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Lupins in European Cropping Systems

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Abstract

The lupins are an interesting group of legume crop species that produce large seeds containing up to 40% protein. The genus *Lupinus* is part of the tribe *Genisteae*. More than 170 species have been described from the New World and only 12 species from Europe, North and East Africa. Wild lupins are bitter and toxic because they produce quinolizidine alkaloids as a means of chemical defence. During domestication, lupins with low alkaloid contents were selected, leading to 'sweet' lupins with alkaloid contents below 0.02% in the protein-rich seeds, which can be used both for human and animal consumption. The domesticated lupins include *Lupinus angustifolius*, *Lupinus albus*, *Lupinus luteus* and *Lupinus mutabilis*. Blue or narrow-leafed lupin (*L. angustifolius*) is the most widely cultivated of them, with a worldwide production of more than 1.3 million t. Several challenges remain for lupin breeding, including the improvement of quantitative and qualitative traits, adaptation to alkaline soil and resistance to fungal pathogens.

Introduction and Taxonomy

Lupin species from the Americas are mostly herbaceous perennials, whereas Old World lupins are generally annuals. All of them host symbiotic nitrogen-fixing *Bradyrhizobium* in root nodules (Sprent and McKey, 1994). Most lupins are 0.3–1.5 m tall; some shrubs reach 3 m in height and a few Andean species grow as trees. Lupin leaves are usually palmately compound, soft and divided into five to 28 leaflets, but a few species in south-eastern North America and Atlantic

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South America carry single leaflets. Flowers appear in dense or open whorls on an erect stem. The pea-like flowers (length 1–2 cm) consist of an upper standard, two lateral wings and a keel (formed from two fused petals). Fruits appear as a typical pod with several hard-coated seeds. Seeds are rather big (in agricultural species), with up to 40% protein, up to 20% lipid, fibre and several secondary metabolites (quinolizidine alkaloids (QA), flavonoids, isoflavones, tannins, saponins, oligosaccharides). The fatty acid profile, particularly the polyunsaturated fatty acids (PUFAs) and n-3:n-6 PUFA ratio (Chiofalo *et al.*, 2012) is considered beneficial for human health (Boschin *et al.*, 2007) and in animal nutrition (Singh *et al.*, 1995; Vicenti *et al.*, 2009). In addition, lupin has been studied as a human foodstuff because of its potential in functional and healthy food products due to its hypocholesterolaemic and antidiabetic potential (El-Adawy *et al.*, 2001; Duranti *et al.*, 2008). Lupins are also cultivated as attractive ornamentals (e.g. Russell hybrids of *Lupinus polyphyllus* and other American species).

Lupinus is a large genus including about 170 species all over the world, only 12 of which are native in Europe or in the Mediterranean Basin: *Lupinus albus*, *Lupinus anatolicus*, *Lupinus angustifolius*, *Lupinus atlanticus*, *Lupinus cosentinii*, *Lupinus digitatus*, *Lupinus hispanicus*, *Lupinus luteus*, *Lupinus micranthus*, *Lupinus pilosus*, *Lupinus palaestinus* and *Lupinus princei* (taxonomy according to GRIN, 2013). Only four species are domesticated and play an important role in agriculture: three come from the 'Old World', *L. albus* (white lupin), *L. angustifolius* (narrow-leafed lupin) and *L. luteus* (yellow lupin); and one comes from the 'New World', *Lupinus mutabilis* (tarwi). Many other lupin species such as *L. cosentinii*, *L. pilosus* and *L. hispanicus* that are underutilized show potential as cultivated plants. Chromosome numbers range from 2n = 32, 36, 38, 40, 42, 50 and 52 in Old World lupins to a more consistent number 2n = 48 or 36 in New World lupins (Käss and Wink, 1997a).

Lupins very likely evolved in the Old World and colonized the Americas via long-distance dispersal almost 15 million years ago. Analysis of DNA sequences (Käss and Wink, 1997a, b; Hughes and Eastwood, 2006; Eastwood *et al.*, 2008) have revealed that three main phylogenetic lineages exist within lupins: (i) lupins of the Old World inhabiting the Mediterranean and African region north of the Sahara (approximately 12 species); (ii) lupins of North, Central and South America (approximately 130 species, depending on the authority) with a recent radiation that was induced by the uplift of the Andes and Rocky Mountains; and (iii) lupins of Atlantic South America (mainly Brazil) (approximately 30 species). A few North American lupins, such as *Lupinus texensis* cluster with Old World lupins. The relationships shown in Fig. 6.1 are important for lupin breeders as they explain why it is impossible or difficult to hybridize Old World lupin species with New World taxa. Hybrids are possible within the North American lupins and between *L. mutabilis* and *L. polyphyllus*.

The word 'lupin' derives from the Latin *lupus* (wolf) with different interpretations: able to grow in very hard environments or able to catch great quantities of nutrient from soil. The oldest record of *L. albus* dates back to around 3500 years BC, in the Late Neolithic, even if without specific evidence of cultivation. Later, clearer evidence of cultivated lupin has been found in the Bronze Age in Greece, Cyprus and Egypt. Seeds of domesticated *L. digitatus* were discovered in

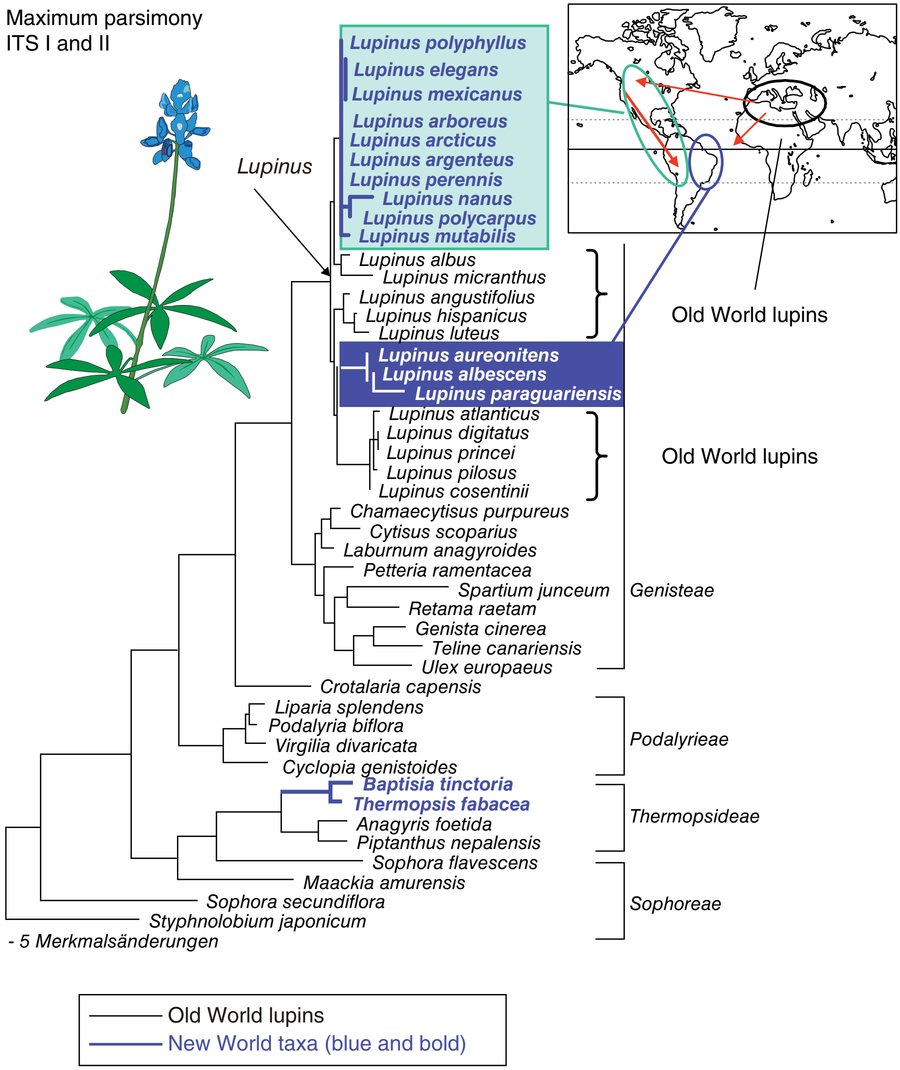

Fig. 6.1. Molecular phylogeny of *Lupinus* in relation to other legumes and to its geographic origin. ITS, Internal transcribed spacer.

the tombs of Pharaohs being over 4000 years old (Zohary *et al.*, 2012). *L. albus* was first cultivated as green manure, as forage and probably also for human consumption.

Cultivation became more widespread when people recognized that the bitter taste due to alkaloids could be removed by a prolonged soaking. Lupin is still a neglected crop species with only about 650,000 ha of cultivated lupins grown (FAOSTAT, 2014). Lupins account for about 1% of all the ten Food and Agriculture Organization of the United Nations (FAO)-recognized grain legume crops, grown largely on land not suitable for beans, chickpea, cowpea, pea, etc. In Europe, lupins are grown on about 150,000 ha, and the area is stable. Poland, the Russian

Federation, Germany, Belarus and Ukraine are the countries in which lupins are cultivated on more than 10,000 ha (FAOSTAT, 2014). In Germany, *L. angustifolius* is the main species grown, while in Poland both *L. angustifolius* and *L. luteus* are cultivated. The main production areas for *L. albus* are found in the south with Italy, France and Spain as the main lupin-producing countries (5000 ha, 3600 ha and 3045 ha, respectively, in 2013; FAOSTAT, 2014).

The main hindrance to the spread of lupins as a crop is low yield, low tolerance to alkaline soil, bitter and toxic alkaloids and anthracnose. Most of these obstacles have been addressed. Considering the low investment, great progress has been achieved in a short period of time.

Secondary Metabolites: Quinolizidine Alkaloids (QA)

Wild lupins are quite toxic due to QA (Wink, 1993) synthesized in the leaves which can account for up to 8% of their dry weight. If a lupin plant is wounded, its alkaloid content can be increased by a factor of four within a couple of hours (Wink, 1992, 1993). Lupins grown at high altitudes usually show lower alkaloid contents than those grown at lower elevations.

It has been postulated that the transfer from the phloem into the growing seeds also requires an alkaloid transporter, providing opportunities to breed lupins with high alkaloid levels in the green parts (to protect against herbivores) but low levels in the seeds. Another important group of secondary metabolites in lupins is the isoflavones, such as genistein, which bind to oestrogen receptors and can be regarded as phyto-oestrogens, a property that might be interesting for nutraceuticals (used to treat menopausal conditions and osteoporosis). Isoflavones also exhibit antifungal activities.

Properties and Uses

In recent years, legumes have established a key position for food and pharmaceutical industries not only for their nutritional role, but also for a number of both adverse and beneficial effects that they may exert on the human body, including food intolerance, allergies and hypolipidemic, hypoglycaemic, hypotensive and anti-obesity activities. In addition, lupin proteins can play important techno/functional roles as witnessed by their increased uses as food ingredients.

Lupin seeds contain two main classes of proteins. These are the albumin and globulin fractions, which account for 10% and 90% of the total protein content, respectively (Blagrove and Gillespie, 1975; Duranti *et al.*, 1981). Prolamins and glutelins are absent.

The most representative albumin protein is δ-conglutin that belongs to the 2S sulfur-rich albumin family (Blagrove and Gillespie, 1975) and accounts for about 5% of the total seed proteins. It is structurally related to cereal bifunctional trypsin/α-amylase inhibitors (Gourinath *et al.*, 2000). The interest in this protein lies in the physiological role, being involved in plant defence against pathogens (Terras *et al.*, 1992; Agizzio *et al.*, 2003).

Three different proteins fall within the globulin group. α-Conglutin belongs to the 11S globulin family (legumin-like) and represents about 35–37% of the total globulins (Duranti *et al.*, 1981). β-Conglutin belongs to the 7S globulin family (vicilin-like) and is the most abundant protein in the seed (about 44–45%) (Duranti *et al.*, 1981). γ-Conglutin is an unusual basic 7S glycoprotein, which accounts for about 4–5% of total proteins in mature lupin seeds (Duranti *et al.*, 1981) and is likely to be involved in plant defence mechanisms (Scarafoni *et al.*, 2010).

From a nutritional point of view, lupin seed proteins have a biological value of about 90% that of egg protein (Egaña *et al.*, 1992). White lupin seeds have a higher essential amino acid index and protein efficiency ratio than narrow-leafed and yellow lupins (Duranti *et al.*, 2008).

Lupin proteins can cause allergic reactions in individuals sensitized to peanut and other legume seeds (Moneret-Vautrin *et al.*, 1999; Parisot *et al.*, 2001). For these reasons, lupin has recently been added to the list of potential allergenic foods and its use as an ingredient should appear on the labelling of foodstuffs (European Commission Directive, 2006/142/ EC) as a precautionary measure for consumer protection.

Because of the low off-flavour, lupin flour and protein isolates are suitable for novel foods. A variety of lupin-based preparations is now available on the market: (i) tofu-like and tempe-like products; (ii) milk and meat product analogues; (iii) bakery products; and (iv) gluten-free pasta, sauces, mayonnaise and salad dressings.

Lupin flours may be used as ingredients in bakery products with up to 20% of inclusion (Dervas *et al.*, 1999; Pollard *et al.*, 2002; Sironi *et al.*, 2005). The use of lupin flour promotes water retention and fat binding in dough and is also considered an excellent egg and butter replacement. Since lupin does not contain gluten, lupin flour is used as a functional ingredient in gluten-free foods (Capraro *et al.*, 2008). Partially purified lupin protein fractions are commercially available (Wäsche *et al.*, 2001).

Beyond the nutritional and technological aspects, lupin is a good source of bioactive compounds, opening up opportunities for new food products. Several molecules, such as proteins, peptides and smaller molecules such as alkaloids, isoflavones and oligosaccharides, isolated from seeds of common and uncommon legume plants have already been investigated for their bioactivities (Scarafoni *et al.*, 2007). The search for novel activities is expanding. Several biological activities have been attributed to the protein fraction, particularly to γ-conglutins. These include glycaemia (Magni *et al.*, 2004; Terruzzi *et al.*, 2011), plasma cholesterol/ triglyceride lowering effects (Sirtori *et al.*, 2004) and anti-hypertensive properties (Yoshie-Stark *et al.*, 2004; Pilvi *et al.*, 2006).

The average oil content amounts ranges from 5% to 6% in *L. angustifolius* and *L. luteus* to about 15–17% in *L. mutabilis*. *L. albus* shows intermediate oil content (9–13%) (Chiofalo *et al.*, 2012). The growing environment (location, time of sowing, climatic variations) affects lipid accumulation, fatty acid quality and phytosterol composition, protein content and composition (Annicchiarico *et al.*, 2014). Lupin oil has a higher omega-3:omega-6 ratio than reported for most vegetable oils (Boschin *et al.*, 2007). All of this makes lupin oil potentially valuable in dietary uses.

The fibre fraction is also relevant. The content of total dietary fibre (TDF) and insoluble dietary fibre (IDF) is considerably higher than in other legumes, including soybean, but soluble dietary fibre (SDF) is slightly lower (Písarikova and Zraly, 2010).

These qualitative traits offer novel business opportunities. Improving knowledge on seed components and their properties is crucial for their optimal exploitation and to develop new products for the food and non-food industries. Proteins seemingly have the greatest potential to be exploited in this respect, owing to the diverse biological activities of their peptides. The potential for use of the lipids is underestimated, possibly because their limited quantities do not encourage industrial extractions and applications. The moderate oil content of white lupin may justify selection work aimed to further increase this characteristic offering the prospect of a dual purpose protein and oil crop like soy.

Genetic Resources, Genomic Tools and Breeding

Ex situ genetic resources

A key aspect underpinning current and future plant breeding efforts is the availability of appropriately conserved and documented germplasm (often referred to as plant genetic resources for food and agriculture, PGRFA).

Westengen *et al.* (2013) provide information on lupin accessions recorded in key databases, namely: (i) the gene bank-level data in the FAO World Information and Early Warning System (WIEWS) on PGRFA; and (ii) accession-level data in GENESYS (a database with information on over 2.3 million accessions from 365 gene banks). For *Lupinus* they found 38,053 reports in WIEWS but only 7503 of these are estimated to represent distinct accessions. Westengen *et al.* (2013) also reported 13,567 accessions in GENESYS. In the Svalbard Global Seed Vault (SGSV) they record a current holding of 591 accessions. The major gene bank holdings of *Lupinus* are in Australia, Germany, Peru, Russia, France and the UK.

The Australian collection holds a significant number of accessions of the major cultivated species (*L. albus*, *L. angustifolius* and *L. luteus*) but also smaller numbers of important related species (e.g. *L. cosentinii*, *L. pilosus* and *L. mutabilis*). None of this germplasm is currently held in the SGSV. Indeed *Lupinus*, as described by Westengen *et al.* (2013), is one of the important non-Annex 1 food crops for which the representation in SGSV is less than 10% of the distinct accessions shown in WIEWS.

The European Cooperative Programme for Plant Genetic Resources (ECPGR) is an important network for conservation and use of PGRFA. The ECPGR database is hosted at the Institute of Plant Genetics, Polish Academy of Sciences. For *L. albus* (3677 accessions listed), the major centres are the Institut National de la Recherche Agronomique (INRA) France, Spain (732 accessions), Germany, USA and Poland, with the biggest collection (979 accessions) in Australia at the Centre for Legumes in Mediterranean Agriculture (CLIMA). A similar pattern is seen for *L. angustifolius* with 3894 accessions including 542 in Spain and 2165 at CLIMA. The listing shows a smaller number of accessions of *L. luteus*: 1799 with 463 at CLIMA and 303 in Spain.

Crop wild relatives or landraces are particularly useful as sources of disease resistance. Adhikari *et al.* (2009) described the situation in *L. albus* with respect to the use of landraces for the introduction of anthracnose resistance into modern cultivars.

Genetic and Genomic Tools

There is now a growing body of genetic and genomic resources available for lupin breeders to increase the speed and precision of their programmes. This is especially true for narrow-leafed lupin, whose genome sequencing is nearing completion in Australia.

Within the last 10 years, genetic maps of white and narrow-leafed lupins have been developed (Nelson *et al.*, 2006; Phan *et al.*, 2007). These studies have gained from work carried out on the model legumes *Medicago truncatula* and *Lotus japonicus* (Zhu *et al.*, 2005). Although lupins are somewhat more taxonomically remote from these models than the other important crop legumes, information on synteny (arrangement of genes on chromosomes) between lupins and these models is useful with respect to both marker and gene discovery. The genome sequencing of both these models opened up important possibilities with regard to alignment to help the sequencing of the lupin genome (Nelson *et al.*, 2010). Quantitative trait loci (QTL) were identified for anthracnose resistance and flowering time by Phan *et al.* (2007). White lupins were used by Croxford *et al.* (2008) to map sequence-tagged sites onto genetic maps using high-resolution melt analysis to identify the sites of sequence variation.

However, the limitations of approaches based only on QTL derived from biparental crosses have become clearer in recent years, so association mapping methods have become more popular. First, it is necessary to analyse the population structure of the species or accessions of that species that are to be used in the mapping. An estimate of the rate of decay of linkage disequilibrium is also required. Iqbal *et al.* (2012) carried out such a study for 122 accessions of white lupin. This work re-emphasizes the importance of *ex situ* collections and the documentation accompanying them.

Anthracnose, caused by the fungus *Colletotrichum lupini*, is the most devastating disease of lupin in most environments, so resistance to this disease has been a major breeding objective. Early flowering to avoid terminal drought is also important under these conditions, and the two traits were combined by Adhikari *et al.* (2013).

Next generation sequencing (NGS) approaches have been used to develop a draft genome sequence of *L. angustifolius* (Yang *et al.*, 2013b) using the high-yielding, anthracnose-resistant cultivar ‘Tanjil’. In addition, these authors developed a restriction-site associated DNA sequencing (RAD-seq) genetic map based on 94 F_8 recombinant inbred lines derived from a cross between ‘Unicrop’ and

'Tanjil'. As compared to a full genome analysis, RAD-seq data cover only part of the genome. NGS also facilitates the development of sequence-specific markers for key traits, with disease resistance genes again being the first exemplars (Yang *et al.*, 2010, 2012, 2013a). Transgenic resources have been developed for *Lupinus*, mostly focused on protein quality. The feasibility of this approach was shown by Molvig *et al.* (1997), who enhanced methionine levels in *L. angustifolius* through the expression of a sunflower seed albumin gene. This addresses the major issue of protein composition with respect to animal diets, namely the deficiency that lupins share with many other grain legumes with respect to the sulfur-containing amino acids cysteine and methionine. Further work showed that a similar approach can also increase the efficiency of wool growth and live-weight gain in sheep fed on such transgenic lupin seed (White *et al.*, 2001). The first report of the production of transgenic plants in *L. luteus* was made by Li *et al.* (2000), who used *Agrobacterium*-mediated transformation to introduce a gene for herbicide resistance. Disease resistance has also been a target for transgenic interventions. Wijayanto *et al.* (2009) reported the use of *Agrobacterium*-mediated transformation to introduce the baculovirus anti-apoptotic *p35* gene to combat fungal necrotrophs with some indications of potential for success. Hamblin *et al.* (2005) concluded that the likelihood of gene flow from a transgenic crop of *L. angustifolius* in Western Australia to wild lupin populations is extremely low, but the situation may differ where wild relatives occur.

Breeding

The history of lupin breeding in general (Cowling *et al.*, 1998), and in Australia specifically, has been reviewed (Cowling and Gladstones, 2000) and will not be repeated here.

An important part of the domestication of lupins has been the reduction of QA content, eliminating the bitterness and making the lupin seed palatable and safe for human consumption. Total seed alkaloid should remain under 0.02%. Nevertheless, alkaloids in lupins are responsible for resistance to herbivorous insects including aphids, which are a major pest and a limiting factor in the development of *L. luteus* as a crop. Adhikari *et al.* (2012) described approaches to the selection of lupin lines with diverse alkaloid profiles to form the basis of a breeding programme in this crop.

In Russia, breeding of *L. albus*, *L. angustifolius* and *L. luteus* draws on the genetic resources of the Vavilov Institute of Plant Industry collection (Lukashevich *et al.*, 2011). Again, anthracnose resistance is a major target along with yield, quality (protein and oil), early maturity and resistance to lodging. However, anthracnose is not the only significant fungal disease of lupin. A survey of soil-borne pathogens of narrow-leafed lupin in north-eastern Germany showed a range of species including *Fusarium* spp., *Rhizoctonia solani* and *Thievalopsis basicola* (Kaufmann *et al.*, 2011). The authors used nested PCR to identify fungal species and highlight the importance of developing robust resistance screens, particularly for *Fusarium oxysporum* and *T. basicola*.

The importance of root structure in nutrient use efficiency, tolerance of abiotic stress and carbon sequestration is becoming increasingly well documented. The modelling of root traits represents one approach to develop a basis for selection of desirable root characteristics from accessions or breeding lines. Chen *et al*. (2012) used *L. angustifolius* accessions with diversity characterized by DArT (Diversity Arrays Technology) to investigate variation in root traits in a semi-hydroponic phenotyping system.

Clearly, lupin species are currently minor crops in many countries and key constraints include unreliable yields, late maturity and poor tolerance of alkaline soils. A programme of breeding of lupins for adaptation to new environments and uses is required in countries where they have considerable potential but are not widely grown currently. Abberton and Mizen (2008) reported some first steps in this regard for the UK. Their major targets were increased yield, earlier flowering and improved tolerance of alkaline soils in *L. angustifiolius* and *L. luteus*. Considerable progress was made after 3 years of phenotypic selection of individual plants, rows and plots. A hydroponic system was used to select for enhanced tolerance of alkaline pH and promising lines were identified. This indicates that even with a narrow range of germplasm, rapid progress can be made with respect to ecogeographic and edaphic adaptation. A further key component is quality for diverse uses including human food and aquaculture as well as an important source of protein in the ruminant diet.

Eickmeyer (2008) enumerated the following objectives for a private-sector breeding programme for narrow-leafed lupin in Germany:

- productivity – seed yield, raw protein yield, number of pods, number of seeds per pod, 1000-kernel weight;
- yield stability – frost tolerance, drought tolerance, lodging resistance, pod shattering resistance, flower dehiscence, soil pH tolerance, early ripening, equal ripening;
- disease resistance – *Colletotrichum*, soil-borne fungal pathogens, *Setoria* beetle; and
- seed quality – protein quality, antinutritive substances, alkaloids, fibre content and quality, phyto-oestrogens, oil quality.

This list shows the range of challenges for the lupin breeder and emphasizes the need for development in high-throughput phenotyping alongside advances in genomics.

Efforts in northern Europe have also focused on narrow-leafed lupin and its adaptations to a range of ecoclimatic conditions, since its growing season is shorter than those of the other domesticated species. Kurlovich *et al*. (2011) reported on the performance of 50 accessions grown across Finland, Russia and Ukraine and the development of cultivars for Finnish conditions.

The study of lupins has clearly advanced into the genomics era, but there is some way to go before the full suite of tools is applied effectively, alongside high-throughput precision phenotyping in breeding programmes, particularly for complex traits such as yield, yield stability and tolerance of edaphic stress. Successful improvement in these traits is necessary if lupins are to expand their role in crop production globally.

Canopy Structures

Domesticated lupin species usually have both branching and non-branching cultivars. As the non-branching cultivars flower only on the main stem, they ripen earlier and more uniformly than branching cultivars. In the latter, the number of branching (and hence flowering and pod-setting) levels is not only genetically determined, but also influenced by environmental and cropping conditions, such as plant density.

Adaptation: Climate and Soil

Of the Old World lupin species, yellow lupin (*L. luteus*) is generally the most drought-resistant and can be grown on the poorest soils with lowest pH (pH 4). However, due to its limited yield potential and high disease susceptibility, yellow lupin production and breeding in Europe is very limited.

Narrow-leafed lupin (*L. angustifolius*) generally requires a minimum soil pH of 5 and maximum pH of 6.5–6.8. It is the main lupin species grown in northern Europe. Non-branching cultivars of narrow-leafed lupin have shorter growing periods than branching cultivars (90–150 days versus 150–180 days), so they can be grown as far north as Finland, whereas branching cultivars are grown no further north than Denmark.

White lupin (*L. albus*) has somewhat wider adaptation to different soil types than the other species, growing also on loamy and light clay soils. Compared with other lupin species, its calcium tolerance can be relatively high, although this property seems to vary widely depending on the origin of the germplasm. Due to its long growing period (140–200 days) it is unsuitable for growing for seed further north than the Netherlands.

Calcium Tolerance

Most commercial cultivars of *L. angustifolius* and *L. luteus* grow poorly on calcareous soils, showing poor nodulation and high levels of chlorosis. Tang and Thomson (1996) suggested this to be the result of low tolerance of *Bradyrhizobium* to high soil pH (pH > 6). However, Nuijten and Prins (2013) successfully inoculated and grew white lupin on soils with pH 7.5 ($CaCO_3$ 8.6%). Other studies suggest that chlorosis in calcium-intolerant cultivars is due to reduced iron uptake (Coulombe *et al*., 1984). However, Raza *et al*. (2001) found no significant differences in iron uptake between tolerant and intolerant *L. albus* cultivars. The latter study did find that intolerant cultivars took up significantly more calcium. High calcium levels in leaf tissue reduce the opening of stomata and hence reduce assimilation rates.

Calcium-tolerant cultivars have been found in *L. pilosus* (Brand *et al*., 2000) and, within *L. albus*, in Egyptian (Christiansen *et al*., 1999; Raza *et al*., 2001) and Italian (Annicchiarico and Thami-Alami, 2012) landrace germplasm. Cultivars of white lupin have been grown successfully on soils with pH values of up to 7.8

and free calcium-carbonate levels of 6.5–8.6% (Gresta *et al.*, 2010; Nuijten and Prins, 2013). Some Egyptian landraces of white lupin are even able to grow on soils with pH values of 8.5 and higher, tolerating free calcium levels of over 10% (Christiansen *et al.*, 1999). On the other hand, there are reports of white lupin grain and biomass reduction already in the presence of soil-free calcium above 1% (Papineau and Huyghe, 2004), which indicates that calcium tolerance varies widely within the species.

The ability to form proteoid roots and excrete citric acid is thought to be one of the main mechanisms of calcium tolerance, as the acids reduce calcium uptake in favour of iron and phosphorus uptake.

Management Techniques

Sowing time and density

In Mediterranean climates, autumn-sown lupin crops often give the best yields (Annicchiarico and Carroni, 2009). Farther north, autumn sowing is not feasible due to the longer and colder winters. Narrow-leafed lupin cultivars tend to have a higher frost tolerance than white lupin (−8/−10°C versus −4/−6°C), so are more suitable for early spring sowing. However, if the weather stays cold, growth is slow and weeds become a problem. Therefore organic lupin growers in the Netherlands tend to sow their crops no earlier than late March or early April (Prins, 2014).

Optimum plant density is cultivar-dependent. In branching cultivars, increasing plant density will generally decrease the level of branching and reduce the number of pods per plant, but ripening will be earlier and more uniform, with less variation in seed number per pod and mean seed weight (Herbert, 1977, 1978). However, dense crops tend to be more susceptible to fungal diseases such as *Sclerotinia* and *Botrytis*.

Fertilization

No significant positive yield responses to nitrogen (N) fertilization were observed in field trials in northern Europe (Prins, 2014). In Dutch field experiments, fertilization with potassium sulfate did not increase yields, but significantly reduced alkaloid levels, thus improving quality. This effect was greater in cultivars with intrinsically high alkaloid levels, and more pronounced in crops grown on soils low in potassium (Prins, 2014; Prins and Nuijten, 2015).

Nodulation

Good nodulation is essential for lupin production and inoculation with *Bradyrhizobium* is nearly always recommended, although lupin has been grown successfully on sandy soils without inoculation or history of lupin cultivation. Inoculation is not considered necessary where lupin or serradella (*Ornithopus sativus* Brot.) has recently been grown successfully, and seems to be more important in soils with less favourable conditions (e.g. pH > 6.5).

Weed control

Weeds compete effectively against lupins. Sufficient plant density helps to reduce weed growth in these crops (Herbert *et al.*, 1978; Isaac *et al.*, 2000), but chemical or mechanical weed control remains necessary. Harrowing four to five times in the first 2 months after sowing has been shown to effectively reduce weed populations to acceptable levels, without damaging the lupin crop (Jensen *et al.*, 2004c). Options for chemical weed control are limited as lupin is susceptible to most post-emergence chemicals. Therefore, weed control often combines pre-emergence herbicides with post-emergence harrowing (Prins, 2015).

Irrigation

The response of lupin to irrigation has been studied in various experiments. In general, irrigation is found to increase seed yield, as long as irrigation levels do not exceed crop water requirements (Herbert and Hill, 1978; Kang *et al.*, 2008, Hill *et al.*, 2011). Nevertheless, irrigation has also been found to increase infestation of lupin by grey mould (*Botrytis cinerea* Pers.), leading to yield losses (Jensen *et al.*, 2004b).

Yields and cultivars

There is little commercial breeding of lupin in Europe. Breeding of narrow-leafed lupin is concentrated in the northern parts of Europe with Saatzucht Steinach in Germany, two breeding companies in Poland (Hodowla Roślin Smolice and Poznańska Hodowla Roślin) and two individual lupin breeders in Denmark, distributing through DLF Trifolium. In Germany, cultivars such as ‘Boregine’, ‘Haagena’ and ‘Sonate’ gave the highest yields in variety trials in 2009–2011 on fine-textured, deep loess soils, with yields of 3.5–5 t/ha (Guddat *et al.*, 2011). The yields of the same lupin cultivars on coarser textured, sandy soils was significantly lower (2.0–3.5 t/ha) and more variable (Guddat *et al.*, 2011). This is supported by field trials on sandy and light-clayey soils, in spring–summer crop cycle, in the Netherlands in 2008 and 2009, where average yields on light-clayey soils exceeded the yield on sandy soils by 16% in 2008 and 67% in 2009 (Prins and Nuijten, 2015). Different cultivars have been developed for different purposes, for example: (i) cultivars with very low alkaloid levels, suitable for human consumption (‘Borlu’ and ‘Vitabor’); (ii) cultivars with very high protein contents (‘Probor’); and (iii) cultivars that exhibit early ripening (‘Haags Blaue’ and ‘Boruta’). In Germany the early ripening, non-branching cultivars are out-yielded on most soil types by the branching cultivars. Further north, in Denmark, early ripening is considered more important as the growing season is restricted, so early ripening is necessary for certainty of harvest. For this reason, breeding has been focused on developing early ripening, branching ('Iris') and non-branching cultivars ('Prima', 'Viol' and 'Primadonna'). In Finland, a breeding programme has targeted non-branching narrow-leafed lupins with an even shorter growing period (85–115 days) to make them suitable for the short Finnish growing season (Kurlovich *et al.*, 2011). In Germany, a lot of attention was given to resistance to *Fusarium* wilt. In the maritime climate regions of Western Europe, *Sclerotinia* and *Botrytis* play a much larger role then in the continental climate regions of eastern Germany and Poland. Hence the Danish cultivar 'Iris' showed much more stable yields than the most productive German cultivars 'Boregine', 'Haagena' and 'Sonate' and Polish cultivars 'Bojar', 'Dalbor' and 'Regent' in variety trials in 2007–2009 and 2011–2013. The yields of the best-performing narrow-leafed lupin cultivars coincides with the yields found in Germany: 3.4–4.8 t/ha on low-calcareous clay soils and 2.5–4.5 t/ha on sandy soils (Prins, 2015; Prins and Nuijten, 2015).

For yellow lupin, breeding is limited to Poland, as breeding activities in other countries (Germany and Denmark) were terminated largely due to the low yield expectations and disease susceptibility (anthracnose). In variety trials in the Netherlands, yellow lupin produced 1.5–2.5 t/ha on sandy soils where the best narrow-leafed lupins produced 1–2 t/ha more.

Breeding activity on white lupin in northern Europe has also declined. Germany had its own white lupin cultivar ('Feodora') bred by Saaten Union, but it is no longer available in Germany and is maintained only in France. Recently, a small Dutch breeder (Globe Seeds) started breeding *L. albus*, but no commercial cultivars are available yet. In the Netherlands, a small breeding programme has been started at the Louis Bolk Institute, looking for calcium-tolerant lupins suitable for young sea-clay soils, using breeding lines from both Globe Seeds and a Danish/Egyptian breeding programme with calcium-tolerant Egyptian germplasm (Nuijten and Prins, 2013). The main breeding activity in white lupin, however, is in France (INRA and, later on, Jouffray-Drillaud), whereas a public breeding programme exists in northern Italy. While breeding of white lupin in northern Europe is focused on spring-sown cultivars, breeding in France and Italy is focused on autumn-sown materials. The French cultivars from INRA have been tested in the UK (at Rothamsted) and showed very good production potential (3–5 t/ha) (Milford and Shield, 1996), although extra focus has been given to earliness of maturity and non-branching character. The non-branching cultivar 'Lucyanne' performed well, although it showed very little tolerance to calcareous soils (Kerley *et al.*, 2004). The French cultivars 'Ludet', 'Luxe' and 'Lucille' have been tested in central (Mediterranean climate) and northern (sub-continental climate) parts of Italy and compared to a local cultivar ('Multitalia') and a landrace from the Molise region. In both locations, the Italian cultivars out-performed the French, yielding up to 5 t/ha when sown during the optimum sowing period (Annicchiarico and Carroni, 2009). Other trials in southern Italy reported lower yield for white (2.2 t/ha) yellow (0.8–1.6 t/ha) and narrow-leafed lupin (0.5 t/ha) (Gresta *et al.*, 2010).

Crop Rotation

Improved yields in the following crop

In field trials in the Netherlands, residual N levels in the soil (0–90 cm) directly after harvest were 40–60 kg/ha, 15–25 kg/ha more than those after the cultivation of spring wheat or barley fertilized with 100 kg/ha of mineral N fertilizer. Crop residues (straw, pods, leaves and roots) leave an extra 40–50 kg/ha of N to be mineralized for the next crop (Prins, 2014).

In field trials in Denmark, unfertilized winter barley grown after lupins showed a 3-year average yield increase of 1.31 t/ha or 77% on sandy soils and 0.87 t/ha or 36% on loamy soils compared with that after oat (1.69 t/ha on sandy soils and 2.42 t/ha on loamy soils). With increased N fertilization of the winter barley crop (120 kg/ha) on sandy soils, the pre-crop advantage of lupin over oat declined to 0.76 t/ha (15% yield increase). A yield increase at such high N-fertilization rates indicates that probably more than just N transfer from the lupin to the barley was responsible for the pre-crop benefit, and phosphorus (P) mobilization or improved soil structure could be involved. On loamy soils, the yield increase in the winter barley after lupin instead of oat was observed up to a N-fertilization rate of 90 kg/ha. In 2 of the 3 years, winter barley yield with 120 kg/ha of fertilizer N after lupin decreased, whereas that after oat increased, probably due to the higher susceptibility of over-fertilized barley to diseases and lodging. Through the entire study, the effect of lupin on the yield of winter barley at different N levels was similar to that of pea, including the decrease in yield on loamy soils at higher N fertilization levels (Jensen *et al.*, 2004b). The observed yield increases of cereals after lupin and pea in this study is confirmed by other studies in Germany with cereals grown after faba bean or pea, where yield increases of 71% were observed at low N fertilization rates, but even at high fertilization rates a yield increase of 10–30% was still found (Entrup *et al.*, 2003).

Diseases and Pests

Soil-borne diseases

If lupins are grown too frequently in a crop rotation, soil-borne diseases can build up and cause substantial yield losses. Within the legumes, the most important soil-borne pathogens are largely host-specific, with lupins mainly affected by *Fusarium oxysporum* and *Fusarium solani* (Jensen *et al.*, 2004a). These effects should be taken into account in crop rotations.

Mammals and birds

The sweet domesticated lupin, with its reduced alkaloid levels, is an attractive food source for deer, rabbits and hares. Crop damage from these herbivores is mostly limited to field edges. Damaged lupin is able to form new shoots, reducing the loss in yield. The forming of new shoots, however, delays the development and ripening of the crop, causing problems of uneven ripening. In contrast to pea, lupin crops are not very susceptible to bird damage.

Insects

Weevils and aphids form the main insect problems in lupin. Leaf weevils (*Sitona* spp.) not only damage the foliage but also the roots and nodules, affecting N fixation and causing severe yield losses (Kaufmann *et al.*, 2009). In areas where the frequency of legumes in crop rotations is high, weevil populations can be a serious problem for lupin production. In contrast, yield losses from aphids (e.g. lupin aphid, black bean aphid) are generally limited.

Fungal diseases

The agricultural lupins are susceptible to various fungal diseases, and fungi are often the principal cause of lupin yield losses. One of the main reasons why lupin breeding in northern Europe focuses on *L. angustifolius* is because of its relative resistance to anthracnose (*Colletotrichum lupini*), which causes great damage in *L. albus* and particularly in *L. luteus*. A second important fungal disease in lupin is caused by species of *Fusarium* (*F. oxysporum* and *F. solani*), which cause emergence problems, growth inhibition and late wilt. Disease pressure is particularly high in narrow crop rotations. The same is true for brown leaf spot (*Pleiochaeta setosa*), which has become a serious problem in narrow crop rotations in Australia (Kaufmann *et al.*, 2009). In wider rotations, brown leaf spot normally occurs as a ripening disease, causing only slight yield losses. Finally, in the moist oceanic climates of Western Europe, lupin production may also be affected by *Botrytis cinerea* and *Sclerotinia sclerotiorum*.

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