also highlighted by Palta et al. [68]. Regarding the ANOVA in each location, specific accessions such as 'Mutitalia', LIB219 and LIB222 (Table 2) presented higher plants by ca. 10 cm in Athens than in Kalamata. The soil in the Athens field contained more potassium, and lower plants of the specific accessions in Kalamata can be attributed to higher needs of these accessions to potassium as also has been observed in other lupin species [69,70]. Among species *L. angustifolius* 'Polo', presented lower plant height than *L. mutabilis* accessions in both locations, probably as a result of the sensitivity of the former to the high soil $CaCO_3$ observed in both locations [71–75].

Days to first flowering (DFF) is one of the most important traits for which lupins are bred, aiming to their adaptation to the European environments [4,29,31]. The accessions 'Multitalia' and LIB223 as well as the population LIB214 were the latest flowering accessions. Hammermeister et al. [76] reported that white lupin results in high yields with a high crude protein content in the seeds, however it is late maturing. The until now evaluated genetic material of *L. mutabilis*, has presented variable days from sowing to flowering ranging from 42 to 140 [29,38,77,78]. Hardy et al. [29] mentioned that time to flowering is a trait that is significantly affected by environmental factors. Under favorable conditions in a greenhouse in Finland, *L. mutabilis* Bolivian and Peruvian accessions flowered at 44 to 64 days [35]. On the other hand, under Mediterranean climate in a field experiment in Portugal, *L. mutabilis* plants flowered at 80.8 to 103.4 days from sowing date [38], values that are smaller compared to the ones measured in the present study where transplantation took place.

Reports in literature regarding the effect of photoperiod on *L. mutabilis* growth and development are contradictory [1,31,34]. Adhikari et al. [79] supported that longer photoperiods combined with higher temperatures, reduced time interval from sowing to flowering, and vernalization affects only late-maturing *L. mutabilis* genotypes. Flowering time along with plant height is also related to the number of mainstem leaves, soil pH and high calcium carbonate which can inhibit the growth of mainstem leaves through Fe deficiency [29,71–74,80]. Hence, increased number of DFF as observed also in Lazaridi et al [20], compared to that mentioned in literature, could be attributed to the lower number of leaves in the main stem and the soil properties. Also, lower precipitation and higher temperatures in Kalamata location, forced some of the accessions (LIB209, LIB212 and LIB214) to flower earlier as it has already been exhibited in other legumes [29].

The majority of the pods and seeds harvested from *L. mutabilis* accessions was observed in the main and first order of inflorescence as it has been reported in other studies in Southern Europe [4,20,38,81]. In addition, more pods and seeds were measured in several *L. mutabilis* accessions compared to the white lupin cultivar in Kalamata location. On the contrary in Athens more pods and seeds were observed in 'Multitalia'. Yield partitioning among the order of branches varies among years as observed by Guilengue et al. [38], indicating that the environmental effect plays an important role in the partitioning of yield, as it is also highlighted in the over-location analysis of the present study (Table 7).

Lower number of seeds per plant can be attributed to the coleoptera *Oxythyrea* sp. and *Tropinota* sp. (Coleoptera: Scarabaeidae, Cetoniinae), observed in the fields of the present study and mostly in Athens location. These coleoptera feed on stamens and pistils of the *Lupinus* spp. flowers among other plant species, while they have a preference on blue color [82]. In this context these Coleoptera presented a preference for *L. mutabilis'* blue colored flowers, thus reduced the number of flowers that can be pollinated. Adomas et al. [33] and Porter et al. [83] mentioned that *L. mutabilis* plants are tender in losing the top flowers in their inflorescences with flower abscission ranging from 44.5 to 74.9%. Furthermore, another factor reducing the number of pods per plant especially in first and second order of inflorescence [27] was the warm weather in spring above 27 °C that resulted in flower abortion [6,35]. However, competition between flowers and branches for nutrients is also accountable for flower loss [4] up to 50%, as Williams, [36] observed. Between locations, plants of *L. mutabilis* accessions in Athens presented more pods in all order of branches than in Kalamata location, probably attributed to the higher temperatures and lower precipitation in Kalamata during and after the flowering stage, as it is also reported by others [6,20,35].

The study of the lodging trait is very important given the intensive way of cultivating the land in modern agriculture and the use of combine harvesters for harvesting lupin. The accessions, from the most sensitive to the less sensitive to lodging, were LIB221, LIB214, LIB219, LIB222, LIB220, LIB212, LIB223, LIB220, 'Polo', 'Multitalia'. While Mikić et al. [84] supports that Andean lupin has a good standing ability, Caligari et al. [14] mention that a *L. mutabilis* mutant (KW-1) with a determinate growth habit was found prompt to lodging.

One of the most important factors for which *L. mutabilis* is not yet cropped commercially in Europe is the low and unstable yields in comparison to the other European originated *Lupinus* spp. such as *L. albus* or *L. angustifolius* [26,27,38,85]. Yields of *L. mutabilis* in literature range from 165 to 6000 kg ha^{-1} in southern Europe and 400 to 6000 kg ha^{-1} in central-northern Europe [4,14,22,27,29,33,85–87] at various sowing densities (20, 25, 35, 50, 55, 60, 90, 120 plants per m^2). Seed yield of *L. mutabilis* has been reported to increase proportionally with sowing density [86]. Under this perspective the yield of *L. mutabilis* obtained in both Athens and Kalamata ranged in similar levels or in some cases above the yields which Lopez-Bellido and Fuentes [27] had reported applying a similar sowing density. Sensitivity of *L. angustifolius* ('Polo') in high soil $CaCO_3$ and pH is higher, compared to *L. albus* ('Multitalia') and *L. mutabilis* that present the same sensitivity [6]. In this context the very low yield of 'Polo' can be attributed to high soil calcium carbonate (15.47 and 17.47%) and alkaline pH values (7.62 and 7.87) [72] of the soil in Athens and Kalamata experimental locations (Table 1). Even though white lupin is tolerant to high soil calcium carbonate [88] compared to other lupin species [6], it produced an extremely low yield in Kalamata location respectively to those mentioned in literature [88–90]. Seed yield in Kalamata location was correlated to both seed and pod number per first and second order of inflorescence, while in Athens seed yield was correlated only to pod and seed number of first order of inflorescence. The low yield of 'Multitalia' in Kalamata was related to the lower number of pods in first and second order inflorescences in comparison to *L. mutabilis* accessions, maybe attributed to higher temperature and lower precipitation levels in spring.

Lupinus mutabilis' seed crude protein is one of the main reasons that European Union is interested in its cultivation [6]. The crude protein content in Andean lupin seeds exceeds this of the native in the Mediterranean region *L. albus* and *L. angustifolius* accessions while it ranges from similar however higher levels than *L. luteus* [31,91]. In an experiment in Poland *L. mutabilis'* seed crude protein content was found to range from 35 g 100 g^{-1} seed in the determinate form to 38 g 100 g^{-1} seed in the indeterminate form [86]. This is explained by Hardy et al. [25] who mentions that indeterminate growth type plants present a preference of assimilates partitioning to vegetative growth at favorable conditions. Caligari et al. [14]

found that *L. mutabilis'* seed crude protein content was higher under European conditions in relation to Andean region, and Neves-Martins [4] mentioned that seed crude protein of *L. mutabilis* is lower in southern than in central-northern Europe. The *L. mutabilis's* seed crude protein in the present study was within literature levels [4,14,33,91]. The seed crude protein of the *L. albus* cv 'Multitalia' was more than 10% lower than in literature [89,90], however in the literature studies, the soil of the experimental field contained only traces of $CaCO_3$ in contrast to the soil of both experimental locations of the present study which was characterized by high levels of $CaCO_3$ (Table 1). Lower levels of white lupin' seed crude protein are also mentioned in literature in other locations [92]. *Lupinus angustifolius* cultivar 'Polo' presented higher crude protein content than other narrow-leafed cultivars in literature [89]. Huyghe, [93] mentions that white lupin crude protein varies among locations, the same was observed in the present study where the over-location analysis presented a statistically significant interaction of accession and location, and a significant effect of both accession and location separately (Table 7). In an experiment in pots, there was no effect of water stress in seed crude protein content of neither *L. mutabilis* nor *L. albus'* seeds [94,95]. However, under water stress such as in Athens and Kalamata due to low precipitation (Figure 2) at flowering stage and high soil porosity in loamy and sandy clay loamy soils [96] growth of lupins was stopped, reducing in this way the time available for the translocation of assimilates from stems and pods to the seeds [68,94,97]. Conclusively, several factors such as high temperatures, low precipitation, high soil pH, pollen eating coleoptera have caused yield loss in the *L. mutabilis* accessions tested.

4.2. Andean Lupin Morphological Plasticity and Performance

Plant morphological descriptors have been reported to vary between different environments in *L. mutabilis* accessions and even among other species cultivars [38,98]. Hence study of their stability is important for breeders who could use them in plant selection for better and more stable performance. In this context the over location analysis revealed a genetic stability for morphological quantitative traits such as stem thickness, petiole length and length of flower indicating that they are not affected by the environmental conditions between the two locations studied. Stipule length was observed to be stable among accessions and locations however the accession LIB214 presented different pattern among locations but still within literature levels [99]. Most morphological qualitative traits presented stable phenotypes between locations, while variability among accessions was recorded. For example, flower color differed among the *L. mutabilis* accessions but not between the two locations (Tables S2 and S9). Kurlovich [52] mentions that the white seeded *L. mutabilis* forms produce light-green plantlets, however this was not observed in the present study as LIB223 that presented white seeds produced medium-green plantlets. To our knowledge stem thickness has not been studied before in *L. mutabilis*, however it has been reported to be negatively correlated to plant density [33] and proportionally correlated to plant height in lupins [77] (as it is also seen in the present study) and heat stress in other legumes [100,101]. Even though several accessions presented thinner stems in Kalamata compared to Athens, the over-location analysis did not present any significant effect of location. However, these differences between Kalamata and Athens locations can partially explain the statistically significant interaction of accession and location regarding this trait. Leaf diameter has not been reported in other studies in literature to our knowledge, however petiole length was within literature levels as reported by Eastwood and Hughes [99]. The height of the lowest primary branch (HlPB) was more stable in Athens than in Kalamata location among the Andean lupin accessions. A within variability of the HlPB has also been reported between years in *L. albus* landraces [102]. The main inflorescence length (IL) of *L. mutabilis* in the present study was within those observed in literature [30,33,38,103]. IL varied among the accessions tested and was positively correlated to pod length as it is also reported in Lazaridi et al [20]. Stem and stipule color differed among the *L. mutabilis* accessions tested. A reddish color at the vegetative organs was observed in LIB209, LIB212, LIB222 accessions, that also present either black color in their seeds or pink-purple flowers. This observation has been previously related to a dominant allele of the *Pur* gene in *L. angustifolius* [52]. Seed length and width values of the various accessions of *L. mutabilis* tested in the present study were similar to those reported in literature [38,99]. Small phenotypic variation as it is also reported in Guilengue et al [38] was observed in the present study. Moreover, in a collection of *L. mutabilis* accessions seed shape presented high total diversity while the diversity of primary seed color was low [28].

4.3. Classification of the Lupinus spp. Accessions in Both and between Environments

As there is a large number of measurements in this study, the analysis of principal components allowed the measurement to be limited to the number of principal components interpreting the largest part of total variance observed. On the one hand, the four different groups of accessions which are defined in Figure 3 biplot depict the different lupin species and the variables contributing the most in each group as described in the results paragraph, whereas on the other hand classification within the *L. mutabilis* accessions can also be seen on the biplot reaching deeper conclusions. For example, the variable seed crude protein content contributes more than the other variables to the description of LIB209 accession which in fact gave the highest percentage of seed crude protein content (Table 6). Two of three *L. mutabilis* populations studied were classified in the group of breeding lines while the other two populations LIB214, LIB223 were grouped together, indicating that there may be a genetic relation among them. The discrimination of the *L. mutabilis* accessions LIB214, LIB223, LIB222 and LIB209 between locations was depicted in the second PCA as a result of the difference in plant architecture between the two locations. The analysis of principal components with agronomical traits between locations exhibited the importance of the environment in phenotypic plasticity for the genetic material of prolonged-indeterminate and semi-indeterminate types in Mediterranean environments [4].

4.4. Insect Pollinators Preferences on Lupinus spp.

From an agricultural perspective, pollinators play a key role in ecosystem sustainability and resilience as well as in productivity enhancement that is in line with the New European Commission's Green Deal Strategy [6,47,48,51,104]. Andean lupin is an entomogamous and partially allogamous crop that through its floral resources can serve the ecosystem by providing forage to the declining population of bees and in parallel increase its seed yield through pollination [6,105,106]. The ways that this synergy is supported are evaluated and highlighted in the present study.

According to Kozin (cited by Williams [54]), bees substantially increase the number and length of the pods as well as the number and weight of the seeds of the majority of the lupin cultivars. As lupin pollen is not transported by wind, bees should be widely used to increase crop yields of lupin seed [107]. In this respect, the lupin flowering period should be synchronized with the activity of the potential lupin pollinators. Even though literature mentions *A. mellifera* and *Bombus* sp. as important visitors of lupin flowers, other wild bee pollinators such as *Andrena* spp., *Anthophora ursina*, *Eucera* sp., *Lasioglossum* spp., *Xylocopa virginica* have also been recorded to visit *Lupinus* spp. flowers [6,54].

In the two cultivation areas of lupin accessions examined in Greece, the pollinating insect species varied mainly with the lupin species, a fact which could probably be associated among others with two phenotypic traits of their flower, the size and the color. However, lupin flower has several functions depending on its flower parts. Standard functions for visual discovery and attraction of pollinators, the wings for the landing of the pollinator and keel handling, and finally the keel enables the efficient transfer of pollen [6,108].

Flowers of *L. mutabilis* accessions are larger than the corresponding *L. albus* and *L. angustifolius* accessions flowers, also highlighted in Cowling et al. [77]. Large flowers such as those of *L. mutabilis* accessions have the potential to accommodate large sized pollinators [109] such as *Bombus* sp., *Xylocopa* spp., and *Anthophora* sp. The smaller flowers of *L. albus* accessions were visited by smaller sized pollinators such as *A. mellifera* and the *Megachile* sp. [56]. Moreover, attraction of more bee genera by *L. mutabilis* flowers compared to *L. albus* and *L. angustifolius* may be associated to the variety of colors (yellow, purple, white, blue, pink) while flowers of the other two varieties are white. The null visits of pollinating bees of *L. angustifolius* accessions could partly be attributed to its early flowering at the mid of March and could be also associated with its smaller (length size) main inflorescence (about 4.3 cm compared to about 15.0 cm for *L. albus* and a range from 6.0–18.5 cm for *L. mutabilis*) and its lower number of flowers in first order inflorescence (about 2 flowers compared with 7–13 flowers for *L. albus* and 5–13 flowers for *L. mutabilis*).

Apis mellifera was not observed to pollinate *L. mutabilis* flowers. This may be the result of both the insect and the flower anatomy [109]. Several species flowers need high mechanical strength to be opened, in many cases honeybees do not possess that strength [110]. This observation is consistent with Coleman's observation (1921), cited by [111]) who stated that lupins are visited by large-scale pollinators (e.g., bumble bees) because of their large flowers but only when lupin flowers are small sized, they can be pollinated by honeybees. More specifically, except from flower and pollinator size, the angle formed between the standard and the wings is also important for pollination efficiency as it is related to the strength needed for the effective transfer of pollen to the pollinator [110]. The correlation of flower color of the lupin varieties tested to the number of pods in main inflorescence observed in Athens locations highlights the importance of floral traits to yield through pollination [54]. In the same line, the advantage of the multicolored (flower) *L. mutabilis* in the crude protein value of the legumes and the attraction of pollinating insect species over the white (flower) varieties support the need for further investigation on the floral traits of *L. mutabilis*, which would enable the selection and creation of varieties with attractive and functional flowers for insect-pollinators [6].

5. Conclusions

To our knowledge this study is the first to describe extensively the agro-morphological traits of Andean lupin accessions phenotype under different Greek soil and climatic conditions. The evaluation of the eight *L. mutabilis* accessions revealed different performance. A large number of descriptors including vegetative, reproductive, yield and seed qualitative and quantitative traits were used. Two accessions were found to combine lateness and yield potential under south European climate conditions (LIB214, LIB223). The variability in the yield performance traits (SPP, 100SW) was affected by the accession x location interaction, while the effect of the environment was not significant for some morphological quantitative traits (ST, PetL). Several qualitative traits of certain populations such as stem color, intensity of stipule color, intensity of petiole color, presented variable frequencies in either locations. Some of the accessions exhibited desirable traits related to yield (LIB209, LIB212, LIB214, LIB222), however not as high as the native *L. albus*. Crude protein content of *L. mutabilis* accessions was affected by both location and accession with LIB209, LIB212, LIB214, LIB220, LIB223 producing seeds with crude protein content above 40 g 100 g^{-1} seed. Insect-pollinators such as *Anthophora* sp., *Bombus* sp., *Megachile* sp., *Xylocopa* spp. recorded for the first-time visiting *L. mutabilis* plants under Greek cultivation. The use of pollinators as agents of crossings may open a way for future breeding strategies. Floral traits such as color and size appear to be related to pollinators diversity, preference and pollination efficiency regarding floral design. By combining pollinator visits with optimized plant-pollinator related traits, farmer will have new tools for yield increasing and biodiversity conservation. Thus, adding a new value to *Lupinus* spp. cropping. The *L. mutabilis* material tested under Greek soil and climatic conditions was more promising in yield terms than the *L. angustifolius* accession tested, while there were no statistically significant differences to the Mediterranean originated *L. albus* in one of the locations (Kalamata). Several factors such as high temperatures in spring in relation to pollen eating coleoptera attributed to the low yields. Further evaluation including more accessions, environments, years and various cropping practices is required to fully describe *L. mutabilis* performance under south European conditions.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0472/11/3/236/s1, Table S1: Vegetative growth traits measured in Athens and Kalamata., and their phenotypic classes or units., Table S2: Reproductive traits measured in Athens and Kalamata, and their phenotypic classes or units., Table S3: Yield and yield related traits at harvest stage traits measured in Athens and Kalamata., and their phenotypic classes or units., Table S4: Seed traits measured in Athens and Kalamata, and their phenotypic classes or units., Table S5: Pod size and yield quantitative traits in Kalamata and Athens locations among different lupin accessions., Table S6: Vegetative quantitative traits in Kalamata and Athens fields., Table S7: Pearson correlations among several studied traits in Athens location., Table S8: Pearson correlations among several studied traits in Kalamata location., Table S9: Flower morphology and insect pollinators in *Lupinus* spp., Figure S1: Two dimensional biplot of the two first components of the principal component analysis for seven *L. mutabilis* accessions between two locations using only agronomical traits.

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