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Lupins

An update on lupin crops and their potential

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Front cover:
A field of Australian narrow-leafed lupin, variety Coyote.
Photo courtesy Lars Kamphuis.

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EDITORIAL

This special issue of Legume Perspectives focuses on the grain legume species of the genus Lupinus commonly known as lupins. Lupins are a member of the Fabaceae (or Leguminosae), widely distributed and the third-largest plant family by the total number of species identified. This makes the species interesting to study from an evolutionary point of view. Despite the large number of species only four have been domesticated. Nevertheless, lupins play an important role in agricultural and ecological systems and are gaining an interest as a human health food or food ingredient. An increased research interest was evident at the International Legume Society Conference held in 2019 in Poznan Poland, where 7 oral and 22 poster presentations focused on lupin research. This led to the idea of having a focus issue on lupins. On behalf of the Legume Society, we wish to thank the authors of the articles in this issue for their concise and well-written contributions.

Lars Kamphuis
Karam Singh
Managing Editors of
Legume Perspectives issue 22

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Carte blanche to...

Lars G. Kamphuis
Karam B. Singh
CSIRO Agriculture and Food and the Centre for Crop and Disease Management, Curtin University

Cultivation of lupins is recorded as early as 2000 BC in Egypt and the crop was also grown in Greek and Roman times. Despite these historic records of lupins in agriculture and human consumption the first lupin species wasn't fully domesticated till the 1960s. To date only four species have been domesticated, namely narrow-leafed lupin (Lupinus angustifolius), white lupin (L. albus), yellow lupin (L. luteus) and pearl lupin (L. mutabilis), with the latter two being niche crops. In agriculture lupins can be used as both a grain and fodder crop, with Australia, Poland and the Russian Federation the largest producers of lupins. The majority of lupins produced end up as animal feed with only a small proportion being used for human consumption. Nevertheless, there is an interest in using lupin grain in human consumption due to its reported health benefits that can help combat obesity and diabetes.

In this special issue we focus on breeding efforts of narrow-leafed and white lupin in Australia and Europe and highlight the current genetic and genomic resources developed for these species. The genetic diversity of the Lupinus species is described and an overview of the INCREASE project to conserve and manage lupin genetic diversity is presented. Furthermore, the current knowledge status of flowering time and other phenology traits in lupins is discussed. The nutraceutical properties of lupin seed storage proteins and research efforts to promote lupins as a food or food ingredient are also presented.

We hope the information in this special issue will increase awareness for the species and its potential to become a protein rich superfood.

Left to right: Dr. Lars Kamphuis and Prof. Karam Singh.

The diversity of lupin species

Colin Hughes[1*]

Abstract: A synopsis of the species diversity in the genus *Lupinus* is presented, incorporating information about the geographical distribution of the genus, including a distribution map, data on relationships of species in the form of a phylogeny of the genus showing the major clades, plus an overview of chromosome groups, and variation in species climatic affinities, life history strategies and functional traits.

Key words: growth form, phylogeny, species diversity, taxonomy, unifoliolate leaves

With five fully or partially domesticated species, the genus *Lupinus* includes an important suite of congeneric pulse crops. Three of these – *L. albus* and *L. luteus* in the Mediterranean region, and *L. mutabilis* in the Andes – were domesticated by indigenous farmers within these regions, probably at least two millennia ago and are still grown as regionally important pulse crops (see e.g. (1,2)). A fourth species, *L. angustifolius*, also originating in the Mediterranean, has been the primary focus of modern domestication and crop breeding and is the most important crop species in the genus (3). Finally, *L. polyphyllus*, a perennial species from the Rocky Mountains of western North America, has been grown and crossed with other species as a garden ornamental, and is now the focus of de novo domestication as a possible crop plant (4).

Despite this diversity of congeneric *Lupinus* crops with their independent parallel origins of domestication on different continents (Figure 1), domestication and breeding of *Lupinus* has tapped in into <2% of species diversity in the genus, which comprises close to 300 species. These 300 or so species are distributed across four continents, span boreal to tropical latitudes, 5000 m of elevation, and Mediterranean to temperate, boreal, subtropical, and tropical alpine climates (Figure 1), and comprise extremely diverse growth forms from perennial woody shrubs and small trees to herbaceous annual plants (Figure 2). This rich diversity of species, traits and ecologies has potential to further enhance the qualities of lupin crops through crossing and hybridization of domesticates with crop wild relatives with promising attributes and traits, and through de novo domestication of promising wild species directly. In this article, I provide a brief synopsis of the diversity of species of *Lupinus*, their phylogenetic relationships, chromosome groups, distributions and attributes.

The estimated number of species in the genus *Lupinus* is between 285 and 300. While the Old World, North American, and Argentinian / Brazilian species are well documented in a series of flora treatments and taxonomic accounts (e.g. (5), Old World; (6), Intermountain Flora, U.S.A.; (7), Argentina), considerable uncertainty remains over the taxonomy, species delimitation and species numbers across the rest of the genus, especially in the Andes and Mexico where no recent or complete taxonomic accounts are available. Work is in progress to complete a taxonomic revision of the large cohort of c. 100 species in the Andes (Hughes, in prep.).

A robust phylogenetic framework has been established for the genus (Figure 3; e.g. (8)). This phylogeny is strongly structured geographically: the Old World species form a group that is sister to a small clade of unifoliolate-leaved species from Florida and the south-east U.S.A; these two clades together are sister to a much larger New

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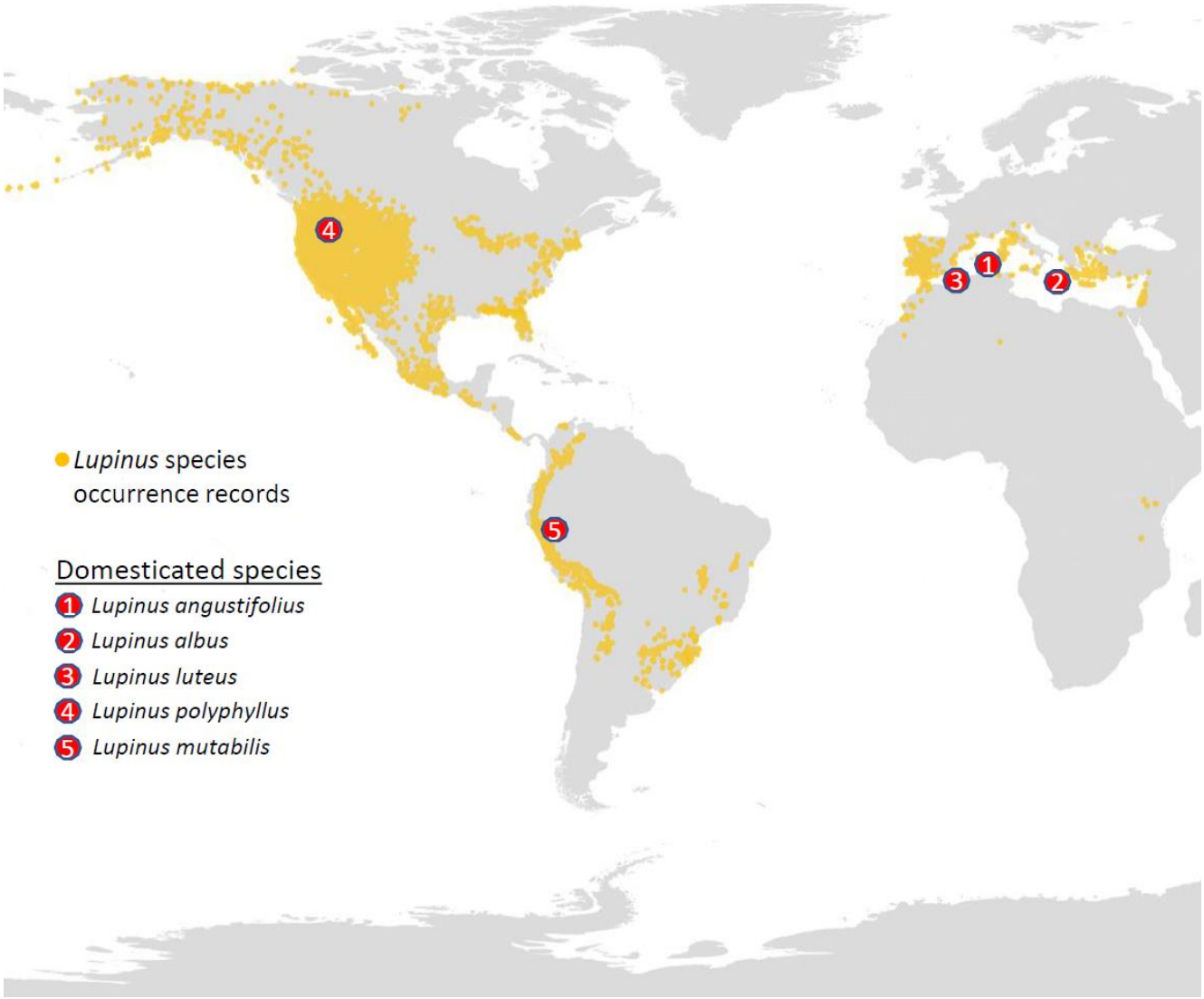

Figure 1. Global native distribution of *Lupinus* species, showing the regional locations of the origins of domestication of the five *Lupinus* crop species. Data are species occurrence points from Cabrera (unpublished data).

World clade comprising the majority of the species (c. 280 spp.) in the genus. This species-rich New World clade is divided into two robust sister groups: an eastern New World, 2n = 36 (32, 34) chromosome clade distributed in the southern U.S.A. and eastern South America comprising c. 35 predominantly lowland species, and a larger western New World, 2n = 48 chromosome clade distributed from Alaska to Chile comprising c. 190 species. This western New World clade has diversified prolifically, especially in montane habitats in the Rocky Mountains, the Sierras of Mexico and Central America, and the Andes in the form of a large evolutionary radiation (8). The domesticated species are distributed across three of these clades (Figure 2) showing that these different *Lupinus* species were domesticated independently from different progenitors on different continents (Figure 1).

The majority of species of *Lupinus* are characterised by digitately compound leaves, which represent the ancestral condition in the genus. Unifoliolate leaves, i.e. leaves reduced to a single leaflet, in some cases also becoming sessile, are derived twice independently within the genus, once in a small clade of c. five species in Florida and the south-east U.S.A. and a second time within the lowland eastern S. American clade of c. 15 species in southern and central Brazil, Uruguay and Argentina. This repeated derivation of unifoliolate-leaves provides a striking example of convergent evolution of lineages apparently adapted to similar fire-prone savanna habitats with very nutrient-poor soils, in the pine barrens of the south-east U.S.A and the Cerrado and other grasslands in eastern S. America.

There is a striking contrast in ages between the Old and New World species. The species-poor Old World clade (c. 14 species) shows deep (3-8 million year, Myr) divergences between species and great variation in chromosome numbers (2n = 32, 34, 36, 38, 40, 48, 50, 52), with just the rough-seeded nested subclade diverging more recently across the Mediterranean basin. In contrast, the large western New World montane perennial clade spanning the Rocky Mountains, the highlands of Mexico and the Andes is much more species-rich,

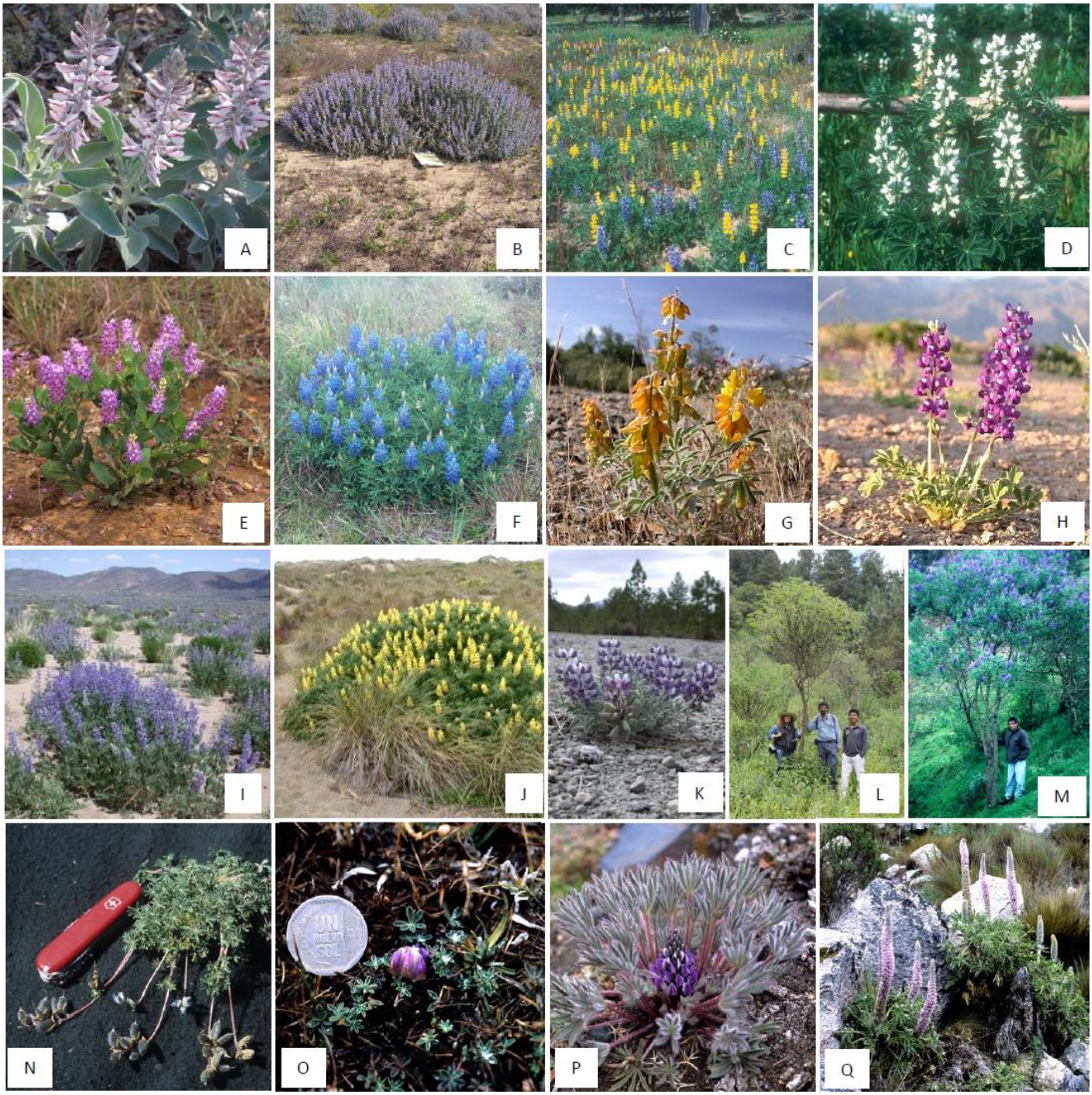

Figure 2. ***Lupinus*** **species diversity, illustrating variation in growth forms, habitats, inflorescences, flower colour and leaves across the genus. A.** ***Lupinus aridorum*****, on pine barren sand ridges in central Florida showing unifoliolate leaves; B.** ***L. cumulicola*****, on pine barren sand ridges central Florida; C.** ***L. luteus*** **and** ***L. micranthus*****, two Old World annual species growing together in cork oak forest, southern Spain; D.** ***L. albus*** **cultivated as a pulse crop in southern Portugal; E.** ***L. crotalarioides*****, a perennial subshrub growing in cerrado (savanna) near Brasilia, Brazil showing the unifoliolate leaf condition; F.** ***L. uleanus*****, a perennial subshrub growing in upland grasslands of Santa Catarina State in S. Brazil; G.** ***L. citrinus*****, an annual species in California, U.S.A.; H.** ***L. odoratus*****, an annual species in California, U.S.A.; I.** ***L. nevadensis*****, a perennial subshrub from the southern Rocky Mountains; J.** ***L. arboreus*****, a perennial woody shrub, on coastal sand dunes, California, U.S.A.; K.** ***L. lepidus*****, a dwarf high elevation perennial subshrub, Rocky Mountains, California, U.S.A.; L.** ***L. jaime-hintonianus*****, a perennial treelet to 4-5 m ht, Sierra de Miahuatlán, Oaxaca, southern Mexico; M.** ***L. semperflorens*****, a perennial treelet to 4-6m ht, in the mid-elevation Andes, Cajamarca, Peru; N.** ***L. chlorolepis*****, a dwarf prostrate perennial in montane forests, Yungas, Bolivia; O.** ***L. pickeringii*****, a minute, prostrate perennial, the inflorescence with a single whorl of flowers, in the high elevation Andes at 4800 m, Junin, Peru; P.** ***L. annaneanus*****, a dwarf perennial acaulescent rosette at 4750 m elevation, La Paz, Bolivian Andes;** ***L. weberbaueri*****, a large perennial acaulescent rosette, with large swollen inflorescences with 500+ flowers, at 4700 m elevation in the Cordillera Blanca, Ancash, Peru. Photos courtesy of Edwin Bridges and Steve Orzell (A & B), Colin Hughes (C-F & M-Q), Chris Drummond (G-K); Guy Atchison (L).**

much younger and has a uniform 2n=48 chromosome complement. This large western New World super-radiation culminated in the Andes where c. 100 species evolved within the last < 2.5 Myr, presenting an exceptionally recent and rapid evolutionary radiation, which has emerged as a model for studying rapid recent plant diversification (9, 10). These contrasting species diversities, ages and chromosome complements have important implications for interspecific hybridization and breeding involving crop wild relatives. In the western New World clade there are c. 190 species of potentially high interspecific crossability, while crossability among the deeply divergent Old World species is very limited and enhancing diversity must rely mainly on intraspecific genetic diversity.

The diversity of growth forms within the genus *Lupinus* is striking (Figure 2). Both annuals and perennials are found, with perenniality, including secondary woodiness, evolutionarily derived multiple times within the genus (11), but most notably subtending the large western New World radiation, where perenniality and occupation of montane habitats coincided with a shift to higher rates of species diversification compared to the closely related grade of annual lineages centred in the lowland Mediterranean climate habitats in California (8). While the annual species display limited variation in growth forms, the perennials encompass very diverse plant habits including herbaceous perennials that die back to the ground each year and are especially prevalent in the Rocky Mountain clade, woody perennials that can be prostrate, small or larger shrubs, or small treelets (at least three treelet species are known – *L. semperflorens* and *L. interruptus* in the northern Andes and *L. jaime-hintonianus* in the highlands of southern Mexico – see Figure 2 L & M), and a suite of unusual acaulescent high elevation perennials (forming rosettes of long-stalked leaves and often with large swollen inflorescences – see Figure 2 P & Q).

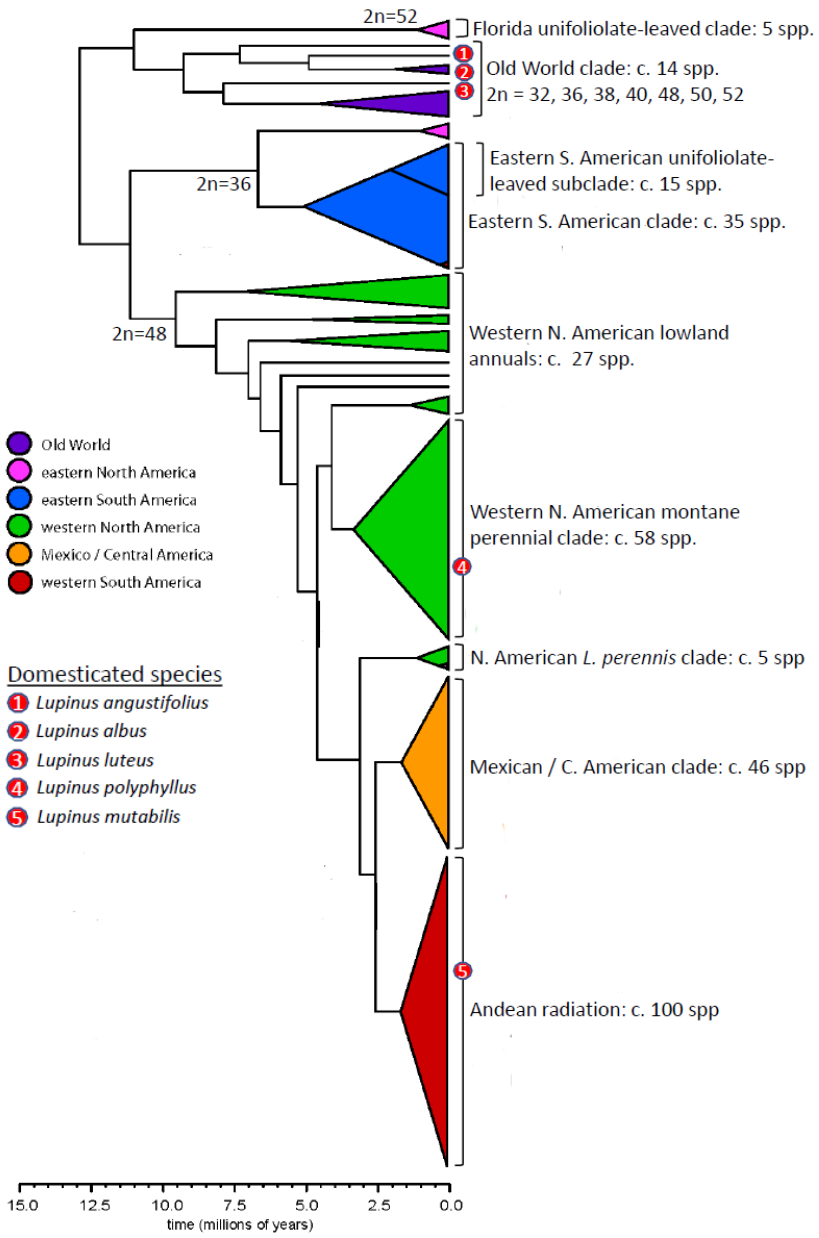

Figure 3. Time-calibrated phylogeny of *Lupinus* modified from (8) based on DNA sequences of nuclear [internal transcribed spacer (ITS) and *cycloidea*] and plastid loci. Clade size is proportional to the approximate number of species in that clade. Clade colours correspond to geographical distributions, as indicated in the legend. Chromosome numbers characteristic of particular clades, and the phylogenetic placements of the five crop domesticates are indicated.

The wider characteristics of the vast majority of *Lupinus* species remain poorly known, especially in terms of traits that are important for crop development and breeding. What is clear though, is that there is tremendous plant functional trait and ecological diversity available among the wild species in the genus. First, the genus spans a remarkable array of latitudes and climates, with an abundance of species adapted to cooler more temperate or even boreal conditions than those of the mainstream currently domesticated species which are concentrated in Mediterranean-type climates, suggesting significant potential to domesticate a cool temperate crop lupin, such as *L. polyphyllus*. Second, there is massive variation in inflorescence size, number of flowers and fruit set among species. In extreme cases, inflorescences with > 500 flowers and 100s of pods per inflorescence suggest ample scope to enhance seed production in crop lupins. It is also clear that many lupin species are well adapted to very nutrient-poor soils and in extreme cases to highly dystrophic acidic nutrient-poor tropical savanna soils (in the Cerrados of S America), or deep, almost pure silicaceous sands (in the pine barren sand ridges of Florida), again suggesting the possibility to extend the potential of crop

lupins to more marginal agricultural soils. The mainstream lupin crop domesticates are annuals (or near-annual in the case of the Andean crop lupin, *L. mutabilis*), but the majority of species in the genus are short-lived perennials, offering scope to develop perennial lupin crops, as envisaged for *L. polyphyllus*, an herbaceous perennial from the Rocky Mountains. One could also envisage scope to develop lupins as tree crops for agroforestry in mid-elevation tropical mountain agricultural systems, once favourable seed traits were introduced to treelet species such as *L. semperflorens* from the northern Andes, perhaps via hybridization with *L. mutabilis*.

There seems little doubt that the rich diversity of lupin species offers a treasure trove of traits and opportunities for future crop development and breeding.

Acknowledgements I thank Victoria Cabrera for the distribution map in Figure 1, and Guy Atchison, Edwin Bridges, Steve Orzell and Chris Drummond for images used in Figure 2.

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Opportunities to improve the value of narrow-leafed lupin in Australian farming systems through breeding

Matthew K. Aubert[1], Dion Bennett[1]

Abstract: Lupins are a valuable part of the Australian farming system due to their roles in atmospheric nitrogen fixation, as a source of protein for animal feed, and as a disease break in crop rotations. Recent changes in production levels can be attributed to increased adoption of canola in farming rotations, increased canola prices, volatile lupin prices and increased prevalence of blue lupin weeds. Future breeding efforts should focus on improving rates of genetic gain for grain yield through technologies such as whole genome selection, creating value for lupins by introducing new herbicide tolerances to aid farming systems, and addressing new market opportunities such as human consumption.

Key words: narrow-leafed lupin, lupin breeding, lupin production, lupin value, Australia

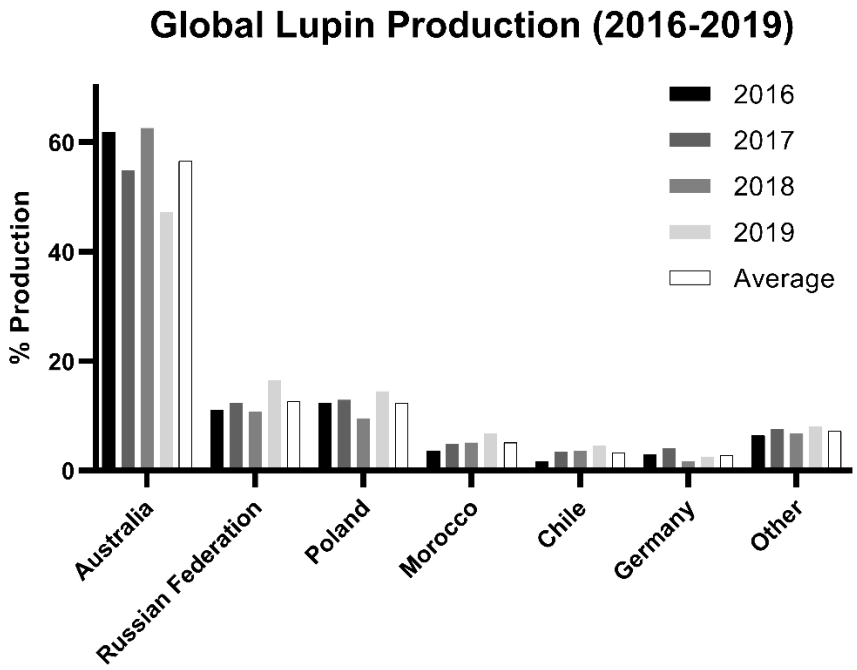

Figure 1. Global lupin production from 2016 to 2019. Includes all species of lupin. Australia on average produces 57% of global lupin production (4).

Past breeding efforts

Domesticated narrow-leafed lupin (*Lupinus angustifolius*) (NLL) is a relatively new crop. In major crops such as wheat, barley and rice, domestication has occurred over thousands of years, and continuous breeding efforts have allowed adaptation to many environments across the globe (1). However, the domestication and breeding of NLL is relatively recent, having only occurred within the last 100 years. This began in Germany in 1928 when Dr. R. von Sengbusch identified two natural mutants with low alkaloid content. Ordinarily, wild lupins have high alkaloids levels, which make them toxic for human and livestock consumption. Since the identification of low alkaloid mutants, subsequent mutants for reduced pod shattering, lower vernalisation requirement and early flowering were identified in 1960 by Dr. J. S. Gladstone (2, 3). By incorporating these mutations into a single cultivar (cv. Uniharvest), NLL was transformed into a modern crop in Western Australia. Since then, breeding efforts have focussed on other key traits to allow for further adaptation to Australian environments and farming systems, including disease resistances (such as anthracnose (*Colletotrichum lupini*), phomopsis (*Diaporthe toxica*), grey leaf spot (*Stemphylium botryosum*), cucumber mosaic virus), herbicide tolerances, soft seeds, white flower colour and speckled seed coat colour. These past

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breeding efforts have resulted in wide NLL adoption across Australia, particularly in Western Australia, where on average 57% of the global NLL crop is produced (4, Figure 1).

Lupin production in Australia

NLL is an important rotational crop to Western Australian growers, especially in the northern agricultural (NAR) region of WA, where NLL are well adapted to the high annual rainfall and sandy acidic soils. However, over the past few years, substantial improvements in overall lupin production have not been observed. This coincides with a steady decrease in NLL production in the north, which has declined on average ~2.4% each year. Interestingly, NLL production within the central agricultural region has steadily increased on average by ~1.3% each year and, as of 2020, the northern and central regions contributed relatively equal proportions of NLL production (5, Figure 2B).

Challenges for lupin production in WA

Three key factors limiting the value of NLL in WA farming systems have emerged in recent years. Firstly, over the past few years, canola production has proven to be more profitable, as well as offering alternative herbicide tolerances contributing to greater adoption by growers in the NAR, at the expense of NLL. Secondly, there has been an increase in prevalence of blue lupin weeds (*L. cosentinii*), in the northern region. With no selective herbicide options for use in NLL crops, as well as the persistence of seeds in the soil bank, growers are reducing their areas sown to NLL, mainly due to a lack of proper control options being available. Finally, particularly in drier seasons, NLL grain yields can be very low, resulting in large variations in profitability of lupins from season to season.

Breeding for improved NLL value in WA farming systems

To improve NLL production in Australia, future breeding efforts need to focus on adding value to lupins, so growers see them as economically viable options. One solution that Australian Grain Technologies (AGT), current lupin breeder in Australia, is working towards is to create more genetic tolerances for alternate herbicide groups within NLL. Currently, NLL have tolerance to herbicides such as Metribuzin, Brodal and Simazine. However, reliance on these herbicide groups has resulted in selection for tolerance in weed populations. In addition, none of these herbicides are selective for control of blue lupins in NLL crops. There is no evidence that NLL possesses the genetic variation for tolerance to any other herbicide groups, so a mutation population has been developed with the aim of identifying individuals carrying the genetic variation required for tolerance to new herbicide groups.

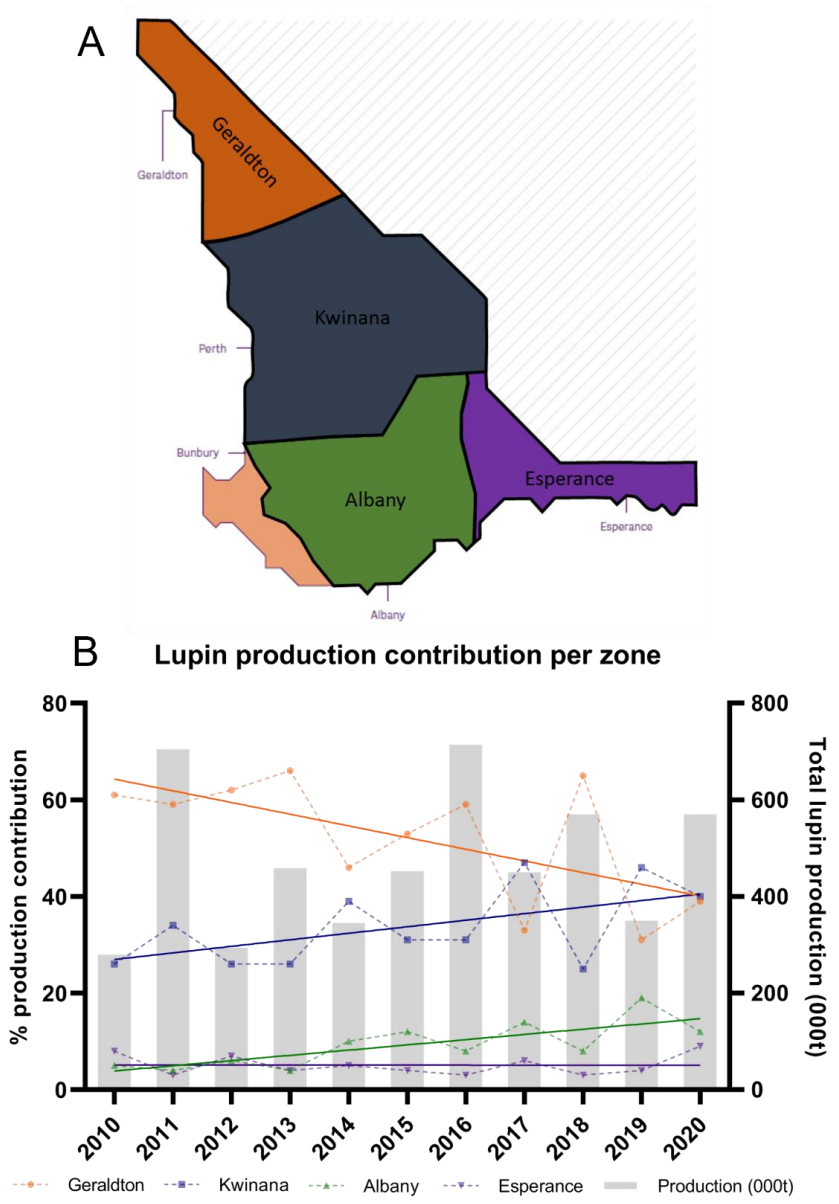

Figure 2. (A) Map of Western Australia's four agricultural regions. (B) Lupin production (NLL and Albus lupin) per zone over the past 10 years (5).

In 2021, a pulse single nucleotide polymorphism (SNP) chip was developed in Australia containing chickpea, lentil, field pea, faba bean and NLL (6). Advancements in SNP chip technology have allowed crop breeding programs to adopt modern breeding techniques (7, 8). However, to date there has been little adoption of this technology in pulse breeding programs and by using this tool the breeding program will be able to utilise whole genome selection (GS) to compliment current breeding strategies in the NLL program. AGT has

successfully applied GS in its wheat and barley breeding programs and piggybacking on this knowledge will enable the NLL program to improve rates of genetic gain for traits that are slow, expensive and/or low throughput to measure phenotypically, such as alkaloid content, disease resistance and particularly grain yield.

Lupin products for human consumption is a burgeoning market and represent a significant opportunity for NLL to improve its price as a commodity. Research into alkaloid distribution, alkaloid components, protein accumulation, allergen reductions as well as starch and oil alterations offer opportunities to identify genetic variation and selection methodologies that improve the value of NLL. Several private lupin food companies are conducting their own research into what makes their lupin products attractive to consumers, and there are numerous opportunities for the breeding program to collaborate with them to ensure their requirements for these traits are selected in future cultivars.

Perspectives

The relatively short period of lupin domestication and breeding has required a tremendous amount of effort. International collaboration, together with the identification of natural mutants, underpinned all current lupin cultivars available today. However, the lupin industry still faces many challenges that will require further breeding efforts to see advancements in lupin production. With an improved overall lupin value, whether that be through increased attractiveness of lupin for human consumption, or increased attraction within the farming system through added herbicide control options or increased commodity prices, the lupin industry is expected to benefit into the future.

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White lupin breeding in Italy

Paolo Annicchiarico[1*], Nelson Nazzicari[1], Barbara Ferrari[1], Tommaso Notario[1], Margherita Crosta[1], Luciano Pecetti[1]

Abstract: Italian white lupin breeding requires adaptation to terminal drought stress and occasional low-temperature stress in winter exacerbated by climate change. Sufficiently low alkaloid content, adaptation to moderately calcareous soil, and tolerance to anthracnose are other important targets. We generated phylogenetic and adaptive information on global landrace genetic resources, identifying agronomically-outstanding parent material used to broaden the crop genetic base for inbred line selection or development of evolutionary populations. Dwarfness may have an interest for indeterminate material grown in favourable environments. Large phenotyping platforms ensuring the reliable assessment of drought or cold tolerance, and genome-enabled prediction of grain yield in specific environments and other traits, are key components of future selection strategies.

Key words: drought tolerance, genomic selection, landrace genetic resources, plant adaptation, plant architecture

Current and perspective cultivation

White lupin (*Lupinus albus* L.) cultivation in Italy dates to the Roman Empire, where its grain had an important role in the diet of people in Rome and the Roman army. While hardly achieving a few thousand hectares of cropped land nowadays, this species has on-going interest for both feed and food use. For feed, it is a valuable high-protein alternative to soybean, whose cultivation in Southern Europe may increasingly be limited by severe drought and scarcity of irrigation water during its summer crop cycle. Indeed, white lupin displayed greater crude protein yield per unit area than other autumn-sown rainfed crops such as pea, faba bean or narrow-leafed lupin in Italian environments with subcontinental or Mediterranean climate (1). White lupin is also an excellent ingredient for vegetarian food due to a combination of favourable nutritional, technological and sensory characteristics, besides possessing nutraceutical properties that can contribute to prevent and possibly

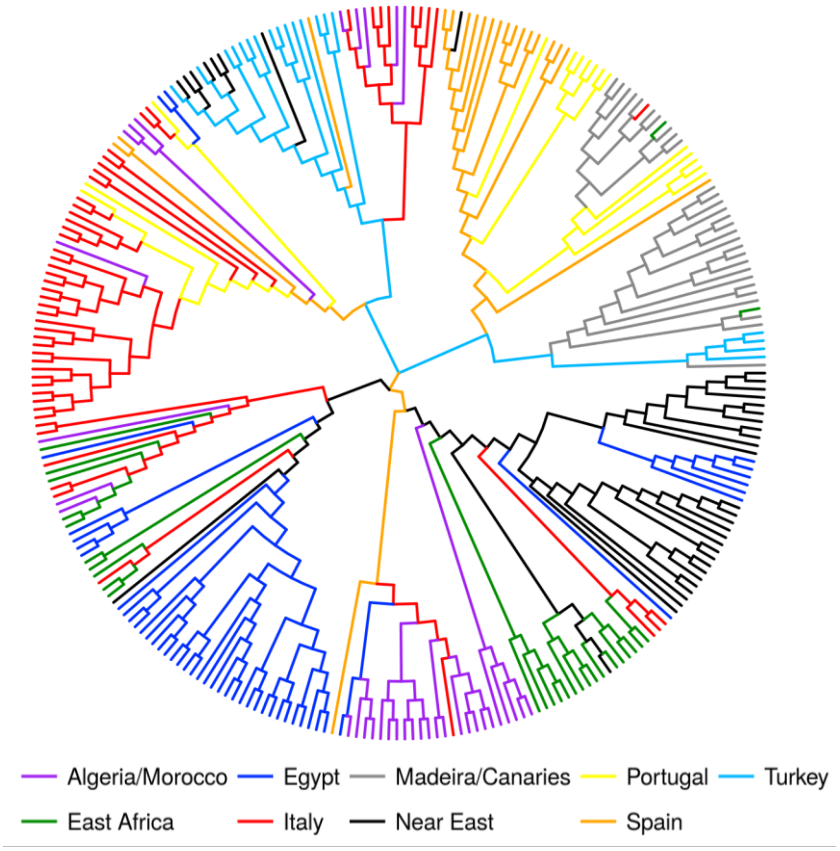

Figure 1. Phylogenetic tree of white lupin landrace accessions belonging to nine major historical cropping regions according to Nei's distance based on 6,198 polymorphic SNP markers issued by *ApeK*I-based genotyping-by-sequencing.

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treat type-2 diabetes mellitus, hypertension and cardio-vascular diseases.

Landrace genetic resources: variation and exploitation

White lupin genetic resources are limited to the primary gene pool and include essentially all landrace germplasm. Landrace genotype molecular diversity according to the phylogenetic tree reported in Figure 1 indicated a fairly distinct separation of germplasm from the northern Mediterranean shore (Italy, Turkey, Spain, Portugal) or the Atlantic Islands from that of the southern shore (Near East, Egypt, Maghreb) or Eastern Africa. These results, along with the outstanding genetic diversity displayed by Near East germplasm (unpublished data) and the fact that various genotypes from Near East clustered with germplasm of the northern Mediterranean shore (Figure 1), suggest a distinct crop introduction path across the two Mediterranean shores starting from material domesticated in the Near East. Additional data under generation for a few additional regions (e.g., Greece) will potentially help provide support for this hypothesis. The concurrent molecular characterization of sweet-seed cultivar germplasm suggested that modern breeding exploited quite a modest portion of the biodiversity represented by landrace germplasm (unpublished data), probably because of breeders' emphasis on crossing work including only sweet-seed (i.e., low-alkaloid) parent germplasm.

The grain yield evaluation of a world landrace collection in autumn-sown subcontinental-climate or Mediterranean-climate environments of Italy and a spring-sown environment of western France suggested an ecological classification of landrace regional pools for European breeding that differs from the molecular classification in Figure 1, owing to the overwhelming importance of phenology as a driver of regional adaptation (2). This work also revealed the outstanding agronomic value of several landrace accessions and some unexploited germplasm sources (for example, that from Madeira and Canary Islands) relative to reference commercial cultivars (3). Other studies highlighted the superiority of some landraces over commercial cultivars for grain yield under severe drought (4), which has increasing interest because of the changing climate, or grain yield in moderately calcareous soil (5), which has great importance to broaden the geographic adaptation of this species.

Our results highlighted the importance of broadening the genetic base for white lupin breeding by exploiting elite landrace germplasm in crossing programmes. Within the project LIVESEED, we generated a large number of inbred lines by factorial crosses of each of four elite landrace genotypes identified by prior evaluation work with each of four elite modern genotypes. This work was also exploited to develop a composite cross population selected for low alkaloid content at an early stage, from which various populations evolved in different European regions (North and Central Italy, Switzerland, Netherlands, Denmark, France) or cropping conditions (pure stand or intercropping) are being developed. These populations could be exploited as a genetic resource and possibly as heterogeneous material introduced for cultivation in organic systems (through a mechanism that could ensure periodic control of alkaloid content).

Breeding targets and challenges

White lupin grain production in Italy is constrained on the one hand by possibly severe terminal drought stress (especially in Mediterranean-climate areas) and on the other hand by occasional low-temperature stress during winter whose effects can be highly damaging on poorly hardened plants exposed to a prior period of mild winter temperatures (6). The increasing within-season and year-to-year climatic variation increases the likelihood and impact of these stresses and hinders a reliable plant evaluation and selection for stress tolerance in agricultural environments. Because of that, we set up various managed environments for genotype evaluation under controlled drought stress levels (Figure 2) and are finalizing a large phenotyping platform for cold tolerance assessment under controlled conditions (which will include a relatively modest hardening period preceding the stress application). For the autumn-sown crop, the contrasting optima of phenology for escape from winter cold stress (late phenology) and terminal drought (early phenology) causes outstanding genotype × environment interaction (GEI) of cross-over type across subcontinental-climate and Mediterranean-climate environments of Italy (3) and poses the dilemma of breeding for wide or specific adaptation to the two climatic regions. While the latter option would maximize the genetic yield gains, the former is currently needed because of the modest crop growing area. The cultivar 'Arsenio' under registration in Italy was selected on the ground of minimal GEI across climatically contrasting regions, as reported in (1) where this cultivar corresponds to the line 7-50. We expect to achieve further progress in breeding for wide adaptation by exploiting the available genetic variation for intrinsic drought tolerance as expressed by yield of material with similar phenology (4), and intrinsic cold tolerance as displayed by young plants that have not commenced any reproductive development.

Early work by INRAE of Lusignan identified new plant architectures featuring dwarf stature to increase the harvest index, semi-determinate habit to increase the yield stability, or the combination of both characteristics. An assessment of the usefulness of these plant types for Mediterranean-climate environments based on near-isogenic lines revealed a complex yield response pattern based on results in (7) presented here for different cropping years (Table 1). Dwarfness may be positive in

Table 1. Grain yield in Mediterranean-climate environments of Sardinia of dwarf and tall near-isogenic white lupin lines belonging to indeterminate or semi-determinate plant types. Results averaged across two sowing densities (35.5 and 25.5 seeds/m^2)

	Indeterminate		Semi-determinate	
Cropping year	**Dwarf**	**Tall**	**Dwarf**	**Tall**
2006-07	2.07 a	1.44 b	1.47 b	1.81 a
2007-08	1.79 a	1.64 a	1.62 a	1.91 a
Over years	1.93 a	1.54 b	1.55 b	1.86 a

Based on results in (7). Row means within plant type followed by different letter differ at $P < 0.05$. The first cropping year was milder-winter and featured, on average, 35% taller plant stature, 37% greater aerial biomass, 30% lower harvest index, and 38% lower proportion of seeds on the main stem than the second year.

indeterminate germplasm in years favouring large biomass production (when the competition between vegetative and reproductive sinks is accentuated), while always tending to be negative in semi-determinate germplasm possibly because of excessive constraint to the plant's source ability (especially for light interception) imposed by its combination with a semi-determinate growth. While currently breeding conventional plant type germplasm, we may foresee also the future breeding of dwarf indeterminate germplasm for climatically favourable areas. It remains to verify, however, whether this plant architecture impacts negatively on the plant ability to compete with weeds or with intercropped small-grain cereals – which are characteristics of great interest for organic farming (whose importance in Europe is continuously raising).

Tolerance to anthracnose (*Colletotrichum lupini*) has crucial importance in most regions of Western and Central Europe, while being seemingly less threatening in Italy possibly because of the modest cropping area. Landrace germplasm from Ethiopia featuring moderate tolerance to this disease has been identified in Australia but its tolerance in Europe was not confirmed by recent work (8), possibly because of different isolate pathogenicity, accession heterogeneity, or different phenotyping method. Recent research in Switzerland has identified other tolerant material and molecular markers for selection (9) by evaluating a large germplasm collection under artificial conditions as described in (8).

Selecting sweet-seed material with alkaloid content below the threshold of 0.200 mg/g that is set for direct grain use as a food can be quite challenging. We observed variation from <0.100 to >0.600 mg/g in elite breeding lines, further complicated by environmental effects (10). We are investigating near-infrared reflectance spectroscopy (NIRS)-based and molecular marker-based procedures that could assist selection, for the challenging aim to distinguish contrasting alkaloid content within sweet-seed material (whereas various procedures are available to distinguish sweet-seed from bitter-seed material). Selection for greater protein content, which may be useful especially for production of high-protein feed, is justified by the fairly large genetic variation indicated by range values of 32.8-43.2% on a dry seed basis that we observed in a recent evaluation of a large set of breeding lines and can exploit pretty accurate NIRS predictions (unpublished data). We found genetic variation for the level of γ-conglutin in the grain (11), and considered a higher trait level, which is useful to produce nutraceuticals that control glycaemia, as a target for selection of our recent cultivar 'Arsenio' [indicated as line 7-50 in (11)].

Figure 2. One of CREA's phenotyping platforms used to study white lupin genotype adaptation to contrasting drought-stress levels, in which a number of genotypes are evaluated under severe stress (central managed environments) and mild stress (outward managed environments). A similar platform with contrasting soil types is used to study the adaptation to moderately calcareous soil.

Novel molecular marker-based selection tools

The development of low-cost, high-throughput genotyping techniques, such as genotyping-by-sequencing (GBS), offered the opportunity to develop marker-assisted selection procedures for key oligogenic traits such as tolerance to anthracnose (based on plant responses assessed in Australia), low-alkaloid content across sweet-seed and bitter-seed germplasm types, and phenology as determined by vernalization requirement (12). In addition, the availability of several thousand polymorphic SNP markers justified the investigation of genomic selection procedures as a means to reduce the phenotypic selection effort for polygenic traits. Two pioneer genomic selection studies have produced quite encouraging results for genomic selection or for genome-enabled identification of promising accessions in germplasm collections. One study focusing on grain yield mainly of landrace germplasm revealed intra-environment predictive ability (as correlation between observed and genomically-predicted values based on cross validations) in the range of 0.47-0.76 for five test environments (13). This study also revealed cross-environment predictive accuracy (as correlation between breeding values and genomically-predicted values when using one environment for model construction and another environment for model validation) in the range of 0.48-0.61 across pairs of distinct environments that displayed positive genetic correlation, namely, autumn-sown subcontinental and Mediterranean sites on the one hand, and moisture-favourable and drought-prone environments on the other (13). A second study focusing on intra-environment prediction of landrace germplasm in Northern Italy revealed high predictive ability for winter survival and onset of flowering (> 0.82) and moderately high predictive ability (0.49-0.63) for several other traits such as pod fertility, individual seed weight, plant height, leaf size and the proportion of seeds on the main stem (14). In the presence of moderate genome-enabled prediction ability, genomic selection may provide greater genetic gain per unit time for the same investment because of lower evaluation cost per genotype and shorter selection cycles, as recently shown for grain yield across Italian environments for pea, another autumn-sown rainfed inbred legume (15).

On-going collaborative work based on the evaluation of a large number (140-180) of GBS-characterized, sweet-seed inbred lines produced by LIVESEED aims to develop genome-enabled prediction models for grain quality traits (protein, oil and alkaloid contents) and for grain yield in specific growing conditions or major agroclimatic regions, exploiting yield evaluation data under severe drought or across contrasting sowing times in Italy, in moderately calcareous soil sites of the Netherlands (by Louis Bolk Institute) and Greece (by Institute of Industrial and Forage Crops of Larissa), across autumn-sown environments of Chile (by Instituto Nacional de Investigación Agropecuaria of Carillanca), and under spring-sowing in Scotland (by James Hutton Institute of Dundee). In the presence of convenient prediction accuracy, plant breeding could exploit specific models to select for adaptation to different target environments, along with general models for traits poorly affected by GEI (as it may be the case for grain quality traits). Albeit developed from data of a broadly-based training population, the applicability of the generated models to completely different genetic bases will require verification.

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A pre-breeding approach for high-yielding and healthy narrow-leafed lupin (*Lupinus angustifolius*)

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Abstract: Livestock management in Europe mainly depends on imported soybean. In recent years, lupin species have gained importance as an alternative protein source for stock feed as well as for food production. Narrow-leafed lupin (NLL) is well adapted to poor soils and dry environments, can help to increase crop rotation and may contribute to adapting European agriculture to climate change. A precondition for this are sufficiently high and secure grain yields. We report on our breeding research efforts which resulted in pre-breeding lines of NLL exhibiting novel growth types with high genetic yield potential or high levels of anthracnose resistance, respectively. Genetic and molecular studies provided molecular markers for anthracnose resistance which may be used in breeding programs to combine high yield and resistance in novel varieties.

Key words: Narrow-leafed lupin, anthracnose resistance, yield, molecular markers

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For the last 15 years, we have been running a program focused on improving germplasm of narrow-leafed lupin with regard to some key traits such as genetic grain-yield potential and resistance to anthracnose, a destructive seed and airborne plant disease of worldwide importance caused by the fungus *Colletotrichum lupini*.

Extensive greenhouse and field tests of genetically diverse plant material have been conducted by us and others to identify novel and effective sources of resistance to anthracnose. An effective resistance was found in the Australian cultivar 'Tanjil'. This resistance was reported to be governed by a single dominant gene, namely *Lanr1* (1). Sequence-based PCR markers for *Lanr1* were developed, enabling to trace this gene in breeding programs (2, 3). Under farming conditions in Germany, the resistance of 'Tanjil' was found to be pronounced, too, however a resistance selected in a local breeding line 'Bo7212' proved to be superior (4). Genetic analysis of this resistance revealed a second dominant resistance gene which was designated *LanrBo*. Using molecular markers published by (5) and (6), respectively, as well as markers developed in our lab, we succeeded in combining the two resistance genes *Lanr1* and *LanrBo* in a common genetic background. Both loci are located on chromosome NLL-11, but the marker alleles closely linked to *LanrBo* were found to map approximately 30 cM distal from *Lanr1* markers (Figure 1). Construction of a physical map confirmed a large distance (5.4 Mbp, Figure 1) between the two genes, thus indicating the existence of two separate loci for anthracnose resistance on the same chromosome. To confirm this hypothesis, a cross derived from lines homozygous for the *Lanr1* and the *LanrBo* resistance was generated and 283 F_2 progeny subjected to allelic tests at the phenotypic and molecular-marker level. Segregation into the resistant vs. susceptible phenotype among the F_2 progeny was 262 to 21, which was consistent with a 15:1 pattern ($\chi^2_{15:1}$= 0.66) as expected if the expression of the resistant phenotype is governed by two independently acting dominant resistance genes. Analysis of this F_2 population at the level of resistance-associated codominant marker *Lanr1_1* and *in silico* mapped marker *LAng31* (for *Lanr1*)

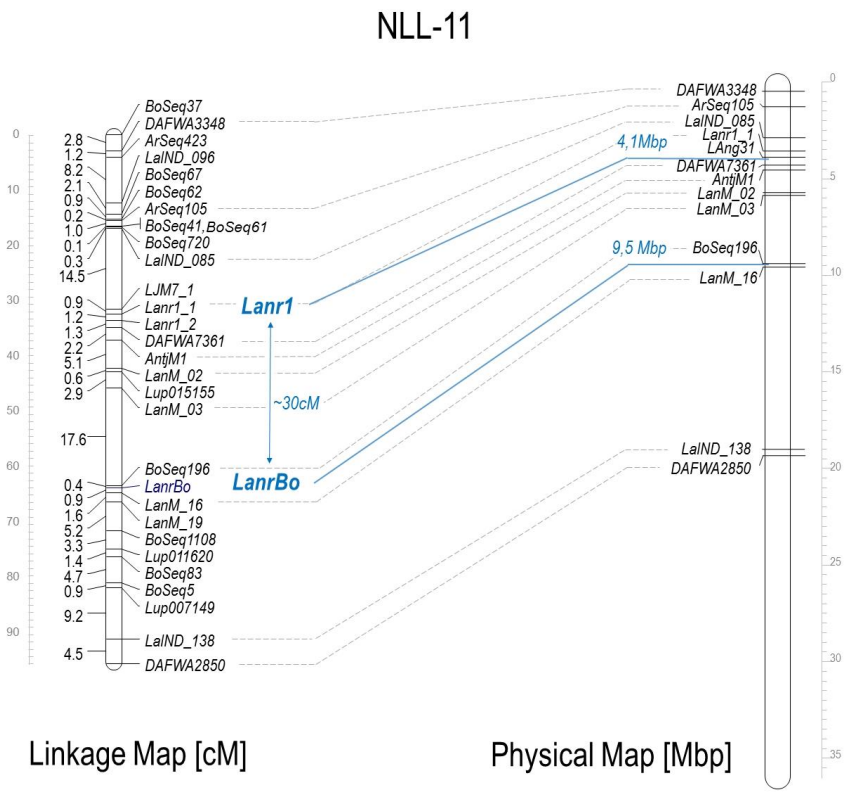

Figure 1. Alignment of the linkage and physical maps of chromosome NLL-11 in narrow-leafed lupin, including reported and *in silico* mapped molecular markers (4-6).

Figure 2. Field trials of a set of M_4 narrow-leafed lupin lines.

and *BoSeq196* (for *LanrBo*) revealed that each of the 262 resistant F_2 plants carried at least one marker allele specific for the dominant allele(s) of one or both resistance loci. Specifically, 151 plants were homozygous or heterozygous for resistance-linked marker alleles at both marker loci and, thus, could be assigned the genotype *Lanr1_ LanrBo_* while another 49 and 62 plants were found to be *Lanr1_ lanrBo lanrBo* and *lanr1 lanr1 LanrBo_*, respectively. The 21 F_2 plants which had been classified as susceptible in the phenotypic screen were found to be homozygous at both marker loci for the allele associated with the non-functional, recessive *lanr1* and *lanrBo* alleles. To summarize, molecular-marker analysis confirmed that the phenotypic 15:1 segregation was based on two dominant resistance loci segregating in a 9:3:3:1 ratio ($\chi^2_{9:3:3:1}$= 2.9).

The combination of high yield and anthracnose resistance seems to be an attractive way to generate new powerful varieties. Genotypes homozygous both for *Lanr1* and *LanrBo* marker alleles were thus used for crosses with high-yielding, susceptible prebreeding lines.

High-yielding NLL lines were obtained from an ethyl methanesulfonate (EMS)-based mutagenesis program. Briefly, seeds of the high-yielding and non-branching cv. 'Boruta' were treated with EMS. Among the M_2 progeny, phenotypic variants occurred and a set of high-branching growth types with promising seed set were selected and propagated to M_4. Phenotypically homogeneous M_4 lines were seed propagated and tested in the field (Figure 2) in 9 environments (3 years, 3 field locations: (i) Groß Lüsewitz conventional, 54°06'N, 12°32'E, (ii) Groß Lüsewitz ecological, 54°07'N, 12°31'E, (iii) Bocksee, 53°N29'N, 12°53'). One line, A4, combining high grain yield and high grain protein content was used as susceptible cross partner in crosses with a line homozygous for both the *Lanr1* and *LanrBo* resistance (Figure 3). F_1 plants were selfed and 43 F_2 genotypes were marker screened with the co-segregating markers for *Lanr1* and *LanrBo*. Among the 43 F_2 plants analysed, all of the nine possible marker genotypes were observed (data not shown). With regard to the two underlying resistance genes, these nine marker genotypes could be conflated to resistance genotypes, giving a segregation of 32 *A_B_* : 3 *A_bb* : 6 *aaB_*: 2 *aabb* (gene symbols according to Figure 3), which again is consistent with a 9:3:3:1 ratio ($\chi^2_{9:3:3:1}$= 6.4). Among the group of *A_B_*

plants, three were determined to be of the *AABB* marker genotype for the *Lanr1* and *LanrBo* resistance genes and are thus potential candidates for further breeding efforts.

These novel breeding lines were transferred to current NLL breeding programs where these lines will be field-tested for their yield potential and seed protein content. High-yielding and high protein content lines may be introduced into a backcross program assisted by molecular resistance markers to allow introduction of the two anthracnose resistance loci into genetic backgrounds of the high-yielding, high-protein NLL breeding lines. Although NLL is thought to be more tolerant to *C. lupini* compared to yellow or white lupin, there is no true anthracnose resistance among current German sweet NLL cultivars. This poses a latent threat to growers as well as to seed producers. The pre-breeding lines outlined here may help to secure lupin production in the future in order to render agriculture more sustainable.

The successful application of marker-assisted tracing of anthracnose resistance genes in NLL provides encouragement to extend this approach to white lupin (*L. albus*) as well as to yellow lupin (*L. luteus*). Yellow lupin is a quite undemanding crop species with high potential especially in regions where light soils and water shortage are prevailing, as is, for instance, the case in northeastern Germany. We launched a research program on *L. luteus* in 2019 and could identify a monogenic anthracnose resistance, which was present in a cultivar bred by Polish breeders (7). Markers are currently being developed to map and to introduce the underlying resistance gene, *Llur*, into high-yielding yellow lupin germplasm.

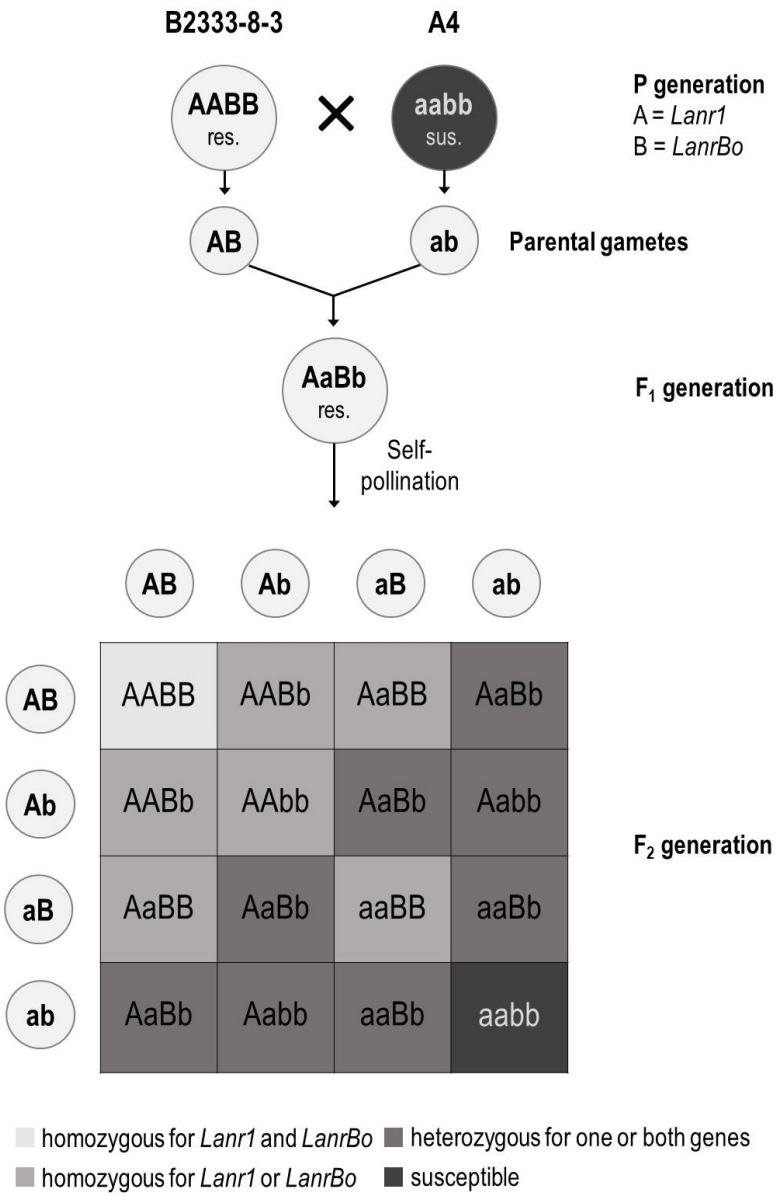

Figure 3. Segregation pattern of F_2 families after crossing resistant line B2333-8-3 with high-yielding line A4.

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Lupin genomics from 2022 and beyond

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Abstract: The sequencing of lupin genomes commenced in the 21st century, with two reference genomes published for narrow-leafed lupin and white lupin, and several others in progress. Furthermore, pan-genomes for white lupin and narrow-leafed lupin have been generated using different approaches. To rapidly introduce new genetic diversity, speed breeding can be utilised, and our team is developing speed breeding protocols for narrow-leafed lupin. Furthermore, our team is generating a reverse genetics population in the reference genetic background of cultivar Tanjil. Together, these genomic resources will be of great value for lupin crop improvement as the world demand for plant-based protein for human consumption is rapidly growing.

Key words: reference genome, pan-genome, *Lupinus* spp., Genistoids

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Lupin species belong to the Genistoid clade of legumes and are estimated to comprise around 300 species. Despite the large number of species, only four have been domesticated. These include three Old World lupin species in narrow-leafed lupin (*Lupinus angustifolius*), white lupin (*L. albus*) and yellow lupin (*L. luteus*) and the New World pearl lupin (*L. mutabilis*). Narrow-leafed lupin (NLL) and white lupin are the two predominant domesticated lupin species grown with Australia and Europe the largest producers. A suite of genomic resources has been generated for these two lupin species (reviewed in (1)) including the first reference genome assemblies with the assembly statistics for the most recent two assemblies presented in Table 1.

For NLL the first survey assembly was published in 2013, which was assembled using short read Illumina sequences and was thus highly fragmented (2). In 2017 an improved assembly was published by our lab that utilised Illumina Paired-End and Mate-Pair data in combination with BAC-end sequence data (3). This approach increased the assembly size from 523 Mb to 609 Mb and reduced the number of scaffolds from 71,995 to 14,379. Over the last few years, the cost of long-read sequencing has considerably come down and this has created opportunities for orphan crops to generate or improve reference assemblies. Wang *et al.* (4), utilised long read PacBio data to improve the assembly for NLL which resulted in a marginal increase to 616 Mb in the genome size, but a significant reduction in the number of scaffolds to 123. Interestingly, BUSCO analysis showed that in terms of the gene content the short-read assembly had more complete and less missing BUSCOs (Table 1). Our lab has developed another iteration of the reference genome by incorporating long read PacBio data and Hi-C data, which has an assembly size of 653 Mb (Garg, Kamphuis and Singh unpublished data) and is developing a pan-genome comprising 55 accessions including domesticated Australian and European varieties and genetically diverse wild germplasm from different parts of the Mediterranean. These resources have also contributed to the generation of a 30k multispecies pulse SNP chip (5), which

includes 5,425 evenly distributed SNPs across the 20 chromosomes of NLL.

For white lupin two reference genomes have been generated in recent years that both have combined short and long read sequence data (6, 7). Both assemblies are from the same cultivar Amiga and differ in their assembly statistics with the assembly from (6) smaller in size (451 Mb), but less fragmented (89 scaffolds) when compared to the assembly of (7) being larger in size (559 Mb) and with 1,580 scaffolds. The cut off value for scaffold length in genome assemblies is user defined and can often explain discrepancies in size and different scaffolds as is the case here. Nevertheless, both assemblies have high BUSCO scores with the shorter assembly being more complete in terms of gene content with a greater number of complete BUSCOs and less missing BUSCOs (Table 1). In addition to the two reference genomes a white lupin pan-genome has recently been published (8). The white lupin pan-genome comprises 39 accessions and include 25 cultivars, ten landraces and four wild accessions from 17 different countries. Each accession was de novo assembled and aligned to the reference genome to identify scaffolds that had less than 90% identity to the reference genome and had a length greater than 2 Kb. This approach yielded 3,663 scaffolds with a total length of 11.74 Mb. Among these novel scaffolds 178 additional genes were predicted. Core and variable gene analysis revealed that 78.5 % of predicted genes (32,068 of 38,443 genes) were present in all 39 accessions. Variable genes from landraces and wild germplasm can serve to increase the gene diversity and improve specific traits in future white lupin varieties.

To rapidly introduce new genetic diversity into crop varieties speed breeding has been employed in crop breeding programs around the globe. Here, a combination of altered light spectrum, intensity, and daylength are used to speed up the generation time from seed to seed. Previous research showed that a combination of vernalisation and speed breeding allowed the rapid generation of a F_6 recombinant inbred line (RIL) population, where four generations of single seed descent occurred in 13 months with an average generation time including vernalisation for 3 weeks of 13.5 weeks (9). The parents of this population (varieties Chittick and Geebung) have different flowering time responses and a range of flowering times were observed in the F_6 RIL progeny indicating that speed breeding doesn't select for early flowering genotypes. Our team at CSIRO is developing glasshouse and controlled environment scale speed breeding protocols for narrow-leafed lupin (Figure 1), where under glasshouse conditions we can reduce the generation time to between ten and twelve weeks, where no labour-intensive embryo rescue is used to shorten the generation time further.

Another activity in our research group is focused on the development and screening of a NLL mutant population for traits of interest to growers, breeders, and end/next users. We use a Targeting Induced Local Lesions in Genomes or TILLING approach, where we use the chemical ethyl

Table 1. Overview of the genome statistics for published narrow leafed lupin (*L. angustifolius*) and white lupin (*L. albus*) genome assemblies.

	Reference genomes Narrow-leafed lupin		Reference genomes White lupin	
	Hane *et al.* 2017	Wang *et al.* 2020	Hufnagel *et al.* 2020	Xu *et al.* 2020
Genome size	609 Mb	616 Mb	451 Mb	559 Mb
Number of scaffolds	13,574	123	89	1,580
N50	11	9	12	14
L50	21.3 Mb	30.8 Mb	17.35 Mb	18.66 Mb
N90	837	18	23	33
L90	37.08 Kb	23.53 Mb	14.55 Mb	1.67 Mb
GC content (%)	33.46	33.27	33.79	36.82
Annotated protein-coding sequences	33,076	33,097	38,258	47,603
BUSCO stats (plantae)				
BUSCOs complete	420	417	418	412
BUSCOs fragmented	1	2	3	2
BUSCOs missing	4	6	4	11

Figure 1. Narrow-leafed lupin TILLING lines under the speed-breeding lights in the CSIRO glasshouse in Floreat, Western Australia.

methanesulfonate (EMS) to promote mutations randomly throughout the genome. This is conducted on many lupin seeds with the aim to develop at least 1,000 M_2 lines. Using low-level re-sequencing and a bioinformatics pipeline we can identify the chemical mutations throughout the genome of each individually mutagenized line. These mutations can reside in non-coding regions, promoters, and genes, where we have a strong interest in mutations that lead to a stop codon, truncating the protein sequence of a gene and rendering it non-functional (depending on the location of the stop mutation). To date we have shallow sequenced 600 M_2 lines, which harbor approximately 3.4 million chemically induced mutations of which 11,554 genic SNP mutations result in a stop-codon in 8,980 genes. The genic stop-codon mutations are potentially useful to remove some undesirable traits such as the thick seed coat, alkaloid content and allergenicity proteins. Such traits are still present in lupin species as they have only been domesticated about 60 years ago. Furthermore, specific amino acid changes in genes can lead to improved tolerance to herbicides. Our current focus is on expanding the TILLING resource and identifying mutants with interesting phenotypes including the ones described above.

The lupin genomic resources including the ones developed in our Lupin Breeders Toolbox project are valuable resources for (pre-)breeders to improve traits in lupins and through speed breeding and effective genotyping using the pulse SNP chip will allow rapid improvement of future varieties. The improvement in yield and other agronomic traits of interest to lupin breeding programs, will help provide a viable grain legume crop for substantial parts of Mediterranean grain growing regions in the world and contribute to the growing demand of plant-based protein.

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A new dimension of lupin genetic resources conservation and management: perspective from the INCREASE project

Magdalena Kroc[1], Magdalena Tomaszewska[1], Katarzyna Czepiel[1], Mohamed Neji[1], Karolina Susek[1*]

Abstract: Characterization of genetic resources is essential for both their conservation and to promote their use for crop improvement. Legumes are of great importance for their high seed protein content and role in food security and climate change mitigation strategies. The aim of the INCREASE project in what refers to lupin is to develop and characterize the lupin 'Intelligent Collections', as a set of nested core collections of different sizes representing the entire diversity of the crop. The strategies used will embrace state-of-the-art approaches for genotyping (genome-wide association studies, population genomics, large-scale data processing) and phenotyping (high-resolution metabolic profiling). This study is carried out within a wide international collaboration as part of the European Union Horizon 2020 'INCREASE' Project. Our studies will further develop lupin research in (pre)breeding programs and contribute to lupin evolution and domestication.

Key words: lupin, food legumes, agrobiodiversity, genotyping, phenotyping

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The lupin (*Lupinus*) genomes appear to be highly rearranged in comparison to other legumes, and they are highly divergent from all the other agriculturally important legumes and model species (1). Recent data has shown that lupins have evolved by duplication and/or triplication (2-5). Furthermore, multiple chromosome rearrangements (4, 6) and epigenetic changes (7) have affected the complex, evolutionary changes within this genus. Therefore, lupin represents an important group to explore and provide further understanding of the mechanisms that underlie genome evolution and domestication within the legume family. Indeed, genome evolution of lupin provides the basis of the biodiversity that underlies its potential as a human food and its adaptation to global environmental changes. *Lupinus* is a large and diverse genus, with ca. 280 species (8). However, three Old World lupins (*L. albus* L., white lupin; *L. luteus* L., yellow lupin; *L. angustifolius* L., narrow-leafed lupin) and one New World lupin (*L. mutablis*, tarwi/pearl lupin) have particular agricultural and food importance.

The INCREASE Project is concentrated on four food legume species: chickpea, common bean, lentil and lupin. To date, exploitation of legume genetic resources in crop breeding has been limited in comparison to the availability of materials, and the potential impact of their use is far from optimal. The main aim of INCREASE is to explore the genetic resources of these food legumes and to promote intensive and effective investment in their research and breeding. Thus, to boost food legume breeding and to attract additional private and public investment, an efficient and innovative genetic resources management system is required (for more information see (9)).

Within INCREASE, our group leads the studies on lupins for the development and characterization of a set of nested core collections of different sizes composed of genetically purified accessions. These core collections will be genetically and phenotypically characterized (at different levels depending on the core collection), towards the creation of the so called, lupin 'Intelligent Collections' (9). Lupin 'Intelligent Collections' will be shared as a tool among genebanks to improve overall management of the genetic diversity of the species. This goal is crucial for many reasons, including: possible heterogeneity of material currently collected in genebanks; the genetic structure of lupin accessions is unknown; the information available for these genetic resources is limited; and passport data for

the traits of interest to breeders and users is scarce and generally limited to morphological descriptors.

Thus, we are focused on two lupin species: the European *L. albus* (white lupin; 2n=50; genome size ~590 Mbp), which is the oldest domesticated lupin, and the American *L. mutabilis* (Andean lupin; 2n=48; genome size ~930 Mbp); these both have among the highest protein contents within the lupin crops. Currently, we are developing the collection of over 3,000 accessions of these two lupin species that will constitute the Reference core (R-core), which are grown under greenhouse and insect-free conditions (Figure 1). All these accessions have been provided as heterogeneous material by worldwide genebanks and seed donors (e.g., research institutes, project stakeholders). Therefore, we are developing singe-seed descent lines for each of these accessions. The harvests from the lines being developed undergo standardized phenotyping procedures during each cycle of seed increase, according to the protocols defined within the INCREASE Project (10). These protocols have been designed as a toolkit to facilitate implementation of best practice for the assessment of seed morphological traits, seed imaging, as well as a broad spectrum of classic morphological traits throughout pants growth and development (for more details see (10)). From the R-core, we are further selecting the Training core (T-core) collection and Hyper core (H-core) collection, which are being characterized at both phenotype and molecular levels. Along with high-quality genome sequences, these data will constitute comprehensive resources to underpin the mechanisms that control various traits.

To extend our knowledge on the variation within lupin genetic resources and to investigate the genotype×environment interactions for lupin adaptation to different environmental conditions, multi-location field trials will be carried out in Europe. Adaptation will be examined not only in terms of agronomic performance, but also taking into account nutritional content and quality traits i.e., amino-acid, starch, mineral quality in seeds as well as technological traits, like seed size, seed coat thickness, etc. Moreover, the combined use of transcriptomic and metabolomic approaches, including gene and metabolite networks, will allow the identification of putative genes responsible for the phenotypic plasticity of the food legume cultivars. The scheme of the studies on lupins within the INCREASE

Figure 1. Growth of the lupin R-core collection in the greenhouse at the Institute of Plant Genetics, Polish Academy of Sciences.

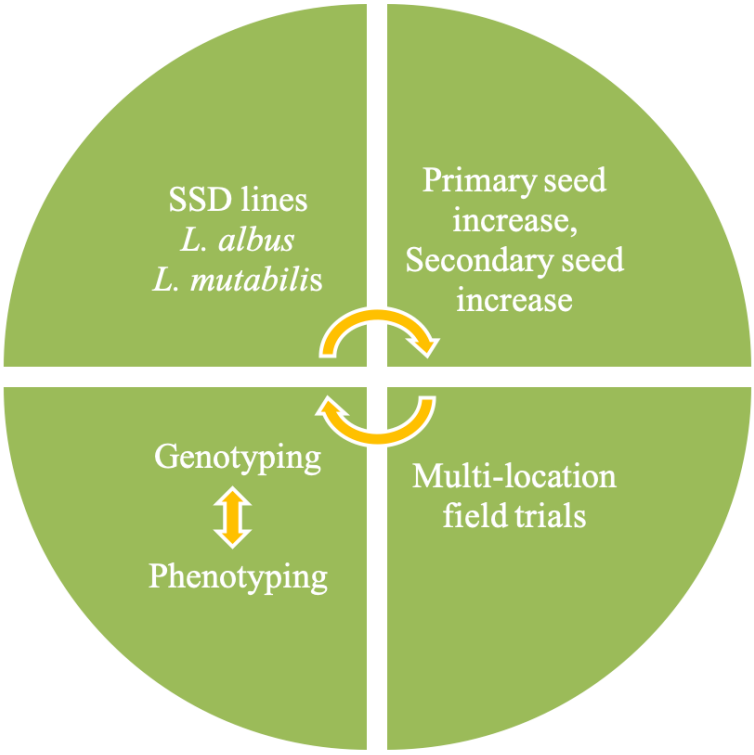

Figure 2. The basic scheme of the studies on lupin within the INCREASE Project. SSD, single-seed descent.

Project is shown on Figure 2.

The results from the INCREASE Project will be useful across the wide community of breeders, farmers, scientists, and many other dedicated research interests, including stakeholders in the exploration of the genetic resources of lupins. For example, currently within national projects (e.g., National Science Centre, Poland: OPUS Project) we are using the data of phenotypic–genotypic variation among white lupin as a useful source to explore the genetic architecture of seed development and seed-size variations in lupin (*Lupinus albus* L.) and common bean (*Phaseolus vulgaris* L.), and to understand the genomic evolutionary processes to identify genes and genomic regions associated with adaptation to environmental conditions.

For more information, please follow us at:
Facebook: facebook.com/pulses.increase
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Phenological diversity in narrow-leafed lupin: why crops need it and where and how to find it

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Abstract: Development of narrow-leafed lupin (*Lupinus angustifolius* L.) varieties with region-specific adaptation is limited by diminished levels of genetic diversity for flowering time and other phenology traits within domesticated relative to wild gene pools. Here, we briefly summarise current progress to address this issue through molecular characterisation of genes that regulate flowering time in response to vernalisation, and the potential for new genetic variation discovered as a result of this research to benefit global production. In addition, we highlight important pre-breeding research objectives moving forward and outline areas of research that will be increasingly important for adaptation in the face of climate change.

Key words: crop adaptation; flowering time; genetic diversity; phenology; vernalisation

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Why is phenological diversity important for narrow-leafed lupin crop production?

Manipulation of phenology (i.e., the timing of life cycle events) is fundamental for domestication and improvement of crops, including narrow-leafed lupin (*Lupinus angustifolius* L.). Throughout the Mediterranean Basin, phenology in the wild relatives of this pulse is overwhelmingly regulated by vernalisation; a physiological process where enduring exposure to cool temperatures promotes (facultative response) or enables (obligate requirement) floral initiation. Vernalisation serves to maximise fitness in wild populations by delaying reproductive development until the arrival of favourable conditions in spring. However, strong vernalisation requirements represent a significant barrier for agriculture, as they greatly restrict in which climates reliable and high grain yields can be achieved.

Narrow-leafed lupin breeders have historically addressed this issue by engaging in strong directional selection for genetic variation circumventing obligate vernalisation requirement. This strategy enables domesticated crops to consistently flower early irrespective of minimum temperature and has permitted cultivation beyond the native distribution of the species, including southern Australia and northern Europe. Almost all varieties bred since the 1960's from both continents now possess one of two vernalisation-independent sources for early flowering time, known as *Ku* and *Jul* (1).

Unfortunately, continuous selection for *Ku* and *Jul* has severely depleted phenological diversity within domesticated gene pools, which restricts further global growth of the crop (2). Inadequate genetic variation for flowering time greatly compromises the adaptive capacity of narrow-leafed lupin and means there is: (i) an inability to develop varieties with specific regional adaptation necessary to achieve high yield potentials across diverse environments, particularly long-season environments; (ii) increased vulnerability to climate change and uncertainty as to whether current yields can be maintained in existing areas of production; (iii) limited opportunity to expand production into new agricultural regions; and (iv) incompatibility with emerging agronomic practices, such as early sowing to take advantage pre-season rainfall events. Supplementing domesticated gene pools with novel genetic variation for phenology is an urgent priority for breeding.

Table 1. Molecular basis of known flowering time alleles and their impacts on phenology in narrow-leafed lupin (*Lupinus angustifolius* L.).

Allele	Phenology	Adoption of allele	Underlying gene and mutation	Source of mutation	Method of discovery	Reference
ku	Strong obligate vernalisation requirement causing late flowering if unfulfilled.	Present in most wild populations in the species' native Mediterranean distribution.	No mutation – it is the *LanFTc1* wild-type allele	NA	NA	(3)
Kulikup; Ku	Early, vernalisation-independent flowering	Heavily selected in Australian and European varieties since the 1960's	1,423 bp deletion in promoter of *LanFTc1*	Natural – arose in a domesticated crop (Borre)	Genetic mapping in RIL population	(3)
Julius; Jul	Early, vernalisation-independent flowering	Heavily selected in European varieties since the 1960's	5,126 bp deletion in promoter of *LanFTc1*	Natural – likely inherited from wild populations from the Near East	Candidate gene allele mining via whole-genome sequencing	(4)
Pal	Mid-season flowering with mild facultative vernalisation requirement	Not yet actively selected in breeding	1,208 bp deletion in promoter of *LanFTc1*	Natural – observed in wild populations from the Near East	Candidate gene allele mining via whole-genome sequencing	(4)
efl	Mid-late-season flowering with mild facultative vernalisation requirement	Selected in one Australian variety (Chittick) released in 1982	Non-synonymous SNP in coding sequence of *LanTGS1-like*	Induced – via ethylene imine mutagenesis of domesticated variety (Borre)	Genetic mapping in RIL population	(8)

Current progress towards improving phenological diversity

Efficiently improving genetic diversity for flowering time begins with identifying genes contributing to this complex trait and understanding the ways in which they are regulated. This makes it possible to: (i) search for natural or induced variation within these genes that may create novel phenology (i.e. "allele mining"); (ii) determine how best to further modify flowering time, for example by combining particular alleles from several interacting or independent genes; and (iii) develop molecular markers that alleviate the technical difficulty, time frames and financial costs associated with introducing and retaining valuable alleles during breeding via marker-assisted selection (MAS).

Concerted efforts have been made to establish the molecular basis of flowering time variation within the vernalisation pathway of domesticated narrow-leafed lupin over the past two decades (Table 1). Large deletions in the proximal regulatory region of *LanFTc1* that de-repress gene expression were found responsible for *Ku* and *Jul* (3, 4). *FT* orthologues, including *LanFTc1*, are well-known central integrating genes in floral initiation pathways and have been implicated in domestication and adaptation of many crops. Identifying the causal mutations for *Ku* and *Jul* has permitted design of a perfectly predictive multiplex PCR marker, which has demonstrated utility for MAS of flowering time (5). More importantly in the quest to expand genetic diversity for phenology, however, it has also led to valuable detection of a third unique deletion of 1,208 bp in the *LanFTc1* promoter (Table 1) (4). This mutation invokes a mild vernalisation response and unique mid-season phenology (4, 6) that could increase annual grain yields by ~390 kg/ha in high-rainfall long-season environments upon introgression into new varieties (7).

A single nucleotide polymorphism (SNP) in *LanTGS1-like*, a gene widely characterised outside the plant kingdom but less so within, meanwhile appears responsible for *efl* (8). The SNP causes a facultative vernalisation response and mid-late phenology by changing an amino acid at an important functional site within the protein product. There is now scope to select *efl* in future varieties through MAS.

Exponential advances in sequencing throughput and corresponding affordability have been instrumental for development of genetic resources allowing insights and applications that would otherwise be beyond the reach of most non-model species with minor global economic significance. Whole-genome and transcriptome assemblies were fundamental to pinpoint the molecular basis of *Ku*, *Jul* and *efl*. Similarly, they've been crucial for discovery of other phenology candidate genes (Table 2) (6, 9, 10) and revealing key genetic architectural differences between narrow-leafed lupin and model species, including the absence of *Arabidopsis* flowering repressor, *FLC* (11). These candidate genes provide necessary targets for further research and potential allele mining beyond *LanFTc1*, particularly in wild germplasm where a far greater breadth of phenological diversity is observed.

Where to from here?

Research to date has predominantly focused on unravelling genetic variation with major effect on vernalisation in domesticated narrow-leafed lupins. This prioritisation has been understandable considering the role *Ku* and *Jul* have shared in shaping broad-acre production and the profound influence this pathway has on phenology relative to others. For instance, photoperiod does not impose similar geographical restrictions, as vernalisation-dependent and -independent varieties can be cultivated across a range of latitudes (12). Genetic variation instilling vernalisation-independent or facultative vernalisation requirements will likely remain an essential requirement for cultivation moving forward, particularly in short-season environments.

It is important that future research continue to characterise the genetic architecture of the vernalisation pathway. This includes elucidating the transcriptional mechanisms of known genes. For example, identifying other deletions and/or resolving key motifs in the promoter of *LanFTc1* may help reveal transcriptional repressors/promoters of *LanFTc1*. Additionally, it would be helpful to uncover other gene components in the vernalisation pathway and assess the extent to which each gene individually contributes phenological diversity through interaction (or a lack thereof) with other endogenous and/or environmental signals. Exploration of the interplay between vernalisation and photoperiod with respect to *LanFTc1* has begun (6). Likewise, the effect of interactions between vernalisation, photoperiod and temperature on phenotype are currently under investigation in domesticated and wild germplasm from diverse origins (J.D. Berger, unpublished data). Understanding where and how temperature-mediated signals integrate with the vernalisation pathway will be crucial for alleviating climate change impacts on crops, particularly as varieties with *Ku* (and *Jul* by extension) are more responsive to ambient temperature than varieties with obligate vernalisation requirements (*ku*) (13).

Table 2. Summary of narrow-leafed lupin (*Lupinus angustifolius* L.) phenology candidate genes revealed through innovative linkage and association mapping approaches with transcriptomes (6, 9, 10).

General functional category	Number of candidate genes
Flowering time initiation, in response to environmental cue signals	24
Various regulatory functions	33
Basic cell life functions	22
Abiotic stress response	25
Photosynthesis	6
Other	42

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Molecular perspective of the nutraceutical properties of narrow-leafed lupin (*Lupinus angustifolius* L.) seed β-conglutin proteins

Elena Lima-Cabello[1], Jose C. Jimenez-Lopez[1*]

Abstract: *Lupinus angustifolius* or narrow-leafed lupin (NLL) is a globally important pulse and an economically affordable alternative source of high-quality protein. NLL β-conglutin proteins exhibit multifunctional properties through regulatory molecular mechanisms promoting health benefits as a result of their unique structure-functional features in comparison with most of the legume proteins from the vicilin family. Multiple disease amelioration properties include antioxidant activity and fighting inflammatory-related diseases such as type 2 diabetes, and deleterious colorectal and mammary cancers, among others. This review outlines updated research about NLL β-conglutin proteins drawing on information gained from biochemical, *in vitro* and *ex vivo* studies on molecular nutraceutics and protein structure insights.

Key words: sweet lupin, β-conglutins structure-functional features, inflammation, cancer, functional foods

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Introduction

Legume-based seed compounds, particularly proteins, are in high commercial everyday demand as functional foods to fight obesity, decrease blood pressure and glucose levels, promote cholesterol- and triglyceride-lowering effects, thus helping to tackle cardiovascular disease and cancer. In this regard, species of the "sweet lupin" group are one of the most relevant alternative sources of plant proteins to soybean, with excellent nutraceutical (Figure 1) and techno-functional properties (1).

Conglutins are the main family of proteins present among lupin seeds, and particularly β-conglutins (7S-globulins or vicilins) the most abundant fraction in lupin seed. They comprise a multigene family with seven functional genes (β1 to β7) (2), following their isolation from seeds accomplished by salt-induced extraction. Overexpression and chromatographic methodologies have allowed to obtain recombinant β-conglutin isoforms (β1 to β4 and β6; purity >95%), to be used in molecular studies, some of them showing a variable number of micro-heterogeneities, likely as a result of their polymorphism which range from 1% to 26% (3), mainly due to their multigene origin (2).

Structure-functional insights of the NLL β-conglutin family

An extensive structural (2D and 3D) variation has been found in NLL β-conglutins (4, 5) as a result of the combination of over 20 polypeptide chains in a large range of molecular masses (10–80 kDa). These are the results of the proteolytic cleavage activity of multiple β-conglutin precursor polypeptides (2).

β-conglutins are unique proteins among the vicilin family since: i) no disulphide bonds compact their structure; ii) stability is achieved by formation of electrostatic interactions and hydrogen bonds (4, 5); iii) the oligomerization process shaping trimeric and tetrameric forms (from 20 to 75 kDa); iv) the combination of different subunits in a multimeric structures that may be different among *Lupinus* cultivars and species; and v) surface glycosylated motifs preventing β-conglutins from proteolysis.

Therefore, structural based molecular modelling analysis of β-conglutins has

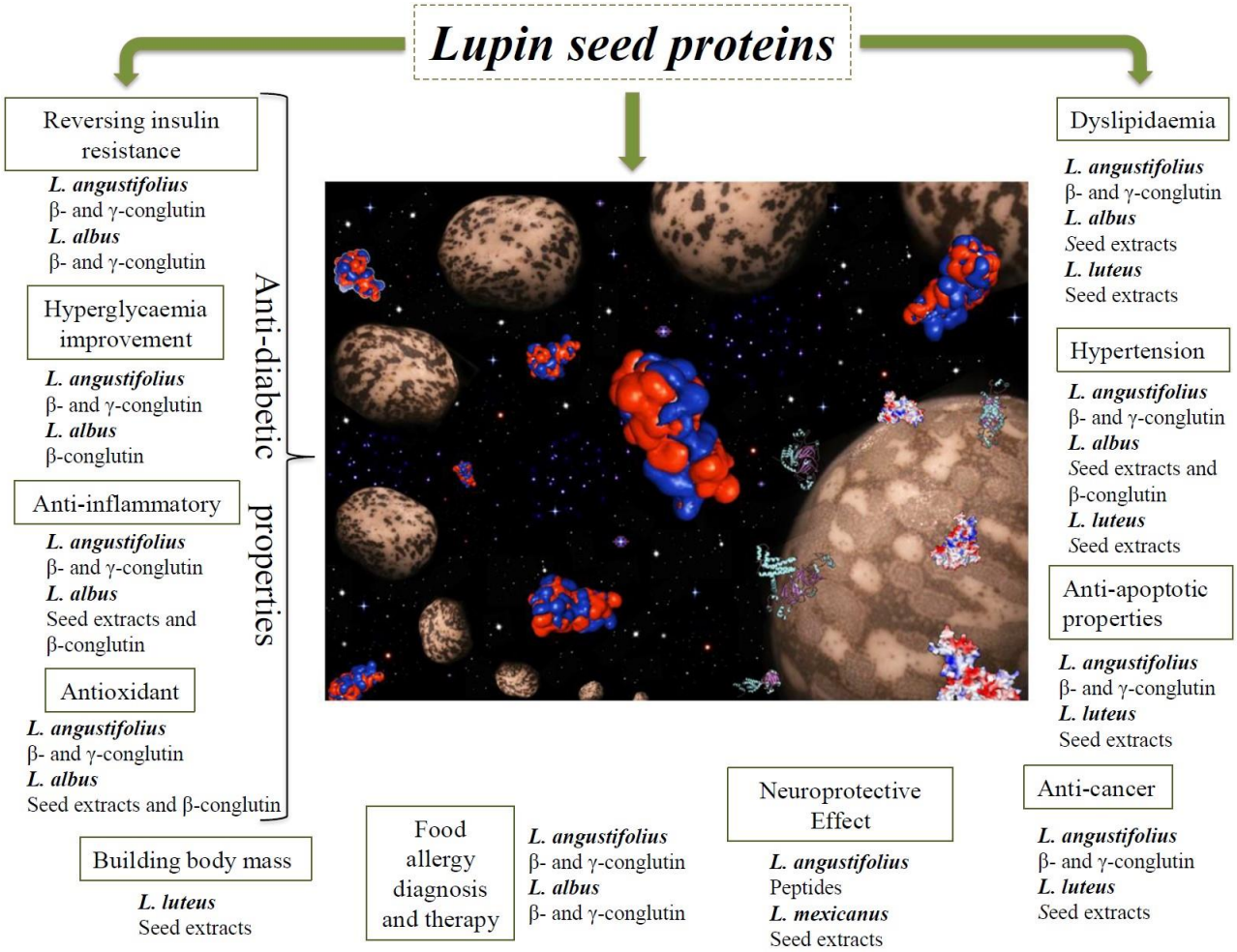

Figure 1. Lupin seed protein main nutraceutical properties. The *Lupinus* seed species (bold) and the protein, seed extract and/or peptide involved in each particular nutraceutical property is detailed in the figure. Central picture named "Lupin Planet" shows lupin seeds of different species at multiple magnifications, and β-conglutin proteins modelled as cartoon and electrostatic potential surface (regular and isocontour value of ±5 kT/e, respectively) on a black stars background.

concluded that substantial differences among isoforms, mostly in the *N*-terminal domain mobile arm, may have large influences on functional differences among NLL β-conglutin isoforms (4, 5). In addition, these studies have showed a conserved metal binding cleft (HYX … R), typical of oxalate oxidase enzymes, suggesting similar functional activities to germin-like proteins, or vicilin-like glucose binding proteins (5).

Regulatory mechanisms underlying molecular nutraceutical properties of NLL β-conglutin proteins

Different and unique structural features of β-conglutins may significantly contribute to regulate functional interactions with proteins integrated in cross-road pathways that regulate health benefits. Thus, these lupin seed proteins are able to constitute innovative ingredients to make functional foods.

Current interest in lupin seed proteins is growingly motivated by recent research outcomes about their nutraceutical properties (6). These include a plethora of molecular mechanisms underlying functional activities (Figure 2) such as antioxidant molecules fighting oxidative stress (decreasing ROS generation), as a main causative factor in the onset and progression of type 2 diabetes (T2DM). β-conglutins are able to: i) improve the antioxidant defence system (glutathione production, superoxide dismutase and catalase level and activities increase); ii) the downregulation of the inducible nitric oxide synthase (*iNOS*) gene expression and reduction of nitric oxide (NO) production (7, 8); and iii) strongly diminishing protein carbonylation damage (9).

Research work accomplished by *in vitro* and *ex vivo* approaches, and the use of β-conglutin proteins purified from recombinant sources have brought out underlying different molecular mechanistic actions performed by these proteins, making them strong new candidates to help fight inflammatory-related diseases such as T2DM and cancer (6, 10).

The molecular mechanisms involved in the anti-inflammatory properties of NLL β-conglutin isoforms 1, 3 and 6 are related to their ability to: (a) decrease the synthesis of cytokines such as IL-1β, INF-γ, TNF-α, IL-2, IL-6, IL-8, and IL-12, and mediators as NF-κB1; (b) regulate the attraction and

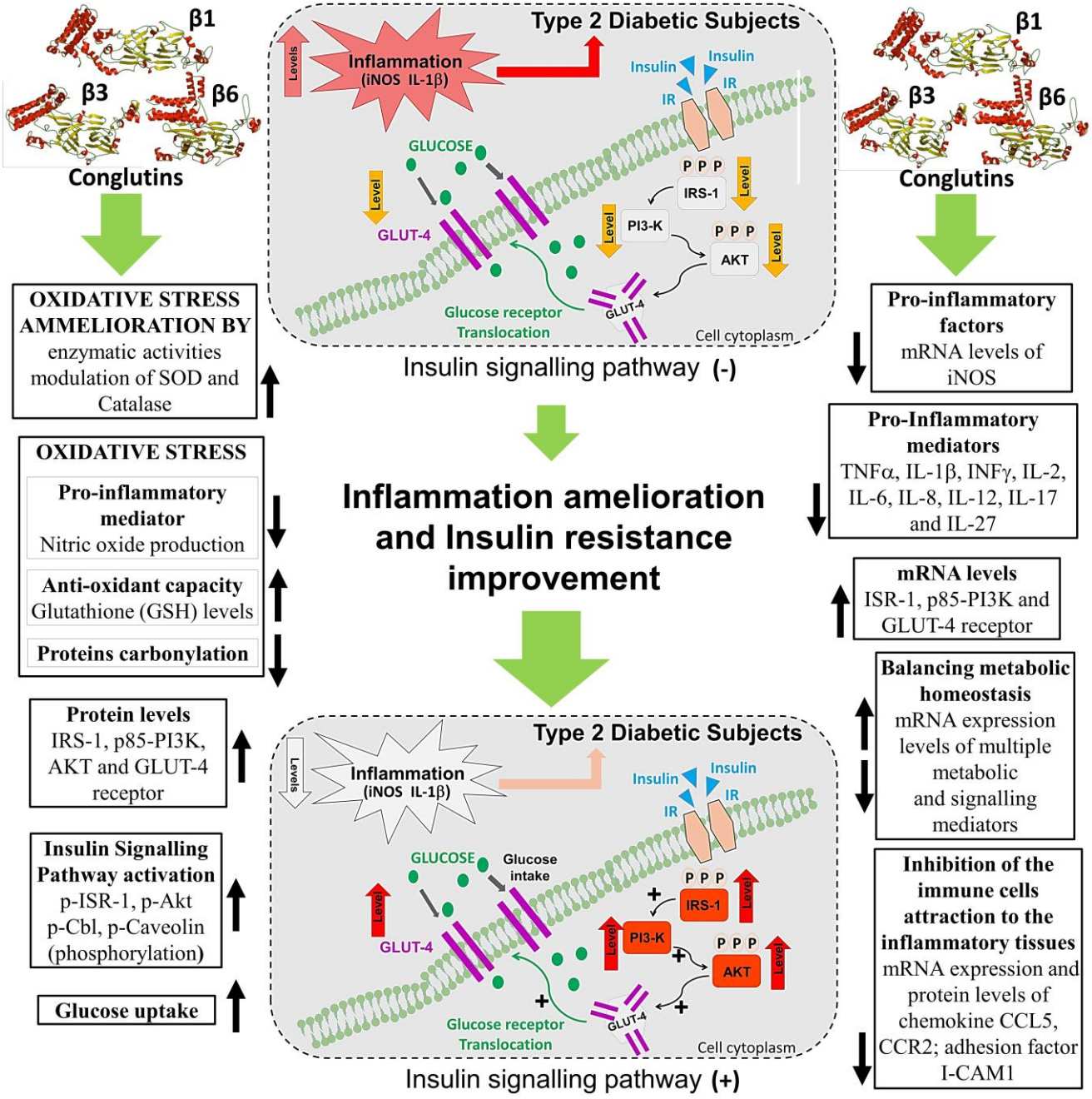

Figure 2. Molecular mechanisms of β-conglutins promoting nutraceutical properties. Insulin signalling pathway activation and type 2 diabetes state improvement are directly linked to the molecular mechanism induced by β-conglutins (6). Black arrows indicate increase (pointing up) or decrease (pointing down) of the action induced by β-conglutins and detailed in the boxes. (-) inhibited, (+) activated.

mobilization of immune system cells (T-cells, NK, dendritic cells, etc.) to the adipose tissues. This is accomplished by the regulation of chemotaxis (i.e., *CCL2* and *CCL5*) and molecular adhesion (i.e., *ICAM-1* and *VCAM-1*) gene expression (9); and (c) decreasing NO production and the *iNOS* gene expression and protein translation (7, 8).

Therefore, T2DM amelioration occurs by the modulation of the insulin signalling pathway activation by NLL β-conglutins. These proteins are able to interact with insulin (9), which may enhance insulin functional properties; they may regulate insulin signalling pathway kinases since β-conglutins: i) increase the protein levels and the gene expression of *AKT*, *IRS-1*, *GLUT-4* and *p85-PI3k* genes; ii) promote their phosphorylation (activation); and iii) decrease *iNOS* and *IL-1β* gene expression levels. Interestingly, β-conglutins promote reverse insulin resistance (7, 8) through different pleiotropic effects such as (a) modulating insulin signalling pathway upstream gene expression (*IRS-1*, *AKT*, *p85-PI3k* and *GLUT-4*); and (b) downstream

mediators (Caveolin, CBL) that modulate activation of vesicular transport to the plasma membrane of glucose transporters; (c) increasing glucose cellular uptake; (d) decreasing cellular oxidative stress; and (e) improving metabolic homeostasis and cell signalling (7-9).

β-conglutin proteins are also chemotherapeutic agents with potential uses for treatment of human colon cancer, since they have been implicated in the viability of colorectal cancer cells and inhibition of their growth, increasing apoptosis and decreasing cell proliferation by inducing cell cycle arrest in the G0/G1 and G2/M phases (10).

Conclusion and future perspective

NLL β-conglutins are confirmed multifunctional proteins with particular structural features making them unique proteins among the vicilin family (Cupin superfamily) and being responsible for a plethora of molecular mechanisms promoting health benefits. Ongoing work is revealing the structural 2D and 3D domains of NLL β-conglutins involved in the accomplishment of their multiple functions, and will bright to light the tight control exerted by NLL β-conglutin proteins over the mechanisms underlying their nutraceutical properties with regards to cardiovascular diseases and cancer. This will make NLL β-conglutins promising alternative new sources of nutraceutical proteins and contribute as excellent candidates for functional foods. In this regard, β-conglutins have been identified as the main allergen protein (Lup an 1) in the lupin seed. Different molecular aspects of β-conglutins involvement in food allergy have been studied, identifying highly reactive isoforms to atopic patient sera; their epitope sequences promoting primary sensitization and cross-allergenicity, etc. However, more molecular but also clinical research have to be done for a better understanding of the role of these lupin seed proteins in the food allergy context for a safety development of lupin derived foods with new health benefits and nutraceutical properties.

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Lupin foods and food ingredients

LiHui Liu[1], Regine Stockmann[1*]

Abstract: As lupin crops form an integral part of sustainable farming systems, the end use of lupin seeds as food for human consumptions could increase and diversify revenue streams for growers and improve farm profitability. Due to the similarity of composition and nutritional values between lupin and soybean, several traditional foods have been attempted with lupins. Recently more extensive investigations have been conducted to effectively fractionate and use lupin protein and kernel fibre as ingredients for various foods.

Key words: food, kernel flour, protein concentrate, dietary fibre, ingredient

Introduction

Lupin is a highly sustainable crop as it is utilised as a profitable break crop in cereal cropping sequences. It fixes nitrogen from air enriching topsoil with nitrogen, mobilises phosphate and other nutrients, breaks disease cycles and controls weed numbers. The lupin industry in Australia is based largely on *Lupinus angustifolius* that contains low alkaloids, has very deep roots and thus low watering needs and grows on infertile, sandy and acidic soils typical of large regions of Western Australia and the southeastern grain belt. This species was developed as a new crop in Australia by John Gladstones in the 1960s (1) and breeding programs have focussed on high protein and crop yields, low alkaloids and dehulling efficiency. Today, Australia is the largest producer and exporter of lupin in the world with production of 475,000 tonnes for 2019/2020 (ABS; Pulse Australia, 2020). Whilst lupins make about 30-40% of the annual Australian pulse crops, they are mostly used in stockfeed and aquaculture and only a small portion of lupin seeds and derivatives are used for human consumption. Therefore, there are opportunities to further explore food uses and allow lupin a greater role in sustainable agro-food systems and human nutrition.

Lupin seeds represent a real food alternative, both as nutritious and healthy whole food and as a source of nutri- and techno-functional ingredients (2). Lupin seeds have high levels of protein and dietary fibre, very low levels of starch and the lowest glycaemic index (GI) among commonly consumed grains and pulses (Table 1). Lupin seeds also contain vitamins and minerals (3), and health functional properties of the globulin protein fraction such as control of blood glucose, hypertension, obesity, satiety and plasma lipid homeostasis (4) have been described. Compared with soybeans, lupin application in food systems has been limited for reasons including price competitiveness and concerns about taste, allergenic or anti-nutrient properties and protein quality and functionality. However, since Food Standards Australia New Zealand recommended lupin as suitable for human consumption in the late 1980s, the use of lupin for human consumption has been extensively investigated over the past 40 years.

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Lupin whole seeds for food use

Lupin seeds or flakes can be eaten as a snack food, or as an ingredient in fresh salads, cereals or soups. Research has shown the utility of lupin seeds in traditional Asian foods, such as lupin bean sprouts (5); bean curd including tofu and tempeh (6), fermented sauce (7, 8) and as an ingredient in drinks (9).

Lupin kernel flour as a food ingredient

The major portion of the lupin seed is the kernel. Lupin kernel flour is derived by milling dehulled seeds. The applications of lupin kernel flour in several food systems have been studied, such as bread and pasta (8, 9). Lupin kernel flour has been used to produce gluten-free foods or high nutritional foods to improve the amino acid balance and protein content of bread (10,11).

Lupin protein concentrate and dietary fibre as food ingredients

While soybean is high in protein and oil, lupin seed is high in protein and dietary fibre. With increasing awareness of the health beneficial effects of dietary fibre, lupins emerge not only as a human health food, but also as an important food additive (12). There are several processing strategies that can be implemented using assets that are commercially available from various vendors to manufacture functional ingredients from lupins. These processing strategies range from dry to wet fractionation with optional additional processing to pre-treat, intensify, refine, stabilise, or formulate. Based on the overall processing flow implemented, the fractions vary in purity, composition and functionality of proteins, fibre, and other molecules such as oligosaccharides, phytates, saponins, tannins, enzyme inhibitors, alkaloids and minerals. CSIRO has led the development of extraction processing and food application of high purity protein concentrate and fibre fractions from lupin.

Protein and dietary fibre fractions from lupin kernels

A simple and efficient process using alkaline extraction at a pilot scale was developed to fractionate both protein and dietary fibre (13, 14), with the wet fractionation process presented in Figure 1. The protein content of the protein concentrate fractions is about 80%. The fibre content in the dietary fibre fraction is about 93%.

Table 1. Chemical compositions of lupin seed, compared to soybean, chickpea, pea and wheat (g/100g)[a]

	Lupin	Soybean	Chickpea	Pea	Wheat
Protein	33-39	30-60	19-24	22-23	9-15
Non-starch polysaccharide	30-40	14-23	9-11	18-23	7-12
Fat	5-8	17-28	4-7	2-3	1-2
Ash	3-5	4-5	3-4	2-3	1-2
Starch	8-16	15-29	39-57	45-65	60-70
Moisture	7-9	9-13	10-12	9-11	12-13

[a] values are presented in a range according to species, cultivars and pedoclimatic conditions

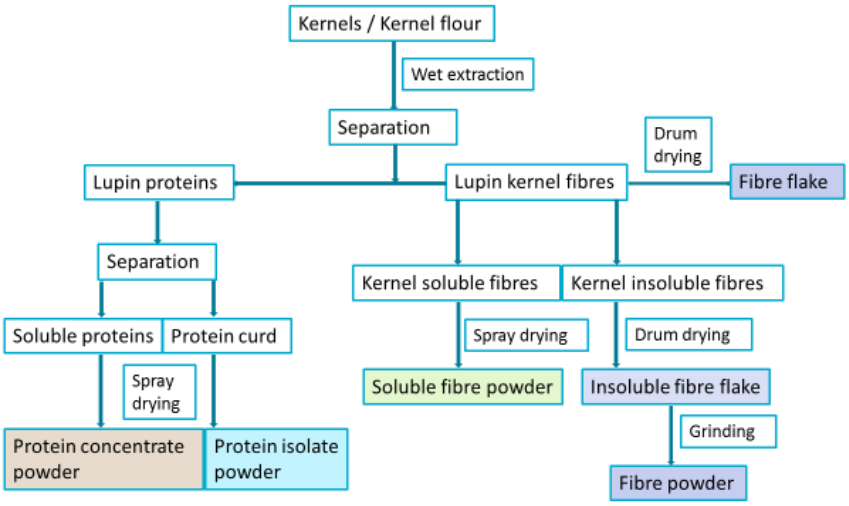

Figure 1. Lupin protein and dietary fibre wet fractionation process.

Food product development using protein concentrate fraction as an ingredient

The functionalities of protein concentrate were evaluated in various foods. The results showed that lupin protein concentrate offered an alternative to soybean, milk, and egg protein products. Lupin protein concentrate was a light cream colour, had excellent functional properties for a wide range of food applications, including bread, pasta, sauces, non-dairy and dairy products, desserts, and beverages (Figure 2). Lupin concentrates would also be suitable as alternative ingredients to soy protein concentrates in protein-based "body-building" health foods (14).

Food product development using fibre fraction as an ingredient

The lupin dietary fibre had lower oil absorption capacity, higher water solubility, swelling power and water absorption capacity, compared to commercial soy fibre. These food functional properties propose lupin fibre as a valuable ingredient in structured meat products, bakery, pasta, soup products and particularly bread, and are important for lupin fibre utilisation as a food ingredients (14).

Food product developments were successful at the commercial scale. Lupin fibre as an ingredient could be incorporated at low levels in a wide range of liquid, semi-

liquid foods, dry foods, and ingredient mixes, without adversely affecting taste and appearance. A range of food products were developed: fibre enriched fruit juices (Figure 2), high fibre fruit bars, a variety of bread and muffins, instant mashed potato, pasta, breakfast cereal, chocolate beverages with various levels of lupin fibre, and fibre tablets with a composition of 30% dietary fibre (14).

Conclusions

Lupin is a highly sustainable crop. The utilization of lupin seeds for human consumption could increase and diversify revenue streams for growers and improve farm profitability. With the world population expected to surge to 10 billion people by 2050, the demand for food will increase by an estimated 70% (15). The diversification of food sources is a crucial challenge in the context of sustainable agro-food systems. Lupins are a promising future source of edible plant proteins and other ingredients to contribute to global food and nutrition security as well as environmentally sustainable food production. With a highly favourable combination of nutritional attributes and food functionality, lupin kernel flour, lupin dietary fibre and protein have potential as new and unique ingredients in a wide range of foods. Separation and fractionation processing of lupins and lupin ingredients must be selected based on composition, functionality, stability, and cost requirements to formulate food products.

Acknowledgements Many aspects of food application of lupin seed and its derivatives have been conducted at CSIRO, Werribee, Victoria. We acknowledge the financial support of the Grains Research and Development Corporation, AusAID, Victoria Government, Australasian Natural Ingredients Pty Ltd and George Weston Foods Ltd.

Figure 2. An example of lupin protein concentrate applied to bakery products and an example of dietary fibre applied to fruit juice products.

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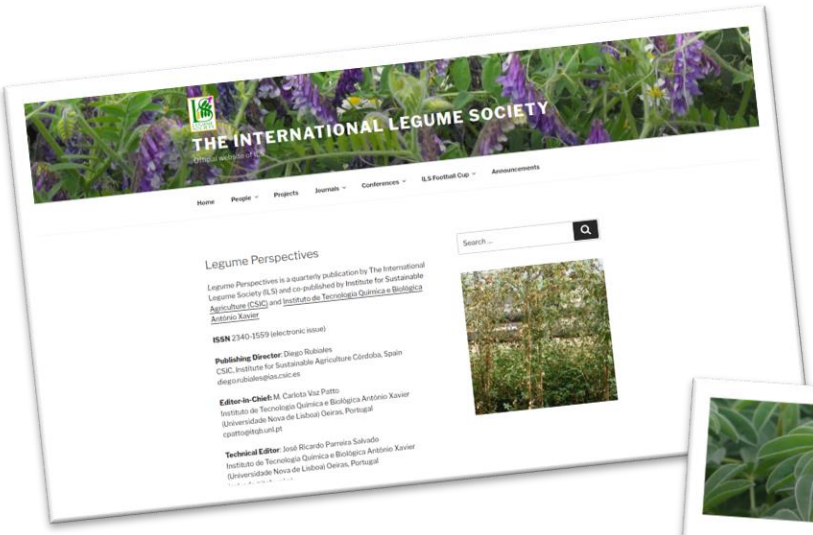

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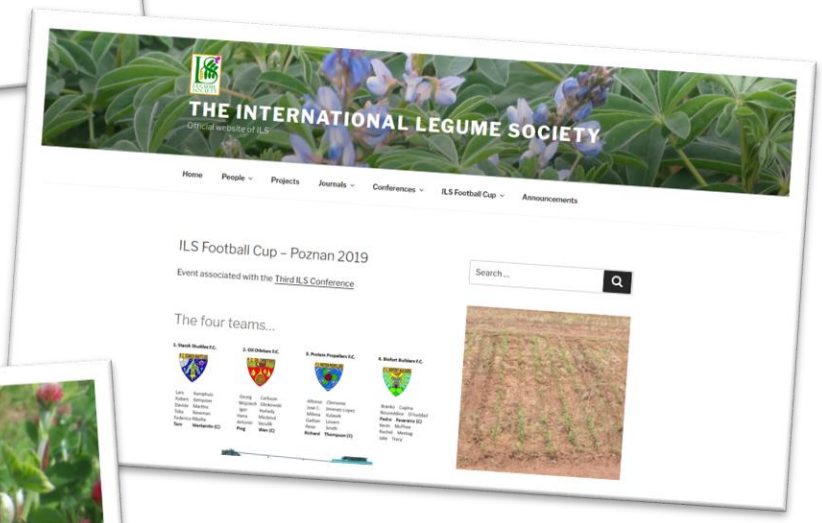

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