Legume Generation

Boosting innovation in breeding for the next generation of legume crops for Europe

Progressing the breeding of lupin

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Legume Generation Report 10

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Contents

Summary

Lupins play an important role in European agriculture for food and feed protein production, especially in regions with poor sandy soils and frequent pre-summer drought. In the Legume Generation project, the Lupin Innovation Community (LUPIC) works together to improve grain yield and yield stability, which is urgently needed to serve farmers´ needs. Since the genepool of sweet lupins is genetically very narrow, the LUPIC approach is to develop new/better traits from bitter lupins to finally breed sweet lupin cultivars.

In our innovation community, we characterise genetic resources with emphasis on agronomically important traits like alkaline soil tolerance, winter hardiness, disease tolerance, heat and drought tolerance, flower and pod non-dehiscence combined with high protein content and grain yield. In several cases we have to develop tools in order to understand inheritance of traits of interest. The tool development as well as the plant material development is in track with our plans and expectations.

We have shared our germplasm to carry out field trials in Germany, Poland, Greece and United Kingdom (Wales) for white lupins and narrow leafed lupins. Due to very limited seed availability Andean lupins could only be tested in Italy.

The grain yield results, lodging- and disease-notations from field observations are complemented by tests under controlled conditions. Here we started to analyse drought tolerance in polythene tunnels, Fusarium- and Sclerotinia-tolerance with a lab test based on q-PCR, alkaline soil adaptability with a pot test in specifically prepared substrate. Heat stress tolerance in climate chambers is in preparation.

In several cases we developed and implemented the phenotyping-tools for testing specific traits.

A crossing programme for narrow leafed lupins has been implemented to produce biparental segregating families for traits with unknown inheritance to be analysed genetically. At the same time these crosses are the start of an introgression approach to bring new variation into sweet lupins. Known molecular markers (SSR, SNP, RNA-Seq) are used for marker assisted selection, e.g. for seed shattering tolerance and new markers are being developed for new traits.

In the phenotyping for agronomic traits, we found useful variation and differentiation for winter survival, drought tolerance, alkaline soil tolerance and fungal disease resistance. Variability of these characters is limited in sweet lupin cultivars. Useful variation for winter survival and alkaline soil tolerance cannot be found in sweet cultivars. Promising variation for these traits has to come from bitter lupin genebank accessions when developing new germplasm of sweet lupins.

In the winter 24/25, we found interesting types for winter survival that match our ideotype thinking: Late flowering genotypes that have never been used in sweet cultivar development seem to be the most winter-hardy types. After winter, these late flowering types are about 4 weeks earlier ripe for grain harvest than spring sown early cultivars. Due to the prolonged vegetation period, they have a much higher potential of pod and seed set, are taller than known sweet cultivars and therefore need lodging tolerance. We will

focus on these types and will prefer autumn sowing instead of spring sowing. These winter hardy genotypes together with above mentioned traits form the ideotype-led type that we aim to breed.

From former projects we have some data about protein content and alkaloid content, especially in narrow leafed lupins. Limited data are so far available for white lupins that show the bitterness of the genebank accessions that we actually work with.

We have not yet started with field testing for demonstration and release. Our material is not ready for cultivar release and our field trials may serve as demonstration for our activities. However, field testing for recognition of specific traits is extremely useful at our actual stage of cultivar development.

With new tools and better understanding of genetics and inheritance we progress in a knowledge-based lupin breeding programme that is able to combine traits systematically and to pyramidise traits using backcrossing and introgression strategies. This work is shared among the LUPIC partners and with added partners beyond the Legume Generation project. We work at different levels in germplasm development with different proximity to cultivar development: Tool development; trait-recognition and -analysis; trait combination; introgression of traits/trait combinations into elite germplasm.

Our final goal is significantly improved sweet lupin cultivars that are welcomed by farmers.

Introduction

This report sets out the progress that has been made in the first two years of Legume Generation in boosting the breeding of lupins. It relates in particular to our plan to boost breeding which was prepared before the project started. This was published in 2024.[1] That report provides a complete description of the background, plans, and organisation of the work within the Lupin Innovation Community.

There are two big areas in Poland and Germany where lupin species are grown in Europe. Just a few breeders run significant breeding programmes to improve lupins for these regions. All breeding programmes exclusively select from sweet (low alkaloid) spring-sown forms. However, the global genetic bases of these sweet lupin types are narrow and consequently breeding progress is slow. Additionally, spring-sown crops are vulnerable to drought. Most of the breeding progress is negated by increasing pre- and early- summer drought in the relevant areas. Our central breeding approach is the selection of winter types in at least three species. To develop these winter types, we use the experience and networks of our Lupin Innovation Community (LUPIC) partners and beyond (our platform is open for scientists and breeders as well as seed merchants) by connecting research in genetic resources and pre-breeding for different traits with an applied breeding programme.

We work mainly with bitter (alkaloid-rich) lupins to make use of the agronomically relevant traits of this so far neglected part of the primary gene pool. However, our mid-term goal is to develop sweet varieties either by crossing and backcrossing to introgress new alleles[2] or by genome editing[3] of the alkaloid transport mechanism.[4] The latter would open the rich variation in the gene pool of bitter lupins to breeding immediately. The geographical distribution of the LUPIC partners is given in the following map (Figure 1).

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[1] Eickmeyer, F., Flüß, H., Susek, K. and Murphy-Bokern D., 2024. The plan for boosting the breeding of lupin. Legume Generation report 2. Available from www.legumegeneration.eu and www.legumehub.eu. DOI: https://doi.org/10.5281/zenodo.17249160

[2] Cowling, W.A., 2020. Genetic diversity in narrow-leaved lupin breeding after the domestication bottleneck. In K. B. Singh et al. (eds.), The Lupin Genome, Compendium of Plant Genomes, https://doi.org/10.1007/978-3-030-21270-4_1.

[3] Pathi, K.M. and Sprink, T. 2025. Lupins in the genome editing era: advances in plant cellculture, double haploid technology and genetic transformation for crop improvement. Front. Plant Sci. 16:1601216. doi: 10.3389/fpls.2025.1601216.

[4] Otterbach SL, Yang T, Kato L, Janfelt C, & Geu-Flores F (2019): Quinolizidine alkaloids are transported to seeds of bitter narrow-leaved lupin. Journal of Experimental Botany, Vol. 70, No. 20 pp. 5799–5808, 2019 doi:10.1093/jxb/erz334.

Figure 1. Map of trial locations and main focus activities in lupins

We have conducted field trials at four locations that are able to carry out plot field trials for grain yield (JKI, IPG, UPM and ESKUSA). In the sowing year 2024 we were only able to sow row or double row trials at these locations and get first impressions on grain yield due to limited amounts of seeds.

In Wales (Aber) and Greece (IHU) we have limited (time and space) testing capacities to complement our grain yield trials with observations regarding fungal diseases and drought and heat stress. Yield does not play the primary role at these both locations.

The testing facilities at SRU, IPK and BOKU focus on specific traits. At BOKU, soil-borne-fungal disease resistance tests are in development. They have given first results on the differences in *Fusarium oxysporum* behaviour of narrow-leaved lupin accessions. *Sclerotinia* results will follow. IPK carried out a first drought tolerance test in greenhouse pots for white and narrow-leaved lupins. The results from IPK will be the basis for the material selection for a heat tolerance test at SRU to be carried out in 2025. We hope to be able to find further testing locations in the southwest and in the Scandinavian area of Europe. A first request for cooperation in white lupins to the company CERIENCE in France (partner of the BELIS-project) has been sent out.

Our ideotype

An ideotype is an idea or conceptual model about an ideal crop plant type. We are applying the ideotype concept[5] to cultivar development for the large areas in Poland and northeast Germany, where lupins are traditionally grown as protein feedstock for animal nutrition. Since these areas are prone to pre-summer drought in the germination phase of spring sown lupins, our main approach is to develop an autumn-sown winter crop. A winter lupin can profit from a prolonged vegetation period which increases the resource capture possibilities of the lupin species. Obviously, winter lupins need frost and snow cover tolerance to survive the winter in these areas. We found these features with promising levels in late flowering genotypes of bitter lupins when we tested lupin sets at IPG in Poznan, at JKI in Groß Lüsewitz and at ESKUSA in Parkstetten. These late types form a rosette before winter and start with the elongation phase in early spring, while early types start with elongation already before winter and are prone to winterkill by frost. Sweet lupins have been selected since their invention about 100 years ago[6] for early maturity and show only limited winter survival.

Our winter type needs very good resistances against soil borne fungal diseases such as *Fusarium* and *Sclerotinia*. We observed a prolonged flowering period that leads to a strongly increased pod set distributed over a long period. Flower dehiscence and pod dehiscence is of less importance when the nutrition of several pods is distributed over a longer period, compared to spring sown lupins that have to form the pods within a very short period. The disadvantage of pod shattering of ripe pods when still new pods are formed within one plant can be overcome by (marker assisted) introgression of pod shattering genes *tardus* and *lentus*, which are known domestication factors[7] in Australian narrow-leaved lupin cultivars. In white lupins pod shattering is less expressed and of minor importance.

In analogy to the usage of different earliness types in soybean in different geographic regions, we can imagine a usage of different levels of winter-hardiness (and/or maturities) in lupins for different European areas.

Our roadmap for cultivar development

We think that there is little scope for useful selection within the sweet lupin genepool because the germplasm that is worldwide available is genetically very narrow. Access to valuable traits from the bitter lupin genepool cannot be achieved on a short-term basis and hence the development of significantly improved cultivars takes time.

Traits have to be identified and described. Their inheritance has to be understood via genetic analysis of well-prepared biparental segregating families or via genome wide

[5] Donald C.M. (1968). The breeding of crop ideotypes. Euphytica 17; 385-403.
[6] Sengbusch Rv, 1930, 1931, 1938. Bitterstoffarme Lupinen I. und II., III. Züchter 2, 1-5; Züchter 3, 93-109; Züchter 10, 219-222.
[7] Buirchell B.J. (2008). Narrow-leaved lupin breeding in Australia – Where to from here? In J.A. Palta and J.B. Berger (eds). 2008. 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008, Fremantle, Western Australia (226-230). International Lupin Association, Canterbury, New Zealand. ISBN 0-86476-153-8.

association studies (GWAS). Positive and negative correlations and linkage drag must be recognised. The valuable traits must be decoupled from the bitterness either by introgression, backcrossing or genome editing to finally get sweet grains for feed and food purposes. For characters that are difficult or cannot regularly be described in the field, e.g., soil borne fungal diseases, drought tolerance, heat tolerance, BOKU, IPK and RU developed tools and/or testing systems with new descriptors to phenotype the plant material regularly and with higher accuracy and reproducibility.

ESKUSA, JKI, and IPG work with biparental crosses to build up segregating populations to understand the genetic basis and the inheritance of traits.

With new tools and better understanding of genetics and inheritance we can form a knowledge-based lupin breeding programme that is able to combine traits systematically and to pyramidise traits using backcrossing and introgression strategies. This work is shared among the LUPIC partners and with partners beyond the Legume Generation project. We work at different levels in germplasm development with different proximity to cultivar development: Tool development; trait-recognition and -analysis; trait combination; introgression of traits/trait combinations into elite germplasm. Field testing for cultivar release is not our actual focus; however, field testing for recognition of specific traits is extremely useful at our actual stage of cultivar development. Figure 2 gives an overview on the roadmap for cultivar development of our LUPIC exemplified with narrow-leaved lupins. We follow similar roadmaps for white lupins and Andean lupins.

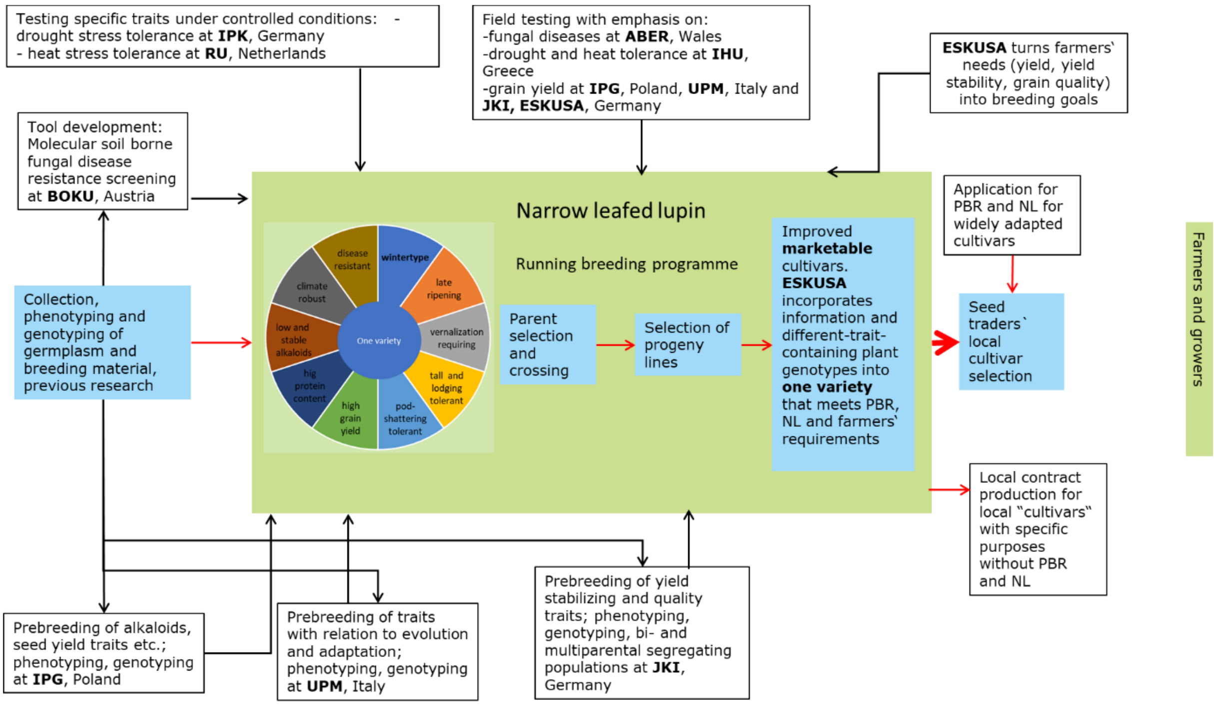


Figure 2. Narrow-leaved lupin cultivar development roadmap

Alternative uses of lupins such as whole-crop silage, green manure, technical use of protein or specific steps such as membrane filtration in protein processing can offer short-cut-alternatives to sweet cultivar development and directly make available bitter lupins for specific uses.

As a long-term income-basis we aim on royalties for seed production of listed varieties. However, we are open for contract breeding of exclusive varieties or contract production for non-listed varieties in a closed loop.

The traits

In our ideotype description we named only a few key characters. The following list shows several important traits that have (additionally) to be included in one cultivar. A good cultivar is always a well-balanced combination of most – if not all – listed traits. The list does not claim to be complete. Certain geographic or climatic regions or certain ways to use the lupins might need to add further traits.

- Winter survival
- Rosette stage before winter
- Late maturity
- Branching
- Soil borne fungal disease tolerance
- anthracnose and leaf spot diseases tolerance
- Resistance against lupin beetle and aphids
- Flower and pod non-dehiscence
- Seed shattering resistance
- Heat tolerance
- Drought tolerance
- Calcareous soil tolerance
- Quick root development
- Increased root system
- Efficient nutrient uptake
- Grain yield
- Grain quality
- High protein content
- Protein quality
- Sulphur-containing amino acids
- Heat tolerance
- Drought tolerance
- Calcareous soil tolerance
- Quick root development
- Increased root system
- Efficient nutrient uptake

We have found these traits with different expression-levels in several accessions of genetic resources and in state-of-the-art cultivars. Sometimes, we just observed the traits. In some cases (winter survival, soil borne fungal disease tolerance, anthracnose tolerance, seed shattering resistance, calcareous soil tolerance) we have built up lines and segregating populations to study inheritance. Development of testing- and phenotyping-tools support the safe characterisation of traits.

Relevant traits can be combined by introgressing them into sweet luplin germplasm through backcrossing, pyramiding characters to trait packages or, in the future, by additionally/alternatively using genome editing tools.

Progress

Phenotyping for agronomic traits

Winter survival

Winter survival as a new trait and selection goal will be a game-changer in lupin variety development. This character will have influence on the whole breeding plans within the project.

We tested several genebank accessions at two locations (ESKU and IPG) for winter survival and found useful variation for this important character. IPK has produced drought stress data. BOKU has produced resistance data with a soil-borne fungal disease test based on molecular data. First field test results are available from JKI, ESKU, ABER. Unfortunately, the field tests at IPG did not perform well in 2024 (probably due to a heavy fusarium attack in a very early stage of the plant development). A heat stress test at RU is in preparation and a field test in Greece (IHU) was sown in December 2024. We added promising genotypes to an existing set of plant-lines with agronomically relevant new traits.

The winter survival test is presented here with Figure 3 of a frozen narrow-leaved lupin and a frozen white lupin. First scoring results are given for narrow-leaved and for white lupins in Figure 4 and Figure 5.

Figure 3. Frosted lupins in January 2025. Left: narrow-leaved lupin; right: white lupin.

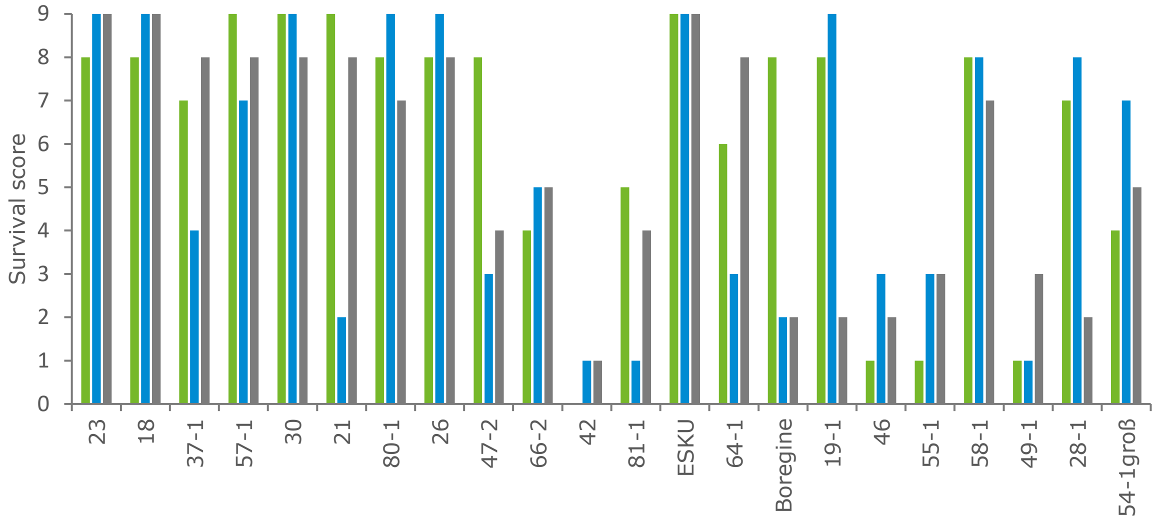


Figure 4. Winter survival scores (1-9) for 22 narrow-leaved lupin varieties (9 = good survival) from ESKUSA field tests in Parkstetten, Germany. The data for each of three replicates are presented.

In the narrow-leaved lupins especially late flowering bitter types with high anthocyan-content (and red cotyledons) seem to be very good in winter hardiness. Sometimes there are strong deviations between the replications. For example, the sweet variety ´Boregine´ didn´t perform well in two of three reps, however performed well in the third replication. At IPG in Poznan the narrow-leaved lupins did not survive the winter 24/25.

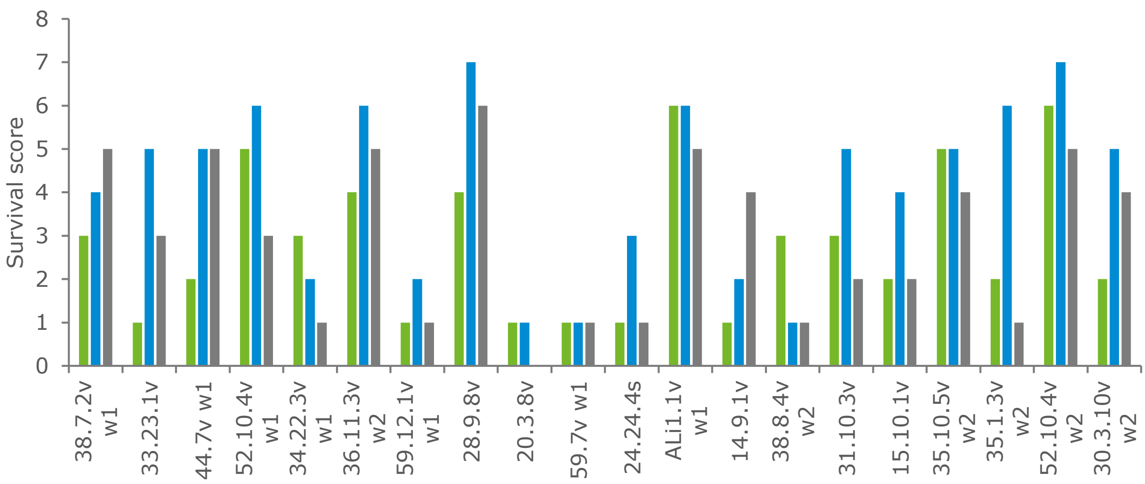


Figure 5. Winter survival scores (1-9) for 20 white lupin varieties (9 = good survival) over three replicates from ESKUSA field tests in Parkstetten, Germany. The data for each of three replicates are presented.

In white lupins single accessions perform quite well for winter hardiness. In most accessions there seems to be still variation (segregation) for winter survival. At IPG in Poznan, several white lupins survived the winter 24/25 (pers. comm. K. Susek).

In yellow lupins (data not shown), single plants in single accessions survived the winter.

The winter survival field tests will be replicated in the winter 2025/26 with an increased set of lines and accessions with ESKUSA and a selected set of winter survivors from above results at JKI and IPG.

Drought tolerance

Twenty-five accessions each of *Lupinus angustifolius* and *L. albus* were grown in a polythene tunnel at IPK Gatersleben (Figure 6 and Figure 7). The entry samples are from different sources and pre-characterised. All accessions were tested for drought stress in particular. In total, 100 plants for control and stress were used, respectively. The material was characterised based on a standardised descriptor.

Figure 6. Drought test - trial set up in a polythene tunnel at IPK.

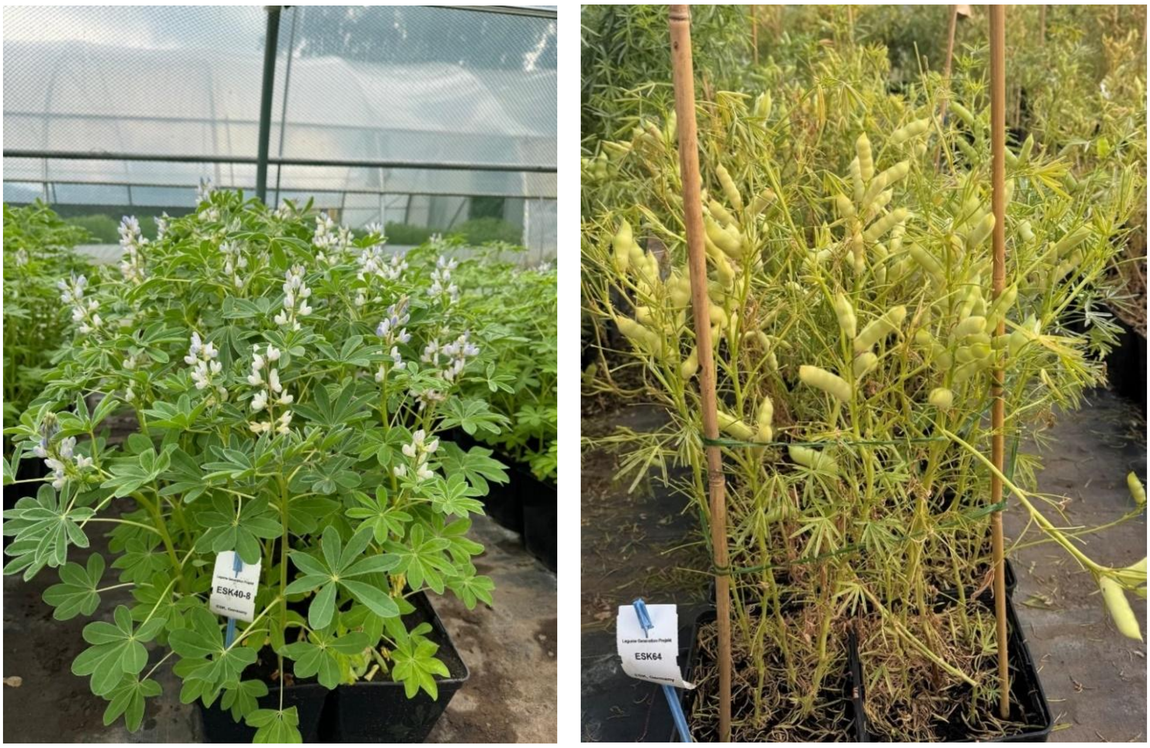


Figure 7. Performance of a white lupin (left) and a narrow-leaved lupin (right) plant in the drought test at time of flowering or seed ripening, respectively

The reduction in grain yield under drought stress is mainly reflected in lower numbers of developed pods (Figure 8).


Figure 8. Reduced set of pods (flower dehiscence) under drought stress conditions

We found two accessions in white lupin (ESK31.9 and cultivar Sulimo in Figure 9 and Figure 10) which reacted with no or just a little bit of seed set reduction under drought conditions during flowering time. However, both accessions show only low seed yields under control conditions. The same is true for three accessions (ESK46, ESK57 and ESK80) of narrow-leaved lupins. The test will be repeated with the same accessions in the next growing season.

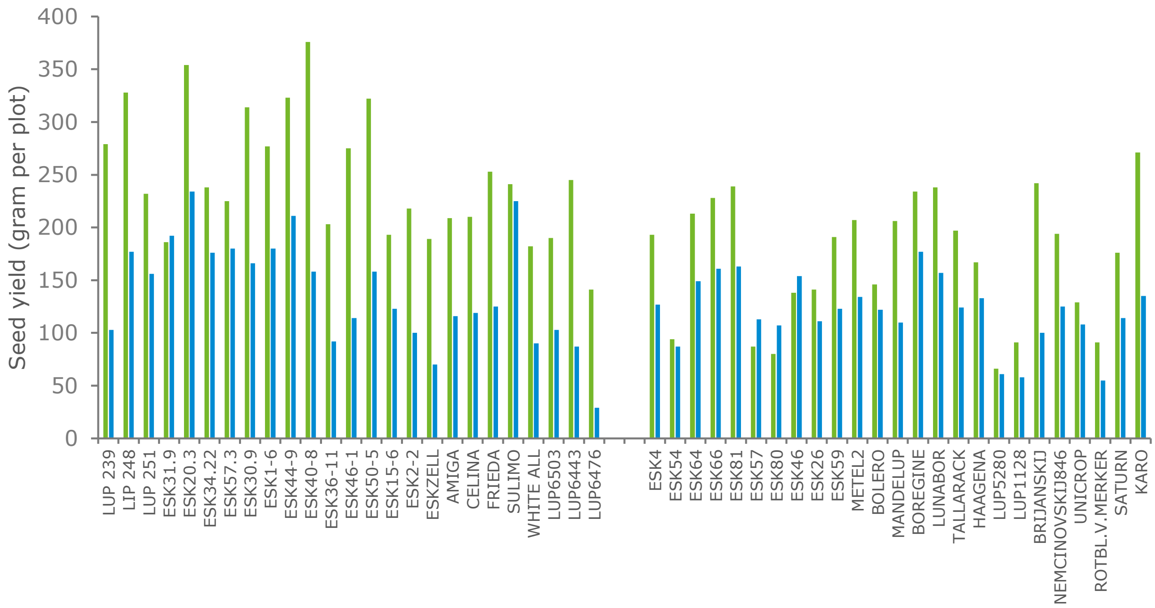


Figure 9. Seed yield of white (left) and narrow-leaved (right) lupins without (green bars) and with (blue bars) drought application.

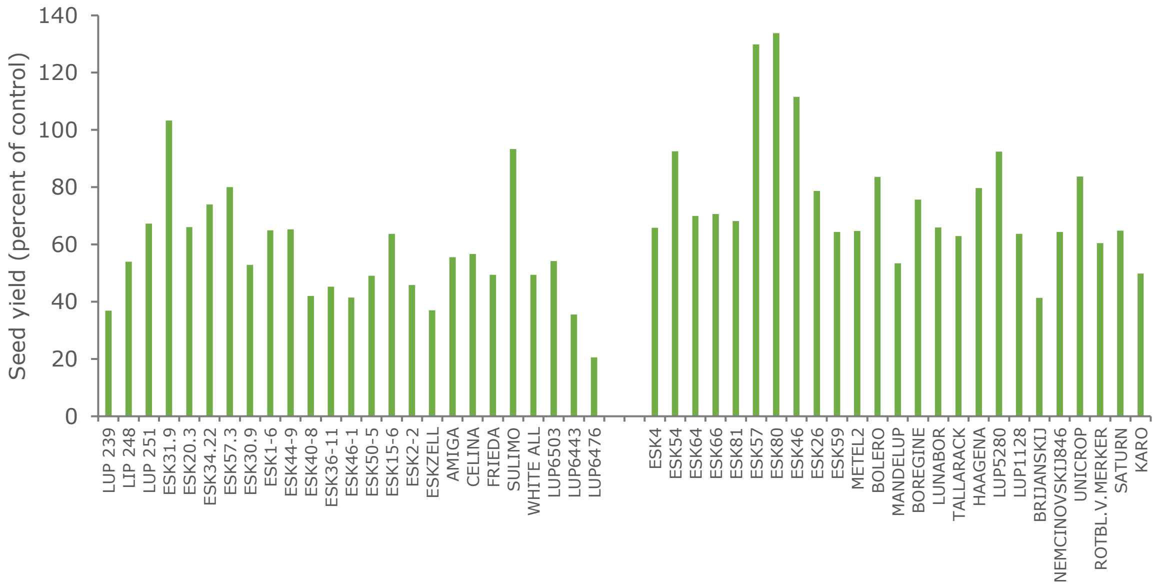


Figure 10. Seed yield of drought treated plants in relation to untreated controls for white (left) and narrow-leaved lupins (right)

Alkaline soil tolerance
In ESKU we have successfully carried out an alkaline soil tolerance pot test (20 g $CaCO_3$ + 2 g CaOH per 1 l culture substrate shifting the pH from approximately 5.8 to 7.4) in the greenhouse with a good differentiation between tolerant and susceptible plants (Figure 11) and identified two tolerance-showing accessions of narrow-leaved lupins.

Figure 11. Alkaline soil tolerance test in pots in the greenhouse. Tolerant plants stay green when grown in alkaline substrate. Susceptible plants turn yellow due to Fe-deficiency-symptoms

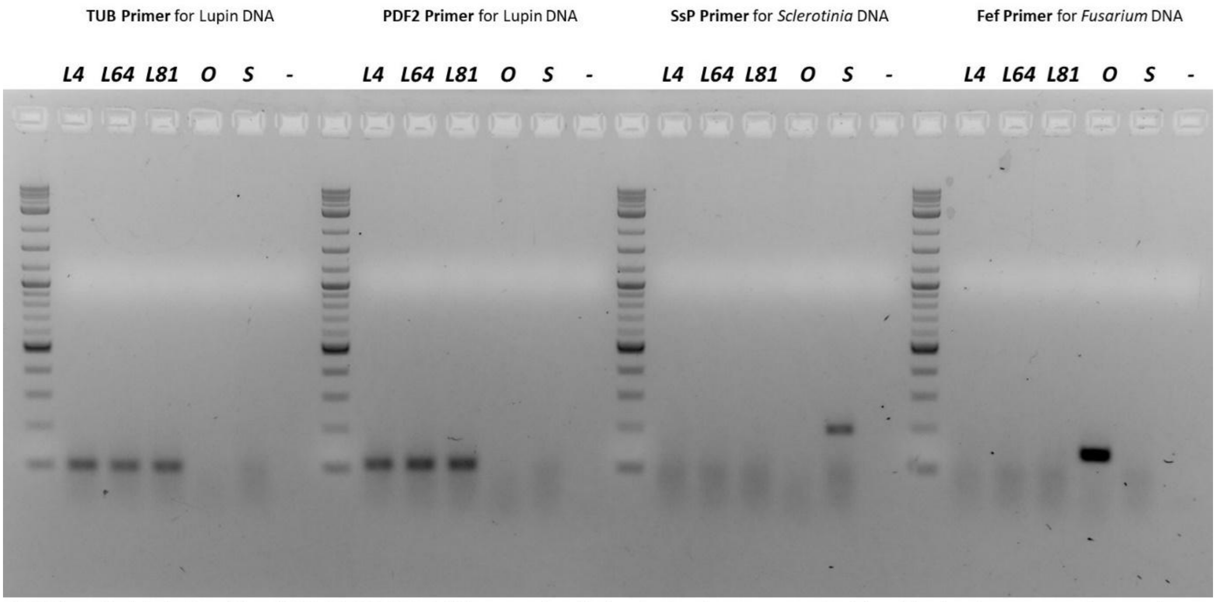
Only two accessions (No. 46 and No. 59) perform very well under alkaline soil conditions. Accession No. 46 went into further analyses in the LupiRhiz-project and in activities at the Heinrich-Heine-University in Düsseldorf to study iron-uptake mechanisms and iron metabolism. Biparental crosses were made at JKI to study the inheritance of this valuable character.

We tried this alkaline soil tolerance pot test also with white lupins and found similar reactions, showing intercostal chloroses in susceptible accessions (Figure 12). A larger set of white lupins is planned for future tests.

Figure 12. Alkaline soil tolerance test in pots with white lupins

Fungal disease resistance

At the beginning of the project a reliable and reproducible infection and screening method was established at BOKU for each of the pathogens and *Fusarium oxysporum* and *Sclerotinia sclerotiorum* independently. Therefore, root infection followed by DNA extraction and quantitative PCR (qPCR) was chosen. The qPCR was done using specific primer pairs for lupin and the respective fungus (Figure 13). By calculating the ratio of the cq values of the fungus and the plant it is possible to assess the progress of the infection and also the resistance level of the plant.



PCR Primer check for lupin and fungal DNA. **L4, L64, L81** = 3 different species of lupins; **O** = *Fusarium oxysporum*; **S** = *Sclerotinia sclerotiorum*; - = negative control

Figure 13. Specificity tests of the primers used for qPCR for lupins, Fusarium and Sclerotinia

Fusarium oxysporum f. sp. lupini

For *Fusarium oxysporum f. sp. Lupini,* the root dipping method, where the root was soaked for 30 minutes in a spore solution, was used to infect the plants. The screening of 20 accessions was done using at least five replicates per accession and condition. After evaluation and statistical analysis, we were able to rank the accessions according to their resistance level (Figure 14). The screened accessions have Cq ratios between 0.75 and 0.92. Since we divide the Cq value of the lupin by the Cq value of the Fusarium we have a higher resistance the lower the ratio is because a high Cq value of Fusarium means that there was less fungal material present.

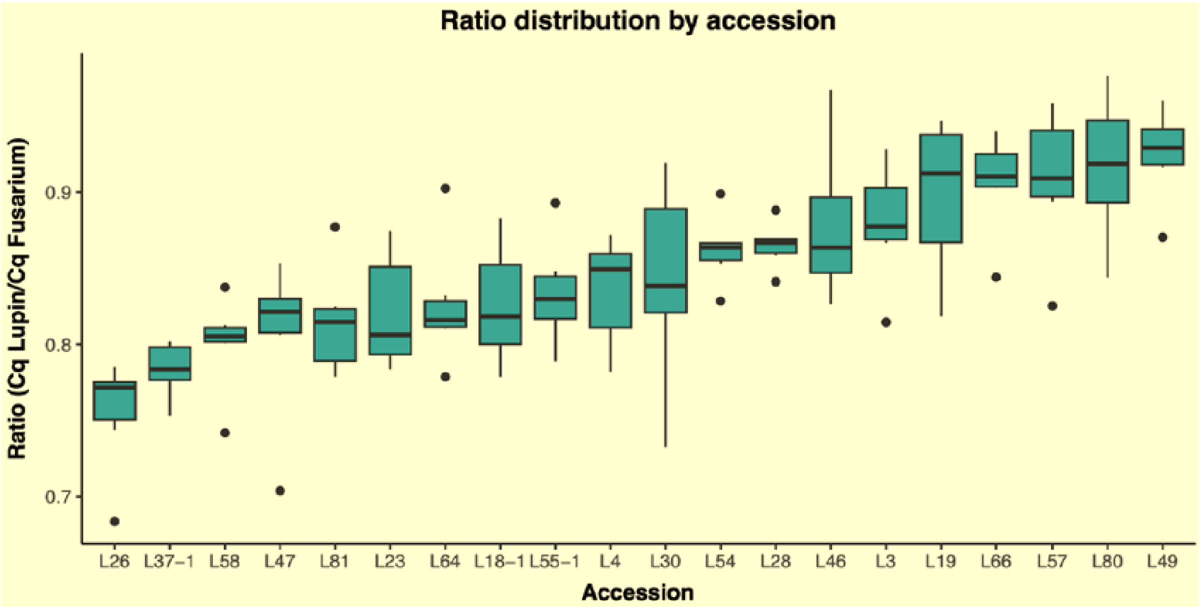


Figure 14. Cq ratios for Fusarium oxysporum of the 20 screened lupin accessions

In a next step we wanted to verify the results by choosing the most resistant accession (L26) and the most susceptible (L49) to determine the progress of infection over a total time period of four days (Figure 15). While after one day of incubation there is no difference between the accessions, from day two on there is a significant difference ($p < 0.05$).

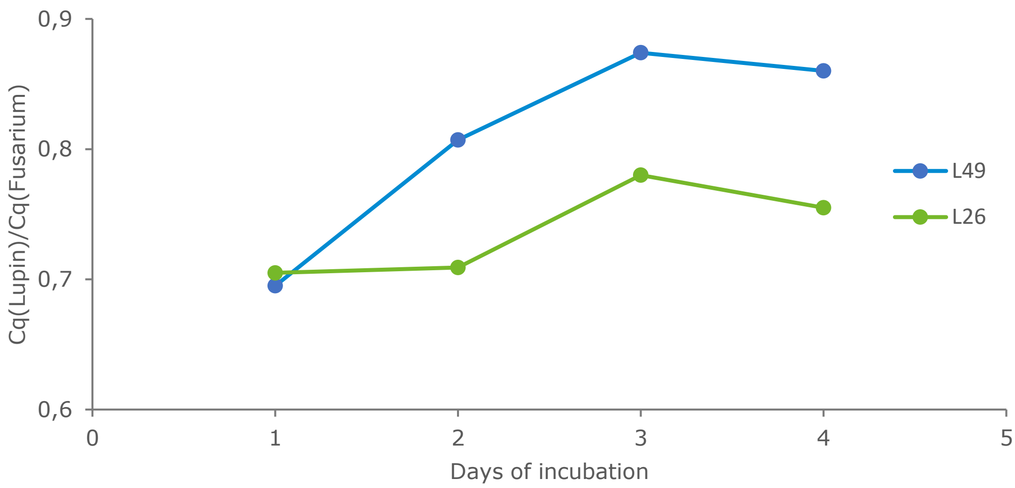


Figure 15. Time line of the accessions previously identified as most resistant (L26) and most susceptible (L49)

It is obvious that the fungus cannot proliferate as fast on L26 as in L49. To find the reason for the differences in growth RNA samples of these four time points were prepared and sent for sequencing. The transcriptome gives us a snapshot of the expressed genes in the plant as well as the fungus at the respective time points. Putting the differentially expressed genes into a biological context will shed light on their role in conferring resistance or susceptibility.

Besides the resistance screening, we tagged *Fusarium oxysporum f. sp lupini* with GFP to trace the spread of the fungus within the plant. The fluorescence protein was ectopically introduced into the fungal genome using *Agrobacterium* mediated transformation. Lupin infection was performed as described before and the roots were evaluated after 2 days using the confocal microscope. Therefore, the root was dissected into thin strips with the aim to assess to which compartments of the root the fungus has spread so far. We were able to localise the site where the fungus penetrated the plant and further confirmed the spread through the xylem (Figure 16).

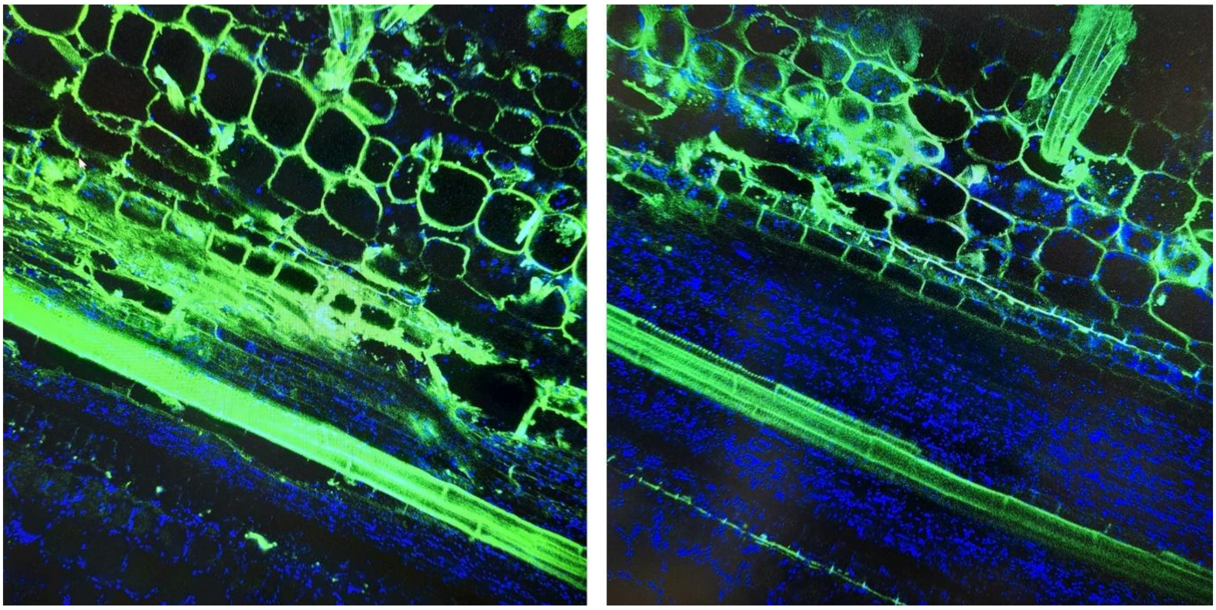


Figure 16. Lupinus angustifolius infected with GFP-tagged Fusarium oxysporum f. sp. lupini; penetration of the root (left); spread through the xylem (right)

Sclerotinia sclerotiorum

The establishment of the infection method for *Sclerotinia sclerotiorum* was a bit more challenging. Root dipping did not lead to reproducible results and also poking the root did not seem to have an impact. Since we aimed to find a method without injuring the plant, we recently tested a new one where an agar plug with mycelium is put on the stem and wrapped around with moistened cotton and parafilm. This infection method has been assessed as reproducible. Currently the first screening of the 20 lupin accessions is in progress.

Field phenotyping for lodging and disease susceptibility

A set of 30 narrow-leaved and 18 white lupin accessions from ESKUSA and JKI was sown in Aberystwyth in early May 2024. Plant height was measured end of July and lodging and disease notations were taken end of August (Table 1, Table 2).

Table 1. Results of the testing of narrow-leaved lupin accessions at Aberystwyth, United Kingdom

Donor	Entry	Height (cm)	Lodging %	Pod shattering %	Brown leaf spot 1=none; 9=severe attack	Rabbit damage
ESK	ESK4	117	100	50	1	0
ESK	ESK5	0		0	1	1
ESK	ESK19	127	100	0	1	0
ESK	ESK26	83	50	60	7	0
ESK	ESK44	87	50	0	2	0
ESK	ESK46	75	100	0	3	0
ESK	ESK54	100	75	0	4	0
ESK	ESK57	107	100	0	1	0
ESK	ESK58	95	60	60	1	0
ESK	ESK59	108	100	0	1	0
ESK	ESK64	93	80	75	1	0
ESK	ESK66	87	100	0	1	0
ESK	ESK68	63	50	0	1	1
ESK	ESK80	117	100	50	1	0
ESK	ESK81	97	100	70	1	0
JKI	LUP 1128	107	40	30	1	0
JKI	LUP 5280	87	100	50	1	0
JKI	NEMCINOVSKIJ 846	30		0	1	1
JKI	Bolero	53		0	1	1
JKI	Boregine	38	100	0	1	1
JKI	Brijanskij	78	90	0	1	0
JKI	Haagena	30		0	1	1
JKI	Karo	93	60	50	1	0
JKI	Lunabor	23		0	1	1
JKI	Mandelup	45		0	1	1
JKI	Metel2	93	80	0	1	0
JKI	Rotbl.v.Merker	97	100	0	1	0
JKI	Saturn	80	70	0	9	0
JKI	Tallarack	73		0	4	1
JKI	Unicrop	0		0	1	1

Table 2. Results of the testing of white lupin accessions at Aberystwyth, United Kingdom

Donor	Entry	Ave. Height (cm)	Lodging %	Seed shattering %	Downey mildew 1=none; 9=severe attack	Rabbit Damage
ESK	Amiga	147	100		1	0
ESK	Celina	102	100	60	1	0
ESK	ESK15-6	117	100		1	0
ESK	ESK1-6	150	100		6	0
ESK	ESK20.3	155	100	60	1	0
ESK	ESK2-2	130	100		7	0
ESK	ESK30.9	152	100		5	0
ESK	ESK31.9	102	100		8	0
ESK	ESK34.22	142	100		7	0
ESK	ESK36-11	133	100	60	1	0
ESK	ESK40-8	113	100		1	0
ESK	ESK44-9	143	100		1	0
ESK	ESK46-1	143	100	50	1	0
ESK	ESK50-5	138	100		1	0
ESK	ESK57.3	127	100		1	0
ESK	ESKZell	143	100		7	0
ESK	Frieda	103	100		1	0
ESK	White All	70	60		7	1

Significant lodging was observed in almost all of the accessions. This could be due to a late sowing date or to prolonged vegetative growth under Atlantic climate conditions. Several accessions of narrow-leaved lupins showed brown leafspot diseases (Figure 17). Some of the white lupins showed downey mildew (Figure 18). Rabbit damage was observed in sweet varieties and in sweet accessions.

Figure 17. Narrow-leaved lupin with brown leafspot disease

Figure 18. White lupin with downey mildew symptoms

Quality traits

Alkaloid content

From former projects (Luprome) we have a good overview about the alkaloid content of our narrow-leaved lupin accessions. We did not analyse any of the accessions so far in Legume Generation, because we already know that any material of interest is bitter. The composition of the alkaloids was also analysed in Luprome and showed lupanin, hydroxylupanine and angustifoline, followed by isolupanine and isoangustifoline as prominent alkaloids. The total alkaloid content was between 1.5 and 2% of the wholemeal.

For white lupins, IPG analysed the first alkaloid contents and compositions for 5 bitter accessions from the ESKUSA material. The alkaloid levels were with values above 2-3% quite high, which was expected for these bitter accessions. The most prominent alkaloid is lupanine, with 70-85% of the total alkaloids. Further important alkaloids in the white lupin are albine, multiflorine and hydroxylupanine. This is in agreement with literature data.

Developing new germplasm and breeding tools

We have multiplied, observed and partly described, harvested, processed and weighed the grain yield of several hundred white lupin lines at ESKUSA. We did the same with a few hundred narrow-leaved lupin lines. We have collected data on earliness, ripeness (figure 19 top), synchronous seed set, health and a few other traits in narrow-leaved and white lupin field trials (e.g. lodging; figure 20 bottom). Three promising lines (Numbers 26, 57 and 80) of narrow-leaved lupins with high protein content and good winter survival and one line of white lupins have been identified for further crosses with sweet lupins.

An autumn sowing has been adopted at UNIVPM. In addition to six T-core lines from the INCREASE project that have been used as control lines, 3 control lines kindly provided by the Società Produttori Sementi were added. Moreover, one additional landrace was considered as control line, that we have previously collected in the local area of cultivation of Monte San Vito (Ancona, Marche region, Italy). The emergency rate (95%) was recorded at the 15 December, and all the plants displayed normal growth. At the beginning of March, during the recording of the 1st flower stage, several plots suffered a fusarium attack, that damaged plants. However, resistant plots have been recorded (i.e., where none of the 3 plants displayed signs of biotic stress), suggesting that Recanati landrace might hold resistant/tolerant alleles. This needs eventually further investigation. While none of the non-adapted controls (INCREASE T-Core lines) have successfully produced seeds, several pure lines from the RECANATI reached full maturity, although yield and plant growth has been strongly affected in some cases by the fusarium attack. Phenotyping has been performed for the date of first flower, first pod and full maturity.

Figure 19. Impressions of material, size and traits of the ESKUSA breeding programme. Top: Different maturity plots of narrow-leaved lupins. Bottom: White lupin double rows (left) and lodging (right) after a heavy storm.

We want to develop and use molecular markers to detect and follow preferred alleles in the variety development process, for example with introgressions into sweet lupin germplasm. SSR-, SNP- and RNASeq-techniques are being developed at JKI, BOKU, IPG and UNIVPM to better characterise the basic material and the breeding lines.

Some crosses have been done and lead to F2-populations; other crosses have to be done. This is an ongoing process. However, we have to connect the molecular developments closer with the variety development. At the moment the molecular tools are used as scientific tools with less application in the breeding process.

Introgression into breeding populations and cultivars

European sweet lupin varieties lack several domestication, yield, and yield-stabilising traits. The three different species have different development stages in breeding and pre-breeding, hence the work to introgress characters into adequate germplasm is at different levels.

In white lupins, we found lines with high grain yields in our first field tests. Here we want to add low alkaloid content and anthracnose tolerance and cross with sweet varieties (IPG, ESKUSA).

In Andean lupins we have still trouble with seed multiplication due to a very late and long ripening period. This material is too under-developed and inadequately characterised to start with crosses or introgressions of characters in advanced germplasm. Well-adapted material is not available.

In narrow-leaved lupin we have to add several agronomic traits to sweet cultivars (ESKU, JKI) and - even more important – to winter types. We want to introgress the following traits: Domestication genes from Australian cultivars (JKI); high protein content (IPG, UNIVPM); seed size (IPG); stable low alkaloids (JKI, IPG); calcareous soil tolerance with higher Fe-uptake (ESKU, UNIVPM, IHU); tall growth / lodging tolerance (ESKU, UNIVPM); plenty pods due to basal branching (ESKU, RU); non dehiscence (RU); synchronous ripeness (ESKU, UNIVPM, IPG); fungal pathogen resistance genes (BOKU, JKI); grain-processing quality (ESKU); stable yield (all partners). Our ideotype includes several of the above traits, which we already observed in different accessions or lines and which have to be combined.

This work runs mostly according to the plan with very positive changes due to the recent discovery of winter types. First sharing of crosses between ESKU and JKI in narrow-leaved lupins has happened. We have not yet managed to cross all above-mentioned traits in narrow-leaved lupins. Some of them have to be analysed genetically or we have to develop a reliable test-system before crossing. However, the partners are expecting further crosses to be done continuously. Further crosses within the white lupins will be shared between ESKU and IPG, based on field results from 2024 and winter survival data. In Andean lupin we still have a lack of seeds due to very late and heterogenous ripening and disease attacks, so no material could be distributed for field tests in our testing network. IPG has started to search for winter survival in this species, too. Target traits including tolerance to alkaline soil, alkaloid content and nutritional value.

Field testing cultivars for demonstration and release

Our first field testing for demonstration and release delivered some data from the locations in Groß Lüsewitz (JKI) and Parkstetten (ESKUSA). In Poznan (IPG) the white lupin and narrow leafed lupin trials (Figure 21) were destroyed by a heavy fusarium attack. The same happened to the trials in Italy (UNIVPM), so that there are no grain yield data available from those locations.

In general, our material is not ready for cultivar release. Our field trials serve as demonstration for our activities and for recognition of specific traits, which is extremely useful at our actual stage of cultivar development.

Figure 20. White lupin field trial at IPG Poznan, mostly destroyed by a heavy fusarium attack

Figure 21. Narrow leafed lupin field trial at IPG Poznan, mostly destroyed by a heavy fusarium attack

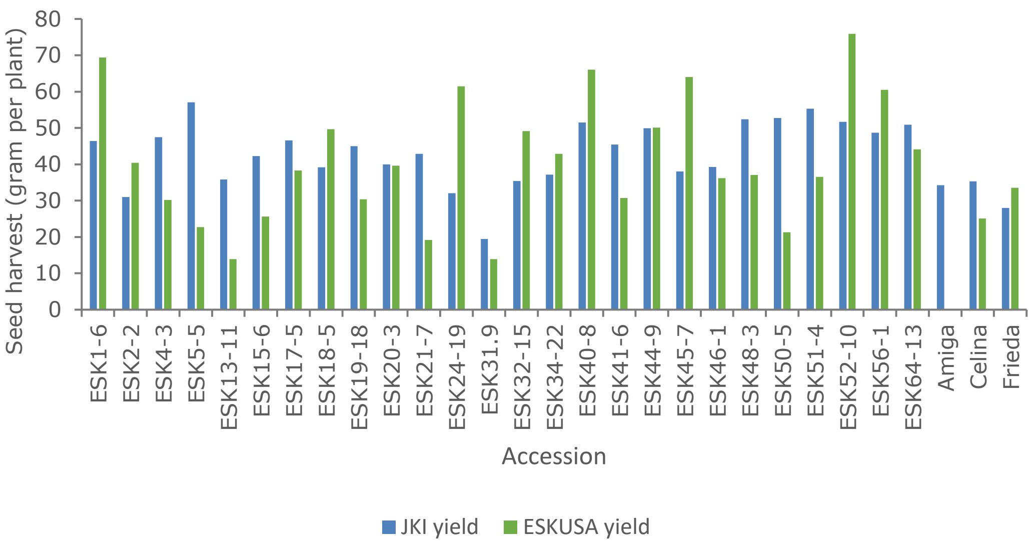


Figure 22. Seed yield (g/plant) of selected white lupin accessions grown in rows at two locations

Phenotyping of Andean lupins has been performed following a common protocol between UNIVPM and IPG, including agronomic, architectural, phenological and morphological traits, such as: date of sowing and of emergence, number of emerged plants, date of first bud, first petal, first flower (detected on a single plant in the row, and at least 50% of the plants displaying the traits bases), both on main stem and on lateral branches, date of full and end of flowering, date of pod setting and full ripening/maturity (detected on a single plant in the row, and at least 50% of the plants displaying the traits bases), on both main stem and lateral branches, flower colour, presence of stem pigmentation, growth habit, number of producing plants/survival/success ratio, number of pods, number of seeds, weight of seeds (total and average per seed), susceptibility to pod shattering (evaluated in the post-harvest during the threshing). Yield and production have been evaluated for the main stem and the lateral branches separately.

UNIVPM is currently analysing the data to identify the best performing lines, and to test them through agronomic trials on 5 m^2 plots, based also on available seeds. Below a few images of the field and plants at different developmental stages (Figure 23).

Figure 23. Field test of Andean lupin and plants in different development stages at Osimo, Italy

UNIVPM also carried out a field trial on a set of SSD purified accessions provided by the INCREASE project derived from a local white lupin landrace from Central Italy known as "Recanati" as a potential source of genotypes tolerant to heavy and alkaline soils. This local variety has been grown traditionally in the surrounding area of the Recanati city (Macerata, Italy), a region characterized by alkaline and heavy soil, that is a typical feature encountered in the soil of the entire Marche Region. Its value resides also on the local utilization (i.e., the population cultivation by farmers has been documented through generations). This includes adaptation to traditional agricultural practices, without formal breeding interventions. Its traditional cultivation requires fewer inputs, such as fertilizers and pesticides, making it a more sustainable option for farmers, particularly in marginal environments. We tested selected genotypes from this locally adapted population through field trials in the CREA research centre (Azienda Settempedana – Osimo, Italy). Before this trial, we had already partially characterized this landrace during previous seed multiplications, done also to obtain SSD lines. A single plant image (Figure 24) from the population RECANATI is provided.

Figure 24. Single plant image from the population RECANATI

In 2023/2024 an agronomic trial with 3 replicated row blocks of 44 pure lines (3 seeds/row) has been performed, with 10 control lines replicated to have a completely repeated randomized block of 60 row/plot.

Network and support

Partners beyond the project

The nine LUPIC partners form a core unit for R&D around lupin species in the project. However, our IC is open for stakeholders beyond the project. Scientific institutes, breeders, seed merchants and food processors are welcome to join our community and support our activities. We have developed or deepened collaboration with the following:

Scientific Institutes

- University of Rostock (root exudates, root microbiome, *Bradyrhizobia*, soil borne fungal diseases)

- University of Münster (iron uptake and -metabolism)
- Fraunhofer IME (gene editing techniques)
- Heinrich Heine University Düsseldorf (iron uptake and -metabolism)
- Technical University of Munich (Technical use of lupin protein; microbial degradation of alkaloids)

Breeders
- Cerience
- Saatzucht Steinach
- DSV

Seed merchants:
- Feldsaaten Freudenberger (seed multiplication, licensing of varieties)

Food processors
- Lupino AG (protein isolates from narrow-leaved lupins)
- Loffee (Lupin coffee from narrow-leaved lupins)

Accompanying projects, activities, and planned actions

The below listed projects deliver basic lupin material from genetic resources for our breeding programmes as well as breeding tools for specific traits and/or processing tools (Figure 25):

LupiRhiz: (High-performance varieties of narrow-leaved lupin (*Lupinus angustifolius*) by unlocking the genetic potential of root traits and rhizosphere interactions) draws on a range of genetic resources and tools to provide data for breeding purposes to transfer the root-related traits from bitter forms to low-alkaloid forms of narrow-leaved lupin and combine them with important domestication and quality traits. Results also include molecular markers for domestication traits such as pod shatter resistance.

Luprocess: (Resource-efficient production of high-quality proteins and fibres from narrow-leaved bitter lupins for (vegan) human nutrition,) aims to obtain high-quality, largely native and functional protein and fiber fractions from narrow-leaved bitter lupin for human nutrition.

Luprome: (Unlocking the potential of narrow-leaved bitter lupin (*Lupinus angustifolius* L.) as a new protein source for human nutrition) developed a process based on sophisticated membrane technology to remove water-soluble alkaloids and produce protein of *Lupinus angustifolius* of highest quality, quantity and on a sustainable basis.

LupiAlk: (Utilisation of genetic resources of narrow-leaved lupin (*Lupinus angustifolius* L.,) to secure low alkaloid content for feed and food usage) focused on the development of new varieties with environmentally stable, low alkaloid contents and high yield potential at the same time.

INCREASE: (Intelligent Collections of Food Legumes Genetic Resources for European Agrofood Systems) uses phenotypic descriptors and molecular tools to identify a set of single seed descent (SSD) lines with a broad genetic variation in white lupin and Andean lupin.

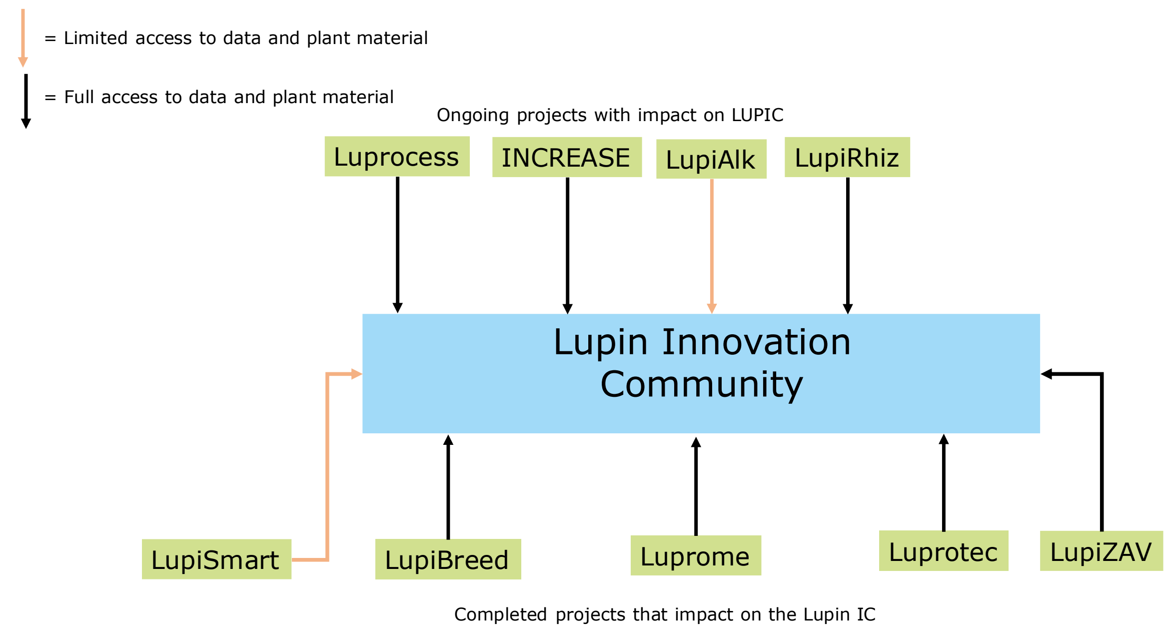


Figure 25. The Legume Generation Lupin Innovation Community provides a route to plant breeding for a range of projects.

Financing breeding and breeding research

We now have a clear picture of a lupin plant ideoptype. Our goal is a winter lupin with sweet kernels and a strongly improved grain yield and yield stability. This goal cannot be reached within a short-term approach of two or three years. We estimate a time frame of 10-15 years to reach a remarkable progress. For this period, we need support: support to transport our ideas and financial support of our interactive network. The financial support can be composed of public money from traditional three-year research projects to solve specific problems in our approach. The financial support can also come from stakeholders that use our future cultivars. Stakeholders like seed merchants, farmers, feed- and food processors. We aim at these stakeholders to engage them in the development of better and competitive lupin cultivars fur European agriculture. We have interested a few of these stakeholders in our activities and we would like to extend this interest to more supporters.

Conclusions and outlook

Since the start of the Legume Generation project in 2023, we have made good progress in boosting the breeding of lupins. We have successfully formed a unit of breeding research, pre-breeding and breeding. We have interested seed merchants and food processors in our activities. Our network is open for further stakeholders.

We have developed - and in some cases we are still developing - breeding tools that are necessary to analyse and describe our traits and germplasm. We have identified a ”game-changer” with the trait winter survival in three lupin species for the large lupin production areas in Europe that helps to address climate change challenges in lupin cropping. We have characterised genetic resources, phenotyped advanced breeding lines and pre-breeding plant material that is able to deliver further progress in plant breeding and finally serve farmers’ needs.

We follow different strategies in developing sweet lupin cultivars or alternatively processing strategies for feed- and food-use of bitter lupins.

We aim at a financially self-supporting system, knowing that for the coming years we need support from public money. Royalties from cultivars and income from contract production will help to finance the activities in future.