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Deliverable: D2.1 Screening of *Lupinus mutabilis* accessions

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Research synthesis

Executive summary

Andean lupin *(Lupinus mutabilis)* is a promising crop for arable farming on marginal lands, for biorefinery purposes and for food and non-food products. Andean lupin is a new crop for Europe with its first appearance in European agricultural research in the 1920's and 1930's in Germany by Professor Von Sengbusch. Andean lupin is a semi-wild crop, rich in proteins (≈45%), oils (≈20%) and oligosaccharides. Andean lupin has virtually no starch and is low in glucose and sucrose. Andean lupin is high in bitter alkaloids, but sweet, low alkaloid varieties are available. Four different promising accessions are agronomically evaluated under different climatic, soil and management conditions in the LIBBIO project. This agronomical evaluation demonstrated the feasibility of Andean lupin as an arable crop in Europe, but also demonstrated its performance limitations under less favourable conditions. This opens up possibilities for establishment of Andean lupin as a recognised arable crop for European farmers but also for continuous crop improvement by modern breeding technologies. A LIBBIO project partner, the seed company Vandinter Semo, succeeded in aquiring Plant Breeder Rights of the first European Andean lupin variety Cotopaxi in December 2020. This is a major success of the LIBBIO project.

In the LIBBIO project the agronomic potential for both seed-production and biomass production (bioenergy and animal feed) was evaluated. A large range of soil types and climatic regions were available to screen the performance of a variety of genetic material and compare this to reference crops that are already cultivated in Europe. The results of all these field trials was very variable, not in the least due to the fact that the weather conditions in the project years have grown increasingly extreme with forst and snow in Greece and severe drought and heavy rainfall occurring in de northern parts of Europe. Due to the large variation of growing conditions and the lack of knowledge on crop optimisation at the start of the project it is still quite difficult to give a complete and reliable image of the agronomic potential, but some of the test results give a positive outlook. Huge biomass productions of 20 t DM/ha reached in Austria and The Netherlands, without the use of any fertilizer does present a very interesting start for further optimisation. Andean lupin for seed production can reach yields of more than 3 t/ha in semi-practical field plots in Portugal using the new variety of Cotopaxi (LIB 221) and a second promising breeding line from Vandinter Semo (LIB220). Andean lupin showed even higher yield potential in irrigated experimental plots in Portugal where yields of more than 6 tons/ha where measured. This was established in triplicate in small plots and based on the seed yield of ten randomly picked plants in the middle of the plot.

A. Crop development in the different climatic regions

From the start of the project, it was decided that due to the fact that *Lupinus mutabilis* is known to be unable to withstand severe frost, sowing was to be performed in spring in the Northern and Eastern European countries (Iceland, The Netherlands, Austria and Romania) while in the Southern European countries (Portugal, Spain, Greece) sowing was to be performed in autumn. Already in the first year of field testing it became clear that plant development in these two regions turned out to be quite different. These differences were not just reflected in the time between sowing and flowering, but also in the height-development of the crop. These differences in crop development were also reflected in the ability to produce either whole plant biomass or dry kernel yields (table I).

Table I. Average crop parameters in 3 years of field data (2017-2019) in 2 Northern European countries (Austria and The Netherlands), Iceland and 3 Southern European countries (Portugal, Spain and Greece)

		Main inflorescence				Yield	
		Flowering	**Ripened**	**Flower to ripened**	**Height**	**Seeds**	**Biomass**
		days after sowing		*days*	*cm*	*t/ha*	*t DM/ha*
Spring sowing	NL/AT	66	131	65	73	0.25	4.5
	IS	100	NA	NA	28	NA	NA
Autumn sowing	PT/ES/GR	116	165	49	42	1,49	3.0

The average time from sowing until flowering was almost double in autumn-sown lupin compared to spring-sown lupin. The exception being Iceland where time to flowering of spring-sown lupin was comparable to the autumn-sown lupin in Southern Europe. A similar effect was seen for the height of the main stem to the base of the primary inflorescence which was much higher for the spring sown crops in Austria and The Netherlands compared to the autumn-sown crops in Southern Europe. The main influencing factor seems to be the temperature during the early growth stages. In both Iceland and in Portugal, the temperature in this growth stage was quite stable around 10°C, whereas the temperatures in Austria and The Netherlands start similarly to Iceland and Portugal but tend to rise substantially in this first part of the growing season (Figure I). In Greece the mean temperature ranges from 10-15°C from planting to flowering with an initial decrease in temperature at the start and an increase afterwards, resulting in reduced growth rate due to fewer degree days (Walker et. al. 2011).

From the same figure (Figure I) it can also be deduced why crop development in Iceland does not progress to much beyond flowering as by the time the crop starts to flower, the temperatures continue to decrease; slowing down further plant development. This is in contrast to the Southern European weather conditions that show quickly rising temperatures after the onset of flowering. The continued low temperatures in Iceland resulted in practically no pod production and also the plant biomass production was very limited.

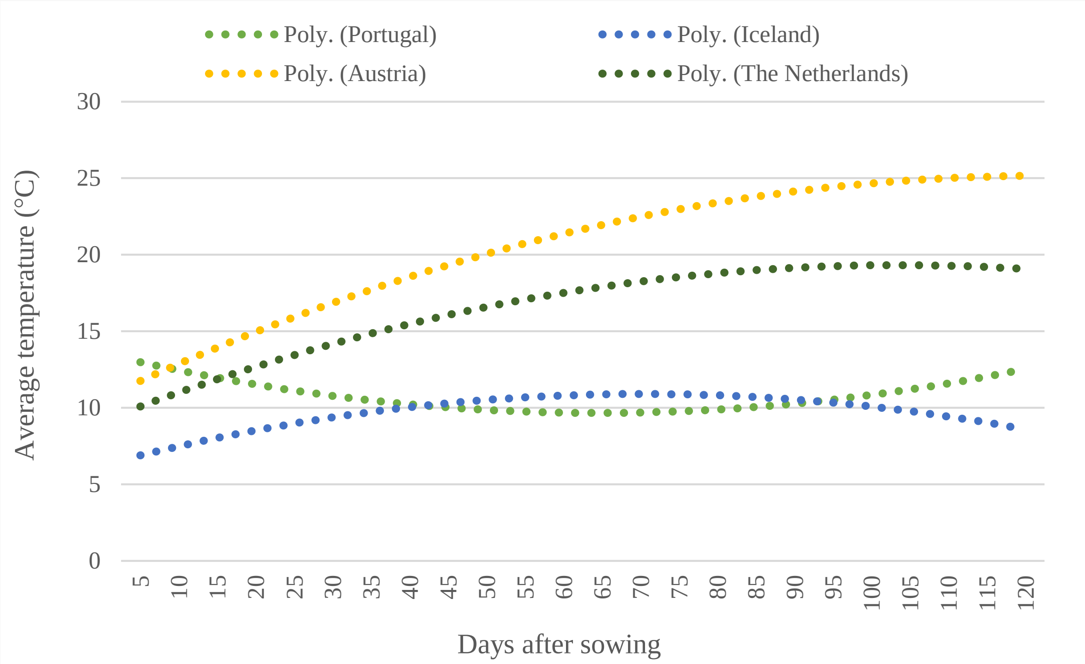


Figure I. The average temperature during the days after sowing.

Not just during the initial stage, but also later in the the growing season, large differences were seen in plant development when comparing climatic regions. The initial height of the main inflorescence seemed to be a good indicator for the final plant height with an average multiplication factor of 1,57 (Figure II). This resulted in a much larger plant height and a higher biomass-production in Austria and The Netherlands than in Portugal, Spain, Greece or Iceland. Especially in Austria, plants were able to grow to a considerable height with a maximum of 207 cm for the Branco accession.

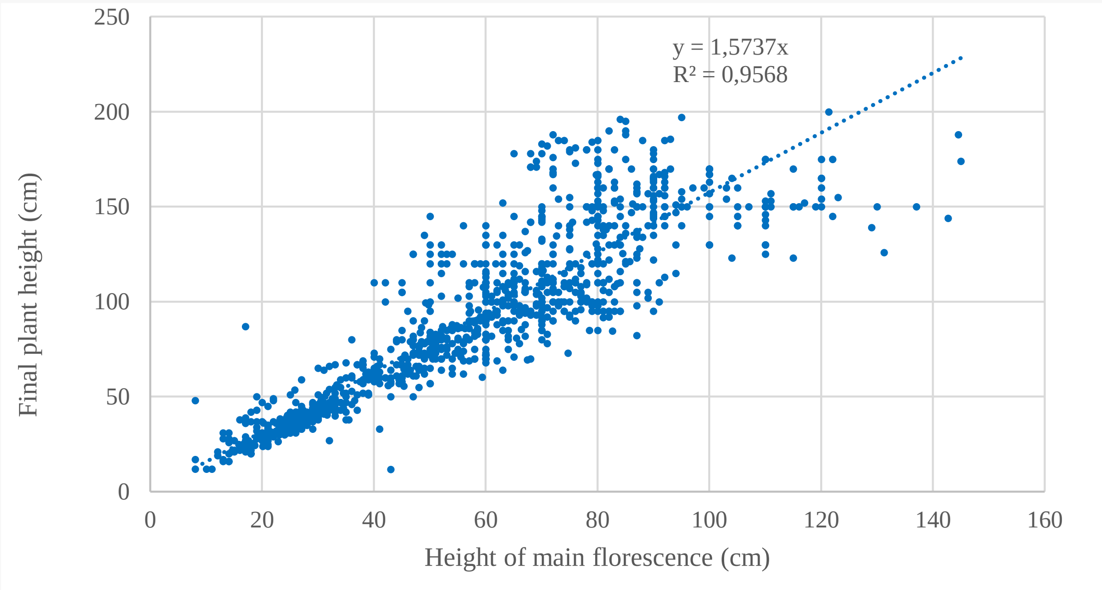


Figure II. The correlation between the main florescence height and the overall plant height.

This bigger plant height in Austria and The Netherlands compared to Portugal, Spain and Greece also resulted in higher biomass production (see table I). The highest biomass production was 24 tonnes of dry matter ha^{-1} in Austria on a eutric cambisol in Lambach and was produced by the vigorously growing accession Branco. In The Netherlands, maximum biomass production was estimated about 20 tonnes of dry matter ha^{-1} in accession LIB220, which in the Netherlands was the tallest accession. Although Branco showed the same kind of vigour as in Austria, the main stem was not able to uphold this biomass and fully lodged in all the years tested. For this reason Branco was nog tested in later years in The Netherlands. Although maximum biomass-productions were very promising, the dry matter yields were very variable over the years and locations (Figure III).

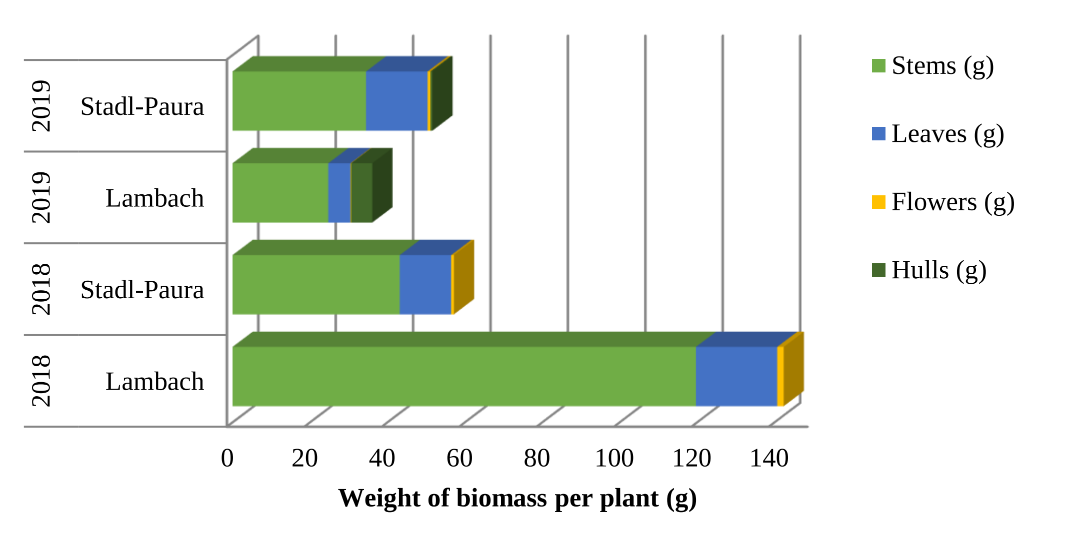


Figure III. Dry matter biomass distribution in Austria during 2 cropping season on 2 locations for accession Branco.

Furthermore, this high level of production was obtained quite late in the growing season (Figure IV).

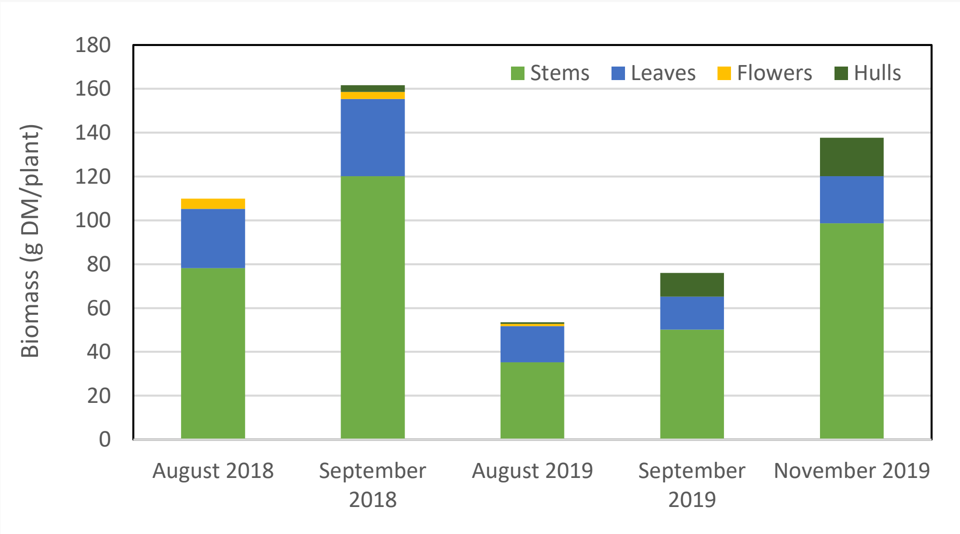


Figure IV. Dry matter biomass production per plant in Trautenfels, Austria in 2018 and 2019.

Most of the accessions tested in Austria and Netherlands kept on growing and flowering in most of the cropping seasons, dry seed yield was low and only reached an average yield of 0,3 tonnes per ha^{-1}. Only one of the early accessions (Cotopaxi) ripened fully and produced dry, harvestable seeds, although the harvest could be as late as October or November. Periods of droughts in late summer induces ripening of Cotopaxi, which results in a harvestable crop in August/September in the Netherlands. In Austria, further crop development was usually stopped by the first nights of frost, usually at the end of October. The best seed yield measured in the field trials in the Netherlands was low (1,3 t ha^{-1}) on an sandy soil, although a relatively high production of 2,7 t ha^{-1} was achieved in a seed propogation field in 2018 which was fully ripened in August and machine harvested.

For the Southern European countries, the situation was quite different. Crop development (main inflorescence height and total crop height) and biomass production was on average a lot lower than in Austria and The Netherlands, as is shown in Table I. This was explained by the fact that the temperature during the early stages of crop development were lower. When temperatures increase substantially in the months following main inflorescence development in March/April, moisture availability starts to decrease. Both high temperatures and decreasing moisture availability induced ripening of the crop, starting from May and continuing to July when the crop was fully ripened.

The semi-determinate ripening does not seem to be a matter of drought and forced ripening of the plants. In an irrigated field trial in Portugal in 2020, plants did still ripen in July, even though shortages in rainfall were compensated by irrigation. Ripening was also not just induced by high temperatures either, as similar temperatures experienced in Portugal, Spain and Greece in May and June did occur during the growing season in Austria as well in June, July and August without inducing ripening of the crop. It therefore seems to be a combination of physiological age (at least some pods need to be formed) and high temperatures/low air humidity that cause full ripening in the Southern European countries.

Even though the climatic conditions in Southern Europe seem to be more favourable for ripening and dry seed harvesting, seed production measured in the years of testing demonstrated large variation between years and locations. Low to very low yields that hardly passed 1 t ha^{-1} in Greece on high calcerous soils were contrasted by seed yields in Portugal of 1,4 to 2,9 t ha^{-1} in a non-irrigated field trial in 2019 and 3,4-6,7 tonnes ha^{-1} in an irrigated field trial in 2020. Two factors that seem to influence the yields are air temperatures/air humidity during flowering and the availability of soil moisture during pod-setting and pod-filling. During flowering, Andean lupins show a lot of flower abortion when temperatures exceed 30°C and air humidity is at or below 50%. But even if some flower abortion occurred during the flowering stage, enough soil moisture is still needed during the pod-setting and pod-filling stage to ensure fully developed seeds. The much lower yields in Greece as compared to Portugal could therefore be explained partly by the higher occurrence of high temperatures during flowering and lower soil moisture availability in May and June during pod-filling in combination with the high calcium content of Greece soils in the LIBBIO experimental fields.

The adaptability of Andean lupin in relation to different climatic conditions in Europe seems limited in the accessions tested in the field trials. Crop development was slowed down when daily mean temperatures were below 10°C, whereas seed production was highly affected when temperatures surpassed 30°C and when rainfall starts to become scarce. The limited adaptability might be explained by the average weather conditions in the highlands of Bolivia, Peru and Ecuador where the Andean lupin originates. During the whole year, average temperatures in this region do not vary much, with night-time temperatures between 10-15 °C and daytime-temperatures between 25-30°C during the whole growing season.

B. Comparing crop development of different lines and accessions

Focussing on a limited number of lines

At the start of the LIBBIO project (2016-2017) we included 21 lines and accessions to be tested in field trials in WP2. These included 4 plant selections from Van Dinter Semo, 14 accessions and 2 varieties from the gene bank in Portugal and one landrace from Latin America (Branco). Most of this material however was only tested during the first growing season. After this initial growing season it was decided to concentrate the field testing on three of the four plant selections from Van Dinter Semo (LIB220, LIB221-now Cotopaxi-, LIB222) and the landrace Branco. The main reason for this focus was due to the fact that none of the material we tested in 2016-2017 were commercial varieties that were available on the market. This made it necessary for the project to organise their own propagation. As propagation of all these accessions and lines was not considered feasible, we had to narrow down the number of accessions and lines to be used in the field trials in the following seasons. Most of the accessions and varieties coming from the gene bank in Portugal showed a high level of heterogeneity in various plant characteristics making it difficult to assess this material agronomically. We therefor decided to concentrate the efforts of the following years on the three more homogenous plant selections in comparison to the landrace Branco.

Flower colours and homogeneity

Andean lupin shows large variation in flower and seed colours from which its name "mutabilis" originated. Flowers tend to change colours after fertilisation, increasing the range of possible colour combinations. To simplify the characterisation of the different accessions available in this project, we distinguished between at least 4 main types of flowers as seen in Figure V. In the first year of testing (2016-2017), it was observed that many gene-bank accessions from ISA-Lisbon showed were heterogeneous for flower colour, whereas the accessions from Van Dinter Semo (LIB220, Cotopaxi and LIB222) were much more homogenous (see Table II).

Type I
Young flower
Heart: yellow
Banner centre: white
Banner edge: blue
Wings: blue
Mature flower
Heart: brownish
Banner and sword: blue

Type II
Young flower
Heart: yellow
Banner centre: white
Banner edge: white
Wings: white
Mature flower
Heart: brownish
Banner and sword: blue

Type III
Young flower
Heart: yellow
Banner centre: blue/purple
Banner edge: blue
Wings: blue
Mature flower
Heart: brownish
Banner and sword: blue

Type IV
Young flower
Heart: yellow
Banner centre: white/pink
Banner edge: pink
Wings: pink
Mature flower
Heart: dark pink/purple
Banner and sword: pink/purple

Figure V. The 4 main flower types distinguished during the LIBBIO project.

Table II. Relative frequency of flower types of the accessions in season 2016-2017 in Kalamata (Greece).

	Type I	**Type II**	**Type III**	**Type IV**	**L.albus/ L.angustifolius**
Accessions	%	%	%	%	%
LIB220		100			
LIB 221 (Cotopaxi)	100				
LIB222				100	
Branco	100				
white lupin					100
blue lupin					100

Height development

There were distinct differences in the height development of the Andean lupin due to the climatic conditions in which they were grown. Apart from the climatic variation, large differences were observed between the main accessions tested. Although differences in height were seen in different countries, the ranking of height development between the different accessions was fairly constant with Branco producing the tallest main stems, followed by LIB220 and then Cotopaxi and LIB222 producing the shortest main inflorescence.

Table III. Main inflorescence height (cm) of the four accessions tested in the different countries in Europe from 2016-2019.

		LIB220	**Cotopaxi LIB221**	**LIB222**	**Branco**	**White lupin**
Spring sowing	***NL/AT***	70,9	52,6	56,1	91,3	55,5
	IS	37,1	27,9	31,5	57,1	NA
Autumn sowing	***PT/ES/GR***	38,9	34,8	32,0	48,5	33,0

When comparing the different accessions of Andean lupin with white lupin, most of the Andean lupin tends to overgrow white lupin, however Cotopaxi has a height development very similar to white lupin.

Table IV. Average plant height (cm) at the end of the growing season (2017-2019).

	LIB220	Cotopaxi LIB221	LIB222	Branco	white lupin
Austria	114,6	82,0	91,1	161,5	91,5
The Netherlands	140,6	93,7	117,6	137,6	72,0

Earliness of flowering and ripening

During the cropping season, the number of days from sowing until flowering and ripening of pods in the main inflorescence was measured for the different accessions of Andean lupin (Table V).

Table V. Average number of days after sowing before 50% of the plants have open flowers in the main inflorescence.

		LIB220	Cotopaxi LIB221	LIB222	Branco	white lupin
Spring sowing	***NL/AT***	69,6	53,7	67,1	99,2	61,4
	IS	121,7	99,9	113,3	NA	NA
Autumn sowing	***PT/ES/GR***	119,3	103,7	118,2	118,5	119,5

As can be seen in other lupin species as well, there seems to be a correlation between shorter plants and early flowering. This seems to be quite clear when looking at the four accessions tested over the whole period. However, the differences in duration of flowering were a lot larger in the spring-sown crops with 45 days between the earliest (Cotopaxi) and latest (Branco), whereas in the autumn-sown crops this difference was only 15 days. When comparing Andean lupin with white lupin in Austria and The Netherlands, the earliest accessions of Andean lupin tend to take an equal amount of time until flowering than the variety of white lupin which would otherwise be used (“Feodora” in The Netherlands and “Nelly” in Austria). In the Southern European countries Cotopaxi and LIB222 are even earlier in flowering than the commercial variety of white lupin (“Multitalia” or “Misak”) that are used locally. This difference in performance of Andean lupin compared to white lupin is not due to a difference in behaviour of Andean lupin, but due to a difference in performance of the varieties of white lupin used locally. Due to breeding efforts, white lupin varieties used in Northern Europe flower and ripen much earlier than varieties used in Southern Europe (see also table VI).

Table VI. Average number of days after sowing until all pods in the main florescence are fully ripened.

		LIB220	Cotopaxi LIB221	LIB222	Branco	white lupin
Spring sowing	NL/AT	138,3	124,1	140,2	151,3[1]	141,2
Autumn sowing	PT/ES/GR	160,8	150,8	159,2	178,2	181,4

[1] Data for Branco in spring sowing only based on Austrian data, as Branco does not produce ripened pods in The Netherlands

The differences in earliness of flowering result in earliness in ripening of the pods on the main inflorescence as well (table VI), although the pod-filling and ripening stage takes much less time in the autumn sown crops than in the spring sown crops (table VII).

Table VII. Number of days between 50% flowering and ripening in the main inflorescence.

		Cotopaxi LIB220	LIB221	LIB222	Branco	white lupin
Spring sowing	NL/AT	68,7	70,4	73,1	52,1[1]	79,8
Autumn sowing	PT/ES/GR	41,5	47,1	41	59,7	61,9

[1] Data for Branco in spring sowing only based on Austrian data, as Branco does not produce ripened pods in The Netherlands

Lodging and pod-shattering
In the field trials, differences were observed in two important agronomic traits for yield stability and cropping risk which are 1) lodging, which can lower the efficiency during harvesting to retrieve the seeds from the field and 2) pod-shattering, which can cause substantial loss of yield in the late stages of crop development (Table VIII).

Table VIII. Susceptibility to pod shattering of the accessions sown in two trial fields in Greece in the 2016-2017 cropping season.

Accessions	Kalamata				Athens			
	No %	Slight %	Medium %	Intense %	No %	Slight %	Medium %	Intense %
LIB220	70,6	11,8	11,8	5,9	59,1	13,7	13,6	13,6
Cotopaxi	65,4	23,1	3,9	7,7	-	-	-	-
LIB222	95,5	4,6			90,0	5,0	5,0	
Branco	84,1	11,4	2,3	2,3	73,9	15,0	7,8	3,3
White lupin	*98,0*	*2,0*			*74,1*	*18,5*	*5,5*	*1,9*
Blue lupin	*80,0*	*8,6*	*5,7*	*5,7*	*52,0*	*12,0*	*4,0*	*32,0*

In a comparison of tendency to pod-shattering in field trials performed in Greece in 2016-2017, large differences were seen among accessions of Andean lupin, but also between two different locations (Table IX).

Table IX. Susceptibility to lodging of the accessions sown on two trial fields in Greece in the cropping season 2016-2017.

Accessions	Kalamata			Athens		
	Light %	Medium %	High %	Light %	Medium %	High %
LIB220	62,8	23,3	13,9	45,0	30,0	25,0
Cotopaxi	2,10	31,2	66,7	-	-	-
LIB222	68,4	28,3	3,3	25,0	45,0	30,0
Branco	40,0	45,0	15,0	10,0	80,0	10,0
White lupin	96,7	1,70	1,6	40,0	55,0	5,0
Blue lupin	51,7	21,7	26,6	85,0	15,0	

The same accessions that were scored for susceptibility to pod-shattering in Greece, were also scored for their susceptibility to lodging in the first cropping season. Cotopaxi was scored as highly susceptible to lodging whereas LIB220 and LIB222 had relatively good resistance to lodging. As the occurrence of lodging was also scored in other countries and in other seasons, more data became available. Both the percentage of plants per plot being lodged and the angle that the plant was hanging (0° being fully erect and 90° being flat to the ground) were scored. The multiplication of these two gives the lodging index with an outcome of 0 for a fully erect crop in the whole plot and 90 for a completely lodged crop (Table X).

Table X. Average lodging index for cropping season 2 and 3.

		LIB220	Cotopaxi LIB221	LIB222	Branco	white lupin
Spring sowing	NL/AT	24,7	31,8	17,8	27,8	0,29
Autumn sowing	PT/ES/GR	6,1	13,7	3,8	7,9	5,1

As expected, lodging occurred much more frequently in the spring sown crops as they grow taller than the autumn sown crops. Cotopaxi was identified as the accession, which is prone to lodging, whereas LIB222 is the least prone to lodge. This shows that the risk of lodging is not just related to crop height, as Cotopaxi is at the same time the smallest line tested. Branco and LIB220 still showed high occurrence of lodging especially in Austria and The Netherlands as these two accessions grew to considerable heights in these countries. When lodging in Andean Lupin was compared to lodging of white lupin, Andean lupin seems in general, more susceptible to lodging than white lupin, especially in Austria and The Netherlands.

Yield potential of the different lines

One of the most relevant agronomic traits for farmers is the yield potential of the different lines and accessions tested. On this matter however we cannot give very definitive results yet. What was concluded earlier is that with the lines and accessions now available, dry seed production is mainly restricted to the southern part of Europe where the crop can be sown in autumn. Provided there is enough soil moisture available in large parts of the months April, May and June, yields can be quite substantial as was demonstrated in field trials performed in Portugal. In the last two years yields of 2-3 t/ha were measured on a sandy loam soil, located in a flood plain. In an irrigated field trial in the season 2019-2020 even higher productions were measured (6.7 t/ha of LIB221/Cotopaxi) although the trial set up consisted of relative small plots, which generally makes the yield estimate unreliable. The majority of the field trials performed in both Portugal and Spain and Greece did not result in such high yields however, so a lot still needs to be learned on how to optimize the cultivation of *L. mutabilis* in Europe.

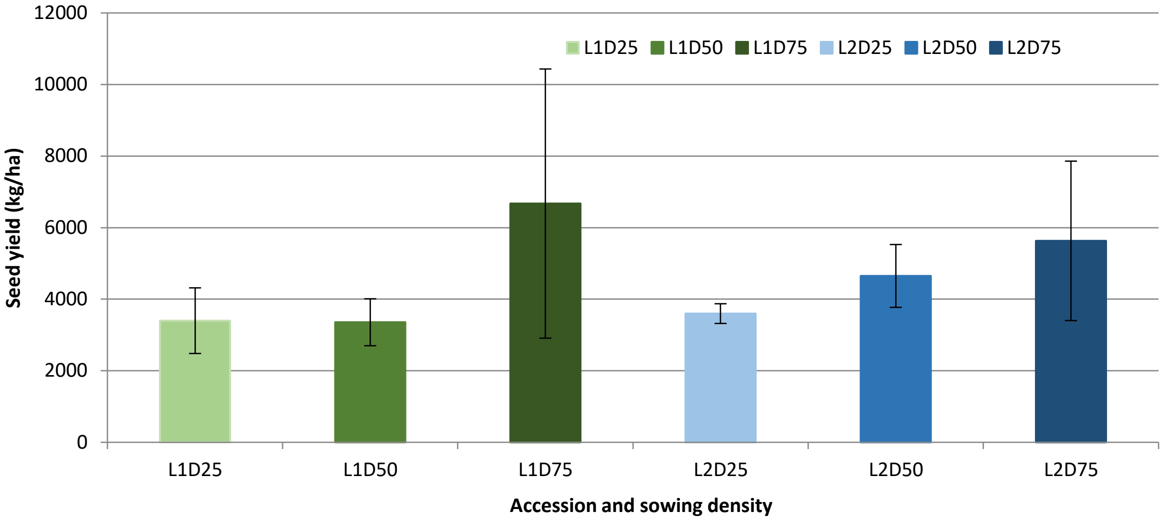


Figure VII. Dry seed yield (kg/ha) of two breeding lines of L. Mutabilis in an irrigated field trial in Santarem in three sowing densities in Portugal in the growing season 2019-2020

As for the biomass production, it seems clear that high biomass production is mainly possible in the northern parts of Europe. Again, very high dry matter yields were obtained in both The Netherlands and Austria of up to 20 t DM/ha by using the tallest lines available (Branco and LIB220). But again, the variation in yields obtained was very high and the majority of the results did not even come close to these yields. Also, in the area of biomass production a lot still needs to be learned.

C. The influence of different soil types on crop development

Andean lupin accessions were tested within the LIBBIO project on a wide variety of soil types, from very sandy soils to heavy clay soils, low to high soil organic matter, high and low pH and high and low $CaCO_3$ levels.

Table XI. List of all the soil types of the field trials in the different countries used within LIBBIO (2016-2020).

		Clay	Silt %	Sand	pH	OM %	CaCO3 %	P-AL mg P/100 g
Iceland								
Eroded volcanic wasteland	Vitrisol	<1	7	93	6,5	<1	<0,2	0,6
Organic reclaimed land	Brown Andosol	2	21	77	5,6	1-12	<0,2	7,5
The Netherlands								
Poor, dry sandy soil	Dystric Regosol	1	7	90	4,0	3,5	<0,2	32
Rich, sandy soil in flood plain	Gleyic Podzol	4	11	83	5,9	3,6	<0,2	55
Rich, young sea clay soil	Calcaric-gleyic Fluvisol	18	51	22	7,4	2,3	6,4	52
Austria								
Well-developed soil on lower slopes	Cambisol	13	35	49	6,0	2,6	<0,2	28
Shallow, poorly developed calcaric soil	Calcaric Skeletic Regosol	22	25	38	7,4	4,5	10,1	112
Low and wet soil in flood plain	Cambisol	10	60	19	7,1	6,2	4,8	26
Romania								

Slightly calcaric clayloam	Chernozem	30	52	13	7,3	3,7	2	92
Slightly acidic clayloam	Phaeozem	33	53	11	5,7	3,7	<0,2	38
Portugal								
Shallow, stoney, sandy soil	Chromic cambisol	7	0	93	7,4	1,3	<0,2	
Sandy loam in flood plain	Fluvisol	Sandy loam			6,3	1,2	<0,2	
Spain								
Sandy loam	Haplic Calcisol	18	33	30	7,5	1,9	17,3	122
Loam	Haplic Calcisol	20	46	23	7,3	2,0	9,5	144
Greece								
Calcaric sandy clay loam flood plain soil	Calcaric regosol	26	15	30	7,9	4,0	25,1	73
Calcaric sandy loam flood plain soil	Calcaric fluvisol	16	31	28	7,6	2,8	22,5	18
Calcaric sandy loam	Calcaric fluvisol	10	39	28	7,4	7,6	15,5	185
Slightly calcaric, shallow clay soil	Calcaric cambisol	51	25	21	7,4	2,9	1,2	4

Soil texture and soil fertility

The variation in soil texture, soil fertility and alkalinity was large. However, distinct soil types under the same climatic conditions were only found in a small number of the countries involved, making it difficult to reach generic conclusions on the influence of soil texture and fertility on crop development in Andean lupin. The cases described here are therefor at best an indication of the influence of soil characteristics.

Soil fertility

In Iceland, trials took place on two different volcanic soils in the south. The first field being heavily eroded, with hardly any soil development, is a sparsely vegetated sandy desert with basaltic parent material and high levels of volcanic glass resulting in a soil with very low organic matter and low levels of nitrogen or phosphorus. The second field was an agricultural field, where organic matter is considerably higher as was the availability of nitrogen and phosphorus. Although another lupin species, the Nootka lupin (*L. nootkatensis*) known to thrive very well on the eroded soils in Iceland, Andean lupin reacted quite strongly to the reduced availability of minerals on the eroded site. Plant height was reduced by almost 50% compared to the plant heights on the organic site (Table XII). This is not surprising as it is known that plant metabolites are needed for both nitrogen fixation (sugars for the *Bradyrhizobium* sp. soil bacteria) and the mobilisation of phosphorus (root exudates), making them unavailable for plant growth. Despite this reduced height however, plants did not show signs of mineral shortages and produced flowers more or less at the same time as the plants on the organic site in the first growing season (Table XIII).

Table XII. Differences in plant height development of four accessions of Andean lupin between an eroded volcanic soil and a fertile agricultural soil in Iceland (2017).

Accession	**Plant height** (cm)		**Factor**	**Significant difference**
	Eroded site	**Organic field**		
Branco	51,7	85,1	1,6	p<0,001
LIB220	38,8	73,4	1,9	p<0,001
Cotopaxi	25,5	48,8	1,9	p<0,001
LIB222	30,1	56,0	1,9	p<0,001
Average	35,9	65,5	1,8	

Table XIII. Differences in flowering time of four accessions of Andean lupin between an eroded volcanic soil and a fertile agricultural soil in Iceland (2017).

Accession	50% flowering: Eroded site (days after sowing)	50% flowering: Organic field (days after sowing)	Factor	Significant difference
Branco	n/a	n/a	n/a	not tested
LIB220	125,0	118,3	0,95	p<0,036
Cotopaxi	100,7	99,0	0,98	NS
LIB222	113,5	113,0	1,00	NS
Average	114,6	111,3	0,97	

The same effect was seen in The Netherlands where crop development was observed on a fertile, low lying sandy soil in a flood plain compared to a drought prone, poor sandy soil on an ancient sand dune (Table XIV).

Table XIV. Differences in plant height and flowering time of three accessions of Andean lupin grown in a dry poor sandy soil and a moist, fertile sandy soil in The Netherlands in cropping season 3.

Accession	Main florescence height: Poor sand (cm)	Main florescence height: Rich sand (cm)	Main florescence height: Factor	Flowering time: Poor sand (days after sowing)	Flowering time: Rich sand (days after sowing)	Flowering time: Factor
LIB220	57,5	91,8	1,6	83	66	0,80
Cotopaxi	57,9	70,1	1,2	68	62	0,91
LIB222	66,2	80,3	1,2	69	67	0,97

Soil texture

As for the influence of the soil texture on plant development, it seems that Andean lupins tend to grow taller on sandy soils when compared to clay or loamy soils. In The Netherlands, the crop development on fertile, young sea clay was compared during several cropping seasons with fertile sandy soils. As can be seen in table XV, plants of different accessions tend to grow 50% taller on sandy soils than on clay soils. What makes it hard to distinguish, however, is the fact that clay soils in The Netherlands also tend to be high in calcium and pH, whereas sandy soils tend to be non-calcareous and with a much lower pH. The main question therefore is if the differences in plant growth are caused by the soil texture or the calcium.

Table XV. Differences in plant height development and flowering time of three accessions of Andean lupin between a fertile young sea clay soil and a fertile sandy soil in The Netherlands (cropping season 2017 and 2018).

Cropping season	Accession	Main florescence height: Clay *(alkaline)* (cm)	Main florescence height: Sand *(acidic)* (cm)	Main florescence height: Factor	Flowering time: Clay *(alkaline)* (days after sowing)	Flowering time: Sand *(acidic)* (days after sowing)	Flowering time: Factor
2018	**LIB220**	70,6	103,3	1,5	60	63	1,05

	Cotopaxi	51,8	77,9	1,5	52	46	0,90
	LIB222	57,3	82,2	1,4	58	63	1,09
2019	**LIB220**	54,8	91,8	1,7	68	66	0,97
	Cotopaxi	54,8	70,1	1,3	61	62	1,02
	Average	57,9	85,1	1,5	60	60	1,01

Calcium and pH

Lupins are generally known for their intolerance to calcareous and high pH soil conditions. Especially blue lupin and yellow lupin (*Lupinus luteus*), that both react strongly to soils with a pH higher than 6,8 and free calcium levels exceeding 0,8%. At the same time, it is known that within the *Lupinus* genus, there are species that have adapted to high pH conditions like *L. pilosus*, *L. atlanticus* and *L. cosentinii* (Tang et al., 1995; Bebeli et al., 2020). It is also known that for white lupin there is a high variation of calcium tolerance among the genetic material collected in the Mediterranean area, with some material being able to grow well on very alkaline soils with pH reaching up to 8,3 and free calcium levels up to 27% (Raza et al., 2000). For this reason, it was particularly interesting to see how much calcium tolerance could be found in the different accessions of Andean lupin tested within this project. As can be seen from Table XII, field trials were performed on a wide variety of soils throughout Europe with the most calcareous soils in Greece and Spain (15-25% of $CaCO_3$). It was therefore decided to score different accessions grown on highly calcareous soils in Greece on chlorosis, plant vigour and chlorophyll content in the leaves (Table XVI, Table XVIII). The aim was to compare this with measurements on another location in Greece with low levels of calcium, but due to weather conditions, this last part was not possible.

Table XVI. Plant vigour, chlorosis and chlorophyll content in the leaves of four accessions of Andean lupin compared to two varieties of white lupin and one variety of blue lupin on high calcareous soils in Greece (2017-2018).

Accession	**Vigour** *(1-9)**	**Chlorosis** *(0-5)***	**Chlorophyll** *(mg/g FW)*
LIB220	4,7	1,4	23
Cotopaxi	4,8	0,8	25
LIB222	3,9	1,1	22
Branco	5,1	0,5	28
white lupin	*4,8*	*0*,4	47
blue lupin	5,2	0,6	25

*** 1: retarded plant, 9: very vigorous plant**
****0: no chlorotic symptoms, 5: severe chlorosis**

There was no severe chlorosis (scores 4-5) observed in the highly calcareous fields of Greece, even in narrow-leafed lupin which is quite sensitive to soil lime. This may have been caused by the relatively dry conditions in Greece, as signs of calcium intolerance tend to increase under wet and cold growing conditions. Also, the lower levels of chlorosis in Table XVI than in Table XVIII are attributed to the fact that plants measured for chlorosis in the first table were pre-germinated, planted in small pots with peat-perlite solution (0% calcium carbonate) and then transplanted to soil in the field. Chlorotic symptoms in lupins due to calcium carbonate induced Fe deficiency, are usually observed in the initial growth stages, hence with transplantation these symptoms were inhibited.

In a field at Athens with very calcareous soil (37,3%), both Andean lupin and white lupin plantlets presented chlorotic symptoms when they were directly sown in the soil. However, at flowering stage, chlorosis symptoms gradually disappeared. The above-ground biomass dry weight obtained from both species was significantly higher in Andean lupin than white lupin with 10,6 and 7,0 g per plant respectively at Athens location. In Kalamata in a field with lower soil $CaCO_3$ (24,8 %) but still high, the respective yields were 8,9 and 7,7 g per plant but not significantly different.

Higher levels of chlorosis were observed in direct planting on the field than transplantation, however vigour was not affected (Table XVII). Chlorosis levels of Andean lupin ranged from the level observed in white lupin up to those of blue lupin but only for the first experiment. The results obtained could not be directly compared among lupin species as white lupin leaves are greener than those of blue lupin and Andean lupin and leaf color is found to be controlled genetically from genes such as aureus or olivaceus in narrowleaf lupin (Kurlovich, 2002). Bebeli et al. 2020, mention that blue lupin is less tolerant to $CaCO_3$ than white lupin and Andean lupin present similar sensitivity. However, by measuring chlorosis levels and vigour, different results were obtained in Greece. Chlorosis is a symptom of either Fe, Mg or nitrogen deficiency (Zohlen et al. 2006).

Table XVII. Chlorosis, vigour and plant height of several *Lupinus* spp. under March or November sowing. Chlorosis was measured at six levels from 0 to 5 (non-chlorotic to very chlorotic), vigour was measured from 1 to 9 (low to high vigour). A deviation from normality was observed, so a non-parametric Kruskal-Wallis test was used with the same results.

Location		**South Greece (Kalamata)**			
Sowing season		**January 2018**		**November 2018**	
Species	**Accession**	**Chlorosis**	**Vigour**	**Chlorosis**	**Vigour**
white lupin	cv Multitalia	0,00a	4,14ab	0,79a	4,22c
	LIB224	-	-	0,92a	4,61c
blue lupin	cv Polo	2,77d	4,33abc	3,83e	3,97c
Andean lupin	LIB220	2,07d	4,07a	3,71e	3,03b
	Cotopaxi	2,00cd	4,50abc	3,43ce	2,67ab
	LIB222	2,00cd	4,60abc	3,40bce	1,93a
	Branco	1,75bcd	5,50c	3,09bc	4,50c
ANOVA (p-value)		0,0000	0,0000	0,0000	0,0000
Kruskal-Wallis (p-value)		0,0000	0,0000	0,0000	0,0000

As to the soil moisture effect on chlorotic symptoms crop performance on calcaric soils in The Netherlands and Austria can be considered. Again however, the more moist conditions in Northern Europe coincided with lower levels of free calcium as compared to Greece (5-10% instead of 20-30%). No severe chlorosis was observed in The Netherlands or Austria. Between the different accessions there were however some differences observed in the chlorotic syptoms. Especially LIB220 and LIB222 showed elevated levels of chlorosis, and especially LIB222 a reduced plant vigour, whereas Cotopaxi and LIB223 only showed mild chlorosis.

Although severe chlorosis was not observed in Greece or any other country where Andean lupin accessions were grown on high pH calcium rich soils, some of the differences in plant growth that were already shown in Table XVI could be attributed to calcium instead of the clay soil texture. Although chlorosis was not extensively scored in The Netherlands it was observed that Andean lupin plants grown on calcareous young sea clays in The Netherlands were lighter in colour compared to the same accessions on acidic sandy soils.

Table XVIII. Differences in plant height development and flowering time of four accessions of Andean lupin between a shallow, poorly developed alkaline soil and a well-developed acidic soil in Lambach, Austria (2018 and 2019).

		Main florescence height			Flowering time		
		Alkaline (Shallow)	**Acidic (Deep)**	**Factor**	**Alkaline (Shallow)**	**Acidic (Deep)**	**Factor**
Year	**Accession**	*cm*			*days after sowing*		
2018	**LIB220**	57,4	83,0	1,4	95	78	0,83
	Cotopaxi	36,4	62,4	1,7	83	75	0,91
	LIB222	38,0	67,1	1,8	94	76	0,81
	Branco	106,0	126,5	1,2	95	89	0,93
2019	**LIB220**	59,5	85,7	1,4	74	72	0,97
	Cotopaxi	30,0	50,9	1,7	74	52	0,70
	LIB222	46,7	54,3	1,2	73	57	0,79
	Branco	77,1	81,3	1,1	73	57	0,79
	Average	56,4	76,4	1,4	82	70	0,84

Differences in growth were also observed between alkaline and a slightly acid soil in Lambach Austria (Table XVIII) in 2018 and 2019. Like in The Netherlands however, the acidity of the soil was not the only variable that could explain the difference between these two sites. The alkaline soil was a shallow, poorly developed soil at the bottom of a mountain slope, whereas the slightly acidic soil was a much better developed agricultural soil in a higher location on the opposite side of the valley. Still, the apparent calcium tolerant accession Branco, seemed to be the least affected in the Austrian trials which would support the hypothesis that calcium tolerance is at least part of the explanation for the reduced growth. Although there is an indication that higher calcium levels in the soil do affect plant development in Andean lupin, they are still able to develop to quite impressive plant heights and produce large amounts of biomass.

D. Abiotic stress

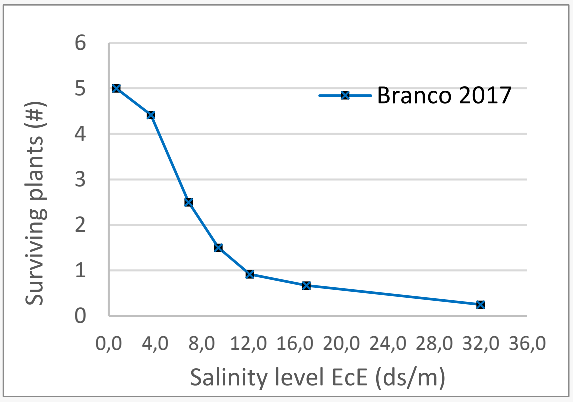
Salinity

Salinization, or the accumulation of soluble salts, poses a serious threat to agricultural production. High salinity limits the normal metabolism of plants (ESBN, 2005) resulting in poor seed production, reduced biomass accumulation, or even failure to establish at all. Recent global mapping by Ivushkin *et al.* 2019 of salt affected soils has indicated that 1069 Mha were salt affected in 2016. In 1986 this was 915 Mha which is an increase of 16,8% in just 30 years. On irrigated lands, the increase is even more severe. The problem of increased salinity, which used to be a problem mainly occurring in the tropical regions is becoming an increasing issue in Europe as well with 25% of the irrigated area in the European Mediterranean being affected by salinity, especially in Spain (Szabolcs, 1990). The screening of crops for tolerance to salinity therefore is becoming increasingly relevant. Crops are divided by the yield loss in the presence of various salinity levels (Table XIX, derived from Abrol *et al*. 1988).

Table XIX. Soil salinity classes and impact on crops.

Ece (dS/m)	Salinity level	Crop classes
0-2	Non-Saline	Only small effects
2-4	Slightly saline	Sensitive crops are restricted
4-8	Moderately saline	Many crops are restricted
8-16	Strongly saline	Only tolerant crops
>16	Very strongly saline	Only very tolerant crops

For this reason, four accessions of Andean lupin were tested for salt tolerance in a controlled environment. The field trials were executed at the Salt Farm Texel in The Netherlands, which is specialized field trial facility where it is possible to irrigate individual small plots with water of variable salinity. In sandy soil, soil salinity reacts quite quickly to these different regimes resulting in soil salinity levels ranging from EcE 1 dS/m to 35 dS/m. Both crop survival and crop development were followed in two consecutive years: 2017 and 2018 (Figures VIII and IX).



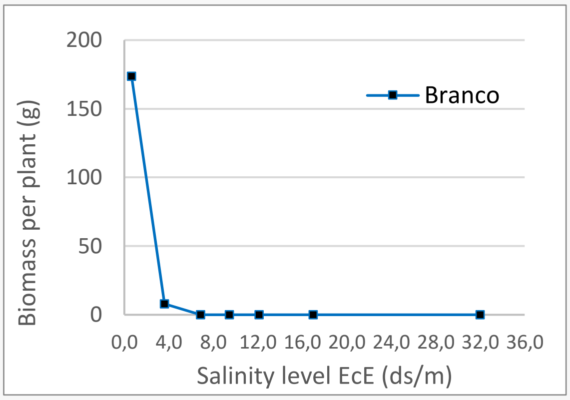
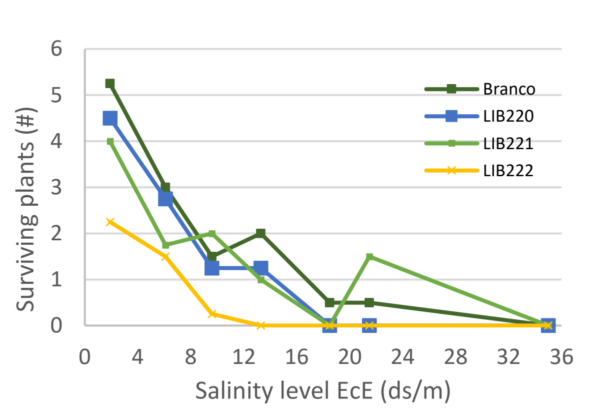


Figure VIIIa and VIIIb. The effect of soil salinity on plant survival (a) and plant biomass (b) in Branco grown at controlled conditions at the Texel Salt farm in The Netherlands in 2017.

In both years of testing, Andean lupin showed little to no salt tolerance. Plant survival dropped quite drastically with salinity levels above the EcE of 4 dS/m, but biomass development reacted even more drastically. In 2017 only Branco was tested (Figure VIII), but LIB220, Cotopaxi and LIB222 showed similar results in 2018 (Figure IX). Of the Andean lupin accessions tested in the field trials, none could be considered salt tolerant.



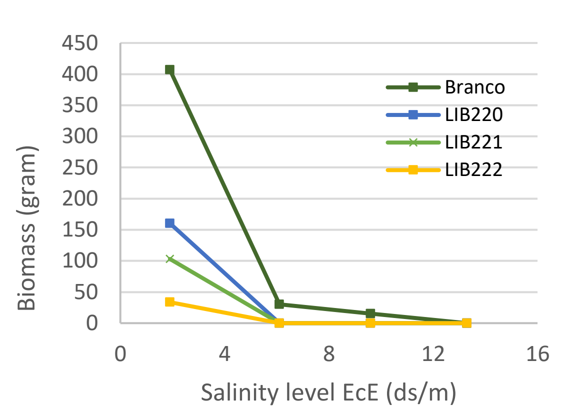


Figure IXa and IXb. The effect of soil salinity on plant survival (a) and plant biomass (b) in 4 Andean lupin accessions grown at controlled conditions at the Texel Salt farm in The Netherlands in 2018. LIB221 is the Cotopaxi accession.

Drought

To observe the tolerance of Andean lupin to drought, several field trials in different countries were designed to gather data. In The Netherlands, one of the field trials was located on an ancient sand dune with shallow anthropogenic soil of 30 cm (SOM 3,5%), below which bare, yellow sand is found. To score drought tolerance, reference crops were sown to see how they reacted to similar growing conditions. Two very dry summers in 2018 and 2019 made it possible to observe the plants reaction to drought, however, the drought in 2018 was so severe that none of the crops survived, and in 2019, deer ate most of the reference crops. The only indication that could be given here was that faba bean, which is known for its low drought-tolerance, seemed to be withering earlier in the growing season of 2018 than white lupin and Andean lupin.

In Romania, an irrigation trial was sown to see what differences in crop development could be seen under irrigated versus non-irrigated circumstances. It was observed that water availability played a major role already during the crop establishment phase (much better germination in the irrigated fields) and in the early crop development, the field trial was then troubled by a heavy infestation of anthracnose (*Colletotrichum lupini*), hindering further crop development.

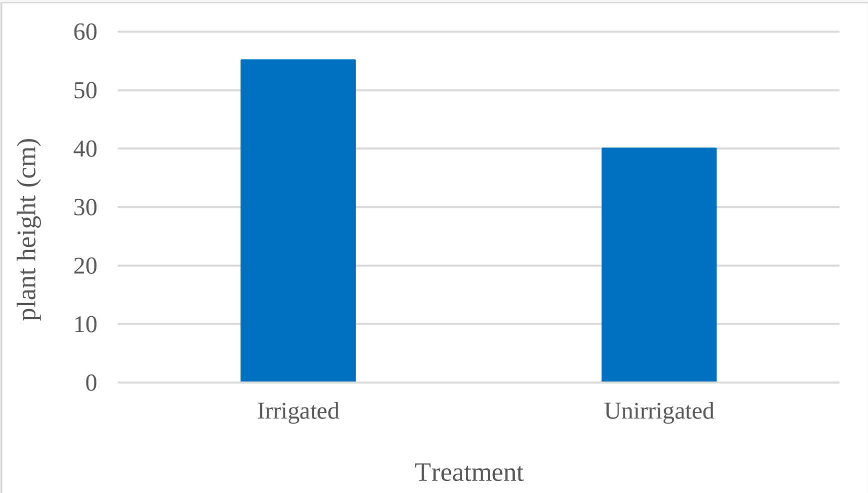


Figure X. The influence of irrigation on plant height development of Andean lupin (LIB220) on a slightly acidic clay loam soil in Romania in 2019.

It was observed in many countries that Andean lupin tends to react quite drastically to periods of hot and dry weather, by completely aborting all flowers. As hot weather often coincides with lack of moisture availability in the soil, it was debated several times within the project, whether it is either drought stress or heat stress that caused this flower abortion. As flower abortion was still observed during hot and dry periods in fields that were irrigated, it was concluded that the high temperatures combined with low air humidity, which nearly always coincides, was the cause of flower abortion rather than the lack of soil moisture.

In the Mediterranean countries, where drought is a common phenomenon in the months of June and July, further crop development after flower production can be cut short by a lack of soil moisture, restricting pod-filling and thereby dry seed production. In Portugal, large differences were observed in seed yield between a non-irrigated field trial in 2018-2019 and an irrigated field trial in 2019-2020 (see Figure IX). As the trials were not performed in the same year, the outcomes of this field trial are only indicative of the importance of water availability during the reproductive stage of crop development.

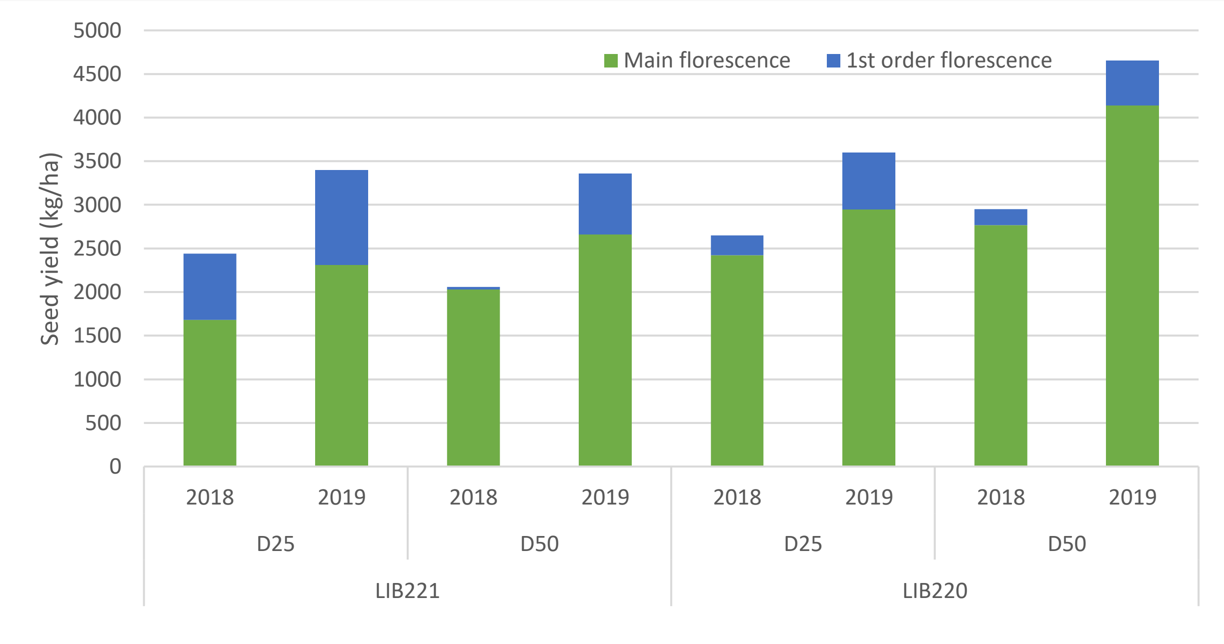


Figure XI. Seed yield of two accessions of Andean lupin in Santarém (Portugal) in two sowing densities (25 and 50 plants/m^2) without irrigation (2018-2019) and with irrigation (2019-2020).

Trials in a controlled environment would be necessary to provide more definitive data on the tolerance to drought of Andean lupin. In the field trials performed during this project, it seems however that Andean lupin needs good moisture availability during emergence, as is usually the case for big seeded crops like other lupin species and faba beans. Drought seems to hinder early crop development as well, although the effects seem to be less drastic than in faba beans. Flower abortion seems to be more an effect of high temperatures and low air humidity than a lack of water, but pod-filling does seem to be affected by the availability of water.

Frost

Andean lupin is known for its low tolerance to frost, making early sowing difficult, especially in Northern Europe. To investigate crop sensitivity and whether genetic variation is available, different accessions were used in a trial performed in Romania, using climate chambers to provide a controlled environment. A controlled environment was necessary to investigate the sensitivity to frost in sufficient detail to determine differences among accessions. Plants from LIB220, Cotopaxi and LIB222 were nursed under optimal growing conditions (15°C), hardened at 5°C for 24 hours and exposed to frost at four different growth stages (Table XX).

Table XX. Description of the four growth stages used for testing frost tolerance in young emerging plants of Andean lupin in climate chambers in Romania.

Growth stage	Description
Phase 1	Cotyledons at breaking soil surface phase
Phase 2	Cotyledons fully above the soil
Phase 3	First leaves visible and erect but folded
Phase 4	First and second leaves have unfolded

The young plants were then subjected to sub-zero temperatures ranging from -2°C to -10°C. After 8 hours of exposure the plants were kept at 15°C for 24 hours and scored on vitality (0 unaffected plant, 1 dead plant). As a comparison, a local variety of white lupin (Mihai) was exposed to the same treatment.

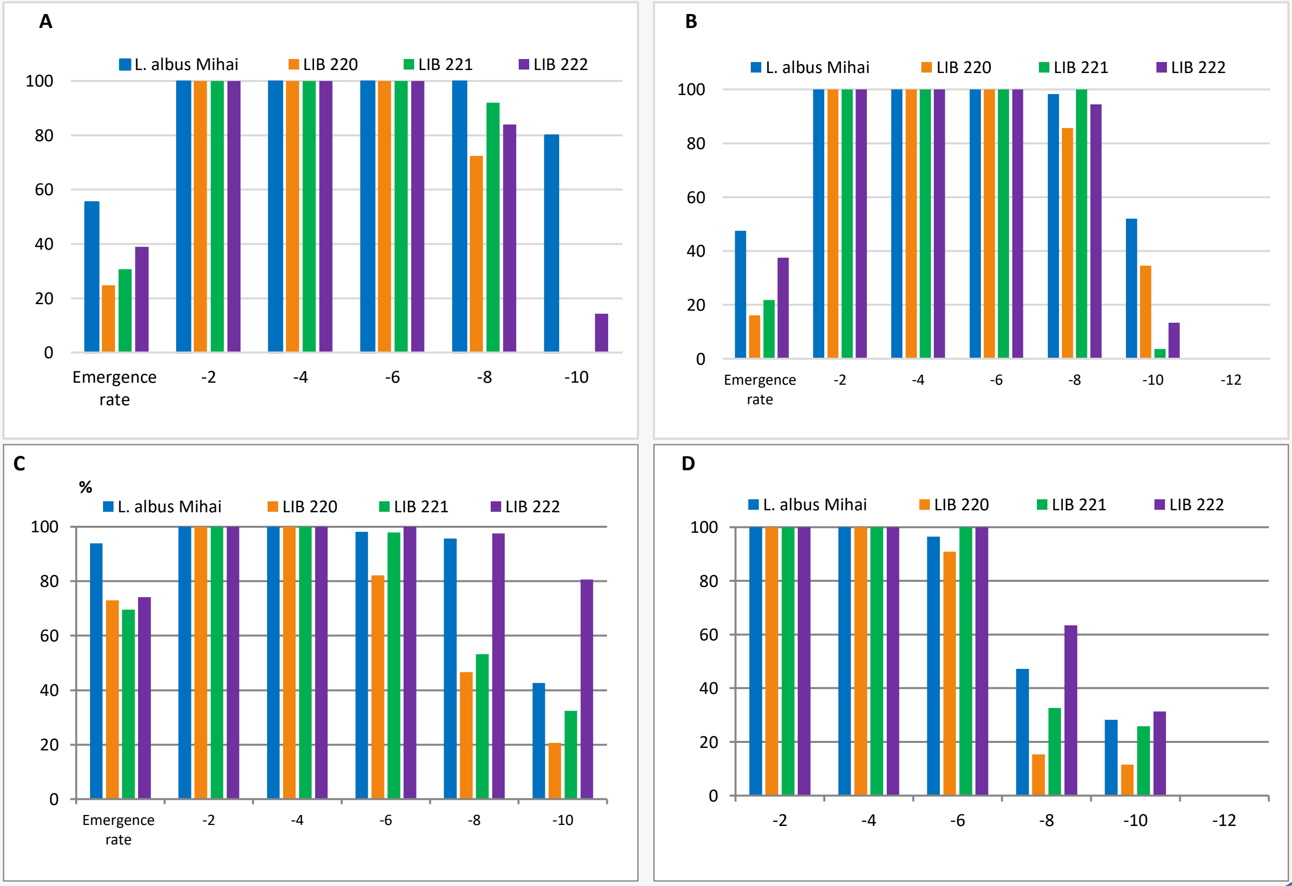


Figure XIII. Effect of severity of frost on the survival of young plants of Andean lupin in different growth stages (stage 1-4, A-D) in a climate chamber in Romania (2020). On x-axis is emergence and temperature in climate chamber, on y-axis is emergence and survival rate. LIB221 is the Cotopaxi accession.

From this trial, no frost damage was observed at temperatures of -4°C or above. Plant damage started to appear at -6°C , increasing both with lower temperatures and with increasing plant phase, although plant survival at -10°C seemed to be less in recently emerged plants (phase 1) than in further developed plants (phase 3 and 4). At nearly all the temperatures and growth stages, LIB222 seemed to be the most frost tolerant and LIB220 being the least frost tolerant. With LIB222 being the purple flowering variety with anthocyanins both in the flowers and in the stem, this could suggest that these anthocyanins play a role in frost tolerance in Andean lupin as previously mentioned in other species (Chalker-Scott, 2002). This could be confirmed by testing more purple flowering accessions. The fact that the white flowering LIB220 seems to be least frost tolerant may provide another clue for the role anthocyanins play in frost tolerance.

The observation of the frost tolerance under controlled conditions is at least partly confirmed by field observations in Portugal and The Netherlands, where a night frost of -2,4°C (January 2019, Portugal) and -3,8°C (November 2020, The Netherlands) caused no damage to young seedlings of Andean lupin. In Greece under night frost conditions (-5°C), LIB220 was observed to be less tolerant than Cotopaxi, LIB222 and Branco with ca. 50% plant loss, when the frost occurred at three leaf stage. However, the parallel effect of other conditions such as high soil moisture and strong winds may have also contributed to this plant loss.

E. Biotic stress

Relevant biotic stress factors affecting Andean lupin were observed in all the field trials performed in the seven countries involved in the work package. Occurrences were registered of both fungal and viral challenges and pests.

Fungal diseases and viruses

1. *Colletotrichum acutatum* spp. *lupin (anthracnose)* The fungal disease anthracnose is a well-known fungal infection that can seriously affect all kinds of lupin species even resulting in near complete crop failure. From the area of origin (Andean highlands) it is known that anthracnose is a serious problem in the cultivation of Andean lupin as well. The detrimental effect of anthracnose on Andean lupin became clear in the third cropping season in Ezareni in Romania (2019). Early infection with anthracnose spread very fast through both white lupin and Andean lupin destroying the entire field trial. Apart from this incident, no serious problems were reported on the other locations. Incidental appearances were reported in Portugal and The Netherlands but were not able to spread through the field, hardly affecting crop performance. In Portugal, the infection was contained through the application of a fungicide, but in The Netherlands the infected plant was simply removed. In Greece, anthracnose was observed in blue lupin (cv. Polo) which did not spread to white lupin or Andean lupin in the same field. For anthracnose to spread actively, high air humidity is needed which coincides with experiences in Portugal of the occurrence of infection directly

following a rainy period at the end of April. A hot and dry period following infection can stop the further spread, but if air humidity due to rainfall stays high it can spread very aggressively, especially if daytime temperatures are higher than 20°C.

2. *Erysiphe martii (Powdery mildew)* is a fungal disease that was observed in nearly all test locations, but especially in the more temperate areas in the second part of the growing season (late summer and autumn). Although especially in The Netherlands and Austria, mildew was observed every season, it did not seem to severely affect plant growth. Powdery mildew appears mainly under moist conditions and lower temperatures but can disappear again when air humidity drops. In Greece, powdery mildew was observed as well, but mainly in *L. angustifolius.*
3. *Root rot and wilt* were observed in several countries mainly during the seedling stage. Several fungi were identified as being the cause of the problems: *Fusarium oxysporum* and *Sclerotinia sclerotiorum* in Austria, *Phytophtora cactorum* in Portugal and *Rhizoctonia* spp. in Greece. In Greece fungicide treatments were applied to prevent major losses due to *Rhizoctonia.*
4. *Viral infections* A viral infection was found in some of the plants in Greece and The Netherlands, which caused symptoms like stunted growth or dwarf growth, curling leaves, and yellowish or mosaic discolouring. The exact virus causing this stunted growth was never found, but the symptoms seemed to be mainly seed borne, it only affected a minor part of the plants and did not seem to spread during the growing season to other plants.

Equally interesting as the fungal diseases that were found in the field trials are the known fungal infections in other lupins that were not, or hardly found in the trial fields. Especially *Pleiochaeta setosa* (brown spot) and *Botrytis cynerea* (grey mold) were not observed. Also, late fusarium wilt, which is known as a damaging phenomenon in Blue lupin was not observed.

Pests and plagues
Pests were observed over the different locations in the different seasons.
Problems in the seedling stage

1. *Elateridea* (wireworms/copperworms) were observed in a trial field in Portugal, causing minor plant loss in both *w*hite lupin and Andean lupin equally in the seedling stage. Presence of wireworms are mainly related to the previous crop in the rotation. Especially perennial crops like grass, grass clover, and alfalfa are known to propagate the click beetles that lay eggs in the sward which produce wireworms as their larvae. Proper design of the crop rotation is key to avoiding major plant loss due to wireworms.
2. *Delia radicum* (cabage fly*)* causes similar problems for young seedlings as wireworms and appeared in a trial field in Portugal with the preceding crop being cabbage. In other lupins other species of Delia were seen to cause damage to young seedlings as well like the wheat bulb fly (*Delia coarctata*). Lowering the risk of damage from these larvae is mainly done by crop rotational choices but are also partly unavoidable as the number of larvae can fluctuate substantially due to weather conditions in the preceding season.

3. Birds like pigeons and crows were reported to have caused damage in young seedlings in Austria and Portugal, although birds preferably targeted faba beans and wheat over Andean lupin in a field trial in The Netherlands.
4. Snails were observed to cause plant loss in young seedlings as well in Portugal. The use of molluscicide prevented plant loss in consecutive years.

Problems during the vegetative and flowering stage

1. *Rabbits, hares, goats and deer.* Damage by some of these animals was reported in Greece, Portugal, Austria and The Netherlands. However, the main damage was done in the sweet varieties of white lupin, blue lupin and yellow lupin. Although the overall bitter accessions of Andean lupin were far less affected, this was also true for the bitter varieties of white lupin and blue lupin. Breeding sweet varieties of Andean lupin will therefore most likely increase the problems of herbivores.
2. *Macrosyphon albifrons* (lupin aphid). In the Dutch trials with sweet blue lupin and white lupin in different regions of the country since 2004, no lupin aphid was ever been seen. From the first year of field trials with the bitter Andean lupin accessions, lupin aphids were observed, re-appearing in every consecutive year. The lupin aphid, which is an exotic insect for Europe, is specialized in using the alkaloids which it takes from the phloem of the bitter lupins to protect itself from attacking insects or birds. The lupin aphid was already introduced in Europe via other bitter lupins like the garden lupin (*Lupinus polyphyllus*). Once these specialized aphids find a suitable crop, they can manifest in large numbers. As the aphids feed on the leaves, stems, and flower buds they can negatively affect plant development and pod-setting. Contrary to the herbivores, damage from lupin aphid is expected to be reduced considerably when sweet accessions of Andean lupin are bred.
3. *Other aphids* were occasionally observed in the field trials, but most of these infections were not observed in Andean lupin. In Greece, *Aphis fabea* were found on white lupin and *Acythosiphon pisum* was found on Blue lupin.
4. *Oxytherea funesta, Tropinata squalida/hirta (Rose beetles)* were mainly observed in early spring in Greece, attacking flowers and reducing pod setting. Large numbers of these beetles were especially observed in the first two growing seasons. The beetles did not just affect Andean lupin but also affected other lupin species.
5. *Sitona lineatus (pea leaf weevil)* was observed only in Greece and affected mainly white lupin and blue lupin. Only mild presence was observed in Andean lupin. The pea leaf weevil is known to cause serious damage to leguminous crops, not because of their damage to the leaves, but due to the fact that the larvae feed on the nitrogen-nodules in the soil. Rates of infestation need to be quite high though to cause substantial damage and this is only reached when a high percentage of the crops in the rotation consists of leguminous crops.

Problems during the podsetting and ripening stage

1. *Lepidoptera* (butterflies and moths) have been reported to cause damage to lupin in various ways. In Greece several larvae were observed appearing from pods and the caterpillars feeding on the leaves. The damage from the caterpillars was not seen as too damaging, but the holes in the pods were then used by ants to further destroy the seeds reducing yields substantially. In Austria larvae of the pea moth (*Cydia nigricana*) were observed in the pods of Andean lupin, damaging the seeds and

thereby reducing the yields. In Iceland, whole plants were stripped completely of their leaves in 2019 by the broom moth (*Melanchra pisi*), which has been adapting itself to feeding on the vast amounts of Nootka lupin (*L. nootkatensis*) that are abundant in large parts of Iceland. This generally happens in late summer in the Nootka lupin, but was observed in Andean lupin as well in 2019.

2. Mice were reported damaging ripened seeds in Austria as the main florescence generally ripens ahead of the pods formed in the other orders of flowering. This caused a serious reduction in the harvestable seeds in 2019.

Andean lupin is a promising crop on marginal soils for Europe. Andean lupin crop (seed) yield of 3 t/ha were reliably measured with indications of even higher yields when crop management is further optimised. Dry biomass production for bioenergy of animal feed or bioenergy can be up to 20 t/ha of dry matter in Austria. The Andean lupin variety Cotopaxi has acquired Plant Breeder Rights. Cotopaxi is an early semi-determinate growing type with seed yields up to 3 tonnes per hectare making it an economical feasible crop for farmers and hence for biorefineries. More breeding efforts are needed for increasing yield, yield stability, disease resistance and determinate growth type.

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Abbreviations

1000K weight	Thousand kernel weight
AEMET	Agencia Estatal de Meteorologia
ANOVA	Analysis of variance
AUA	Agricultural University of Athens
CHha	Haplic Chernozem
CL	Clay loam
CRF-INIA	Spanish Gene Bank
CSIC	Consejo Superior de Investigaciones Científicas
CV	Coefficient of variation
EC	Soil electrical conductivity
EcE	Electrical conductivity of saturated soil paste
ESAC	Escola Superior Agrária de Santarem (Coimbra)
ESAS	Escola Superior Agrária de Santarém (Santarem)
ESDAC	European Soil Data Centre
EU	European Union
FW	Fresh weight
GPS	Global Positioning System
Gst	Inter- population genetic diversity
GWAS	Genome-wide association study
He	*Nei's* genetic diversity index
HI	Harvest index
Hp	Mean phenotypic diversity within each population
Hs	Intra-population phenotypic diversity
Ht	Total phenotypic diversity of the collection
IBPGR	International Board for Plant Genetic Resources
ISA	Instituto Superior de Agronomia
K	Potassium
KNMI	Royal Dutch Metereological Insitute
LBI	Louis Bolk Institute
Lv	Vertic Luvisols
N	Nitrogen
N/A	Not applicable
NS	Not significant
OM	Organic matter
P	Phosphorus
PCA	Principal Component Analysis
RCBD	Randomized Complete Block Design
REML	Restricted Maximum Likelihood
RM	Mean rain
SCL	Sandy Clay Loam

SE	Standard Error
SL	Silt loam
SPAD	Soil Plant Analysis Development
T	Temperature
TKW	Total kernel weight
TM	Mean temerature
USAMV	University of Agronomic Sciences and Veterinary Medicine of Bucharest

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1 Introduction

The Andean lupin (*Lupinus mutabilis*) is a legume that has been cropped for centuries by small holder farmers in South America. The LIBBIO project is designed to explore the possibilities of cropping Andean lupin in Europe and its potential uses. It has potential as food, feed, green manure, and for non-food applications. The seeds of Andean lupin are remarkably rich in protein, contain little starch, and contain rich oils with potential applications in the food, cosmetics and biofuel industries. Since the Andean lupin is a legume, it has the potential of being developed into a sustainable low-input resilient cropping system where nitrogen (N) fixation is a key function. Lupin is also known for its ability to release tightly bound phosphorus (P) thus enhancing phosphorus availability. As such, the Andean lupin could be beneficial for consecutive crops, perhaps minimizing the need for additional fertilizer.

Due to climatic differences among the participating countries in the LIBBIO project, the Andean lupine is cropped in summer in central and northern parts of Europe, but in the Southern countries, it is cropped in winter. There is considerable knowledge within Europe on cropping broad and narrow leaved lupin (also referred to as white and blue lupin), so lupins are not an entirely new crop to Europeans.

This deliverable describes the screening of accessions of Andean lupin under various conditions in seven countries in Europe, focusing on marginal land and diverse environmental conditions. The southern countries were represented by the Lusosem agricultural products in Portugal (LUS), the Spanish National Research Council in Spain (CSIC), the Agricultural University of Athens in Greece (AUA), the University of Agricultural Sciences and Veterinary medicine of Iasi in Romania (UAI). The central and northern countries were represented by the Agricultural research and Education Centre Raumberg-Gumpenstein in Austria (AREC), The Louis Bolk Institute (LBI) and Vandinter Semo BV (VDS) in the Netherlands and in Iceland by the Soil Conservation Service of Iceland (SCSI). In the southern countries, the cropping season starts in the fall and completes in the early summer. In the northern countries the cropping season starts in the spring and concludes in the fall. Due to the different locales, the Andean lupine was tested in highly diverse environments, e.g. during the third growing season, maximum average temperature of 32,4°C, and a minimum average temperature of 1,2°C were recorded.

By testing Andean lupin in field trials, data could be collected on how susceptible or adaptable Andean lupin is to different biotic (e.g. diseases and pests) and abiotic (e.g. frost, drought, salinity) stress factors. Besides Andean lupin, reference crops were included like white and blue lupin, wheat and faba bean to obtain information on how Andean lupin compares to currently cropped commercial varieties.

This deliverable was due in July 2020 when the fourth project season was ongoing. The findings of the first three cropping seasons are therefore presented. Covid-19 related delay of the project gave the opportunity to delay submission of the document a few months for reviewing the conclusions in the light of fourth season findings. The results were very similar to the previous ones, they are to some extend included in the text, those results from The Netherlands are included in an annex. Also, the accession LIB221 got registered with the trade name Cotopaxi during the delay and the text reflects that to some extent.

2 Cotopaxi (LIB221)

Plant Breeders Rights were granted to Vandinter Semo BV on 29th December 2020 for the Andean Lupin variety Cotopaxi. Cotopaxi is result of research in LIBBIO project. Andean Lupin (Lupinus mutabilis) has its origin in South America and is one of the four lupin species for human consumption. Andean Lupin is a sustainable alternative for soybean because of its comparable oil and protein content and its contribution to biodiversity and soil improvement. Cotopaxi is the first Andean Lupin variety in Europe that has been granted with Plant Breeder Rights.

Andean Lupin is one of the "lost crops of the Incas" like quinoa and chia. Andean lupin grows in the same ecozone as potato. Andean lupin bean composition is comparable with soybean.
Andean lupin is one of the "lost crops of the Inca's". Andean lupin beans are rich in protein (45%) and vegetable oil (20%). This makes Andean lupin (Lupinus mutabilis) a potential important crop. There are only four lupin species available for human consumption. Three of them are from the old world, the white, blue and yellow lupin, while the Andean lupin comes from the new world. Research on lupins, also the Andean lupin, started in the 1920's and 30's in Europe by the legendary Professor Dr. Reinhold von Sengbusch. He succeeded to breed sweet lupin cultivars for all the four lupins, including the Andean lupin, with acceptable crop yields. Unfortunately, the sweet Andean lupin variety went lost in Europe's dark times.

Cotopaxi is an early ripening crop which grows well on sandy well drained soils. Its yield potential is between 2-7 tonnes dry beans per hectare in different locations in Europe. It is suited for winter cropping in S-Europe and for summer cropping in N-Europe. This is the earliest and most promising accession for EU cropping. It is one of the earliest emerging accessions and it ripens fast with relative high yields.

Cotopaxi or LIB221 is propagated in season 2019 in different locations, including Portugal and the Netherlands. Cotopaxi was sown on the 23rd of April on a sandy soil in Scheemda with a seed desnisty of 34 pl/m2. Seed emergence was on the 4th of May, 50% flowering main stem was on the 24th of June, 50% ripe pods on main stem was on 25th of July. Height of main stem was 93 cm and total plant height was 118 cm. The field (830m2) was machine harvested on 27th of August and it yielded about 2,0 tonnes/ha. Photographic impressions are shown in Figure 1.


Figure 1a


Figure 1b

Flower and pods of L.mutabilis Cotopaxi (LIB221), season 2019, Scheemda, Netherlands.


Figure 1c


Figure 1d

Hull and seeds of Cotopaxi (LIB221) at 26-7-2019 and 12-8-2019 respectively.

Propagation field Cotopaxi (LIB221), 26 July 2019, Scheemda The Netherlands.

Propagation field Cotopaxi (LIB221), 12 August 2019, Scheemda The Netherlands

Figure 1 (a,b,c,d,e and f). Photographic impressions of Cotopaxi grown in a propagation field in 2019 in the Neterlands.

3 General conditions during the cropping seasons

3.1 Seed availability

Key to all agronomy studies is access to propagation material and land. Multiple sites were available for all the project partners, but the Andean lupin seeds were not freely available at the start of the LIBBIO project. As the crop is not grown commercially in Europe, Andean lupin seed is not multiplied by commercial seed companies. As a result, no substantial amount of seeds was available for conducting large agronomic field trials. Because of this, the design of the field trials had to be adjusted and simultaneously, propagation of Andean lupin had to be organized. Initially, the propagation was directed to Vandinter Semo in The Netherlands. However, the first growing season of 2017 showed that the Netherlands is not the most suitable location due to climatic constraints that prevent sowing in the autumn. The propagation was therefore redirected to Lusosem in Portugal.

In the field trials, material was tested from three different sources. The accession 'Branco' could be directly imported from South-America. Accessions from preceding pre-breeding activities of the Julius Kühn Insitute in Germany of which Vandinter Semo in the Netherlands provided the seeds and gene bank accessions were provided by the Instituto Superior de Agronomia in Lisbon, Portugal.

During the first cropping season, only a few hundred seeds were available for testing. During the second cropping season, the most promising accessions were multiplied by Lusosem and made available for the project, but the seed quantity was still limited and the germination quite poor. During the third cropping season, the quantity of seeds and quality did improve. In the fourth and final cropping season, an ample amount of good quality seeds was available for testing.

Both seed quantity and quality have made it difficult to perform all the planned field trials in all of the subtasks of Work package 2. Plot sizes were therefore relatively small in most of the cropping seasons. Also, the different research tasks needed to be divided among the partners to be able to answer most of the research questions posed in the proposal.

3.2 Climate conditions in general

The overall success of the LIBBIO project was influenced by unusual weather conditions throughout Europe. Under "normal" conditions, the southern countries have warmer and drier conditions than the northern countries which then are generally cooler and wetter.

In Austria, the climate is alpine with normally a long and cold winter. Moderate temperatures during spring and autumn and not too hot during the summers. However, recently, the Austrian climate has changed. Now, there is less snow in the winter and it is not as cold as it used to be. Spring is almost absent resulting in more severe changes in temperature. Also, the autumn is now very warm with the temperatures varying considerably.

The Greek climate is characterised as hot-summer Mediterranean. But as the field locations are at quite a distance from each other, the climate depends also on terrain resulting in different micro-climates with higher and lower temperatures ranging from -1.1 to 43,8 ºC among locations and years. It came as a surprise, that in one of the locations there was a prolonged snow cover in the winter of 2019/2020.

The climate of Iceland is a maritime climate with cool summers. The weather in the first cropping season was typical, but the second was unusually wet and the third unusually dry.

Depending on the field location, the Portuguese climate was temperate, with rainy winters and hot, dry summers and the other location equally dry and rainy but less warm. Portugal was hit by floods in early 2020.

Romania is characterized by a temperate continental climate. The field trial area was cooler and rainfall frequent. During the LIBBIO project the trials faced higher temperatures, especially in June and August, with a rise of 4,2 °C higher than the multiyear average in one location and in addition, drought was a problem. These were some of the limiting factors that affected severely the field trials.

In Spain the climate is Mediterranean subtropical. During the first cropping season a higher maximum average temperature was recorded, during the second cropping season it was the coldest. During the third growing season a maximum average temperature of 32,4 °C and a minimum average temperature of 1,2 °C were recorded.

In the Netherlands the climate is characterized as temperate maritime, with cool summers and cool winters, but this has been changing in recent years. The second cropping season was most extreme with a warmer and drier summer than previously recorded during the last 30 years. During the third cropping season, Dutch temperatures above 40 °C were registered for the first time in recorded history.

In the next paragraphs the unusual weather events are highlighted. The overall measurements recorded are presented in Appendix I.

3.2.1 Austria

In Austria, the two locations are in different climatic regions, one belongs to the alpine region in Northern Styria; Trautenfels; the other to the pre-alpine region in Upper Austria; Lambach and Stadl-Paura, both belonging to the branch of the department for Organic Arable Farming of the Institute for Organic Farming and Biodiversity.

Lambach has a pre-alpine climate: mild winters with little snow and usually enough rain in the cropping season. The last years the weather had longer dry-time intervals with occasional heavy rains in a short time. In 2017, spring came early but snow returned in April while July was hot and dry. The autumn had more rain and mild temperatures. During the winter there was almost no snow, the spring of 2018 warm and dry and almost no rain in June and July, the autumn was relatively wet. Also, little snow in 2019 and a warm dry spring. May was very cold, June dry and warm but in July enough rain fell for cropping.

The climatic conditions at Trautenfels in general can be characterized as: long winters with normally much snow, springtime with moderate temperatures, summers with much rainfall and not too hot temperatures and autumns with a slow decrease of the temperatures until winter. Theses climatic conditions have changed a lot during the last centuries. The winters are mostly mild with less snow and not too cold temperatures. Spring starts early and sometimes the temperatures are quite high in April or May, sometimes combined with drought. The summers have higher temperatures and less rainfall but if it is raining the amount of precipitation is very high and causes often damages by water and mud flow. In autumn warm days may occur and snow mostly falls to non-frozen soil.
The climatic conditions at Lambach/Stadl-Paura can be described as: moderate winters with little snowfall, moderate temperatures in spring, warm summers and moderate temperatures in autumn. At that location climatic conditions have changed a lot. Winters have almost no snow, springtime is beginning in the end of March sometimes with high temperatures and drought in May or June. Summers can be very dry as well, there were many years with a rainfall deficiency.

The first cropping year (2017) was characterized by an early spring that changed in the second half of April to very cold temperatures and snowfall even at Lambach and Stadl-Paura, right after sowing the field trials. In early summer, a period of drought occurred at Lambach and Stadl-Paura lasting until the beginning of August followed by small amounts of precipitation. In autumn there was no frost so the Andean lupines were able to grow until the field was cleared for the next cropping season. At Lambach and Stadl-Paura the Andean lupins were incorporated into the soil. At Trautenfels the Andean lupin residuals were removed from the field.
At Trautenfels the weather in 2017 was similar but we started the field trial later. The summer was not as dry as at Lambach as we had moderate rainfall. A thunderstorm with hail in the middle of July damaged the Andean lupin a little bit but they were not destroyed completely. There was no frost until the middle of November, so the plants still had green leaves, buds and flowers until that date.

The weather conditions of 2018 were different from 2017 and were characterized by a mild winter and an early spring with warm temperatures in March. The next month’s moderate temperatures and more rainfall in Trautenfels occurred, but also a lack of rainfall in Lambach until the beginning of July. The drought in Lambach resulted in reduced growth of the plants. In July rainfall came again but not too much. In autumn of the year 2018 we had an early frost at Trautenfels in the end of September, all plants of Andean lupineswere stressed severely and were lost. In Lambach there was no frost until November, but the weather was wet and foggy. In winter 2018 - 2019 almost no snow and precipitation were recorded, followed by a warm and dry spring at Lambach and Stadl-Paura.

The growing season 2019 for the Austrian field trials was different from the two years before. In 2019, winter snowfall was high in the Alpine region (Trautenfels) while lower in the pre-alpine region (Lambach/Stadl-Paura). During the early spring, a short period of warm

weather occurred followed by a very cold and wet period on all locations. Snowfall occurred during the first week of May on both locations. At the end of May weather changed again and a dry and warm period followed. During June and the first half of July it was very warm. At the end of July it rained for a short period, however intensely. In autumn, the weather changed very often, we had warm and cold periods, always lasting a few days.
Because of the wet and cold weather in spring, the field trials could be sown in the end of May and beginning of June. Therefore, the germination was very quick, especially at Trautenfels. At Lambach the situation was quite different because the drought caused was the problem with germination, not the seed-quality. The vegetation period was long because no frost came in autumn until we stopped growing by ploughing the fields in the middle of November.

3.2.2 **Greece**

The climate in Greece is Mediterranean with a variety of micro-climates at different locations as a result to the great diversity of different terrain (Founda and Giannakopoulos, 2009). The experimental fields were placed in locations that are stated to be hot-summer Mediterranean areas by Beck et al. (2018), however these sites are characterized by different microclimates. More specifically, Kalamata is usually hotter and rainier than Athens, while on the other hand, Erythres is colder and rainier than the other two. Yearly meteorological data for the three locations during the experimental cropping seasons are presented in Table 1. Another experimental field was also established in Mani in 2016-2017, but Andean lupin accessions failed to germinate and grow as sowing took place in the middle of December when low temperatures dominate in this location (Table 2.) Some accessions (LIB219, LIB220, LIB222) were tested for their germination and growth in Andros. The meteorological data were collected from the commercial meteo.gr webpage that receives data from the meteorological stations of the National Observatory of Athens (www.noa.gr).

Table 1. Mean main air temperature and total precipitation per year in the different experimental locations in Greece.

Location	**Kalamata**			**Athens**			**Erythres**
Cropping season	2016/17	2017/18	2018/19	2016/17	2017/18	2018/19	2017/18
Mean air (T ºC)	17,3	17,0	16,0	17,0	17,4	17,0	12,9
Max air (T ºC)	43,8	36,4	34,0	41,3	33,9	35,2	32,0
Min air (T ºC)	1,2	5,3	2,8	-1,1	3,5	-0,1	-0,8
Precipitation (mm)	532,8	666,2	622,2	352,2	638,6	455,3	658,0

Table 2. Monthly mean, max and min air temperature and total precipitation in Mani during 2016-2017 cultivation period.

Month	**Mean air**	**Max air**	**Min air**	**Precipitation**
	T °C	T °C	T °C	mm
December	9,2	21,7	0,0	32,4
January	7,2	18,7	-2,0	57,6
February	10,9	21,7	2,1	61,5
March	13,3	25,7	5,7	71,7
April	15,8	29,6	7,0	6,3
May	21,8	37,4	13,0	4,5
June	26,1	44,5	15,6	1,5
July	30,1	44,0	19,3	0,0

3.2.3 **Iceland**

Iceland is situated in the North Atlantic between latitude 63°23'N 66°32'N and longitude 13°30'W and 24°32'W. The climate of Iceland is classified as maritime with cool summers and mild winters (Einarsson, 1984). Average annual rainfall in Iceland is about 1200 mm/year (Arnalds et al. 2013). Annual mean temperature ranges from 2,0-5,7 °C in the lowlands, but generally 4-5 °C in the southern part where LIBBIO trials have taken place. The three growing seasons 2017, 2018 and 2019 were all very different from each other with the first being more normal, the second being incredibly wet and the third being incredibly dry, but temperatures did not vary greatly (table 3).

Table 3. Climatic data for study sites in Iceland from May to September in 2017-2019. Temperature was logged at the study sites by a HOBO USB Micro station data logger. But precipitation data were obtained from the Icelandic Met office.

	2017			**2018**			**2019**		
	min	**max**	**mean**	**min**	**max**	**mean**	**min**	**max**	**mean**
Temperature (°C)	-1,6	23,8	10,0	-2,2	23,4	10,2	-2,45	23,9	11,0
Precipitation (mm/month)	-	-	73,7	-	-	106,9	-	-	68,1

During field trials, soil water content, soil temperature (2cm soil depth) and air humidity were recorded with a HOBO USB Micro station data logger (https://www.onsetcomp.com/hobo-micro-station) (table 4).

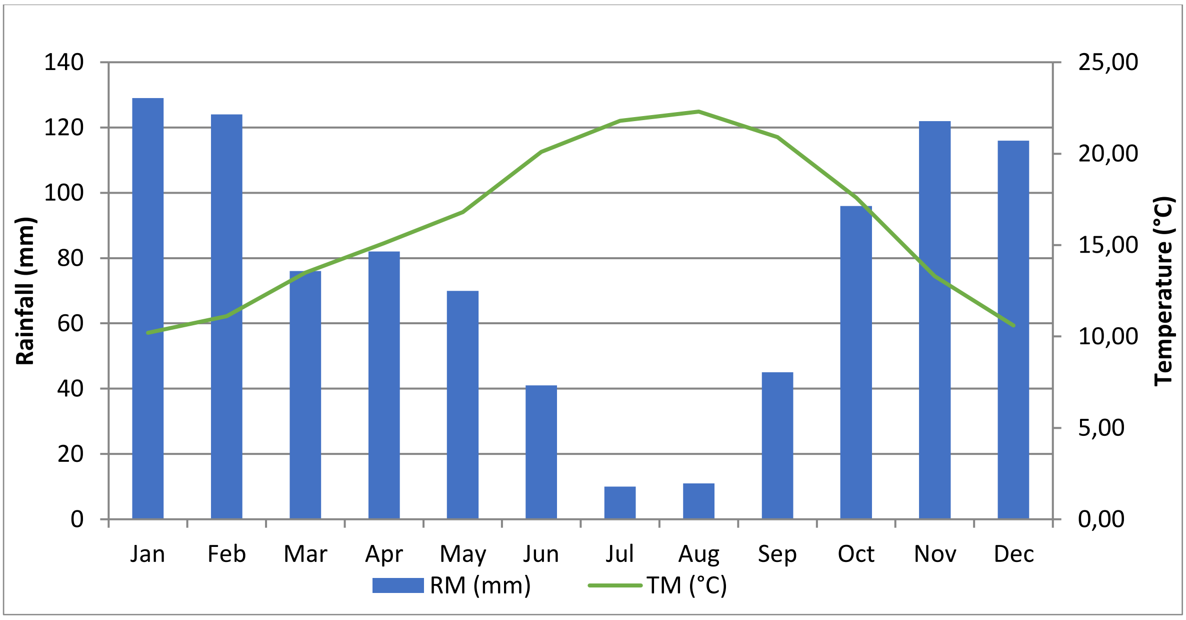
Table 4. Soil humidity, soil temperature and air humidity for study sites in Iceland 2017-2019. In 2017, studies were performed on two plots (on eroded soil and on organic soil), but in 2018 and 2019 they were performed in one location (eroded soil). Air humidity was recorded at the eroded soil site since these conditions were not expected to vary considerably since the sites were only 6,9 km apart.

	2017	**2018**	**2019**

	Eroded soil	Organic soil	Eroded soil	Eroded soil
Soil water content (m^3/m^3)	0,02-0,12	0,04-0,20	0,05-0,09	0,03-0,13
Mean water content (m^3/m^3)	0,070	0,113	0,068	0,066
Soil temperature range (°C)	-0,2-24	2,3-30,2	0,4-27,2	0,7-32,9
Mean soil temperature (°C)	10,6	12,2	11,1	12,9
Air humidity range (%)	26,7-100		38,0-100	18,5-100
Mean air humidity (%)	86,6		88,1	70,8

3.2.4 **Portugal**

The climate of mainland Portugal according to the Koppen classification is divided into two regions: one with temperate climate with rainy winter and dry and hot summer (Csa) and the other with temperate climate with rainy winter and dry and not very hot summer (Csb (IPMA). According to this characterization, one LIBBIO trial location, Coimbra, was located in a Csb region and the other two locations: Santarém and Lisbon – in a Csa region.
Lupins in Portugal are mainly winter crops. They can be sown from the beginning of October up to late January, crops incorporated in soil in March for sideration or harvested in June for grain production. Short cycle varieties are used, and late sowing can result in lower yields (Talhinhas et al. 1999). Poorly drained soils are common in Portugal and considering that higher yields are usually obtained when dry winters occur or when rainfall is evenly distributed during the plant growth cycle. During the late winter and spring rainfall, which greatly influences non-irrigated winter crops such as winter cereals and lupins in Portugal is irregular. In June, when temperatures are higher, lack of rainfall that is common, determines the end of the cropping season (Figure 2).



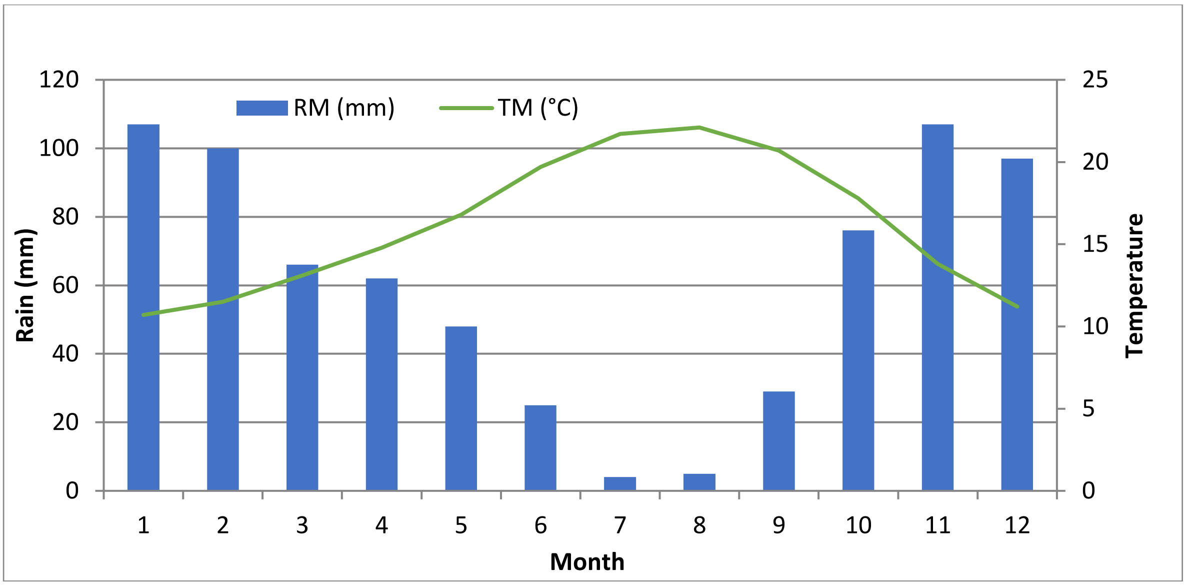


Figure 2 Coimbra (above) and Santarém (below) 30 years series climate data 1982-2012.

3.2.5 **Romania**

During the 2017 growing season the temperature was higher than the multi-annual average in all the three locations, especially in June and August at Ezareni and Secuieni. At Suceava location, the monthly average temperature was very high, with a very high differences (up 4,2°C) in July in relation to the long-term average. The precipitation during the growing season was relatively normal, except the Secuieni location, where the sum of monthly precipitation was lower than the multi-annual average.

The trails of 2018 for all five locations faced harsh climate conditions. During the beginning of March 2018, the rainfall was 56,8 mm, more than double than the average, and the temperatures were low, 1,2˚C which is 2,1˚C lower than average. These conditions caused a delay in sowing of about 2 weeks. After this period temperatures increased suddenly and for about seven weeks precipitation was very limited. For example, in April and May 2018 rainfall was 30% less than the average. In the meantime, the temperature was up to 5,3 °C higher than average. In June and July the temperature was normal but rainfall was up to three times higher than usual.

The trials of 2019 for all three location generally were challenged by much more rain than is normal for April and May and much less in June, July and August, The rainfall regime made the trial with irrigation to be unusual and facilitate the development of the diseases.

3.2.6 **Spain**

Córdoba (Andalucía, Spain) is characterized by subtropical Mediterranean climate conditions. At the experimental sites, the trend in climatic conditions affecting field experiments during the three consecutive growing seasons 2016-2017, 2017-2018 and 2018-2019 is reported in Figure 3-5.

During the 2016-2017 growing season, the following climate data were recorded: maximum average temperature of 35,6 °C, minimum average temperature of 1,6 °C and an average temperature range from 7,6 °C to 27, 4 °C with an average of relative humidity, albeit not uniformly distributed during the growing season, between 41% and 78%.

During the 2017-2018 growing season, the recorded data were: maximum average temperature 31,1 °C, minimum average temperature 2,1 °C and mean temperature ranging from 8,5 °C to 23,3 °C, with a mean relative humidity between 55,8% and 86,6 % .

During the 2018-2019 growing season, the data were: maximum average temperature 32,4 °C, minimum average temperature 1,2 °C and average temperature ranging from 7,2 °C to 23,9 °C, with a mean relative humidity, not uniformly distributed during the growing season, of 43,5% to 79,8%.

The highest maximum average temperature was recorded during the 2016-2017 growing season, while the minimum was recorded during the 2018-2019 growing season.

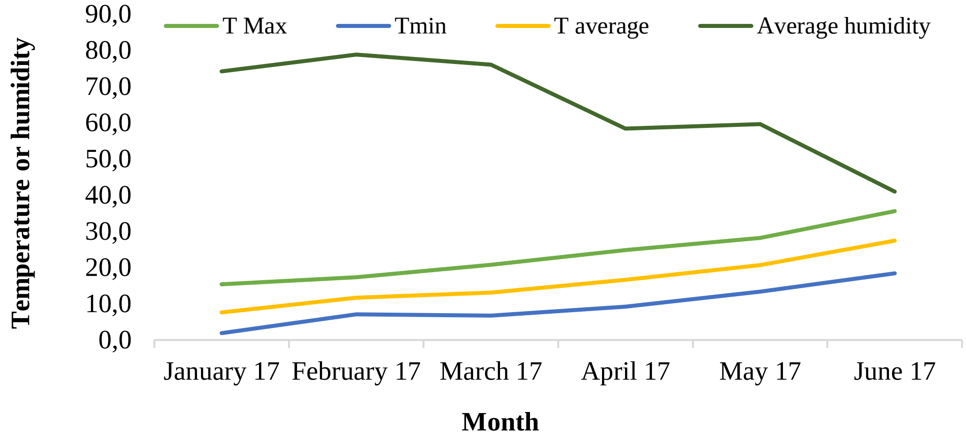
Table 5. Temperature and humidity during the field experiments in the 2016-2017 growing season (data obtained from the nearest weather station in Cordoba, provided by AEMET). Climatic descriptors: temperature (°C) (maximum, minimum and average values) and average relative humidity.

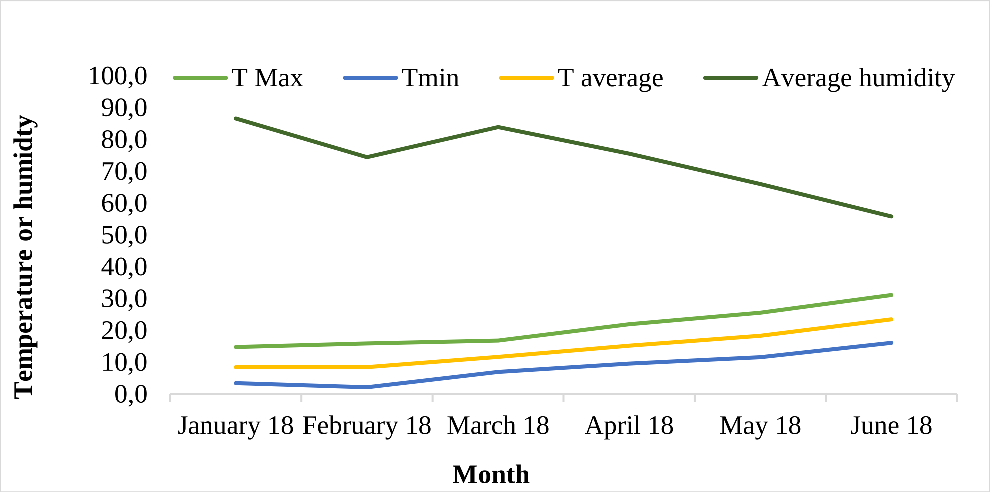
Month	**T Max**	**Tmin**	**T average**	**Average humidity**
January 17	15,4	1,9	7,6	74,2
February 17	17,3	7,1	11,7	78,8
March 17	20,8	6,7	13,1	76,0
April 17	24,8	9,2	16,6	58,4
May 17	28,2	13,3	20,7	59,6
June 17	35,6	18,4	27,4	41,0

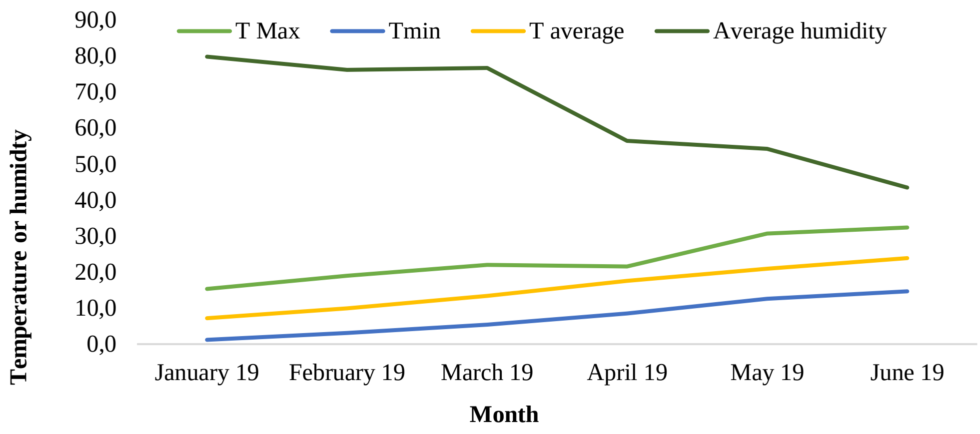
Month	**T Max**	**Tmin**	**T average**	**Average humidity**
January 18	14,8	3,4	8,5	86,6
February 18	15,9	2,1	8,5	74,4
March 18	16,8	6,9	11,7	83,9
April 18	21,9	9,5	15,2	75,5
May 18	25,6	11,6	18,3	66,0
June 18	31,1	16,1	23,5	55,8

Month	T Max	Tmin	T average	Average humidity
January 19	15,3	1,2	7,2	79,8
February 19	19,0	3,1	9,9	76,1
March 19	22,0	5,4	13,4	76,6
April 19	21,6	8,5	17,5	56,5
May 19	30,7	12,6	20,9	54,2
June 19	32,4	14,7	23,9	43,5

Figure 3-5 (below). Temperature and humidity during the field experiments in three consecutive growing seasons 2016-2017, 2017-2018 and 2018-2019 (data are taken from the nearest weather station in Cordoba provide by AEMET). Climatic descriptors: temperature ºC (maximum, minimum and average values) and average relative humidity.







3.2.7 The Netherlands

When starting the LIBBIO project temperature and rainfall on the trial fields were initially monitored using Hoboware and Thermofisher detectors in possession of the Louis Bolk Institute. However, comparison of these data to nearby formal weather stations owned by the Royal Dutch Metereological Insitute (KNMI) showed a strong difference between our 'fairly simple' kit and the formal measurement equipment. It was therefore decided to abandon our own technology and focus on the data provided by the KNMI. Detailed weather data can be found in appendix I.III.

The first experimental year (2017) was a warm, sunny and relatively wet year. The year started with a very cold January, fluctuating temperatures in February and a warm mild March. In March beside mild temperatures and a peak in the sun hours also rainfall was a bit lower (62mm) than the long-term average (67mm) recorded. April started with cold temperatures which caused damage to orchard crops. April was dry with only 24 mm of rainfall versus the common 42 mm. May and June were warmer than is common for this time of the year and in May only about half of the usual rainfall was recorded making it exceptionally dry. June was very warm but July, August and September were slightly cooler. July was very wet with a national rainfall average of 110 mm versus the common 78 mm and September turned out the be the wettest for 15 years. The autumn and winter were warm.

In the Netherlands 2018 was the most exceptional. Figures 6 and 7. show the average daily temperature and rain from sowing to harvest in the De Bilt where an automated weather station operated and owned by the Royal Netherlands Meteorological Institute is located. De Bilt is the nearest station to the main field location in 2018. Note how the 2018 season started considerably warmer compared to the long-term average and how the precipitation was lower for most of the cropping season.

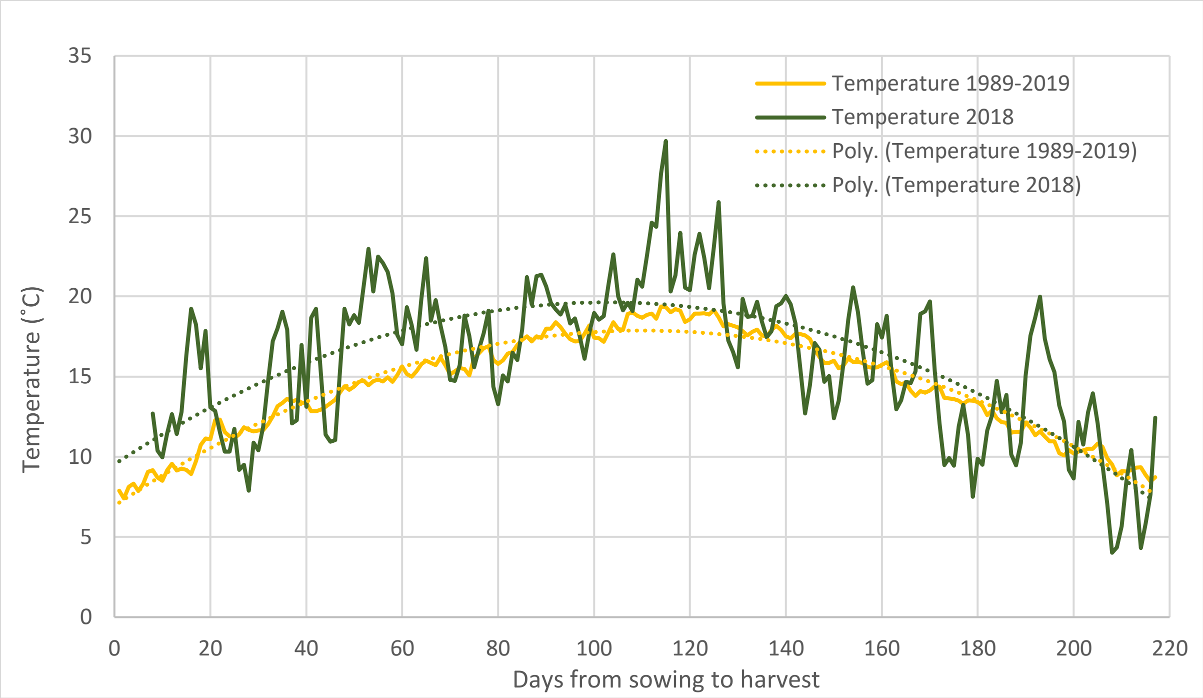


Figure 6. The daily average temperature during the last 30 years and the average temperature in 2018. And the general trend of the last 30 years and 2018. Data provided by KNMI.

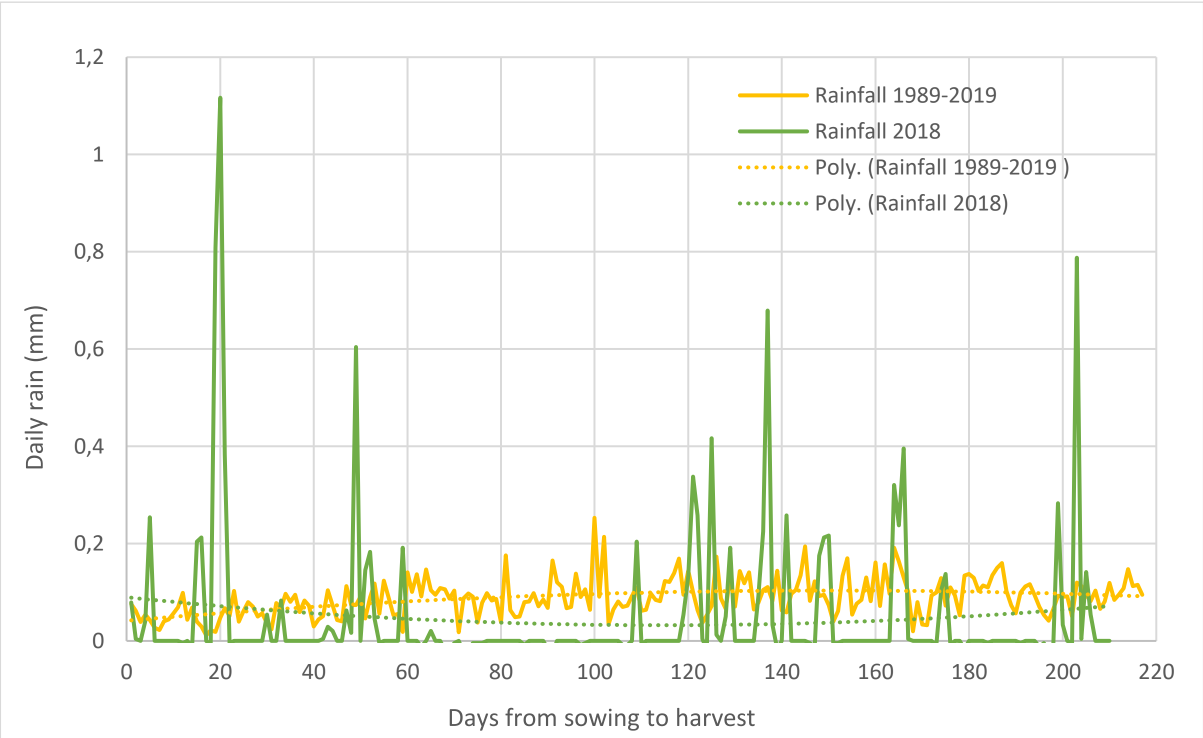


Figure 7. The daily average rainfall during the last 30 years and the average rainfall in 2018. And the general trend of the last 30 years and 2018. Data provided by KNMI.

The month of April 2018 was characterized by multiple days with more than >25°C which is quite uncommon. In May and June, various records were set for high temperatures. In addition, this summer was very dry. The last rainfall occurred on the 15th of May and the drought continued until the 28th of July. In July only 5,3 mm versus the average 68 mm of rainfall was measured. In August and September were warm but rainfall in August reignited the lupin growth which had halted in June-July. October was very sunny with 180h of sunshine versus the usual 113h. Rainfall was low with 37 vs 83 mm. Lupins were still flowering in October and to maximise seed setting the cropping season was continued until the middle November. However, in November regular weather conditions returned and the season was competed.

Like the previous years, 2019 was warm and fairly dry. A mild winter was followed by variable weather. March was mild and wet; April was very mild with exceptionally high temperatures during the the second half of the month. May was quite cold and in some parts of the country some snow fell. During the summer months the weather varied between fairly cool and extremely high temperatures. For the first time in the Netherlands the 40 °C barrier was crossed in the South. The autumn was mild and rainy periods were exchanged with drier intervals.

4 Accessions in the trials

The LIBBIO project started with building a foundation of seed material. No commercial varieties were available and prior to performing detailed trials, a general exploration of various accessions was performed. As a result, during the first cropping season in the autumn of 2016 in the Mediterranean countries and in the spring-summer of 2017 for the Central and Northern European countries, a large number of accessions were tested. The outcome of this initial screening was the selection of accessions to test in the following cropping seasons which explored crop traits in more detail.

Only 100-200 seeds from each accession and 500 seeds from the accession ‘Branco’ were distributed to each partner. The first season aimed at acquiring familiarity with the crop, where it concerned the general development, flowering and maturation under different soil and climatic conditions. This was necessary as most partners had no previous experience with Andean lupin cultivation and its climatic conditions requirements.

Table 6. Cropping seasons and timing.

Cropping season	Mediterranean countries	Central and Northern Europe
1	Autumn 2016-Summer 2017	Spring 2017-Fall 2017
2	Autumn 2017-Summer 2018	Spring 2018-Fall 2018
3	Autumn 2018-Summer 2019	Spring 2019-Fall 2019
4	Autumn 2019-Summer 2020	Spring 2020-Fall 2020

Table 7. Accessions used included in LIBBIO

Accessions	Origin
Branco	Latin-America
Inti/LIB202	Instituto Superior de Agronomia
LIB200	Instituto Superior de Agronomia
LIB201	Instituto Superior de Agronomia
LIB203	Instituto Superior de Agronomia
LIB204	Instituto Superior de Agronomia
LIB205	Instituto Superior de Agronomia
LIB207	Instituto Superior de Agronomia
LIB208	Instituto Superior de Agronomia
LIB209	Instituto Superior de Agronomia
LIB210	Instituto Superior de Agronomia
LIB211	Instituto Superior de Agronomia
LIB212	Instituto Superior de Agronomia
LIB214	Instituto Superior de Agronomia
LIB217	Instituto Superior de Agronomia
LIB218	Instituto Superior de Agronomia
LIB219	Vandinter Semo
LIB220	Vandinter Semo

LIB221	Vandinter Semo
LIB222	Vandinter Semo

During the first cropping season, it appeared that the material from the gene bank in Portugal was very diverse in plant morphology which hindered phenotyping. The Vandinter accessions were more homogeneous and performed better with regards to earliness and productivity. As a result, the Vandinter accessions were selected for cropping seasons 2 and 3 for the overall analyses. For some countries, a few additional accessions looked promising which were also included in the trials.

4.1 Austria

In Austria, screening trials were performed at two locations, normally with four replications of each line (LIB 220, 221,222 and Branco) per plot except in the first year when there was a lack of seeds.

As Branco performed well in Austria in 2017, and more seeds were available from Branco than any other accession, the second and third cropping season focussed on Branco.

In the table below (table 8) the plant architecture of Branco in 2018 can be seen. Data of two field trial locations (Lambach and Stadl-Paura) were taken. Plants with an average height of 163 cm often show 12-13 side branches. The length of these branches reaches about 90,90 cm in length. Furthermore, plants in Lambach grew better compared to those in Stadl-Paura. In Lambach Branco grew higher and had longer side branches.

Table 8. Plant morphology of Branco in cropping season 2.

Field	**Line**	**Date**	**Plant height (cm)**	**Average number of side branches per plant**	**Average side branch length (cm)**
Lambach	Branco	13.09.2018	154,20	19,00	76,78
Lambach	Branco	05.10.2018	171,40	19,00	78,25
Lambach	Branco	22.10.2018	163,60	12,80	90,90
Stadl-Paura	Branco	20.08.2018	124,00	16,20	42,25
Stadl-Paura	Branco	17.09.2018	121,80	14,00	58,10
Stadl-Paura	Branco	05.10.2018	171,20	19,00	78,25
Stadl-Paura	Branco	23.10.2018	140,00	13,60	59,20

The figure 8 below shows the average dried biomass distribution of an Andean lupine plant of Branco line. What can be seen is the inhomogeneity of biomass per plant over the years and locations. In 2018 we had heavier plants in Lambach and Stadl-Paura than in 2019. One reason for this might be the earlier sowing date of 2.5.2018. Whereas, in 2019 the Andean lupin was sown on 3.6.2019 due to heavy rainfall in May. As a result the growing season was shortened. However, with respect to hull production, the growing season in 2019 was better than in 2018. In 2018 the average plant did not produce a noticeable hull amount. That had a strong effect on the corn yield of 2018.

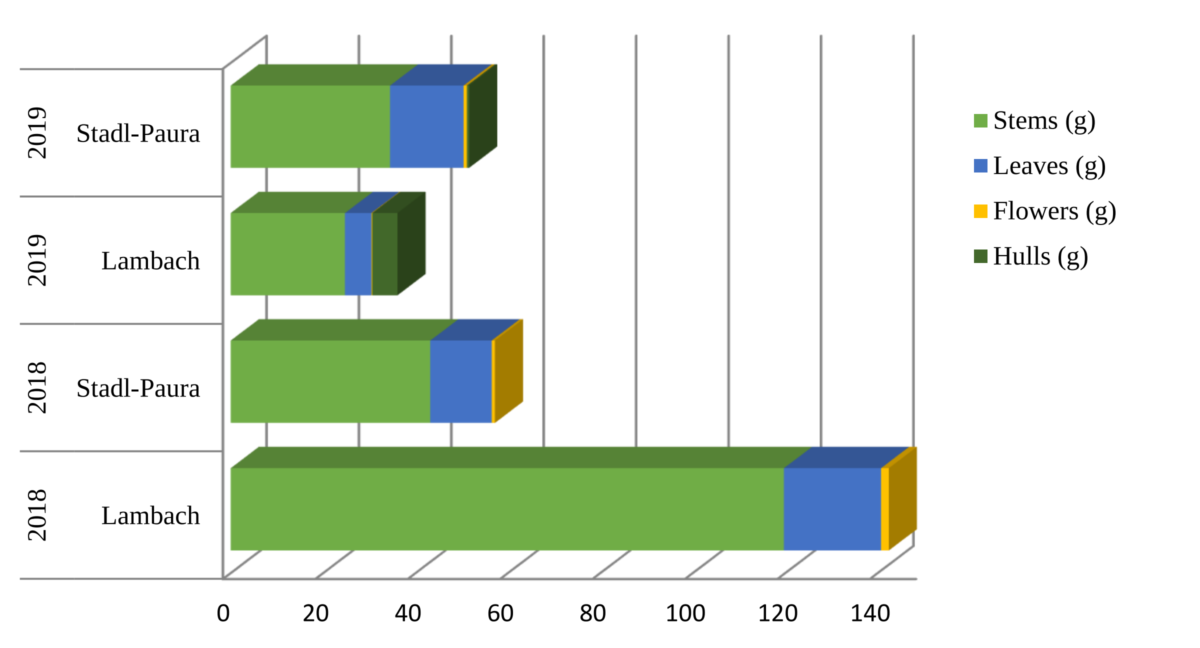


Figure 8. Distribution of dried biomass in 2018 and 2019.

Table 9. Material sampled for biomass distribution analyses.

Line	Sampling date	Year	Field	Stems (g)	Leaves (g)	Flowers (g)	Hulls (g)
Branco	05.10.2018	2018	Lambach	119,7	21,0	1,7	
Branco	05.10.2018	2018	Stadl-Paura	43,2	13,3	0,7	
Branco	23.09.2019	2019	Lambach	24,7	5,7	0,2	5,5
Branco	01.10.2019	2019	Stadl-Paura	34,5	15,9	0,7	0,5

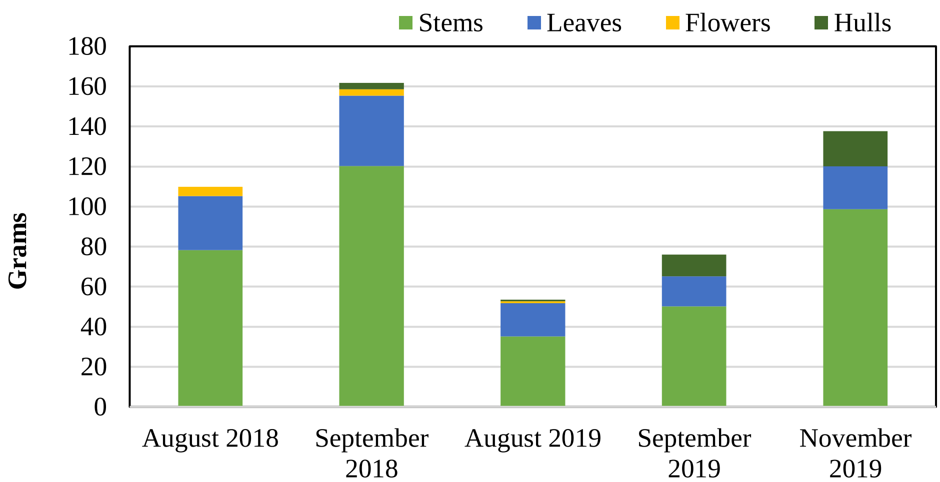


Fig. 9. Average dried biomass distribution at Trautenfels.

The situation at Trautenfels was quite different. There were few plants in the plots in 2018, so the stems got really thick. An early frost prevented sampling in October because all plants were killed. Despite sampling at similar dates, the results are quite different. In 2018 only a few pods on Branco were found in the middle of September despite sowing the trial in the beginning of May. The sowing in the end of May resulted in hull production in the middle of August but only a few hulls were produced. The later the sampling the higher the number of hulls. Flowers were set at each sampling, but the weight was too low for measuring. In 2019 we had stems at an average therefore the weight is not so high than in the year before.

The other accessions, beside Branco, also performed well during the growing season in 2019. Different parameters have been investigated, like the time of flowering and the canopy height. The following tables (Table 10) show the canopy height of the accessions that were sown during the growing season 2019. The plants in Lambach needed less time to flower compared to Stadl-Paura. Furthermore, they reached a higher number of the orders of flowering than in Stadl-Paura. That may be caused by the better soil in Lambach, which has a higher ability to store water and contains less coarse material than the soil in Stadl-Paura.

Table 10. Flowering date in Lambach and Stadl-Paura 2019.

Beginning of flowering (DAS) in Lambach 2019

Varieties	Main flowering	1st order flowering	2nd order flowering	3rd order flowering
Branco	54	72	112	-
LIB222	57	72	85	-
LIB221	54	72	75	85
LIB220	72	78	112	-

Beginning of flowering (DAS) in Stadl-Paura 2019

Varieties	Main flowering	1st order flowering	2nd order flowering	3rd order flowering
Branco	71	92	-	-
LIB222	71	92	101	-
LIB221	71	92	101	-
LIB220	71	101	-	-

Beginning of flowering (DAS) at Trautenfels 2019

Varieties	Main flowering	1 st order flowering	2 nd order flowering	3 rd order flowering
Branco	56	76	102	125
LIB222	63	79	105	130
LIB221	48	72	98	120
LIB220	67	88	117	147

Furthermore, the plant development was investigated. It was found that in Lambach the plants grew higher than in Stadl-Paura. Moreover, at the end of the growing season the Andean lupin varieties in Lambach reached their maximum height at which they started lodging due to the heavy canopy weight. In Stadl-Paura the plants did not reach that level of growth at all.

4.2 Greece

A total number of ten experimental fields were established in Greece during the first three cropping seasons aiming to evaluate the performance and suitability of various Andean lupin accessions during winter in a typical Mediterranean climate and under low-input cropping systems (Table 11). The genetic material that was studied varied among the cropping seasons and locations (Table 12). In all field trials the varieties *L. albus* cv. Multitalia and *L. angustifolius* cv. Polo were included as reference lupin species as they are native to the Mediterranean. In Mani, also a local landrace of *L. albus* was used, which is traditionally cultivated in the region (Table 12).

Table 11. Experimental fields established for screening Andean lupin accessions in Greece.

Cropping season	**Location**	**Geographical coordinates**	**Details**
2016-2017	Athens (exp 1)	37º59'03.5"N, 23º42'10.0"E, 24 m asl	transplantation
	Athens (exp 2)	37º59'03.5"N, 23º42'10.0"E, 24 m asl	Portuguese entries, transplantation
	Kalamata	37°03'39.3"N, 22°03'49.5"E, 7 m asl	transplantation
	Mani	36°49'36.4"N, 22°26'04.5"E 475 m asl	direct sowing
2017-2018	Athens	37°59'03.5"N, 23°42'10.0"E, 24 m asl	In parallel to Task 2.2, transplantation
	Kalamata	37°03'33.9"N, 22°02'46.8"E, 41 m asl	Aithaia, in parallel to Task 2.2, transplantation
	Kalamata	37°03'39.3"N, 22°03'49.5"E, 7 m asl	TEI, in parallel to Task 2.2, direct sowing
	Erythres	38°12'55.6"N, 23°18'47.2"E, 376 m asl	In parallel to Task 2.2, transplantation
2018-2019	Athens	37°59'03.5"N, 23°42'10.0"E, 24 m asl	In parallel to Task 2.2, transplantation
	Kalamata	37°03'39.3"N, 22°03'49.5"E, 7 m asl	TEI, in parallel to Task 2.2, direct sowing

Table 12. Material evaluated in each location.

LIBBIO code/ varieties	**2016-2017**				**2017-2018**				**2018-2019**	
	Athens (exp 1)	Athens (exp 2)	Kalamata TEI	Mani	Athens	Kalamata Aithaia	Kalamata TEI	Erythres	Athens	Kalamata TEI
LIB200					X	X		X	X	X
LIB201		X								
LIB203		X								
LIB206		X								
LIB208		X								
LIB209	X		X	X	X	X	X	X	X	X
LIB212	X		X				X			
LIB213					X					

LIB214	X		X		X	X	X	X	X	X
LIB217		X								
LIB218					X			X		
LIB219	X		X	X			X			
LIB220	X		X	X	X	X	X	X	X	X
LIB221			X	X	X	X	X	X	X	X
LIB222	X		X	X	X	X	X	X	X	X
Branco	X		X	X	X	X	X	X	X	X
LIB224		X			X	X		X	X	X
cv. Polo	X	X	X	X	X	X	X	X	X	X
cv. Multialia	X	X	X	X	X	X	X	X	X	X
Kokkala Manis				X						

All experiments were conducted using a Randomized Complete Block Design (RCBD). In the experiments were transplantation took place, seeds were pre-germinated in rolled filter papers after being treated with 20% hypochlorite solution for 2-3 min and incubated at room temperature (25 °C). In all experiments, except from direct sowing experiments in Mani and in Kalamata in 2018-2019, as soon as the radicle reached 1 cm in length, a wet application procedure of *Bradyrhizobium* inoculum (HiStick® Lupin, BASF) was applied.

4.2.1 **First cropping season, all accessions, 2016-2017**

Regarding the first experiments in Kalamata and Athens (experiment Athens 1) during the first cropping season (2016-2017) some loss of seeds was recorded due to *Fusarium oxysporum* infestation. Seed pre-germination was performed for both experiments. Percentages of seed germination for each accession are presented in Table 13. Due to increased seed loss because of *Fusarium oxysporum* and the very low germination percentage of LIB221, this accession was included solely in the field trial in Kalamata. In Mani, as reported previously, the seeds were sown directly in the field. Emergence of all the fields that were sown directly were recorded till 14 days after sowing. Germination percentages of each accession are presented in Table 14.

Table 13. Germination percentages (%) of accessions used in Athens exp. 1 and in Kalamata in cropping season 1 (2016-2017).

LIBBIO code	**Acc. code**	**Germination** %
LIB209		88,1
LIB212		96,4
LIB214		89,4
LIB219		93,1
LIB220		95,8
LIB221		47,9
LIB222		95,6
Branco		94,4
	cv. Polo	98,6
	cv. Multitalia	97,2

Table 14. Emergence percentages (%) of accessions sowed in Mani during 2016-2017.

LIBBIO code	Acc. code	Emergence %
LIB209		18,3
LIB219		36,7
LIB220		40,0
LIB221		6,7
LIB222		45,0
Branco		18,3
	cv Multitalia	60,0
	cv Polo	70,0
	Kokkala Manis	72,5

For LIB221 a very low seed germination percentage was also recorded in Mani, as well as during the pre-germination treatments that were applied, probably due to *Fusarium oxysporum* infestation. Overall, a higher germination percentage of *L. albus* and *L. angustifolius* entries was recorded in comparison to Andean lupin genotypes that were sown directly in soil. Low germination percentages of Andean lupin genotypes could be attributed to the late sowing as low temperatures and snowfall predominated in December and January in the area, which do not promote Andean lupin development (Neves-Martins et al., 2016).

The Portuguese entries were tested in the second experiment conducted in Athens (exp. 2) cropping season 1 and were characterized by a high germination percentage with the exception of LIB208 (Table 15).

Table 15. Germination percentages of Portuguese entries during 2016-2017 in Athens.

LIBBIO code	Acc. code	Germination %
LIB217		93,3
LIB208		68,3
LIB203		93,3
LIB206		91,7
LIB201		83,3
LIB224		100,0
	cv Multitalia	100,0
	cv Polo	100,0

Cropping season 2, 2017-2018

Regarding the second cropping season (2017-2018) the percentages of germination of pre-germinated seeds used in the three experiments (Athens, Aithaia, Erythres) was above 77% with the exception of Potosi Alemao (LIB213) (54,1%) and Branco (66,7%) (Table 16). Branco is characterized by a rough seed testa that did not absorb the water easily which could have influenced the germination.

Table 16. Germination percentages (%) of accessions used in 2017-2018.

LIBBIO code	Acc. code	Germination %
LIB200		80,6
LIB209		79,4
LIB213		54,1
LIB214		77,8
LIB218		79,7
LIB220		83,3
LIB221		88,1
LIB222		88,3
Branco		66,7
LIB224		77,8
	cv. Polo	91,1
	cv. Multitalia	100,0

In Kalamata, at the Technological Education Institute (TEI) direct sowing was performed. Due to high precipitation after sowing many seeds did not emerged, so the trial was re-sown. The results of emergence (of both the sowing times combined) for each accession are presented in Table 17. LIB212 presented extremely low emergence probably because it was a 2-year-old seed lot. LIB222 also emerged poorly mainly because of *Fusarium oxysporum* infection of the seeds.

Table 17. Germination rates after direct sowing in Kalamata, TEI during 2017-2018.

LIBBIO code	Acc. code	Germination %
LIB209		90,0
LIB212		12,2
LIB214		87,8
LIB219		96,7
LIB220		72,2
LIB221		88,9
LIB222		44,4
Branco		28,9
	cv. Polo	100,0
	cv. Multitalia	21,1

Cropping season 3, 2018-2019

In the third cropping season, in Athens, pre- germinated plants were used. The germination is presented in the following table (Table 18).

Table 18. Germination rates in Athens during 2018-2019.

LIBBIO code	Acc. code	Germination %
LIB200		98,7

LIB209		99,0
LIB214		99,3
LIB220		100,0
LIB221		87,8
LIB222		73,3
Branco		76,5
LIB224		100,0
	cv Polo	99,3
	cv Multitalia	100,0

In Kalamata the sowing was done directly in the field. The results of germination are presented in Table 19. Variable seed germination was observed among the seed lots used in each year. In addition, the depth of the sowing applied was 1 cm in the first year and 3-4 cm in the second year. LIB222 and Branco showed a lower germination percentage than the other accessions that were used.

Table 19. Germination rates after direct sowing in Kalamata, TEI during 2018-2019.

LIBBIO code	**Acc. code**	**Germination**
		%
LIB200		74,45
LIB209		62,20
LIB214		77,75
LIB220		69,40
LIB221		65,55
LIB222		25,55
Branco		19,40
LIB224		84,95
	cv. Polo	76,65
	cv. Multitalia	61,05

Remarks- Conclusions

- Germination of Andean lupin seeds is strongly affected by the duration of seed lots of preservation and storage.
- Germination of Andean lupin seeds is strongly affected by the infection of pathogens like *Fusarium oxysporum* (e.g LIB221 during the 2016-2017 cultivation period).
- Overall, Andean lupin emergence (%) after direct sowing was lower than the *L. albus* and *L. angustifolius* entries that were used.
- Branco seeds presented difficulties in absorbing water and germination-emergence were inhibited, especially when applying the direct sowing method.
- A sowing depth of 3-4 cm decreased severely the percentages of seed emergence that was recorded, probably due to the heavy and rocky soils in these trials
- Heavy rain also negatively affected the seed germination that was recorded, especially supplementary to a greater sowing depth.

- LIB222 presented higher emergence than other Andean lupin accessions after direct sowing in sowing in Mani during 2016-2017, but presented very low emergence rates due to infestation during 2017-2018 and 2018-2019 cropping season.
- LIB214 also resulted in mediocre germination rates in many of the experiments conducted, either applying transplantation or direct sowing.
- LIB220 presented as the most stable accession of Andean lupin regarding germination-emergence rates across the experiments conducted.
- LIB200, LIB214, and LIB221 also presented generally good germination rates.

4.2.1.1 Crop development and homogeneity/ diversity of accessions

In this section additional findings of the accessions tested in Greece regarding crop development and homogeneity/diversity are presented. Especially during cropping season 1 a detailed characterization and primary evaluation of the accessions was performed aiming to screen the accessions and define which accessions were promising and therefore used for future evaluation in our edaphoclimatic conditions.

Athens 1st Experiment and Kalamata (2016-2017)

In this first cropping season, transplantation in Kalamata and Athens took place in December 2016. The extra characterization performed was based on the IBGRI descriptors list for Lupin (IBPGR, 1981). Data on fifty morphological and agronomical traits were recorded during the 2016-2017 cropping season in Athens (exp. 1) and in Kalamata. In Mani, Andean lupin entries germinated poorly and did not manage to grow so no measurements were taken.

The measurements taken referred to 30 vegetative traits, namely seedling cotyledon color, the intensity of cotyledon color, cotyledon length (cm), seedling hypocotyl color, the intensity of hypocotyl color, the color of hypocotyl, seedling hypocotyl length (cm), growth habit, plant habit, stem formation, stem pubescence, stem color, the intensity of stem color, stem waxiness, stem thickness (cm), branching, the height of lowest primary branch (cm), the diameter of the leaf (cm), leaflet shape, central leaflet tip, pubescence of leaflet upper surface, pubescence of leaflet lower surface, leaf color, the intensity of leaf color, stipule length (cm), stipule color, the intensity of stipule color, petiole length (cm), petiole color, the intensity of petiole color.

Nineteen referred to reproductive stage, namely days to first flowering, flower color, the height of the main inflorescence, the height of flowering in every order of flowering (cm), number of (first order, second, etc.) side branches with flowers when a certain order of flowering has fully developed, length of side branches (cm), number of leaves on side branches, number of flowers in first-order side branches, length of principal inflorescence (cm), length of flower (cm), the height of first pod (cm), green pod pubescence, mature pod pubescence, pod shattering, number of pods in the main inflorescence, number of pods in first, second and third-order of pod setting, number of pods per plant, pod length, pod width.

In addition, twelve traits related to seed yield and biomass production were recorded, lodging of the plants during ripening, number of seeds in the main inflorescence, number of seeds in every other order of pod setting, seeds per pod, the weight of seeds/plant (g), total above-ground weight after harvest (g), stem weight after harvest (g), stem dry weight (g), root weight after harvest (g), root dry weight (g), hundred seed weight (g), harvest index and nine traits referring to seed morphology, namely seed shape, seed luster, seed primary color, the intensity of seed primary color, seed secondary color, the intensity of seed secondary color, seed secondary color distribution, seed length (cm), seed width (cm).

Additionally, thirteen agro-morphological traits were measured, namely height of flowering in every order of flowering (cm), length of side branches (cm), number of leaves on side branches, number of (first order, second, etc.) side branches with flowers, number of pods in every order of pod setting, number of seeds in each order of pod setting, number of seeds per pod, weight of seeds/plant (g), total above-ground weight after harvest (g), stem weight after harvest (g), stem dry weight (48 h, 80 °C) (g), root weight after harvest (g) and root dry weight (g), harvest index. All traits were recorded for each one of the sixty plants per accession. Relative frequency (percentage %) of the values of each of the 36 qualitative characteristics of the accessions examined was calculated using Microsoft Excel 2007 and presented by order regarding the developmental stage of the plants in which received as well as traits related to seed yield measured are presented as a separate category. Finally, the seed crude protein content of each accession for each one of the two locations estimated through Kjeldahl-N method was used to determine the nitrogen content of lupin accessions in dry seed samples (Kjeltec™ 8400 Analyzer unit, FOSS). Crude protein content was calculated by multiplying N by the factor 6.25.

4.2.1.2 Qualitative and vegetative trait results

All lupin material tested was characterized as “herb” and not “shrub” as well as all Andean lupin accessions characterized by glabrous stem and waxiness. LIB214 central leaflet tip presented as “not acuminate”, whereas in the rest of the accessions was “acuminate”. No pubescence in leaflet upper or lower surface was observed in Andean lupin entries, while leaf color was green in all Andean lupin accessions. Furthermore, stipule color did not vary among the Andean lupin accessions and characterized by yellow color, while its intensity as medium with the exception of LIB221 and LIB222 that were characterized as pale. Seedling qualitative traits are presented in the following table both for Athens (exp.1) and for Kalamata as all the seed pre-germinated together (Table 20).

Table 20. Seedling’s dominant cotyledon and hypocotyl color and cotyledon and hypocotyl length of the accessions both used in Athens and in Kalamata.

Accessions	Seedling traits							
	Cotyledon color	Intensity of cotyledon color	Cotyledon length	Hypocotyl color	Intensity of hypocotyl color	Red color of hypocotyl	Intensity of red coloring of hypocotyl	Hupocotyl length
			cm					cm
LIB209	Green	Dark	1,90	Green	Medium	Yes	Pale	4,57
LIB212	Green	Dark	2,06	Green	Medium	Yes	Pale	4,15
LIB214	Green	Dark	2,18	Green	Medium	Yes	Pale	3,98
LIB219	Green	Medium	2,06	Green	Medium	Yes	Pale	4,95
LIB220	Green	Medium	2,29	Green	Medium	No		3,63
LIB221	Green	Medium	2,52	Green	Medium	Yes	Pale	3,57
LIB222	Green	Medium	1,95	Green	Medium	Yes	Pale	4,24
Branco	Green	Dark	2,68	Green	Medium	Yes	Pale	4,83
cv. Multitalia	Green	Dark	1,99	Green	Medium	No		1,66

cv. Polo	Green	Medium	1,37	Green	Medium	No		3,79

The qualitative descriptor of vegetative traits that were studied presented as percentages (%) in the following tables (Tables 21-25) for each accession in each location, where Field A. Kalamata and Field B. Athens (exp.1) during cropping season 1.

Table 21. Relative frequency (percentage %) of the values corresponding to plant habit of the accessions.

Accessions	**Plant habit**					
	Field A			Field B		
	Erect	Semi-erect	Prostrate	Erect	Semi-erect	Prostrate
	%	%	%	%	%	%
LIB209	83,3	15,0	1,7	65,0	26,7	8,3
LIB212	81,7	13,3	5,0	50,0	32,7	17,3
LIB214	63,3	30,0	6,7	47,0	31,4	21,6
LIB219	75,0	18,3	6,7	51,0	34,7	14,3
LIB220	55,0	31,7	13,3	47,4	30,6	22,0
LIB221	13,3	78,4	8,3	-	-	-
LIB222	91,7	3,3	5,0	81,5	14,8	3,7
Branco	56,7	38,3	5,0	49,2	38,9	11,9
cv. Multitalia	100,0			100,0		
cv. Polo	100,0			75,0	19,6	5,4

Table 22. Relative frequency (percentage %) of the values corresponding to stem formation of the accessions.

Accessions	**Stem formation**			
	Field A		Field B	
	Not prominent	Prominent	Not prominent	Prominent
	%	%	%	%
LIB209	100,0		44,8	55,2
LIB212	52,6	47,4	29,8	70,2
LIB214	100,0		38,9	61,1
LIB219	100,0		41,7	58,3
LIB220	21,7	78,3	23,9	76,1
LIB221	100,0		-	-
LIB222	100,0		49,1	50,9
Branco	100,0		82,5	17,5
cv. Multitalia	100,0		92,9	7,1
cv. Polo	100,0		70,3	29,7

Table 23. Relative frequency (percentage %) of the values corresponding to intensity of leaf color of the accessions.

Accessions	**Intensity of stem color**					
	Field A			Field B		
	Pale	Medium	Dark	Pale	Medium	Dark
	%	%	%	%	%	%
LIB209	33,3	13,4	53,3	13,7	22,5	63,8

LIB212	65,9	24,3	9,8	87,0	1,9	11,1
LIB214	96,5	3,5		92,6	5,5	1,9
LIB219	100,0			100,0		
LIB220	100,0			100,0		
LIB221	100,0			-	-	-
LIB222	78,6	19,6	1,8	71,4	28,6	
Branco	100,0			100,0		
cv. Multitalia	100,0			100,0		
cv. Polo	100,0			100,0		

Table 24. Relative frequency (percentage %) of the values corresponding to petiole color of the accessions.

Accessions	**Petiole color**					
	Field A			Field B		
	Green	Red	Green/ Red	Green	Red	Green/ Red
	%	%	%	%	%	%
LIB209	26,3	73,7			66,6	33,4
LIB212	60,0	13,3	26,7	83,6	16,4	
LIB214	53,6	10,7	35,7	100,0		
LIB219	100,0			100,0		
LIB220	100,0			100,0		
LIB221	100,0			-	-	-
LIB222	90,9	9,1		100,0		
Branco	100,0			100,0		
cv. Polo	100,0			100,0		
cv. Multitalia		100,0			100,0	

Table 25. Relative frequency (percentage %) of the values corresponding to the intensity of petiole color of the accessions.

Accessions	**Intensity of petiole color**					
	Field A			Field B		
	Pale	Medium	Dark	Pale	Medium	Dark
	%	%	%	%	%	%
LIB209	24,4		75,6		100,0	
LIB212	86,7	1,7	11,6	94,5	5,5	
LIB214	5,0	95,0			100,0	
LIB219	100,0			100,0		
LIB220	98,3	1,7		100,0		
LIB221	100,0			-	-	-
LIB222	71,4	28,6		100,0		
Branco		100,0			100,0	
cv. Multitalia	100,0			100,0		
cv. Polo	100,0			100,0		

4.2.1.3 **Reproductive stage traits results**

Table 26. Relative frequency (percentage %) of flower color of the accessions in Kalamata.

Accessions	Color of flower						
	Field A						
	Type 1	Type 2	Type 3	Type 4	Type 5	Type 1 discolored	Type 3 purple
	%	%	%	%	%	%	%
LIB209	24,4						75,6
LIB212	15,8				8,3	32,2	43,7
LIB214	26,20					57,1	16,7
LIB219						100,0	
LIB220		100,0					
LIB221	100,0						
LIB222					100,0		
Branco	100,0						
cv. Multitalia					100,0		
cv. Polo					100.0		

Table 27. Relative frequency (percentage %) of flower color of the accessions in Kalamata, field B.

Accessions	Color of flower						
	Field B						
	Type 1	Type 2	Type 3	Type 4	Type 5	Type 1 discolored	Type 3 purple
	%	%	%	%	%	%	%
LIB209					1,7		98,3
LIB212					3,3	50,0	46,7
LIB214	19,5					80,5	
LIB219						100,0	
LIB220		100,0					
LIB221	-	-	-	-	-	-	-
LIB222					100,0		
Branco	100,0						
cv. Multitalia					100,0		
cv. Polo	100,0						

Table 28. Relative frequency (percentage %) of the values corresponding to green pod pubescence of the accessions.

Accessions	Green pod pubescence							
	Field A				Field B			
	Absent	Light	Medium	Heavy	Absent	Light	Medium	Heavy
	%	%	%	%	%	%	%	%
LIB209		1,7	67,2	31,1			68,3	31,7
LIB212			68,3	31,7			10,9	89,1
LIB214			100,0				100,0	
LIB219		100,0				100,0		
LIB220			100,0				100,0	
LIB221		100,0			-	-	-	-
LIB222				100,0				100,0
Branco		100,0				100,0		
cv. Multitalia			100,0				100,0	
cv. Polo			100,0				100,0	

Table 29. Relative frequency (percentage %) of the values corresponding to mature pod pubescence of the accessions.

Accessions	Mature pod pubescence							
	Field A				Field B			
	Absent	Light	Medium	Heavy	Absent	Light	Medium	Heavy
	%	%	%	%	%	%	%	%
LIB209		15,8	39,5	44,7		9,1	40,1	50,8
LIB212		13,0	45,7	41,3		20,0	33,4	46,6
LIB214		18,8	62,5	18,7		17,.	51,1	31,1
LIB219		7,7	73,1	19,2		35,6	42,2	22,2
LIB220			11,8	88,2		13,6	22,7	63,7
LIB221		41,7	45,8	12,5	-	-	-	-
LIB222			18,2	81,8		27,5	25,0	47,5
Branco		29,6	54,5	15,9		29,5	48,6	21,9
cv. Multitalia		52,3	45,5	2,2		82,9	17,1	
cv. Polo		15,2	75,8	9,0	24,0	32,0	40,0	4,0

Table 30. Relative frequency (percentage %) of the values corresponding to pod shattering of the accessions.

Accessions	Pod shattering							
	Field A				Field B			
	No	Slight	Medium	Intense	No	Slight	Medium	Intense
	%	%	%	%	%	%	%	%
LIB209	84,2	10,5	2,6	2,6	67,3	16,4	7,3	9,1
LIB212	82,6	8,7	4,4	4,3	62,2	17,8	13,3	6,7
LIB214	56,3	31,3	3,1	9,4	77,8	15,6	6,7	
LIB219	92,3	3,9		3,8	91,1	4,4	2,3	2,2
LIB220	70,6	11,8	11,8	5,9	59,1	13,7	13,6	13,6
LIB221	65,4	23,1	3,9	7,7	-	-	-	-
LIB222	95,5	4,6			90,0	5,0	5,0	
Branco	84,1	11,4	2,3	2,3	73,9	15,0	7,8	3,3
cv. Multitalia	98,0	2,0			74,1	18,5	5,5	1,9
cv. Polo	80,0	8,6	5,7	5,7	52,0	12,0	4,0	32,0

4.2.1.4 **Traits related to seed yield results**

Susceptibility to lodging was recorded as it is a trait that can negatively affect seed yield. The results of each accession are presented in Table 31 separately for each one of the two field trials.

Table 31. Relative frequency (percentage %) of the values corresponding to susceptibility to the lodging of the accessions.

Accessions	Lodging					
	Field A			Field B		
	Light	Medium	High	Light	Medium	High
	%	%	%	%	%	%
LIB209	42,9	39,3	17,8	70,0	20,0	10,0

LIB212	28,8	49,2	22,0	45,0	40,0	15,0
LIB214	14,0	29,9	56,1	35,0	30,0	35,0
LIB219	35,8	32,1	32,1	30,0	45,0	25,0
LIB220	62,8	23,3	13,9	45,0	30,0	25,0
LIB221	2,10	31,2	66,7	-	-	-
LIB222	68,4	28,3	3,3	25,0	45,0	30,0
Branco	40,0	45,0	15,0	10,0	80,0	10,0
cv. Multitalia	96,7	1,70	1,6	40,0	55,0	5,0
cv. Polo	51,7	21,7	26,6	85,0	15,0	

4.2.1.5 Seed morphological traits results

Seed morphological traits were recorded. Each sample studied consisted of 100 seeds. Seed luster was recorded as “shiny” for all the accessions except for cv. Polo which characterized as “dull”. Seed primary color appeared to be pale cream to pale orange for cv. Multitalia, white for Branco and pale cream for the accessions LIB219, LIB220, LIB221, LIB222, LIB209, LIB212, LIB214, and cv. Polo. Seed secondary color was medium brown for cv. Polo, dark brown for LIB222, while LIB209 seeds characterized by 20% absence of secondary color and 80% by a secondary dark brown color. LIB212 seeds characterized by 84% absence of secondary color and 16% dark brown. The rest of accessions did not present seed secondary color. The distribution of secondary color on seeds of each accession is presented in Table 32. The seed shape of the accessions is also presented in Table 32.

Table 32. Relative frequency (percentage %) of the values corresponding to seed secondary color distribution of the accessions.

Accessions	**Seed secondary color distribution**									
	Crescent	Eyebrow	Back	Spotted	Moustache	Marbled	Marbled crescent	Marbled eyebrow	Moustache -hilum line	Absent
	%	%	%	%	%	%	%	%	%	%
LIB209		24,0					28,0	28,0		20,0
LIB212							16,0			84,0
LIB214										100,0
LIB219										100,0
LIB220										100,0
LIB221										100,0
LIB222						100,0				
Branco										100,0
cv. Multitalia										100,0
cv. Polo									100,0	

Table 33. Relative frequency (percentage %) of the values corresponding to seed shape of the accessions.

Accessions	**Seed shape**						
	Spherical	Flattened spherical	Oval	Flattened Oval	Cuboid	Flattened cuboid	Reniform
	%	%	%	%	%	%	%
LIB209	24,0			76,0			

LIB212			100,0		
LIB214		100,0			
LIB219		100,0			
LIB220			100,0		
LIB221		100,0			
LIB222			100,0		
Branco	100,0				
cv. Multitalia				100,0	
cv. Polo					100,0

4.2.1.6 Quantitative trait results

The quantitative traits followed the normal distribution within the same experimental field with the exception of some traits. However, the corresponding balance charts all characterized by homoscedasticity. All quantitative traits subjected therefore to ANOVA ($\alpha = 0.05$), followed by a *Tukey-Kramer* (HSD) ($\alpha = 0.05$) means comparison method. The *p*-values for the factor accession for every quantitative characteristic are shown in table 34. On the following tables, each quantitative trait is presented (tables 35-53) by order regarding the plant developmental stage of which the measurement was received, while traits that are related to seed yield and biomass production are included in a separate group. Within each column means followed by the same letter do not show statistically significant differences for the same experimental field. Where no letters appear, no significant difference was recorded.

Table 34. *P*-values for each quantitative trait studied and for each field.

Developmental stage	Quantitative traits	Field A	Field B
Vegetative stage	Cotyledon length (seedlings)	< 0,0001	< 0,0001
	Hypocotyl length (seedlings)	< 0,0001	< 0,0001
	Stem thickness	< 0,0001	< 0,0001
	Plant height after harvest	< 0,0001	< 0,0001
	Height of lowest primary branch	< 0,0001	< 0,0001
	Diameter of leaf	< 0,0001	< 0,0001
	Stipule length	< 0,0001	< 0,0001
	Petiole length	< 0,0001	< 0,0001
Reproductive stage	Time of flowering (Days after sowing)	< 0,0001	< 0,0001
	Number of branches with first order flower	< 0,0001	< 0,0001
	Number of branches with second order flower	< 0,0001	0,0145
	Number of branches with third order flowering	0,042	-
	Height of main flowering	< 0,0001	< 0,0001
	Height of first order flowering	0,0012	< 0,0001
	Height of second order flowering	0,3201	< 0,0001
	Length of first order side branches	0,0093	< 0,0001
	Length of second order side branches	0,194	< 0,0001

	Number of leaves in first order side branches	0,1212	< 0,0001
	Number of leaves in second order side branches	0,2407	0,0011
	Number of flowers in first order flowering	0,4455	< 0,0001
	Number of flowers in second order flowering	0,3339	0,0177
	Length of principal inflorescence	< 0,0001	< 0,0001
	Flower length	< 0,0001	< 0,0001
	Height of first pod	< 0,0001	< 0,0001
	Pod length	< 0,0001	< 0,0001
	Pod width	< 0,0001	< 0,0001
Seed traits	Seed length	< 0,0001	< 0,0001
	Seed width	< 0,0001	< 0,0001
Seed yield-Biomass traits	Pods/plant	< 0,0001	< 0,0001
	Number of pods in main inflorescence	< 0,0001	< 0,0001
	Number of pods in first order flowering	< 0,0001	0,0012
	Number of pods in second order flowering	0,0028	0,0148
	Number of pods in third order flowering	0,1186	0,5274
	Above ground plant weight	< 0,0001	< 0,0001
	Above ground plant weight without pods	< 0,0001	< 0,0001
	Dry above plant weight without pods	< 0,0001	< 0,0001
	Root fresh weight	< 0,0001	< 0,0001
	Root dry weight	< 0,0001	< 0,0001
	Number of seeds in main flowering	0,0009	< 0,0001
	Number of seeds in first order flowering	< 0,0001	< 0,0001
	Number of seeds in second order flowering	0,0015	0,3427
	Number of seeds in third order flowering	0,1732	-
	Seeds/pod	< 0,0001	< 0,0001
	Seeds/plant	< 0,0001	< 0,0001
	Weight of 100 seeds	< 0,0001	< 0,0001
	Harvest index (HI)	< 0,0001	< 0,0001

4.2.1.7 Vegetative stage trait results

Seedlings cotyledon and hypocotyl length of the plants were measured, presented for both experiments which were grown in parallel in the greenhouse (table 35). The rest of the vegetative traits are presented separately for Field A (Kalamata) and Field B (Athens exp.1) (tables 35-38).

Table 35. Mean seedling's cotyledon and hypocotyls length of the accessions.

Accessions	**Cotyledon length**	**Hypocotyl length**
	cm	cm
LIB209	1,90 d	4,56 abc
LIB212	2,05 cd	4,15 cd
LIB214	2,18 bc	3,98 cd

LIB219	2,06 cd	4,95 a
LIB220	2,29 b	3,62 d
LIB221	0,25 f	0,43 f
LIB222	1,95 d	4,24 bcd
Branco	2,67 a	4,83 a
cv. Multitalia	1,99 cd	1,66 e
cv. Polo	1,37 e	3,79 d

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 36. Mean stem thickness for Field A and Field B.

	Stem thickness	
Accessions	Field A	Field B
	cm	cm
LIB209	0,64 b	0,57 b
LIB212	0,63 b	0,58 b
LIB214	0,61 bc	0,60 b
LIB219	0,41 ef	0,56 b
LIB220	0,52 cd	0,57 b
LIB221	0,50 cde	-
LIB222	0,46 def	0,55 b
Branco	0,78 a	0,64 b
cv. Multitalia	0,60 bc	0,86 a
cv. Polo	0,38 f	0,31 c

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 37. Mean plant height after harvest and height of first primary branch of the accessions.

	Plant height after harvest		**Height of first primary branch**	
Accessions	Field A	Field B	Field A	Field B
	cm	cm	cm	cm
LIB209	35,44 bc	37,94 b	12,62 cd	11,20 b
LIB212	33,53 bcd	38,92 b	9,75 de	12,13 b
LIB214	39,50 b	40,23 b	13,21 bc	14,23 ab
LIB219	24,5 e	35,66 b	14,69 bc	12,77 b
LIB220	36,91 bc	38,62 b	14,55 bc	12,00 b
LIB221	29,14 cde	-	8,21 ef	-
LIB222	27,65 de	36,83 b	5,94 f	11,62 b
Branco	52,17 a	49,04 a	21,63 a	12,02 b
cv. Multitalia	36,28 b	54,88 a	24,22 a	19,10 a
cv. Polo	27,33 de	26,07 c	16,13 b	17,59 ab

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 38. Mean diameter of leaf and stipule length of the accessions.

	Diameter of leaf	**Stipule length**	**Petiole length**

Accessions	Field A	Field B	Field A	Field B	Field A	Field B
	cm	cm	cm	cm	cm	cm
LIB209	5,50 cd	5,95 cd	0,40 c	0,39 c	4,70 abc	4,77 de
LIB212	6,06 bcd	7,19 b	0,40 c	0,40 c	3,95 de	6,04b
LIB214	6,16 bc	6,10 cd	0,20 e	0,39 c	4,36 bcd	5,42 bc
LIB219	4,46 f	5,67 d	0,30 d	0,30 d	4,11 cd	4,50 ef
LIB220	5,40 de	6,41 c	0,30 d	0,30 d	4,73 ab	5,50 bc
LIB221	5,38 cde	-	0,30 d	-	3,75 de	-
LIB222	4,25 f	4,93 e	0,20 e	0,20 e	3,46 e	4,04 fg
Branco	6,98 a	5,80 d	0,30 d	0,30 d	5,29 a	5,19 bcd
cv. Multitalia	6,26 b	8,72 a	1,50 a	1,51 a	5,29 a	7,16 a
cv. Polo	4,78 ef	4,03 f	0,60 b	0,55 b	4,16 bcd	3,45 g

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

4.2.1.8 Reproductive stage trait results

All accessions produced up to two orders of side branches and therefore to the second order of flowering. Only LIB214 had some plants that reached the third order of side branches in Kalamata (Field A) and therefore produced some pods and seeds in the third order of flowering. The number of seeds in second-order of flowering was also very small and many of the accessions did not produce seeds in that order. LIB214 produced on average the most seeds of second-order (6,44) followed by LIB222 (3,72), LIB221 (1,11) and Branco (0,92). The rest reproductive traits that were recorded are presented in Tables 39-44.

Table 39. Mean time of flowering in days after sowing (DAS) and number of first and second order of side branches of accessions.

	Time of flowering		**Number of first order side branches with inflorescence**		**Number of second order side branches with inflorescence**	
Accessions	Field A	Field B	Field A	Field B	Field A	Field B
LIB209	112,00 c	125,00 b	1,50 bc	1,75 b	0,28 b	2,66 b
LIB212	113,00 c	126,00 b	1,23 cd	2,37 b	0,08 b	1,00 b
LIB214	116,00 c	126,00 b	1,12 cd	1,87 b	1,00 a	4,00 ab
LIB219	123,00 b	126,00 b	0,23 e	1,63 b	0,02 b	0,00 c
LIB220	121,00 b	127,00 b	0,75 cde	1,57 b	0,00 b	0,00 c
LIB221	104,00 d	-	1,09 cd	-	0,56 ab	-
LIB222	125,00 b	120,00 c	0,65 de	1,71 b	0,26 b	1,50 b
Branco	135,00 a	139,00 a	2,75 a	2,12 b	0,53 ab	1,66 b
cv. Multitalia	132,00 a	138,00 a	2,20 ab	3,24 a	0,03 b	7,00 a
cv. Polo	106,00 d	111,00 d	2,30 a	1,99 b	0,61 ab	1,50 b

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 40. Mean height of main, first order and second order inflorescences.

Accessions	Height of main flowering		Height of first order flowering		Height of second order flowering	
	Field A	Field B	Field A	Field B	Field A	Field B
	cm	cm	cm	cm	cm	cm
LIB209	23,90 bc	23,70 cd	25,10 c	31,10 d	32,50 bc	33,50 b
LIB212	23,00 bc	25,80 cd	23,90 cd	30,99 de	31,20 bcd	40,00 ab
LIB214	23,80 bc	26,95 bc	29,90 b	35,22 cd	32,50 b	35,37 b
LIB219	20,80 cd	24,91 cd	21,00 cd	30,21 de	19,00 d	-
LIB220	26,40 b	26,53 bc	35,10 a	38,68 bc	-	-
LIB221	20,10 cd	-	24,80 cd	-	26,80 cd	-
LIB222	18,80 d	22,81 d	22,60 cd	30,06 de	28,30 bcd	38,60 b
Branco	37,30 a	35,68 a	36,70 a	43,13 b	40,87 a	39,40 b
cv. Multitalia	25,10 b	29,89 b	30,43 b	49,69 a	36,80 a-d	59,60 a
cv. Polo	18,90 d	18,57 e	21,30 d	26,08 e	25,50 cd	31,60 b

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0.05$ by *Tukey's* HSD.

Table 41. Mean length of first and second order side branches.

Accessions	Length of first order side branches		Length of second order side branches	
	Field A	Field B	Field A	Field B
	cm	cm	cm	cm
LIB209	7,98 de	6,57 b	8,01 ab	4,50 cd
LIB212	8,04 cd	7,43 ab	9,40 ab	5,00 bcd
LIB214	7,41 ef	7,36 ab	8,29 ab	6,00 bc
LIB219	9,10 ab	7,27 ab	9,00 ab	-
LIB220	8,65 bc	6,93 b	-	-
LIB221	6,55 f	-	7,59 b	-
LIB222	8,80 bc	6,89 b	7,00 b	10,33 a
Branco	9,85 ab	8,37 a	9,12 a	7,20 ab
cv. Multitalia	9,30 abc	8,17 a	7,00 ab	6,85 b
cv. Polo	10,25 a	7,23 ab	7,06 b	2,30 d

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0{,}05$ by *Tukey's* HSD.

Table 42. Mean number of leaves in the first and second order branches and number of flowers of first order branches with flowers.

Accessions	Number of leaves in first order side branches		Number of leaves in second order side branches		Number of flowers in first order flowering	
	Field A	Field B	Field A	Field B	Field A	Field B
LIB209	7,98 de	6,57 b	8,01 ab	4,50 cd	9,40 bc	9,66 ab
LIB212	8,04 cd	7,43 ab	9,40 ab	5,00 bcd	8,30 b-e	7,35 b

LIB214	7,41 ef	7,36 ab	8,29 ab	6,00 bc	8,70 bcd	7,18 bc
LIB219	9,10 bc	7,27 ab	9,00 ab	-	5,50 cde	5,00 bc
LIB220	8,65 bcd	6,93 b	-	-	10,40 ab	7,61bc
LIB221	6,55 f	-	7,59 b	-	5,30 de	-
LIB222	8,80 bcd	6,89 b	7,00 b	10,33 a	13,60 a	7,67 b
Branco	9,85 ab	8,37 a	9,12 a	7,20 ab	8,70 bcd	10,37 ab
cv. Multitalia	9,30 bc	8,17 a	7,00 b	6,85 b	7,30 cde	13,50 a
cv. Polo	10,25 a	7,23 ab	7,06 b	2,30 d	2,70 e	2,19 c

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 43. Mean length of principal inflorescence, mean flower length and mean high of first pod.

Accessions	**Length of principal inflorescence**		**Flower length**		**Height of first pod**	
	Field A	Field B	Field A	Field B	Field A	Field B
	cm	cm	cm	cm	cm	cm
LIB209	11,74 a	18,81 a	1,35 d	2,03 cd	23,27 bc	25,59 cd
LIB212	10,73 a	16,65 a	2,03 bc	2,13 bc	21,92 bc	27,06 c
LIB214	8,33 b	11,22 bc	2,15 b	2,18 ab	23,79 bc	28,76 bc
LIB219	5,34 c	8,62 c	3,01 a	2,06 cd	19,75 c	27,19 c
LIB220	12,78 a	13,10 b	2,25 b	2,25 a	25,37 b	28,94 bc
LIB221	7,97 b	-	2,07 bc	-	19,63 bc	-
LIB222	6,40 bc	8,35 c	1,93 bc	1,99 d	18,70 c	23,14 de
Branco	11,98 a	12,49 b	2,26 b	2,26 a	33,70 a	37,28 a
cv. Multitalia	11,29 a	18,46 a	1,79 bcd	1,72 e	22,21 bc	31,93 b
cv. Polo	4,63 c	3,95 d	1,40 cd	1,38 f	21,14 bc	20,15 e

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0.05$ by *Tukey's* HSD.

Pod length and pod width determined by measuring one representative pod of main inflorescence of each plant (Table x).

Table 44. Mean number of pod length and pod width of the accessions.

Accessions	**Pod length**		**Pod width**	
	Field A	Field B	Field A	Field B
	cm	cm	cm	cm
LIB209	5,71 bcd	5,79 cd	1,24 cd	1,15 cd
LIB212	5,90 bc	6,60 b	1,32 bc	1,29 abc
LIB214	6,09 bc	6,21 bc	1,44 ab	1,35 a
LIB219	5,04 de	6,01 bcd	1,17 cd	1,17 bcd
LIB220	6,29 ab	6,04 bcd	1,34 bc	1,18 bcd
LIB221	5,31 cde	-	1,32 bc	-
LIB222	4,77 ef	5,53 cd	1,09 d	1,11 de

Branco	5,66 bcd	5,26 d	1,54 a	1,32 a
cv. Multitalia	7,01 a	8,70 a	1,34 bc	1,30 ab
cv. Polo	3,95 f	3,37 e	1,12 d	0,96 e

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD

4.2.1.9 Seed traits

Seed length and seed width of each accession measured by measuring one hundred seeds per accession from the beginning seed lots provided (Table 45).

Table 45. Mean seed length and width of the accessions.

Accessions	**Seed length**	**Seed width**
	mm	mm
LIB209	7,80 fg	6,28 fg
LIB212	8,32 ef	6,58 efg
LIB214	9,28 c	7,52 bc
LIB219	8,48 de	6,84 def
LIB220	10,00 ab	7,48 bcd
LIB221	9,00 cd	7,12 cde
LIB222	7,44 gh	5,10 h
Branco	9,40 bc	7,86 b
cv. Multitalia	10,12 a	9,54 a
cv. Polo	6,96 h	6,12 g

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

4.2.1.10 Traits related to yield and biomass production

Traits that relate to seed yield and biomass production were recorded (Tables 46-53). For measuring the number of seeds per pod, one representative pod of the main inflorescence per plant was measured. For the hundred seed weight, the average of two random lots per accession and location was measured.

Table 46. Mean number of pods per plant of each accession.

Accessions	**Pods/plant** Field A	Field B
LIB209	8,42 ab	9,04 a
LIB212	5,55 abc	7,87 ab
LIB214	10,84 a	7,76 ab
LIB219	1,92 c	7,29 ab
LIB220	5,17 abc	9,33 a
LIB221	6,45 abc	-
LIB222	9,60 a	10,23 a
Branco	6,47 abc	4,94 bc

cv. Multitalia	2,05 c	8,39 a
cv. Polo	2,84 bc	2,31 c

Values are given as means in columns with different letters indicated significant differences among accessions at *p* ≤ 0,05 by *Tukey's* HSD.

Table 47. Mean number of pods in main, first order and second order of flowering.

	Number of pods in main inflorescence		**Number of pods in first order flowering**		**Number of pods in second order flowering**	
Accessions	Field A	Field B	Field A	Field B	Field A	Field B
LIB209	3,83 a	6,08 ab	4,39 ab	2,66 ab	0,19 b	0,27 a
LIB212	2,85 abc	6,83 ab	2,73 abc	1,03 b	0,00 b	0,00 b
LIB214	2,54 abc	6,19 ab	4,94 a	1,60 ab	2,75 a	0,06 ab
LIB219	2,62 abc	5,71 ab	0,44 c	1,46 ab	0,00 b	0,00 b
LIB220	4,03 a	7,89 a	1,34 bc	1,43 b	0,00 b	0,00 b
LIB221	2,66 abc	-	3,20 abc	-	0,58 ab	-
LIB222	3,51 ab	7,87 a	4,99 ab	2,37 ab	1,50 ab	0,00 b
Branco	3,28 ab	2,59 c	2,58 abc	2,08 ab	0,61 ab	0,07 ab
cv. Multitalia	2,15 bc	4,95 b	0,13 c	3,38 a	0,00 b	0,06 ab
cv. Polo	1,48 c	1,54 c	1,34 bc	0,73 b	0,02 b	0,00 ab

Values are given as means in columns with different letters indicated significant differences among accessions at *p* ≤ 0,05 by *Tukey's* HSD.

Table 48. Mean above ground plant weight, above ground plant weight without pods and dry plant weight without pods.

	Above ground plant weight		**Above ground plant weight without pods**		**Dry plant weight without pods**	
Accessions	Field A	Field B	Field A	Field B	Field A	Field B
	g	g	g	g	g	g
LIB209	8,30 bc	6,68 bc	3,98 bc	2,79 cd	2,59 b	2,37 cd
LIB212	5,07 c	7,41 bc	2,34 c	2,52 cd	1,79 b	2,14 cd
LIB214	18,01 a	9,16 b	8,29 ab	3,80 bc	6,83 a	3,29 bc
LIB219	0,08 c	5,86 bc	0,09 c	2,40 cd	0,06 b	1,87 cd
LIB220	3,94 c	7,03 bc	1,50 c	2,84 cd	1,37 b	2,46 cd
LIB221	5,56 bc	-	2,02 c	-	1,74 b	-
LIB222	6,42 bc	7,19 bc	2,87 c	2,77 cd	2,56 b	2,28 cd
Branco	12,79 ab	7,48 bc	8,59 a	5,02 b	6,70 a	4,44 b
cv. Multitalia	5,22 c	23,45 a	2,30 c	7,82 a	1,98 b	7,22 a
cv. Polo	1,79 c	1,69 c	0,82 c	0,80 d	0,75 b	0,73 d

Values are given as means in columns with different letters indicated significant differences among accessions at *p* ≤ 0,05 by *Tukey's* HSD.

Table 49. Mean root fresh and dry weight.

	Root fresh weight		**Root dry weight**	
Accessions	Field A	Field B	Field A	Field B

	g	g	g	g
LIB209	1,06 bc	0,63 b	0,83 bc	0,56 bc
LIB212	0,59 c	0,70 b	0,49 c	0,61 bc
LIB214	2,00 ab	0,94 b	1,80 ab	0,83 bc
LIB219	0,07 c	0,51 b	0,06 c	0,43 bc
LIB220	0,33 c	0,78 b	0,30 c	0,69 bc
LIB221	0,41 c	-	0,25 c	-
LIB222	0,36 c	0,60 b	0,35 c	0,52 bc
Branco	2,41 a	1,11 b	2,10 a	1,00 b
cv. Multitalia	1,07 bc	3,37 a	0,88 c	3,00 a
cv. Polo	0,40 c	0,26 b	0,40 c	0,22 c

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 50. Mean number of seeds in main and first order of flowering.

	Number of seeds in main inflorescence		**Number of seeds in first order flowering**	
Accessions	Field A	Field B	Field A	Field B
LIB209	9,26 a	14,01 c	9,58 ab	3,38 b
LIB212	6,18 ab	16,76 abc	5,73 abc	2,24 b
LIB214	5,60 ab	15,57 bc	12,71 a	3,04 b
LIB219	5,10 ab	17,64 abc	0,00 c	2,59 b
LIB220	6,93 ab	10,94 cd	1,72 bc	1,84 b
LIB221	6,40 ab	-	7,41 abc	-
LIB222	8,28 a	21,88 ab	14,05 a	4,67 b
Branco	6,32 ab	5,31 de	4,41 abc	3,08 b
cv. Multitalia	6,92 a	22,97 a	0,37 c	11,44 a
cv. Polo	2,76 b	2,65 e	1,92 bc	1,43 b

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 51. Mean number of seeds per pod and number of seeds per plant of the accessions.

	Seeds/pod		**Number of seeds per plant**	
Accessions	Field A	Field B	Field A	Field B
LIB209	3,17 ab	3,23 c	19,16 abc	17,64 cd
LIB212	2,91 bcd	3,43 bc	11,84 cde	19,01 c
LIB214	3,07 abc	3,09 c	26,09 a	18,66 c
LIB219	2,55 bcd	3,41 bc	3,33 e	20,24 c
LIB220	2,72 bcd	2,25 d	8,12 de	12,79 de
LIB221	2,94 a-d	-	14,85 bcd	-
LIB222	3,33 ab	4,14 b	26,07 ab	26,55 b
Branco	2,32 cd	2,06 d	11,64 cde	8,47 ef

cv. Multitalia	3,71 a	4,95 a	7,14 de	34,56 a
cv. Polo	2,11 d	1,84 d	4,54 de	4,10 f

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

Table 52. Mean hundred seed weight and harvest index (HI) of the accessions.

Accessions	**Weight of 100 seeds**		**Harvest index (HI)**	
	Field A	Field B	Field A	Field B
	g	g	g	g
LIB209	10,20 def	9,13 cd	0,26 ab	0,24 c
LIB212	11,43 cde	12,21 bcd	0,27 ab	0,27 bc
LIB214	15,15 bc	13,09 bcd	0,27 ab	0,28 bc
LIB219	7,65 ef	7,60 d	0,22 bc	0,25 bc
LIB220	14,09 bcd	13,94 bcd	0,23 b	0,23 cd
LIB221	10,34 c-f	-	0,26 ab	-
LIB222	6,75 f	7,86 cd	0,25 ab	0,29 bc
Branco	16,13 b	16,02 b	0,15 c	0,17 d
cv. Multitalia	21,74 a	28,29 a	0,32 a	0,40 a
cv. Polo	9,85 def	15,71 bc	0,22 b	0,34 ab

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

4.2.1.11 Seed crude protein content

The seed crude protein content of each accession for each experimental location is presented in Table 53.

Table 53. Mean seed crude protein content (%) of each accession.

Accessions	**Seed crude protein**	
	Field A	Field B
	%	%
LIB209	43,84 a	42,12 a
LIB212	43,57 ab	42,70 a
LIB214	43,74 a	41,91 ab
LIB219	39,59 d	37,32 bc
LIB220	42,99 b	42,18 a
LIB221	41,68 c	-
LIB222	37,55 e	38,92 abc
Branco	43,45 ab	42,47 a
cv. Multitalia	35,59 f	32,26 d
cv. Polo	31,99 g	34,45 cd

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

4.2.1.12 **Principal Component Analysis (PCA)**

A Principal Component Analysis (PCA) was performed in each one of the two experimental locations. In the analysis, due to the high number of the traits studied, a simplification was needed in which; if a trait had a high positive relation (> 0,80) with another trait, the trait represented in the analysis from the main trait. In Kalamata (Field A) the first three axes explained the 58,69% of the total variability, while in Athens (Field B) the 72,71%, respectively (Table 54). Traits related to the first three principal axes for each location are presented in Table 55, 56.

Table 54. Eigenvalues, percent and cumulative percent of each principal axis for Kalamata (Field A) and Athens exp. 1 (Field B).

Axes	**Field A**			**Field B**		
	Eigenvalue	Percent	Cum Percent	Eigenvalue	Percent	Cum Percent
1	8,5933	25,275	25,275	7,5624	31,510	31,510
2	6,5942	19,395	44,669	5,6973	23,739	55,248
3	4,7666	14,091	58,688	4,1917	17,465	72,714
4	4,3335	12,746	71,434	3,3291	13,871	86,585
5	3,7697	11,087	82,521	1,2242	5,101	91,686
6	2,1368	6,285	88,806	0,9145	3,810	95,496
7	1,8642	5,483	94,289	0,7590	3,162	98,659
8	1,3410	3,944	98,233	0,3219	1,341	100,000
9	0,6008	1,767	100,000			

Table 55. Eigenvectors of traits related to the first three principal axes in Kalamata (Field A).

Traits	**Eigenvectors**		
Plant height after harvest	0,18115	0,2368	-0,1286
Pod length	0,20389	0,23914	0,17814
Seeds per plant	0,16169	-0,0900	0,02835
Mature pod pubescence	0,06316	-0,2307	0,18896
Harvest Index	-0,0174	0,06531	0,2565
Above ground plant weight	0,19795	0,1135	-0,1470
Plant habit	0,04365	-0,0883	-0,1871
Stem formation	0,06477	-0,0429	0,18833
Branching	-0,0870	0,12983	0,03476
Leaflet shape	0,01853	0,27076	0,04077
Central leaflet tip	0,18243	-0,2047	0,0499
Pubescence of leaflet lower surface	-0,2926	0,18698	0,03755
Leaf diameter	0,15151	0,30319	-0,1256
Stipule color	0,01321	0,28826	0,20591

Intensity of stipule color	0,17881	0,19057	0,08655
Petiole color	-0,0307	0,21602	0,19388
Intensity of petiole color	0,12832	0,08676	-0,0882
Green pod pubescence	-0,0732	-0,0420	0,31582
Length of principal inflorescence	0,19171	0,20812	0,14239
Flower length	0,15974	-0,0786	-0,0674
Number of first order side branches with flowers	-0,1291	0,27555	-0,1600
Number of second order side branches with flowers	0,00241	0,02277	-0,3327
Number of leaves on first side branches	-0,1873	0,04219	0,09438
Number of flowers on first side branches	0,19038	-0,0267	0,26585
Flower color	0,16352	-0,1750	0,22044
Time to flowering	0,07901	0,20574	0,1226
Seed shape	-0,2756	0,05274	0,22315
Seed luster	0,30338	0,04838	0,13058
Seed primary color	-0,0111	-0,3170	0,0701
Intensity of seed primary color	-0,1670	0,07458	-0,2662
Seed secondary color distribution	-0,2179	-0,1250	0,04221
Stem waxiness	0,29259	-0,1870	-0,0376
Lodging	0,19798	-0,0309	-0,2955

Table 56. Eigenvectors of traits related to the first three principal axes in Athens (exp. 1) (Field B).

Traits	**Eigenvectors**		
Plant height after harvest	0,34786	0,05138	0,04404
Number of pods on first side branches	0,23221	-0,0748	0,28904
Seeds/ plant	0,18778	-0,1026	0,21896
Plant habit	0,07065	0,32257	-0,2568
Stem color	0,06789	0,17558	0,0898
Intensity of stem color	-0,0521	0,05326	0,41996
Stem waxiness	-0,078	0,3859	0,08209
Leaflet shape	0,24557	-0,105	0,00291
Petiole length	0,25736	-0,2766	0,05222

Intensity of stipule color	0,22649	0,2493	0,06331
Petiole color	0,17749	-0,1414	0,36743
Intensity of petiole color	0,06487	0,21168	0,11949
Green pod pubescence	-0,1816	-0,1949	0,07827
Length of principal inflorescence	0,22038	0,10362	0,30287
Lodging	0,17831	0,16403	-0,1854
Number of leaves on first side branches	0,24597	0,00419	-0,2694
Pubescence of leaflet lower surface	0,07798	-0,3859	-0,0821
Above ground plant weight without pods	0,35259	-0,0408	0,04246
Seed length	0,30183	0,12964	-0,0976
Intensity of seed primary color	-0,0546	-0,0811	-0,2670
Intensity of seed secondary color	-0,2345	-0,1844	0,22414
Seed primary color	-0,2818	0,02011	0,10698
Flower color	-0,1451	0,21826	0,30256

Correlations among main traits obtained from PCA

Greece, as well as all the other Southern countries, focused on seed production and therefore correlations of main traits with the number of seeds per plant are presented for Field A and B separately (Tables 57). Seed protein content was also included aiming to define the possibility of a negative correlation to seed yield traits that could affect selections for increased seed yield and high seed protein content.

Table 57. Correlations among main traits in the experimental Field B (Athens exp 1).

Correlations (Pairwise method) Kalamata	leaves in first order branches	Days to flowering	Seed protein content	Diameter of the leaf	first order side branches	Petiole length	Length of first order side branches	pods of first order side branches	# of seeds of first order side branches	# of seeds in MF	Pods per plant	Pod length	Seeds per pod	Weight of seeds/ plant	Over ground weight of the plant after harvest	Seeds per plant
# of leaves in first order branches	1,000															
Days to flowering	0,488	1,000														
Seed protein content	-0,541	0,021	1,000													
Diameter of the leaf	-0,198	0,339	0,483	1,000												
# of first order side branches	0,371	0,205	-0,253	0,567	1,000											
Petiole length	0,282	0,619	0,111	0,682	0,618	1,000										
Length of first order side branches	-0,333	0,374	0,726	0,698	0,061	0,380	1,000,									
# of pods of first order side branches	-0,500	-0,250	0,404	0,003	-0,211	-0,427	0,455	1,,000								
# of seeds of first order side branches	-0,506	-0,208	0,311	-0,08	-0,296	-0,484	0,395	0,982	1,000							
# of seeds in MF	-0,314	0,240	0,452	-0,024	-0,370	-0,003	0,399	0,511	0,526	1,000						
Pods per plant	-0,504	-0,116	0,504	0,053	-0,307	-0,334	0,593	0,967	0,957	0,547	1,000					
Pod length	-0,319	0,446	0,482	0,647	-0,037	0,581	0,579	-0,075	-0,065	0,438	0,057	1,000				
Seeds per pod	-0,453	0,186	0,082	0,061	-0,303	-0,010	0,181	0,316	0,420	0,708	0,331	0,608	1,000			
Weight of seeds/plant	-0,596	0,022	0,427	0,451	-0,035	0,062	0,684	0,693	0,703	0,252	0,773	0,387	0,401	1,000		
Over ground weight of the plant after harvest	-0,448	0,165	0,521	0,562	0,117	0,183	0,907	0,985	0,658	0,235	0,772	0,330	0,212	0,947	1,000	
Seeds per plant	-0,525	-0,086	0,354	-0,020	-0,334	-0,382	0,490	0,952	0,983	0,587	0,966	0,077	0,510	0,774	0,721	1,000

Correlations (Pairwise method) _Athens	# of leaves in first order branches	Days to flowering	Seed protein content	Diameter of the leaf	# of first order side branches	Petiole length	Length of first order side branches	# of pods of first order side branches	# of seeds of first order side branches	# seeds in MF	Pods per plant	Pod length	Seeds per pod	Weight of seeds/ plant	Over ground weight of the plant after harvest	Seeds per plant
# of leaves in first order branches	1,000															
Days to flowering	0,672	1,000														
Seed protein content	-0,130	0,185	1,000													
Diameter of the leaf	0,424	0,717	-0,052	1,000												
# of first order side branches	0,618	0,475	-0,517	0,718	1,000											
Petiole length	0,509	0,758	0,024	0,982	0,695	1,000										
Length of first order side branches	0,517	0,942	0,272	0,607	0,372	0,659	1,000									

# of pods of first order side branches	0,057	0,574	-0,216	0,490	0,468	0,426	0,663	1,000								
# of seeds of first order side branches	0,304	0,534	-0,555	0,688	0,793	0,631	0,531	0,838	1,000							
# of seeds in MF	-0,202	0,217	-0,199	0,539	0,293	0,435	0,243	0,552	0,637	1,,000						
Pods per plant	-0,491	0,215	0,270	0,419	-0,105	0,348	0,349	0,499	0,327	0,,780	1,,000					
Pod length	0,279	0,683	-0,114	0,949	0,623	0,905	0,604	0,610	0,769	0,,752	0,,594	1,,000				
Seeds per pod	-0,050	0,280	-0,373	0,598	0,531	0,488	0,590	0,681	0,807	0,,952	0,,630	0,,775	1,,000			
Weight of seeds/plant	0,358	0,528	-0,222	0,807	0,843	0,762	0,467	0,697	0,960	0,,611	0,,292	0,,843	0,,763	1,,000		
Over ground weight of the plant after harvest	0,413	0,668	-0,405	0,856	0,806	0,829	0,622	0,746	0,951	0,,612	0,,356	0,,893	0,,745	0,,981	1,,000	
Seeds per plant	-0,050	0,346	-0,336	0,639	0,490	0,541	0,364	0,703	0,818	0,,964	0,,697	0,,825	0,,987	0,,784	0,,782	1,,000

4.2.1.13 **Comparison between the experimental locations**

Due to statistically significant heterogeneity of the experimental error between the two experimental fields and the different sets of observations between the two experimental fields used, a combined analysis over location used the Mixed Model Analysis to define the effects of random effects. Specifically, the following model was adapted:

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

where y_{ijk} (i = 1, 2, 3, 4, 5, 6, 7, 8, 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 Xiyuan et al. (2013).
For the estimation of variance components, the REML (Restricted Maximum Likelihood) method was used. Some selected traits studied were those for which the method could be applied (Table 58).

Table 58. *P*- values for location, accession and their interaction for each trait studied, derived from the Mixed Model Analysis by the REML.

Traits	**Location**	**Accession**	**Accession x Location**
Diameter of leaf	0,3567	< **0,0001**	< **0,0001**
Stem thickness	0,7821	< **0,0001**	< **0,0001**
Petiole length	0,1882	< **0,0001**	< **0,0001**
Length of flower	0,7762	< **0,0001**	< **0,0001**
Plant height after harvest	0,6777	0,2619	0,3040
Number of pods in main inflorescence	0,0003	< **0,0001**	<**0,0001**
Number of pods per plant	0,0262	< **0,0001**	< **0,0001**
Number of seeds in main inflorescence	0,0004	< **0,0001**	< **0,0001**
Number of seeds per plant	0,0305	< **0,0001**	< **0,0001**
Above ground plant weight	0,4586	< **0,0001**	< **0,0001**
Hundred seed weight	< **0,0001**	< **0,0001**	< **0,0001**

4.2.1.14 **Remarks and Conclusions all accessions**

- Regarding stem formation LIB212 presented a high variability as characterized by both not prominent and prominent plants. LIB220 also presented some variability.

- A higher variability in stem formation presented in Athens than in Kalamata's experimental field. None of the Andean lupin accessions presented solely prominent stem formation.
- Regarding Andean lupin accessions, LIB209 was characterized by higher within variability, as presented variability regarding many qualitative traits studied as in seed shape, seed secondary seed color, and petiole color. Observations showed that this accession is composed of two different morphotypes.
- The rest of the Andean lupin accessions used were characterized by homogeneity regarding most of the quantitative traits studied with the exception of LIB209 as mentioned above.
- Among the Andean lupin accessions, Branco, LIB209, LIB212, and LIB214 had the thickest stems, resulting in the best genotypes for breeding programs targeting biomass.
- Branco and cv. Multitalia were the latest flowering accessions, while cv. Polo, LIB221 (in Kalamata), and LIB222 (in Athens) were the earliest flowering ones.
- In the present study, the number of first-order side branches with flowers for the Andean lupin accessions ranged from 0,65 to 2,75. The number of second-order side ranged from 0 to 4, while the corresponding number in the study by Clements et al. (2008) ranged from 0 to 3,3. Andean lupin is susceptible both to the presence of frost (Neves-Martins et al., 2016) during vegetative growth and drought stress during flowering that prevailed during this study in both experimental locations in Greece.
- Lodging sensitive accessions (from the most sensitive to less sensitive) were LIB221, LIB214, LIB219, LIB222, LIB220, LIB212, Branco, and LIB220. Consequently, Andean lupin was found more susceptible to lodging than *L. angustifolius* and *L. albus* under the present study cultivation conditions.
- The number of pods per plant for the Andean lupin accessions ranged from 1,92 to 10,84 which is a lower value than the observations of Clements et al. (2008) (11 to 20 pods per Andean lupin plant). LIB222, LIB214, LIB212, LIB209, gave a higher number of branches at the experimental field of Kalamata, as well as Branco, LIB222, LIB209, LIB214 gave a higher number of seeds per plant in Kalamata compared to Athens.
- The number of seeds on the main stem and the number of seeds on first-order side branches contributed strongest the final seed yields, which is in agreement with the observation of Neves-Martins et al. (2016) where the main stem and the first-order side branches produced the highest seed numbers under Portuguese crop conditions, contributing 95% to total seed yield. Neves-Martins (1992) stated that under European climatic conditions (particularly in Portugal) late maturating Andean lupin accessions show higher seed yield, which was observed in the case of LIB214 in our study. Finally, seed yield of Andean lupin was low in both experimental fields, which is consistent with the observation of Talhinhas et al. (1999) that the production of Andean lupin is relatively low and with the findings of Caligari et al. (2000) that the yields of Andean lupin in Europe are much lower than those of other leguminous crops.

- The harvest index of Andean lupin accessions ranged from 0,15 to 0,29. These findings are in agreement with Clements et al. (2008) that reported a harvest index of Andean lupin genotypes ranging from 0,13 to 0,33. The harvest index for Andean lupin accessions presented lower than the two other lupin species implemented in this study.
- Seed color is probably associated with plants anthocyanin's, as LIB209, LIB212, LIB214, and LIB222 which had red color in the hypocotyl and the stem were those that presented a variety of seed colors having, all except for LIB214, secondary seed colors.
- Generally, plants in Kalamata appeared more vigorous and had better growth than in Athens. However, a higher coefficient of variation recorded for most quantitative traits of plants in Kalamata (Field A) than in Athens (Field B).
- The highest seed yielded Andean lupin accessions in Athens were LIB214 and LIB209, produced 1167,63 kg ha^{-1}, and 1159,77 kg ha^{-1}, respectively, while the lowest was LIB219 that produced 1122,39 kg ha^{-1}. In Kalamata, the highest yielded accession was LIB209 203,44 kg ha^{-1}followed by Branco (201.09 kg ha^{-1}), while again LIB219 produced the lowest amount of seeds (160,33 kg ha^{-1}).
- A high variability was observed for many quantitative traits, such as number of pods on the first and second order of branches, number of seeds on first order of branches, number of pods per plant, and number of seeds per plant, that are primarily correlated to seed yield. So, there are many promising but also less promising plants within many accessions available for further selection aiming to increase seed yield.
- LIB209, Branco and LIB212 were characterized by high seed protein content (%), while LIB219 and LIB222 by low, among the Andean lupin accessions tested.
- A higher overall variability was found explained by the first three primary axes through PCA in Athens (72,71%) in comparison to Kalamata (58,69%).
- More traits contributed to the variability explained from the first three axes in Kalamata in comparison to Athens experimental field.
- No significant negative correlation was obtained between seed protein content and the number of seeds per plant.
- Strong positive correlations were obtained between number of seeds per plant and pods per plant, seed weight per plant, above-ground biomass weight, number of pods on first side branches and number of seeds on first side branches in both experimental locations.
- A significant interaction of location x accession was recorded, while there were significant differences among the accessions for many traits, therefore probably the selection should focus in promising Andean lupin accessions for each location.

4.2.2 First cropping season Portuguese collection, 2016-2017

A specific analysis was performed regarding the Portuguese entries only during 2016-2017 (Athens exp. 2), as they were mainly considered as landrace material, without any formal breeders' selection so far. Transplantation of plants in their final position on the field was performed on the 6th of March 2017. Therefore, were probably consisting of mixtures of populations. In this respect, forty-eight agro-morphological traits were recorded according to the IBGRI descriptors list for Lupin (IBPGR, 1981) as well as seventeen traits proposed from LIBBIO protocol (presented *in italics*) regarding all developmental stages of the plants. The traits recorded are presented in Appendix II.I. All traits were recorded for each one of the forty-five plants per accession.

All continuous traits were transformed into ordinal by dividing their range into equal classes and thus formed discrete ranks (Terzopoulos et al., 2008; Lazaridi et al., 2016). The frequency of each rank within each trait was calculated. Data were used to characterize the entire collection and each population separately, as well as for the calculation of phenotypic diversities, based on *Nei's* genetic diversity index (*He*) (Nei, 1973). For each trait, total phenotypic diversity of the collection (*Ht*), inter- population (*Gst*) and intra-population phenotypic diversity (*Hs*), as well as its average across all populations ($\dot{H}s$) were calculated. Mean phenotypic diversity within each population across all traits ($\dot{H}p$) was also calculated and used for the estimation of heterogeneity of each population. Comparisons of all populations $\dot{H}p$ were carried out applying Duncan's multiple range test ($p \leq 0.05$) using the statistical program (Statgraphics Centurion, Version 1.0.1.C). Principal component analysis (PCA) was also performed in order to examine the contribution of each trait to the total diversity using the statistical software JMP-8 (SAS Institute Inc., 2008).

4.2.2.1 **Characterization of Portuguese collection**

Days to 50% emergence of seeds ranged from 12 to 16 days, with LIB208 to be the latest one to emergence, followed by cv. Multitalia (14 days), while all the other accessions had 50% germination within 12 days from sowing. All the plants in the collection had an herb growth habit (100%), while their plant habit was characterized mostly as semi-erect (59,36%) to erect (38,16%). In most of the plants, the stem was prominent (73,40%), while most of them were glabrous (74,19%). The majority of plants were characterized by green stem color (99,65%) and pale intensity (95,74%). Stem waxiness was present for 61,79% of the plants and absent for 38,21%. Stem thickness of the collection characterized as medium (56,88%), while 84,82% of the plants were not branched, with most of the plants branched to have 1 (5,94%) or 3 branches (5,94%).

The diameter of the leaves was characterized as medium (74,18%) and all the leaves as acuminate (100%) and by an elliptical shape (100%). Leaf pubescence was absent in the upper surface of all leaves, while 25,81% presented to be pubescent in the lower surface, Most of the plants had 578,68-824,01 leaflets. The green color was the main leaf color (79,84%) with medium intensity (43,62%), while some plants had yellow leaves (20,16%). Petiole length for most of the plants ranged from 3,67 to 5,33 cm and was characterized as medium and had green color (93,45%) with pale intensity (72,73%). Hypocotyl color was either green (77,45%) or red (22,45%).

Flower wing and keel color were mainly white for 96,42% and 88,21%, respectively. The marginal band of the standard petal was white in all plants (100%), while the color of central spots of the standard was either yellow (61,07%) or white (38,93%) with the pale intensity of the color (100%). Intermediate region color of the standard was characterized primarily as white (74,64%) with pale intensity (92,55%). The length of principal inflorescence was medium (9,67-19,33 cm) and regarding the main inflorescence, the height from the ground was small (52,68%) with only 1,26% of the plants being characterized from a large height.

The number of pods per plant ranged from 0 to 6 for 56,52% of the plants, followed by 38,65% that presented 7 to 13 pods. Most of the pods (69,42%) had 4,3-7 cm length and

1,07-1,63 cm width (93,20%). Also, most of them had light pubescence at maturity (73,30%) and did not present shattering (91,22%) or shedding (100%).

Seed shape was variable, with 39,08% of the seeds to be oval, 23,56% flattened spherical, 21,26% flattened oval, 14,37% cuboid, and 1,15% flattened cuboid and 0,57% spherical. Seed color was primarily white (98,85%) while some seeds had brown color (1,15%). Seed secondary color was absent in 77,91% of the seeds with 22,09% of the seeds to present white or brown secondary coloration. The secondary color distribution was either moustache (96,88%) or formed an eyebrow (3,13%).

Days to first flowering were 97-118 for most of the plants (52,72%), while 33,80% was characterized by early flowering, thus need only 75-96 days till flowering. The growth rate of the collection recorded as normal for 64,91% of the plants and seedling growth type was erect in most cases (86,77%). Plant height was characterized as small, medium, and large for 33,69%, 37,78%, and 28,33% of the plants, respectively.

The number of seeds in primary pods ranged from 0 to 7, with most pods to include 7 seeds (34,16%). The number of pods in the main inflorescence recorded was 7-13 to 49,76% of the plants, while the number was very small (0-6) to 45,37%. The number of pods in the 1st order of inflorescence was remarkably smaller than on the main inflorescence and ranged from 1 to 6, with most of the plants to present 1-2 pods of 1st order. The number of seeds in the main inflorescence as well as in the 1st order of flowering characterized as small and ranged from 0 to 11 and 1 to 2, respectively. Finally, 50% of the plants in the collection had 0-11 healthy seeds.

Total plant fresh weight of the collection was small (0,49-8,15 g) (59,80%), as well as stem fresh weight (0,34-7,64 g) (55,81%) and dry weight (0,32-6,35 g) (66,80%). Root fresh and dry weight was also characterized as small. Seed weight per plant in most of the plants ranged from 0,000 to 1,763 g and characterized as small. Hundred seed weight was characterized as medium (39-77 g) for 58,55%.

4.2.2.2 Phenotypic diversity

Seed shape and number of seeds per pod were the traits that contributed most to the total phenotypic diversity of the collection (Ht = 0,726 and 0,766, respectively). The number of seeds per pod was also the trait that ranged the most within populations (Hs = 0,715) (Table 59.). The highest mean phenotypic diversity within each population across all traits ($\dot{H}p$ = 0,26) presented by LIB201 and LIB203 (Table 60).

Table 59. Total phenotypic diversity (*Ht*), mean intra- population diversity (*Hs mean, Hs max* and *Hs min*) and among populations diversity (*Gst*) recorded in the collection.

Traits	*Ht*	*Hs (mean)*	*Hs (min)*	*Hs (max)*	*Gst*
Growth habit	0,223	0,068	0,000	0,345	0,718
Plant habit	0,501	0,422	0,310	0,555	0,159
Stem formation	0,395	0,335	0,089	0,475	0,144
Stem pubescence	0,388	0,015	0,000	0,060	0,962
Stem color	0,007	0,006	0,000	0,045	0,195
Intensity of stem color	0,082	0,057	0,000	0,451	0,309
Stem waxiness	0,473	0,007	0,000	0,056	0,985
Stem thickness	0,548	0,465	0,342	0,525	0,151
Branching	0,257	0,237	0,000	0,391	0,083

Primary branch	0,273	0,252	0,000	0,428	0,077
Diameter of leaf	0,416	0,209	0,000	0,459	0,496
Leaflet shape	0,000	0,000	0,000	0,000	0,000
Central leaflet tip	0,000	0,000	0,000	0,000	0,000
Pubescence of leaflet upper surface	0,000	0,000	0,000	0,000	0,000
Pubescence of leaflet lower surface	0,383	0,015	0,000	0,061	0,962
Leaflet number per plant	0,486	0,036	0,000	0,280	0,926
Leaf color	0,322	0,277	0,000	0,497	0,142
Intensity of leaf color	0,630	0,526	0,000	0,647	0,166
Petiole length	0,595	0,000	0,000	0,000	1,000
Petiole color	0,124	0,117	0,000	0,274	0,048
Intensity of petiole color	0,404	0,256	0,056	0,464	0,365
Flower wing color	0,069	0,051	0,000	0,408	0,261
Intensity of flower wing color	0,000	0,000	0,000	0,000	0,000
Flower keel color	0,208	0,115	0,000	0,495	0,447
Intensity of flower keel color	0,000	0,000	0,000	0,000	0,000
Marginal band color of standard	0,000	0,000	0,000	0,000	0,000
Color of central spots of standard	0,476	0,018	0,000	0,049	0,975
Intensity of color of central spot of standard	0,000	0,000	0,000	0,000	0,000
Intermediate region color of standard	0,379	0,377	0,000	0,750	0,005
Intensity of intermediate region color of standard	0,138	0,104	0,000	0,397	0,248
Length of principal inflorescence	0,594	0,515	0,399	0,649	0,133
Pod number per plant	0,528	0,432	0,062	0,654	0,183
Pod length	0,471	0,415	0,132	0,609	0,129
Pod width	0,129	0,098	0,000	0,290	0,236
Mature pod pubescence	0,427	0,406	0,208	0,568	0,050
Pod shattering	0,000	0,000	0,000	0,000	0,000
Pod shedding	0,000	0,000	0,000	0,000	0,000
Seed shape	0,726	0,387	0,000	0,662	0,467
Seed primary color	0,023	0,021	0,000	0,165	0,090
Seed secondary color	0,349	0,154	0,000	0,438	0,558
Seed secondary color distribution	0,295	0,132	0,000	0,272	0,554
Hypocotyl color	0,348	0,223	0,000	0,450	0,359
Days to first flowering	0,558	0,202	0,000	0,495	0,639
Growth rate	0,517	0,487	0,329	0,548	0,056
Seedling growth type	0,230	0,011	0,000	0,045	0,952
Plant height	0,662	0,467	0,043	0,648	0,295
Height of flowering in every order of flowering (from soil to lowest flower on fully	0,511	0,325	0,000	0,470	0,363

developed flowering)-main flowering					
Height of flowering in every order of flowering (from soil to lowest flower on fully developed flowering)-1st order flowering	0,000	0,000	0,000	0,000	0,000
Seeds per pod (1 primary pod)	0,766	0,715	0,643	0,778	0,066
Number of pods in main inflorescence	0,544	0,337	0,000	0,660	0,382
Number of pods in first order of pod setting	0,000	0,000	0,000	0,000	0,000
Number of seeds in main inflorescence	0,489	0,408	0,000	0,648	0,167
Number of seeds in first order of pod setting	0,000	0,000	0,000	0,000	0,000
Number of seeds in second order of pod setting	0,000	0,000	0,000	0,000	0,000
Healthy seeds per plant	0,567	0,486	0,350	0,640	0,142
Total plant fresh weight	0,515	0,422	0,000	0,595	0,182
Stem fresh weight	0,535	0,407	0,000	0,599	0,240
Stem dry weight	0,460	0,339	0,000	0,505	0,263
Root fresh weight	0,179	0,146	0,000	0,343	0,185
Root dry weight	0,117	0,091	0,000	0,261	0,227
Seed weight per plant	0,319	0,267	0,000	0,517	0,156
Hundred seed weight	0,531	0,456	0,401	0,501	0,142

Table 60. Means comparison for phenotypic diversity within each population across all traits ($\dot{H}p$).

Accessions	Mean
LIB201	0,26 b
LIB203	0,26 b
LIB206	0,23 b
LIB208	0,21 ab
LIB217	0,23 b
LIB224LI	0,21 ab
cv. Multitalia	0,17 ab
cv. Polo	0,13 a

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0,05$ by *Tukey's* HSD.

4.2.2.3 **Principal Component Analysis (PCA)**

The first three principal axes of PCA explained 51,61% of the total phenotypic diversity (Table 61). In the first principal axis were related traits such as petiole color and leaflet shape, while in the second, traits like seed shape, seed secondary color, and leaf diameter. Seed yield traits were related to the first and third axes (Table 62). Andean lupin populations discriminated from the other lupin species, with the exception of LIB208 (Figure 10).

Table 61. Eigenvalues, percent and cumulative percent of each principal axis for Portuguese accessions.

Axes	Eigenvalue	Percent (%)	Cum. percent (%)
PCA1	16,7875	29,978	29,978
PCA2	6,5952	11,777	41,755
PCA3	5,5225	9,862	51,616

Table 62. Eigenvectors of traits with relation with the first three PCA components.

Traits	PCA1	PCA2	PCA3
Growth habit	-0,01592	0,32736	-0,13034
Plant habit	0,11892	0,04382	-0,04358
Stem formation	0,06534	-0,00591	0,17418
Stem pubescence	-0,05519	0,18959	-0,04949
Stem color	0,1536	0,26135	0,09599
Intensity of stem color	0,10684	0,21793	0,20384
Stem waxiness	0,13648	-0,12467	-0,13954
Stem thickness	0,17183	0,04675	-0,09759
Branching	0,04336	-0,00077	0,04435
Primary branch	0,04365	0,02271	0,01404
Diameter of leaf	0,14095	0,11913	-0,19629
Leaflet shape	0,10957	0,16246	-0,02127
Central leaflet tip	0,161	0,25854	0,09184
Pubescence of leaflet lower surface	-0,05519	0,18959	-0,04949
Leaflet number per plant	0,12788	0,01863	-0,25929
Leaf color	0,07922	0,1298	0,0207
Intensity of leaf color	0,08783	0,16656	-0,08049
Petiole length	0,12208	0,14632	-0,19335
Petiole color	0,13485	0,16941	0,10494
Intensity of petiole color	0,06491	0,13326	0,23185
Flower wing color	0,05148	0,00107	0,01094
Flower keel color	0,04232	0,00121	0,08809
Intensity of flower keel color	0,17159	0,23334	0,10497
Marginal band color of standard	0,15029	-0,05485	-0,01663
Color of central spots of standard	0,17168	0,23345	0,10452
Intermediate region color of standard	0,09355	-0,04948	-0,06016
Intensity of intermediate region color of standard	0,14376	0,15891	0,04599
Length of principal inflorescence	0,18175	-0,03973	-0,00442
Pod number per plant	0,17749	-0,15255	-0,0203
Pod length	0,19988	-0,09965	0,10202
Pod width	0,19445	-0,06531	0,13172
Mature pod pubescence	0,13195	-0,06996	0,16456
Pod shattering	0,02971	-0,02887	0,06376
Seed shape	0,15163	-0,062	0,16522

Seed primary color	0,1072	-0,06403	0,09163
Seed secondary color	-0,01774	0,02228	0,29493
Seed secondary color distribution	-0,03223	0,03005	0,32303
Hypocotyl color	-0,01058	0,21652	-0,09787
Days to first flowering	0,09381	0,21407	-0,06155
Growth rate	0,12107	0,10265	-0,00757
Seedling growth type	0,12868	-0,1867	0,20272
Plant height	0,21402	-0,0069	-0,0146
Height of flowering in every order of flowering (from soil to lowest flower on fully developed flowering)-main	0,20794	0,0308	-0,05827
Seeds per pod	0,13938	-0,09336	0,18612
Number of pods in main inflorescence	0,17437	-0,15154	-0,00416
Number of pods in first order of pod setting	0,06548	-0,02449	-0,12349
Number of seeds in main inflorescence	0,16254	-0,15147	0,03762
Number of seeds in first order of pod setting	0,04554	-0,00542	-0,06944
Healthy seeds per plant	0,16218	-0,14731	0,03526
Total plant fresh weight	0,20065	-0,13636	-0,10569
Stem fresh weight	0,166	-0,07277	-0,20949
Stem dry weight	0,164	-0,07267	-0,20695
Root fresh weight	0,17371	-0,05998	-0,20128
Root dry weight	0,17431	-0,05675	-0,20103
Seed weight per plant	0,17253	-0,11768	0,1141
Hundred seed weight	0,16715	-0,07103	0,10348

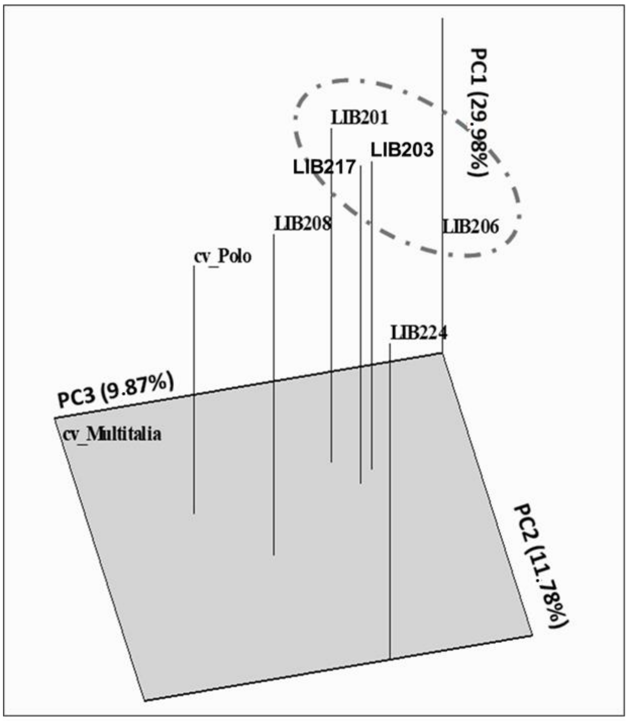


Figure 10. Grouping of Andean lupin Portuguese entries.

4.2.2.4 Remarks and Conclusions

- A remarkable amount of among and within diversity defined, therefore these populations consist a valuable source of desirable traits for breeding.
- The highest mean phenotypic diversity within each population across all traits ($\dot{H}p$ = 0,26) presented by LIB201 and LIB203, which means that they were the populations containing more variability within them.
- LIB208 presented to differentiate from the other Portuguese entries.
- LIB208 presented to be more homogeneous ($\dot{H}p$ = 0,21) than the other Andean lupin assessed.

4.2.3 Second cropping season, 2017-2018

During cropping season 2017-2018 and 2018-2019, screening of Task 2.1 was performed in parallel to Task 2.2. in three experimental locations (Athens, Kalamata, Erythres). Four experimental fields (Athens, Kalamata-Aithaia, Kalamata-TEI, and Erythres) were established during 2017-2018. In this report, the plants that were cultivated during early sowing treatments are presented, the early sowing allowed plants to fully develop and express their characteristics. During the late sowing of Kalamata-TEI many plants did not manage to reach maturity and to be productive due to high temperatures at the beginning of spring and soil $CaCO_3$. Therefore, results were not considered valid due to the low number of plants and observations received regarding the characterization of the accessions.
Transplantation took place on the 5th and 6th of December in Erythres, on the 12th and 13th of December in Kalamata-Aithaia, and on the 30th of November in Athens in 2017-2018. Sowing took place in Kalamata-TEI on the 23rd of January 2018. During 2018-2019, two experimental fields were established (Athens, Kalamata-TEI), where transplantation in Athens took place on the 7th of November 2018, while sowing in Kalamata-TEI on 14th of November 2018 and resowed on 5th of December 2018.
In 2017-2018, twenty-six qualitative traits were recorded, named stem color at the vegetative stage, cotyledon color, leaf color, the intensity of leaf color, leaflet shape, central leaflet tip, growth habit, plant habit, stem color at flowering phase, stem color intensity, stem waxiness, stem pubescence, pubescence leaflet upper surface, pubescence leaflet lower surface, petiole color, petiole color intensity, stipule color, stipule color intensity, stem formation, presence of leaves at maturity, color of flower (type), pod shattering, seed shape, seed primary color, seed secondary color and seed pattern. Methods and protocol used were the same implemented in the first cropping season in Experiment 1 in Athens and in Kalamata.

4.2.3.1 Growth habit and cotyledons

All plants characterized by "herb" growth habit in all experiments. All Andean lupin accessions characterized by stem waxiness and absence of stem pubescence with the exception of LIB209 that presented 33,33% of plants that had pubescence in their stems and light pubescence in their leaflets lower surface. Cotyledon color recorded for all accessions and for each experimental location in the greenhouse, with the exception of the field Kalamata-TEI. Cotyledon color percentages for each accession for the plants transplanted are presented in Table 63.

Table 63. Percentages (%) of cotyledon color for each accession for fields (Athens, Kalamata-Althaea, Erythres).

Accessions	Yellow	Green	Grey	Green-Red	Green-Red margin
	%	%	%	%	%
LIB200		69,44			30,56
LIB209		63,98		5,56	30,56
LIB213		97,22			2,78
LIB214		94,44			5,56
LIB218		80,56		5,56	13,89
LIB220		100,00			
LIB221		100,00			
LIB222		94,44			5,56
Branco		88,89			11,11
LIB224		100,00			
cv. Multitalia		100,00			
cv. Polo		97,22			2,78

4.2.3.2 **Athens**

In the following tables qualitative traits measured in Athens during 2017-2018 are presented (Tables 64-76).

Table 64. Percentages of leaf color and intensity of leaf color of the accessions used.

Accessions	Leaf color					Intensity of leaf color		
	Yellow	Green	Grey	Green-Red	Green-Red nerves	Pale	Medium	Dark
	%	%	%	%	%	%	%	%
LIB200		72,22		27,78			100,00	
LIB209		69,44		30,56			66,67	33,33
LIB213		88,89		11,11		33,33	66,67	
LIB214		80,56		19,44		0,00	100,00	
LIB218		75,00		25,00		33,33	66,67	

LIB220	100,00			100,00	
LIB221	100,00			100,00	
LIB222	94,44	2,78	2,78	100,00	
Branco	91,67	8,33		100,00	
LIB224	100,00				100,00
cv. Multitalia	100,00				100,00
cv. Polo	100,00			33,33	66,67

Table 65. Percentages of leaflet shape and central leaflet tip of each accession.

Accessions	**Leaflet shape**			**Central leaflet tip**	
	Elliptical	Widest towards the extreme	Other	Not acuminate	Acuminate
	%	%	%	%	%
LIB200	100,00			50,00	50,00
LIB209	100,00			52,78	47,22
LIB213	100,00			19,44	80,56
LIB214	50,00	50,00		61,11	38,89
LIB218	66,67	33,33		33,33	66,67
LIB220	66,67	33,33		33,33	66,67
LIB221	66,67	33,33		33,33	66,67
LIB222	100,00			33,33	66,7
Branco	100,00			33,33	66,7
LIB224	100,00			33,33	66,7
cv. Multitalia		66,67	33,33	100,00	
cv. Polo	5,56	27,78	66,67	100,00	

Table 66. Percentages of plant habit and stem formation.

Accessions	**Plant habit**			**Stem formation**	
	Erect	Semi-erect	Prostrate	Not prominent	Prominent
	%	%	%	%	%
LIB200	97,22	2,78			100,00
LIB209	100,00			33,33	66,67

LIB213	100,00				100,00
LIB214	86,11	13,89		2,78	97,22
LIB218	100,00				100,00
LIB220	100,00				100,00
LIB221	91,67	8,33			100,00
LIB222	100,00				100,00
Branco	97,22	2,78		2,78	97,22
LIB224	100,00			80,56	19,44
cv. Multitalia	97,22		2,78	26,47	73,53
cv. Polo	94,44	5,56		5,56	94,44

In the experimental field in Athens, stem color was recorded at the vegetative phase and at flowering. In Table 67, plants stem color at the vegetative phase are presented. Stem color remained the same for the two developmental stages.

Table 67. Percentages of the different colors of stem during vegetative stage in Athens.

Accessions	**Stem color vegetative**		
	Green	Red	Green-Red
	%	%	%
LIB200	27,78	33,33	38,89
LIB209	8,33	13,89	77,78
LIB213	13,89	25,00	61,11
LIB214	88,89	11,11	
LIB218	11,11	61,11	27,78
LIB220	100,00		
LIB221	100,00		
LIB222	61,11	33,33	5,56
Branco	58,33	41,67	
LIB224	100,00		
cv. Multitalia	100,00		
cv. Polo	100,00		

Table 68. Percentages of stem color intensity, petiole color and petiole color intensity.

Accessions	**Intensity of stem color**			**Petiole color**					**Intensity of petiole color**		
	Pale	Medium	Dark	Yellow	Green	Grey	Green-Red	Red	Pale	Medium	Dark
	%	%	%	%	%	%	%	%	%	%	%
LIB200		100,00			13,89		50,00	36,11	25,71	51,43	22,86
LIB209		66,67	33,33		11,11		47,22	41,67	2,86	48,57	48,57
LIB213		100,00			22,86		60,00	17,14	31,43	54,29	14,29
LIB214		100,00			30,56		50,00	19,44	44,44	47,22	8,33
LIB218		100,00			16,67		41,67	41,67	16,67	58,33	25,00

LIB220	100,00		100,0				100,00	
LIB221	100,00		16,67	83,33		52,78	44,44	2,78
LIB222	100,00		0,00	88,89	11,11	52,78	38,89	5,56
Branco	100,00		8,33	55,56	36,11	33,33	50,00	16,67
LIB224	,	100,00		100,00		13,89	58,33	27,78
cv. Multitalia	33,33	66,67	33,33	66,67			86,11	13,89
cv. Polo	100,00		80,56	16,67	2,78	16,67	83,33	0,00

Table 69. Percentages of stipule color and intensity of stipule color.

Accessions	**Stipule color**							**Intensity of stipule color**		
	Yellow	Green	Grey	Green-Blue	Green-Red	Yellow-Blue	Yellow-Red	Pale	Medium	Dark
	%	%	%	%	%	%	%	%	%	%
LIB200	33,33				61,11			55,56	5,56	38,89
LIB209	19,44				80,56			25,00	8,33	66,67
LIB213	20,00				80,00			32,35	52,94	14,71
LIB214	50,00				50,00			75,00	13,89	8,33
LIB218	25,00				69,44		2,78	38,89	19,44	41,67
LIB220	100,00							100,00		
LIB221	55,56				30,56		13,89	100,00		
LIB222	77,78				22,22			86,11	5,56	8,33
Branco	8,33				91,67			11,11	77,78	11,11
LIB224				100,00					33,33	66,67
cv. Multitalia	33,33			,	66,67				33,33	66,67
cv. Polo	77,78			22,22				30,56	66,67	2,78

Table 70. Percentages of flower color (type) of each accession.

Accessions	**Flower color (type)**						
	Type 1	Type 2	Type 3	Type 4	Type 5	Type 1 discolored	Type 3 purple
	%	%	%	%	%	%	%
LIB200						30,56	69,44
LIB209	33,33			2,78		8,33	55,56
LIB213		2,86	5,71	20,00		71,43	
LIB214	2,78	2,78				38,89	55,56
LIB218		2,78		58,33		36,11	2,78
LIB220		100,00					

LIB221	100,00				
LIB222	2,78		97,22		
Branco	75,00	5,56			19,44
LIB224				100,00	
cv. Multitalia				100,00	
cv. Polo				100,00	

Table 71. Percentages of existence of leaves at maturity stage and pod shattering.

Accessions	**Leaves at maturity**			**Pod shattering**	
	Green	Yellow	No leaves	Non shattering	Shattering
	%	%	%	%	%
LIB200	41,67		58,33	66,67	33,33
LIB209	47,22		52,78	72,22	27,78
LIB213	34,29		65,71	82,35	17,65
LIB214	77,78		22,22	75,00	25,00
LIB218	41,67		58,33	77,14	22,86
LIB220	16,67		83,33	82,35	17,65
LIB221	25,00		75,00	100,00	
LIB222	16,67		83,33	88,89	11,11
Branco	94,44		5,56	77,14	22,86
LIB224	94,12		5,88	100,00	
cv. Multitalia	65,71		34,29	85,29	14,71
cv. Polo	11,11		88,89	86,11	13,89

Table 72. Percentages of green and mature pod pubescence.

Accessions	**Green pod pubescence**			**Mature pod pubescence**		
	Light	Medium	Heavy	Light	Medium	Heavy
	%	%	%	%	%	%
LIB200	73,91	26,09		27,78	44,44	27,78
LIB209	26,09	21,74	52,17	11,43	25,71	62,86
LIB213	62,86	37,14		2,94	55,88	41,18
LIB214		68,57	31,43	41,67	41,67	16,67
LIB218	52,17	47,83		41,67	22,22	36,11
LIB220	66,67	33,33		11,76	50,00	38,24
LIB221	76,19	23,81		63,89	36,11	
LIB222	47,62	9,52	42,86		27,78	72,22
Branco	33,33	66,67			58,82	41,18
LIB224	60,61	39,39		66,67	9,09	24,24
cv. Multitalia	26,47	73,53		48,57	22,86	28,57
cv. Polo	18,18	81,82		91,43	8,57	

Table 73. Percentages of seed shape of each accession.

Accessions	Seed shape					
	Spherical	Flattened spherical	Oval	Flattened Oval	Cuboid	Flattened cuboid
	%	%	%	%	%	%
LIB200				71,43		28,57
LIB209			16,67	50,00	8,33	25,00
LIB213			25,71	65,71		8,57
LIB214			5,56	58,33		36,11
LIB218			5,71	80,00		14,29
LIB220					100,00	
LIB221				80,56		19,44
LIB222			13,89	66,67		19,44
Branco			14,29	80,00		5,71
LIB224					24,24	75,76
cv. Multitalia			29,41	2,94	26,47	41,18
cv. Polo			66,67	27,78		5,56

Table 74. Percentages of seed primary color of each accession.

Accessions	Seed primary color								
	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%
LIB200	100,00								
LIB209	100,00								
LIB213	100,00								
LIB214	97,22								2,78
LIB218	97,14								2,86
LIB220	100,00								
LIB221	100,00								
LIB222	94,44								5,56
Branco	100,00								
LIB224	63,64	36,36							
cv. Multitalia	32,35	67,65							
cv. Polo		100,00							

Table 75. Percentages of seed secondary color of each accession.

Accessions	Seed secondary color									
	Absence	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown

	%	%	%	%	%	%	%	%	%	%
LIB200	60,00									40,00
LIB209	55,56									44,44
LIB213	82,86									17,14
LIB214	75,00	2,78								22,22
LIB218	51,43	2,86								45,71
LIB220	100,00									
LIB221	100,00									
LIB222	2,78	5,56								91,67
Branco	82,86									17,14
LIB224	100,00									
cv. Multitalia	67,65	32,35								
cv. Polo	30,56									69,44

Table 76. Percentages of seed secondary color distribution of each accession.

Accessions	**Seed secondary color distribution**									
	Crescent	Eyebrow	Back	Spotted	Moustache	Marbled	Marbled crescent	Marbled eyebrow	Moustache-hilum line	Absent
	%	%	%	%	%	%	%	%	%	%
LIB200			2,86					34,29	2,86	60,00
LIB209							5,56	38,89		55,56
LIB213			2,86					14,29		82,86
LIB214		8,33						13,89	2,78	75,00
LIB218		2,86			2,86		2,86	37,14	2,86	51,43
LIB220										100,00
LIB221										100,00
LIB222					13,89		80,56	2,78		2,78
Branco		2,86					2,86	11,43		82,86
LIB224										100,00
cv. Multitalia						32,35				67,65
cv. Polo						66,67		2,78		30,56

4.2.3.3 Kalamata

In the following tables qualitative traits measured in Kalamata during 2017-2018 are presented (Tables 77-89).

Table 77. Percentages of leaf color and intensity of leaf color of the accessions used.

Accessions	Leaf color					Intensity of leaf color		
	Yellow	Green	Grey	Green-Red	Green-Red nerves	Pale	Medium	Dark
	%	%	%	%	%	%	%	%
LIB200		85,71		14,29			100,00	
LIB209		83,33		16,67			100,00	
LIB214		100,00					100,00	
LIB220		100,00					100,00	
LIB221		100,00					100,00	
LIB222		88,89		11,11				
Branco		100,00				22,22	77,78	
LIB224		100,00						100,00
cv. Multitalia		100,00						100,00
cv. Polo		100,00						100,00

Table 78. Percentages of leaflet shape and central leaflet tip of each accession.

Accessions	Leaflet shape			Central leaflet tip	
	Elliptical	Widest towards the extreme	Other	Not acuminate	Acuminate
	%	%	%	%	%
LIB200	100,00			8,82	91,18
LIB209	100,00			8,33	91,67
LIB214	100,00			11,11	88,89
LIB220	100,00				100,00
LIB221	100,00				100,00
LIB222	100,00				100,00
Branco	100,00				100,00
LIB224	100,00				100,00
cv. Multitalia		100,00		100,00	
cv. Polo			100,00	100,00	

Table 79. Percentages of plant habit and stem formation.

Accessions	Plant habit			Stem formation	
	Erect	Semi-erect	Prostrate	Not prominent	Prominent
	%	%	%	%	%
LIB200	100,00				100,00
LIB209	100,00				100,00
LIB214	94,44	5,56			100,00
LIB220	100,00				100,00
LIB221	100,00				100,00
LIB222	100,00				100,00
Branco	97,14	2,86			100,00
LIB224	100,00			100,00	

cv. Multitalia	100,00		100,00
cv. Polo	100,00	27,78	72,22

Table 80. Percentages of color of stem during vegetative and flowering stage in Kalamata.

Accessions	Stem color vegetative			Stem color flowering		
	Green	Red	Green-Red	Green	Red	Green-Red
	%	%	%	%	%	%
LIB200	5,71	28,57	65,71	31,43	31,43	37,14
LIB209	5,56	55,56	38,89	20,59	35,29	44,12
LIB214		5,56	94,44	58,33	16,67	25,00
LIB220	100,00			100,00		
LIB221	2,78	2,78	94,44	69,44	13,89	16,67
LIB222		55,56	44,44	2,94	61,76	35,29
Branco	2,78	25,00	72,22	8,82	52,94	38,24
LIB224	100,00			100,00		
cv. Multitalia	100,00			100,00		
cv. Polo	100,00			100,00		

Table 81. Percentages of stem color intensity, petiole color and petiole color intensity.

Accessions	Intensity of stem color			Petiole color					Intensity of petiole color		
	Pale	Medium	Dark	Yellow	Green	Grey	Green-Red	Red	Pale	Medium	Dark
	%	%	%	%	%	%	%	%	%	%	%
LIB200		100,00			41,18		23,53	35,29	23,53	52,94	23,53
LIB209		100,00			22,22		50,00	27,78	22,22	30,56	47,22
LIB214		100,00			30,56		2,78	66,67	52,78	38,89	8,33
LIB220		100,00			100,00				100,00		
LIB221		100,00			32,35			67,65	67,65	32,35	
LIB222		100,00			100,00				75,00	16,67	8,33
Branco		100,00			8,82		23,53	67,65	44,12	29,41	26,47
LIB224	100,00							100,00	25,00	33,33	41,67
cv. Multitalia	100,00							100,00	58,82	26,47	14,71
cv. Polo	100,00				100,00				100,00		

Table 82. Percentages of stipule color and intensity of stipule color.

Accessions	Stipule color							Intensity of stipule color		
	Yellow	Green	Grey	Green-Blue	Green-Red	Yellow-Blue	Yellow-Red	Pale	Medium	Dark
	%	%	%	%	%	%	%	%	%	%
LIB200	26,47	14,71			41,18		17,65	73,53		26,47
LIB209	5,56	19,44			61,11		13,89	41,67	11,11	47,22
LIB214	8,33	13,89			22,22		55,56	72,22	27,78	
LIB220	58,33	22,22					19,44	100,00		
LIB221	11,76	44,12					44,12	97,06	2,94	
LIB222	36,11	16,67			13,89		33,33	86,11	5,56	8,33
Branco	2,94	32,35			55,88		8,82	35,29	47,06	17,65
LIB224				11,11	88,89				,	100,00
cv. Multitalia	2,94			23,53	73,53				2,94	97,06
cv. Polo	100,00							8,33	91,67	,

Table 83. Percentages of flower color (type) of each accession.

Accessions	Flower color (type)

	Type 1	Type 2	Type 3	Type 4	Type 5	Type 1 discolored	Type 3 purple
	%	%	%	%	%	%	%
LIB200	76,47	2,94	17,65			2,94	
LIB209	30,56					16,67	52,78
LIB214	16,67		2,78			77,78	2,78
LIB220		97,22				2,78	
LIB221	100,00						
LIB222				97,06			2,94
Branco		71,88					28,13
LIB224					100,00		
cv. Multitalia					100,00		
cv. Polo					100,00		

Table 84. Percentages of existence of leaves at maturity stage and pod shattering.

Accessions	**Leaves at maturity**			**Pod shattering**	
	Green	Yellow	No leaves	Non shattering	Shattering
	%	%	%	%	%
LIB200	9,38	3,13	87,50	96,55	3,45
LIB209	14,81	3,70	81,48	100,00	
LIB214		11,76	88,24	96,67	3,33
LIB220	2,78		97,22	96,55	3,45
LIB221	8,70		91,30	100,00	
LIB222	17,86	3,57	78,57	100,00	
Branco	51,85	3,70	44,44	100,00	
LIB224	13,89	19,44	66,67	96,97	3,03
cv. Multitalia	82,35	11,76	5,88	78,57	21,43
cv. Polo			100,00	100,00	

Table 85. Percentages of green and mature pod pubescence.

Accessions	**Green pod pubescence**			**Mature pod pubescence**		
	Light	Medium	Heavy	Light	Medium	Heavy
	%	%	%	%	%	%
LIB200	5,88	67,65	26,47	65,52	17,24	17,24
LIB209		76,67	23,33	56,52	26,09	17,39
LIB214	2,78	88,89	8,33	40,00	50,00	10,00
LIB220		100,00		31,03	58,62	10,34
LIB221	100,00			80,95	14,29	4,76
LIB222	14,29	77,14	8,57	9,52	47,62	42,86
Branco		100,00		71,43	14,29	
LIB224			100,00	100,00		
cv. Multitalia		100,00		78,57	3,57	
cv. Polo	100,00			90,91	9,09	

Table 86. Percentages of seed shape of each accession.

Accessions	**Seed shape**					
	Spherical	Flattened spherical	Oval	Flattened Oval	Cuboid	Flattened cuboid
	%	%	%	%	%	%

LIB200				73,08		26,92
LIB209			9,09	77,27		13,64
LIB214				92,86		7,14
LIB220				10,71		89,29
LIB221				27,27		72,73
LIB222				33,33		66,67
Branco				85,71		14,29
LIB224		17,65				82,35
cv. Multitalia					100,00	
cv. Polo			52,63	47,37		

Table 87. Percentages of seed primary color of each accession.

Accessions	**Seed primary color**								
	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%
LIB200	100,00								
LIB209	95,45						4,55		
LIB214	100,00								
LIB220	100,00								
LIB221	100,00								
LIB222	100,00								
Branco	100,00								
LIB224	100,00								
cv. Multitalia		100,00							
cv. Polo		100,00							

Table 88. Percentages of seed secondary color of each accession.

Accessions	**Seed secondary color**									
	Absence	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%	%
LIB200	72,22									27,78
LIB209	72,22									27,78
LIB214	94,44									5,56
LIB220	100,00									
LIB221	100,00									
LIB222	41,67									58,33
Branco	100,00									
LIB224	100,00									
cv. Multitalia	100,00									
cv. Polo										100,00

Table 89. Percentages of seed secondary color distribution of each accession.

Accessions	**Seed secondary color distribution**									
	Crescent	Eyebrow	Back	Spotted	Moustache	Marbled	Marbled crescent	Marbled eyebrow	Moustache-hilum line	Absent
	%	%	%	%	%	%	%	%	%	%
LIB200			11,11		2,78		5,56	5,56	2,78	72,22
LIB209								27,78		72,22

LIB214	5,56		2,78	,				2,78	94,44
LIB220									100,00
LIB221									100,00
LIB222					5,56	,	52,78		41,67
Branco									100,00
LIB224									100,00
cv. Multitalia									100,00
cv. Polo	100,00	,							

4.2.3.4 **Erythres**

In the following tables qualitative traits measured in Erythres during 2017-2018 are presented (Tables 90-100). In Erythres, due to waterlogging, stem color in the vegetative and flowering phase, the intensity of stem color, green pod pubescence and presence or absence of leaves at maturity were not recorded as it was not possible to enter the experimental field.

Table 90. Percentages of leaf color and intensity of leaf color of the accessions used.

Accessions	**Leaf color**					**Intensity of leaf color**		
	Yellow	Green	Grey	Green-Red	Green-Red nerves	Pale	Medium	Dark
	%	%	%	%	%	%	%	%
LIB200		90,00			10,00		100,00	
LIB209		84,00		4,00	12,00		96,00	4,00
LIB214		100,00				6,90	93,10	
LIB218		83,33			16,67		88,89	11,11
LIB220		100,00					66,67	33,33
LIB221		100,00					100,00	
LIB222		97,22			2,78		100,00	
Branco		100,00				2,86	94,29	2,86
LIB224		100,00						100,00
cv. Multitalia		100,00						100,00
cv. Polo		100,00						100,00

Table 91. Percentages of leaflet shape and central leaflet tip of each accession.

Accessions	**Leaflet shape**			**Central leaflet tip**	
	Elliptical	Widest towards the extreme	Other	Not acuminate	Acuminate
	%	%	%	%	%
LIB200	100,00			20,00	80,00
LIB209	100,00			8,00	92,00
LIB214	100,00			25,00	75,00
LIB218	100,00			38,89	61,11
LIB220	100,00				100,00
LIB221	100,00				100,00
LIB222	100,00				100,00
Branco	100,00			5,71	94,29
LIB224	100,00				100,00

cv. Multitalia	100,00		100,00
cv. Polo	,	100,00	100,00

Table 92. Percentages of plant habit and stem formation.

Accessions	Plant habit			Stem formation	
	Erect	Semi-erect	Prostrate	Not prominent	Prominent
	%	%	%	%	%
LIB200	70,00	23,33	6,67		100,00
LIB209	80,00	20,00		33,33	66,67
LIB214	62,07	34,48			100,00
LIB218	100,00				100,00
LIB220	61,76	29,41			100,00
LIB221	86,11	13,89			100,00
LIB222	97,22	2,78			100,00
Branco	80,00	20,00		2,50	97,50
LIB224	100,00			95,00	5,00
cv. Multitalia	100,00			10,00	90,00
cv. Polo	66,67	33,33			100,00

Table 93. Percentages of petiole color and petiole color intensity.

Accessions	Petiole color					Intensity of petiole color		
	Yellow	Green	Grey	Green-Red	Red	Pale	Medium	Dark
	%	%	%	%	%	%	%	%
LIB200		17,39			60,87	65,22	21,74	13,04
LIB209	27,27				72,73	45,45	9,09	45,45
LIB214	41,67				58,33	83,33	16,67	
LIB218	16,67	33,33			50,00	41,67	25,00	33,33
LIB220	25,00	75,00				100,00	0,00	
LIB221	58,82	23,53			17,65	44,12	55,88	
LIB222	20,69	34,48			44,83	93,10	6,90	
Branco		8,57		23,53	91,43	22,86	74,29	2,86
LIB224					100,00		100,00	
cv. Multitalia					100,00	58,82	26,47	14,71
cv. Polo		100,00				100,00		,

Table 94. Percentages of stipule color and intensity of stipule color.

Accessions	Stipule color							Intensity of stipule color		
	Yellow	Green	Grey	Green-Blue	Green-Red	Yellow-Blue	Yellow-Red	Pale	Medium	Dark
	%	%	%	%	%	%	%	%	%	%
LIB200	8,70	17,39	,		65,22		8,70	69,57	21,74	8,70
LIB209	27,27				72,73			27,27	27,27	45,45
LIB214	8,33				91,67			41,67	58,33	
LIB218					33,33		50,00	41,67	25,00	33,33
LIB220	42,86	50,00	,				7,14	100,00		

LIB221	2,94				91,18	50,00	44,12	5,88
LIB222		6,90		93,10		75,86	17,24	6,90
Branco	2,86			97,14		20,00	45,71	34,29
LIB224			50,00	50,00			50,00	50,00
cv. Multitalia	2,50		7,50	90,00			5,00	95,00
cv. Polo	100,00						100,00	

Table 95. Percentages of flower color (type) of each accession.

Accessions	**Flower color (type)**						
	Type 1	Type 2	Type 3	Type 4	Type 5	Type 1 discolored	Type 3 purple
	%	%	%	%	%	%	%
LIB200	76,19		4,76				14,29
LIB209	63,64		0,00				36,36
LIB214	42,86		57,14				
LIB218	61,54			7,69			30,77
LIB220		100,00					
LIB221	76,93					23,08	
LIB222		3,57		96,43			
Branco							100,00
LIB224					100,00		
cv. Multitalia					100,00		
cv. Polo					100,00		

Table 96. Percentages of mature pod pubescence and pod shattering.

Accessions	**Mature pod pubescence**			**Pod shattering**	
	Light	Medium	Heavy	Non shattering	Shattering
	%	%	%	%	%
LIB200		30,00	70,00	96,55	3,45
LIB209	10,00	70,00	20,00	100,00	
LIB214	25,00	75,00		96,67	3,33
LIB218		27,27	63,64		
LIB220	5,60	22,22	72,22	96,55	3,45
LIB221		72,73	27,27	100,00	
LIB222		50,00	50,00	100,00	
Branco	16,67	50,00	33,33	100,00	
LIB224	25,00	65,00	10,00	96,97	3,03
cv. Multitalia			100,00	78,57	21,43
cv. Polo	100,00			100,00	

Table 97. Percentages of seed shape of each accession.

Accessions	**Seed shape**					
	Spherical	Flattened spherical	Oval	Flattened Oval	Cuboid	Flattened cuboid
	%	%	%	%	%	%
LIB200				100,00		
LIB209				100,00		

LIB214		83,33		16,67
LIB218		66,67		33,33
LIB220				100,00
LIB221		100,00		
LIB222		100,00		
Branco	11,11	88,89		
LIB224			35,00	65,00
cv. Multitalia			100,00	
cv. Polo	72,73	27,27		

Table 98. Percentages of seed primary color of each accession.

Accessions	**Seed primary color**								
	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%
LIB200	100,00								
LIB209	100,00								
LIB214	100,00								
LIB218	100,00								
LIB220	100,00								
LIB221	100,00								
LIB222	100,00								
Branco	100,00								
LIB224	100,00								
cv. Multitalia		100,00							
cv. Polo		100,00							

Table 99. Percentages of seed secondary color of each accession.

Accessions	**Seed secondary color**									
	Absence	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%	%
LIB200	70,00									30,00
LIB209	60,00									40,00
LIB214	100,00									
LIB218	22,22									77,78
LIB220	100,00									
LIB221	100,00									
LIB222										100,00
Branco	100,00									
LIB224	100,00									
cv. Multitalia	100,00									
cv. Polo										100,00

Table 100. Percentages of seed secondary color distribution of each accession.

Accessions	Seed secondary color distribution									
	Crescent	Eye-brow	Back	Spotted	Moustache	Marbled	Marbled crescent	Marbled eyebrow	Moustache -hilum line	Absent
	%	%	%	%	%	%	%	%	%	%
LIB200			10,00				20,00			70,00
LIB209								40,00		60,00
LIB214										100,00
LIB218							66,67	11,11		22,22
LIB220										100,00
LIB221							9,09			90,91
LIB222							100,00			
Branco										100,00
LIB224										100,00
cv, Multitalia										100,00
cv, Polo	100,00									

4.2.3.5 Remarks and Conclusions

- Late sowing is not appropriate for performing characterization assessments in our country because due to predominately high air temperatures in spring, plants are stressed, leading to many plant losses so the amount of data obtained in many cases is not valid.
- A high variability was observed for LIB200, LIB209, and LIB218 regarding many of the qualitative traits studied.
- Most of the plants were characterized by a prominent stem formation, however, LIB209 presented a high percentage of not prominent plants.
- LIB220, LIB221, and LIB222 presented a high homogeneity regarding flower color (type) and characterized by a Type 2, Type 1, and Type 4, respectively.
- LIB209, LIB214, LIB213 and LIB200 were characterized by different flower colors.
- LIB209 characterized by either a Type 1 of flower or by a Type 3 (purple) supporting the findings of the 2016-2017 cropping season that LIB209 is actually a mix of two morphotypes.
- LIB221 was characterized by completely non-shattering pods.
- LIB220 was characterized by a stable seed shape, characterized as a cuboid or flattened cuboid that discriminated this accession from all the other Andean lupin accessions used in this study.
- In LIB214 and LIB218 variability were observed regarding secondary seed color and pattern.
- LIB221, LIB220, and Branco were presented to be completely homogeneous regarding seed color (white).
- In LIB222 however some seeds were more or less brownish-grey, it was definitely a stable trait that discriminates this accession from all the others used in this study.
- Overall, LIB220, LIB221, and LIB222 were characterized by a very good level of homogeneity among the Andean lupin accessions tested.
- Accessions LIB209, LIB218, LIB200 were found not to be homogeneous.

4.2.4 **Third cropping season, 2018-2019**

In 2018-2019, some main quantitative traits descriptors were recorded, methods and protocol that was used were identical to the previous years. Traits measured referred to stem color at the vegetative stage and at flowering stage, flower color (type), the existence of leaves at maturity, mature pod pubescence, pod shattering, seed shape, seed primary color, seed secondary color and seed secondary color pattern. Two experimental fields were established in Athens and in Kalamata-TEI. However, in Kalamata-TEI many plants did not reach maturity or to be productive due to high temperatures at the beginning of spring and soil $CaCO_3$. Therefore, results were not considered valid due to the low number of plants and observations regarding the characterization of the accessions, as it was in the 2017-2018 cropping season.

4.2.4.1 **Results**

In the following tables qualitative traits measured in Athens during 2018-2019 are presented (Tables 101-108).

Table 101. Percentages of color of stem during vegetative and flowering stage.

Accessions	**Stem color vegetative**			**Stem color flowering**		
	Green	Red	Green-Red	Green	Red	Green-Red
	%	%	%	%	%	%
LIB200	33,33	38,89	27,78	33,33	38,89	27,78
LIB209	13,89	77,78	8,33	13,89	77,78	8,33
LIB214	91,67		8,33	91,67		8,33
LIB220	100,00			100,00		
LIB221	100,00			100,00		
LIB222	63,89	5,56	30,56	63,89	5,56	30,56
Branco	58,33		41,67	58,33		41,67
LIB224	100,00			100,00		
cv. Multitalia	100,00			100,00		
cv. Polo	100,00			100,00		

Table 102. Percentages of flower color (type) of each accession.

Accessions	**Flower color (type)**						
	Type 1	Type 2	Type 3	Type 4	Type 5	Type 1 discolored	Type 3 purple
	%	%	%	%	%	%	%
LIB200			100,00				
LIB209	100,00						
LIB214			100,00				
LIB220		100,00					
LIB221	100,00						
LIB222				100,00			
Branco	100,00						

LIB224	100,00
cv. Multitalia	100,00
cv. Polo	100,00

Table 103. Percentages of existence of leaves at maturity stage.

Accessions	**Leaves at maturity**		
	Green	Yellow	No leaves
	%	%	%
LIB200	91,67	8,33	
LIB209	100,00		
LIB214	97,22	2,78	
LIB220	81,82	18,18	
LIB221	100,0		
LIB222	100,00		
Branco	100,00		
LIB224	97,22	2,78	
cv. Multitalia	100,00		
cv. Polo	100,00		

Table 104. Percentages of mature pod pubescence and pod shattering.

Accessions	**Mature pod pubescence**			**Pod shattering**	
	Light	Medium	Heavy	Non shattering	Shattering
	%	%	%	%	%
LIB200	64,71	35,29		73,53	23,53
LIB209	10000			60,71	35,71
LIB214	75,00	25,00		94,44	5,56
LIB220	100,00			87,88	12,12
LIB221	77,14	22,86		91,43	8,57
LIB222	95,65	4,35		91,30	8,70
Branco	100,00			95,83	4,17
LIB224	77,42	9,68	12,90	93,55	6,45
cv. Multitalia	70,59	29,41		85,29	14,71
cv. Polo	87,10	12,90		100,00	

Table 105. Percentages of seed shape of each accession.

Accessions	**Seed shape**					
	Spherical	Flattened spherical	Oval	Flattened Oval	Cuboid	Flattened cuboid
	%	%	%	%	%	%
LIB200			58,06	41,94		
LIB209			89,66	10,34		
LIB214	7,69		57,69	34,62		
LIB220					100,00	
LIB221		10,71		89,29		
LIB222				28,57	71,43	
Branco			68,42			31,58

LIB224	32,26	67,74
cv. Multitalia	23,53	76,47
cv. Polo	16,67	83,33

Table 106. Percentages of seed primary color of each accession.

Accessions	Seed primary color								
	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%
LIB200	100,00								
LIB209	100,00								
LIB214	100,00								
LIB220	100,00								
LIB221	100,00								
LIB222	100,00								
Branco	100,00								
LIB224	100,00								
cv. Multitalia		100,00							
cv. Polo		100,00							

Table 107. Percentages of seed secondary color of each accession.

Accessions	Seed secondary color									
	Absence	White	Yellow (Beige)	Orange	Rose	Red	Green	Blue (Black)	Violet	Brown
	%	%	%	%	%	%	%	%	%	%
LIB200	41,94									58,06
LIB209	3,45									96,55
LIB214	100,00									
LIB220	100,00									
LIB221	100,00									
LIB222								100,00		
Branco	100,00									
LIB224	100,00									
cv. Multitalia	100,00									
cv. Polo										100,00

Table 108. Percentages of seed secondary color distribution of each accession.

Accessions	Seed secondary color distribution									
	Crescent	Eyebrow	Back	Spotted	Moustache	Marbled	Marbled crescent	Marbled eyebrow	Moustache-hilum line	Absent
	%	%	%	%	%	%	%	%	%	%
LIB200		22,58			3,23	6,45	12,90	12,90		41,94
LIB209	3,45	6,90			3,45	3,45	3,45	75,86		3,45
LIB214										100,00
LIB220										100,00
LIB221										100,00
LIB222						100,00				
Branco										100,00

LIB224		100,00
cv. Multitalia		100,00
cv. Polo	100,00	

4.2.4.2 Remarks and Conclusions

- Stem color did not change between vegetative and flowering stage.
- Accessions presented had a uniform flower color.
- LIB214 and Branco were characterized by a high percentage of non-shattering pods, followed by LIB221 and LIB222.
- All the entries used had leaves, green or yellow, during harvesting.
- LIB220 had a uniform and discrete seed shape.
- All accessions were characterized by the homogeneity of seed primary color.
- LIB200 and LIB209 presented variability regarding seed secondary color and pattern.

4.2.4.3 Correlations among qualitative traits studied

Pearson Correlation Coefficients calculated among the qualitative traits studied, using STATISTICA 8 statistical package (Table 109) taking into consideration all lupin accessions studied during the 2017-18 and 2018-19 cropping season. Furthermore, Pearson Correlation Coefficients calculated among the qualitative traits studied (Table 109) taking into consideration only Andean lupin accessions studied during the 2017-18 and 2018-19 cropping season. Traits not mentioned in the two tables that correlations are presented, like growth habit and Pubescence leaflet upper surface, did not varied among the accessions.

Table 109. Pearson correlations among qualitative traits studied across all lupin accessions.

Traits	Cot. colour	Leaf colour	Leaf colour intensity	Leaflet shape	Central leaflet tip	Plant habit	Stem colour	Stem colour intensity	Stem waxiness	Stem pubescence	Pubescence leaflet lower surface	Petiole colour	Petiole colour intensity	Stipule colour	Stipule colour intensity	Stem formation	Maturity leaves	Flower colour	Green pod pubescence	Mature pod pubescence	Pod shattering	Seed shape	Seed primary colour	Seed secondary colour	Seed pattern
Cot. colour	1.000	.510***	-0.131**	-.134**	.156***	-.001	.143***	.015	.171***	-.171***	-.171***	.147**	.214***	.104*	.225***	.112**	.002	-.016	.0435	.116**	.073	-.100*	-.042	.207***	.044
Leaf colour		1.000	-.176***	-.128**	.099*	.021	.122**	.016	.183***	-.183***	-.183***	.144**	.213***	.103*	.213***	.120**	.042	.019	.034	.102*	.060	-.099*	-.076	.289***	.048
Intensity of leaf colour			1.000	.407***	-.286***	-.042	-.420***	-.083	-.930***	.930***	.930***	.087*	.094***	.048	.547***	-.589***	-.218***	.724***	.251***	-.332***	-.023	.180***	.360***	-.034	-.128**
Leaflet shape				1.000	-.712***	.093*	-.381***	-.206***	-.486***	.486***	.486***	-.265***	-.099*	-.301***	.114**	.071	-.016	.419***	-.132**	-.273***	.032	-.241***	.252***	.241***	.135**
Central leaflet tip					1.000	-.086*	.275***	-.022	.408***	-.408***	-.408***	.068	.035	.104*	-.185***	-.012	.233***	-.235***	.075	.171***	-.043	.088*	-.352***	.019	-.254***
Plant habit						1.000	.010	.010	.061	-.061	-.061	-.052	.031	-.014	-.037	.013	-.020	-.031	.015	-.063	-.023	-.126**	-.044	.036	.040
Stem colour							1.000	.138**	.419***	-.419***	-.419***	.170***	.012	.260***	-.136**	.212***	.248***	-.452***	.032	.185***	-.040	-.122**	-.205***	.119**	-.140**
Stem colour intensity								1.000	.087*	-.090*	-.090*	-.103*	.188***	.100*	-.047	-.085*	-.217***	-.178***	-.192***	.319***	-.004	.189***	-.375***	-.094*	.462***
Stem waxiness									1.000	-1.000***	-1.000***	-.123*	-.113**	-.051	-.616***	.632**	.214***	-.769***	-.188***	.335***	.041	-.143**	-.385***	.036	.088*
Stem pubescence										1.000	1.000***	.123**	.113**	.051	.616***	-.632***	-.214***	.769***	.188***	-.335***	-.041	.143**	.385***	-.036	-.088*
Pubescence leaflet lower surface											1.000	.123**	.113**	.051	.616***	-.632***	-.214***	.769***	.188***	-.335***	-.041	.143**	.385***	-.036	-.088*
Petiole colour												1.000	.122**	.532***	.463***	-.212***	-.224***	.073	.141**	-.060	.039	.036	.252***	-.147**	-.207***
Petiole colour intensity													1.000	.176***	.430***	-.161***	-.138**	.102*	.096*	.087*	.029	-.013	-.064	.081*	.139**
Stipule colour														1.000	.411***	-.170***	-.180***	-.030	.156***	-.008	-.046	.166***	.106*	-.156***	-.157***
Stipule colour intensity															1.000	-.442***	-.303***	.561***	.227***	-.099*	.031	.067	.330***	.129**	-.047
Maturity leaves																	1.000	-.148**	-.038	-.072	-.167***	-.175***	-.262***	.231***	-.289***
Flower colour																		1.000	.107*	-.175***	-.001	.048	.340***	.309***	-.061
Green pod pubescence																			1.000	-.111**	-.038	.099*	.076*	-.047	-.321***
Mature pod pubescence																				1.000	.092*	-.042	-.260***	.141**	.326***
Pod shattering																					1.000	-.048	.081	-.014	.118**
Seed shape																						1.000	.099*	-.387***	-.121**
Seed primary colour																							1.000	-.165***	-.314***
Seed secondary colour																								1.000	.095*
Seed pattern																									1.000

*. Correlation significant at the 0.05 level, **. Correlation significant at the 0.01 level, ***. Correlation significant at the 0.001 level

Table 110. Pearson correlations among qualitative traits studied across Andean lupin accessions.

Traits	Cot. color	Leaf color	Intensity of leaf color	Leaflet shape	Central leaflet tip	Plant habit	Stem color	Stem color intensity	Stem waxiness	Stem pubescence	pubescence leaflet lower surface	Petiole color	Petiole color intensity	Stipule color	Stipule color intensity	Stem formation	Maturity leaves	Flower color	Green pod pubescence	Mature pod pubescence	Pod shattering	Seed shape	Seed primary color	Seed secondary color	Seed pattern
Cot. color	1.000	.484***	0.028	-.094*	.137**	-.007	.078	-.053	.053	-.053	-.053	.191***	.270***	.120*	.409***	.058	-.025	.217***	.087	.075	.056	-.110*	.106*	.233***	.031
Leaf color		1.000	-.0466	-.065	.050	.019	.043	-.057	.057	-.057	-.057	.190***	.270***	.120*	.402***	.062	.020	.302***	.074	.050	.045	-.110*	-.024	.333***	.035
Intensity of leaf color			1.000	-.258***	.094	.017	.046	.613***	-.613***	.613***	.613***	-.033	.059	.061	.120*	-.567***	-.101***	-.061	.402***	-.017	.003	.262***	.007	-.064	-.036
Leaflet shape				1.000	-.639***	.182***	-.270***	-.066	.066	-.066	-.066	-.077	-.057	-.116*	-.117*	.030***	-.207***	-.069	-.099	-.074	.058	.079	-.028	-.142**	.293***
Central leaflet tip					1.000	-.125*	.113*	-.296***	.296***	-.296***	-.296***	-.029	-.018	.014	-.041	.290***	.248***	.162***	-.048	-.006	-.006	-.016	-.125*	.201***	-.351***
Plant habit						1.000	-.013	-.032	.032	-.032	-.032	-.003	.039	.012	.024	.035	-.087	.017	.022	-.099*	-.020	-.127*	-.013	.011	.037
Stem color							1.000	.193***	-.193***	.193***	.193***	.310***	.063	.359***	.213***	-.147**	.214***	-.082	.118*	-.022	-.071	-.119*	-.026	.149**	-.273***
Stem color intensity								1.000	-1.000***	1.000***	1.000***	.062	.141**	.099*	.312***	-.917***	-.083	-.160***	.326***	.051	-.060	.207***	-.012	-.107*	.145**
Stem waxiness									1.000	-1.000	-1.000	-.062	-.141**	-.099*	-.312***	.917***	.083	.160**	-.326***	-.051	.060	-.207***	.012	.107*	-.145**
Stem pubescence										1.000	1.000	.062	.141**	.099*	.312***	-.917***	-.083	-.160**	.326***	.051	-.060	.207***	-.012	-.107*	.145**
pubescence leaflet lower surface											1.000	.062	.141**	.100*	.312***	-.917***	-.083	-.160**	.326***	.051	-.060	.207***	-.012	-.107*	.145**
Petiole color												1.000	.107**	.438***	.416***	-.067	-.086	-.044	-.031	-.020	.014	-.306***	-.033	.143**	-.033
Petiole color intensity													1.000	.137**	.529***	-.099	-.131*	.068	.054	.077	.044	-.166**	.073	.200***	.203***
Stipule color														1.000	.425***	-.057	-.027	-.105*	.120*	-.049	-.082	-.082	-.023	.090	-.142**
Stipule color intensity															1.000	-.268***	-.164**	.198***	.188***	.124*	.023	-.176***	.041	.387***	.150**
Stem formation																1.000	.087	.146**	-.312***	-.030	.065	-.159**	.013	.117*	-.158**
Maturity leaves																	1.000	.048	-.134**	-.105*	-.198***	.078	.050	.071	-.397***
Flower color																		1.000	.035	.303***	.039	-.081	.009	.683***	.106*
Green pod pubescence																			1.000	.029	-.015	.075	.137**	.064	-.102*
Mature pod pubescence																				1.000	.086	-.122*	-.089	.253***	.268***
Pod shattering																					1.000	-.091	-.025	-.003	.191***
Seed shape																						1.000	-.043	-.203***	-.160**

Seed primary color	1.000	-.036	-.101*
Seed secondary color		1.000	.030
Seed pattern			1.000

*. Correlation significant at the 0.05 level, **. Correlation significant at the 0.01 level, ***. Correlation significant at the 0.001 level.

4.2.4.4 Remarks and Conclusions

- The growth habit and pubescence of the leaf upper surface did not vary among the accessions.
- Strong positive correlations were obtained for cotyledon color with leaf color, stem color, stem waxiness, petiole color, stipule color, and secondary seed color ($p \leq 0{,}001$) for all the accessions used.
- Strong positive correlation among cotyledon color, leaf color, petiole color, flower color, and secondary seed color ($p \leq 0{,}001$) were recorded for Andean lupin accessions.
- Plant habit exhibited no significant correlation with the other traits studied for Andean lupin entries.
- Stem waxiness and stem pubescence are completely inversely analogous traits.
- Stem color correlated positively with stem pubescence ($p \leq 0{,}001$) of Andean lupin entries.
- Stem formation exhibited some negative correlations with leaf color intensity, stem color, intensity of stem color, stem pubescence, and leaflet pubescence on their upper surface, stipule color intensity, green pod pubescence, and secondary color seed pattern.
- Flower color correlated positively to seed secondary color ($p \leq 0{,}001$) and pattern ($p \leq 0{,}05$) but not to seed primary color.
- Pod shattering correlated strongly and negatively with the absence or presence of leaves at the maturity stage of plants ($p \leq 0{,}001$), while positively with seed secondary color pattern ($p \leq 0{,}001$) of Andean lupin accessions.

4.3 Iceland

Due to lack of seed in 2018 and 2019, trials on multiple accessions were only performed in 2017. The varieties that were tested in 2017 are listed in table 111.

Table 111. Andean lupin accessions tested in Iceland 2017. The accessions shaded in grey were tested at two sites with four replications at each. The non-shaded accessions could not be planted in four replications due to lack of seed.

Accession	1000K weight (g)	Seeds available	Eroded soil (replicates)	Organic soil (replicates)
Branco	266,1	3.758	4	4
LIB219	140,5	500	4	4
LIB220	223,4	500	4	4
LIB221	177,9	500	4	4
LIB222	91,1	500	4	4
LIB200	183,7	231	1	1
LIB201	359,1	91	1	1

LIB202	195,2	210	1	1
LIB203	272,1	125	1	1
LIB204	187,0	124	1	1
LIB207	171,9	206	1	1
LIB208	164,2	205	1	1
LIB209	120,7	201	1	1
LIB210	195,0	201	1	1
LIB205	234,3	173	1	1
LIB211	217,8	195	1	1
LIB212	150,4	209	1	1
LIB213	159,4	217	1	1
LIB214	203,9	208	1	1
LIB218	149,3	121	1	1
LIB217	193,0	219	1	1

The accession trials in 2017 were performed at two locations about 6,9 km apart: 1) at an eroded site with no organic soil (Figure 11) in an old field previously used for barley production with organic soil, sheltered by wind-breaks (Figure 12). These sites are close to the headquarters of the Soil Conservation Service of Iceland at Gunnarsholt.

Figure 11. The eroded site.

Figure 12. The organic field site.

The organic soil field has been used for hay and barley production for decades and is considered one of the more productive fields in the area. By testing the accessions at an eroded site in comparison, the ability of Andean lupin to grow on extremely poor soil at a challenging site, can be demonstrated.

In 2017, most accessions flowered, but none of them produced any seed at all. In light of that and the fact that seed for further accession trials was limited, the LIBBIO consortium decided to focus on accession trials in the Southern countries where the plant performance was more promising, therefore making better use of the limited amount of seed available. All experiments in 2018 and 2019 in Iceland were therefore performed with Branco. Luckily, this was also logical since Branco grew faster in Iceland than other accessions in 2017. The tallest Branco individual measured well above 1 m tall at the organic field site (Figure 13).

Figure 13. An individual inflorescence of Branco measuring well over 1m tall at the organic field site.

The seeds were sown on May 16, 2017 at both sites according to the common experimental protocol for accession testing provided to participants in the trials. Measurements were performed as dictated in the same protocol. Descriptive data for germination are summarized in table 112. Germination percentages were compared with a two-way ANOVA after square-root transforming the data, with accessions and germination as main factors, but only for those accessions where four replications were present for each (Figure 14). No significant differences were found between field sites, but germination percentages varied significantly among accessions (F=65,53; p<0,001)

Table 112. Germination of accessions tested at the eroded site and at the organic field site. ANOVA on square-root transformed data was performed on the accessions where four replications were present for each (shaded grey).

Accession	**Germination at eroded site (%)**	**Germination at organic field site (%)**	**Significant difference**
Branco	62	73	NS
LIB219	93	91	NS
LIB220	88	87	NS
LIB221	50	40	NS
LIB222	88	91	NS
LIB200	32	0	not tested
LIB201	76	68	not tested
LIB202	56	36	not tested
LIB203	32	44	not tested
LIB204	56	36	not tested
LIB205	16	0	not tested
LIB207	40	36	not tested
LIB208	24	8	not tested
LIB209	56	60	not tested
LIB210	64	20	not tested
LIB211	52	60	not tested
LIB212	52	40	not tested
LIB213	60	12	not tested
LIB214	36	48	not tested
LIB217	44	20	not tested

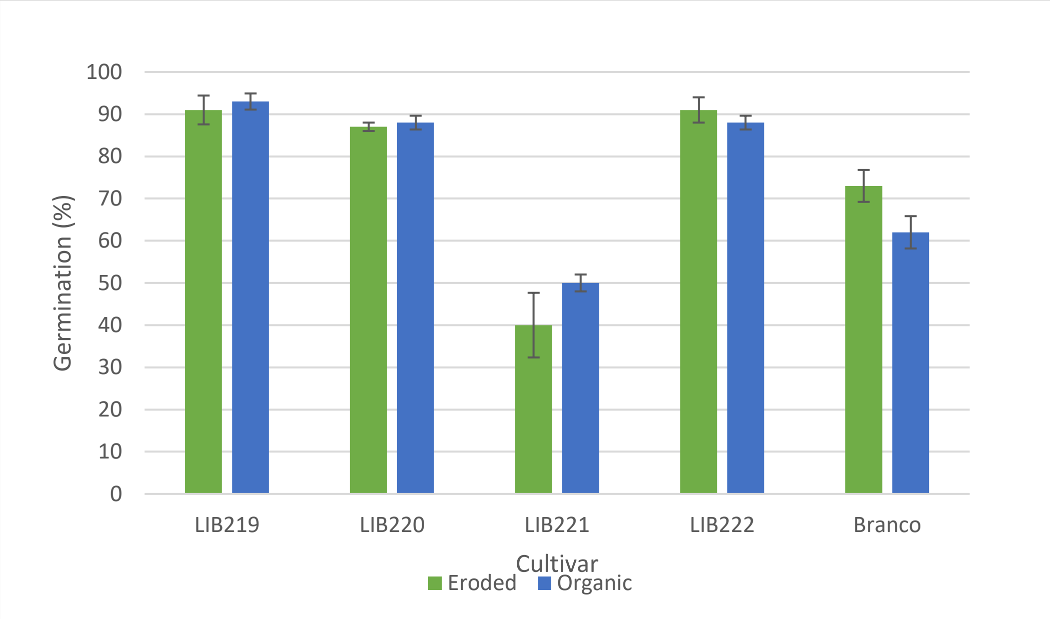


Figure 14. Germination of Andean lupin accessions at the eroded site and at the organic field site 2017. Bars indicate the mean germination of each accession at each site. Error bars indicate the standard error (SE).

Height measurements were performed according to the common protocol. Descriptive data on final height measurements are summarized in table 113. A two-way ANOVA was performed on the height measurements with accessions and site as main effects. In all cases, plant height was greater at the organic field site (Figure 15) than the eroded site ($p<0,001$) and accessions varied significantly ($p<0,001$). The interaction between accession and site was non-significant.

Table 113. Plant height of accessions tested at the eroded site and at the organic field site in 2017. ANOVA was performed on the accessions where four replications were present for each (shaded grey).

Accession	Plant height at eroded site (cm)	Plant height at organic field site (cm)	Significant difference
Branco	51,7	85,1	p<0,001
LIB219	33,3	64,2	p<0,001
LIB220	38,8	73,4	p<0,001
LIB221	25,5	48,8	p<0,001
LIB222	30,1	56,0	p<0,001
LIB200	32	0	not tested
LIB201	76	68	not tested
LIB202	56	36	not tested
LIB203	32	44	not tested
LIB204	56	36	not tested
LIB205	16	0	not tested
LIB207	40	36	not tested
LIB208	24	8	not tested
LIB209	56	60	not tested

LIB210	64	20	not tested
LIB211	52	60	not tested
LIB212	52	40	not tested
LIB213	60	12	not tested
LIB214	36	48	not tested
LIB217	44	20	not tested

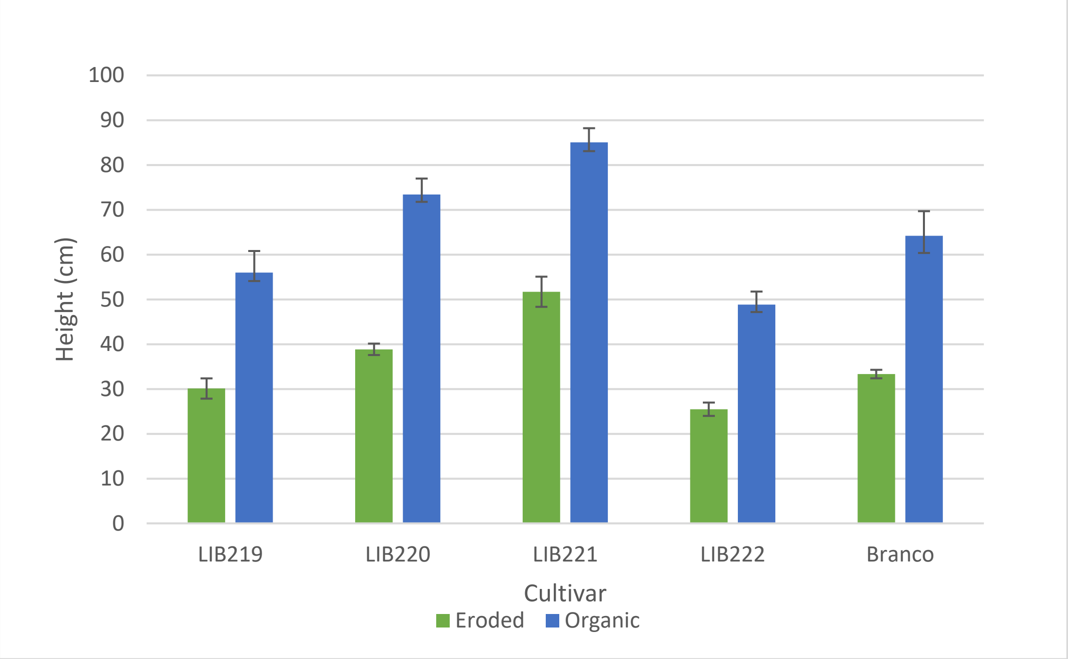


Figure 15. Height of Andean lupin accessions at the eroded site and at the organic field site as measured on September 13, 2017. Bars indicate the mean height of each accessions at each site. Error bars indicate the standard error (SE).

Days after sowing to 50% flowering (DAS 50% in flower) were measured according to the common protocol and are summarised in table 114. A two-way ANOVAwas performed on DAS 50% in flower with accession and site as main effects. Flowering in Branco never reached 50% and was not included in the test. DAS 50% in flower was significantly less at the organic field site (figure 16) than the eroded site (p<0,036) and accessions varied significantly (p<0,001). The interaction between accession and site was non-significant.

Table 114. Days after sowing to 50% flowering (DAS 50% in flower) of all accessions tested in 2017 at the eroded site and at the organic field site. ANOVA was performed on the accessions where four replications were present for each (shaded grey) excluding Branco which never reached 50% flowering at either site (N/A: not applicable, since the accessions never reached this stage).

Accession	DAS 50% in flower at eroded site	DAS 50% in flower at the organic field site	Significant difference
Branco	n/a	n/a	not tested
LIB219	119,0	115,0	p<0,036

LIB220	125,0	118,3	p<0,036
LIB221	100,7	99,0	NS
LIB222	113,5	113,0	NS
LIB200	n/a	n/a	not tested
LIB201	107	113	not tested
LIB202	n/a	n/a	not tested
LIB203	121	n/a	not tested
LIB204	121	121	not tested
LIB205	n/a	n/a	not tested
LIB207	107	113	not tested
LIB208	n/a	n/a	not tested
LIB209	113	113	not tested
LIB210	107	113	not tested
LIB211	121	n/a	not tested
LIB212	107	n/a	not tested
LIB213	113	121	not tested
LIB214	113	113	not tested
LIB217	n/a	n/a	not tested

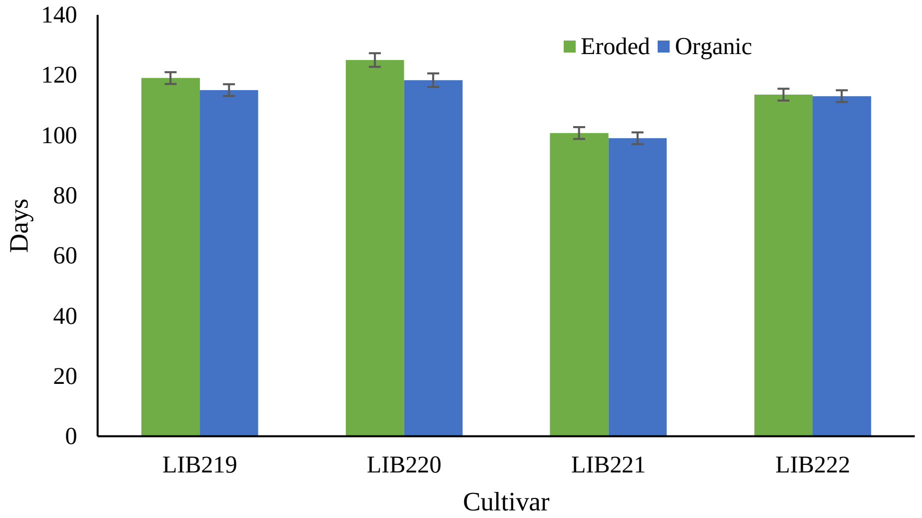


Figure 16. Days until 50% flowering of Andean lupin accessions at the eroded site and at the organic field site. Error bars indicate the standard error (SE).

4.4 Portugal

Andean lupin in Portugal was evaluated at two different locations in the 2016/2017 cropping season and at three different locations in 2017/2018 and 2018/2019 cropping seasons.
In the 2016/2017 cropping season, four accessions from the Vandinter Semo were tested, 15 accessions from Instituto Superior de Agronomia (ISA) and a landrace were tested in Coimbra (Loreto) and Lisbon (ISA).

Although in ISA sowing took place and in Loreto transplantation, plants evolved equally. In both locations white lupin was also sown. Andean lupin plants with about -4-6 leaves were higher with light green leaves when compared to white lupin with lower plants but with dark green leaves (Figure 17).

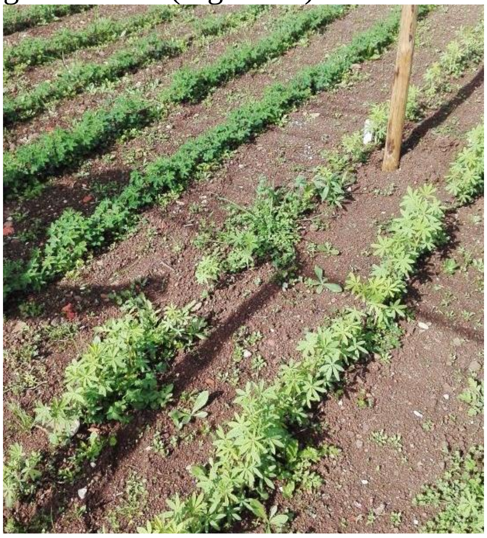


Figure 17. Left rows white lupin and right rows Andean lupin (ISA, 2017).

The earlierst flowering occurred in white lupin var. Misak (98 DAS) when compared to all LIBBIO accessions. LIB221 showed the shortest cycle up to flowering, but 10 days longer (108 DAS) than white lupin. Precipitation in October and November in Portugal, specially north of the Tagus river, can often push sowing dates to late December or January therefor precocity is a key issue.

Figure 18. 1st order of flowers in white lupin (right), and main inflorescence in LIB222 (left), (Loreto, 2019).

During crop season observations, LIB accessions proved to be stable, adapted to local conditions, when life cycle, pest and diseases tolerance are considered. Although the ISA accessions, are very well adapted to local conditions, they proved to be difficult for cropping evaluation because they are still heterozygous. Different stems colour and different flower colour occurred in the same plot. |The accession Branco showed poor adaptation to the local conditions with only a few flowers and pods being produced. It was also the only accession were lodging was observed.


Figure 19. Different flower and steam colours in an ISA line (Loreto, 2017).


Figure 20. Absence of flowers on Branco accession in Coimbra/Loreto.

The yield was also evaluated and results proved that they were acceptable for a new crop. When the average yield per plot was analysed (based on 10 plants/plot) values varied between 0,679 and 1,103t ha^{-1}, between LIB222 and LIB221, and up to 2,424 and 2,732 t/ha^{-1} kg/ha between LIB220 and LIB221 (no significant difference between values).
In the 2017/2018 cropping season, propagation of four LIB accessions (LIB219, LIB220, LIB221 and LIB222) was conducted in Loreto and ISA accessions in Lisbon/ISA.
On a marginal land in Loreto, productivity from LIB accessions proved to be more irregular. LIB219 accession lost the majority of the plants due to fungal infections.

An herbicide screening trial was installed in Santarém/Escola Superior Agrária de Santarém (ESAC) in April. Spring sowing with drip irrigation allows for a very fast plant development, flowering occurred but only a few seeds were found in the pods. LIB220, LIB221 and LIB222 were tested.

Figure 21a. Herbicide Screening trial, phytotoxicity (ESAC, 2018), Figure 21b. Spring sowing, plants with pods on 2nd order of pods (ESAC, 2018).

In the first two years plant size, canopy height and good behaviour of the accession was observed concerning disease tolerance. Next competition with weeds and productivity were two important issues to be solved. Densities from 25 to 33 plants per m^2 have been used so far. In Portugal white lupin is usually sown at densities from 20 to 25 plants per m^2 and faba bean at 8 to 10 plants per m^2.
In 2018/2019 a density trial was installed in ESAS to better understand the accessions response to these factors. Density levels of25, 50 and 75 plants per m^2 were tested with LIB220 and LIB221.
Although there were no significant differences between the tested accessions and densities, both accessions have responded positively to higher densities, both in productivity and in ground cover. Potential productivity on 10 of 18 trial plots where above 2 tonnes ha^{-1}. One plot of LIB220 exceeded 4 tonnes ha^{-1}. Only in a plot of LIB221 at 75 plants per m^2 anthracnosis was detected. Results indicate that a better performance with densities around 50 plants per m^2 may be obtained.

Seed density experiments in Portugal in 2020 demonstrated dramatic desnity effects, probably due to reduced branching at higher plant densities. Andean lupin has a strong tendency for branching, thereby allocating energy to a continuous pod and seed production on first and second order side branches. Increasing plant density forces the crop to invest energy in the main stem causing it to be higher. Total plant height (including side branches) however was hardly ot not affected by plant density. As expected, total kernel weight per plant decreased because of increased plant crop competition for resources, but his was outweighed by increased production due to more plants per square meter. Andean lupin variety Cotopaxi obtained its highest seed yields of 6,7 tonnes/ha at the highest plant densities.

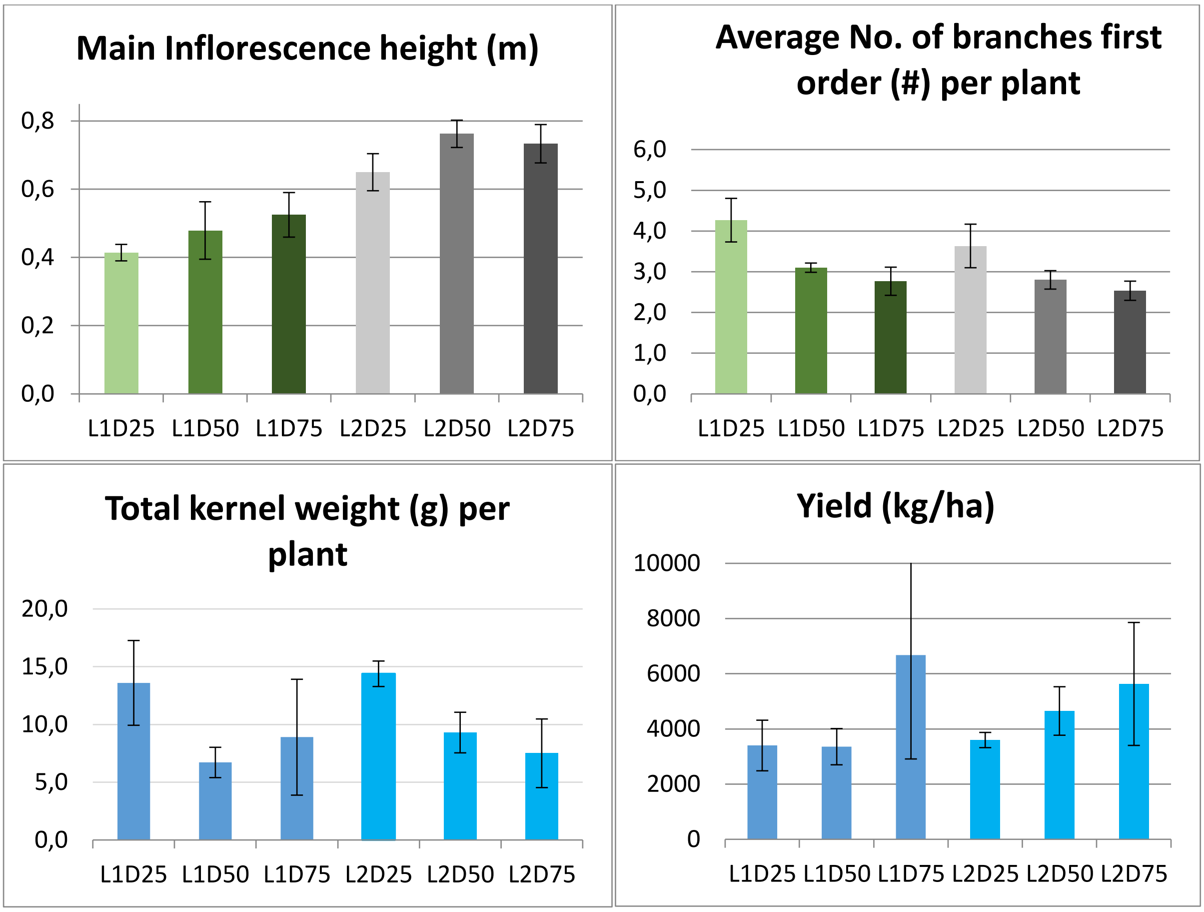


Figure 22. L1 Cotopaxi and L2 LIB220 seed densities respectively 25, 50 and 75 plants/m2, cropping season 2020 in Santarém Portugal.

Also, in the 2018/2019 cropping season, propagation of LIB220, LIB221 and LIB222 was conducted in Loreto. After sowing the propagation in Loreto on the 10th December and density trial in ESAS on the 21st December, frost occurred between the 2nd and the 14th of January 2019. In both locations, plants showed good resistance to frost. This subject is decribed in more detail in chapter 9.3. Although more information and results are needed, this observation opened an opportunity for Andean lupin to be grown in Portugal, were frost occurs during the winter in some of the potential production sites.

4.5 Romania

4.5.1 First cropping season, 2017

In 2017, at USAMV Iasi applied three field experiments, in three locations, which are part of the Moldavian Plateau, but with different soil and climatic conditions. These locations are 110-150 km apart.

The GPS location was between $46^0$51'07" and $47^0$37'52" north latitude, between $26^0$14'40" and $27^0$30'41" eastern longitude and the altitude from sea level was between 113 m and 358 m (Table 115).

Table 115. The GPS locations of the Andean lupin experiments in Romania, 2017.

Location	County	North Latitude	Eastern Longitude	Altitude from sea level (m)
Ezareni	Iasi	$47^0$07'22"	$27^0$30'41"	113
Secuieni	Neamt	$46^0$51'07"	$26^0$51'50"	182
Suceava	Suceava	$47^0$37'52"	$26^0$14'40"	358

The number of emerged plants after three weeks from sowing (Figure 23) was very low (2-9 % from total number of seeds/plot), comparative with *Lupinus albus* cultivars. Only Branco reached 20% in the Suceava location.

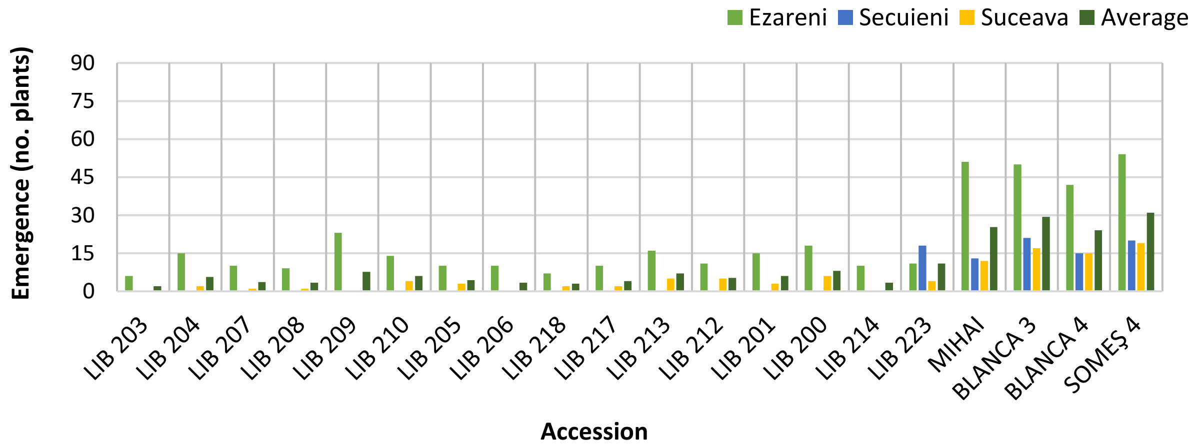


Fig. 23. Number of emerged plants three weeks after sowing.

Five weeks after sowing, the average emergence percentage increased to 21 – 45 % in Andean lupin but remained lower than *L. albus* emergence (Figure 23). At the Suceava location the highest emergence was observed for all the accessions of Andean lupin.

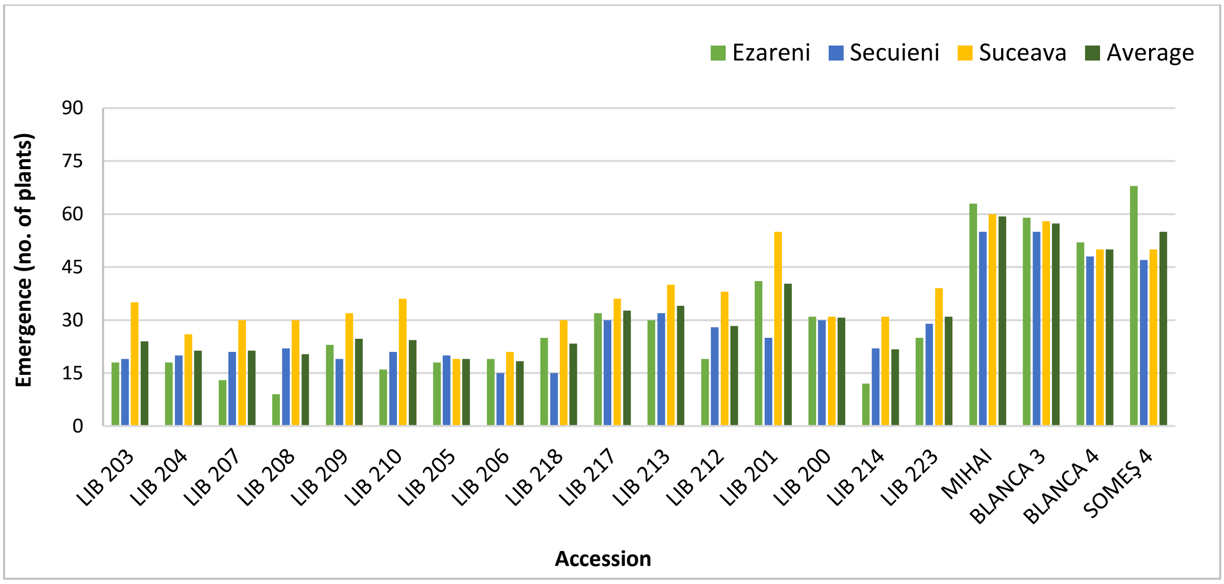


Figure 24. Number of emerged plants five weeks after sowing.

Table 116. Average results of measurements done at Ezareni, Iasi, 2017.

	Height (cm)					Pods size (cm)		# seeds		Weight (g)		
Accession	**Canopy**	**First ordes**	**Pod level**	**Flowering Branches #**	**Pods #**	**Length**	**Width**	**Total**	**Good quality**	**Pods**	**seeds**	**Good quality seeds**
LIB 203	116	70	45	16	88	15	4	155	66	50	19	9
LIB 204	112	66	46	15	83	16	4	154	90	57	23	15
LIB 207	103	61	43	17	83	13	4	130	59	47	18	9
LIB 208	112	65	46	12	52	15	4	113	47	37	16	8
LIB 209	102	55	47	15	110	14	4	191	78	54	20	11
LIB 210	116	60	56	12	80	14	4	153	85	45	20	11
LIB 205	113	71	43	6	22	11	3	37	11	13	6	2
LIB 206	115	63	51	15	71	14	4	114	57	43	16	9
LIB 218	108	57	50	21	125	13	3	218	105	52	21	12
LIB 217	109	64	45	10	82	5	1	149	68	57	19	11
LIB 213	103	57	46	16	100	13	4	194	76	56	28	13
LIB 212	111	65	46	13	66	5	1	118	45	40	15	7
LIB 201	102	64	38	10	61	4	1	113	64	42	17	10
LIB 200	113	60	52	16	74	5	12	122	47	47	19	9
LIB 214	110	72	38	11	70	4	2	68	14	37	7	2
Branco	135	81	19	5	13	3	1	15	0	7	2	0
AVERAGE	**111**	**64**	**45**	**13**	**74**	**10**	**3**	**128**	**57**	**43**	**17**	**9**

Table 117. Average results of measurements done at Secuieni, Neamt, 2017.

	Height (cm)					Pods size (cm)		# seeds		Weight (g)		
Accession	**Canopy**	**First ordes**	**Pod level**	**Flowering Branches #**	**Pods #**	**Length**	**Width**	**Total**	**Good quality**	**Pods**	**seeds**	**Good quality seeds**

LIB 203	125	73	48	18	120	5	1	309	48	93	288	288
LIB 204	129	64	56	15	93	6	1	195	25	53	182	182
LIB 207	124	71	42	11	74	4	1	180	30	59	167	167
LIB 208	120	70	51	19	104	5	1	234	34	74	216	216
LIB 209	109	55	50	17	122	5	1	275	34	63	261	261
LIB 210	121	64	57	19	118	5	1	252	41	76	250	250
LIB 205	137	75	76	32	190	5	1	384	57	109	349	349
LIB 206	110	70	41	13	65	6	1	171	28	55	156	156
LIB 218	116	56	52	22	171	5	1	413	53	90	322	322
LIB 217	133	75	57	15	107	6	1	244	39	82	228	228
LIB 213	118	66	50	24	132	4	1	261	39	74	249	249
LIB 212	116	61	51	16	109	5	2	285	40	65	275	275
LIB 201	127	68	48	10	52	5	1	120	20	41	113	113
LIB 200	147	76	60	19	99	5	1	243	44	81	233	233
LIB 214	142	65	69	18	91	5	1	207	37	75	196	196
Branco	168	107	52	12	30	5	1	55	8	21	45	45
AVERAGE	**128**	**70**	**54**	**17**	**105**	**5**	**1**	**239**	**36**	**69**	**221**	**221**

Table 118. Average results of measurements done at Suceava, 2017.

	Height (cm)					Pods size (cm)		# seeds		Weight (g)		
Accession	**Canopy**	**First ordes**	**Pod level**	**Flowering Branches #**	**Pods #**	**Length**	**Width**	**Total**	**Good quality**	**Pods**	**seeds**	**Good quality seeds**
LIB 203	104	69	35	7	46	5	1	100	100	32	14	14
LIB 204	98	66	31	7	60	4	1	166	161	44	24	24
LIB 207	98	63	31	7	69	3	1	153	148	41	22	22
LIB 208	100	65	35	8	58	4	1	136	136	40	22	22
LIB 209	94	57	35	7	68	3	1	186	184	40	23	23
LIB 210	92	59	33	8	45	5	1	110	104	36	19	19
LIB 205	110	76	33	9	58	4	1	122	110	38	18	16
LIB 206	98	69	29	6	41	4	1	111	105	38	21	20
LIB 218	85	52	32	7	62	4	1	155	152	35	19	19
LIB 217	92	61	31	6	39	5	1	96	96	29	14	14
LIB 213	90	57	32	7	62	4	1	131	120	31	15	14
LIB 212	86	57	28	5	42	4	1	113	110	27	14	14
LIB 201	92	61	30	5	43	4	1	134	128	32	18	18
LIB 200	98	64	30	5	37	4	1	97	88	31	16	15
LIB 214	104	64	39	6	47	4	1	126	121	40	22	21
Branco	123	104	18	4	19	3	1	36	30	13	6	5
AVERAGE	**98**	**65**	**31**	**7**	**50**	**4**	**1**	**123**	**118**	**34**	**18**	**17**

The growing and development of Andean lupin accessions in all the three location was slower during the first stages, when compared to *L. albus* cultivars.A possible reason may be that these accessions were poorly adapted to the local conditions and the extreme weather conditions that occurred during this season. In the second half of the season, all accessions showed an explosive vegetative growth and a long flowering period. The general tendency was, in all locations, indeterminate growth. Resulting in orders in flower while the main inflorescence was ready for harvest.

4.5.2 Second cropping season, 2018, seed multiplication

In 2018, at USAMV Iasi five fieldvexperiments were organised, at three locations, which are part of the Moldavian Plateau from Romania, with different soil and climatic conditions. These locations are 110-150 km apart.
The GPS location was located between 46°51'07" and 47°37'52" North latitude, between 26°14'40" and 27°33'15" Eastern longitude and the altitude from sea level was between 109 m and 358 m (Table 119). The 2018 experiments focused on seed multiplication of the LIB accessions.

Table 119. The GPS locations of the Andean lupin experiments in Romania, 2018.

Location	County	North Latitude	Eastern Longitude	Altitude from sea level (m)
Ezareni	Iasi	47°07'25"	27°30'53"	109
Iasi	Iasi	47°11'42"	27°33'15"	159
Horlesti	Iasi	47°15'23"	27°27'39"	156
Secuieni	Neamt	46°51'07"	26°51'50"	182
Suceava	Suceava	47°37'52"	26°14'40"	358

Table 120. Germplasm available for the experiments in Romania, 2018.

		Good seeds				
	Available (g)	**g**	**#**	**%**	**TKW**	**Location**
LIB 220	2800	825,3	4600	30	182	Suceava 600
LIB 221	4600	1530	12200	33	125,4	Iasi 1100
LIB 222	1050	900,5	10306	85,8	83,3	Secuieni 750
LIB 219	300	84,3	720	28	116,6	Horlesti 100
LIB 227	470	148,7	880	31,6	130,8	Iasi tent
LIB 228	40					Iasi tent
LIB 229	100					Iasi tent
LIB 202 (Inti)	463	463,7	2791	100	166,2	Iasi tent

Table 121. Germplasm of Andean lupin used for the second sowing in Romania, 2018.

	Available (g)	Location and m²	Emergence (%)

LIB 220	578	Suceava 600	20%
LIB 221	612+3000	Iasi 1100	1%
LIB 222	178	Secuieni 750	20%
LIB 219	6,5	Horlesti 100	3%
LIB 227	104	Iasi tent	10%
LIB 228	1,4	Iasi tent	10%
LIB 229	3,4	Iasi tent	10%
LIB 202 (Inti)	307	Iasi tent	50%

As a consequence of the very low emergence, the accessions were resown at the 19th of June. Unfortunately, these also performed very poorly.

Figure 25 and 26. Andean lupin plants covered to prevent cross pollination and genetic contamination (left) and the plants at harvest (right).

Table 122. Seeds produced in the 2018 seed multiplication.

	Good seeds			
	g	**No.**	**Total**	**Location**
LIB 220	728,5	4769	5769	Ezareni Iasi
LIB 222	211	2810	3810	Secuieni
LIB 221	-	1000	1000	Suceava

Table 123. Number of plants during the growing season and harvested, Ezareni, 2018.

	Seeds #	Germ inatio n	Emerged plants (#)		Harvested plants (#)				
Accession	**Plants /plot**	**%, 31 DAS**	**Total**	**Total**	**from which, isolated plants**	**With pods**	**Without pods**	**To tal**	**Plants to harvest vs. seeds nr. (%)**	**Plants to harvest vs. emerged plants (%)**
LIB 220	90	29	26	26	16	4	18	22	4	15

LIB 221	90	18	16	11	4	6	6	12	7	38
LIB 222	90	28	25	19	5	5	10	15	6	20
LIB 219	90	24	22	19	6	7	9	16	8	32
LIB 227	90	7	6	5	0	1	1	2	1	17
LIB 228	90	22	20	16	4	6	12	18	7	30
LIB 229	90	2	2	2	0	1	0	1	1	50
LIB 202 (INTI)	90	56	50	38	4	10	32	42	11	20
Misak Variety	90	32	29	17	0	0	12	12	0	0
Misak Segreg.	90	30	27	19	0	3	13	16	3	11
Bitter Segreg.	90	50	45	39	0	4	30	34	4	9
Sweet Population	90	42	38	38	0	35	0	35	39	92
***L. albus* Mihai**	220	50	110	106	0	104	0	104	47	95

Table 124. Plants architecture measurement, Ezareni-Iasi, 2018.

Accession	**Plant height (cm)**	**First order flowering height (cm)**	**Height main inflorescence (cm)**
LIB 220	115,0	62,5	
LIB 221	67,5	51,5	16,0
LIB 222	67,0	50,5	16,5
LIB 219	71,0	53,5	17,5
LIB 227			
LIB 228	75,0	44,0	31,0
LIB 229			
LIB 202 (Inti)	106,0	77,0	29,0
***L. albus* Blanca 3**	55,5	20,5	35,0
***L. albus* Somes 4**	95,0	32,5	62,5
***L. albus* Mihai**	74,0	31,5	42,5

Table 125. Main inflorescence architecture, Ezareni-Iasi, 2018.

Accession	**MF (#)**	**Pods (#)**	**Pods length (cm)**	**Pods width (cm)**	**Pods weight (g)**	**Total no. of kernels**	**Total weight of kernels (g)**	**No. of normal kernels**	**Weight of normal kernels (g)**
LIB 220	1,0	9,5	5,0	1,4	2,5	10,5	0,6	6,5	0,4
LIB 221	1,0	4,5	5,3	1,7	2,1	7,0	0,4	4,0	0,3
LIB 222	1,0	5,0	3,8	1,2	1,1	3,5	0,2	3,5	0,2
LIB 219	1,0	5,0	4,9	1,2	1,6	11,0	0,4	6,5	0,3
LIB 227									
LIB228	1,0	5,0	4,1	1,2	0,9	8,5	0,3	8,5	0,3
LIB 229									
LIB 202 (Inti)	1,0	3,5	4,9	1,5	1,5	5,5	0,4	0,0	0,0
***L. albus* Blanca 3**	1,0	8,5	7,7	1,4	8,4	36,5	4,1	10,0	1,6
***L. albus* Somes 4**	1,0	7,0	8,5	1,5	8,2	30,0	3,5	9,0	1,4

***L. albus* Mihai**	1,0	2,5	7,3	1,4	3,8	9,5	2,4	9,5	2,4

Table 126. The 1st+2nd+3rd order architecture.

Accession	No. of inflores -cences	No.of pods	Pods length (cm)	Pods width (cm)	Pods weigh t (g)	Total nr. of kernels	Total weight of kernels (g)	No. of normal kernels	Weight of normal kernels (g)
LIB 220	0,0	0,0	-	-	0,0	0,0	0,0	0,0	0,0
LIB 221	1,5	5,5	4,9	1,5	2,2	10,0	0,6	5,5	0,4
LIB 222	3,0	8,0	3,0	0,9	1,0	10,0	0,3	6,0	0,2
LIB 219	0,0	0,0	-	-	0,0	0,0	0,0	0,0	0,0
LIB 227									
LIB 228	5,0	8,5	4,4	1,2	2,2	12,5	0,4	7,5	0,3
LIB 229									
LIB 202 (Inti)	3,5	17,5	3,7	1,2	4,5	16,5	0,8	7,5	0,5
***L. albus* Blanca 3**	9,0	24,5	7,3	1,3	18,2	85,5	8,3	42,5	5,3
***L. albus* Somes 4**	11,5	24,0	5,8	1,3	15,7	45,0	4,9	16,0	2,7
***L. albus* Mihai**	3,5	4,0	6,6	1,4	6,8	11,5	3,9	10,0	3,6

Table 127. General productions architecture, Ezareni-Iasi, 2018.

Crt.no	No. of inflores cences	No. of pods	Pods length (cm)	Pods width (cm)	Pods weight (g)	Total nr. of kernels	Total weight of kernels (g)	No. of normal kernels	Weight of normal kernels (g)	TKW (g)
LIB 220	1,0	9,5	5,0	1,4	2,5	10,5	0,6	6,5	0,4	66,9
LIB 221	2,5	10,0	3,4	1,6	4,3	17,0	1,0	9,5	0,7	69,5
LIB 222	4,0	13,0	2,4	1,1	2,1	13,5	0,5	9,5	0,4	38,9
LIB 219	1,0	5,0	4,9	1,2	1,6	11,0	0,4	6,5	0,3	45,4
LIB 227										
LIB 228	6,0	13,5	2,7	1,2	3,1	21,0	0,6	16,0	0,6	35,0
LIB 229										
LIB 202 (Inti)	4,5	21,0	3,0	1,4	6,0	22,0	1,2	7,5	0,5	61,3
***L. albus* Blanca 3**	10,0	33,0	4,5	1,3	26,7	122,0	12,4	52,5	6,9	131,0
***L. albus* Somes 4**	12,5	31,0	4,9	1,4	23,8	75,0	8,3	25,0	4,1	162,0
***L. albus* Mihai**	4,5	6,5	4,4	1,4	10,6	21,0	6,3	19,5	6,0	309,0

Due to limited seed availability and seed quality issues the strategy and field designs had to be adapted in order to obtain optimal results for multiplication. Unfortunately, the crops emergence and performance of the first sowing was very poor. None of the fields were initially irrigated. For the second sowing a drip irrigation system was installed on the field in Iasi. Despite the additional effort, the crop performed too badly to obtain a desirable seed production.

4.5.3 Third cropping season, 2019, seed multiplication

In 2019, at USAMV Iasi five fields were organised with experiments, in three locations from three different counties. All the fields were in a part of the Moldavian Plateau from Romania, with some different soil and climatic conditions.
These locations are 110-150 km apart. and all the fields were located in the area of State Research Stations.The GPS location was between 46^{0}51’09” and 47^{0}37’52” North latitude, between 26^{0}14’40” and 27^{0}30’54” Eastern longitude and the altitude from sea level was between 105 m and 358 m (table 3.5.3).

In 2019 we received seeds from the accessions LIB220, 221 and 222.

The quality of the seeds received from Portugal and of our own production was much better this year than in 2018.


Figure 30 and 31. Multiplication of LIB222 in Secuieni 2019.

In addition to seed multiplication phenotyping of the Andean lupin accessions was performed. The year 2019 starting very promising. Good quality of the seeds, rain after sowing, good emergence of plants. The conditions (warm and wet) affected especially LIB 220 which was used for the main trials in Ezareni Iasi. Development of Andean lupin accessions in all the locations was slower in the first stages, compared to *L. albus* cultivars. Possibly the Andean lupin accessions were poorly adapted to the to the local climate conditions, the unusually high temperatures combined with less rain than is usual for these areas.
In the second half of the season, all sixteen accessions from Andean lupin displayed an explosive vegetative growth and a long flowering period. Generally, in all locations, the growth type was indeterminate growth, with new inflorescences continuing to set while the primary inflorescence was already harvest ready.

4.6 Spain

The set of material included in the field trials consisted of different types of accessions: landraces, breeding accessions, germplasm accessions and mixtures.

The accessions were Branco, four breeding accessions produced by Vandinter Semo and fifteen entries from the Germplasm collection of the Portuguese Instituto Superior de Agronomía (ISA). In addition, a collection of 20 accessions from the Spanish gene bank, CRF-INIA, were sown, and 25 entries from the Vavilov institute were also included. However, the viability of the seeds was extremely low (Appendix IV.I).

Considering that seed mixtures of accessions with different characteristics have the potential to optimise adaptability and as well in stabilizing crop yield in different environments, CSIC explored the different germplasm combinations in 20 different mixtures. Each of the 20 mixtures were composed of the same number of seeds for four entries. The design of the mixtures was carried out during the first growing season (2016-2017) at one of the locations. The mixtures combined material fromVandinter Semo, ISA and accession 'Branco' and the combinations were selected at random.

In order to improve the field trial results of the first growing season, the seeds used in each new growing season were harvested at the same site of the previous season, which led to a high level of performance. In this way, the plants that CSIC used in the ongoing seasons were from seeds that had been harvested during the previous seasons and had developed for 3 consecutive years. By doing this, CSIC was using its own multiplied germplasm to favour the process of adaptation to local conditions. As a result, plant development improved which allowed for the morphological and agronomic traits to be expressed more clearly, resulting in a satisfactory evaluation (Table 129).

The activities related to the deliverable 2.1 included the evaluation of these accessions in multi-field trial experiments (different locations and years).

4.6.1 **Experimental design and results**

The field experiments were carried during three growing seasons at three locations, depending on the year: two main locations with conventional and organic cropping systems and a third location in the 2017-2018 growing season. We now have the full data for the 2016-17, 2017-2018 and 2018-2019 trials, while the 2019-2020 experiments are still ongoing. When the seeds became available, the breeding material was grown in a replicated randomized block designs at two locations: Culturhaza, an organic site and ENDL, a conventionally-managed site.

The trials included 40-45 plots per replicate, location and year. The most common plant density was 10 plants/m^2, with plots 3 m apart and rows of 5m in length. This standard plot was subjet to change because of seed limitations and the farmer´s suggestions. For instance, our field trials started with a set of unreplicated accessions, with very few seeds. Among the plots, the faba bean gene-pools from CSIC germplasm collection constituted the reference crop, randomly replicated, although in the 2019-2020 growing season, two *Lupinus albus* accessions were also included. Weed control was performed both manually and mechanically using a tractor drawn coulter cultivator.

Phenotyping of all the field trials for morpho-phenological and reproductive traits was performed based on a common phenotyping protocol during the three growing seasons. This protocol includes the phenology, distribution and determinants of yield data (pods per plant, seeds per plant and seed size). Tables 128 and 129 show a summary of the data for several adaptability traits (emergence, plant density, plant height and days to flowering) and yield determinants per plant (number of pods and seeds and seed weight) of the main CV of each germplasm group for the three growing seasons.

One-way ANOVA testing was used to test the variation among entries for each variable of adaptability and determinants of yield traits. The analyses were conducted separately for each growing season and site. For each variable, F-test was used to determine significant differences beween entries with at least 5% of probability

Regarding adaptability traits, significant variations were found for plant height among the entries across the growing seasons and sites, with LIBs the lowest CVs. Remarkably, no significant differences among entries were noted for days to flowering at each location and site. Considerable variation, in each site, was found for determinants of yield. Regarding the differences among entries, ANOVAS indicated significant differences for pods, per plant, seeds per plant and seed size at the ENDL site for the 2017-2018 growing season, while LIB accessions had the lowest number of seeds per plant and seed weight. However, in the 2018-2019 growing season, the LIB accessions produced the higher number of seeds per plant at Culturhaza. In short, CSIC is using its own multiplied germplasm which is adapting to local conditions. As a result, there was an improvement in plants development permiting a clear expression of morphological and agronomic traits, which produced a satisfactory evaluation (Tables 128 and 129).

Table 128. Mean evaluation values of main adaptability traits evaluation in phenotyping trials.

Growing season 2018-2019

Site: ENDL

CV	Emerged plant density	Plant height	Days to flowering
LIB221	10	48,95	118
LIB222	10	56,45	128
POTOSI-ALEMAO	10	59,45	126
POTOSI-ISA	10	56,45	123
25	10	66,05	132
38	10	68,60	127

Site: CULTURHAZA

CV	Emerged plant density	Plant height	Days to flowering
LIB221	7,45	19,80	121
LIB222	4,15	23,29	126

POTOSI-ALEMAO	6,90	30,20	136
POTOSI-ISA	7,35	30,55	124
25	4,30	38,45	138
38	7,10	36,90	137

Growing season 2017-2018

Site: ENDL

CV	Emerged plant density	Plant height	Days to flowering
LIB221	6,7	33,00	156
LIB222	6,2	29,00	152
POTOSI-ALEMAO	5,9	56,90	142
POTOSI-ISA	3,4	61,20	144
25	6,7	53,10	151
38	7,2	55,20	148

Site: CULTURHAZA

CV	Emerged plant density	Plant height	Days to flowering
LIB221	1	25,00	164
LIB222	1,1	29,83	175
POTOSI-ALEMAO	1,6	34,00	172
POTOSI-ISA	1,6	34,80	180

Growing season C2016-2017

Site: ENDL

CV	Emerged plant density	Plant height	Days to flowering
LIB221	0,4	27,85	124
LIB222	0,7	29,00	116
POTOSI-ALEMAO	0,2	59,00	118
POTOSI-ISA	0,1	58,00	115
25	0,2	52,17	128
38	0,4	45,00	126

Site: CULTURHAZA

CV	Emerged plant density	Plant height	Days to flowering
LIB221	0,2	25,95	97
LIB222	0,7	28,30	106
POTOSI-ALEMAO	0,2	43,90	109

POTOSI-ISA	0,1	42,89	112

Table 129. Mean evalution values of main determinants of yield evaluation in phenotyping trials.

Growing season 2018-2019

Site: ENDL

CV	Pods per plant	Seeds per plant	Seed weight (g/seed)
LIB221	46,00	131,80	0,015
LIB222	48,70	133,15	0,012
POTOSI-ALEMAO	45,50	112,80	0,014
POTOSI-ISA	44,36	108,44	0,014
25	42,50	119,25	0,014
38	52,20	142,25	0,013

Site: CULTURHAZA

CV	Pods per plant	Seeds per plant	Seed weight (g/seed)
LIB221	10,67	18,33	0,009
LIB222	7,60	18,70	0,006
POTOSI-ALEMAO	5,45	11,67	0,010
POTOSI-ISA	4,22	7,77	0,011
25	5,92	8,03	0,013
38	4,74	7,52	0,011

Growing season 2017-2018

Site: ENDL

CV	Pods per plant	Seeds per plant	Seed weight (g/seed)
LIB221	4,00	9,78	0,013
LIB222	11,83	25,00	0,009
POTOSI-ALEMAO	23,50	55,30	0,015
POTOSI-ISA	21,70	51,50	0,016
25	18,60	39,50	0,015
38	24,20	47,60	0,014

Site: CULTURHAZA

CV	Pods per plant	Seeds per plant	Seed weight (g/seed)
LIB221	5,75	10,50	0,010

LIB222	4,67	11,80	0,006
POTOSI-ALEMAO	5,11	7,56	0,009
POTOSI-ISA	3,50	5,50	0,011

Growing season 2016-2017

Site: ENDL

CV	Pods per plant	Seeds per plant	Seed weight (g/seed)
LIB221	8,00	19,63	0,010
LIB222	8,00	17,56	0,006
POTOSI-ALEMAO	25,30	47,40	0,012
POTOSI-ISA	16,00	39,00	0,015
25	7,30	22,80	0,006
38	8,80	14,20	0,011

Site: CULTURHAZA

CV	Pods per plant	Seeds per plant	Seed weight (g/seed)
LIB221	5,60	12,30	0,011
LIB222	4,70	9,56	0,007
POTOSI-ALEMAO	6,90	11,00	0,013
POTOSI-ISA	6,22	12,00	0,014

4.7 The Netherlands

Over the course of 2017-2019 various field trials were performed to study the accessions. In the first season a general analysis of 20 accessions was made on three soil types. In the second and third cropping season a more detailed assessment was made of LIB220, 221, 222 and Branco on three soil types ranging from fertile to marginal soils. None of the sites could be irrigated except for the site in Wageningen in 2019.

4.7.1 First cropping season, 2017, Germination and overall performance

To test which accessions most was promising during this first cropping season in 2017, 3 soil types were selected. However insufficient seed was available. As a consequence, not all accessions were tested on all soil types. Two replicates were sown of each accession if the seed quantity allowed it, and the plots were randomized.

The accessions from Vandinter Semo performed considerably better than other accessions. The accession Branco was irregular in its germination but of this line a high quantity of seeds could be obtained in South-America so this accession is of interest as well (Table 130).

Table 130. Germination percentages on three soil types in 2017.

Accession	Sand	Marginal sand	Clay
Branco	10,9	17,4	50,7
Inti	3,1		16,0
LIB200	1,0		15,0
LIB201	12,2		41,0
LIB203	0	5,1	17,0
LIB204	1,0		19,0
LIB205	0		4,0
LIB207	3,1		15,0
LIB208	1,0		10,0
LIB209	10,2		23,0
LIB210	1,02	2,0	11,0
LIB211	5,1		23,0
LIB212	1,0		4,2
LIB214	1,0		10,0
LIB217	2,0		8,0
LIB218	4,1		32,0
LIB219	46,9	30,6	81,0
LIB220	63,3	19,4	71,0
LIB221	27,6	14,3	42,0
LIB222	30,6	22,5	79,0

Table 131. Average days after sowing to 50% of the line is in flower and pods are ready for harvest.

	Sandy soil		Marginal sand		Clay	
Line	**50% in flower**	**Pods harvest ready**	**50% in flower**	**Pods harvest ready**	**50% in flower**	**Pods harvest ready**
Branco	71	143	100	x	82	x
LIB219	71	141	100	x	65	x
LIB220	71	x	100	x	62	x
LIB221	55	118	x	x	61	122
LIB222	69	133	100	x	63	122

x = no pod setting

Due to the LIB accessions outperforming the other accessions, the LIB accessions were selected for the next cropping seasons.

4.7.2 Second cropping season, 2018

In all cases a split plot design was used, the seeds were sown by hand at a sowing depth of about five cm, and a row distance of thirty centimetres was applied. Plots had a surface area of 4,5m^2. None of the sites could be irrigated.

The trails of 2018 started on the 11th of April on the sandy soil at Gooijer-wetering. Andean Lupin LIB221, broad leaved lupin Boros, Faba bean Fuego and Wheat Lavett were sown. This was the early sowing of the Andean lupin. In May Andean Lupin LIB221, LIB222 and LIB220 were sown on all three locations. This represented the middle sowing in the sandy soil, and the standard sowing in the marginal sandy soil of Noordhout and the clay soil at Kraggenburg. In June the final sowing of Andean lupin was performed on the sandy soil.

4.7.2.1 Emergence

It was a challenge to obtain a proper crop density at all test sites. To obtain a back-up plants were also sown at the nursery Gitzels, in Wervershoof, The Netherlands, as well. At the nursery the seeds were germinated and the plants grown under optimal conditions.

The emergence rates were very low, even at the nursery. Emergence is the percentage of plants that emergenced in relation to the number of plants that were sown. The early sowing of LIB221 was completely lost since only 2 plants emerged. Due to the limited number of plants the influence of early sowing time could not be studied. Except Branco none of the Andean lupin accessions exceeded 35% emergence at the nursery (Figure 32). In the field only about 9% germination was found in LIB221. In May 2018 seedlings from the nursery were transplanted on all test sites to obtain the required density.

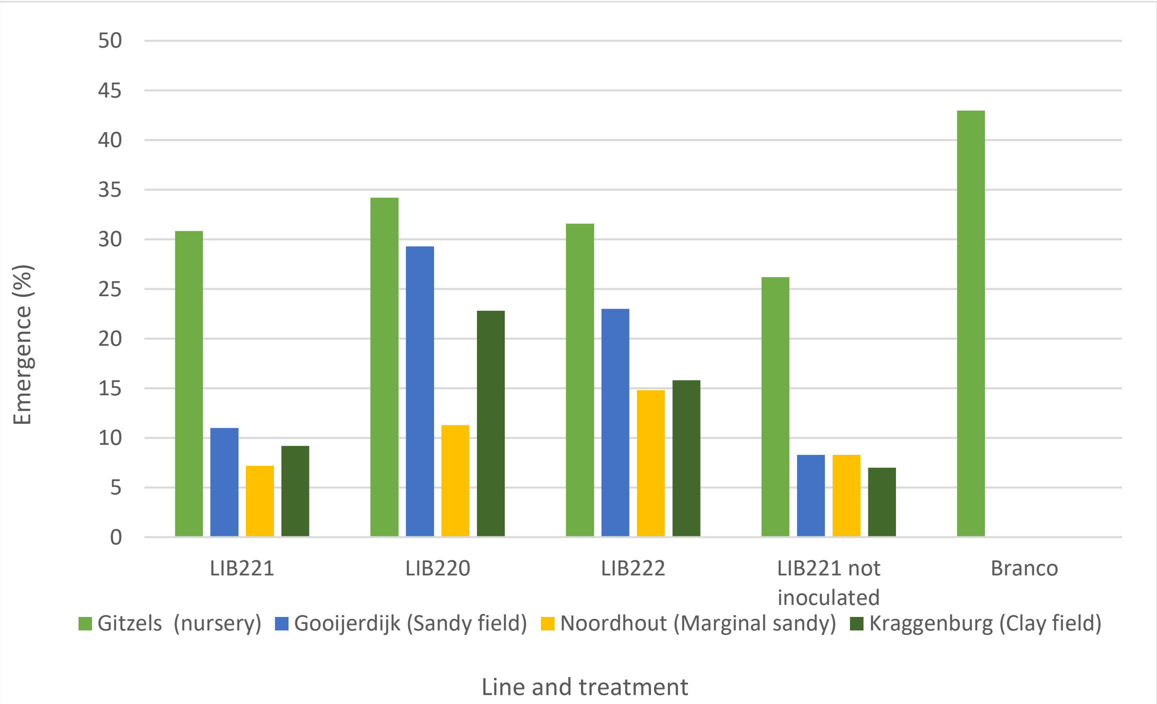


Figure 32. Average emergence at three test sites and at Gitzels a nursery.

The poor emergence is believed to be due to very poor seed quality. Seed batches showed mixing of different accessions, poor sorting of debris and broken seeds and were polluted with what are believed to be sclerotinia (sclerotia was observed during the seed production). The emergence of the reference crops broad leaved lupin 'Boros', Faba bean 'Fuego' and Wheat 'Lavett' was very well on the sandy location. It is therefore believed that soil and climatic conditions were not the cause of the poor germination of the Andean lupin.

Figure 33. Above 'waste fraction', below clean fraction.

4.7.2.2 **Crop establishment**

The crop establishment after transplanting seedlings was well on both the sandy and clay soil. However due to the prolonged drought most of the plots on the marginal sand location Noordhout were lost. In the other sites flower and pod abortion was observed during the peak of summer but the crops survived.

Figure 34 and 35. Noordhout test site, R>L Late June and the 8th of August.

4.7.2.3 **Crop development and homogeneity**

The height of the main florescence (MF) LIB220 is significantly higher than LIB222 and LIB221 (Figure 36) Inoculation does not significantly influence the crop height. Due to the drought most of the plots at the marginal sand location were lost. Therefore, no direct comparison can be made to determine a location influence, but the data does suggest a soil influence is present when assessing crop height. The first orders were always higher that the main florescence. Also, the comparison between the accessions was the same.

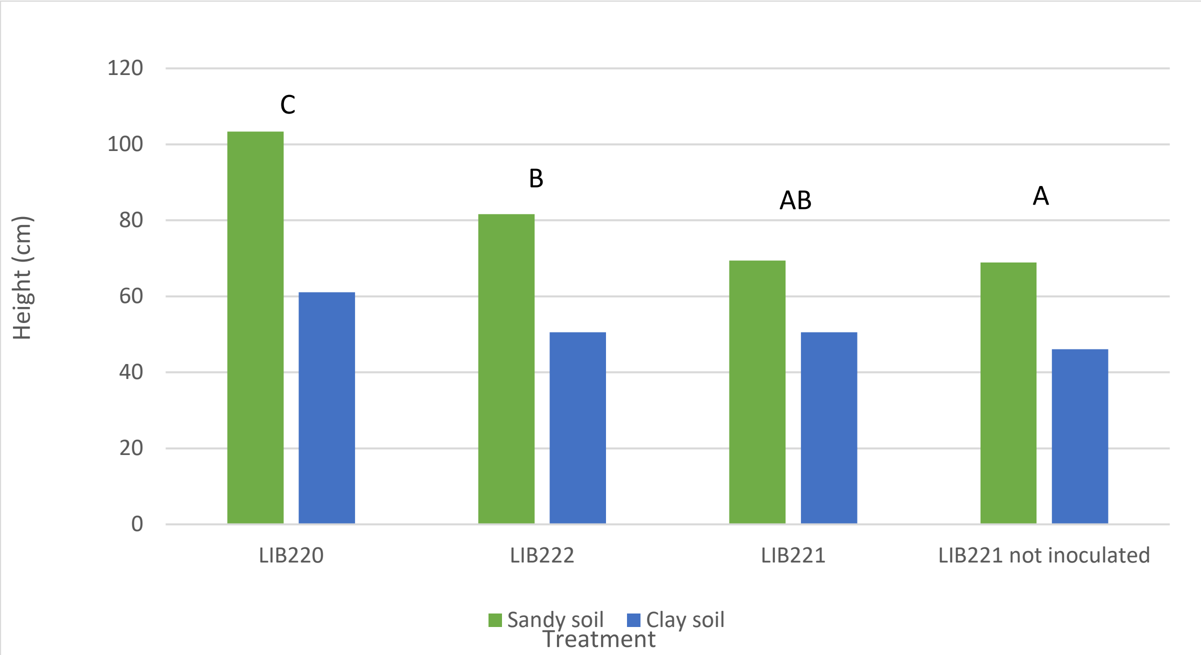


Fig 36. Average MF heights on two soil types in 2018.

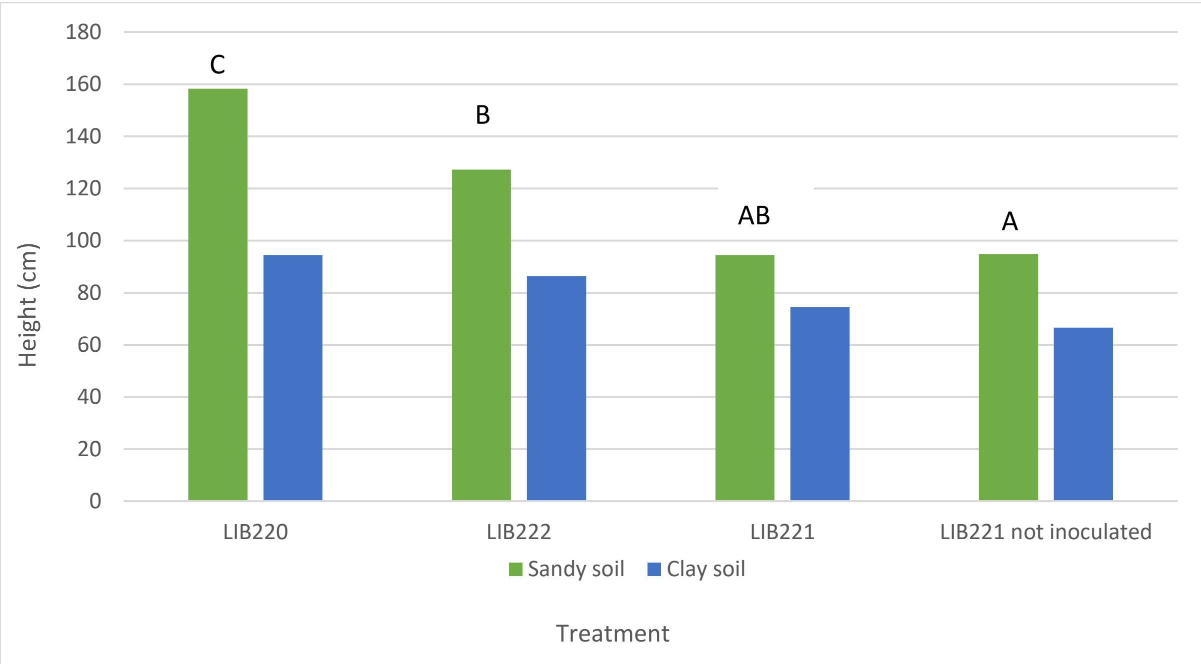


Figure 37. Average 1st order heights on two soil types in 2018.

Crop homogeneity

Stem colour in LIB221 was almost constant before and during flowering. LIB220 was fully constant. LIB222 showed variation in stem colour within the line and slightly changes during flowering.

Table 132. Stem colour type before and during flowering (1=green, 9=purple, 1-9 shades between 1 and 9).

Line	Before flowering			During flowering		
	% Type 1	**% Type 9**	**% Type 1-9**	**% Type 1**	**% Type 9**	**% Type 1-9**
LIB221	96		4	100		
LIB220	100			100		
LIB222	13,3	66,7	20	28,6	52,4	19
Branco	100			100		

Flower colour of Andean lupin was quite varied and colour intensity seems to differ per soil type (Table 133). More flower colours were found than the previously defined I-IV. In addition, types defined as V and VI were found. V having white to pink flowers where the heart colour did not change after fertilization and type VI that had a yellow heart, and white wings and banners. The days required to obtain 100% flowering was shortest in LIB221; 43 days after sowing

Table 133. Flower types of young and fertilized flowers.

Line	Flower type young flowers						Flower type fertilized flowers					
	% type I	**% type II**	**% type III**	**% type IV**	**% type V**	**% type VI**	**% type I**	**% type II**	**% type III**	**% type IV**	**% type V**	**% type VI**
LIB221	69,4	8,3		22,2			71,2	11,5		15,4	1,9	
LIB220		61,1			5,6	33,3		40,9			9,1	50,0
LIB222			5,6	94,4				14,3		85,7		

LIB221 flowers earliest when sown at the middle sowing date (Table 134). Earlier or later sowing does not positively influence flowering time. When intercropped with maize, the accession LIB221(L309) flowers later and equals mono- and intercropped LIB220 and monocropped LIB222. After 100 days still not all Branco plants flowered.

Table 134. Days after sowing to reach 100% flowering. Unless otherwise stated middle sowing was applied.

Line	DAS at 100% flowering
LIB221 early sowing	78*
LIB221	44
LIB221 late sowing	58
LIB221 not inoculated	44
LIB221 low density	44
LIB221 high density	44

LIB221 intercropped	63
LIB220	63
LIB220 intercropped	63
LIB222	63
Branco	>100

*just 2 plants present

4.7.2.4 Crop yield

Biomass production (fresh weight) was measured by harvesting 1 row per plot of which the pods had not been harvested. From every harvest the total fresh biomass was measured. Per harvest per plot, three plants were measured for fresh and dry biomass. From LIB221 early sowing and all the treatments on marginal sand insufficient plants were available for biomass measurements.

On sand only the fresh biomass of LIB220 intercropped with maize was significantly higher than LIB221 and LIB221 not inoculated (Figure 38).

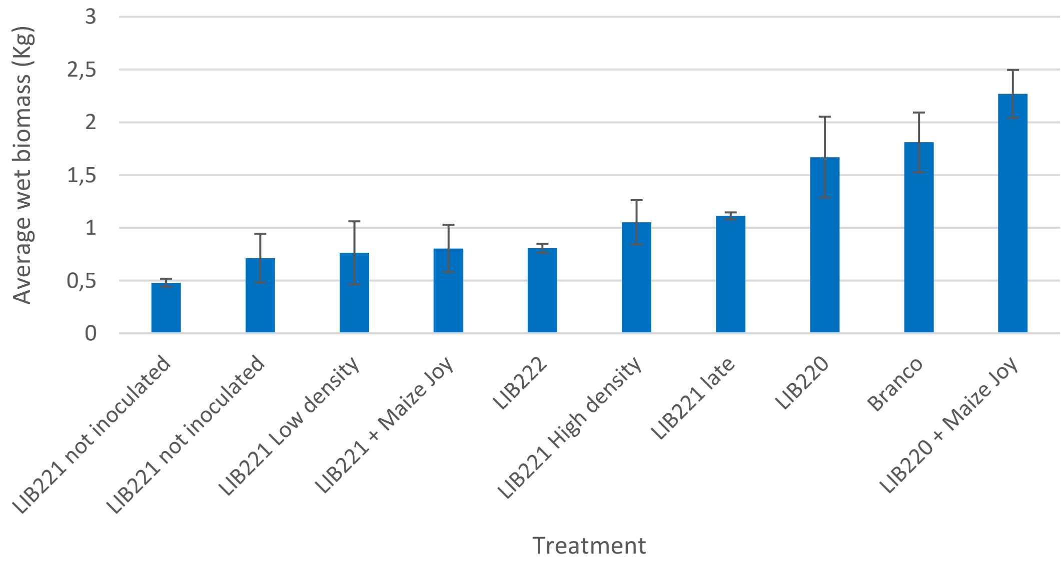


Figure 38. The average fresh weight of three plants per plot on sandy soil. Error bars indicate the standard error (SE).

When the biomass per 3 plants per plot was converted to tonnes per hectare a potential biomass production of 74 tonnes ha^{-1} was obtained for LIB220 which compared to sugar beet, another major biomass producing crop, at 60 tonnes ha^{-1} was high. However, converting the harvest of small plots to a hectare is notoriously inaccurate. Hence the high biomass production of LIB220 only hinted at its potential. Large scale trials will be needed for accurate assessment of biomass potential.

The dry biomass on sandy soil was only significantly different for LIB221 not inoculated and LIB220 intercropped with maize (Figure 39). The lack of significant differences was probably due to the high variation among the plots. For example for Branco we measured 356, 320 and 193 grams of dry weight per 3 plants per plot. In addition, the extensive lodging caused a lot of contamination in the samples with sand. Therefore, the fresh vs the dry weight was inconsistent as well.

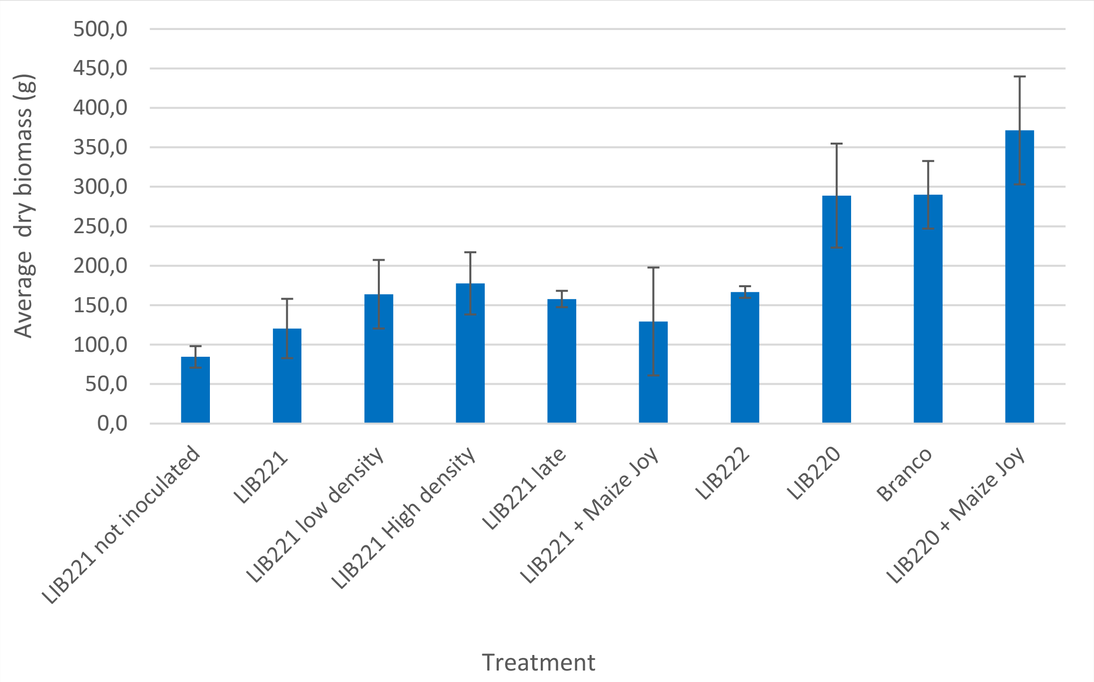


Figure 39. Dry biomass production based on three plants per plot on sandy soil. Error bars indicate the standard error (SE).

For pod production the centre three rows were harvested, dried at room temperature with forced air and pods counted. At the marginal sandy soil, the number of plots that survived the drought was too small to make a reliable assessment. At the sandy soil no pods were harvested of the late sowing of LIB221, because after setting the pods aborted due to drought, also the early sowing of LIB221 was excluded because this were only 2 plants. No significant differences were found between pod setting.

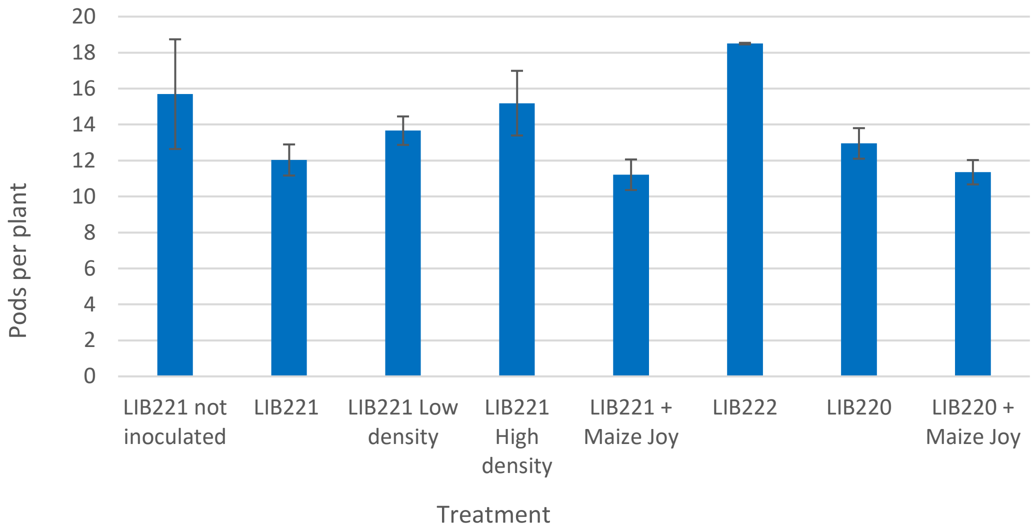


Figure 40. Average pod setting of the main florescence on a sandy soil. Error bars indicate the standard error (SE).

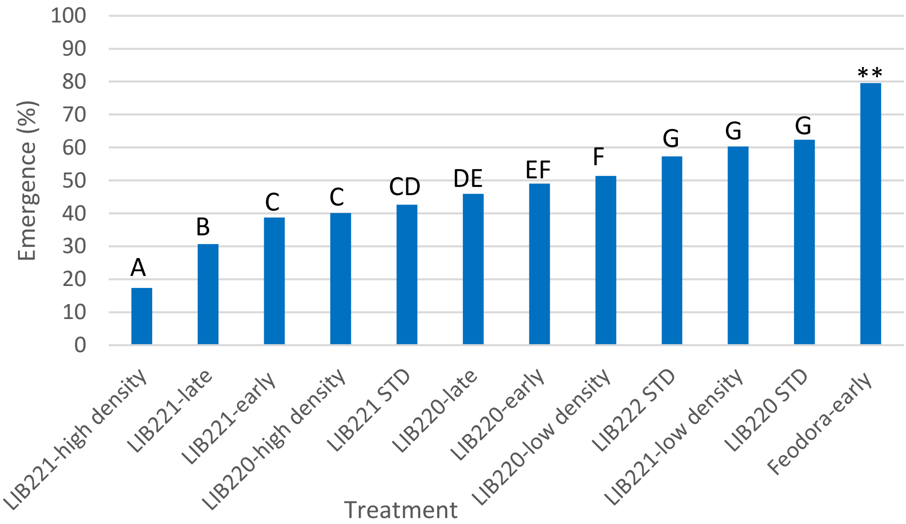
4.7.3 Third cropping season, 2019

Like in the second cropping season three test sites were used. A sandy soil location at the university farm Wageningen, a clay soil location in Kraggenburg and a location with a marginal sandy soil in Noordhout. In all cases a split plot design was used, the seeds sown manually at a sowing depth of about five cm, and a row distance of thirty centimetres was applied. On the sandy and marginal sandy soil, the plots had a surface area of 4,5m^2. On the clay soil the plots were 10,5m^2 to match neighbouring trials. Sowing started in April and continued into May. In Wageningen and Noordhout the trail electric fenching was installed to prevent rodents and deer from entering the field. In Wageningen various measures were also taken to deter birds. Wageningen was the only location were irrigation was possible. It was decided that the timing of the irrigation would be synchronised to the other trials on the same field. During this season on the main location, the sandy soil location, the influence of soing date and density was studied. For sowing three moments of sowing were applied; early (early April), normal (middle April) and late sowing (early May) at which 25plant per m^2 were sown. At mid April 3 levels were used for sowing density: 12,5, 25 and 50 plants per m^2. The standard treatment was sowing in the middle April using a density of 25 plants m^2 (STD).

4.7.3.1 Emergence and homogeneity

For 2019 the amount of seeds available of LIB220 was insufficient. As a result for LIB220 no multi-location analyses can be made. For the general analyses it was therefore decided to focus on the sandy soil test site in Wageningen, the main location. This site could be sown completely using freshly produced seeds from Lusosem. The clay and marginal sand test site had to be sown with seeds left over from previous years. Unfortunately, germination was so poor that LIB220 had to be excluded from the analyses.

Emergence on sandy soil in 2019 was considerably better that in 2018. (Figure 41). Still some treatments failed to establish properly but was inconsistent when comparing line by line. For example, LIB221 germinated quite well at 60% a low density but only 42% at the standard density. For LIB220 standard density germinated better than the low density. Feodora, a commercially available white lupine obtained 80% germination whereas the best germinating Andean lupin LIB220 only obtained 62%. Efforts to improve seed quality for Andean lupin are desirable.



Early= early sowing date and regular sowing density. Late = late sowing date and regular sowing density. Low density= low sowing density at the regular sowing date. High density = high sowing density at the regular sowing date. STD = regular sowing date and density.

Figure 41. Emergence on sandy soil.

Stem and flower colour were assessed to determine if the crop was genetically homogeneous. On sandy soil the stem colour was quite intense. Ranging from bright green (type 1) to dark indigo/purple (type 9). On clay soil the colour intensity was much lower and assessment was difficult. In Table 135 the data is presented for the sandy soil. Seed cultivation in cages to prevent cross pollination by Lucosem has greatly improved the crops homogeneity in LIB220 and LIB221 in comparison to 2018. In LIB222 the stem colour was inconsistent, but the flower colour was more consistent compared to 2018.

Table 135. The stem and flower colour on Sandy soil, averages of all plots per line.

	Stem colour %			**Flower colour %**				
Line	**Type 1**	**Type 9**	**Type 1-9**	**Type I**	**Type II**	**Type III**	**Type VI**	**Type V**
LIB220	99,98	0,02	0		99,4	0,6		
LIB221	99,81	0,19	0	87,2		0,8	11,9	
LIB222	43,66	56,34	0			4	96	

4.7.3.2 Crop development and yield

With regards to the main florescence height LIB221 was not influenced by the timing of sowing. The sowing density did influence LIB221, at a high density of 50pl/m^2 and low density of 11plants/m^2, compared to the standard of 25 plants/m^2, the MF was higher. At the high density this was likely caused by competition for light. At a low density this may be caused by a higher nutrient availability so the plants could grow more vigorously. Also, for LIB220 the timing of sowing had no influence on the MF height. Just focusing on the height, the LIB220 was largest at the standard density of 25 plants/m2. Figure 42 shows that LIB220, 221 and 222 were all significantly different which was also observed for other phenotypical traits addressed later in this chapter. Apart from LIB221 at a low density in all cases the first order branching was higher than the MF. This correlates to the undetermined growth type of Andean Lupin.

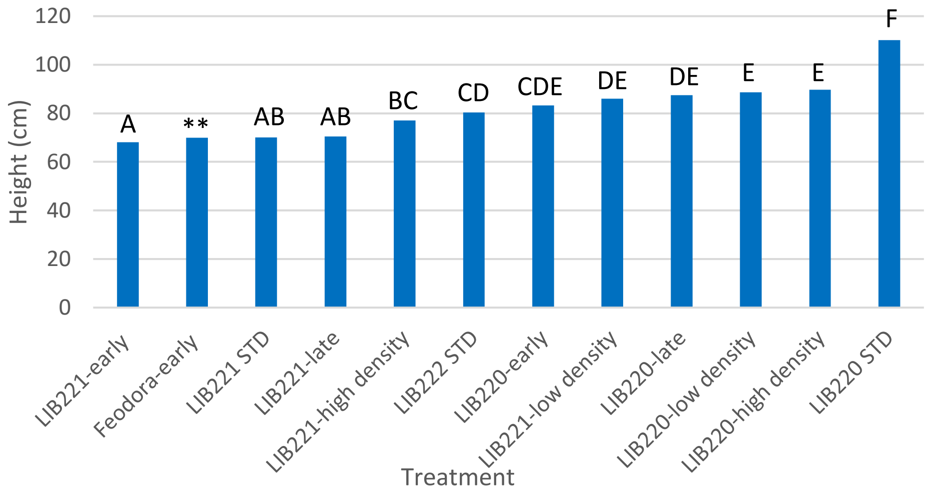


Figure 42. The main inflorescence height on sandy soil.

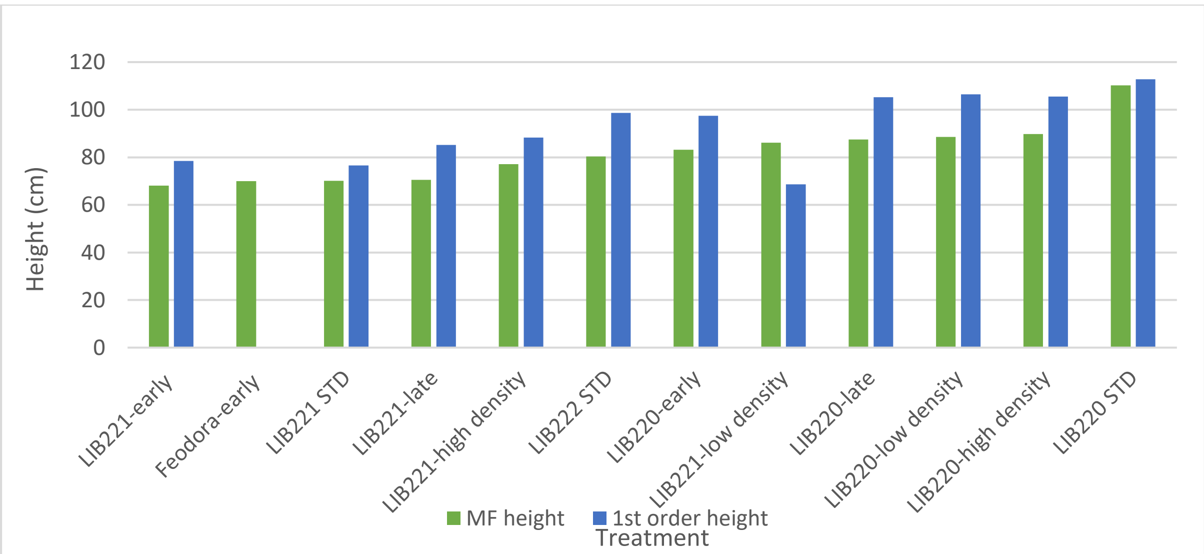


Figure 43. The main florescence height versus the height of the first order.

When cropping Andean Lupin for seed (protein) production it is vital to have a proper pod setting. All accessions obtained flowers and again the accessions reacted differently like it was the case for crop height. Interestingly late sowing of LIB220 and LIB221 resulted in the earliest flowering, early being divined as the lowest amount of days from sowing to obtain 50% of the flowers in open but not yet fertilized stage. LIB220 reacted less strongly to sowing density than LIB221; only cropping at low density delayed flowering, potentially because of the higher vegetative biomass production was observed as more stretching of the plant (Figure 43). LIB221 reacted stronger where the standard sowing density flowered later than low and high density. LIB222 was comparable to LIB220. Interestingly white lupin Feodora which is cropped commercially was average so Andean lupin might, when sown late offer, a benefit in areas with a short cropping season (Figure 44).

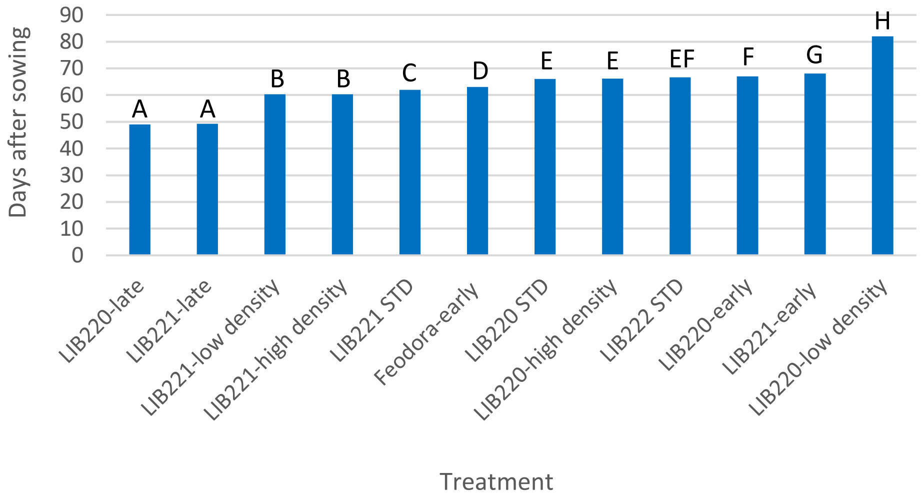


Figure 44. DAS until 50% of the MF is in flower on sandy soil.

Early flowering has no purpose if it does not result in early ripening of the pods. The variation among the treatments was low so the results are only indicative. The trend was that early flowering also resulted in earlier ripening of the MF pods (Figure. 45). However, it must be stated that this is only a beneficial trait if the pods are harvested manually. For mechanised harvesting the MF pods can only be harvested if the other orders have ripened and the plants are dry enough to be combine harvested.

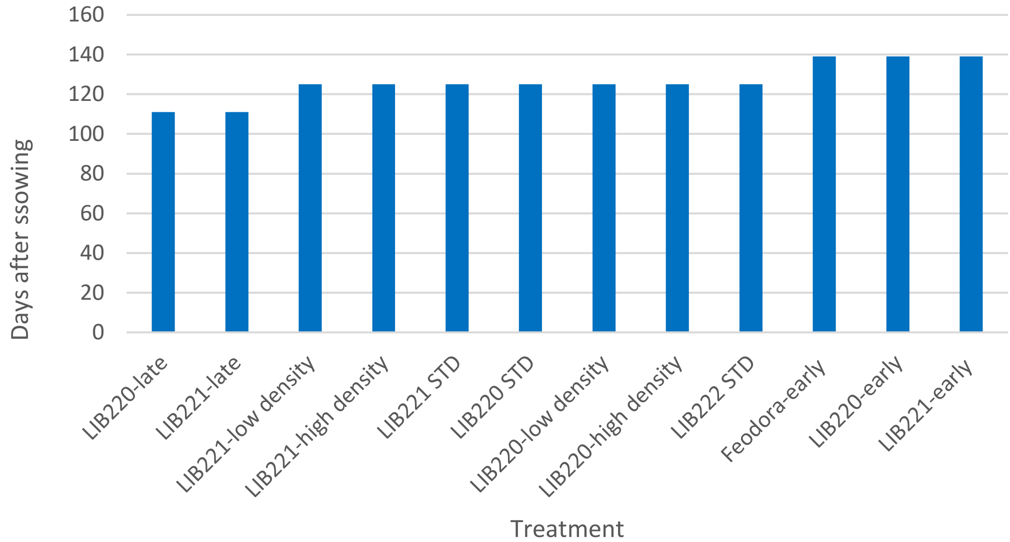


Figure 45. DAS until 50% of the MF is harvest ready on sandy soil.

For LIB220 early sowing resulted in the highest number of pods so the faster flowering of late sown plants has no benefit for MF pod production (Figure 46). For pod production LIB221 was not influenced by the time of sowing. However, it was influenced by sowing density where a low density resulted in less pods than standard or high density sowing. LIB222 was again comparable to LIB221. All Andean lupin accessions compared to Feodora which is interesting. Feodora has been purposely breed for pod production where Andean lupin is in a less developed breeding stage. This may mean that Andean lupin has potential to compete with Feodora as it was bred to be have a more determinate growth type like Feodora has.

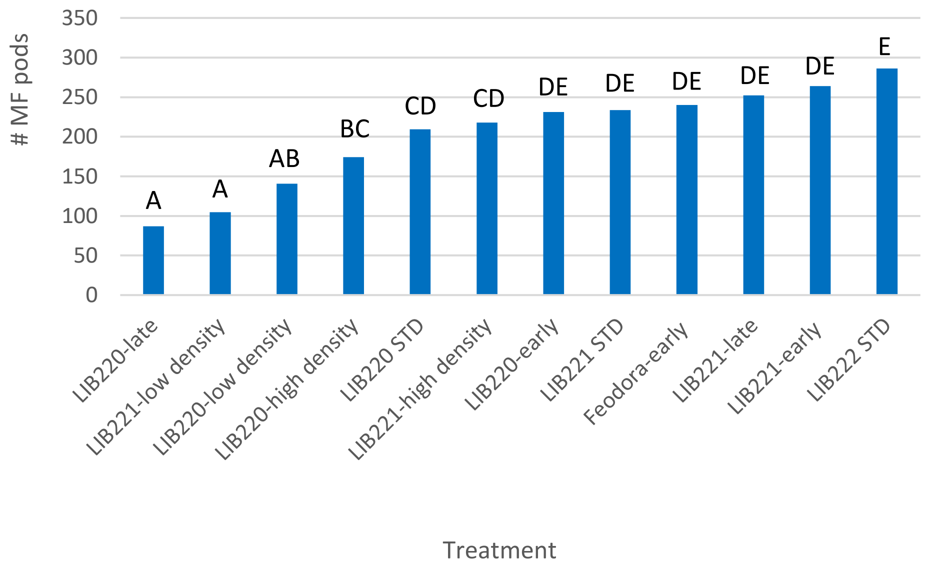


Figure 46. The pod production of the main florescence on sandy soil.

To determine if a higher pod production also results in a higher seed production per hectare that pods were dried for 48h at 35 °C after harvesting. This was mostly to remove any access moisture to enable storage until the pods could be combined using a Wintersteiger stationary combine. Due to unbalanced plots only a mean could be estimated. In addition, it must be stated that subsampling small plots and converting this data to tonnes per hectare is notoriously inaccurate and is only an indication of possible yields when cropping a large area. The results however do indicate that of the Andean lupin the early sown LIB221 has the most potential as both in MF kernel production and kernel yield per hectare of this treatments performed best in comparison to the white lupin Feodora (Figures 47 and 48). However, a yield of only 1,6 tonnes ha^{-1} was very poor for a white lupin which more commonly exceeds > 3 tonnes ha^{-1}, so repeating this field trial would be needed to truly compare Andean lupin to commercial white lupin.

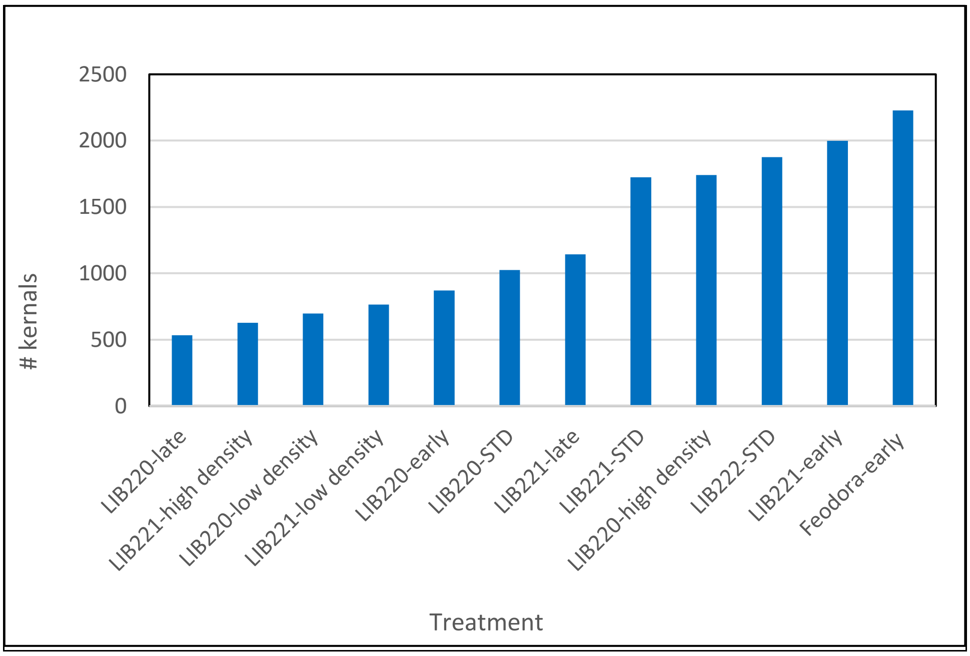


Figure 47. The kernel yield of the MF on Sandy soil.

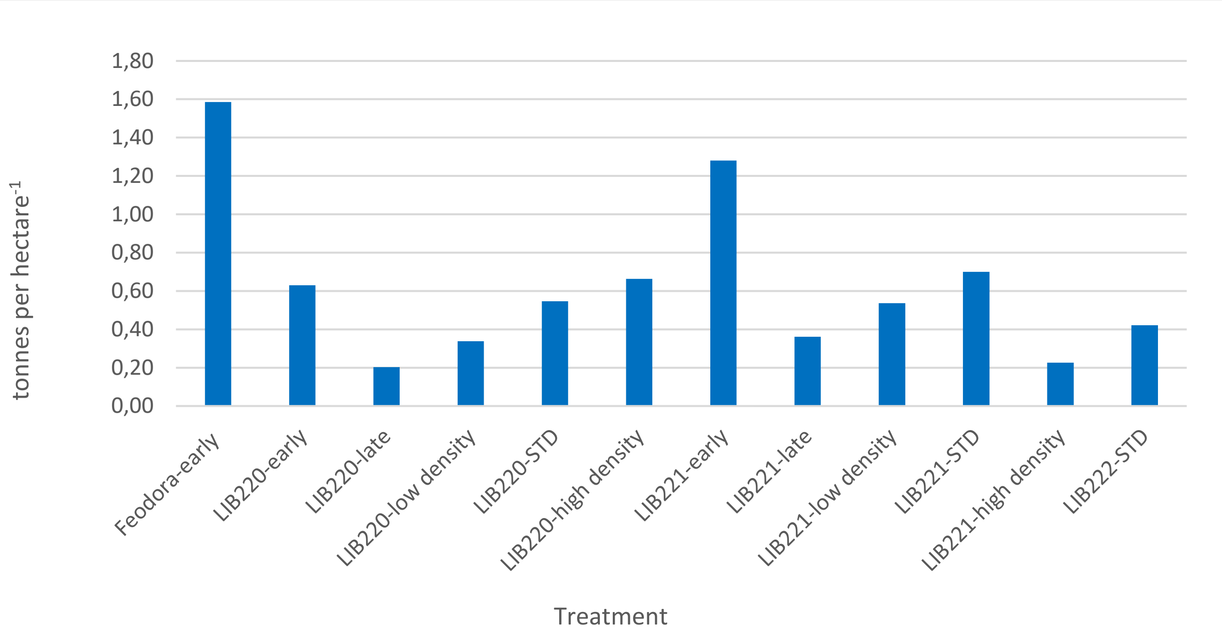


Figure 48. The grain yield in tonnes ha^{-1}.

4.7.4 Conclusions

In 2017 the accessions from the Vandinter Semo outperformed all the gene bank accessions with regards to germination and these accessions were more homogeneous. These findings resulted in the decision to continue with the Vandinter Semo accessions for the next cropping seasons.

In 2018 more advances were made in getting to know the crops behaviour but the seed quality and quantity was still limiting.

In 2019 the seed quantity was limiting but the quality and homogeneity of the crop was much improved.

5 Soil types

To obtain a clear indication about at which soil Andean lupin would thrive we aimed at obtaining a diverse set of soils for the field trials. Within soil classification 24 reference groups are defined. In addition, soil features like the presence or absence of layering or specific components are added. A poorly developed soil is a soil that lacking the clear horizontal layering that some older or more mature soils do have. It is not an indication of how much the soil is influenced by human activity.

Table 136. The soil types and main features of the LIBBIO field trial locations.

Country	Location	Soil Classification	Characteristics
Austria	Lambach	Cambisol	Pale brown soil, lacks strong layering, proper water holding capacity usually favourable for agriculture. Cambisol of loamy-silty topset beds with three soil horizons: A/Bt1/Bt2. The A-horizon is about 20 to 30 cm mighty and contains silt. The Bt1-horizon is between 30 and 70 cm and contains loamy silt. The Bt2-horizon is between 70 and 100 cm and contains silty clay.
Austria	Stadl-Paura	Calcaric Skeletic Regosol	Soil that is poorly developed, rocky and high in calcium carbonate. Low water holding capacity. Less suitable for agriculture. Finely granulated and coarse terrace-material with two horizons: A/C. The A-horizon is built from loamy sand with a high percentage of coarse material and is about 20 to 30 cm mighty. The C-horizon is between 30 and 100 c, and is built of predominant gravel and broken stones and contains much lime.
Austria	Trautenfels	Skeletic Cambisol	Rocky pale brown soil, lacks strong layering, has proper water holding capacity. Skeletic Cambisol of secondary foliate with four horizons:

			A/AB/Bv/C. The A and AB-horizon contain sandy silt with a moderate percentage of coarse material; the A-horizon reaches until 25 cm. The AB-horizon is between 25 and 45 cm. The Bv-horizon is built from sandy silt with a high percentage of coarse material and reaches until 60 cm. The C-horizon has been built of weathered rocks.
Greece	Athens	Clay loam, 17.5% caco3. Lcr, Rhodic luvisol or calcaric fluvisol	Rodic luvisol: soil with a different textures and high clay activity. Fluvisol: young soil in a flood plain.
Greece	Erythres	Clay, 0.2%, $CACO_3$, lc, calcaric-lithic leptosol	A shallow soil layer (to about 25cm deep) over a rocky or gravelly layer
Greece	Kalamata TEI	Calcaric fluvisol Silt loam, 8.8%caco3	A young soil soil with a higher calcium carbonate content with fluvic soil material
Greece	Kalamata Aithea	Calcaric regosol. Sandy loam	Poorly developed soil rich in secondary calcium carbonates.
Greece	Mani-Agios Nikolaos, Lakonias	Sandy loam, calcaric-lithic leptosol	A shallow soil over a hard rock or gravelly layer, rich in secondary calcium carbonates.
Iceland		Andosol	Young mineral soil in volcanic regions. Sensitive to erosion.
Netherlands	Driebergen, Gooijer- wetering	Gleyic podzol	Low laying soils with a humus layer. Need drainage due to the close proximity of the groundwater. Loamy sand.
Netherlands	Driebergen, Noordhout	Sandy soil often found in dune areas. Dystric Regosol	Soil that is poorly developed, Sandy soil found mostly in dune or beach areas. Pool soil fertility.
Netherlands	Kraggenburg	Calcaric-gleyic Fluvisol	Low lying young soil high in calcium carbonate, little layering. Can be highly fertile.
Netherlands	Texel	Eutric Regosol	Weakly developed soil, little layering. Sandy soil found mostly in dune or beach areas. Poor soil fertility.
Netherlands	Wageningen	Loamy sand/gleyic Podzol	Low laying soils with a humus layer. Need drainage due to the

			close proximity of the groundwater. Loamy sand.
Portugal	Quinta do Loreto, Coimbra	Sandy loam, Eutric cambisol or a Chromic cambisol	Relatively young soil, lacks strong layering, proper water holding capacity usually favourable for agriculture.
Portugal	ESAS, Santarém	Calcaric fluvisol (Lusosem stated sandy)	Young soil usually found in areas that are commonly flooded, rich in secondary calcium carbonates
Portugal	ISA, Lisboa	Clay, chromic vertisol	Soil rich in clay minerals, vertisols shrik and swell when getting dry or wet, deep cracks can occur.
Romania			
Spain	Cortijo Frías - ENDL	Haplic Calcisol	Soil rich in secondary calcium carbonates. No other striking features
Spain	Culturhaza	Haplic Calcisol	Soil rich in secondary calcium carbonates. No other striking features.

5.1 Austria

In Austria we had two different locations with different climatic conditions and different soil types. In the pre-alpine region, we had a good soil at Lambach and a poor soil at Stadl-Paura. At Trautenfels in the alpine region the soil is well suitable for grassland but not so well suitable for crops.

The soil types of the year 2017 were the following:
The soil type of Trautenfels: Skeletic Cambisol without lime of secondary foliate with four horizons: A/AB/Bv/C. The A and AB-horizon contain sandy silt with a moderate percentage of coarse material; the A-horizon reaches until 25 cm. The AB-horizon is between 25 and 45 cm. The By-horizon is built from sandy silt with a high percentage of coarse material and reaches until 60 cm. The C-horizon has been built with weathered rocks. The soil is moderately dry.
The soil type of **Stadl-Paura, field 5/1**: Calcaric Skeletic Regosol, near to Calcaric Skeletic Leptosol with a very high percentage of coarse soil in horizon C; dry; very high soil permeability; horizon A (until 25 cm), then horizon C (until 100 cm); soil texture: horizon A = loamy sand with a high percentage of coarse soil, horizon C: only gravel;
The soil type of **Lambach, field 12/3**: Calcaric Skeletic Regosol, near to Calcaric Skeletic Leptosol with a very high percentage of coarse soil in horizon C; dry; very high soil

permeability; horizon A (until 25 cm), then horizon C (until 100 cm); soil texture: horizon A = loamy sand with a high percentage of coarse soil, horizon C: only gravel
The soil types in the year 2018 were the following:
The soil type of **Trautenfels**: Eutric Gleysol, from silty alluvial soil; Ag-horizon (until 10 – 15 cm), then CG-horizon (until 40 cm) and C (until 100 cm); a very humus soil; soil texture: silt; only weak chalky, soil permeability moderately; influenced by ground-water
The soil type of **Stadl-Paura, field 2/1**: Calcaric Skeletic Regosol, near to Calcaric Skeletic Leptosol with a very high percentage of coarse soil in horizon C; dry; very high soil permeability; horizon A (until 25 cm), then horizon C (until 100 cm); soil texture: horizon A = loamy sand with a high percentage of coarse soil, horizon C: only gravel;
The soil type of **Lambach, field 12/3**: Eutric Cambisol from loamy-silty overburden without lime; A-horizon (until 20 – 30 cm), Bt1-horizon (until 60 – 70 cm) and then Bt2-horizon (until 100 cm); soil texture: A-horizon = silt, Bt1-horizon = loamy silt; Bt2-horizon = silty loam, no lime

The soil types in the year 2019 were the following:
The soil type of **Trautenfels**: Skeletic Cambisol without lime of secondary foliate with four horizons: A/AB/Bv/C. The A and AB-horizon contain sandy silt with a moderate percentage of coarse material; the A-horizon reaches until 25 cm. The AB-horizon is between 25 and 45 cm. The By-horizon is built from sandy silt with a high percentage of coarse material and reaches until 60 cm. The C-horizon has been built with weathered rocks. The soil is moderately dry.
The soil type of **Stadl-Paura**, **field 33**: Calcaric Skeletic Regosol, near to Calcaric Skeletic Leptosol with a very high percentage of coarse soil in horizon C; dry; very high soil permeability; horizon A (until 25 cm), then horizon C (until 100 cm); soil texture: horizon A = loamy sand with a high percentage of coarse soil, horizon C: only gravel;
The soil type of **Lambach, field 7:** Eutric Cambisol from loamy-silty overburden without lime; A-horizon (until 20 – 30 cm), Bt1-horizon (until 60 – 70 cm) and then Bt2-horizon (until 100 cm); soil texture: A-horizon = silt, Bt1-horizon = loamy silt; Bt2-horizon = silty loam, no lime

Over the years it has been observed that the Andean lupine growth was heavily affected by the drought caused by the coarse soil and high permeability of the soil at Stadl-Paura. In 2019 up to 60 % of the line LIB 221 emerged plants were lost due to drought.

5.2 Greece

In Greece, the experimental location of Athens was classified as Regosols according to FAO classification profiling (Yassoglou et al. 2017), while in Kalamata as Fluvisols (Misopollinos et al. 2015), in Erythres Cambisol (FAO, 2012) and in Mani Vertic Luvisols (Lv) (FAO, 1992). A large part of Greek agricultural soil territory contains a high percentage of calcium carbonate ($CaCO_3$) (Anagnostopoulos et al. 2003; Zamanidis and Evangelogiannis 2017) that can inhibit lupin growth and vigor, although regions with low $CaCO_3$ can be found in some regions (Figure 49). Also, soil alkalinity (pH) ranges from 6,4 to 8,8 (Figure 50) and a high soil alkalinity can affect negatively lupin plants development and cause chlorotic symptoms.

Soil samples were taken in depths of 0-30 cm and 30-60 cm according to the common soil analysis LIBBIO protocol. Soil analysis regarding full soil profile of each experimental field texture $CaCO_3$, pH, EC (μS cm^{-1}), OM%, total N, NO_3, NH_4 and plant-available soil P and K analysis were conducted. Soil samples concentration in total and available S (mg kg^{-1}). Available Mg (mg kg^{-1}) and available Na (mg kg^{-1}) was also measured. The soil parameters obtained from a depth of 0-30 cm are presented as the plants in Greek rocky soils did not expand their roots in a greater soil depth (Table 137).
The clay loam (CL) soil in Erythres. In combination with the high rainfall that characterizes the location. Resulted in plant stress and losses due to water lodging (Figures 51, 52).

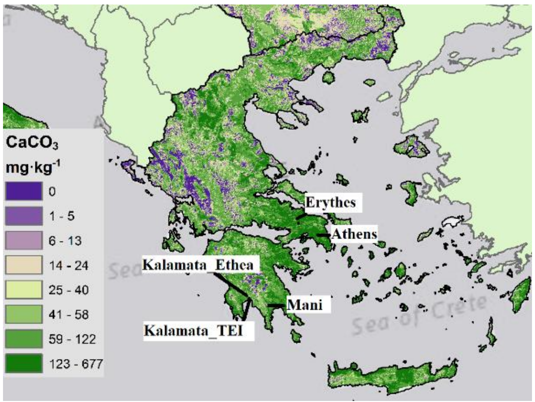


Figure 49. Soil carbonate content of Greece (ESDAC., 2020).

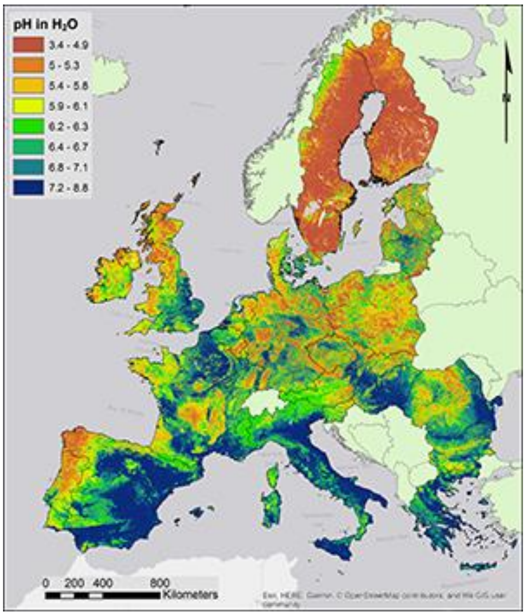


Figure 50. Soil alkalinity (pH) of EU countries (ESDAC, 2020).

Table 137. Main mean soil parameters in a depth of 0-30 cm of the experimental fields established in Greece.

Location	Soil type	$CaCO_3$ (%)	pH	EC (μS cm^{-1})	OM (%)	Total N (%)	NO_3 (ppm)	NH_4 (ppm)	Avail. P (ppm)	Avail. K (ppm)
Athens (37º59'03.5"N. 23º42'10.0"E. 24 m asl)	SCL	17,5	8,1	304,33	3,2	0,196	6,35	6,97	45,46	881,33
Kalamata (TEI) (37°03'39.3"N. 22°03'49.5"E. 7 m asl)	SL	14,3	7,8	266,3	1,2	0,117	10,03	2,78	41,37	198,00
Kalamata (Aithaia) (37°03'33.9"N. 22°02'46.8"E. 41 m asl)	SL	15,5	7,4	171,6	7,4	0,144	6,22	2,44	37,00	172,86
Erythres (38º12'55.6"N.	CL	0,2	7,3	*(not measured)*	3,1	0,114	7,30	1,20	0,30	43,00

23°18'47.2"E. 376 m asl)											
Mani (36°49'36.4"N. 22°26'04.5"E 475m a.s.l)	SL	0,2	6,0	140,0		0,8	0,285	19,68	15,20	37,52	172,66

Figure 51. Stressed plants of cv. Multitalia. in Erythres (2017-2018). due to increased precipitation.

Figure 52. Completely water logged plots in Erythres (2017-2018).

5.3 Iceland

The accessions tested in 2017 were planted into two different soil types, 1) a barren eroded site with little or no organic material and 2) a field with organic soil that has for decades been used for hay production and barley.

The eroded site is a sparsely vegetated sandy desert, freely drained with basaltic parent material and high levels of volcanic glass (Arnalds 2008, Arnalds et al. 2013). Upper layers of the soil are sandy loam/loamy sand, though the surface layer is gravelly due to winter frost heaves (Arnalds et al. 2013). The soils are low in carbon (<1%), have low water retention, and are classified as Vitrisols by Arnalds (2013) or as Andosols by the World Reference Base (IUSS Working Group WRB, 2015). The organic soil site is classified as Brown Andosol (Arnalds, 2013) or Haplic Andosol (IUSS Working Group WRB, 2015) with 1-12% carbon, freely drained with high levels of Allophane (15-30%).

To identify further the physical and chemical properties of the soil, samples were taken from each site and analysed. A soil auger with a 5cm diameter was used to extract samples to a depth of 10cm. Ten samples were collected from randomly selected points at the sites and mixed. This was repeated three times resulting in six samples in total that were analysed for bulk density pH, N and P, summarized in table 138.

Table 138. Physical and chemical properties of soil at the experimental sites.

	Bulk density (g/m3)	**pH**	**Total soil N (%)**	**P (Al) (mg P/100g soil)**
Eroded site	1,46	6,34	0,24	0,25
Eroded site	1,50	6,47	0,18	0,74
Eroded site	1,53	6,75	0,21	0,81
Organic field	0,85	5,72	0,71	8,20

Organic field	0,83	5,54	0,90	7,89
Organic field	0,79	5,52	1,50	6,50

5.4 Portugal

In Portugal, acid soils are predominant (Figure 1). Large areas of neutral soils are low calcium soils. Cambisols are dominant along with Luvisols. Vertisols are also wide-spread (Figure 2). For these reasons, lupines are widely spread all over the country. Yellow lupin (*Lupinus luteus*) is the most common and is used for soil sideration, in some cases in large areas as in cork trees plantations, like in Podzols soils. White lupin (*Lupinus albus*) is also cultivated, usually in small parcels by small farmers and hand harvest for auto-consumption as snack or sold in small quantities to industries, also for snacks production. Only some areas of Calcic Vertisols aren't proper for lupincultivation.

LIBBIO trials were performed in 3 different types of soil. A Eutric Fluvisol (associated to Calcic Fluvisols) with neutral pH in Loreto/Coimbra, a Calcic Cambisol with neutral pH, but with severe anthropomorphic influence resulting on a non-calcic sandy light soil in ESAS/Santarém and a Cromic Vertisol in ISA (Figure 2).

In all 3 locations Andean lupin proved to be well adapted. ESAS soil can be considered as a marginal soil and since the 2016/2017 winter crop season plants behaviour and potential productivity were positive. In 2018/2019 winter crop season, on a density trial performed in this location, several plots yielded above 2.000 kg/ha and one plot reached 4.060 kg/ha. These potential yields weren't significant different from others in better quality soils.

Poor drainage is common on soil adapted for lupins in Portugal. This must be taken on account when soil preparation for lupins to improve soil drainage.

Table 139. Soil charcteristics in Portugal.

Country	Name	Field coordinates	Texture	pH	% OM	P2O5 (mg/kg)	K2O (mg/kg)	Total Ca
PT	Quinta do Loreto Coimbra	40°13'49.67" N 8°26'38.53"W	Sandy-Loam	6,6	2,0	279,0	205,0	Low
PT	ESAS, Santarém	39°15'5.84"N 8°42'6.47"W	Sandy	7,4	1,6	622,0	129,5	Low
PT	ISA, Lisboa	38°42'25.40"N 9°10'52.08"W	Clay					Low

5.5 Romania

In 2017, at USAMV Iasi three field experiments were set up in three locations, which are part of the Moldavian Plateau, but with some different soil and climatic conditions. The distances between these 3 places is about 110-150 km.

The GPS location was contained between 46°51'07" and 47°37'52" north latitude, between 26°14'40" and 27°30'41" eastern longitude and the altitude from sea level was between 113 m and 358 m (table 140).

Table 140. The GPS locations of the Andean lupin experiments in Romania, 2017.

Location	County	North Latitude	Eastern Longitude	Altitude from sea level (m)
Ezareni	Iasi	47˚07'22"	27˚30'41"	113
Secuieni	Neamt	46˚51'07"	26˚51'50"	182
Suceava	Suceava	47˚37'52"	26˚14'40"	358

General description of the soil type per location (FAO classification) and a brief description of the soil profile (Table 141).

Table 141. The main characteristics of soil for Andean lupin experiments in Romania, 2017.

Location	Ezareni	Secuieni	Suceava
Soil type	Haplic Chernozem (CHha)	Haplic Chernozem (CHha)	Cambic Phaeozem
General soil profile	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-AB-Bv-B/C-Cca
Thickness of A-horizont (cm)	43	40	40
Dominant surface textural class	Clay loam	Clay loam	Clay loam
% organic mater	1,56	1,58	2,96
% clay	33,0	15,0	40,0
% silt	28,5	48,2	32,0
% sand	39,0	36,8	*28,0*
pH	6,69	6,79	5,10
P (ppm)	19,90	24,35	19,00
K (ppm)	299,4	453,8	245,2
Humus (%)	2,69	2,73	3,47
Sum of the bases meq (%)	28,7	24,1	14,2
Hydrolitic acidity	2,34	1,44	6,20
Saturation level V (%)	92,5	94,3	69,5
Nitrogen index	2,49	2,57	2,41

In 2018, at USAMV Iasi organised five fields with experiments, in three areas (counties), which are part of the Moldavian Plateau from Romania, with different soil and climatic conditions. The distances between these 3 areas are 110-150 km from one to another.
The GPS location was contained between 46˚51'07" and 47˚37'52" North latitude, between 26˚14'40" and 27˚33'15" Eastern longitude and the altitude from sea level was between 109 m and 358 m (table 1 and Figure 1).
The main target was established by the members of Consortium to be **seeds multiplication**.

Table 142. The GPS locations of the Andean lupin experiments in Romania, 2018.

Location	County	North Latitude	Eastern Longitude	Altitude from sea level (m)
Ezareni	Iasi	$47^0$07'25"	$27^0$30'53"	109

Iasi	Iasi	47^{0}11’42”	27^{0}33’15”	159
Horlesti	Iasi	47^{0}15’23”	27^{0}27’39”	156
Secuieni	Neamt	46^{0}51’07”	26^{0}51’50”	182
Suceava	Suceava	47^{0}37’52”	26^{0}14’40”	358

Figure 53. The map with the locations of the Andean lupin experiments in Romania, 2018.

Most of the analyses were provided by Eurofins thanks to the help of Louis Bolk Institute (Table 143).

Table 143. The main characteristics of soil for Andean lupin experiments in Romania, 2018.

Location	Ezareni	Iasi	Horlesti	Secuieni	Suceava
Soil type	Haplic Chernozem (CHha)	Haplic Chernozem (CHha)	Haplic Chernozem (CHha)	Haplic Chernozem (CHha)	Cambic Phaeozem
General soil profile	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-AB-Bv-B/C-Cca
Thickness of A-horizon (cm)	43	47	50	40	40
Dominant surface textural class	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam
% organic mater	3,00	2,80	5,2	3,1	4,4
% clay	35	29	30	30	*30*

% silt	55	53	50	49	*53*
% sand	7	11	9	18	*15*
pH	6,2	7,6	7,4	7,0	5,2
P plant available mg P/kg	4,0	1,4	12,2	13,8	5,2
P soil stock mg P_2O5/kg	36	41	113	121	39
P total mg P_2O5/100g	142	115	304	216	222
K mg K/kg	337	153	365	231	180
S plant available mg S/kg	4,8	4,1	≤1,1	≤1,1	8,5
S total in soil mg S/kg	215	215	330	245	295
Mg mg Mg/kg	337	153	365	231	180
Na mg Na/kg	13	6	8	6	7
Organic matter (%)	3,0	2,8	5,2	3,1	4,4
C inorganic (%)	0,07	0,65	0,09	0,07	0,65
C total g C/100g	2,0	2,2	3,1	2,0	2,7
CaCO3 (%)	≤0,2	4,7	1,7	0,4	0,2

No special problems were expected related to the subsoil composition. One field (Iasi) was used for the first time for arable cropping after 20 years of grape production so it was plougd prior to sowing.

In 2019, at USAMV Iasi organised five fields with experiments, in three locations in three different regions. All the fields were in a part of the Moldavian Plateau from Romania, with different soil and climatic conditions. The distances between these 3 areas are 110-150 km from one to another and all the fields were State Research Stations property.
The experiments were located between 46°51’09” and 47°37’52” North latitude, between 26°14’40” and 27°30’54” Eastern longitude and the altitude from sea level was between 105 m and 358 m (table 144 and Figure 1).
The main target was established by the members of Consortium to be seeds multiplication.

Table 144. The GPS locations of the Andean lupin experiments in Romania, 2019.

Location	**County**	**North Latitude**	**Eastern Longitude**	**Altitude from sea level (m)**
Ezareni	Iasi	47°07’22”	27°30’54”	105
Secuieni	Neamt	46°51’09”	26°51’49”	184
Suceava	Suceava	47°37’52”	26°14’40”	358

Most of the analyses were provided by Eurofins via the Louis Bolk Institute in 2018 - table 2. In 2019 we placed the trials nearly in the same places as in 2018.

Table 145. The main characteristics of soil for Andean lupin experiments in Romania, 2019.

Location	Ezareni	Secuieni	Suceava
Soil type	Haplic Chernozem (CHha)	Haplic Chernozem (CHha)	Cambic Phaeozem
General soil profile	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-Bv1-Bvk-Cca1-Cca2	Ap-Atp-Am-AB-Bv-B/C-Cca
Thickness of A-horizon (cm)	43	40	40
Dominant surface textural class	Clay loam	Clay loam	Clay loam
% organic mater	3,00	3,1	4,4
% clay	35	30	*30*
% silt	55	49	*53*
% sand	7	18	*15*
pH	6,2	7,0	5,2
P plant available mg P/kg	4,0	13,8	5,2
P soil stock mg P_2O_5/kg	36	121	39
P total mg P_2O_5/100g	142	216	222
K mg K/kg	337	231	180
S plant available mg S/kg	4,8	≤1,1	8,5
S total in soil mg S/kg	215	245	295
Mg mg Mg/kg	337	231	180
Na mg Na/kg	13	6	7
Organic matter (%)	3,0	3,1	4,4
C inorganic (%)	0,07	0,07	0,65
C total g C/100g	2,0	2,0	2,7
CaCO3 (%)	≤0,2	0,4	0,2

No special problems related to the subsoil composition. One field (Iasi) was use for the first for ploughing time after a wine plantation (more than 20 years).

5.6 Spain

The percentage of clay, silt and sand reported in the analysis indicated that the soil samples were all in the silty loam class. Generally, this type of soil has intermediate physical properties between sandy soil and clay soil with a good water retention capacity, good drainage, no hardening and a compaction typical of clay. Soil acidity, with a slightly basic pH, ranking from (7,7 to 7,2), is within the range suggested for the plants' optimum growth. The value detected in the soil analysis indicates that soil pH did hinder nutrient availability. More details in the results of the soil analysis are found in Table 146.

Table 146. Results of the soil analysis.

Sections	Resultaat	Enheid	C-R1	C-R2	ENDL-R1	ENDL-R2

1	N-soil stock	mg N/kg	1020	1130	1170	1170
	S-plant available	mg S/kg	10	6,5	16,4	10
	S-soil stock	mg S/kg	425	<150	<150	<150
	P-plant available	mg P/kg	1	2,5	1,6	1,7
	P-soil stock	mg P_2O_5/kg	98	145	165	122
	K-plant available	mg k/kg	121	155	129	108
	Mg-plant available	mg Mg/kg	162	157	157	164
1.2	Na-plant available	mg Na/kg	13	14	12	15
2	Acidity (pH)			7,3	7,2	7,3
	Acidity (pH)		7,7			
	Acidity (pH-KCl)		7,7	7,3	7,2	7,3
3	Organic matter	%	2,2	0,6	2,1	1,8
	C-inorganiccarbonated lime	%	2,11	2,34	1,6	0,89
	Carbonated lime	%	16,4	18,2	12,3	6,6
4	Clay (<2 µm)	%	17	19	20	20
	Silt (2-50 µm)	%	36	30	44	48
	Sand (>50 µm)	%	28	31	22	24
5	P-total	mg P_2O_5/ 100g	198	183	163	157
	C-total	g C/ 100g	2,8	2,9	2,4	1,8

5.7 The Netherlands

5.7.1 Field trial locations

In the Netherlands the Noordhout location is the most challenging location for crop growth. Located on a sandy ridgeline dating back to the last ice age, this soil provides the lowest amount of nutrients for plant growth and has the lowest water holding capacity, hence this location was our ‘marginal soil test site’. The soil could only be sampled to a depth of 25 cm, below this you hit a rock-hard sand layer. The soil was comprised of 90% sand, a little silt and hardly any clay. The pH was only 4,0. The soils had a calcium carbonate percentage of 0,05%. No soil born or root diseases were observed but the low water holding capacity did result in crop losses due to drought.

The Gooijer-wetering, the main location in 2017 and 2018, was classified as a loamy sand with 10% clay, 39% silt and 28% sand. The pH was 5,6 and the calcium carbonate was 0.08%. Hence no issues with pH or calcium intolerance were expected. At a depth of 25-30 cm a layer of yellow coloured sand was found which was very impermeable. At this location, no problems were expected except in case of extreme rain when water logging could occur.

However, no extreme rainfall occurred during the project. In addition, no soil-born or root diseases were observed.

The Kraggenburg location had the highest clay percentage and highest water holding capacity. The soil had 18% clay, 51% silt and 22% sand generally this area is considered to have some of the best soils for agriculture in the Netherlands. In Kraggenburg the soil depth before you hit yellow sand was about 40cm, so slightly deeper than Noordhout and comparable to Gooijer-Wetering. Kraggenburg did have a higher calcium carbonate content at 6,4% and a pH of 7,4. This pH could reduce the vigour of lupin growth; when soil pH exceeds 7,2 nutrients can be partly immobilized. However, compared to the soils of other partners the Dutch soils were decent. No root borne diseases were observed during any of the cropping seasons.

The Wageningen location, the main location of 2019, also offered a loamy sandy soil, with little clay, 11% silt and 85% sand. The pH was 6,1 and the calcium carbonate level was 0,04. So like Gooijer-Wetering no issues were expected. At a depth of 30 cm the soil layering becomes more unclear and more yellow sand was found when probing. Like the other locations no soil born or root diseases were found.

5.7.2 Crop performance

In general crop growth was faster and most vigorous at the loamy soil locations. 2017 was an exploratory year were the amount of seeds was lacking, 2018 was extremely dry resulting in premature plant death, so as a result 2019 was the cropping season selected for general analyses of crop performance.

In 2019 not enough seeds were available from LIB220 for the marginal and clay soil test site. It is important to note, that in table 147, the performance of LIB220 was included but since this is seed from the year before it cannot be compared to LIB221 and LIB222 on marginal and clay soils but it does offer a hint to its potential performance. The analyses were performed in Genstat version 19, using an anova for unbalanced design with a LSD < 0,05 Bonferoni test.

On marginal sandy soil, germination was always poorest for all accessions. On sandy soil the canopy height of all accessions were significantly higher than on clay or marginal soil. The number of days after sowing to obtain 50% of the main florescence in flower was fastest for LIB221 on sandy and clay soils, on marginal sands in was slower but still the fastest to flower of all accessions on marginal soil. Also, the number of days to be able to harvest the pods of the main inflorescence was fastest for LIB221 on clay and sand. For marginal soils no differences were found between the accessions. The biomass production on sandy soil was highest for LIB220 however, since this location was sown using a higher quality of seed than on clay and marginal soils, we cannot determine if a soil x genotype interaction was present for LIB220. For LIB221 no significant differences were found between clay and sandy soils, on sandy soils LIB222 outperforms LIB221. In general, we can state that LIB221 flowers and ripens early but does not produce much biomass, LIB220 and LIB222 took longer to flower and set pods but were stronger biomass producers. These results show that different accessions need to be selected for seed production than for biomass yield.

Table 147. The crop performance of Andean lupin on marginal sand, sand and clay soil.

	Germination	**Canopy height cm**	**DAS 50% MF in flower**	**DAS harvest ready MF**	**Biomass dry tonnes/ha**
LIB220 Marginal sand	9,55	75,93	79,22	167,9	0,409
LIB220 Sand	45,22	131,83	65,52	127,5	9,991
LIB220 Clay	27,19	89,28	65,31	141,0	6,472
LIB221 Marginal sand	9,55	50,87	63,37	167,9	0,223
LIB221 Sand	25,87	92,48	53,90	120,0	2,167
LIB221 Clay	25,97	63,34	54,22	117,2	0,652
LIB222 Marginal sand	26,82	59,52	69,87	167,9	0,194
LIB222 Sand	45,22	106,58	125,2	125,2	5,733
LIB222 Clay	44,54	43,00	122,0	122,0	6,472

6 Calcium tolerance and pH

Calcium tolerance was studied by including calcareous field locations.

High levels of calcium in the soil are commonly found in soils with a higher pH (pH 6,3 or higher). The site in Athens combined the high $CaCO_3$ level with a pH of 8,1. Soil pH influences soils fertility. In soil with a pH>7,2 immobilization of nutrients may occur. As the pH exceeds 8,5 the soil structure may be lost due to dispersion of soils particles (Fao 2015). Since high pH and high calcium carbonate levels were found side by side testing, it was not possible to test these separately in a field trial. To study the effect individually, experiments in a controlled environment would have to be performed.

6.1 Greece

Lupin, prefers acid to neutral soils with a pH from 5,5 to 7,0 while they are sensitive to $CaCO_3$ and react by inducing intensive chlorotic symptoms (White 1990; Tang et al. 1995). In Greece, calcium carbonate and alkalinity response of different Andean lupin genotypes were evaluated in three different locations (Athens, Kalamata, and Erythres) that were all characterized by high soil pH values but with different levels of $CaCO_3$ (%). Specifically, the experimental locations in Athens and Kalamata were characterized by a high $CaCO_3$ concentration (14,3-17,5) while much lower in Erythres (0,2%).
Plants response evaluated through measurements regarding chlorotic symptoms in fully developed new lupin leaves, using an optical scale 0-5 (0: no chlorotic symptoms, 5: severe chlorosis) and plant vigor on a scale (1-3-5-7-9) (1: retarded plant, 9: very vigorous plant). These measurements were recorded during the 2017-2018 cropping season in the three experimental locations, while they were received in twelve central plants per plot when they were in their early vegetative stage (one month from sowing). LIB213 and LIB218 were not included in the analysis as they were not cultivated in all three locations. SPAD measurements were also used to calculate total chlorophyll content (mg g^{-1} FW). SPAD measurements also received in twelve central plants per plot.

6.1.1 Results

Chlorosis and vigour measurements
A strong negative correlation observed for plant vigour with chlorosis, $CaCO_3$ and pH level (Table 148.) taking into consideration the data received from the three experimental locations. $CaCO_3$ as expected correlated positively to soil pH level.

Table 148. Pearson correlation among vigour, chlorosis, soil $CaCO_3$ and pH level for the three experimental locations.

Traits	Vigour	Chlorosis	$CaCO_3$	PH
Vigour	1,000	-0,342***	-0,151***	-0,194***

Chlorosis	1,000	-0,042	0,008
$CaCO_3$		1,000	0,940***
PH			1,000

Chlorotic symptoms however did not obtain a significant correlation either to $CaCO_3$ or to pH level. This could be attributed to those chlorotic symptoms observed in Erythres are not a consequence of $CaCO_3$ concentration since its practical 0%, waterlogging could be considered as the most possible reason for them. Calculating Pearson correlation between two locations (Athens and Kalamata) chlorosis symptoms correlated positively to $CaCO_3$ and pH level (Table 149) enhancing this finding.

Table 149. Pearson correlation for two locations (Athens, Kalamata).

Traits	Vigour	Chlorosis	$CaCO_3$	PH
Vigour	1,000	-0,345***	-0,203***	-0,203***
Chlorosis		1,000	0,133***	0,133***
$CaCO_3$			1,000	1,000***
PH				1,000

For this reason, further data analysis performed, taking into consideration only data obtained from the experimental field in Athens ($CaCO_3$: 17,5, pH 8,1) and in Kalamata ($CaCO_3$: 14,3, pH: 7,8). A statistically significant experimental trial x accession interaction obtained from ANOVA (α = 0,05) for vigor and chlorosis of plants. Means for plant vigour for each accession and for each location are presented in Table 150.

Table 150. Mean vigor and chlorosis for each accession and location.

Location	Accessions	Vigor	Chlorosis
Athens	**LIB200**	3,71±0,27i	2,10±0,23a
	LIB209	4,81±0,13c-g	0,19±0,04fg
	LIB214	4,54±0,12d-h	0,63±0,09def
	LIB220	4,19±0,12f-i	1,54±0,12b
	LIB221	4,61±0,10d-h	0,82±0,09cde
	LIB222	3,89±0,12ghi	1,34±0,13bc
	Branco	4,78±0,13b-g	0,43±0,07efg
	LIB224	4,76±0,09c-g	0,65±0,09def
	cv Multitalia	4,41±0,12e-i	0,39±0,07efg
	cv Polo	5,02±0,11b-e	0,77±0,10de
Kalamata	**LIB200**	6,20±0,30a	0,11±0,05fg
	LIB209	4,72±0,18c-g	0,37±0,07efg
	LIB214	5,58±0,23ab	0,65±0,08def
	LIB220	5,35±0,27a-d	1,12±0,14bcd
	LIB221	5,00±0,18b-f	0,87±0,09cde
	LIB222	3,96±0,17ghi	0,82±0,11cde
	Branco	5,52±0,29abc	0,59±0,12d-g
	LIB224	5,56±0,22abc	0,00±0,00fg
	cv Multitalia	5,04±0,19a-e	0,00±0,00g
	cv Polo	5,49±0,15abc	0,42±0,10efg
Main effects			
Athens		4,47±0,05b	0,88±0,04a

Kalamata	5,19±0,07a	0,56±0,03b
LIB200	4,32±0,23cd	1,62±0,19a
LIB209	4,77±0,11abc	0,27±0,04f
LIB214	5,03±0,13ab	0,64±0,06de
LIB220	4,71±0,14bc	1,35±0,09ab
LIB221	4,78±0,10abc	0,84±0,07cd
LIB222	3,92±0,10d	1,14±0,09bc
Branco	5,06±0,14ab	0,49±0,06ef
LIB224	4,96±0,09ab	0,49±0,07ef
cv Multitalia	4,62±0,11bc	0,25±0,05f
cv Polo	5,24±0,09a	0,61±0,07def
Significance		
Location	***	***
Accession	***	***
Location x Accession	***	***

*: significant at 0,05 level, **: significant at 0,01 level, ***: significant at 0,001 level

SPAD Index and chlorophyll measurements

In the second cropping season, SPAD measurements were performed in order to calculate the chlorophyll content of plants as a response to the soil $CaCO_3$ effect. After defining a positive correlation among SPAD measurements and chlorophyll content in all experimental locations. By using STATISTICA 8.0 software (Figure 54) chlorophyll measurements were then subjected in ANOVA and Tukey-Kramer means' comparison method (Table 151.).

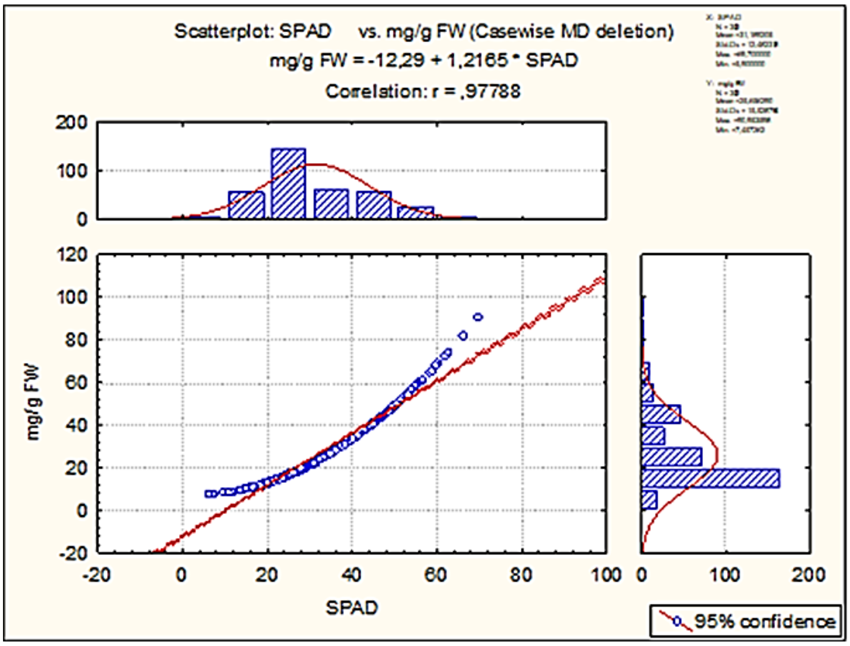


Figure 54. Defining strong positive correlation among chlorophyll content (mg g^{-1} FW) and SPAD measurements.

Table 151. Mean chlorophyll content (mg g^{-1} FW) for each accession and location.

Location	Accessions	Chlorophyll (mg g^{-1} FW)

Athens	**LIB200**	35,64±1,42c
	LIB209	34,82±1,63cd
	LIB214	31,81±1,27cde
	LIB220	22,06±1,01fgh
	LIB221	31,56±1,12cde
	LIB222	26,37±1,12def
	Branco	34,27±1,10cd
	LIB224	45,66±2,32a
	cv Multitalia	45,43±2,07ab
	cv Polo	13,28±0,90h
Kalamata	**LIB200**	31,78±1,38cde
	LIB209	24,48±2,53efg
	LIB214	33,05±2,30cde
	LIB220	24,01±1,70efg
	LIB221	18,41±1,95fgh
	LIB222	16,92±1,63gh
	Branco	20,79±2,38fgh
	LIB224	45,61±1,38a
	cv Multitalia	50,48±2,68a
	cv Polo	36,48±2,78bc
Main effects		
Athens		32,09±0,67a
Kalamata		30,36±0,88b
LIB200		33,76±1,01b
LIB209		29,65±1,62bcd
LIB214		32,43±1,31bc
LIB220		23,03±0,99f
LIB221		25,27±1,35def
LIB222		21,71±1,13f
Branco		27,63±1,52cde
LIB224		45,64±1,34a
cv Multitalia		47,96±1,71a
cv Polo		24,88±2,00def
Significance		
Location		*
Accession		***
Location x Accession		***

*: significant at 0.05 level, **: significant at 0.01 level, ***: significant at 0.001 level

Pearson correlation coefficients were calculated also including chlorophyll data obtained (Table 152).

Table 152. Pearson correlation including chlorophyll data.

Traits	**Vigour**	**Chlorosis**	**$CaCO_3$**	**PH**	**Chlorophyll ($mg\ g^{-1}$ FW)**
Vigour	1,000	-,075*	-,459***	-,459***	,056
Chlorosis		1,000	,212***	,212***	-,177***
$CaCO_3$			1,000	1,000***	,060

PH	1,000	,060
Chlorophyll (mg g^{-1} FW)		1,000

6.1.2 Conclusions

Due to environmental factors (e.g. precipitation) and the variability of $CaCO_3$ in the soil, tolerance to $CaCO_3$ should be evaluated also in the most controlled environment e.g. in pots.
In Athens, among Andean lupin accessions, higher vigor observed in LIB209 and Branco followed by LIB221. Low vigor was recorded for LIB200.
In Kalamata, among Andean lupin accessions, higher vigor was observed in LIB200 and Branco and LIB214, while low vigor recorded for LIB222.
In Athens, among Andean lupin accessions severe chlorotic symptoms were observed in LIB200, LIB220, and LIB222, while in Kalamata in LIB220 and LIB221.
Among Andean lupin accessions less chlorotic symptoms were observed in LIB209 and Branco in both locations. In Kalamata also LIB200 presented few chlorotic symptoms.
A significant location x accession interaction was observed for chlorophyll content, mainly due to a significant accession impact.
In Athens among Andean lupin accessions, a higher chlorophyll content was recorded for LIB200, LIB209, and Branco, while in Kalamata for LIB214, LIB200 and LIB209.
In Athens, among Andean lupin accessions, a lower chlorophyll content was recorded for LIB220 and LIB222, while in Kalamata for LIB222 and LIB221.
Overall, Branco and LIB209 presented in both locations, vigorous plants with not extended chlorotic symptoms and with high chlorophyll content. LIB200 performed very well regarding these three parameters studied only in Kalamata.
LIB222 and LIB220 presented more intense chlorotic symptoms, lower chlorophyll content, and not so vigorous plants than the other Andean lupin accessions.
Entries of *L. albus* used presented higher amounts of chlorophyll content and less chlorotic symptoms and overall a better appearance than *L. angustifolius* cv Polo and Andean lupin accessions.
Lupinus angustifolius cv Polo exhibited lower vigor and less chlorophyll content in the experimental field in Athens.
There was not a significant correlation between vigor and chlorophyll content, while there was a significant negative correlation between chlorosis and chlorophyll content as expected.
Vigor and optical chlorotic record correlated significant to $CaCO_3$ and pH soil level, whereas chlorophyll content obtained no correlation to soil $CaCO_3$ and pH. For this reason, the optical record of vigor and chlorosis constitute reliable methods and cannot be substituted by chlorosis measurements. Chlorosis measurements in lupin leaves were probably affected by the pubescence of leaves in many of the accessions tested.

6.2 The Netherlands

In the Netherlands the highest percentage of calcium carbonate was found in the clay soil Kraggenburg at 6,4-6,9% $CaCO_3$ at a pH of 7,4. When comparing this to the sandy soil locations in 2018 Gooijerdijk and 2019 Wageningen theses sites had a $CaCO_3$ level of 2% and a pH of 7,4 at the Gooijerdijk and 0.04% $CaCO_3$ and pH of 6,1 at Wageningen. Comparing the crop performance at the clay and sandy location offered a clue. However, since these locations are 70 km apart many other parameters could have influenced the crop performance, so these results are indicative only. Due to a shortage of seeds available, only LIB221 was included in the analyses.

Gooijerdijk offered a hint at the $CaCO_3$ sensitivity since the pH is the same(Table 153). Wageningen offered the location where both the $CaCO_3$ level and pH were more desirable.

6.2.1 Results and conclusion

Table 153. Comparing averages on Clay (High $CaCO_3$) with sand (low $CaCO_3$) .

Treatment	MF height	MF pods	MF # kernals	kernels tonnes ha^{-1}
LIB221-2018-Clay	55,5	239	1331	0,6687
LIB221-2018-Sand	71,7	186,5	2255	0,5212

At a lower $CaCO_3$ level it was found that the plants were significantly larger and produced more kernels, but the pod production and overall kernel yield was not influenced by $CaCO_3$ level.

Table 154. Comparing averages on Clay (High $CaCO_3$ and high pH) with sand (low $CaCO_3$ and pH).

	MF height	MF pods	MF # kernals	Kernels tonnes ha^{-1}
LIB221-2019-Clay	44,3	301,5	1845	0,4728
LIB221-2019-Sand	68,45	242,5	1794	0,7794

When comparing clay soil with a higher $CaCO_3$ and high pH with sandy soil at a low $CaCO_3$ and pH, sandy soil produced higher plants and a higher overall yield.

When the pH was identical, the kernel production in tonnes per hectare showed no differences, when both the pH and $CaCO_3$ was low, kernel production was higher. More extensive studies with a greater number of accessions must be applied and preferably at locations closer to each other to make a strong statement on Andean lupin sensitivity to $CaCO_3$ and pH however these results do suggest that Andean lupin performs better at a neutral, low $CaCO_3$ soil than an alkali and calcareous soil.

7 Salinity in the Netherlands

Soil salinization, or the accumulation of soluble salts, poses a serious threat to agricultural production. High salinity limits the normal metabolism of plants (ESBN, 2005) resulting in poor seed production, reduced biomass accumulation or even failure to establish at all. Although soil salinization can be the result of various natural processes, primary salinization, like weathering, salinization induced by human activity, secondary salinization, contribute to declining soil fertility and productivity. Secondary salinization can be the result of e.g. fertilizer application or an over-extraction of ground water for irrigation.

A recent global mapping by Ivushkin *et al.* 2019 of salt affected soils indicates that 1069 Mha were salt affected in 2016. In 1986 this was 915 Mha so an increase of 16,8% in just 30 years. On irrigated lands this increase is even more severe. Hamilton 2014 estimated that up to 2000 hectares of irrigated farm land per day are lost due to salt related soil degradation, an increase of over 30% since the early 1990's. These data illustrate that solutions must be found to enable cropping on salt affected soils. Beside various modifications in agricultural practises like cropping on raised beds also the use of salt tolerant crops has potential.
Crops are divided by the yield loss in the presence of various salinity levels (Table 155, derived from Abrol *et al*. 1988).

Table 155. Soil salinity classes and impact on crops.

Ece (dS/m)	Salinity level	Crop classes
0-2	Non-Saline	Only small effects
2-4	Slightly saline	Sensitive crops are restricted
4-8	Moderately saline	Many crops are restricted
8-16	Strongly saline	Only tolerant crops
>16	Very strongly saline	Only very tolerant crops

Edible plant species that are known for their tolerance to salt are Ice Plant, *Mesambryanthemum crystallinum* which stores water and salt ions in the epidermal bladder cells (Agarie *et al.* 2007) which appear like crystals on the leaves (Figure 55) and Marsh Samphire, *Salicornia europaea.* The nutritional content and the yield per hectare of these crops is low so advances on more nutrient dense and higher yielding crops are welcomed. Advances have been made in more common food crops such a potato and wheat. Adding a protein source would be a welcome addition. Within LIBBIO, the potential of Andean lupin for cropping on saline soils was explored.


Figure 55. Ice Plant.

7.1 Material and Methods

The salt tolerance of Andean lupin was studied at Salt Farm Texel, The Netherlands. This location offers 7 salinity levels in 4 replicates in a sandy soil. Salt Farm Texel is organic farm. Trials were conducted in 2017 and 2018. Salinity in the LIBBIO experiments is expressed in EcE (ds/m), Electrical conductivity of saturated soil paste. To measure the EcE, soil is saturated with water till the soil has a paste-like consistency, of this paste the water is extracted and salt levels are measured with a salinity meter. Soils with a salinity of 4 ds/m or higher are considered saline soils.

In 2017 and 2018 experiments were conducted using different salinity levels in the irrigation water namely 1, 4, 8, 12, 16, 20 and 35 ds/m. 35 ds/m corresponds to pure seawater. The Salt Farm Texel frequently checked if the salinity in the salinity level in the soil at 0-10, and 20-30cm depth (2017) and 5-15 and 25-35cm depth (2018) (table 156)

In 2017 the soil salinity was slightly lower and in 2018 slightly higher than the irrigation water.

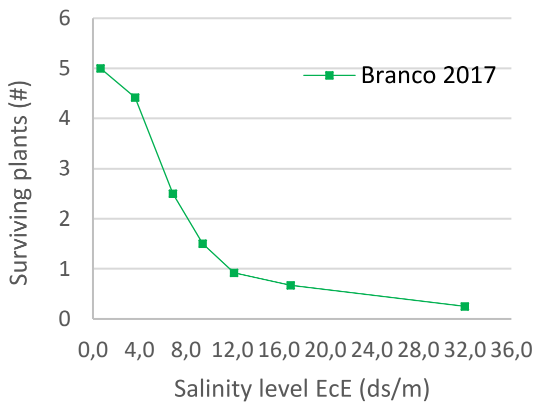
Table 156. The salinity in the irrigation water and the resulting soil salinity.

Irrigated salinity EcE (ds/m)	Soil salinity 2017	Soil salinity 2018
1	0,7	1,9
4	3,6	6,1
8	6,9	9,6
12	9,5	13,3
16	12,1	18,5
20	17,0	21,5
35	32,0	‘35’

In 2017 only Branco was used for testing. In 2018 Branco, LIB220, LIB221 and LIB222 were used.

7.2 Results

Due to the slight differences in soil salinity between 2017 and 2018 the years cannot be compared however the trends observed in 2017 and 2018 can be compared. In 2017, Branco showed a decreasing plant survival and reduction of biomass production at an increasing salinity level (Figures 56 and 57).



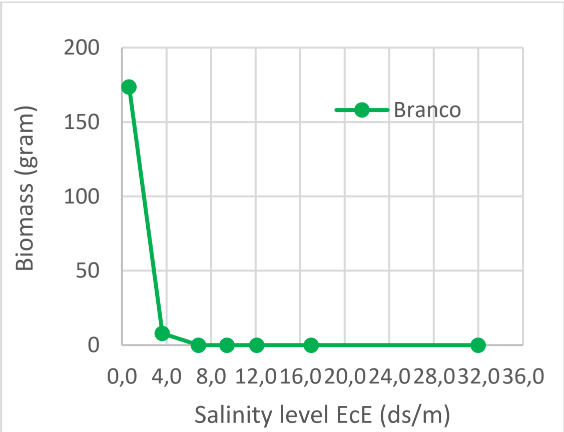


Figure 56. Surviving plants of Branco in 2017. Figure 57. Plant biomass of Branco in 2017.

In 2018, beside Branco LIB220, 221 and 220 were also used in testing salt tolerance of Andean lupin. As indicated in table 156, the soil salinity was slightly higher in 2018, but still a suitable range was obtained. Like in 2017, in 2018 a strong reduction in plant survival and biomass was observed at an increasing soil salinity (Figure 58 and 59).

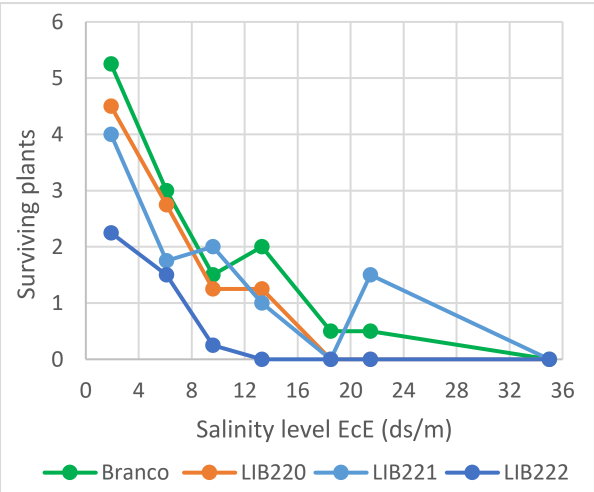


Figure 58. Surviving Andean Lupin in 2018.

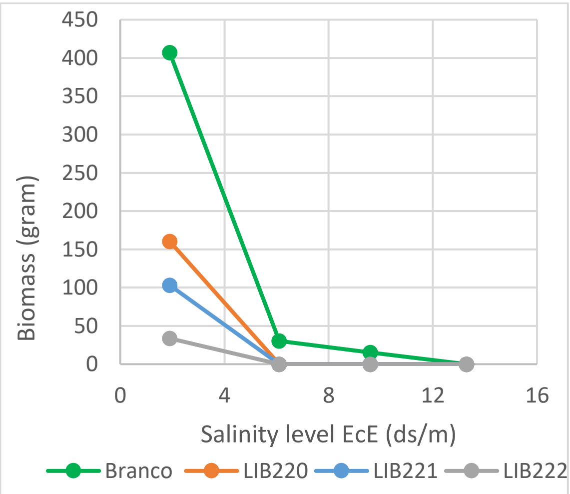


Figure 59. Plant Biomass in 2018.

Figure 60. Branco at 1 ds/m (left) and 4 ds/m (right), note the significant loss in biomass production 2018.

7.3 Discussion

The marked decline in both the survival and biomass of the Andean lupin at 6 ds/m, which is characterised as only moderately saline, indicated that these Andean lupin accessions were not salt tolerant. But the results did hint at a presence of genetic variation among the 4 accessions in relation to salt sensitivity, but a much larger dataset would be required to make reliable statements on that. What is important to examine is if the levels of soil salinity applied in these trials were comparable to the actual situation of salinity. Ivushkin *et al.* 2019 states that 1069 Mha was salt affected in 2016. However, the fast majority at 1036 Mha is only slightly saline between 2-4 ds/m. Studying Andean lupin at a more extensive range between 0-4 ds/m combining diverse genotypes may yet yield a genotype that could be cropped on slightly saline soils. But for any soils with a moderate or high soil salinity Andean lupin has shown no potential.

8 Drought

8.1 Portugal

No specific trials were performed in Portugal to determine drought sensitivity. However, white lupin was used in propagation fields as a biological barrier in between Andean lupin accessions. Yield was not compared at a statistical level, but this Andean lupin showed no signs of stress during short periods of light drought during the cropping seasons, with similar behaviour to Portuguese white lupin variety Misak. This variety is well adapted to local conditions and showed resistance to drought, hinting at a possible drought tolerance to Andean lupin.

8.2 Spain

No drought stress was observed. However, extreme weather events, occurring during the critical emergence, flowering, pod development, and pod-filling growth stages can have detrimental effects on crop performance. Considerations of the potential impacts of abiotic factors on Lupins should be based not only on the values of expected climatic parameters, such as drought, but also on the probability, frequency and severity of other possible extreme events. We therefore examined, the different abiotic factors which had the potential to influence crop performance.

8.2.1 First cropping season 2017-2018

Damage was caused by early frosts after emergence and by late frosts at flowering. Abiotic stresses represented a major constraint to emergence, due to temperature stress, and was one of the major determining factors for low emergence. Susceptibility to low temperatures was identified as the most critical environmental variable which affected emergence. Figure 61 shows damage to leaves, derived from early frosts after emergence, and additional damage derived from late frosts at flowering, resulting in the lost of the first order flowering. It should be noted, in relation to abiotic stresses, that while there was damage due to temperature stress in the first order of flowering, the second order of flowering developed well.

Figure 61. Pictures showing abiotic stresses in growing season 2017/2018.

8.2.2 Second cropping season 2018 -2019

Low temperature stress in the early stages constituted a serious constraint. Additionally, damage from hail and accidents caused by strong wind were registered, while extreme temperature events included damage derived from heat-waves, such as pod shattering. Heat-waves occurring during the spring period had the most dramatic impact on plant productivity, and under these dry and hot conditions, even one day delay´s in harvest resulted in substantial losses of seeds (Figure 62).

Figure 62. Pictures showing abiotic stresses in the 2018-2019 growing season.

8.3 The Netherlands

To study if Andean lupin is tolerant to drought, trials were performed at Noordhout. Noordhout is set within a national park ‘Utrechtse Heuvelrug’, this park is comprised of moraines and basins that date back to the last ice age and characterised by a hilly, forested landscape. The Noordhout field is located on the moraine and features a Dystric regosol, a poor sandy soil with low fertility. Due to its location within the national park, this site cannot be irrigated.

8.3.1 Second cropping season 2018

The Noordhout location has, with the marginal sandy soil, the lowest water buffering capacity. On this location the LIB220, LIB221, LIB222 and Branco were tested in relation to *Lupinus albus* cv. 'Boros', Faba bean cv. 'Fuego' and Rye. Germination was poor ranging from 7,2% for LIB221 to 15% for LIB222, 13,3% for Faba bean and 27,9% for *L. albus*. Due to the low germination the results would be indicative at most. However, during this cropping season the drought was so severe that many plots were lost (Figures 63 and 64). This site has no access to any water source making irrigation impossible. As a consequence to the high plant mortality, no comparison could be made between the drought susceptibility of the various accessions.

Figure 63 and 64. Noordhout test site, R>L Late June and the 8th of August.

8.3.2 **Third cropping season 2019**

In 2019 the drought trials were continued in Noordhout. The trial was expanded to include a wider range of lupin species (table 157). The aim was to find if there were genetic differences between the Andean lupin accessions and to find how Andean lupin would compare to other lupin species. Unfortunately, seed availability was still an issue so LIB220 was sown using B-grade seeds. This resulted in very poor germination so LIB220 was excluded from the comparison. Also, Branco did not establish well. The other accessions established sufficiently.

Table 157. The crops, varieties and germination used on Noordhout 2019.

Crop	Accession	Germination %
Andean Lupin	LIB220	5,2
Andean Lupin	LIB221	33,7
Andean Lupin	LIB222	39,7
Andean Lupin	Branco	5,2
White lupin/ Broad leaved lupin	Feodora	47,4
Blue lupin / Narrow leaved lupin	Regent	76,3
Yellow lupin	Puma	(Abscent.)
Faba bean	Fuego	82,1

Where in 2018 the experiment was cut short due to prolonged drought, in 2019 the trial was damaged badly by herbivory by deer. The trial field was located in a nature conservation area; fencing options were therefor limited because of local regulations in fauna management.

After the deer had fed on the trial, regrowth was observed but with every regrowth the deer returned as well. The yellow lupin was the most favoured which never established. Eventually all lupin species except the Andean lupin were destroyed. Only LIB221 and LIB222 remained in sufficient quantity to make an assessment.
This limited dataset did not allow for statistical analyses, but the results indicates that in LIB221, the main inflorescence height was lower and it also flowered earlier than LIB222. LIB222 was the only Andean lupin line to produce pods but the vast majority of the pods were empty.

8.3.3 Fourth cropping season 2020

In the final season 2020 high alkaloid accessions of Andean, blue and white lupin will be compared to study drought sensitivity. The high alkaloid accessions are predicted to deter herbivory allowing for a proper comparison between the accessions and other lupin species.

8.3.4 Conclusion

Cultivation on Noordhout was met with various challenges. Observations have indicated that lupin in general was more tolerant to drought than faba bean. However, with the drought and herbivory it could not be determined whether Andean lupin is more adapted to dry conditions than white, blue or yellow lupin.

9 Frost tolerance

When cropping on arable fields rather than in a greenhouse, many climate challenges can occur: desiccation due to strong winds, drought, flooding or frost. In Southern Europe Andean lupin may face frost in January and February. In the Northern countries frost can occur as late as May. Experience with other lupin species like *Lupinus albus* suggested that lupin was most susceptible when the cotyledons are about to break the soil surface or just above the soil surface (Prins, personal communication 2019). For ease of communication, the plant developmental stages were divided in 4 phases. Phase 1: the cotyledons have just broken the soil surface. Phase 2: the cotyledons are above the soil and the stem is stretching. Phase 3: The first leave pair is visible but still folded. Phase 4: the first leave pair has unfolded. At phase 1 and 2, the seedlings were expected to be least tolerant to frost.

To determine if frost tolerance occurs in Andean lupin, a climate chamber experiment was performed in Romania where all 4 phases could be tested in a controlled environment.
In the Netherlands a field trial was performed, in Greece and Portugal no specific frost trial was designed but the trials did face multiple days of frost.

9.1 Romania: controlled environment

Spring frost poses a challenge for all major crops in Romania. At the moment, a protocol for large scale screening of lupin germplasm under frost-simulating conditions is not yet available. But an initial exploration on a smaller scale was made. A series of tests were conducted in order to develop a protocol for screening lupin germplasm under artificial frost-simulation conditions using a plant growth chamber.

9.1.1 Material and methods

Four lupin accessions, one *L. albus* and three Andean lupin were used in the tests (*L. albus* cv. Mihai, LIB220, LIB221, LIB222). All seeds were planted simultaneously in containers with potting soil and germinated at 15°C and a light regime of 16 hours light/8h dark. The plants were transferred to 5°C for 24h for hardening. After this the plants were exposed to various levels of frost.

To test the frost sensitivity in relation to plant developmental all phases 4 stages were assessed. The seedlings were exposed to five levels of frost: − 2°C, − 4°C, − 6°C − 8°C, or − 10°C for 8 hours (table 158). Afterwards, the seedlings were transferred to 15°C for 72h to recover. After these 72 h, the plants were scored for frost tolerance. Frost damage was scored on a 0-1 scale, where 0 denotes healthy plants and 1 are dead plants (Table 159).

Table 158. Plant developmental phase and testing temperatures.

Plant phase	Germination temperature	Test temperature (day/night)
Cotyledons at breaking soil surface phase[1]	15°C	-2°C
	15°C	-4°C
	15°C	-6°C
	15°C	-8°C
	15°C	-10°C back-up
	15°C	5°C control
Cotyledons fully above the soil[2]	15°C	-2°C
	15°C	-4°C
	15°C	-6°C
	15°C	-8°C
	15°C	-10°C back-up
	15°C	5°C control
First leaves visible and erect but folded[3]	15°C	-2°C
	15°C	-4°C
	15°C	-6°C
	15°C	-8°C
	15°C	-10°C back-up
	15°C	5°C control
First and second leaves have unfolded[4]	15°C	-2°C
	15°C	-4°C
	15°C	-6°C
	15°C	-8°C
	15°C	-10°C back-up
	15°C	5°C control

Table 159. Frost damage scale.

Scale	Visual symptoms
0	Plant healthy
1	Whole plant died

9.1.2 **Results**

Generally, seedling tolerance decreased proportionally with the decline in temperature. For phase 1 and phase 2, no differences were observed for temperatures up to -6°C (Figure 65 and Figure 66). However, plants started to be affected from -8°C, suggesting that a specific cold tolerance threshold lies between -6 and -8°C for phase 1 and 2 seedlings.

Interestingly, for the later development stages: phase 3 and phase 4, notable differences were observed starting at -6°C (Figure 67 and 68). Frost of −8°C resulted in differences in seedling damage relative to the other temperatures, while −10°C resulted in the highest overall seedling damage.

Differences were observed between genotypes. For phase 1 and 2 *L. albus* (Mihai) and LIB221 were most tolerant. Notably, in phase 3 and 4, beside *L. albus* (Mihai), LIB222 outperformed the other genotypes. This indicates that the tolerance threshold has a genetic factor. Interestingly LIB222 is a genotype that is characterised by a higher level of anthocyanin in the tissue. Mainly visible as a purple coloured stem and darker flower colour. Since anthocyanin is induced in some crop species when exposed to the cold (Chalker-Scott 1999), this may provide a clue to the LIB222 frost tolerance compared to the other Andean lupin accessions.

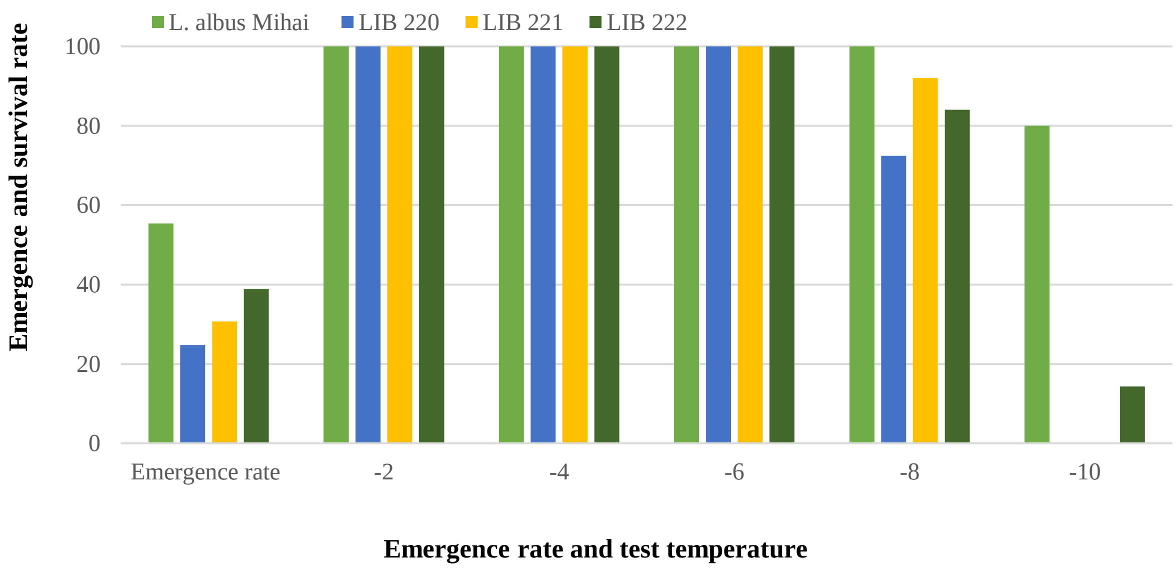


Figure 65. Phase 1 (cotyledon) phase seedling survival.

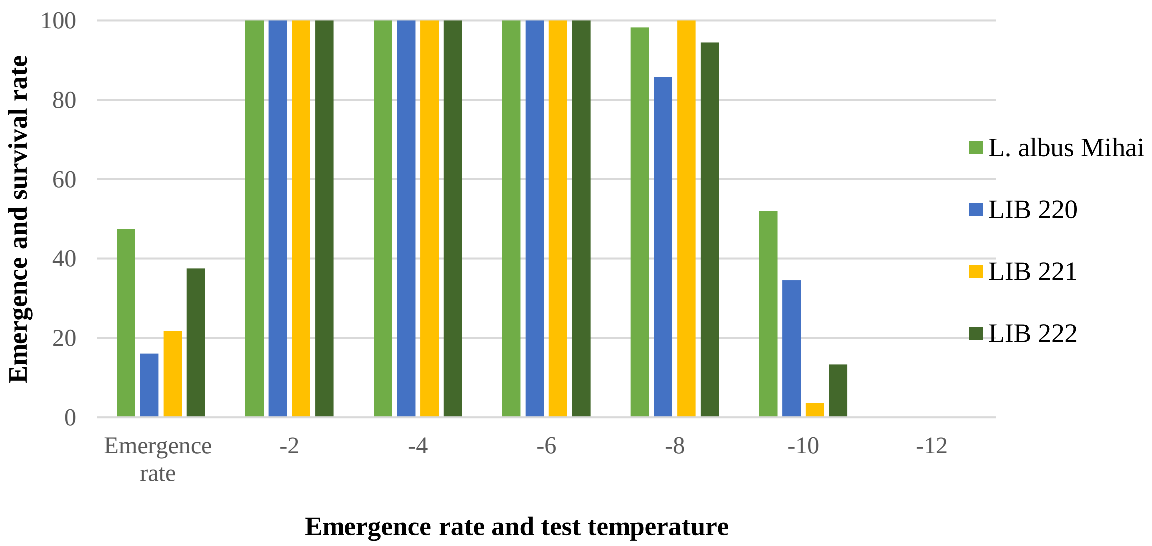


Figure 66. Phase 2 (cotyledon) phase seedling survival.

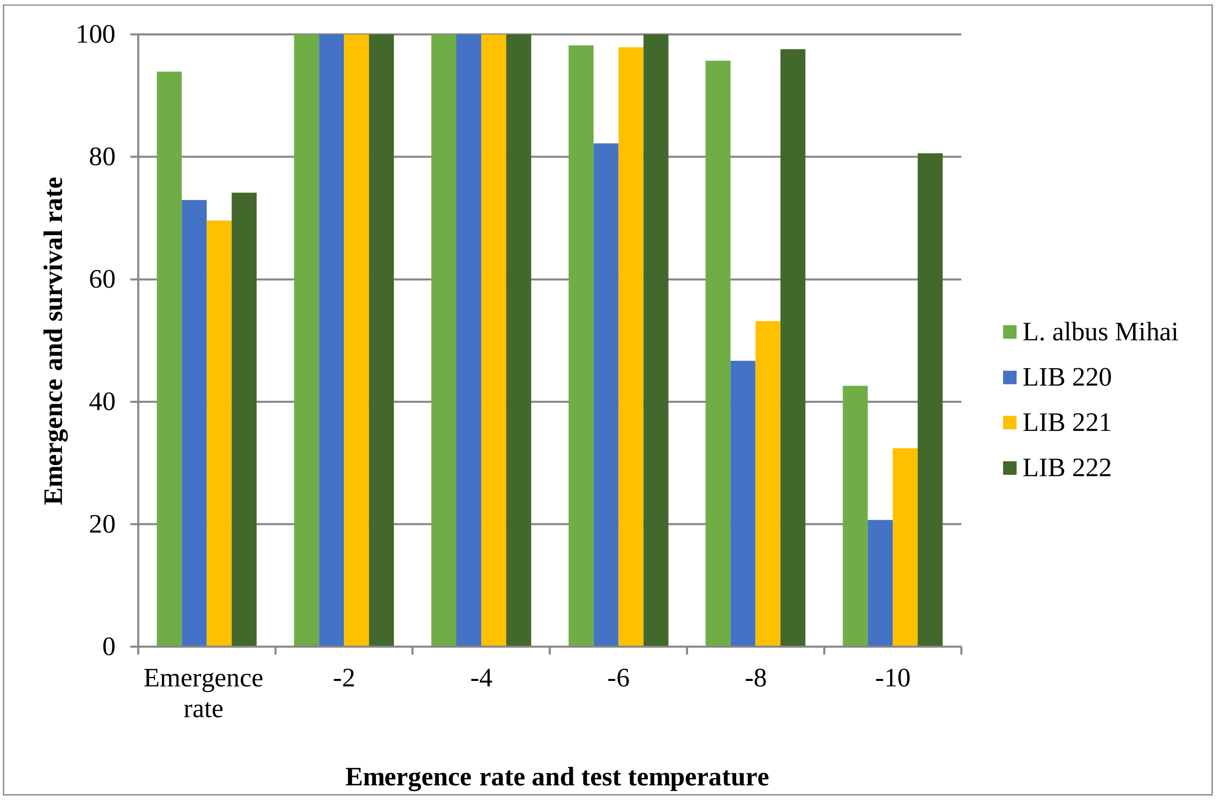


Figure 67. Phase 3 phase seedling survival.

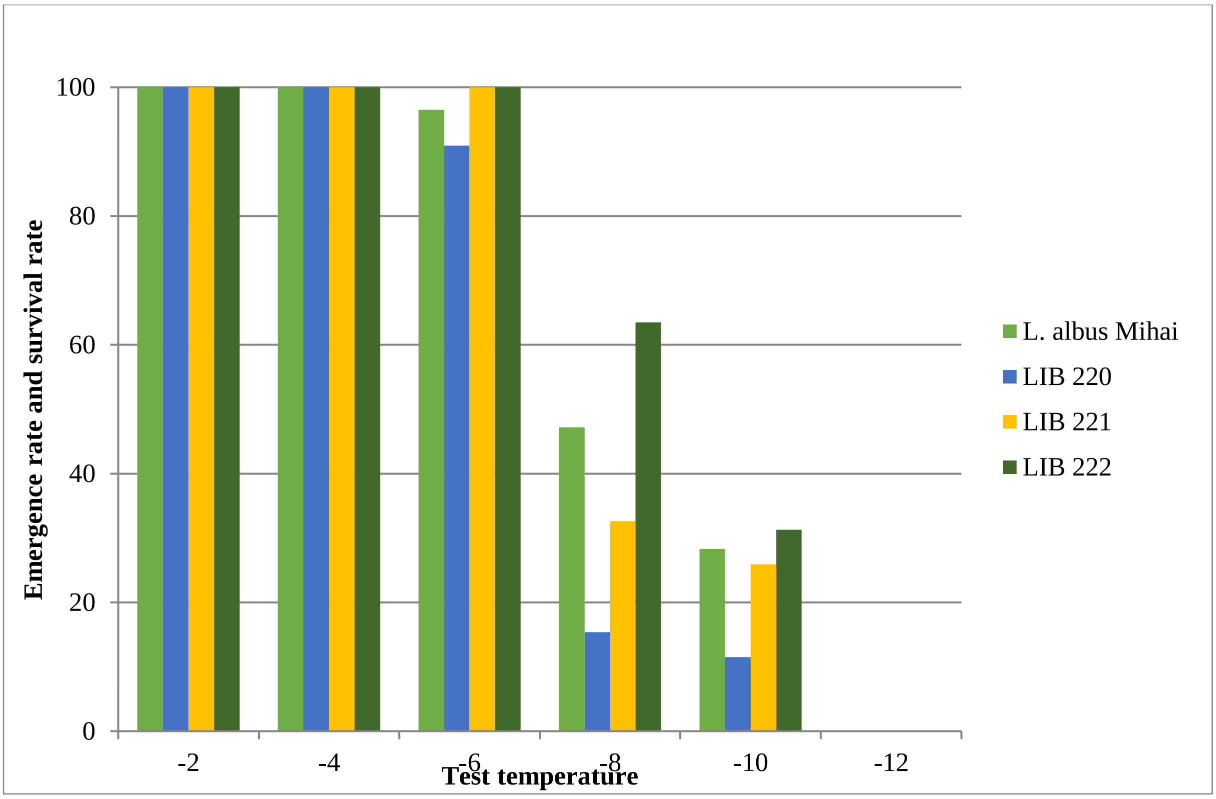


Figure 68. Phase 4 phase seedling survival.

The seedling tolerance decreased proportionally with the decline in temperature. Frost of − 6°C resulted in differences in seedling damage relative to the other temperatures, while − 10°C resulted in the highest overall seedling damage.

These results indicate that the plant growth stage is an important criteria for screening frost tolerant germplasm. Plants are more susceptible to freezing at the cotyledon stage. We accordingly used seedlings development stage and exposed them to severe cold stress in order to show the most variation in response to different freezing temperatures.
The tests are still in progress and will be presented at the project end.

9.2 Greece: field environment

Low temperatures from -3 to-5C during the first developmental stages of Andean lupin have been mentioned to be detrimental for its growth and development (Raabe and Von Sengbusch, 1935; Von Sengbusch and Zimmermann, 1937; Lopez-Bellido and Fuentes, 1986; Gulisano et al. 2020). In Greece, during the 4th cropping season in a sowing density experiment (direct soil sowing) Andean lupin plants suffered three waves of low temperatures <0°C, and also a two-week snow cover while they were in the first developmental stages (three-leaf stage, phase 4).

All the Andean lupin accessions tested (LIB220, LIB221, LIB222, and Branco) presented plant losses higher than 25% (Table 168, A.II.II). However, LIB220 exhibited significantly more losses than the other accessions of 55,8%, indicating a higher frost sensitivity.

In the same location and experimental year, however, in an inoculated and non-inoculated experiment (transplantation in the three-leaf stage) *L. albus* and *L. angustifolius* were also tested while Branco was excluded.

In the latter experiment, all Andean lupin accession LIB220 again had a higher loss than *L. albus* (Table 169, A.II.II).
From the above-mentioned there was not a specific frost tolerant *L. mutabilis* accession, however, LIB220 seems to be the most sensitive.

9.3 Portugal: field environment

In the third cropping season, in Coimbra/Loreto, where LIB220, LIB221 and LIB222 propagation fields were sown on the 10th of December and in Santarém/Escola Superior Agrária de Santarém (ESAS) where the density trial with the same LIB accessions was installed on the 21st of December, frost was registered for a few days in January 2019. Figures 69 and 70 show minimum temperatures registered in Loreto and ESAS. After plants emerged, 6 days with temperatures below 0°C (1,5 m air T) were recorded and frost was

observed in the field (Figure 6 and 7). In Loreto plants were in between the stages of having just cotyledons (phase 2) to 2 unfolded leaves (phase 4) at 23 to 35 DAS and in ESAS from emergence to phase 2 from 12 to 24 DAS. No visible damage was observed.
No differences between LIB accessions were observed but other crops, like potato, did show frost damage (Figure 71-74).

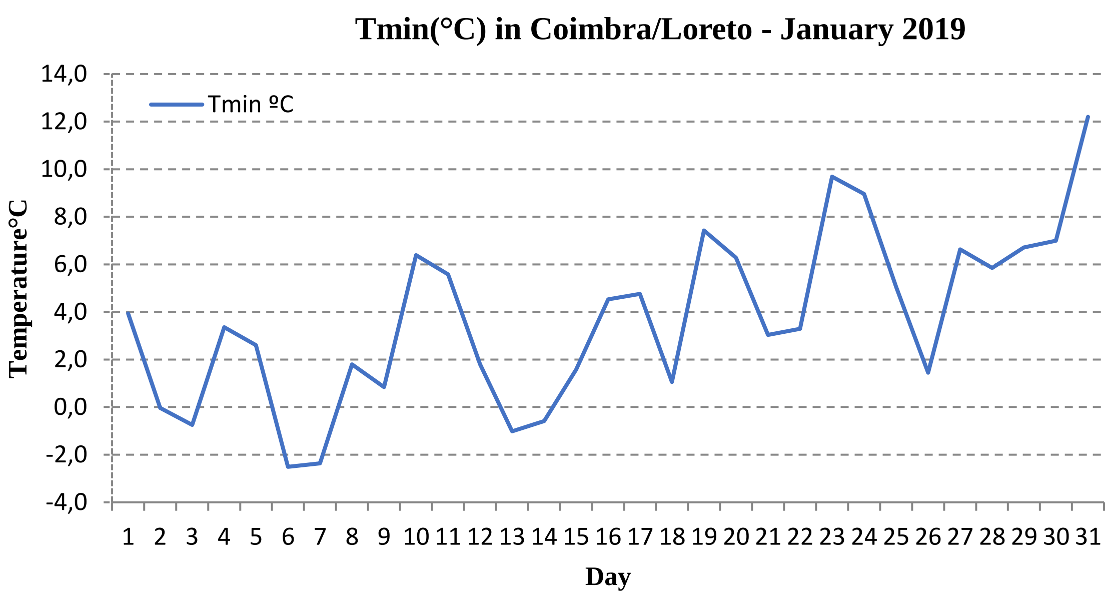


Figure 69. Minimum Temperature in Coimbra/Loreto in January 2019 (source – ESAC station).

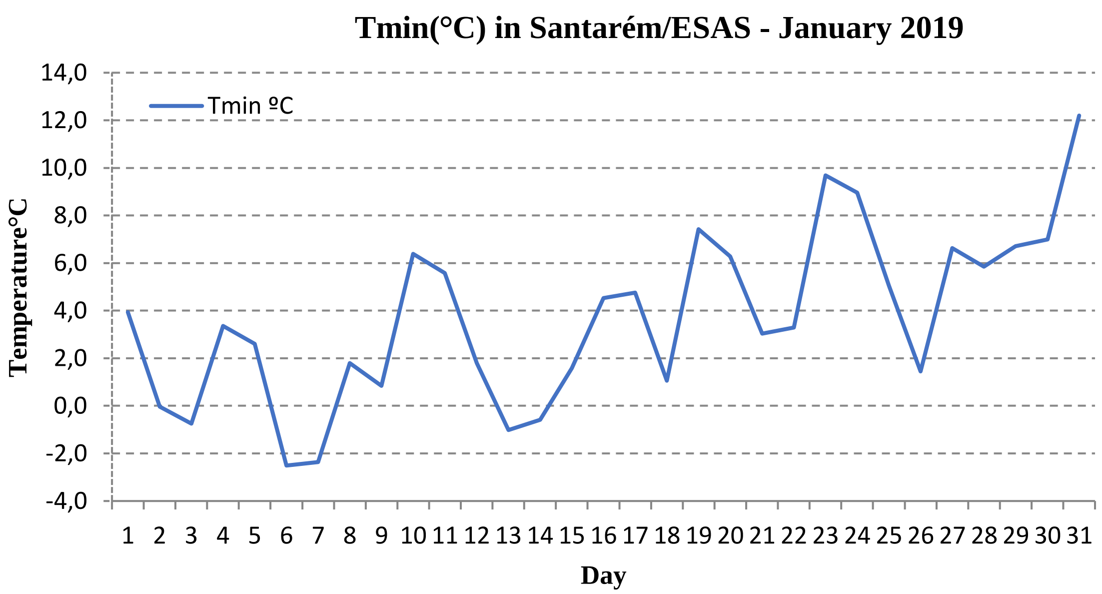


Figure 70. Minimum Temperature in Santarém/ESAS in January 2019 (source – ESAS station).


Figure 71. Frost on Andean lupine cotyledons.


Figure 72. Frost on Andean lupine leaves.


Figure 73. Potato plant destroyed by frost.


Figure 74. Cyperus spp destroyed by frost.

9.4 The Netherlands: field environment

In the clay soil of Kraggenburg during cropping season 3 a split plot design was used to study frost tolerance in the field. Andean LIB220 and LIB221 were compared to the frost tolerance of soy. The trial was sown at the 24th of April. Since frost can occur until the 8th of May in the Netherlands, the aim was to have seedlings exposed to frost when they were in phase 1 or 2.

When frost was forecasted, for the night of the 6th to the 7th of May, one half of the field was covered with thermic sheet and one half left uncovered. The thermic sheets are frequently used in arable cropping to protect frost sensitive seedlings as it raises the temperature under the sheets for a few degrees (Figure 75). Temperature sensors were installed at the soil surface and at 5cm deep, both on open soil and the under the thermic sheet. Unfortunately, +2˚C was the lowest recorded temperature on bare soil so it is not known what frost would do to Andean lupin. Since frost in the Netherlands in spring is getting rarer it was decided not to repeat the experiment in the final cropping season.

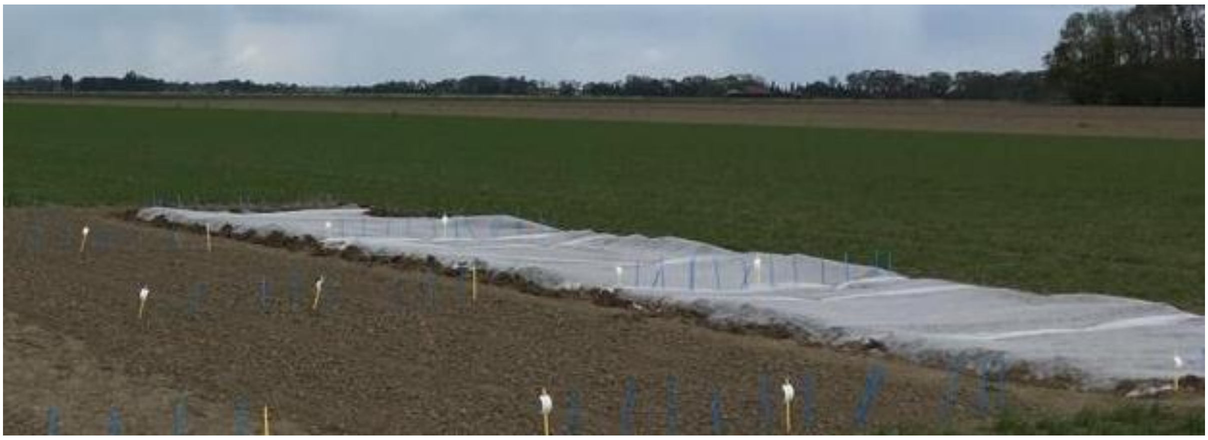


Figure 75. The Kraggenburg field showing the exposed and covered plots.

10 Susceptibility to diseases

Various pathogens are known to infect lupin spp. Like *Colletotrichum acutatum, Pleiochaeta seitosa, Fusarium oxysporum, Botrytis cinerea and Sclerotinia, sclerotiorum*. To study the succepibility of Andean lupin the crop was monitored during all the cropping season for naturally occurring diseases.

10.1 Austria

In Austria the field trials were observed very carefully, especially for anthracnose which is caused by an infection with *Colletotrichum lupini*. In the field trials 2017-2019 several suspects were identified but after analyses these proved to be negative. In the field trials 2020 all lupin varieties and almost all plants were severely infected with anthracnosis , on both locations. This circumstance is connected with the weather conditions in 2020, characerised by high rainfall as documented in Appendix I.I.
Other diseases that were observed in the field caused by:
Pleichaeta setosa (brown leaf spot): was not present
Fusarium oxysporum (fusarium wilt): One infection was confirmed in the laboratory together with other infections of *Fusarium redolens, Fusarium solani, Rhizoctonia solani* and *Pythium ultimum*
Botrytis cinerea (grey mold): was not present
Sklerotinia sklerotiorum (white delay): only some seedlings were infected
Erysiphe martii (mildew): was observed on most of the plants in autumn 2017 and from summer to autumn 2018. In the year 2019 mildew was observed at Trautenfels very late in autumn. The infection with mildew had no effect to the yield of the plants because the infection was only at a light level.
Fusarium root rot was observed (*Fusarium spp.*) on young seedlings. Some of these infected plants died afterwards, not only because of this disease but maybe because of a mixed infection with other diseases such as damping off (*Pythium ultimum*)In Figure 76 a-d some of infected plants are shown.

Figure 76 a. *Pythium ultimum* on a seedling of LIB223 at Trautenfels in 2018.

Figure 76 b. *Colletotrichum lupini* on a plant of LIB221 at Trautenfels in 2020 in the middle of September.

Figure 76 c. *Colletotrichum lupini* on a plant of LIB221 at Trautenfels in 2020 in the middle of July.

Figure 76 d. *Colletotrichum lupini* on a plant of LIB221 at Trautenfels in 2020 in the end of September.

Figure 76.a-d: Infected Andean lupin plants.

10.2 Greece

Disease monitoring took place in Greece in all experimental fields across all years. The main fungal disease recorded was an infection with *Rhizoctonia* spp. in the early developmental stage of plants. This was observed in all locations and caused significant plant losses in the 2016-2017 and the 2017-2018 cropping season. In 2016-2017, increased problems were observed on LIB219 in the experimental field in Athens. While in 2017-2018 many of the accessions presented symptoms of stem rot, stem lesions and damping-off (Table 160). Symptoms of *Rhizoctonia* spp. infestation was also recorded during 2018-2019 but suppressed successfully to prevent the spread of the disease by watering the plants with Folio Gold® (200 ml/ 100 L H_2O) in Athens and with Ortiva Opti® 8/40 (200 ml/100 L H_2O) in Erythres.

Table 160. Losses of plants per accession in each experimental location during 2017-2018.

LIBBIO code	Erythres	Kalamata	Athens	Acc. total
LIB200	1	5	4	***10***
LIB209	3		4	***7***
LIB213				***0***
LIB214	1	1	2	***4***
LIB218	1		4	***5***
LIB220	2	2		***4***
LIB221	2	5	1	***8***
LIB222	3	3	4	***10***
Branco	2	2		***4***
LIB224		3		***3***
cv. Polo	2	2	3	***7***

cv. Multitalia				***0***
Totals	***17***	***23***	***22***	***62***

Colletotrichum acutatum (Anthracnose) symptoms were observed in Kalamata. Aithaia in the second cropping season in *L. angustifolius* cv. Polo with plots that had severely infected plants, however Andean lupin and *L. albus* genotypes fortunately did not present any symptoms.

Fusarium oxysporum symptoms were observed in *L. albus* cv. Multitalia in Athens also during the second cropping season (Figure 77), however, it did not affect Andean lupin plants as Andean lupin is reported to be susceptible to a different strain. *Fusarium oxysporum* was also observed in the seed lots of LIB221 that were received at the beginning of the project (Figure 78) as well as of LIB222 the second season.

Figure 77. *Colletotrichum acutatum* (Anthracnose) symptoms on a *Lupinus angustifolius* cv. Polo plant in Kalamata. Aithaia in 2017-2018.

Figure 78. *Lupinus albus* cv. Multitalia plant affected by *Fusarium oxysporum.*

Figure 79. Infected seed lot of LIB221 in the beginning of the project.

Figure 80. Infected plant of cv. Polo from *Oidium* spp. in Kalamata. TEI in 2018-2019.

Figure 81. Infected plant of LIB209 from *Oidium* spp. in Kalamata. TEI in 2018-2019.

Figure 82. A LIB200 plant come from a seed borne virus (Athens. 2017-2018).

Incidence of *Oidium* spp. was also recorded in all locations during the third cropping season, which mostly affected cv. Polo plants (Figure 81) but also some Andean lupin genotypes

(Figure 80). No chemical treatment was applied in this case as the plants were already in, or had entered their maturity stage.
Regarding virus disease incidences. only one plant of LIB200, in Athens during 2017-2018, was observed to be infected by a seed-borne virus, thus it was observed to have mosaic symptoms in the new leaves and presented (Figure 82).

10.3Portugal

No specific trials were performed in Portugal for disease testing. However, monitoring of diseases took place in all fields. In the winter of 2016/2017 loss of plants due to fungal infection was high in early stages. *Phytoptora cactorum* was identified as the cause. In the fourth cropping season although it was present in all fields, plants losses from *Phytoptora cactorum* infection were always under 2%. An improved seed quality may have helped in reducing these infections.
Also in 2017, in a late stage of the crop, powdery mildew was identified in the Loreto trial. Both Vandinter and ISA accessions were infected. Irrigated crops nearby, such as potatoes irrigated by sprinkler irrigation or corn irrigated using gravity irrigation, could have produced abnormal humidity conditions propitious for the disease. Up to today, no more powdery mildew was registered.
In the third cropping season a sowing density trial was performed in ESAS/Santarém. Anthracnose was identified in a single plot of LIB221 of the highest density (50 pl/m^2) on both the steams and pods (Figure 83 and 84), 109 DAS.

Figure 83. Anthracnose infected stem.

Figure 84 Anthracnose infected pods.

The infection with anthracnose was identified on 10/04/2019. Temperature and precipitation on the previous days can be indicative to Anthracnose development.

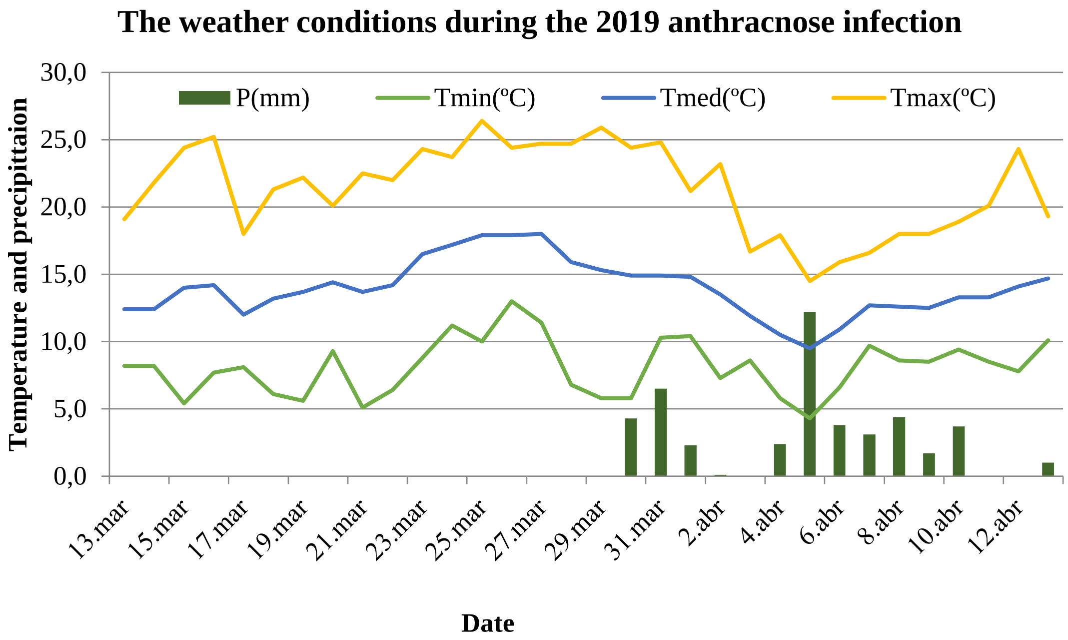


Figure 85. The weather conditions in during the 2019 anthracnose infection.

After the disease was identified, a treatment with azoxistrobyn was made and this proved to be effective against preventing new infections.

10.4 Romania

In 2018 anthracnose was observed in the reference drop *Lupinus albus* however not in Andean lupin. Excess moisture could have contributed to disease incidence in the field trails (Figure 86).

Figure 86. Plants affected by diseases, pests and excess moisture at Ezareni farm, Romania; 89 DAS, 11 July 2018; a. *L. albus*, dead plants; b. *L. albus*, delayed germination and growth; c. L. albus, anthracnose (*Colletotrichum gloeosporioides); d. L. albus*, Misak cv., *virus symptoms.*

Using a "wet-room" method some micromycetes were observed - *Fusarium* spp. and *Penicillium* spp.. For isolation of *Fusarium* spp. and *Penicillium* spp., samples were taken from symptomatic plant fragments. The samples were surface-sterilized with a 1% sodium hypochlorite solution for 3 minutes and rinsed using several changes of sterile water. The sterilized samples were placed onto a general medium (water agar) and a semi-selective medium for *Fusarium*, i.e., peptone-pentachloronitrobenzene agar (PPA) plates. The plates were incubated under a standard growing conditions. The resulting colonies were single-spored and transferred onto potato dextrose agar (PDA) for morphological identification. Images were captured using a Leica microscope (Figure 87).

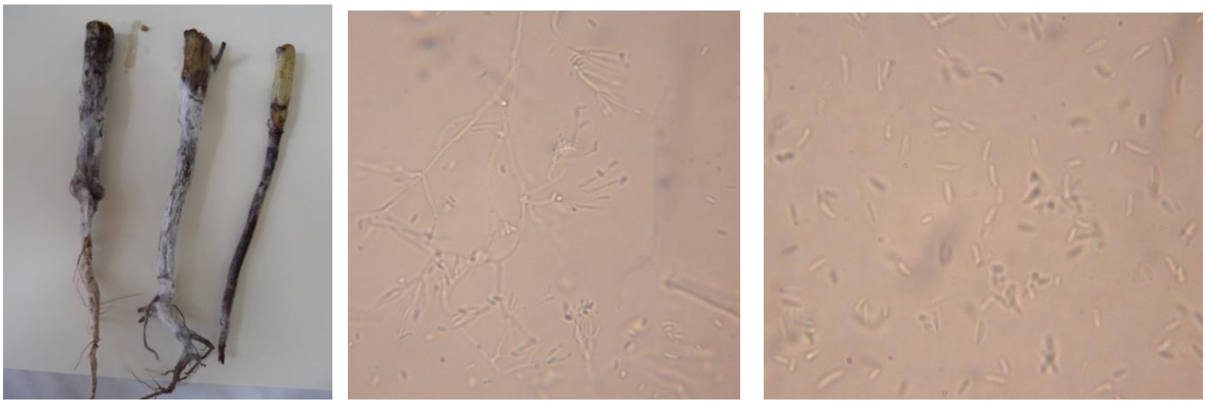


Figure 87. A: visual simptoms visible on Lupin roots: b. *Penicillium* spp. under microscopic observation of plant material harvested from the field trails; c. *Fusarium* spp conidia under microscopic observation of plant material harvested from the field trails (left to right).

In 2019, the Ezareni field was affected by diseases as shown in figure 88.

Figure 88. Ezareni 2019 field experiment, LIB 220 affected by diseases, especially anthracnose (*Colletotrichum gloeosporioides).*

In 2019, at Ezareni, Iasi, the emergence rate was good, compared to previous years, but in one field the disease incidence was very high, resulting in almost all plant being infected and no harvest. Similar symptoms were observed also for *L. albus* cultivars, which was the first time since studies on with Lupinus spp. started more that 25 years ago. We suspect that initial seed infection with the pathogen could have been the cause of the observed damage.

10.5 Spain

No significant disease damage was observed in any of the locations during any of the cropping seasons.

10.6 The Netherlands

During all seasons it was observed that after rainfall in the late summer (August/September) mildew affected all the plants regardless of genotype or species. The plants continued to grow and set pods, so the infection was not considered detrimental. When dry weather returned the mildew disappeared again. Beside mildew a few plants in 2018 and 2019 were affected by what is believed to be a seed born virus. The exact nature of the virus is not known; it has a slight mosaic appearance and the overall plant growth is stunted and yield is strongly reduced. No spread of this virus to other plants was observed so this too is not considered detrimental, but seed born infections are a reason to be alert if aiming to produce seed at a professional scale. During the second cropping season 2 plants were observed to have anthracnose symptoms. These plants were removed and the infection did not spread nor was it observed again.

11 Susceptibility to pests

Just like diseases pest could cause severe crop damage. Not only as a primary cause due to direct feeding of the pest but also because pests can act as vectors for diseases.

11.1 Austria

Pests were observed but was mostly herbivory that caused damage. In 2018 and 2019 herbivory by rabbits caused damage but only in the sweet lupin. Birds like pigeons and crows picked out the seeds which were not planted deep enough. This problem was observed in each year and on each location. In 2019 damaged pods were found, it was assumed that mice were responsible.

Figure 89. Damages on pods caused by mice on the Andean lupins LIB221 at Trautenfels 2019.

In 2018 and 2019 as well, pea moths (*Cydia nigricana*) were found in the hulls. They caused severe damage of the pods and seeds and sometimes small larvae could be observed in the pods. Figure 90 shows these larvae's and damaged grains.

Figure 90. Damaged Andean lupin pods and *Cydia nigricana* larvaes.

11.2 Greece

A serious problem in Andean lupin was caused by the attacks from *Oxytherea funesta*. *Tropinota squalida* (Figures 91 and 92) and *Tropinota hirta* (Figures 93-95) in all experimental locations in 2017-18 and 2018-19. Incidences in Athens were more intense than

in Kalamata and Erythres. Observations and sampling took place two to three times per week during the morning hours.
In Athens, a large population was collected. Reaching up to 979 bugs during the second cropping season, while it reached up to 1150 bugs in the third cropping season. In Kalamata, 172 and in Erythres 75 bugs were collected feeding from lupin flowers. During the third season, only a non-significant number of 40 bugs were observed. These bug species were active during early spring, therefore they affected the plants of early sowing treatments more that were already flowering in that period. *Lupinus albus* entries included in the experiment were also attacked but most of the incidences were recorded in Andean lupin flowers and therefore a clear preference for Andean lupin was present. The bugs were also found to be more active during 11.00-14.00 p.m. as reported previously by Yaşar and Sağdag (2014).

Figure 91. *Tropinota squalida* in Athens. observed in LIB224 (Misak) during 2017-2018.

Figure 92. *Tropinota squalida* in Kalamata. Aithaia observed in LIB221 during 2017-2018.


Figure 93. *Tropinota hirta* in Athens. observed in LIB224 (Misak) during 2017-2018.


Figure 94. *Tropinota hirta* in Athens. observed in LIB220 during 2017-2018.


Figure 95. *Tropinota hirta* in Athens. observed in LIB221 during 2017-2018.

During all the three applied cropping seasons and in all locations, aphids were recorded in *L. angustifolius* cv. Polo and *L. albus* (LIB224) but not in the Andean lupin accessions that were tested (Figures 96-98).


Figure 96. Aphids recorded in *L. angustifolius* cv. Polo in 2016-17 (*Acyrthosiphon pisum*) in Athens.


Figure 97. Aphids recorded in LIB224 in 2016-17 (*Aphis fabae*) in Athens.

Figure 98. Aphids recorded in LIB224 in 2018-19 (*Aphis fabae*) in Kalamata.

Figure 99. Aphrophoridae on cv. Polo plants.

Furthermore, spittlebugs of the Aphrophoridae family were observed on *L. angustifolius* cv. Polo in Athens and in Kalamata during the first and second cropping season (Figure 99). Although not causing a major threat, these insects' incidences were recorded as they can transmit bacterial and viral diseases to plants. Another pest observed to visit Andean lupin was a weevil (*Sitona lineatus*). Weevil incidences were recorded mainly in the experimental area of Erythres feeding from leaves of cv. Polo and cv. Multitalia. Regarding the Andean lupin accessions weevil were observed only in Branco plants in Athens during the 2019-2020 seed emergence test and in 2017-2018 when plants were in the vegetative and flowering phase (Figure 100-102).

Figure 100. Weevil symptoms on cv. Polo leaves in Erythres during 2017-2018 cultivation period.

Figure 101. Weevil feeding from Branco in Athens during 2019-2020 cultivation year.

Figure 102. Weevil feeding from Branco in Athens during 2017-2018 cropping season.

Figure 103. *Thrips* sp. symptoms on leaves of LIB224 in Erythres in 2017-2018.

Thrips sp. symptoms on *L. albus* leaves (cv. Multitalia. LIB224) were observed in Erythres. in 2017-2018 cropping season (Figure 103) and treated the insecticide Laser® 240 SC (50 ml/100 L H_2O). Some snails were also found feeding from lupin flowers (Figure 104) while some *Liriomyza* sp. were observed on the leaves (Figure 105) they were not causing any noteworthy problems. Some incidences of various Lepidoptera larvae were recorded. These were feeding from lupin flowers (Figure 106) and pods (Figure 106) across all years and locations without having a major impact on seed yield.

Figure 104. Snail feeding from flowers of LIB222 in Athens during 2016-2017.

Figure 105. *Liriomyza* sp. in leaves of LIB224.

Figure 106. Lepidoptera larvae feeding by a flower of LIB220.

Figure 107. Lepidoptera pupae in a pod of LIB220 and a destroyed seed.

Figure 108. Lepidoptera larvae leaving holes in pods of LIB224.

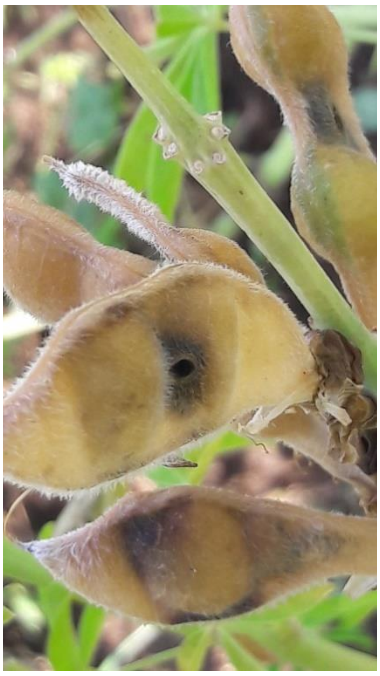


Figure 109. Lepidoptera larvae leaving holes in pods of LIB224.

Figure 110. Ants feeding from a pod of cv. Polo in Kalamata. Aithaia (2017-2018).

Figure 111. Ants feeding from pods of LIB224 in Athens (2017-2018).

Finally, in sweet entries, used during our experiments, namely cv. Polo, cv. Multitalia and LIB224, ants severely were attacking the pods and seeds after Lepidoptera had been feeding (Figures 108-111.).

Conclusively, among the pest incidences, the most serious problem for Andean lupin genotypes was caused by the three bugs (*Oxytherea funesta*, *Tropinota squalida*, *Tropinota hirta*) by attacking their flowers and therefore reducing the seed yield. For the other two lupin species (*L. albus*, *L. angustifolius*) used in experiments, conducted in Greece, Lepidoptera constituted as the most serious pest, as they were followed by attacks of ants severely reducing seed yield.

11.3 Iceland

Only one pest was identified as having a severe effect on Andean lupin. In 2019 in one experiment in WP5, *Melanchra pisi*, the caterpillar of the Broom moth had stripped Branco plants, in a 2 x 4m plot, of most of their leaves in August (Figure 112). This was only observed in one location and therefore was not a problem in other places. The caterpillar is known for its affinity for *Lupinus nootkatensis* in the same area and often strips the plants entirely late in the summer.

Figure 112. Branco plants in an experiment 2019 where most of their leaves had been eaten by *Melanchra pisi.*

11.4 Portugal

Slugs and snails, wireworms (*Agriotes* sp.), cabbage fly (*Delia radicum*) birds and rabbits were identified in Portuguese fields.
Slugs were always present in ISA/Lisbon, from 2016 to 2020 winter sowing and the application of 0,3 g/ha of molluscicide (4% p/p metaldehyde grains) was necessary to prevent major plant losses. In 2017/2018 in propagation fields in Loreto/Coimbra and 2018/2019 density trial in ESAS/Santarém these pests were also present and the same treatment was made in both. These treatments proved to be effective to prevent plant losses.

Wireworms were present in vegetation boxes in ISA/Lisbon, also in Loreto/Coimbra and ESAS/Santarém. No phytopharmaceutical products were applied but loss of plants was registered. Andean lupine did not appear to be more susceptible than white lupine. Rotation with non-susceptible crops must be considered when this pest is present.
Cabbage fly was present which caused severe loss of plants in LIB221 in cropping season 2 in Loreto/Coimbra. This proved to be the only severe attack. The previous crop was cabbage and was probably the cause. Rotation with non-susceptible crops must be considered when this pest is present.
Birds can cause loss of plants from plant emergence to the second leaf stage (Figure 113). After this stage no damage was registered. On small sized field trials the damage was greater than in bigger fields like the propagation fields in Loreto (2017/2018, 2018/2019 and 2019/2020 campaigns) and the GWAS field in ISA in 2018/2019 campaign.
Rabbits were present in Loreto in the 2019/2020 multi-cropping trial (Andean lupin, fava bean, and wheat). Andean lupin plants were damaged but with no severe losses occurred (Figure 2). Both faba bean and wheat were not affected by rabbits.


Figure 113. plant damaged by birds.


Figure 114. plants damaged by rabbits.

11.5 Romania

In June 2018 a lot of plants were discoloured and looked poor Figure 114). Some of them were very easy to be removed from the soil. *Otiorhynchus ligustici* (alfalfa beatles), were found (Figure 115). Also, aphids were present in Suceava.


Figure115. Symptomes of the plants.

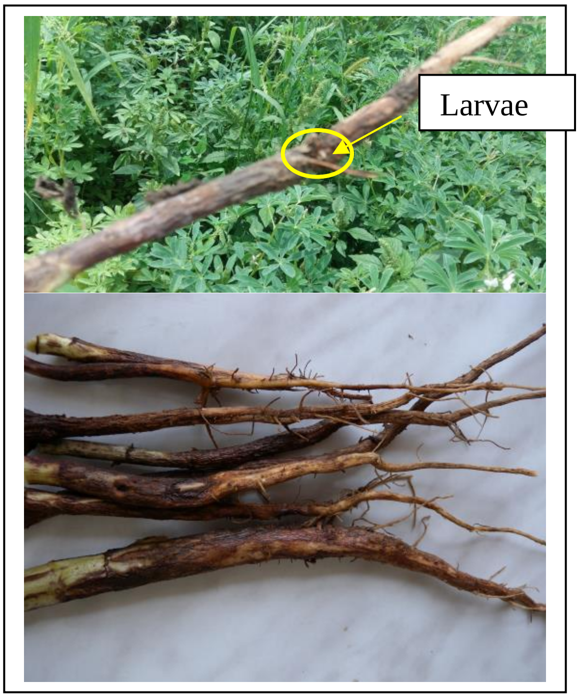


Figure 116. Roots affected by *Otiorhynchus ligustici*.

11.6 Spain

No significant pest damage was observed in any environment during any of the growing seasons.

11.7 The Netherlands

To assess pest's assessments were made at a frequent interval by scoring pest presence. A 1-5 scale was used on a plot level. Score 1 = low pest pressure in the plot, scale 5 = a highly invested plot. During the second cropping season *Macrosiphum albifons*, the lupin aphid, was found on all accessions. Only intercropping with maize seems to reduce aphid infestation. This makes sense since maize is not a host plant for the lupin aphid *Macrosiphum albifons.* However, the differences are small (Figure 117) so in general Andean lupin is considered susceptible to aphid infestation.

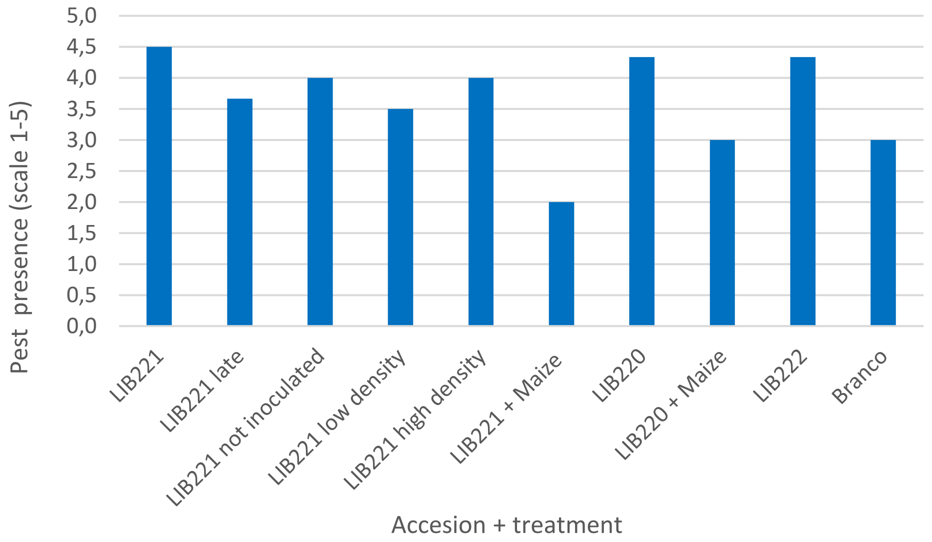


Figure 117. Aphid infestation on sandy soil 2018.

In the third cropping season in 2019, the predominant pest observed in the Andean lupin was again the lupin aphid *Macrosiphum albifons.* Aphids were found on all accessions and no significant differences were found among the treatments. Few young plants were severely affected by the lupin aphid but the aphids did not spread through the whole crop.

What did cause problems was herbivory by birds and deer. In 2019 on the Wageningen test site the reference line Faba bean cv. 'Fuego', and Wheat cv. 'lavett', had to be resown several times due to bird damage. Netting and scarecrows did not deter the birds. Eventually a proper density of faba bean was obtained but the wheat had to be replaced with maize.

On the Noordhout test site, the site with a marginal soil in a forested environment, deer feeding destroyed almost all reference material of the *L. albus*, *L angustifolius* and *L. luteus*.

However, in all cases the Andean lupin was not damaged by deer. Because of the deer's favour for the sweet white, blue and yellow lupin versus the bitter, unpalatable, Andean lupin, the fourth cropping season only bitter lupin accessions were sown on Noordhout.

Figure 118. (Left) lupin aphids on the tip of LIB220.
Figure 119. (Right) Broad leaved lupin on the marginal sand location stripped bare by deer.

12 Conclusions

12.1Austria

Conclusions from this project for Austria can be:
Andean lupin is an interesting new crop.
Andean lupin is a crop with high biomass yield especially Branco, almost higher than with silage-maize.
Andean lupin contains much fibre in its stems, which could be interesting for industrial production for making products from fibre.
Andean lupin in Austria is in direct competition to soya-beans which are very common in Austria and can be grown in many regions with arable land
Andean lupin with such a high amount of alkaloids cannot be used as fodder or as food so all other plants are more interesting for farmers.
Maybe Andean lupins can be used for industrial fallow areas for reclaiming them again for using as arable land.
Maybe Andean lupins can be used for making biogas together with silage maize. A possible approach could be intercropping maize and Andean lupin Thus maize can benefit from the nitrogen the lupines are assimilating and maize can support the lupine plants against lodging.

12.2 Greece

Overall conclusion for Task 2.1 in Greece regarding Andean lupin is a lupin species promising for seed production cultivation in Greece, as plants are reaching only the second order of branches and therefore could not be grown easily for biomass production.
Care should be taken regarding seed lots storage and infection, as well as the sowing depth application in Greek heavy and rocky soil in addition to the heavy rainy weather that predominates during the autumn-winter sowing period in the area. Therefore, a sowing depth of maximum 2 cm should be applied. Among, Andean lupin accessions tested, LIB220 presented as the most stable regarding germination rates across the experiments conducted. LIB200, LIB214 and LIB221 also presented generally good germination rates. Branco presented difficulties in absorbing water and germinate early.
Various disease and pest incidences recorded, but the most serious problems for Andean lupin genotypes caused *Oxytherea funesta*, *Tropinota squalida* and *Tropinota hirta* by attacking flowers and reducing therefore, seed yield production as well as *Rhizoctonia* spp. during the early vegetative stage and *Fusarium oxysporum* in seeds leading in significant plant losses.
Andean lupin accessions managed to grow and be productive under the very high percentage of $CaCO_3$ (up to 17,8) and pH value (up to 8,1) of our experimental fields. Mainly after transplantation, probably because created a special microclimate around the rhizosphere.
Overall, Branco and LIB209 presented in both locations, vigorous plants with not extended chlorotic symptoms and with high chlorophyll content. LIB220 performed very well regarding these three parameters studied only in Kalamata. LIB222 and LIB220 presented more intense chlorotic symptoms, lower chlorophyll content and not so vigorous plants than the other Andean lupin accessions.

Screening Andean lupin accessions, LIB200, LIB218, and LIB209 were characterized by high within variability, as presented variability regarding many qualitative traits studied as in seed shape, seed secondary color and petiole color. Observations showed that LIB209 is formed by two different morphotypes. A remarkable amount of among and within diversity also defined, in Portuguese populations, LIB201 and LIB203, while LIB208 presented to differentiate from the other Portuguese entries and be more homogeneous. These accessions therefore, could consist a valuable source of desirable traits for breeding and should be further evaluated. On the other hand, LIB220, LIB221, and LIB222 characterized by a very good level of homogeneity among the Andean lupin accessions tested taking into consideration the qualitative traits studied.
Branco and cv. Multitalia were the latest flowering accessions, while cv. Polo, LIB221 the earliest flowering ones. The highest yielded accessions were LIB214, LIB209 and Branco enhancing Neves-Martins (1992) stated that under European climatic conditions late maturating Andean lupin accessions show higher seed yield. However, a lower harvest index (H.I.) of Andean lupin accessions obtained in comparison to the other *L. angustifolius* and *L. albus* entries.
Strong positive correlations were obtained among many seed yield traits as well as qualitative traits studied indicating feasibility for implementing them in breeding programs. A significant interaction of location x accession recorded, while there were significant differences among the accessions for many traits, therefore probably selection should focus on promising Andean lupin accessions for each location.

12.3 Iceland

In 2017, most accessions of Andean lupin germinated well at both experimental sites. Most of them flowered at both sites also, but none produced any seeds. Only one accession, LIB221, started producing seed pods, but did not even come close to maturity. There was a clear difference in the overall performance of Andean lupin between the two experimental sites in 2017 where plants grew taller and flowered sooner in the organic field site. The fact that none of the accessions produced any seeds, even in the organic field site, demonstrated clearly that Andean lupin will never become an important crop species in Iceland. There was a certain level of optimism after the first season in 2017, that it might be useful in land reclamation since some of the accessions grew to about 1m in height (Branco) and could be important biomass producers.

In the following years, 2018 and 2019, no accession comparisons were performed due to the overall poor performance in 2017 and the general lack of seed in the LIBBIO project. However, since Branco seemed to outperform all other accessions in 2017 in terms of vegetative growth, and the fact that there was plenty of seed available, it was selected for further experiments in 2018 and 2019 under WP5.

12.4 Portugal

After 3 completed crop seasons and with half of 4th crop season, some Andean lupine accessions evaluated in Portugal indicate that this crop is adapted to Portuguese conditions. Important and necessary trials were performed to better understand some key issues like behaviour on different soils and regions. Response to waterlogging indicates an equivalent behaviour when compared to white lupine or wheat necessary in winter crops. Adaptation to late sowing is a key issue when precipitation in November and December don´t allow sowing, a common situation for winter crops, especially north of Tagus river. LIB221, the shorter cycle from LIBBIO accessions, is the most adapted for late sowing. Density trial allowed better understanding LIB accessions response to different densities and indicates a possible improvement on potential yield. Drought tolerance was not evaluated. Like other winter crops such as wheat, barley or white lupine, whose flowering and pod setting occur usually in Mars and if in low water holding capacity soils, yield can be affected, especially if precipitation is low on February and Mars. Weed management in the first two years was only achieved with resorting to manual hoeing. After an herbicide screening trial which provide information concerning selective and efficient pre-emergence herbicides and the increase of plants density, weed management was successfully achieved. However, none of the tested herbicides is available for Andean lupine in EU. Phytophtora and anthracnose were the most significant diseases affecting this crop on the project fields. The use of quality seeds decreased the number of infected plants on early stages. No significate differences in between tested accessions were observed when anthracnose was present. Concerning pests, only soil warms such as wireworms and cabbage fly were identified. When Andean lupine followed cabbages crop in Loreto/Coimbra, almost all plants were destroyed by cabbage flies. Previous crop tolerance to this pest can possibly decrease damages.

12.5 Romania

The conditions in the 2017 season were normal. This season was the first contact for Andean Lupin and this year Andean lupin seemed promising for Romanian conditions in all three locations. The main purpose for this year, screening of 16 accessions, was done in order to assess the feasibility of cropping this species in particular soil and climate conditions. Results indicated that Andean lupin could be a feasible alternative for a protein crop in Romania.

Due the conditions in 2018, low seed quality, extreme climate conditions, no relevant data could be obtained.

The year 2019 started very promising. Good quality of the seeds, rains after sowing, good emergence of plants. The conditions (warm and wet) stimulated severe disease incidence in the fields and especially LIB 220 which was used for the main trials in Ezareni Iasi was affected. Due the fact that the line did not set any pods, the results were poor and a field meeting for the farmers /students was not possible.

In conclusion, under normal climatic conditions, using high quality seeds and genotypes adapted to the local conditions, Andean lupin could be a serious competitor for *L. albus* in Romania. Under biotic and abiotic stresses, Andean lupin however did appear more sensitive than other legumes. This suggests that more research is needed to improve accessions and breed for high stress tolerance and constant yields.

12.6 Spain

Suitable germplasm for Andean lupins. has been evaluated and developed by CSIC partner The data obtained has allowed the description and comparison of different germplasms, which has finally led to the selection of materials for recommendation to farmers. Phenotypic evaluation permitted the identification of improved genetic pools, in particular certain open-pollinated populations and mixtures which will be crucial for farmers and breeders to create new breeding varieties.

Further studies are needed to discover if Andean Lupin is a crop which offers an advantage over other available protein crops. In addition, it is clear that farmers would be interested in growing Andean lupine if the bio-refineries were prepared to pay for the seeds.

12.7 The Netherlands

After 3 cropping seasons in the Netherlands on sandy, clay and marginal sandy soil it we can conclude the following:

From the various genotypes available the genotypes obtained via the Vandinter Semo seem most adapted to Dutch cropping conditions.
Andean lupin performs best at sandy soil.
Germination in the laboratory setting does not correlate to field germination so care must be taken when deciding to sow this crop on a large scale. Andean lupin germinates very poorly in relation to commercially available brad leaved lupin.
LIB220 and 221 are fairly homogeneous, LIB222 is still very heterogeneous so more selection is needed to obtain a line suitable for large scale studies.
LIB221 is the earliest to flower when sown it a time that is common for lupin in the Netherlands, early or late sowing does not positively influence the time of sowing to pod production.
LIB220 has potential for biomass production if the quality for industrial purposes proofs desirable. Due to the bitterness Andean lupin is not yet suited as a food or feed product.
Protein yield in the Netherlands is far too low to be competitive with other protein crops.

12.8 Overall conclusion

Andean lupin is a promising crop for Europe, also growing on marginal soils. Andean lupin crop (seed) yield can be high up to 6,7 t/ha in experimental fields in Portugal and 2,0 t/ha in a machine harvested field in the Netherlands. Dry biomass production for bioenergy of animal feed or bioenergy can be up to 30 t/ha on dry matter base in Austria. The Andean lupin variety Cotopaxi has acquired Plant Breeder Rights. Cotopaxi is an early semi-determinate growing type with seed yields up to 6,7 tonnes per hectare making it an economical feasible crop for farmers and hence for biorefineries. More breeding efforst are needed for increasing yield, yield stability, disease resistance and determinate growth type.
The LIBBIO project clearly demonstrates the potential of Andean lupin as a real double purpose crop for Europe with respectively seed and biomass production where potential yields can be achieved of almost 7 tonnes/ha and 30 tonnes dm/ha. This makes Andean lupin attractive for farmers and biorefineries.

13 References

1. Abrol I.P., Yadav J.S.P., and Massoud F.I. 1988. Salt-Affected Soils and their Managemnet. FAO soils bulletin 39.
2. Agarie S, Shimoda T, Shimizu Y, et al. Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant Mesembryanthemum crystallinum. *J Exp Bot*. 2007;58(8):1957-1967. doi:10.1093/jxb/erm057
3. Anagnostopoulos A, Koukis G, Sabatakakis N and Tsiambaos G (2003) Empirical correlations of soil parameters based on Cone Penetration Tests (CPT) for Greek soils. Geotechnical and Geological Engineering. 21:377-387
4. Arnalds, O. (2008). Soils of Iceland. Jökull, 58, 3-20.
5. Arnalds, O., Orradottir, B., & Aradottir, A.L. (2013). Carbon accumulation in Icelandic desert Andosols during early stages of restoration. *Geoderma*, *193–194*, 172-179.Einarsson, M. 1984. Climate of Iceland. In: H. van Loon (editor): World Survey of Climatology: 15: Climates of the Oceans. Elsevier, Amsterdam, 1984, pp 673-697.
6. Beck H., Zimmermann N., Mc Vicar T., Vergopolan N., Berg A., and Wood E., (2018) Present and future Köppen-Geiger climate classification maps at 1-km resolution. Sci Data. 5:180-214. doi: https://doi.org/10.1038/sdata.2018.214
7. Bianchi, F. J., Booij, C. J. H., & Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proceedings of the Royal Society B: Biological Sciences, 273(1595), 1715-1727.
8. Caligari P.D.S., Römer P., Rahim M.A., Huyghe C., Neves-Martins J.A., and Sawicka-Sienkiewicz E.J., (2000) The potential of *Lupinus mutabilis* as a crop. Linking research and marketing opportunities for pulses in the 21st century. Current Plant Science and Biotechnology in Agriculture. 34:568-573
9. Cambell J.W., Irvin, A., Irvin, H., Stanley-Stahr C., Ellis J.D., 2016. Insect visitors to flowering buckwheat, fabopyrum esculentum (Polygonales:polygonaceae), in North Centra Florida. Florida Entomologist, Vol 99, No.2.
10. Chalker-Scott L., Environmental Significance of Anthocyanins in Plant Stress Responses. In: PhPhotochemistry and Photobiology, 1999, 70(1): 1-9
11. Clements J., Prilyuk L., Quealy J., and Francis G., (2008) Interspecific crossing among the new world lupin species for *Lupinus mutabilis* crop improvement. In: Lupins for health and wealth. Proceedings of the 12th International Lupin Conference, Palta JA and Berger JB eds, International Lupin Association, Canterbury, New Zealand, pp. 324-327
12. ESDAC, (2020) https://esdac.jrc.ec.europa.eu/content/chemical-properties-european-scale-based-lucas-topsoil-data (*accessed on 20/2/2020*)
13. FAO, 1992. Soil Map Of The World – Europe (http://www.fao.org/fileadmin/user_upload/soils/docs/Soil_map_FAOUNESCO/acrobat/Europe_V.pdf)
14. FAO/IIASA/ISRIC/ISSCAS/JRC, 2012. Harmonized World Soil Database (version 1.2). FAO, Rome, Italy and IIASA, Laxenburg, Austria.
15. For FAO soil classification see http://www.fao.org/soils-portal/soil-survey/soil-maps-and-databases/faounesco-soil-map-of-the-world/en/

16. Founda D., and Giannakopoulos C., (2009) The exceptionally hot summer of 2007 in Athens. Greece - A typical summer in the future climate? Global and Planetary Change. 67:227-236. doi: 10.1016/j.gloplacha.2009.03.013
17. Gulisano, A.,; Alves, S.,; Martins, J.N.,; Trindade, L.M., Genetics and Breeding of *Lupinus mutabilis*: An Emerging Protein Crop. *Front. Plant Sci.* **2019**, *10*, 1–13, doi:10.3389/fpls.2019.01385.
18. Hamilton, 2014. https://unu.edu/media-relations/releases/world-losing-2000-hectares-of-farm-soil-daily-to-salt-induced-degradation.html
19. Harrison, T., Gibbs, J., & Winfree, R. (2019). Anthropogenic landscapes support fewer rare bee species. Landscape Ecology, 34(5), 967-978.
20. Heather M. Whitney, Georgina Milne, Sean A. Rands, Silvia Vignolini, Cathie Martin, Beverley J. Glover. The influence of pigmentation patterning on bumblebee foraging from flowers of Antirrhinum majus. Naturwissenschaften, 2013; DOI: 10.1007/s00114-013-1020-y
21. HOBO software: https://www.onsetcomp.com/hobo-micro-station)
22. http://www.soya-uk.com/wp-content/uploads/2017/09/LupinContracts.pdf accessed on 25-3-2020
23. https://erkendstreekproduct.nl/product/ijskruid/. Accessed january 2020.
24. https://unu.edu/media-relations/releases/world-losing-2000-hectares-of-farm-soil-daily-to-salt-induced-degradation.html Hamilton United Nations University. 2014. http://unu.edu/media-relations/releases/world-losing-2000-hectares-of-farm-soil-daily-to-salt-induced-degradation.html
25. IBPGR, (1981) International Board for Plant Genetic Resources. Lupin Descriptors. Rome, Italy
26. Icelandic Met Office, 2020. Data obtained directly from officials at the Icelandi Met office in jan. 2020.
27. IPMA,. https://www.ipma.pt/pt/educativa/tempo.clima/).
28. IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.
29. Kanswohl, N., (2018): Schlussbericht zum Verbundvorhaben: Züchterische Evaluierung der Andenlupine im Vergleich zur weißen und zur blauen Lupine für die Bioenergienutzung; Teilvorhaben 2: Ermittlung Methanausbeute. Unter https://www.fnr-server.de/ftp/pdf/berichte/22407312.pdf. Acessed on 04.12.2019.
30. Lazaridi E., Ntatsi G., Savvas D., Bebeli P.J., (2016) Diversity in cowpea (*Vigna unguiculata* (L.) Walp.) local populations from Greece. Genet Resour Crop Evol. 64:1529-1551. doi: 10.1007/s10722-016-0452-6
31. Lopez-Bellido, L.,; Fuentes, M., Growth, Yield, and Yield Components of Lupin Cultivars. *Agron. J.* **1990**, *82*, 1050–1056, doi:10.2134/agronj1990.00021962008200060004x.
32. Misopollinos N., Sillaios N., Mpilas G., Karapetsas N., Dionisiou N., Misopollinos L., Pazarlogou M., Strati S., Cherif I., Ovakoglou I., Kosmas K., Karamesouti M., Kounalaki A., Kolovos H., Zaglis G., Kairis O., Tsigas V., Kouroupis S., Papadakis G., Printsos V., Kontidimos G., Gianousios A., Stampoulidou M., 2015. Developement of an integrated system for soi geographic data delineation of agricultural zones in Greece. AUTH, Thessaloniki. (http://appliedsoilab.web.auth.gr/images/GRSoilMap_ENG_500k_poster_oversize_May2016_v2_300dpi.pdf)

33. Nei M., (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U.S.A. 70: 3321-3323
34. Neves-Martins J., Talhinhas P., and Sousa R.B., (2016) Yield and seed chemical composition of *Lupinus mutabilis* in Portugal. Revista de Ciências Agrárias 39:518-525. doi: 10.19084/RCA16079
35. Ozinga, W. A., Scheper, J. A., de Groot, A., Reemer, M., Raemakers, I., van Dooremalen, C., & Kleijn, D. (2018). Wilde bijen en zweefvliegen per landschapstype (No. 2920). Wageningen Environmental Research.
36. Raabe, A.,; von Sengbusch, R. Züchterisch wichtige Beobachtungen an einigen Lupinenarten. *Der Züchter* **1935**, *7*, 244–248, doi:10.1007/BF01812317.
37. Raza S., Abdel-Wahab, A., Jørnsgård B. and Christiansen, J.L.; 2000; Calcium tolerance and ion uptake of Egyptian lupin landraces on calcareous soils; African Crop Science Journal; Vol. 9. No. 2; p. 393-400
38. Roux, S., Höppner F., Wiedow D., and Kanswohl N., (2017): Züchterische Evaluierung der Andenlupine im Vergleich zur weißen und zur blauen Lupine für die Bioenergienutzung. Vortrag beim 2. Kongress der FNR "Mit Pflanzenzüchtung zum Erfolg", 3.-4. April 2017, Berlin.
39. SAS Institute Inc. (2007) JMP/Sales Department. Cary, NC, USA
40. Soil Atlas of Europe, European Soil Bureau Network European Commission, 2005, 128pp, Office for Official Publications of the European Communities, L-2995 Luxembourg.
41. StatSoft, Statistica package release 8 (2007) StatSoft, Inc. 1984-2007, Tulsa, USA
42. Szabolcs I.; 1990; Impact of climatic change on soil attributes: Influence on salinization and alkalinisation; In: Soils on warmer Earth; Sharpenseel et al. ed.; p.61-71; Elsevier.
43. Talhinhas P., Vaz Patto M.C., Martins J.M., Römer P., Huyghe C., Rahim M., and Caligari P., (1999) Evaluation of *Lupinus mutabilis* Sweet cultivars under Mediterranean conditions. In: Proceedings of the 8th International Lupin Conference, Hill G ed, International Lupin Association, Canterbury, Asilomar (CA), USA. pp. 87-93
44. Tang C., Robson A.D., Longnecker N.E., and Buirchell B.J., (1995) The growth of *Lupinus* species on alkaline soils. Australian Journal of Agricultural Research. 46:255-268. doi: https://doi.org/10.1071/AR9950255
45. Terzopoulos P.J., Kaltsikes P.J., and Bebeli P.J., (2008) Determining the sources of heterogeneity in Greek faba bean local populations. Field Crop Research 105**:**124-130. doi: 10.1016/j.fcr.2007.08.006
46. Von Sengbusch, R.,; Zimmermann, K., Die Auffindung der ersten gelben und blauen Lupinen (*Lupinus luteus* und *Lupinus angustifolius*) mit nichtplatzenden Hülsen und die damit zusammenhängenden Probleme, insbesondere die der Süßlupinenzüchtung. *Der Züchter* **1937**, *9*, 57–65, doi:10.1007/BF01812469.
47. White P.F., (1990) Soil and plant factors relating to the poor growth of Lupinus species on fine-textured. alkaline soils - a review. Australian Journal of Agricultural Research. 41:871. doi: 10.1071/ar9900871
48. Xiyuan H., Shiwei Y., and Kunlun S. (2013) Heterogeneity of error variance and its influence on genotype comparison in multi-location trials. Field Crop Res. 149:322-328
49. Yaşar B., and Sağdag A., (2014) The Capturing of the Apple Blossom Beetle. *Tropinota hirta* (Poda) (Coleoptera: Scarabaeidae). by Different Traps in Afyonkarahisar. Turkish Journal of Agricultural Research. 1:29-34

50. Yassoglou N., Tsadilas C., Kosmas C., 2017. The soils of Greece. Hartemink, A.E. Ed.; Springer International Publishing AG, Switzerland.
51. Zamanidis P.K., and Evangelogiannis D.I., (2017) The effect of climate in Greek soil. Easaterina. Eurasian union of scientists 04/30/2017_04 (37). https://euroasia-science.ru/selskoxozyajstvennye-nauki/the-effect-of-climate-in-greek-soil-paschalidis-ch-d/ (*accessed on 18/2/2020*)
52. Zubr J., 1997. Oil-Seed crop: Camelina sativa. Industrial crops and Products, Volume 6:113-119.

Appendix

A.I Austrian weather data

The following figures illustrate the average temperature and precipitation per day from May to the end of November. Data were recorded by the meteorological station (Pessl Instruments GmbH, iMetos 3.3) directly at the trial site in Stadl-Paura (station coordinates 48.087077°N, 13.876294°E). Data were retrieved from the online platform “Field Climate”.
Annual rainfall in Lambach/ Stadl-Paura in 2019: 744 mm
Average annual temperature in Lambach/ Stadl-Paura in 2019: 10,39 °C

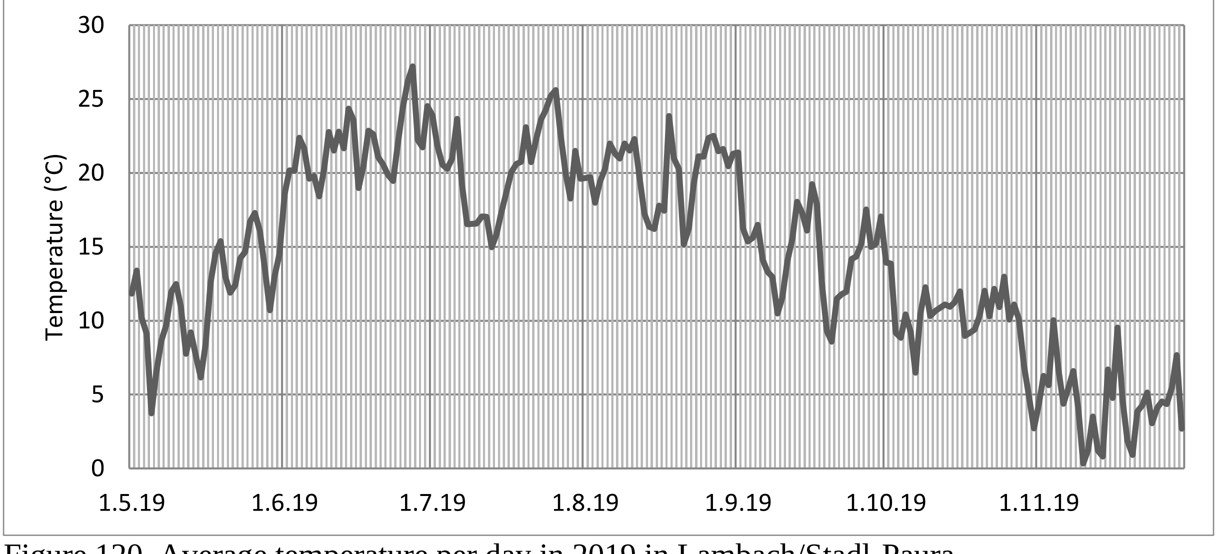


Figure 120. Average temperature per day in 2019 in Lambach/Stadl-Paura.

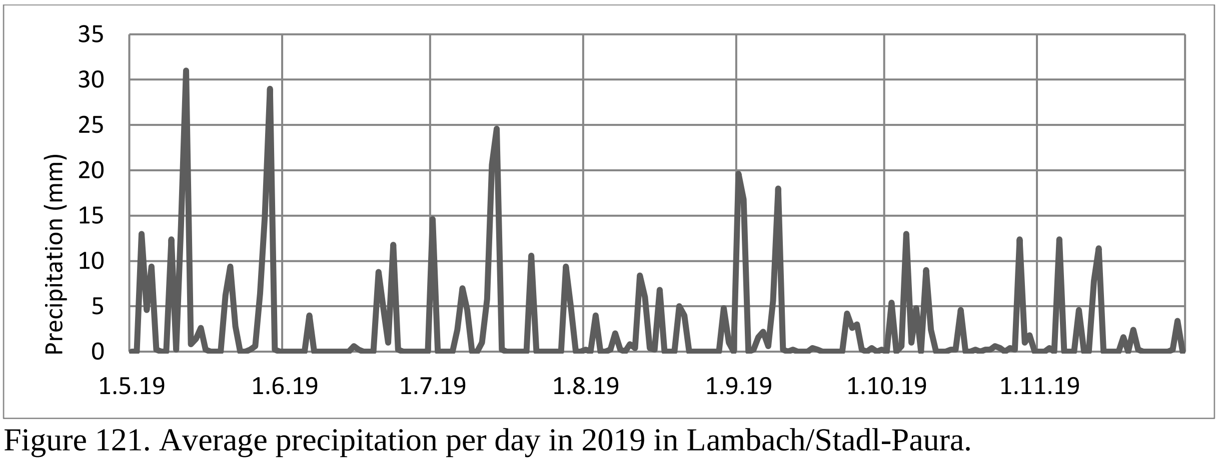
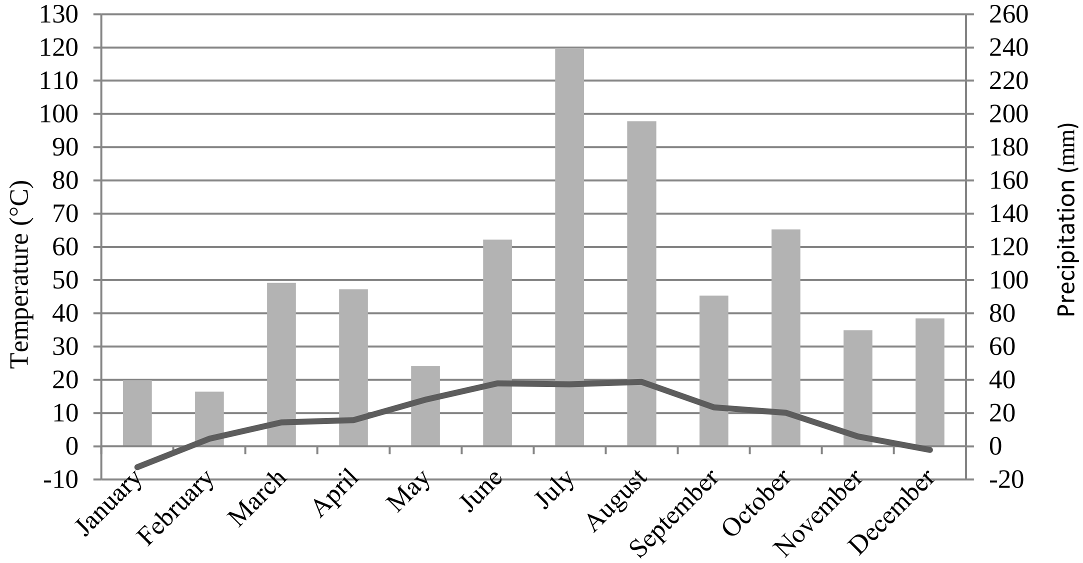
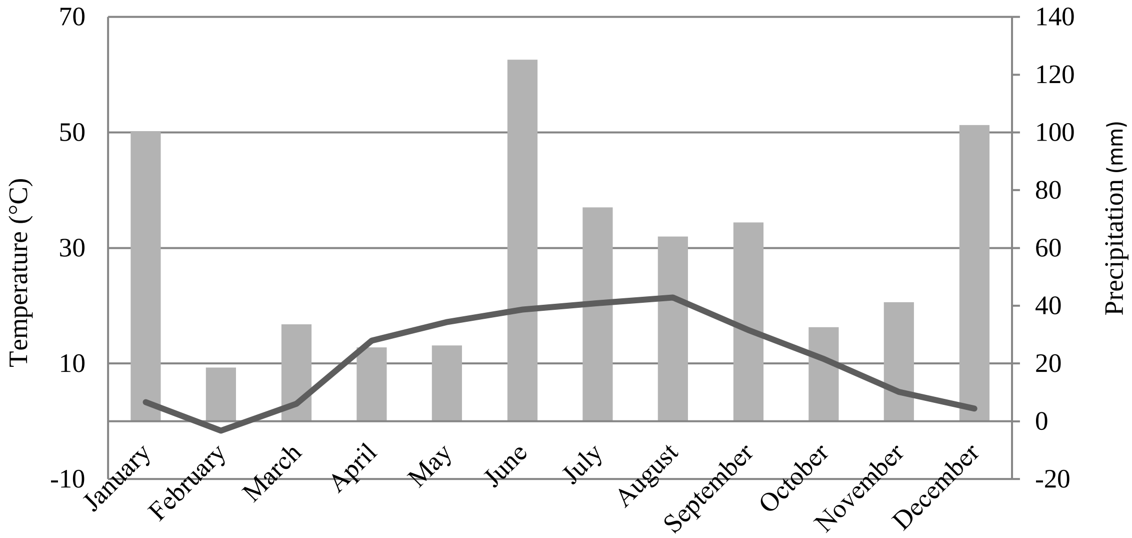


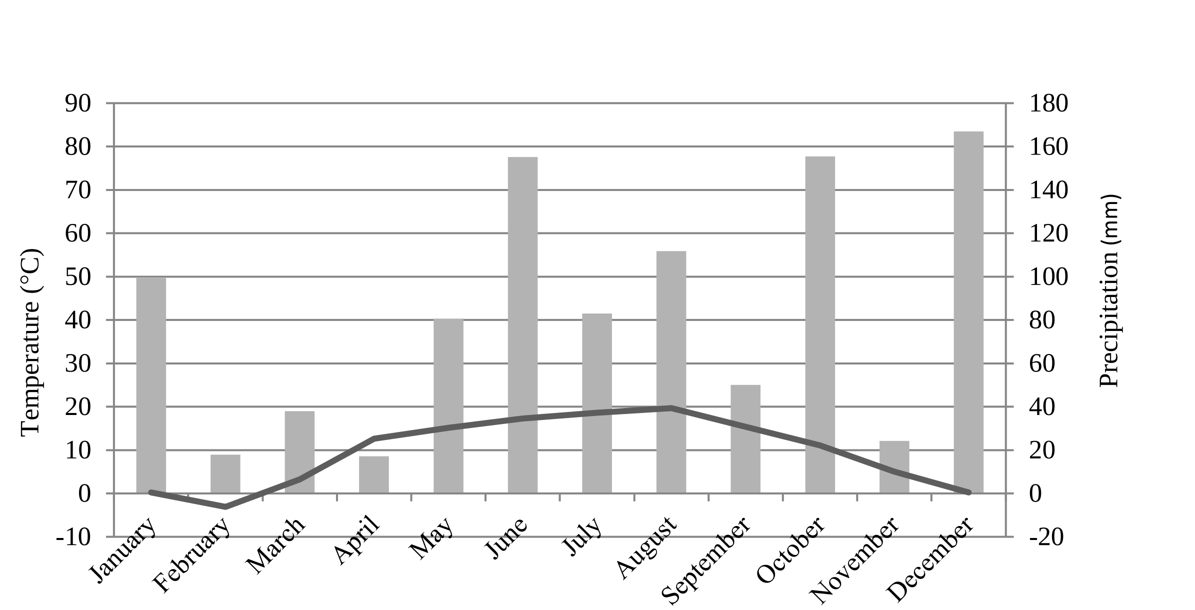
Figure 121. Average precipitation per day in 2019 in Lambach/Stadl-Paura.

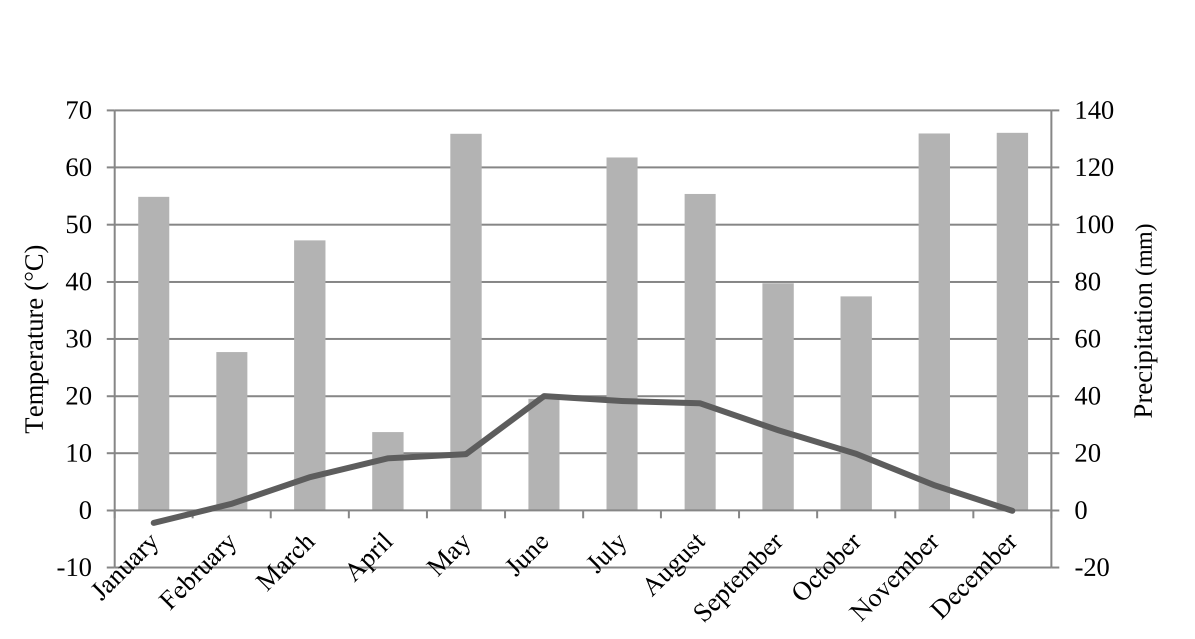
Annual rainfall in Trautenfels in 2019: 1111 mm
Average annual temperature in Trautenfels in 2019: 9,1 °C
Although we have no weather-station at Trautenfels we use the data of the weather-station from Gumpenstein. This is about 3 km far away from Trautenfels and situated at the opposite site of the Enns-valley. The weather station at Gumpenstein is an official weather station of

the ZAMG (Zentralanstalt für Meteorologie und Geodynamik) and is a partly automated weather station.






90
80
70
60
50
40
30
20
10
0
-10
Temperature (°C)
180
160
140
120
100
80
60
40
20
0
-20
Precipitation (mm)
January
February
March
April
May
June
July
August
September
October
November
December


70
60
50
40
30
20
10
0
-10
Temperature (°C)
140
120
100
80
60
40
20
0
-20
Precipitation (mm)
January
February
March
April
May
June
July
August
September
October
November
December

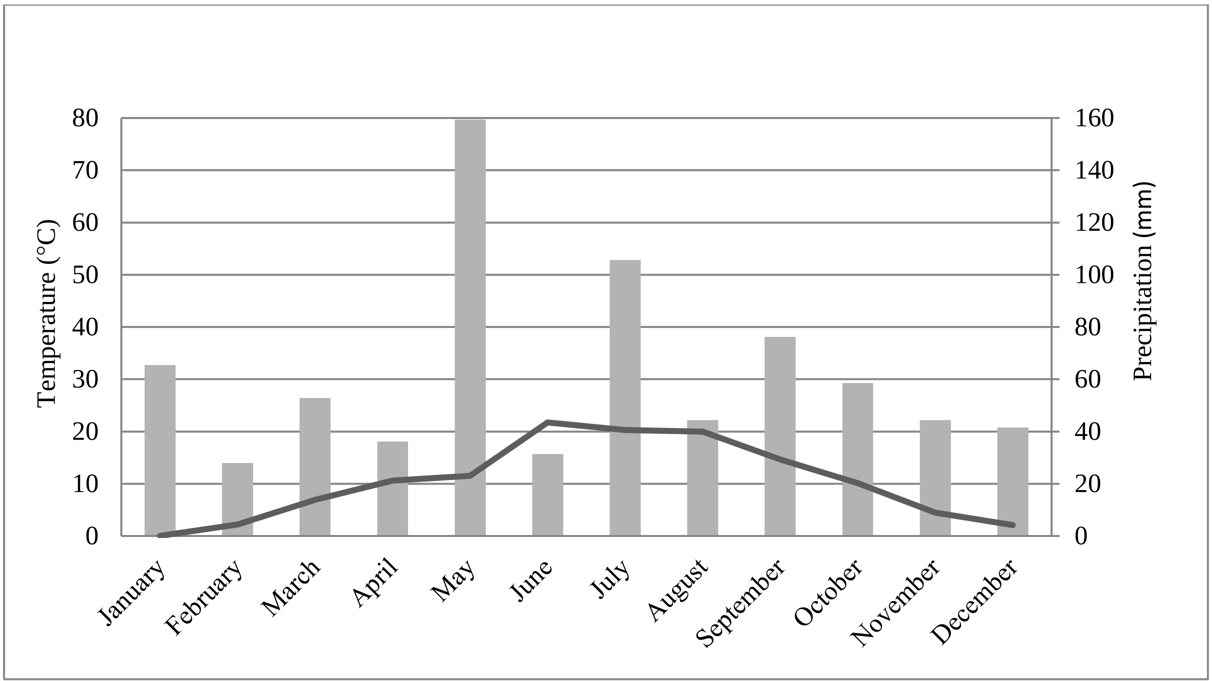


Figure 122-126 All temperature and rainfall data of each year of Lambach, Stadl-Paura and Trautenfels. From top to bottom Temperature in Lambach 2019, rainfall in Lambach 2019, Tratenfells 2017, Lambach 2018, Trautenfels 2018, Trautenfels 2019, Lambach 2019.

The year 2018 was quite different. We started sowing in the beginning of May at Lambach/Stadl-Paura. Then we had a very dry spring with a period of drought in June. In summer there was a little bit more rainfall and high average temperatures. The weather conditions in autumn were more wet but not too cold. The field trials were mulched in the middle of November.

At Trautenfels the weather conditions were similar but there is more precipitation because of the condensate on the plants in the morning. In spring we had enough rainfall for growing a lot of weeds but too much for using a hoe during the youth development of the plants. In summer we had a mix of hot days and rainfall. In autumn we had a light frost in the end of September but it was enough to kill all Andean lupines plants.

The weather conditions in the year 2019 were quite different from the both years before. After warm temperatures in April May started with cold and wet weather, even with snowfall in the pre-alpine region of Lambach. The wet period lasted almost the whole May. The soils were rain-drenched that no machine could go into the fields. Therefor the sowing date at Lambach and Stadl-Paura was very late but the earliest was possible. Afterwards a period without rainfall started that caused drought especially at Stadl-Paura. In the end of July moderate rainfall started, good for the plants. No frost came until the field trial was mulched in the middle of November.

At Trautenfels we had similar weather conditions in spring but we could start sowing about 10 days earlier. The summer months showed a mix of hot days and rainfall and that was the same in autumn. There was no frost so the plants were mulched in the middle of November to prepare the field for the next year.

A.II Romania weather data

2017

Ezareni, Iasi

Table 161. Climatic conditions at Ezareni – Iasi, 2017.

Ezareni - IS	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.	Oct.
	Temperature (^{0}C)									
Average	-4,9	-0,8	8,0	10,1	16,1	21,1	21,6	21,9	17,2	10,9
Min.	-17,1	-21,9	-1,2	-2,5	3,0	8,6	9,7	5,9	-1,4	-1,0
Max.	7,3	15,2	22,7	25,5	29,6	33,1	25,6	37,9	32,2	28,0
Multi-annual avg.	-3,6	-1,9	3,3	10,1	16,1	19,4	21,3	20,6	16,3	10,1
Departure from normal	**-1,3**	**1,1**	**4,7**	**0**	**0**	**1,7**	**0,3**	**1,3**	**0,9**	**0,8**
	Precipitation (mm)									
Sum	323,6	13,8	107,0	140,4	72,8	71,6	84,4	61,8	23,2	62,4
Multi-annual avg.	28,9	27,4	28,1	40,3	52,5	75,1	69,2	57,6	40,8	34,4
Departure from normal	**294,7**	**-13,6**	**78,9**	**100,1**	**20,3**	**-3,5**	**15,2**	**4,2**	**-17,6**	**28,0**
	Relative humidity (%)									
Average	97,2	82,1	89,5	92,1	94,8	94,7	94,9	93,8	91,1	94,8

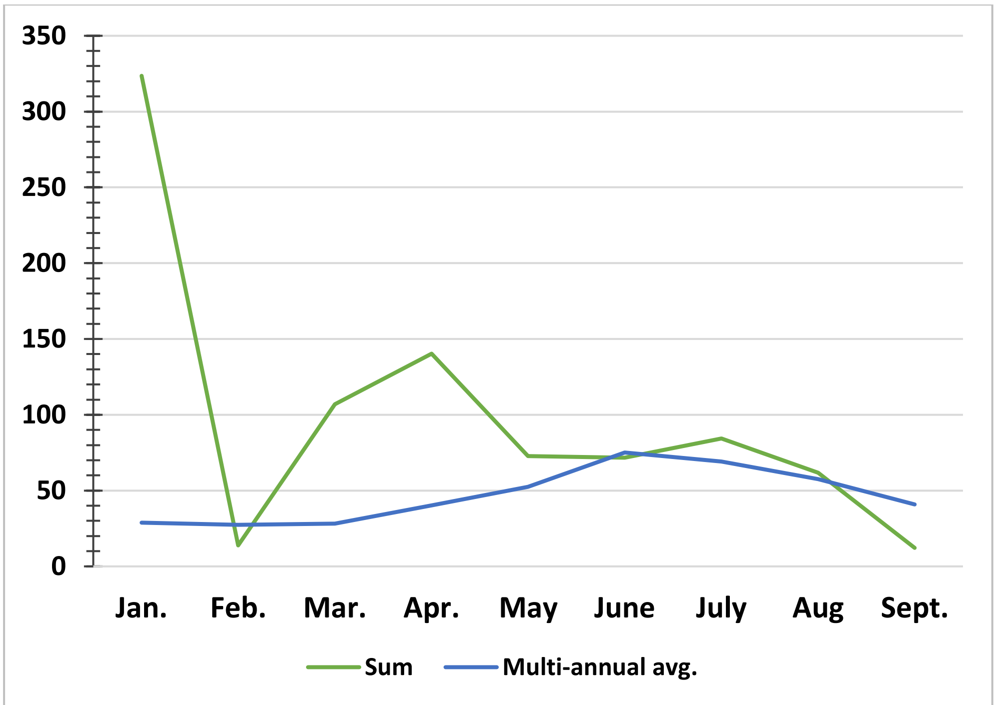


Figure 127. Rainfall in Iasi 2017.

Secuieni, Neamt

Table 162. Climatic conditions at Secuieni – Neamt, 2017.

Secuieni – NT	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.
	Temperature (^{0}C)								
Average	-5,7	-1,8	7,0	9,1	15,8	20,3	20,4	21,2	18,5
Min.	-17,8	-22,1	-1,2	-1,3	2,9	8,9	9,5	5,2	8,6
Max.	7,9	14,6	23,2	25,8	29,0	34,1	36,5	35,9	31,5
Multi-annual avg.	-3,8	-2,3	2,6	9,4	15,4	18,8	20,3	19,5	14,8
Departure from normal	**-1,9**	**0,5**	**4,4**	**-0,3**	**0,4**	**1,5**	**0,1**	**1,7**	**3,7**
	Precipitation (mm)								
Sum	7,3	17,0	101,6	54,4	59,4	49,4	72,2	23,0	28,8
Multi-annual avg.	20,5	19,6	25,4	46,8	64,8	84,3	84,0	61,4	45,4
Departure from normal	**-13,2**	**-2,6**	**76,2**	**7,6**	**-5,4**	**-34,9**	**-11,8**	**-38,4**	**-16,9**
	Relative humidity (%)								
Average	-	-	87	78	80	80	85	78	81

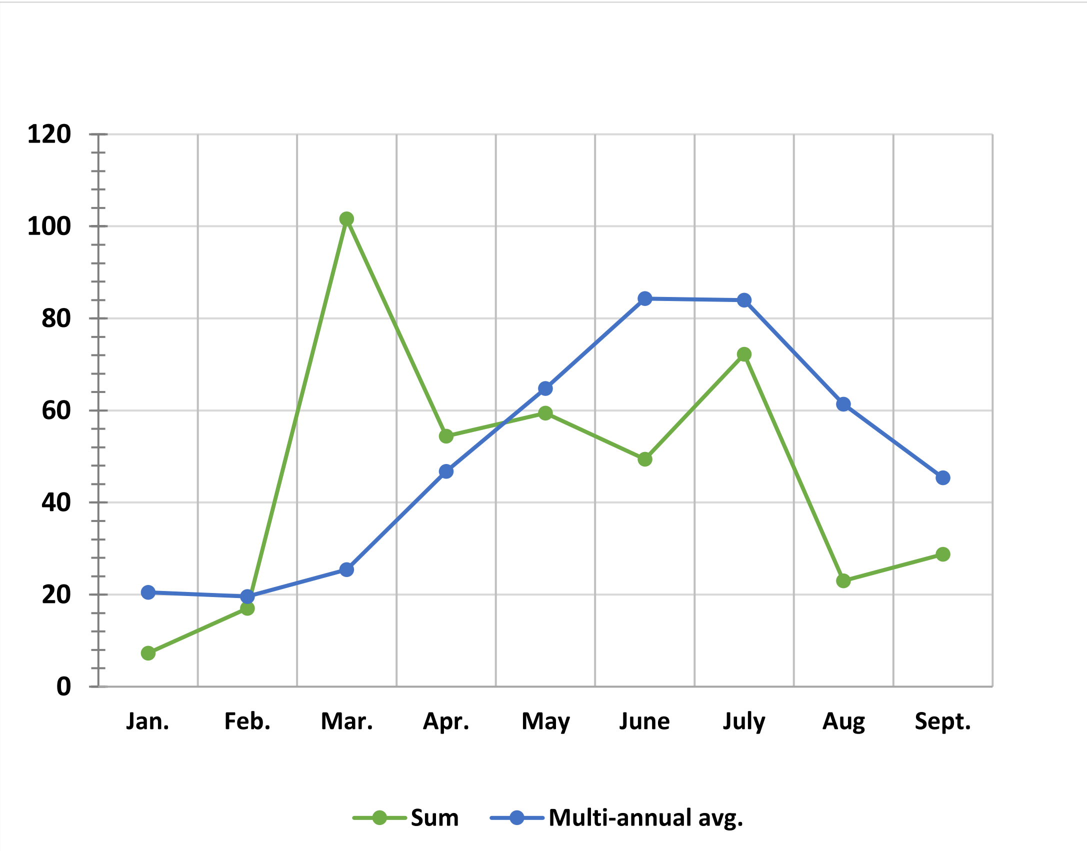


Fig. 128. Rainfall at Secuieni 2017.

Table 163. Climatic conditions at Suceava, 2017.

Suceava - SV	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.
	Temperature (0C)								
Average	-4,7	-1,5	6,5	8,6	13,4	16,2	22,6	20,5	16,7
Min.	-17,3	-22	-1,3	-1,5	2,8	8,7	9,9	7,2	7,8
Max.	7,8	14,7	23,5	24,7	30,2	35,5	37,7	36	28,9
Multi-annual avg.	-4,1	-2,9	1,2	8	13,7	16,9	18,4	18,3	14,2
Departure from normal	-0,6	1,4	5,3	0,6	-0,3	-0,7	4,2	2,2	2,5
	Precipitation (mm)								
Sum	4,7	14,6	38,9	35,5	30	55	28	19	25
Multi-annual avg.	24,2	25,6	36,2	48,2	80,2	93,6	88,6	62,8	40
Departure from normal	-19,5	-11	2,7	-12,7	-50,2	-38,6	-60,6	-43,8	-15

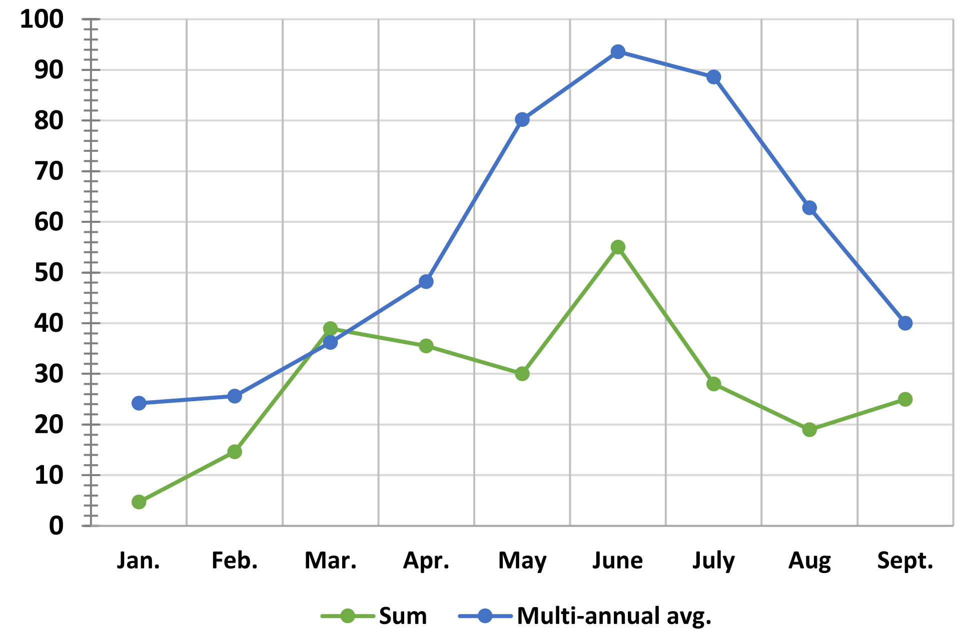
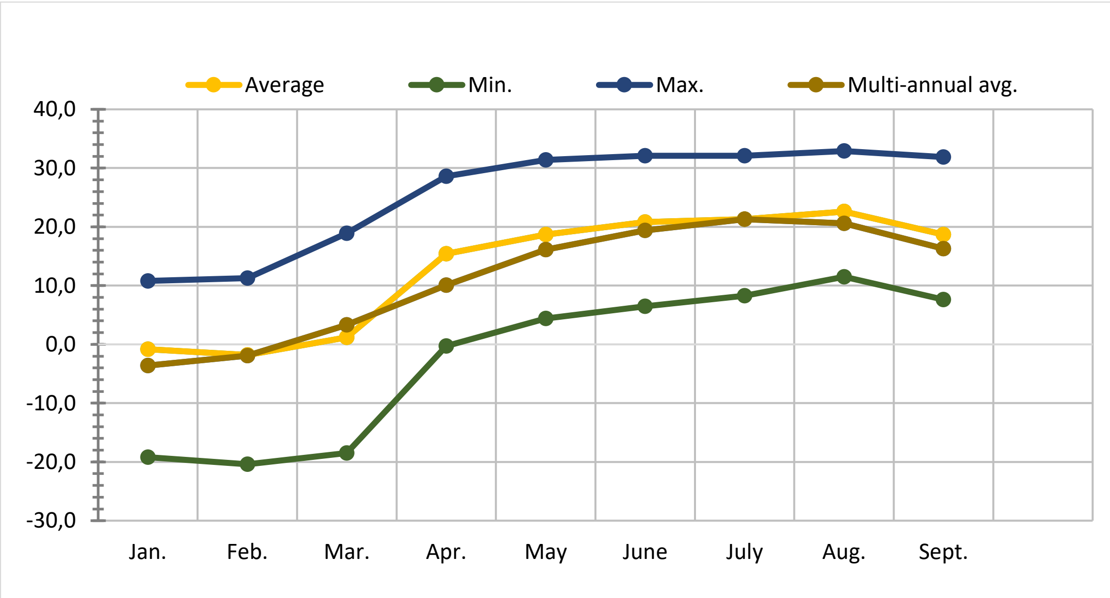


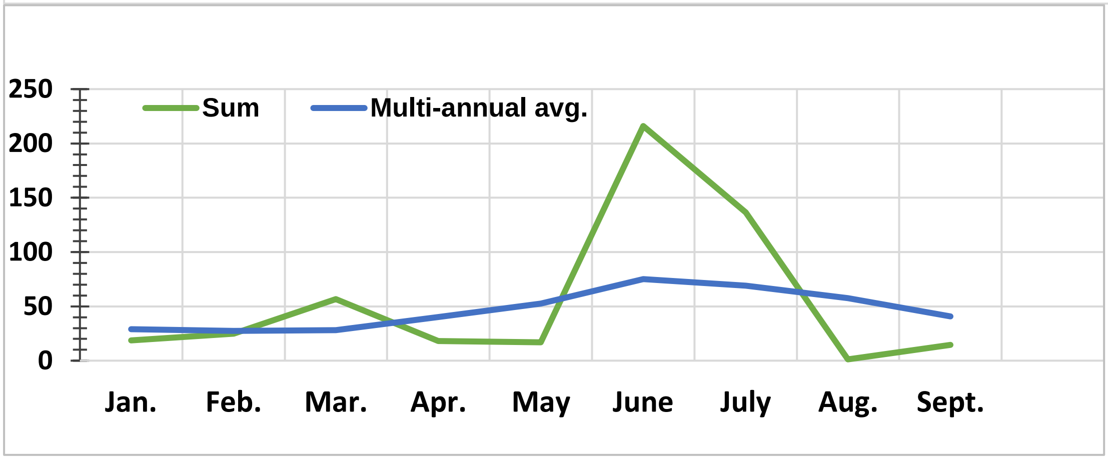
Figure 129 Rainfall at Suceava 2017.

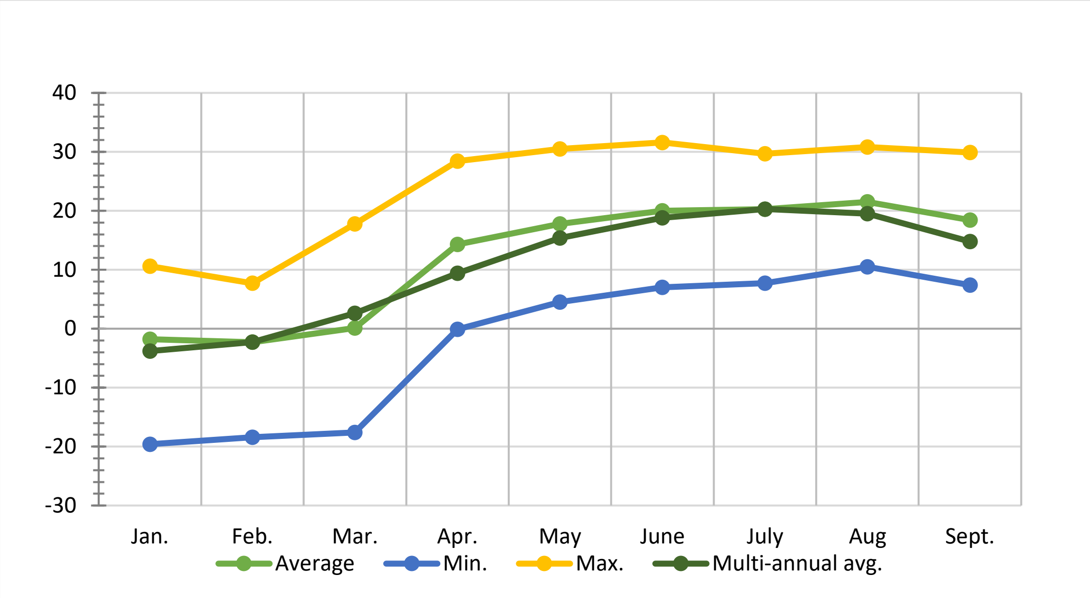
Table 164. Climate conditions at Ezareni Iasi 2018.

Ezareni - IS	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.
	Temperature (^{0}C)								
Average	**-0,8**	**-1,8**	**1,2**	**15,4**	**18,7**	**20,8**	**21,3**	**22,6**	**18,7**
Min.	-	-	-	-0,3	4,4	6,5	8,3	11,5	7,6
Max.	10,8	11,3	18,9	28,6	31,4	32,1	32,1	32,9	31,9
Multi-annual avg.	-3,6	-1,9	3,3	10,1	16,1	19,4	21,3	20,6	16,3

Departure from normal	**2,8**	**0,1**	**-2,1**	**5,3**	**2,6**	**1,4**	**0**	**2**	**2,4**
	Precipitation (mm)								
Sum	**18,8**	**24,8**	**56,8**	**18**	**16,8**	**216**	**136,6**	**1,2**	**14,4**
Multi-annual avg.	28,9	27,4	28,1	40,3	52,5	75,1	69,2	57,6	40,8
Departure from normal	-10,1	-2,6	28,7	-22,3	-35,7	140,9	67,4	-56,4	-26,4
	Relative humidity (%)								
Average	**99,5**	**99,8**	**98,6**	**91,2**	**90,9**	**93,7**	**98,5**	**93,6**	**93,6**







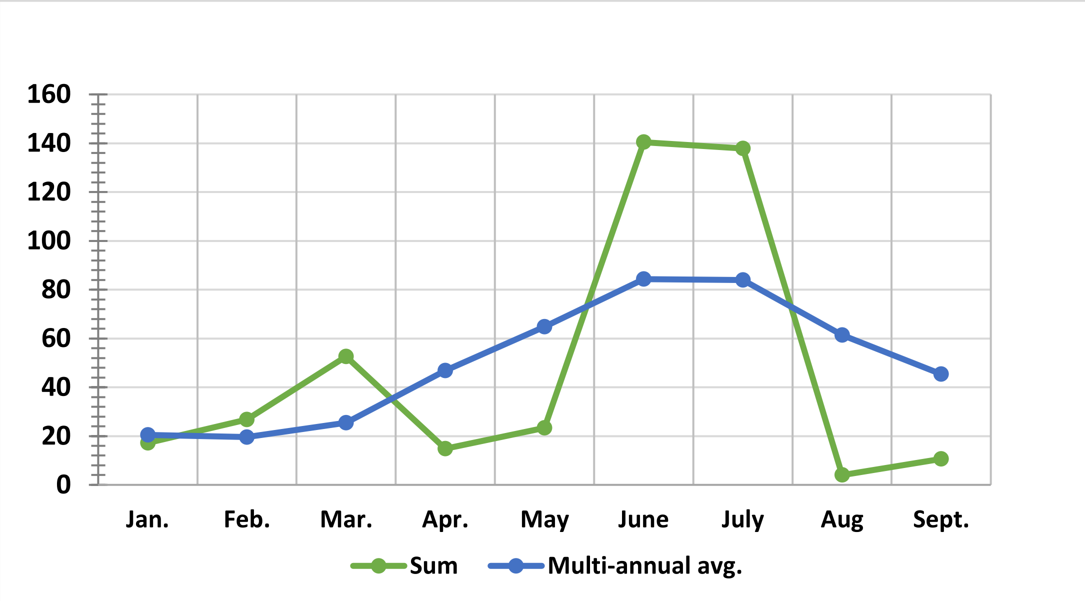
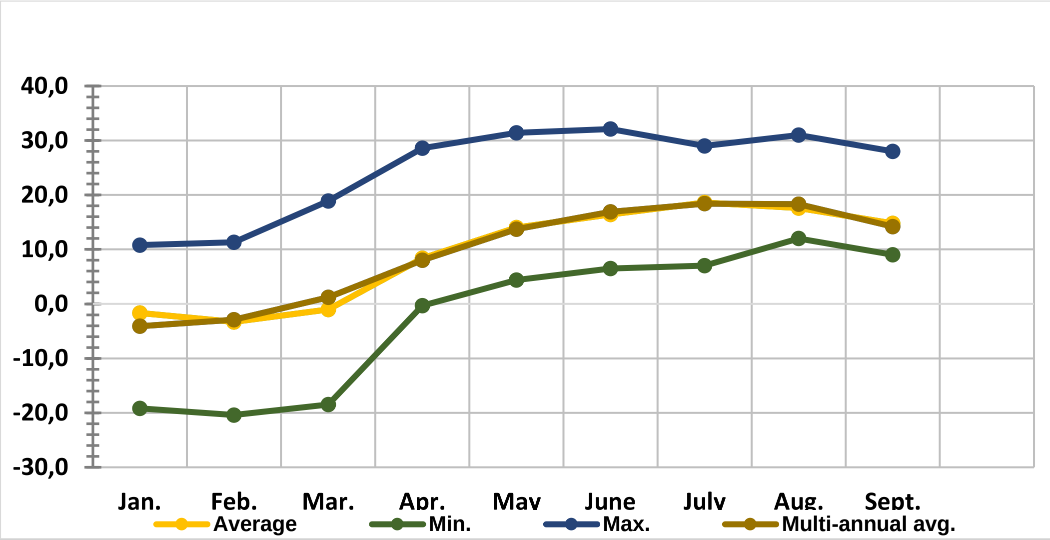


Figure 130-133.Temperatures and rainfalls in Iasi area 2018 versus the multi-annual averages.

For the field in Suceava the climatic conditions are presented in table 6 and figure 7.
Table 165. Climatic conditions and Suceava, 2018.

SUCEAVA, 2018	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
	Temperature (^{0}C)								
Average	-1,7	-3,3	-1,0	8,4	14,0	16,4	18,6	17,6	14,8
Min.	-19,2	-20,4	-18,5	-0,3	4,4	6,5	7,0	12,0	9,0
Max.	10,8	11,3	18,9	28,6	31,4	32,1	29,0	31,0	28,0
Multi-annual avg.	-4,1	-2,9	1,2	8,0	13,7	16,9	18,4	18,3	14,2
Departure from normal	2,4	-0,4	-2,2	0,4	0,3	-0,5	0,2	-0,7	0,6
	Precipitation (mm)								

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Sum	30,7	36,6	58,3	17	31	92	218	61	2
Multi-annual avg.	24,2	25,6	36,2	48,2	80,2	93,6	88,6	62,8	40
Departure from normal	6,5	11	22,1	-31,2	-49,2	-1,6	129,4	-1,8	-38



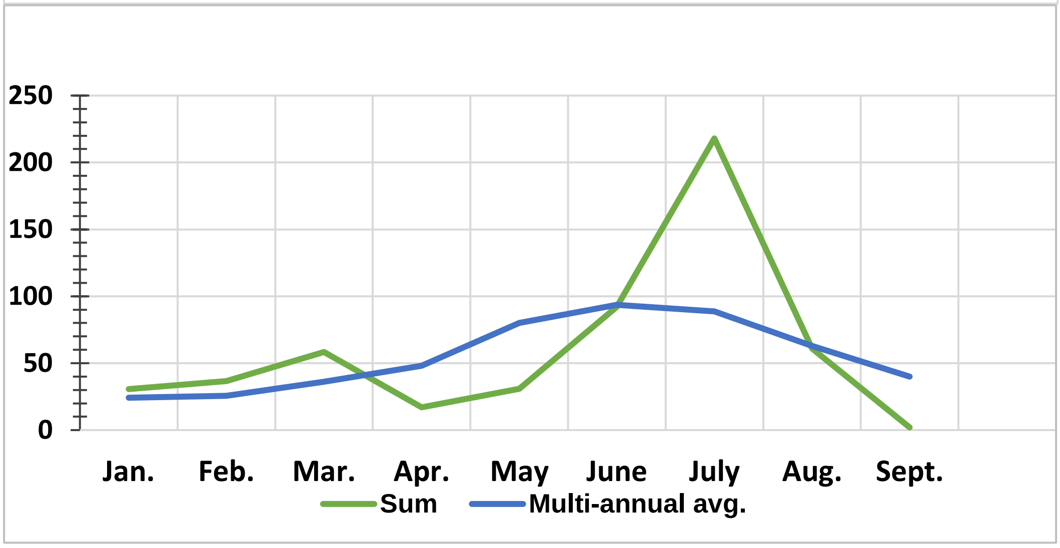


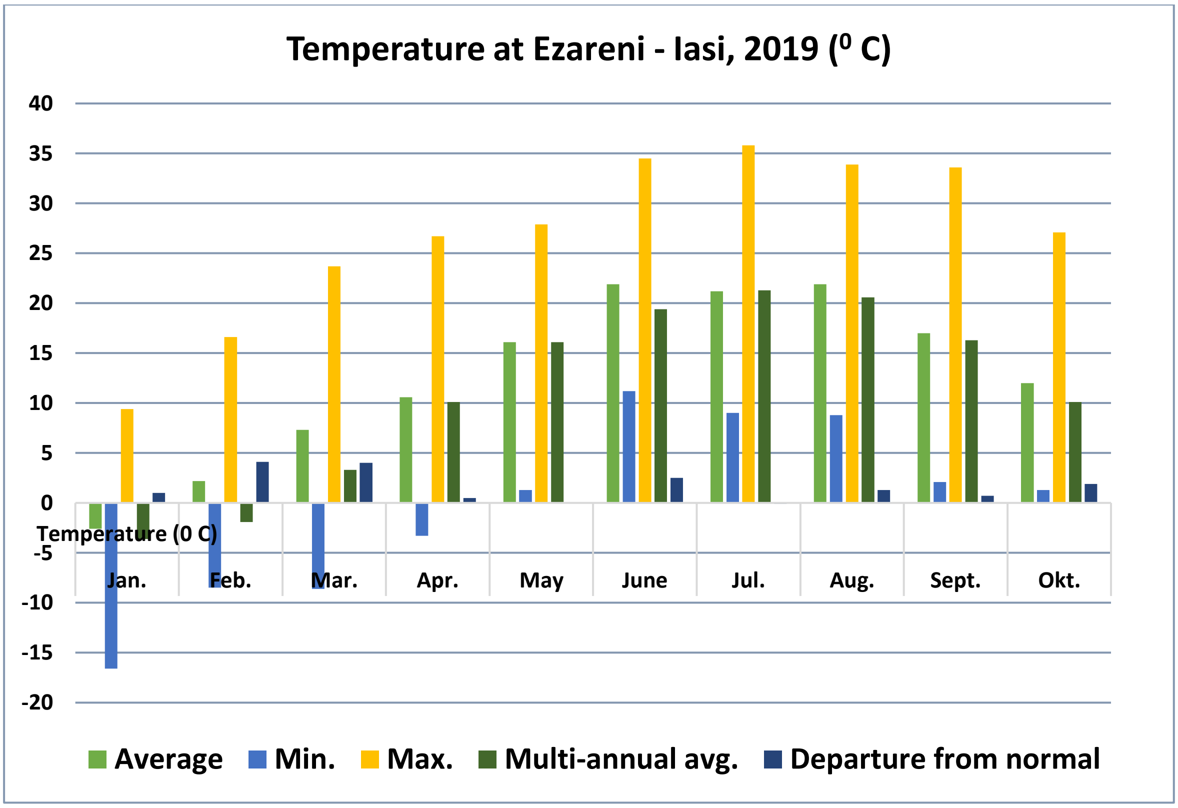
Figure 134 and 135. Temperature and rainfall in Suceava 2018.

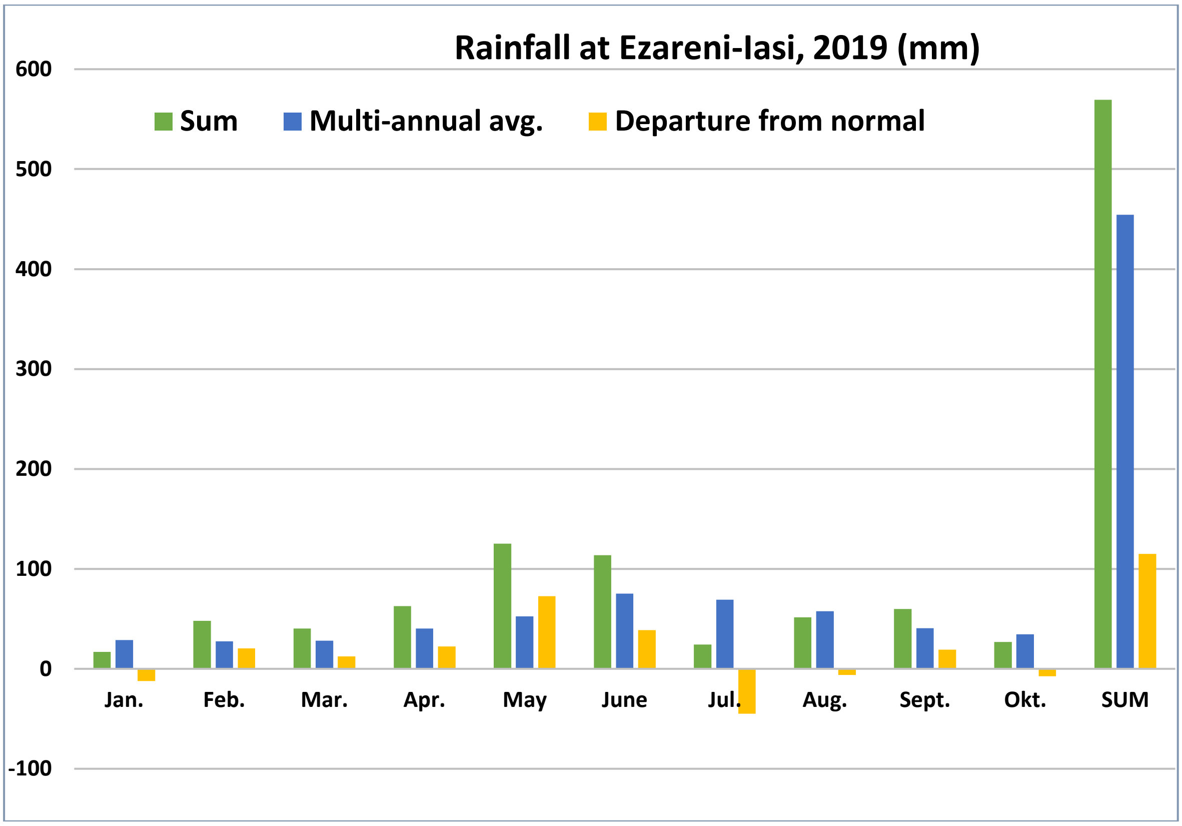
Table 166. Climatic conditions at Secieni 2018.

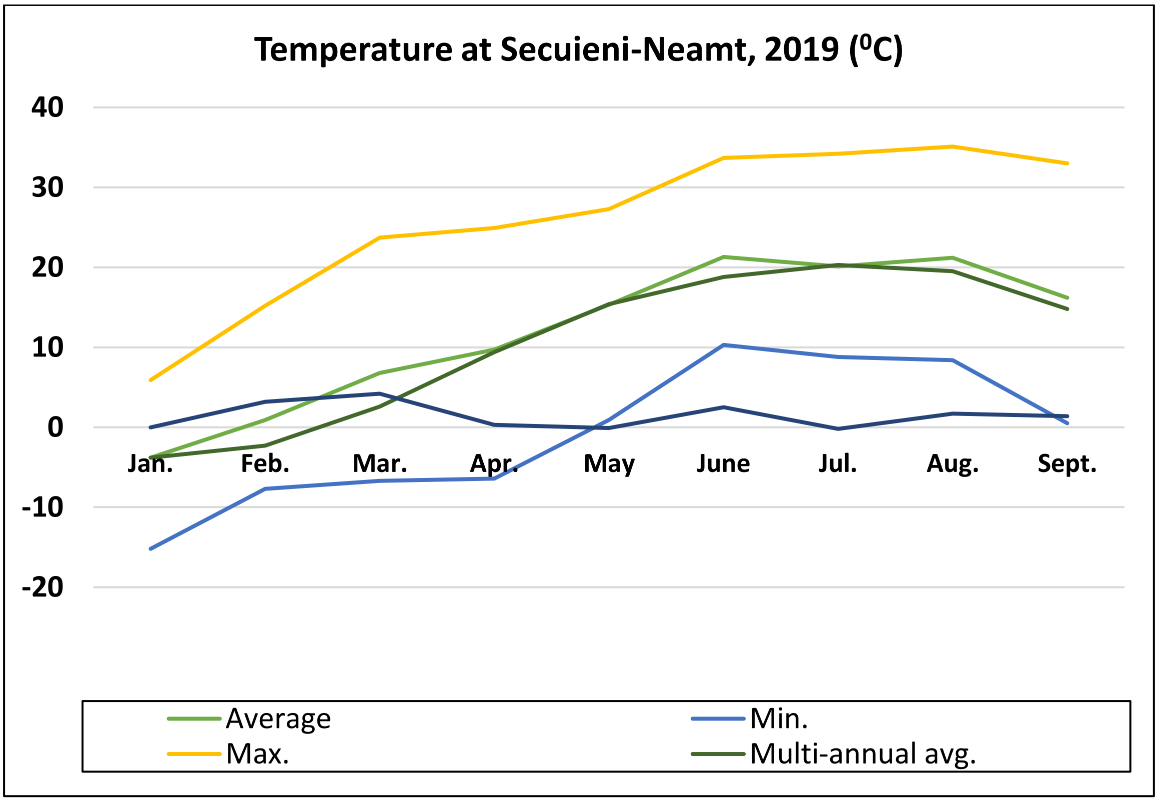
Secuieni - NT	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**June**	**July**	**Aug**	**Sept.**
	Temperature (^{0}C)								
Average	-1,8	-2,3	0,1	14,3	17,8	20,0	20,3	21,5	18,4
Min.	-	-	-17,6	-0,1	4,5	7,0	7,7	10,5	7,4
Max.	10,6	7,7	17,8	28,4	30,5	31,6	29,7	30,8	29,9
Multi-annual avg.	-3,8	-2,3	2,6	9,4	15,4	18,8	20,3	19,5	14,8
Departure from	2	0	-2,5	4,9	2,4	1,2	0,0	2,0	3,6

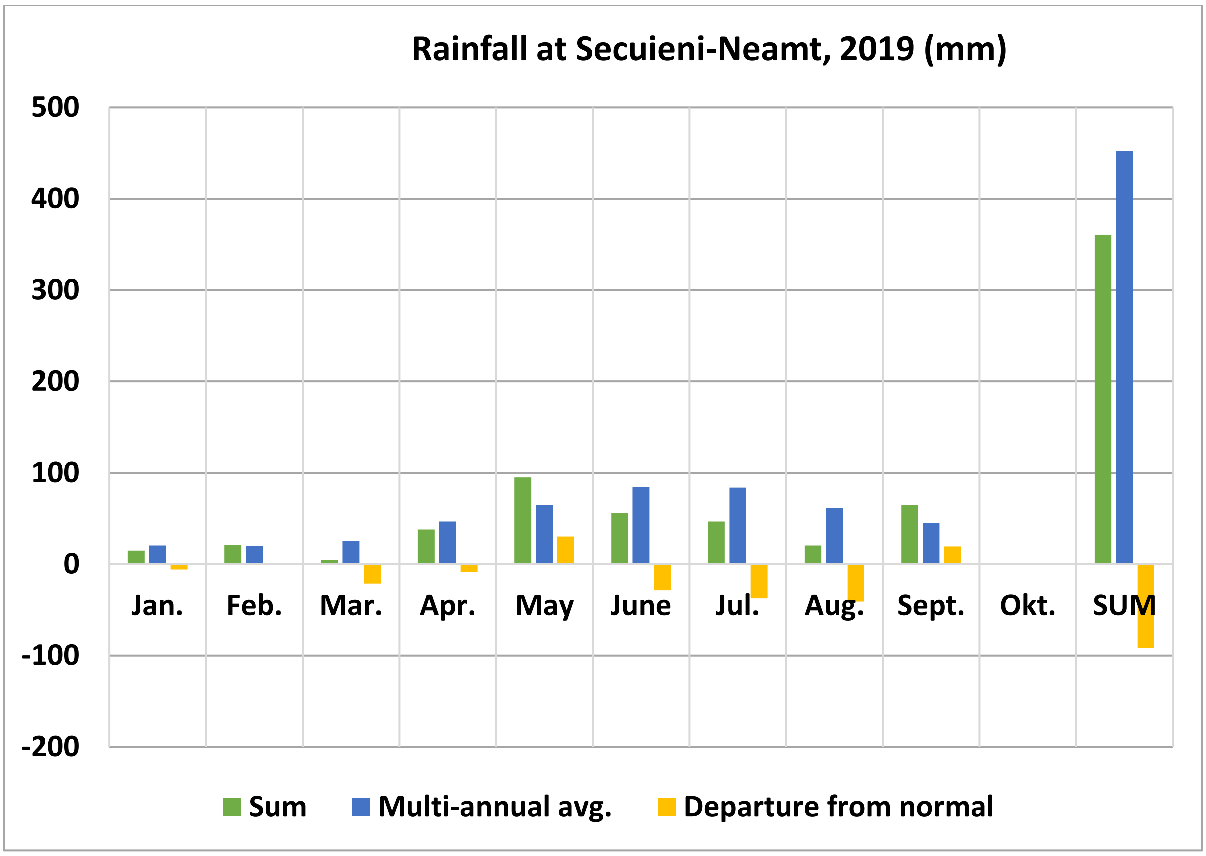
	Precipitation (mm)								
Sum	17,2	26,8	52,6	14,8	23,4	140,4	137,8	4,0	10,6
Multi-annual avg.	20,5	19,6	25,4	46,8	64,8	84,3	84	61,4	45,4
Departure from normal	-3,3	7,2	27,2	-32	- 41,4	56,1	53,8	- 57,4	-34,8
	Relative humidity (%)								
Average	97	96	94	76	74	85	91	84	83

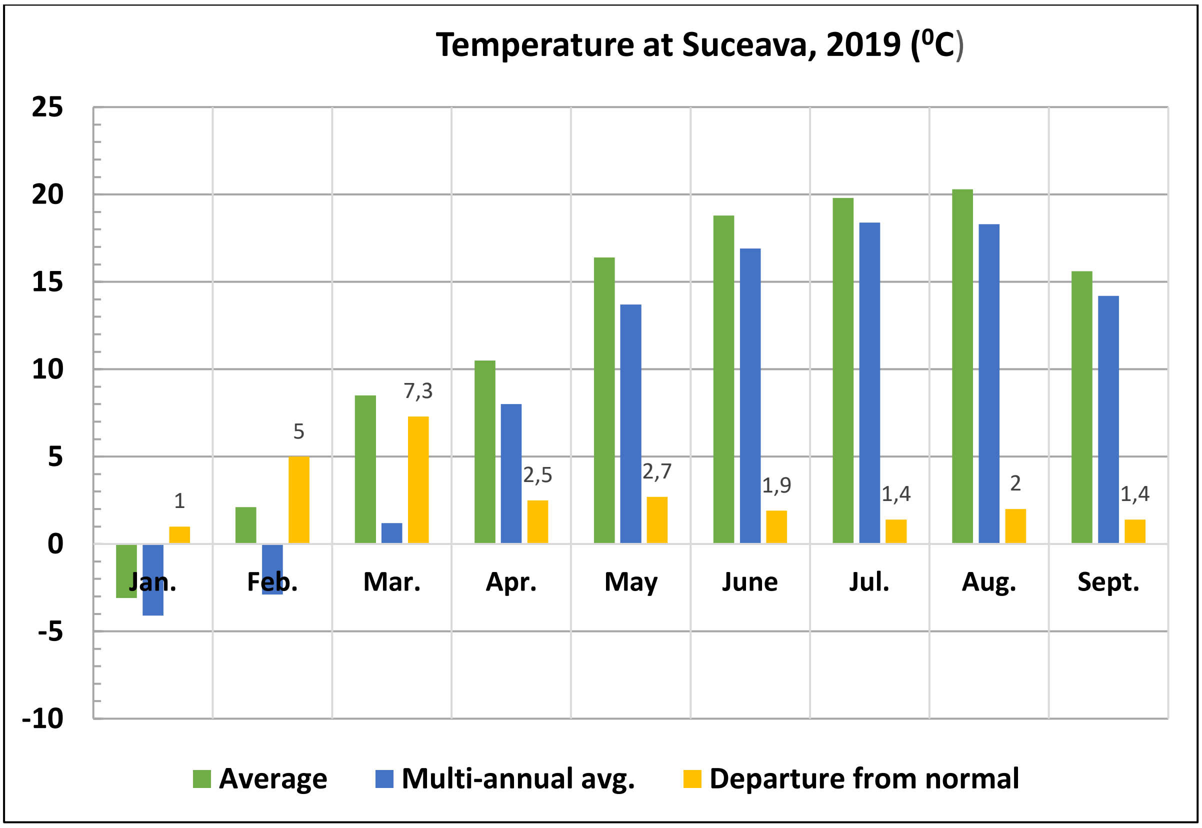
For the both places (Secuieni and Suceava) the general presentation of the climate conditions made for Iasi are the same (hot and dry April and May 2018, wet and very wet in June and July for Secuieni and only July for Suceava). Comparing with Iasi, the conditions for *Andean lupin*plants were a little bit less harsh.




Rainfall at Ezareni-Iasi, 2019 (mm)
Sum
Multi-annual avg.
Departure from normal
600
500
400
300
200
100
0
-100
Jan.
Feb.
Mar.
Apr.
May
June
Jul.
Aug.
Sept.
Okt.
SUM


Temperature at Secuieni-Neamt, 2019 (⁰C)
40
30
20
10
0
-10
-20
Jan.
Feb.
Mar.
Apr.
May
June
Jul.
Aug.
Sept.
Average
Min.
Max.
Multi-annual avg.


Rainfall at Secuieni-Neamt, 2019 (mm)
500
400
300
200
100
0
-100
-200
Jan.
Feb.
Mar.
Apr.
May
June
Jul.
Aug.
Sept.
Okt.
SUM
Sum
Multi-annual avg.
Departure from normal


Temperature at Suceava, 2019 (0C)
25
20
15
10
5
0
-5
-10
1
5
7,3
2,5
2,7
1,9
1,4
2
1,4
Jan.
Feb.
Mar.
Apr.
May
June
Jul.
Aug.
Sept.
Average
Multi-annual avg.
Departure from normal

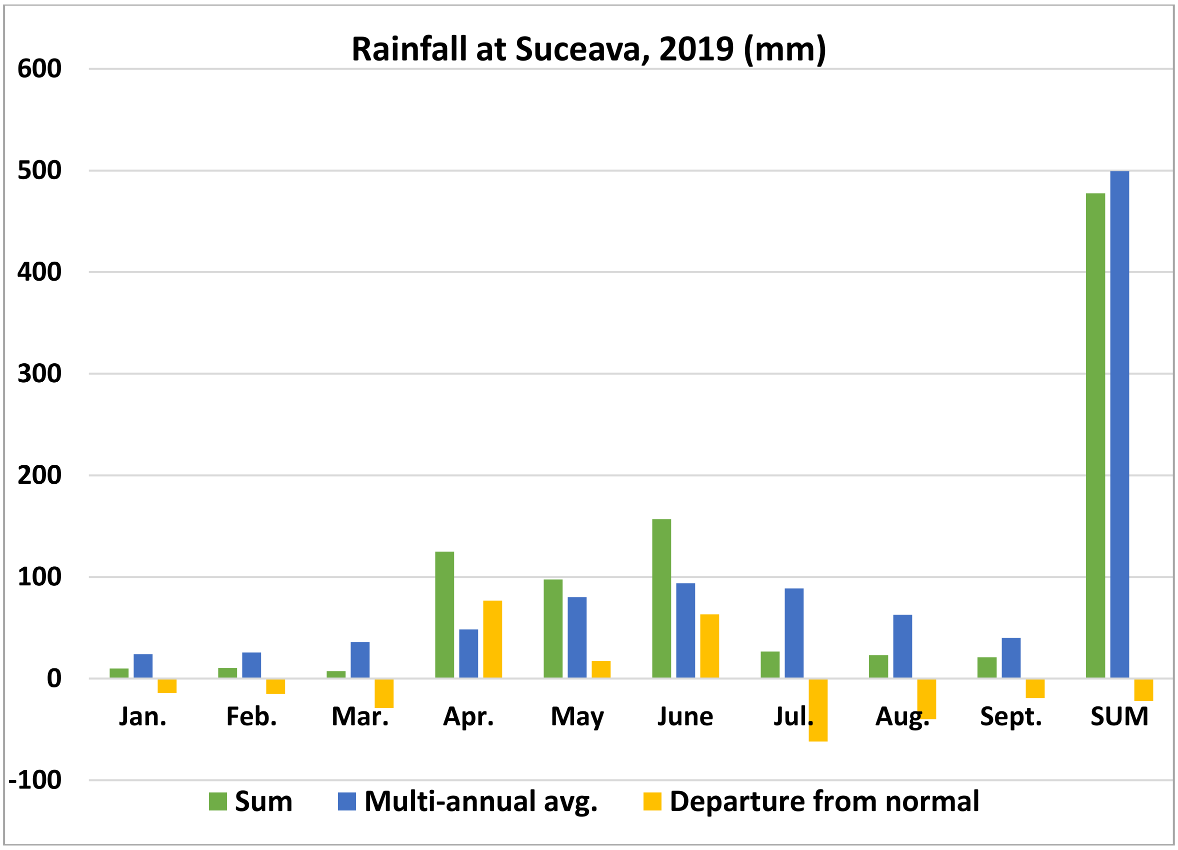


Figure 136-141. Temperatures and rainfalls in Secuieni 2018 versus the multi-annual averages.

A.III The Netherlands, climate data overview

The climate data presented is provided by the Royal Meteorological institute (KNMI) and represent the daily average temperatures recorded by fully automated weather stations.

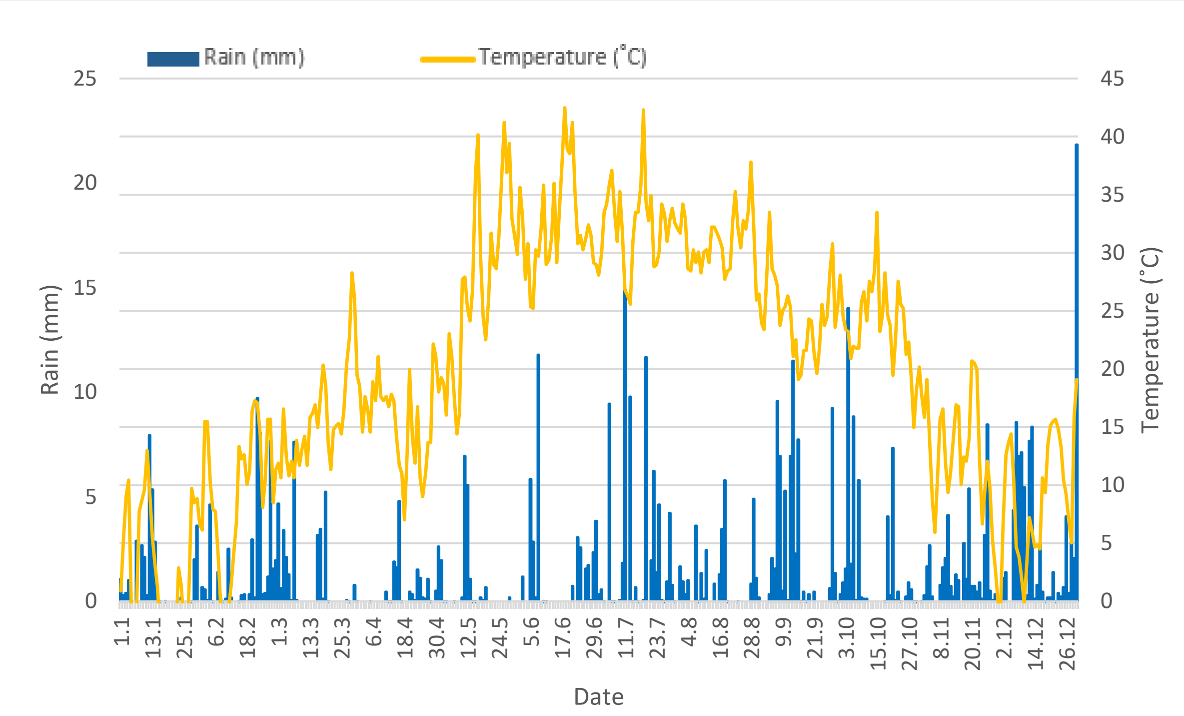


Figure 142. The daily average rainfall and temperature at the De Bilt weather station in 2017. De Bilt is the nearest the main test site in Driebergen.

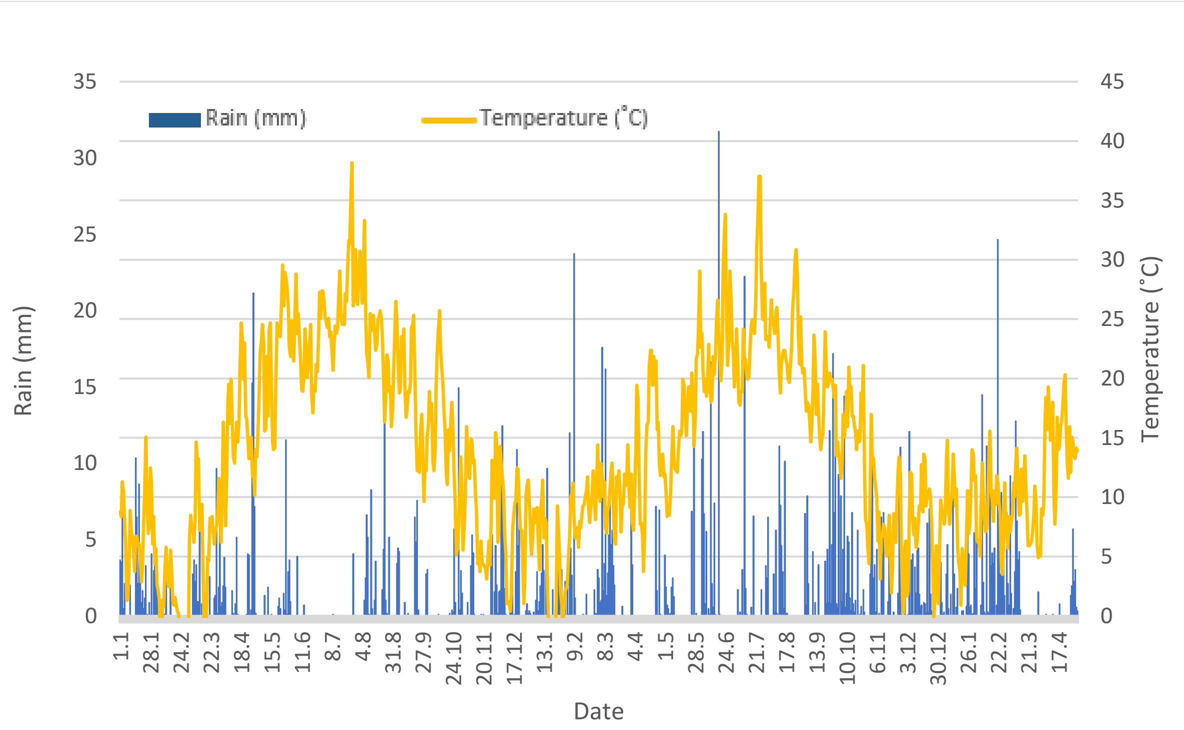


Figure 143. The daily average rainfall and temperature at the De Bilt weather station in 2018. De Bilt is the nearest the main test site in Driebergen.

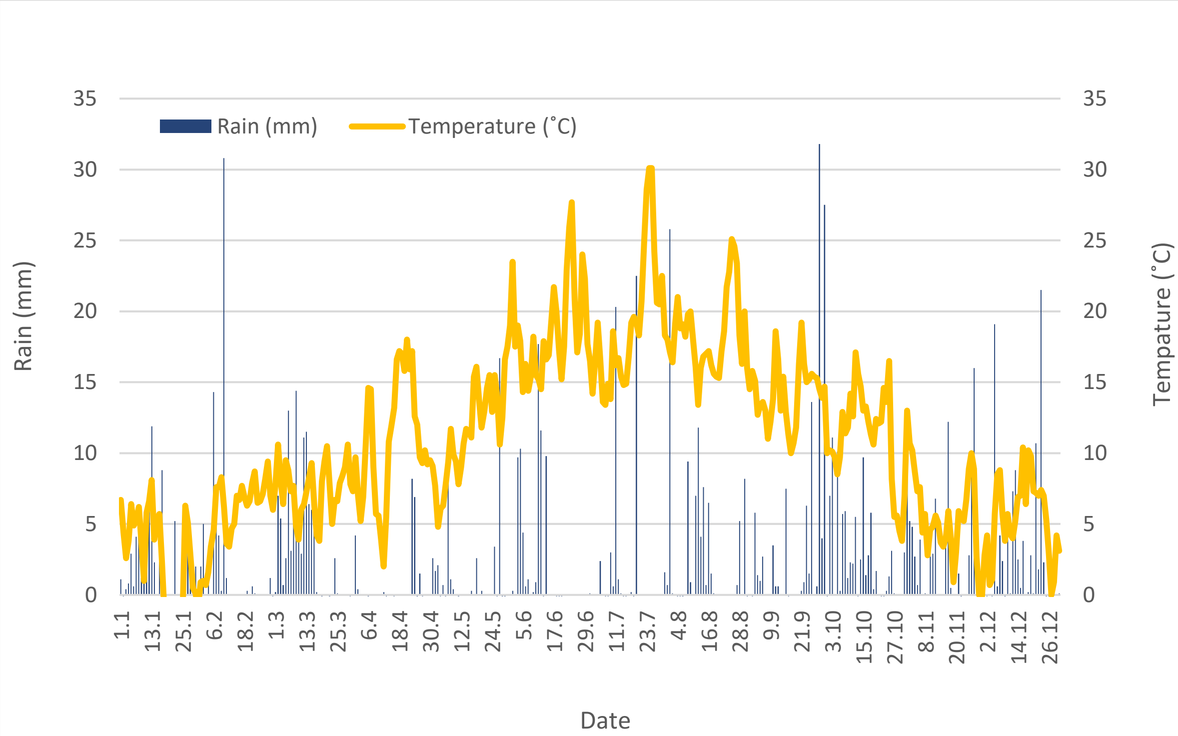


Figure 144. The daily average rainfall and temperature at the Deelen weather station in 2019. Deelen is the station nearest the main test site in Wageningen.

A.IV.I Greece Agro-morphological traits

Table 167. Agro- morphological traits recorded in the Portuguese collection during 2016-2017.

Traits	Classes	Remarks	Growth stage
Growth habit	1 = herb 2 = shrub		vegetative
Plant habit	1 = erect 2 = semi-erect 3 = prostrate		vegetative
Stem formation	0 = main stem not prominent 1 = main stem prominent		vegetative
Stem pubescence	0 = glabrous 1 = pubescent		vegetative
Stem color	1 = yellow 2 = green 3 = grey		vegetative
Intensity of stem color	3 = pale 5 = medium 7 = dark		vegetative

Stem waxiness	0 = absent 1 = present		vegetative
Stem thickness (cm)	1 = small 2 = medium 3 = large	1 = 5,00-5,26 2 = 5,27-5,53 3 = 5,54-5,80	vegetative
Branching	0 = unbranched 1 = branched		vegetative
Primary branch (number of primary branches)	(0-5 branches)	(0-5 branches)	vegetative
Diameter of leaf (cm)	1 = small 2 = medium 3 = large	1 = 5,00-7,33 2 = 7,34-9,67 3 = 9,68-12,01	vegetative
Leaflet shape	1 = elliptical 2 = widest toward the extreme 3 = other		vegetative
Central leaflet tip	1 = not acuminate 2 = acuminate		vegetative
Pubescence of leaflet upper surface	0 = absent 1 = present		vegetative
Pubescence of leaflet lower surface	0 = absent 1 = present		vegetative
Leaflet number per plant	1 = small 2 = medium 3 = large	1 = 88,00-333,33 2 = 333,34-578,67 3 = 578,68-824,01	vegetative
Leaf color	1 = yellow 2 = green 3 = grey		vegetative
Intensity of leaf color	3 = pale 5 = medium 7 = dark		vegetative
Petiole length (cm)	1 = small 2 = medium 3 = large	1 = 2,00-3,66 2 = 3,67-5,33 3 = 5,34–7,00	vegetative
Petiole color	1 = yellow 2 = green 3 = grey		vegetative
Intensity of petiole color	3 = pale 5 = medium 7 = dark		vegetative
Flower wing color	1 = white 2 = yellow 3 = orange 4 = rose 5 = red 6 = green 7 = blue 8 = violet	Just before opening	reproductive

	9 = brown		
Intensity of flower wing color	3 = pale 5 = medium 7 = dark	just before opening	reproductive
Flower keel color	1 = white 2 = yellow 3 = orange 4 = rose 5 = red 6 = green 7 = blue 8 = violet 9 = brown	just before opening	reproductive
Intensity of flower keel color	3 = pale 5 = medium 7 = dark	just before opening	reproductive
Marginal band color of standard petal	0 = marginal band absent, 1 = white, 2 = yellow, 3 = orange, 4 = rose, 5 = red, 6 = green, 7 = blue, 8 = violet, 9 = brown	just opened flower	reproductive
Color of central spots of standard petal	0 = central spots absent, 1 = white, 2 = yellow, 3 = orange, 4 = rose, 5 = red, 6 = green, 7 = blue, 8 = violet, 9 = brown	just opened flower	reproductive
Intensity of color of central spots of standard	3 = pale 5 = medium 7 = dark	just opened flower	reproductive
Intermediate region color of standard	0 = intermediate region absent, 1 = white, 2 = yellow, 3 = orange, 4 = rose, 5 = red, 6 = green, 7 = blue, 8 = violet, 9 = brown	just opened flower	reproductive
Intensity of intermediate region color of standard	3 = pale 5 = medium 7 = dark	just opened flower	reproductive
Length of principal inflorescence (cm)	1 = small 2 = medium 3 = large	1 = 0,00-9,66 2 = 9,67-19,33 3 = 19,34-29,00	reproductive

Pod number per plant	1 = small 2 = medium 3 = large	1 = 0-6 2 = 7-13 3 = 14-17	pod
Pod length (cm)	1 = small 2 = medium 3 = large	1 = 1,5-4,2 2 = 4,3-7,0 3 = 7,1-9,8	pod
Pod width (cm)	1 = small 2 = medium 3 = large	1 = 0,50-1,06 2 = 1,07-1,63 3 = 1,64-2,20	pod
Mature pod pubescence	0 = absent 3 = light 5 = medium 7 = heavy		pod
Pod shattering	0 = non shattering 3 = slight shattering 5 = moderate shattering 7 = complete shattering		pod
Pod shedding	0 = non-shedding 3 = slight shedding 5 = moderate shedding 7 = complete shedding		pod
Seed shape	1 = spherical 2 = flattened spherical 3 = oval 4 = flattened oval 5 = cuboid 6 = flattened cuboid 7 = other		seed
Seed primary color	1 = white 2 = yellow 3 = orange 4 = rose 5 = red 6 = green 7 = blue 8 = violet 9 = brown		seed
Seed secondary color	0 = no secondary color 1 = white 2 = yellow 3 = orange 4 = rose 5 = red 6 = green 7 = blue 8 = violet		seed

	9 = brown		
Seed secondary color distribution	1 = crescent 2 = eyebrow 3 = back 4 = spotted 5 = moustache 6 = marbled 7 = marbled crescent 8 = marbled eyebrow 9 = other		seed
Hypocotyl color	1 = yellow 2 = green 3 = grey 4 = red		vegetative
Days till emergence		number of days from sowing to 50% emergence	vegetative
Days to first flowering	1 = few 2 = medium 3 = many	1 = 75-96 2 = 97-118 3 = 119-140	vegetative
Growth rate	3 = slow 5 = normal 7 = rapid		vegetative
Seedling growth type	1 = rossette 9 = erect		vegetative
Plant height (cm)	1 = short 2 = medium 3 = tall	1 = 0-31 2 = 32-63 3 = 64-95	reproductive
Height of flowering in every order of flowering (from soil to lowest flower on fully developed flowering)- in main inflorescence (cm)	1 = short 2 = medium 3 = tall	1 = 0,00-25,66 2 = 25,67-51,33 3 = 51,34-77,00	vegetative
Height of flowering in every order of flowering (from soil to lowest flower on fully developed flowering)-1st order of flowering	1 = short 2 = medium 3 = tall	1 = 11,00-33,33 2 = 33,34-55,67 3 = 55,68-78,01	vegetative
Seeds number per pod (in one primary pod per plant)	(0-7 seeds)	(0-7 seeds)	after harvesting

Number of pods in main inflorescence	1 = few 2 = medium 3 = many	1 = 0-6 2 = 7-13 3 = 14-20	after harvesting
Number of pods in first order of pod setting	1 = few 2 = medium 3 = many	1 = 1-2 2 = 3-4 3 = 5-6	after harvesting
Number of pods in second order of pod setting	1 = few 2 = medium 3 = many	1 = 1-2 2 = 3-4 3 = 5-6	after harvesting
Number of seeds in main inflorescence	1 = few 2 = medium 3 = many	1 = 0-11 2 = 12-23 3 = 24-35	after harvesting
Number of seeds in first order of pod setting	1 = few 2 = medium 3 = many	1 = 1-2 2 = 3-4 3 = 5-6	after harvesting
Number of seeds in second order of pod setting	1 = few 2 = medium 3 = many	1 = 1-2 2 = 3-4 3 = 5-6	after harvesting
Number of harvested healthy seeds per plant	1 = few 2 = medium 3 = many	1 = 0–11 2 = 12-23 3 = 24-35	after harvesting
Total plant fresh weight (g) (including pods)	1 = small 2 = medium 3 = large	1 = 0,49-8,15 2 = 8,16-15,82 3 = 15,83-23,49	after harvesting
Stem fresh weight (g) (without pods)	1 = small 2 = medium 3 = large	1 = 0,34-7,64 2 = 7,65-14,95 3 = 14,96-22,26	after harvesting
Stem dry weight (g) (without pods)	1 = small 2 = medium 3 = large	1 = 0,32-6,35 2 = 6,36-12,39 3 = 12,40-18,43	after harvesting
Root fresh weight (g)	1 = small 2 = medium 3 = large	1 = 0,070-1,673 2 = 1,674-3,277 3 = 3,278-4,881	after harvesting
Root dry weight (g)	1 = small 2 = medium 3 = large	1 = 0,060-1,536 2 = 1,537-3,013 3 = 3,014-4,490	after harvesting
Seed weight per plant (g)	1 = small 2 = medium 3 = large	1 = 0,000-1,763 2 = 1,764-3,527 3 = 3,528-5,291	after harvesting
Hundred seed weight (g)	1 = small 2 = medium 3 = large	1 = 0-38 2 = 39-77 3 = 78-116	after harvesting

A.IV.II Greece Supplementry tables

Table 168. Plant loss after snow cover in plants grown with direct sowing (frost after cotyledon stage).

Accession	Mean %
LIB220	55,8 b
LIB221	28,5 a
LIB222	30,3 a
Branco	30,8 a
Significance	***

Means between accessions were separated by Tukey-Kramer (HSD) test at a significance level of α = 0,05. Within each column the means followed by the same letter do not show statistically significant differences

Table 169. Plant loss after snow cover was in transplanted plants (frost after three leaf stage).

Accession	Mean %
cv Multitalia	3,5 a
cv Polo	19,0 ab
LIB220	25,5 b
LIB221	20,8 ab
LIB222	16,8 ab
Significance	*

Means between accessions were separated by Tukey-Kramer (HSD) test at a significance level of α = 0,05. Within each column the means followed by the same letter do not show statistically significant differences

A.V Portugal field locations

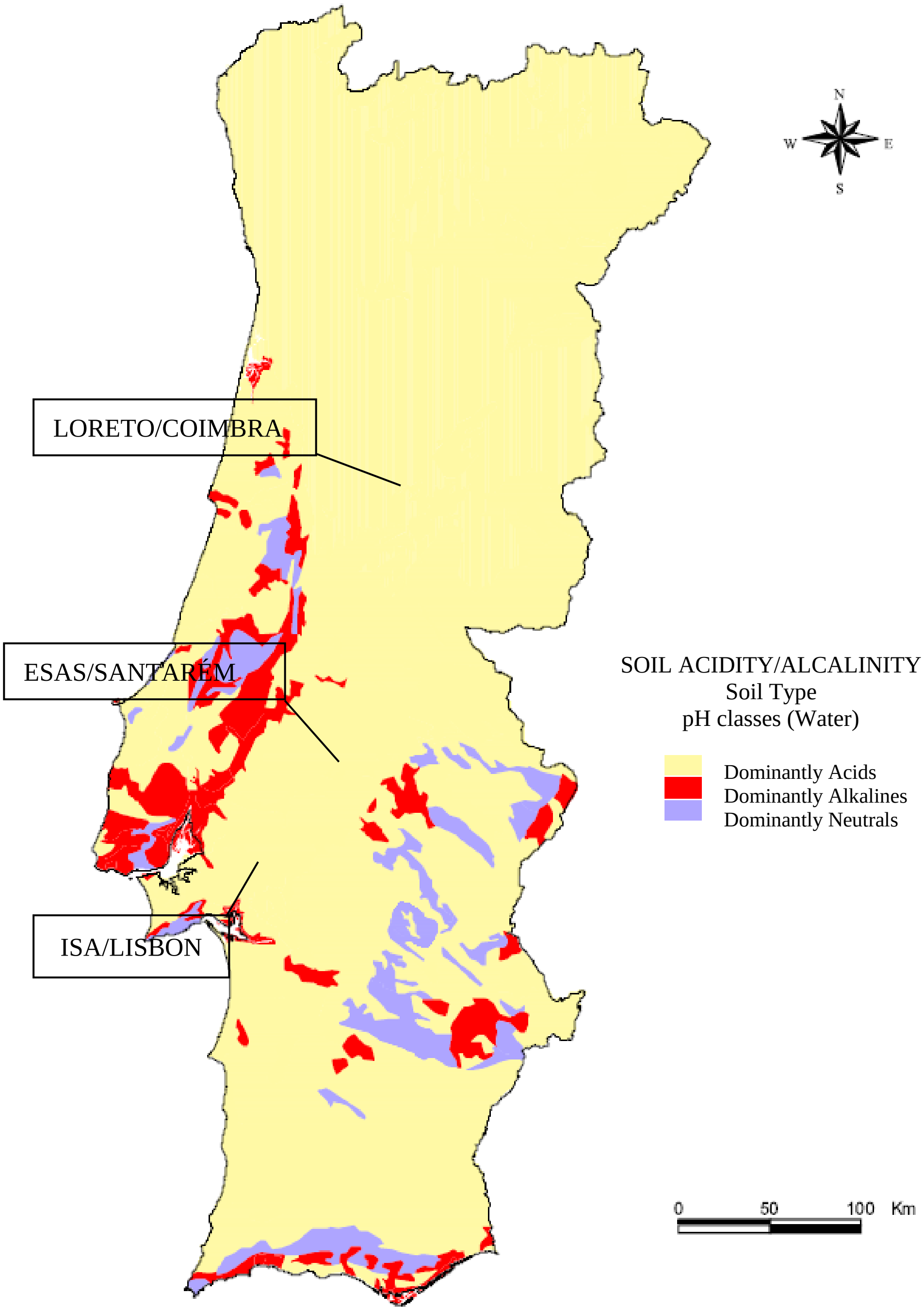


Figure 170. Map of Portuguese type of Soils, pH classes (Adapted from Atlas do Ambiente Digital).

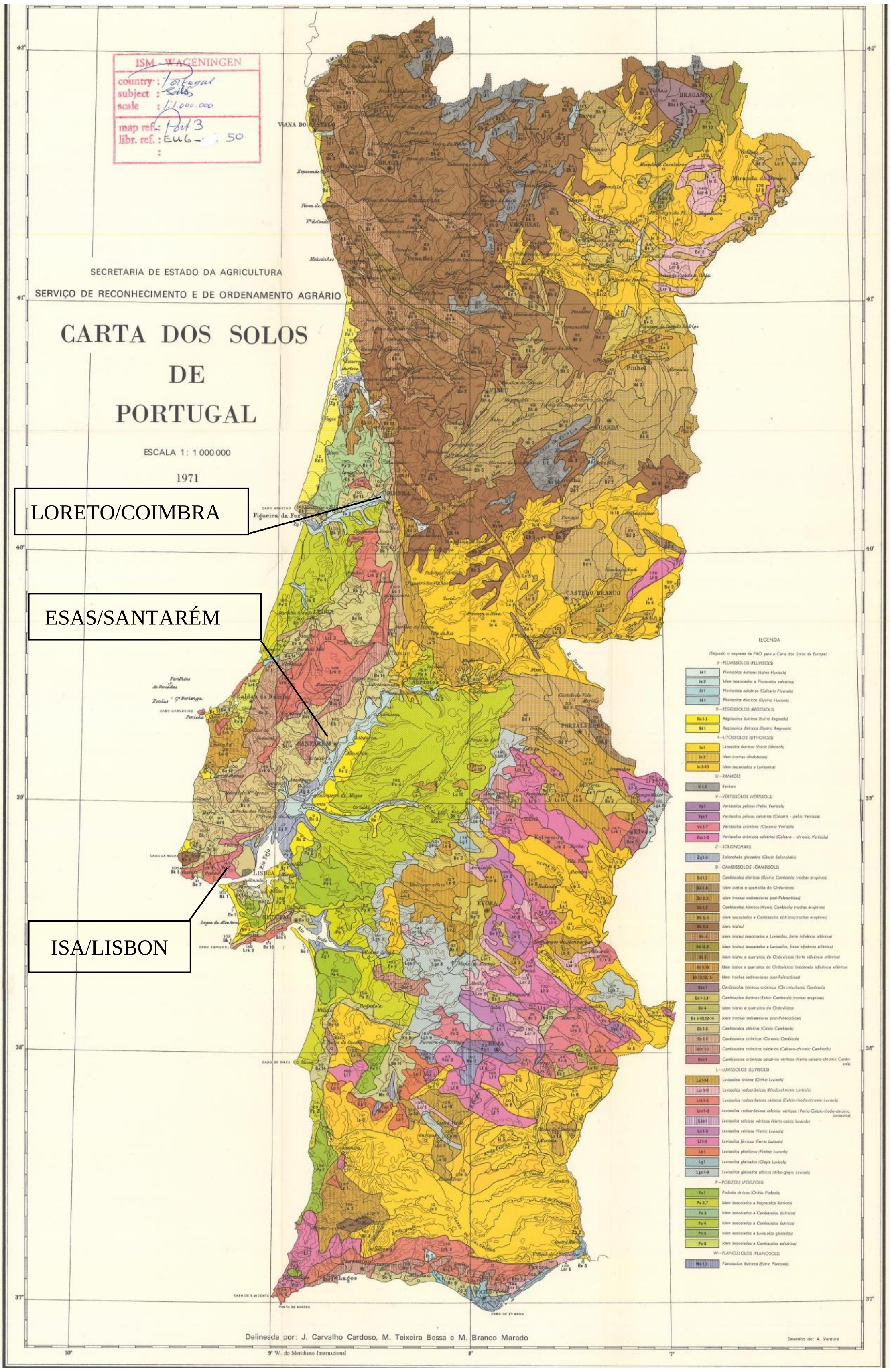


Figure 171. Map of Portuguese Soils - FAO classes (Adapted from ESDAC – European Soil Data Centre.

A.VI Spanish accessions

Lupinus mutabilis germplasm available by Spain
Land races: accession Branco

Table 172. Vandinter Semo Accessions.

LIB219
LIB220
LB221
LIB221

Table 173. Germplasm collection from the Spainish Centro de Recursos Fitogenéticos.

BGE 009695
BGE 009790
BGE 010271
BGE 010272
BGE 010273
BGE 010274
BGE 010275
BGE 010276
BGE 010277
BGE 010278
BGE 010279
BGE 010280
BGE 010281
BGE 010282
BGE 010283
BGE 010284
BGE 010285
BGE 010286
BGE 010287
BGE 010288

Table 174. Germplasm collection from the N.I.Vavilov Institute of Plant Genetic Resources (VIR).

1, nam1472, Eo915	6O R2 C16-17	20-nam 2907 Eo915
1, nam1472, Eo915	6O R2 C16-17	6O R2 C16-17
6O R2 C16-17	11-nam 2280 Eo915	6O R2 C16-17
6O R2 C16-17	11-nam 2280 Eo915	6O R2 C16-17
6O R2 C16-17	6O R2 C16-17	21-nam 2908 Eo915
2- nam 1566 Eo915	6O R2 C16-17	21-nam 2908 Eo915
2- nam 1566 Eo915	6O R2 C16-17	6O R2 C16-17
6O R2 C16-17	12-nam 2343 Eo915	6O R2 C16-17

6O R2 C16-17	12-nam 2343 Eo915	6O R2 C16-17
6O R2 C16-17	6O R2 C16-17	22-nam 3732 Eo915
3-nam 1624 Eo915	6O R2 C16-17	22-nam 3732 Eo915
3-nam 1624 Eo915	6O R2 C16-17	6O R2 C16-17
6O R2 C16-17	13-nam 2348 Eo915	6O R2 C16-17
6O R2 C16-17	13-nam 2348 Eo915	6O R2 C16-17
6O R2 C16-17	6O R2 C16-17	23-nam 3733 Eo915
4-nam 1914 Eo914	6O R2 C16-17	23-nam 3733 Eo915
4-nam 1914 Eo914	6O R2 C16-17	6O R2 C16-17
6O R2 C16-17	14-nam 2349 Eo915	6O R2 C16-17
6O R2 C16-17	14-nam 2349 Eo915	6O R2 C16-17
6O R2 C16-17	6O R2 C16-17	24-nam 3911 Eoe 14
5-nam 1918, Eoe15	6O R2 C16-17	24-nam 3911 Eoe 14
5-nam 1918, Eoe15	6O R2 C16-17	6O R2 C16-17
6O R2 C16-17	15-nam 2451 Eo915	6O R2 C16-17
6O R2 C16-17	15-nam 2451 Eo915	6O R2 C16-17
6O R2 C16-17	6O R2 C16-17	25-nam 3912 Eoe 14
6-nam 2051 Eo915	6O R2 C16-17	25-nam 3912 Eoe 14
6-nam 2051 Eo915	6O R2 C16-17	6O R2 C16-17
6O R2 C16-17	16-nam 2781 Eo915	6O R2 C16-17
6O R2 C16-17	16-nam 2781 Eo915	6O R2 C16-17
6O R2 C16-17	6O R2 C16-17	
7-nam 2137 Eo 914	6O R2 C16-17	
7-nam 2137 Eo 914	6O R2 C16-17	
6O R2 C16-17	17-nam 2783 Eo915	
6O R2 C16-17	17-nam 2783 Eo915	
6O R2 C16-17	6O R2 C16-17	
8-nam 2141 Eo915	6O R2 C16-17	
8-nam 2141 Eo915	6O R2 C16-17	
6O R2 C16-17	18-nam 2784 Eo915	
6O R2 C16-17	18-nam 2784 Eo915	
6O R2 C16-17	6O R2 C16-17	
9-nam 2159 Eo915	6O R2 C16-17	
9-nam 2159 Eo915	6O R2 C16-17	
6O R2 C16-17	19-nam 2785 Eo915	
6O R2 C16-17	19-nam 2785 Eo915	
6O R2 C16-17	6O R2 C16-17	
10-nam 2161 Eo915	6O R2 C16-17	
10-nam 2161 Eo915	6O R2 C16-17	
6O R2 C16-17	20-nam 2907 Eo915	

A.VII Material sampled at Trautenfels 2019

Table 175. Material sampled at Trautenfels 2019.

Line	Sampling date	Year	Field	Stems (g)	Leaves (g)	Flowers (g)	Hulls (g)
Branco	22.08.2018	2018	Trautenfels	78,2	27	4,67	
Branco	12.09.2018	2018	Trautenfels	120,21	35,1	3,25	3,17
Branco	19.08.2019	2019	Trautenfels	35,18	16,65	0,94	0,78
Branco	12.09.2019	2019	Trautenfels	50,2	15		10,8
Branco	07.11.2019	2019	Trautenfels	98,72	21,42		17,53

A.VIII Cropping season 4, the Netherlands

The alignment of the WP2 deliverables was chosen to align with the trials sown in autumn in Southern Europe. Hence the main document delivers only cropping season 1, 2 and 3. The results of the spring sown trials of the fourth cropping season in the Netherlands are presented in this appendix.

A.VIII.I Materials & Methods

In this season two trials were performed. A larger scale trial in Kraggenburg, on an arable organic farm with fertile clay soils. And a small-scale trial on Noordhout, near Utrecht. A site located in the Utrechtse Heuvelrug national park characterised by shallow, poor sandy soils. This was the marginal soil test site. Details on the soil conditions can be found in chapter 5 of this deliverable.

A.VIII.I.I Clay soil

In Kraggenburg the largest Andean lupin trial, so far, was performed by the Louis Bolk Institute. For the first time the quantity of seeds that was available allowed for larger, machine sown plots. In this trial both the influence of sowing depth and the pollinator visits to the crop were studied. The trial was sown using a custom build sowing machine, designed to sow small trial fields, owned by the Field Crops unit of Wageningen University & Research, location Lelystad, the Netherlands. The plots were 15m^2 in size and the trials were sown using two or three replicates. The trial was scored regularly. Weeding was performed manually when needed. Netting was installed to deter herbivores. Due to the uniform ripening both Faba bean ‘Fuego’ and white lupin ‘Boros’ could be threshed using the combine harvester that was present on site. The other lines were harvested by hand and later on threshed by a Wintersteiger LD350 stationary thresher, owned by Unifarm, Wageningen University, the Netherlands.

A.VIII.I.I.I Sowing depth

For the sowing depth trial 3 replicates were sown of 4 accessions (Table 176). The standard sowing depth is about 5 cm deep (depending on site specific differences). The minimum sowing depth that can be applied is about 2 cm deep. Like in previous years general traits like emergence and crop performance were scored. The pods were harvested separating them in main florescence and first and second order (if present). To prevent decay the pods were dried at 30˚C for 48 hours. The pods were counted, threshed and the seeds counted using a Pfeuffer Contador Seed Counter. The data was analysed by with an ANOVA $p<0,05$ anova, Bonferroni test. Data analysis was performed in Genstat 19th edition.

Table 176. Sowing depth treatments of Andean lupin accessions and White lupin in a trial at Kraggenburg in the fourth cropping season.

Accession	Depth of sowing
Andean lupin LIB220	2 and 5cm
Andean lupin LIB221	2 and 5cm
Andean lupin LIB222	2 and 5cm
White lupin 'Boros'	2 and 5cm

A.VIII.I.I.II Functional Agrobiodiversity

During the third cropping season the pollinator trail was also located at this location. Besides repeating part of this experiment a research question that remained was the influence of quinolizidine alkaloids on pollinator flower visits.

For this pollinator trial 8 accessions and 5 crops were used (Table 177). Andean Lupin (*Lupinus mutabilis*) 220 and 221 are both accessions which are high in quinolizidine alkaloids, also referred to as bitter accessions since these alkaloids induce a bitter taste to the lupin seeds. Earlier trials have shown that LIB221 ripens early and LIB 220 ripens late. Andean lupin 'Inti' has a much lower level of alkaloids and acts as a "Andean lupin control', since its flower appearance is identical to LIB221. Two white lupin (*Lupinus albus*) varieties were included, 'Mihai' which is fairly bitter and 'Boros' which is a sweet, or low alkaloid variety. White lupin flowers have a much paler colour do not change colour post-anthesis. Faba bean 'Fuego' (*Vicia faba*) was included because it used to be a fairly common legume in the Netherlands. Rapeseed (*Brassica napus*) was a prime pollinator attractant in earlier years so was included again. Camelina (*Camelina sativa*) has been cultivated as an oil seed crop for thousands of years and in the last decades has seen renewed interested due to omega 3 content in the oil (Zubr 2006).

The protocol applied for the insect surveys was identical to the third cropping season. Two rounds of transect walks were performed per plot at the flowering time of the corresponding accession. On the plots, insects were observed per plot within an observation time of ten minutes. Pollinators were only recorded when they interacted with the flower and all pest predators and parasitoids on the plant were recorded. Insects were identified on the wing or taken for identification by an entomologist of Bureau FaunaX, Badweg 40B, 8401 BL Gorredijk, the Netherlands. Data was analysed by comparing total insect numbers per accession with an ANOVA, or when there was no equality of variances, a Kruskal Wallis test with a post hoc Tukey HSD or Dunn test respectively for multiple comparison. Data analysis was performed in R.

Table 177. Pollinator test treatments at Kraggenburg fourth cropping season, two replicates per treatment.

Crop	Accession	Origen
Andean lupin	LIB220	VanDinter Semo, The Netherlands
Andean lupin	LIB221	VanDinter Semo, The Netherlands
Andean lupin	Inti	Univeristy of Lisbon, Portugal
White lupine	Mihai	University of applied Life sciences and Environment, Romania
White lupin	Boros	Agrifirm, the Netherlands
Faba bean	Fuego	Wiersum Plant Breeding, the Netherlands
Rapeseed	Helga	Vreekens Zaden, the Netherlands
Camelina (False Flax)	Zuzana	Vreekens Zaden, the Netherlands

A.VIII.I.II Marginal soil

At Noordhout, to study drought tolerance and agrobiodiversity three replicates were sown. All sowing was performed by hand. In this trial Andean lupin, blue and white lupin, camelina, rapeseed and buckwheat were included (Table 178). The plot size was 4,5m^2. The sowing depth for the lupins was 5cm, which is standard for lupin in the Netherlands. The camelina, rapeseed and buckwheat were sown at about 2 cm deep. To prevent damage from hares and rabbits netting was installed. To prevent herbivory by deer, which caused damage during the previous season, this season only bitter lupin lines were used. Throughout the season general traits were scored like emergence and crop height. The harvest was performed by hand and the threshing was done using a stationary thresher, Wintersteiger LD350, at Wageningen at Unifarm, Wageningen university.

Table 178. Test treatments in the fourth cropping season, Noordhout 2020.

Crop	Accession	Origen
Andean lupin	LIB220	VanDinter Semo, The Netherlands
Andean lupin	LIB221	VanDinter Semo, The Netherlands
Andean lupin	LIB222	VanDinter Semo, The Netherlands
White lupin	Mihai	University of applied Life sciences and Environment, Romania
Blue lupin	Azuro	Vreekens Zaden, the Netherlands
Buckwheat	Lileja	Vreekens Zaden, the Netherlands
Rapeseed	Helga	Vreekens Zaden, the Netherlands
Camelina	Zuzana	Vreekens Zaden, the Netherlands

A.VIII.I.II.I Functional Agrobiodiversity

Like in previous years general traits like emergence and crop performance were scored. The pods were harvested separating them in main florescence and first and second order (if present). To prevent decay the pods were dried at 30˚C for 48 hours. The pods were counted, threshed and the seeds counted using a Pfeuffer Contador Seed Counter. The data was analysed by with an ANOVA $p<0,05$ anova, Bonferroni test. Data analysis was performed in Genstat 19th edition.

A.VIII.I.II.II Drought tolerance

The Noordhout test site is a sandy soil with a low water holding capacity. This site was therefore suitable to test drought tolerance. To assess drought tolerance the lines were scored visually using a 1-5 scale. 1 = drought sensitive, 5= no sign of drought stress. Drought sensitive lines show wilting of the leaves, flower and/or pod abortion or overall decay. In the Netherlands August is usually a dry warm month so assessment was done in August.

A.VIII.I.II.III Functional Agrobiodiversity

For the biodiversity assessment at the marginal site in Noordhout was included. Due to issues with herbivors in previous years only bitter lupin varieties were used. Also included was ‘Azuro’ a bitter narrow leaved or blue lupin. Rapeseed and camelina were included as well. Buckwheat is often mentioned to be an attractant to pollinators (Cambell et al. 2016) and it is known to grow well on poor soils. The protocol applied for the insect surveys was identical to the one used in the previous season and identical to the Kraggenburg protocol. Two rounds of transect walks were performed per plot at the flowering time of the corresponding crop (accession). On the plots, insects were observed per plot within an observation time of ten minutes. Pollinators were only noted when they interacted with the flower and all pest predators and parasitoids on the plant were noted. Insects were identified on loaction or taken for identification by a researcher of the Louis Bolk Institute. Data was analysed by comparing total insect numbers per accession with an ANOVA, or when there was no equality of variances, a Kruskal Wallis test with a post hoc Tukey HSD or Dunn test respectively for multiple comparison. Data analysis was performed in R.

A.VIII.II Results

A.VIII.II.I Clay soil

In this final year only basic features were assessed like height and yield. In earlier seasons a more extensive phenotyping was performed.

A.VIII.II.I.I Sowing depth trial

Initially, 27 days after sowing, the emergence of the lupin sown at 5 cm deep was higher than sown at 2 cm deep. However at 47 days after sowing the emergence was no longer different between the various treatments (figures 145A and 145B). The likely explanation for this behaviour is that soil moisture availability in the top 2 cm was not sufficient at the start of the growing season to trigger all the viable seeds to germinate. Rainfall later on in the season made up for this deficit and finally triggered the germination of nearly all of the viable seeds in both sowing depths, resulting in comparable emergence rates after 47 days. This resulted in an overall emergence of 43 to 58%, which is acceptable for Andean lupin, but still quite low for commercial seeds. The unwanted side effect of delayed germination in crops however is that you get a large variety in crop development stages simultaneously present in the field. This makes timing of crop management measures like harrowing or hoeing more difficult. Uniformity of emergence is therefor always preferable, which seems to be increased by using a bigger sowing depth. Still, in the end for all lines a decent crop density was obtained.

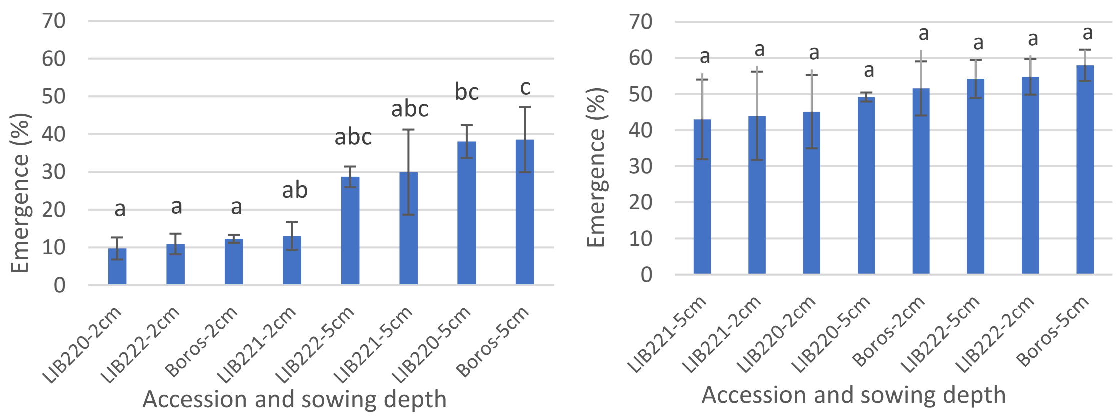


Figure 145. A (left) emergence at 27 days after sowing, B (right) emergence at 47 days after sowing. Error bars indicate standard deviation.

After 164 days after sowing the height of the crop was measured for the final time (Figure 146). The white lupin variety Boros performed very poorly this year. The Andean lupin performed well but only the height of LIB221 sown at 5 cm deep was significantly different from LIB220 at 2 cm deep. But previous seasons showed that LIB221 is general shorter that LIB220 because it ripens earlier, therefore completing its vegetative growth earlier as well.

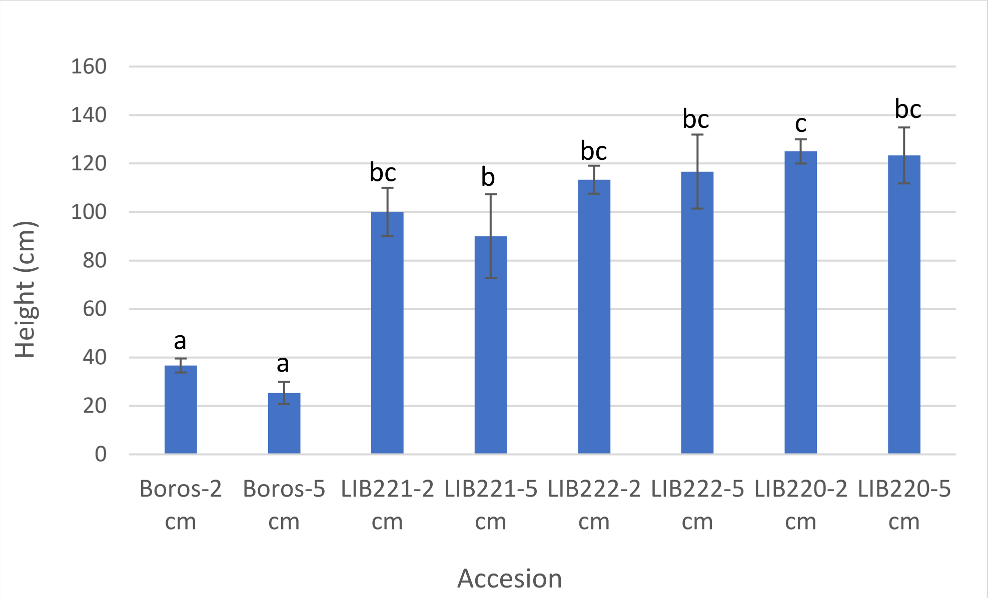


Figure 146. The height of the lupin crop at the final canopy height measurement at 164DAS.
Error bars indicate standard deviation.

When assessing the yield of the treatments (Figure 147) no significant differences were detected. This means that on the overall production the depth of sowing had no influence. As a conclusion we recommend that on clay soil in the Netherlands a sowing depth of 5cm is recommendable. As results have shown, sowing at 2 cm deep tends to cause more heterogeneity in the timing of emergence, complicating further crop management, but is also more risky if the spring is even dryer causing an overall decrease in emergence. However it has to be stated that a maximum yield of only 0,75 tonnes ha^{-1} which was obtained for the deep sowing of LIB220 and Boros is still very low compared to a successful white lupin crop in the Netherlands which could exceed 3 tonnes ha^{-1} although this is probably caused by other factors than sowing depth.

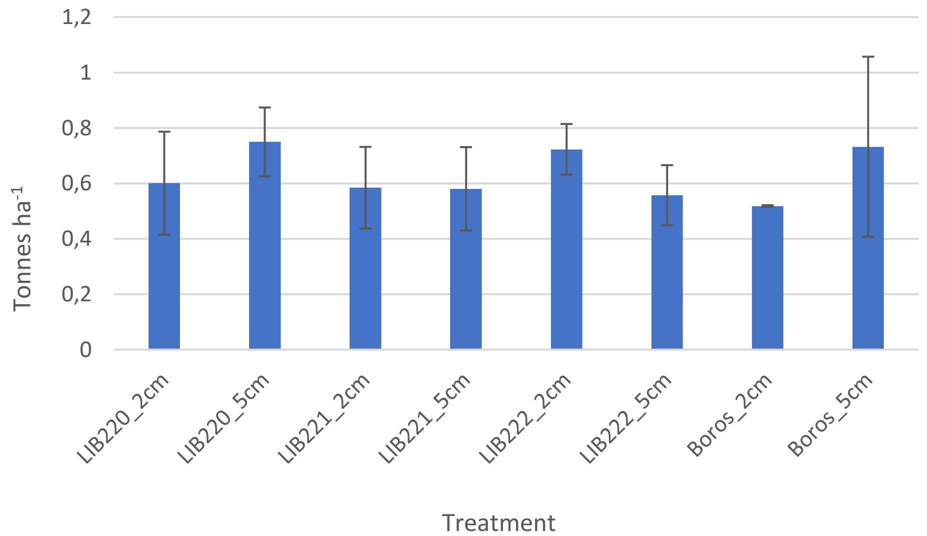


Figure 147. The yield of the Andean lupin crop
Error bars indicate standard deviation. No significant differences at p<0,05 anova, Bonferroni test.

During the season no diseases, pests or problems with lodging were observed in the trial.

A.VIII.II.I.II Functional Agrobiodiversity

At the Kraggenburg trial field, 432 insects were counted visiting the different crops. Functional biodiversity observed at the Kraggenburg test site can be divided in functional groups; pollinators as bumblebees, wild bees, honeybees, and butterflies and moths, pest predators, pest parasitoids and syrphids, which are pest predators as larvae and pollinators as adults. Like in 2019, syrphids were the most abundant species group at this site (Figure 148). Also, rapeseed was the most visited crop in 2020. Different than in 2019, the main visitors of this crop were honeybees. These were absent in 2019 and are probably found this year because beehives were placed nearby. The non-leguminous crops camelina and rapeseed have significant higher insect visitor numbers than the Andean lupin accessions. Largest part of their insect visits is compromised by syrphids, while the visiting community of most legumes is more diverse. Faba bean was the most visited legume, with significant higher insect numbers than “Inti” and LIB220. Faba bean was visited by large numbers of bumblebees and butterflies and moths. LIB221 is, like in 2019, the most visited Andean lupin. There is no clear preference for the sweet Andean lupin cultivar “Inti” visible. Only the predatory wasp *Dolichovespula saxonica* was observed more on this cultivar than on the other crops.

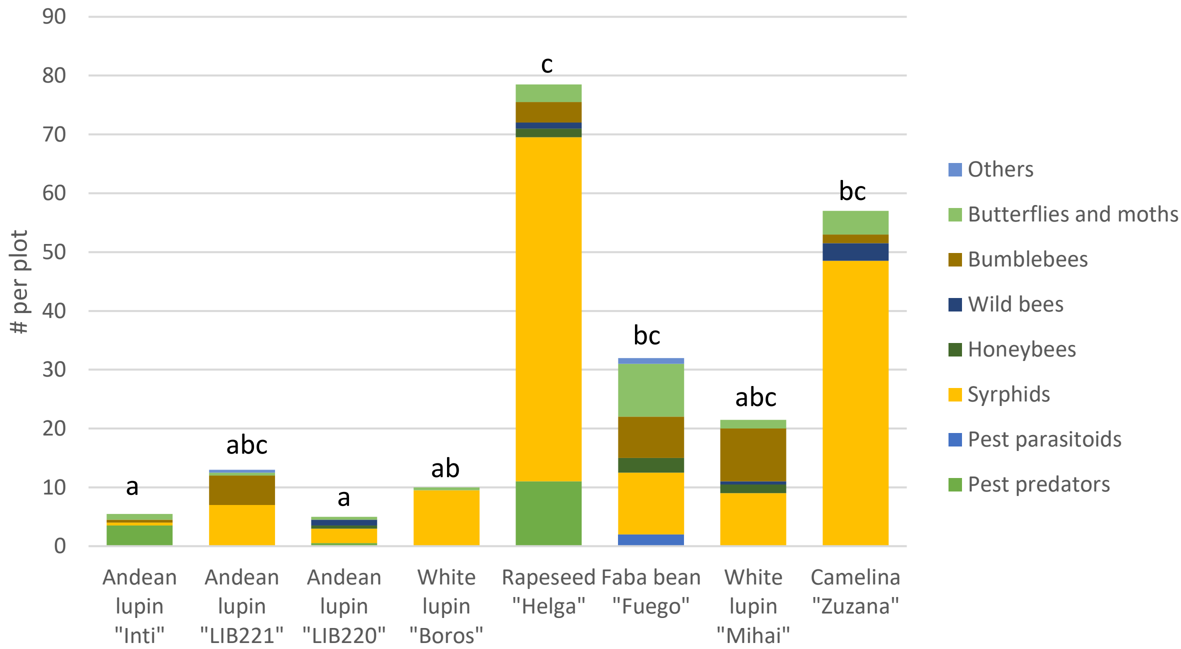


Figure 148. Number of observed insects per species group per plot, counted over two transects on clay soil. Letters indicate significant differences among accessions at $p \leq 0.05$ with Dunn (1964) test.

Table 178 shows that syrphids are the most numerous and also the most diverse group in the trial. Highest species diversity is found in the crop’s oilseed, fieldbean and camelina. All species observed are rather common in the Dutch agricultural landscape, including the bumblebees *Bombus terrestris, Bombus lapidarius* and *Bombus pascuorum*. Wild bees observed on lupin include two species of the Megachilidae; *Megachile centuncularis* and *Megachile willughbiella.* Most observed syrphids on the lupins were *Eupeodes corrollae* and *Sphaerophoria scripta*. Like in 2019, rather few ladybirds (*Coccinellidae*) were observed on the Andean lupin.

Table 178. Total number of observed insect species per species group in the different crops.

	Andean lupin "Inti"	Andean lupin "LIB221"	Andean lupin "LIB220"	White lupin "Boros"	Oilseed "Helga"	Fieldbean "Fuego"	White lupin "Mihai"	Camelina "Zuzana"	Total
Pest predators	2	0	1	0	2	0	0	0	4
Pest parasitoids	0	0	0	0	0	2	0	0	2
Syrphids	1	3	2	3	9	4	4	8	13
Honeybees	0	0	1	0	1	1	1	0	1
Wild bees	0	0	1	0	2	0	0	1	3
Bumblebees	1	2	0	0	1	2	3	2	3
Butterflies and moths	1	1	1	1	1	4	3	2	4
Others	0	1	0	0	0	1	0	0	2
Total	**5**	**7**	**6**	**4**	**16**	**14**	**11**	**13**	**32**

A.VIII.II.II Marginal soil

A.VIII.II.II.I General traits

In this final year, only basic features were assessed like height and yield. In earlier seasons a more extensive phenotyping was performed.

A.VIII.II.II.II Emergence

Unfortunately, the blue lupin 'Azuro" germinated only 1% so this treatment was cancelled. The emergence of the Andean lupin was reasonable. For LIB220 a density of 12 plants/m^2 was obtained, for LIB 221 a density of 11 plants/m^2 and for LIB222 16 plants/m^2. This is a slightly better establishment than the previous season. These densities correlated to the low sowing density used on the locations with fertile soils in the previous season. The white lupin 'Mihai' obtained a density of 40 plants/m^2. The emergence of the buckwheat, camelina and rapeseed were assessed on a 1-5 scale since these are sown in far greater density than lupin which makes counting troublesome. 1 = no or hardly any emergence, 5=is high density and uniform appearance. Buckwheat scored a 4 on average, camelina was disappointing at 1,7 and rapeseed was acceptable at 3. Buckwheat was grazed upon by deer early in the season, however regrowth occurred and this treatment could be included in the trial.

A.VIII.II.II.III Crop growth and development

The canopy height 113 days after sowing, the final height measurement was not significantly different for the various lines. Like is previous years LIB221 flowered the earliest being comparable to the white lupin cv. Mihai. LIB220 was the last to flower (Table 179).

Table 179. General crop growth features.

Accession	Canopy Height (cm) at 113 DAS, final measurement	% Main Florescence in flower at 80DAS
LIB220	136.7 a	43.3 a
LIB221	110.0 a	100.0 b
LIB222	123.3 a	65.0 ab
Mihai	113.3 a	100.0 b

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0.05$ Bonferroni

A.VIII.II.II.IV Yield: pod and seed production

The pod production on the marginal soil site was not significantly different for the Andean lupin. In the pods of Mihai we found shattering when drying. Although no seeds were lost because of the shattering it was not possible to accurately count them. LIB222 produces the highest number of pods per plot and also the highest number of seed per plot (Table 180). However the seed yield was considerably higher for white lupin 'Mihai' than for the Andean lupin accessions. This hints at the potential that lupin species have for cropping at marginal soils.

Table 180. Number of pod and seeds and seed yield on marginal soil.

Accession	Average no. of pods MF per plot	Average no. seeds MF per plot	Seed yield tonnes ha^{-1}
LIB220	80a	224a	0,21
LIB221	105a	236ab	0,38
LIB222	198b	728b	0,44
Mihai	Shattering	649ab	1,71

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0.05$ Bonferroni

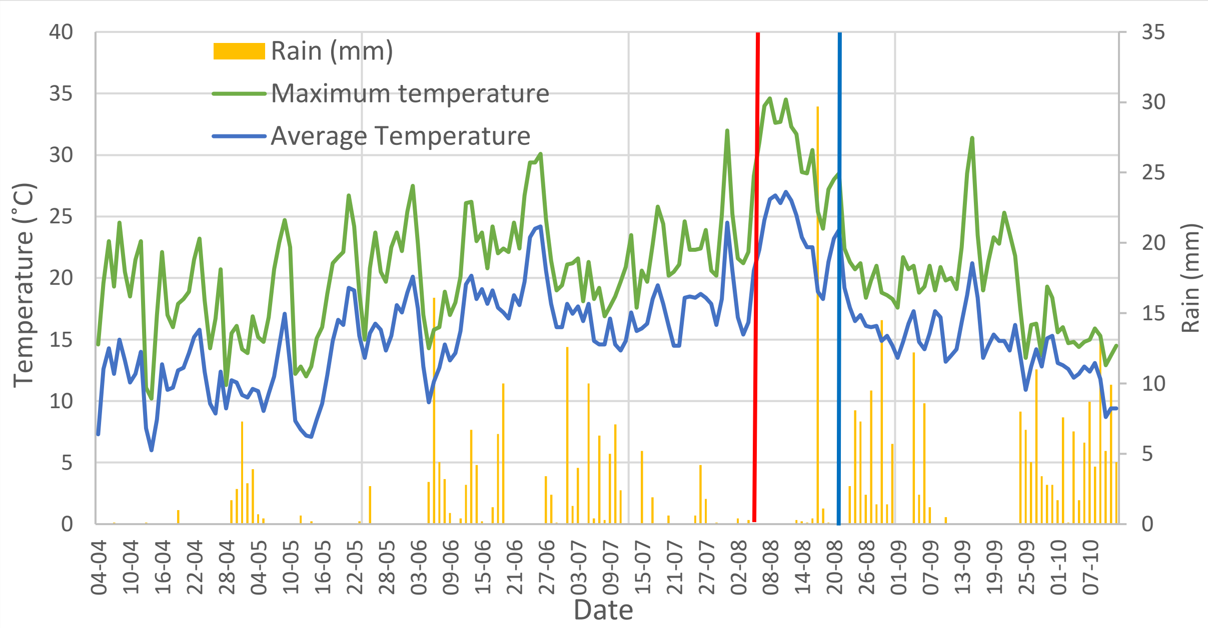
A.VIII.II.II.V Diseases & Pests

During the season occasionally some lupin aphids (*Macrosiphum albifrons*) were observed on LIB220. However numbers were small and did not negatively affect the trial. In late July mildew was observed on all lupin plants. This is a common occurrence on many crops in the Netherlands, and was observed in all previous seasons as well, it did not negatively influence the trial.

A.VIII.II.II.VI Drought tolerance

In 2019 the drought tolerance test was lost due to herbivore feeding. Some indications were present that LIB222 was more tolerant to harsh conditions than white lupin. On the 5th and the 20th of August (113 and 128 days after sowing), usually the peak of the Dutch summer, the assessments were made (Figure 149).

Figure 149. Temperature and rainfall in De Bilt, the nearest weather station of the Noordhout test site. Indicated in red line is 113 DAS, in blue line 128 DAS. Average temperature is the daily average, maximum temperature was the highest temperature measured per day.



Andean lupin LIB222 seems to be most tolerant to harsh conditions but the differences are small. Only after 113 days a significant difference was detected between white lupin 'Mihai' and the Andean lupin lines. 128 days after sowing at the 20th of August no differences were detected (Table 181). Combined with the frost tolerance seen earlier in LIB222 it is believed that assessing Andean lupin LIB222 in more detail could be interesting for future research efforts into climate adaptability.

Table 181. Drought tolerance

Accession	Drought tolerance 113DAS	128DAS
LIB220	2,7a	2,3a
LIB221	3,3ab	2,7a
LIB222	4,7b	3,3a
Mihai	3.33ab	3,3a

Values are given as means in columns with different letters indicated significant differences among accessions at $p \leq 0.05$ Bonferroni

A.VIII.II.II.VII Functional Agrobiodiversity

At the Noordhout trail field 487 insect visitors were counted. Similar as at the Kraggenburg test site, these insects are categorised in functional species groups, which are assessed per crop (accession) (Figure 150). Like in Kraggenburg, syrphids were the most abundant species group, followed by pest predators with a very high abundance of ladybirds (*Coccinella species*) (134, 27% of the total insect community observed). Wild bees were more abundant at the Noordhout test site than in Kraggenburg. The large difference between the number of insect visitors of non-legumnious versus the leguminous crops seen in Kraggenburg is not visible in Noordhout. Here, legumes have equally or higher insect numbers than the other crops, with white lupin "Mihai" and Andean lupin LIB221 as the most visited crops. Low number of visits to camelina can be attributed to the low emergence of the crop (see 5.2.1.1 Emergence). Pest predators (mainly ladybirds) were mostly found on the lupins. Parasitoids were observed in very low numbers at the trail field. Syrphids and wild bees were observed in relatively high numbers on all crops, while honeybees were only observed in rapeseed. The largest numbers of bumblebees were found on white lupin and rapeseed.

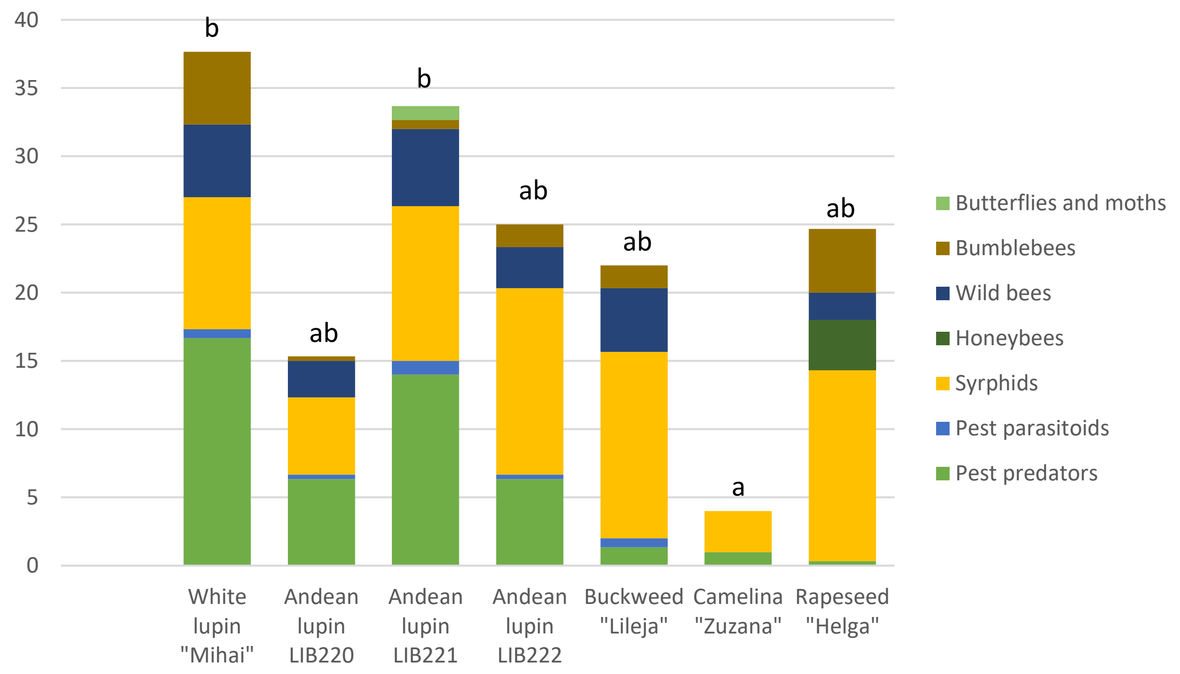


Figure 150. Number of observed insects per species group per plot on marginal soil, counted over two transects. Letters indicate significant differences among accessions at $p \leq 0.05$ with a Tukey HSD post hoc test.

Syrphids were an abundant species group with a high diversity of 12 different observed species (Table 182). Other diverse groups were that of bumblebees and wild bees. Bumblebees *B. terrestris, B. pascuorum, B. hypnorum, B. hortorum, B. lucorum* and *B. pratorum* were observed. Observed wild bees were *Megachile centuncularis, Megachile willughbiella*, two species of *Hylaeus*, a *Lasioglossum spp*. and an *Andrena spp*. Except for camelina all crops had a rather high insect species diversity with 10-14 species observed. Most abundant species, next to ladybirds, were the syrphids *Sphaerophoria scripta* and *Episyrphus balteatus*. Most abundant pollinator was the bumblebee *B. pascuorum*. Like in Kraggenburg, LIB221 was the Andean lupin with the highest insect numbers and species numbers. With four different species LIB220 and LIB221 had a relatively high diversity of wild bees visiting. As in Kraggenburg, main bee visitors were the *Megachile* species. Bumblebees visiting Andean lupin were *B. terrestris, B. pascuorum,* and *B. hortorum*.

Table 182. Total number of observed insect species per species group in the different crops.

	White lupin "Mihai"	Andean lupin LIB220	Andean lupin LIB221	Andean lupin LIB222	Buckweed "Lileja"	Camelina "Zuzana"	Oilseed "Helga"	Total
Pest predators	1	1	1	1	1	0	0	**1**
Pest parasitoids	2	1	1	2	1	1	1	**2**
Syrphids	4	3	6	4	6	3	6	**12**
Honeybees	0	0	0	0	0	0	1	**1**
Wild bees	3	4	4	3	2	0	2	**6**
Bumblebees	2	1	1	2	4	0	2	**6**
Butterflies and moths	0	0	1	0	0	0	0	**1**
Total	**12**	**10**	**14**	**12**	**14**	**4**	**12**	**29**

A.VIII.III Conclusion and Discussion

The sowing depth trial on clay soil has indicated that the common sowing depth, for spring sown legumes, of 5 cm deep is also possible for Andean lupin. Deeper sowing, which is used for winter faba beans or for summer faba beans on very drought prone soils has not been tested for Andean lupins, so no statements can be made on the possibility to sow Andean lupins at this sowing depth. Although the overall yield was not influenced by sowing depth the emergence was faster and more uniform at 5 cm deep compared to 2 cm deep. Possibly this is caused by the availability of soil moisture. At 5 cm deep, at this trial location, usually still some soil moisture available. At 2 cm deep the germination is more dependent on rainfall to obtain moisture. But the very low yield of maximum 0.75 tonnes ha^{-1} would not be competitive versus other legume crops in the Netherlands.

The drought tolerance trial on marginal sandy soil seemed to indicate that LIB 222 was most tolerant after 113 days after sowing. However, after 128 days the differences were no longer significant. These results match earlier observations where LIB222 seems to be more tolerant to both drought and frost stress. Interestingly LIB222 is the accessions which is the richest in anthocyanin. It would therefore be interesting to perform additional studies to determine if anthocyanin in Andean lupin relates to tolerance to adverse weather conditions.

On both locations the Andean lupin accessions did not obtain seed yields that were competitive in comparison to other lupin species or legumes in the Netherlands.

Assessing insect biodiversity at the two different experimental sites revealed that the surrounding landscape is important to explain the use of Andean lupin by insects. In Kraggenburg, the non-leguminous crops rapeseed and camelina were most visited, whereas in Noordhout the legumes “Mihai” and LIB221 were the most visited accessions. This can be explained by the local insect community. Insect communities are shaped by the surrounding landscape (Bianchi et al., 2006). In a forested landscape like at Noordhout different species of wild bees, bumblebees and syrphids can be expected compared to the species in Kraggenburg, an agricultural landscape. In open agricultural landscapes with a low abundance of natural elements, the insect community is dominated by highly mobile and generalist species which are able to survive in a landscape with few species of forage plants and a high level of disturbance (Ozinga et al., 2018). These species, mainly syrphids, generally forage on easily accessible flowers as that of Brassicae. In more natural landscapes also more specialised insects are able to survive (Harrison et al., 2019). Specialised insects, as several bumblebees (*B. pascuorum, B. hortorum*) and wild bees (*Megachilidae*) prefer legumes. High numbers of insect visits on leguminous crops like Andean lupin are thus more likely in areas where these specialised insects are present.
Between the different Andean lupin accessions in Kraggenburg there was no clear preference for the low alkaloid “Inti” compared to the other accessions. Possibly, differences in alkaloid levels are not detected by insects but because of the low insect numbers visiting Andean lupin no hard conclusions can be drawn. Like in 2019, LIB221 was the most visited Andean lupin accession at both locations (although differences were not significant). This accession, having contrasting purple flowers with a yellow heart, might be more attractive to pollinators than other accessions with less contrasting flowers (Heather et al., 2013).