A pan-genome and chromosome-length reference genome of narrow-leafed lupin (Lupinus angustifolius) reveals genomic diversity and insights into key industry and biological traits

Gagan Garg1,†, Lars G. Kamphuis1,2,3,4,*,†, Philipp E. Bayer4, Parwinder Kaur5, Olga Dudchenko6,7,*,†, Candy M. Taylor2,5, Karen M. Frick1,8, Rhonda C. Foley1, Ling-Ling Gao1, Erez Lieberman Aiden5,6,7,9,10, David Edwards2,4 and Karam B. Singh1,2,3,1*  
1CSIRO Agriculture and Food, Floreat, WA 6014, Australia, 2UWA Institute of Agriculture, University of Western Australia, Crawley, WA 6009, Australia, 3Centre for Crop and Disease Management, Curtin University, Bentley, WA 6102, Australia, 4The School of Biological Sciences, University of Western Australia, Crawley, WA 6009, Australia, 5School of Agriculture and Environment, University of Western Australia, Crawley, WA 6009, Australia, 6Center for Genome Architecture, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA, 7Center for Theoretical Biological Physics, Rice University, Houston, TX 77005, USA, 8Section for Plant Biochemistry and Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark, 9Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech, Pudong, China, and 10Broad Institute of MIT and Harvard, Cambridge, MA, USA  
Received 27 March 2022; revised 15 June 2022; accepted 23 June 2022; published online 2 July 2022.  
*For correspondence (e-mail karam.singh@csiro.au; lars.kamphuis@csiro.au).  †These authors contributed equally to this work.

SUMMARY  
Narrow-leafed lupin (NLL; Lupinus angustifolius) is a key rotational crop for sustainable farming systems, whose grain is high in protein content. It is a gluten-free, non-genetically modified, alternative protein source to soybean (Glycine max) and as such has gained interest as a human food ingredient. Here, we present a chromosome-length reference genome for the species and a pan-genome assembly comprising 55 NLL lines, including Australian and European cultivars, breeding lines and wild accessions. We present the core and variable genes for the species and report on the absence of essential mycorrhizal associated genes. The genome and pan-genomes of NLL and its close relative white lupin (Lupinus albus) are compared. Furthermore, we provide additional evidence supporting LaRAP2-7 as the key alkaloid regulatory gene for NLL and demonstrate the NLL genome is underrepresented in classical NLR disease resistance genes compared to other sequenced legume species. The NLL genomic resources generated here coupled with previously generated RNA sequencing datasets provide new opportunities to fast-track lupin crop improvement.

Keywords: legume, pan-genome assembly, quinolizidine alkaloids, seed storage proteins, resistance genes, Genistoids.

INTRODUCTION  
Narrow-leafed lupin (NLL; Lupinus angustifolius L.) is a grain legume grown as a break crop in rotation with cereal crops, thereby reducing the need for fertilisers, increasing cereal yields and importantly providing disease breaks (Seymour et al., 2012). NLL and other lupins thrive on nutrient-poor soils due to their symbiosis with beneficial bacteria to fix atmospheric nitrogen and to efficiently mobilise phosphorus from soils (Lambers et al., 2013). NLL is a relatively young pulse crop, having only begun the process of domestication in the early 20th century in Germany, which was concluded by the release of the first fully domesticated cultivar with low alkaloid content, permeable seeds, early flowering and non-shattering pods in the 1960s in Australia (Gladstones, 1970). To date, four lupin species have been domesticated, including white lupin (Lupinus albus), yellow lupin (Lupinus luteus), pearl lupin (Lupinus mutabilis) and NLL, with NLL being the
predominant lupin grown worldwide (85% of all lupins) (FAO, 2021). NLL is grown on over 750,000 hectares, predominantly in Australia, but also in other countries, such as Poland, Russia and Germany (FAO, 2021).

The lupin grain is mainly used for animal and aquaculture feed (White et al., 2007), but in recent years it has gained interest as a human health food and food additive. This is because lupin grain is rich in protein (30–40% of whole seeds), has low amounts of undesired starch compared to other pulses and is high in dietary fibre (25–30%), low in fat and carbohydrates and gluten-free (Kohajdova et al., 2011). In human food products, lupin kernel flour is predominantly used as a food additive in bread and pasta (Kohajdova et al., 2011) and has been shown to reduce insulin resistance (Lee et al., 2006). Its attractiveness as a gluten-free, non-genetically modified alternative to soybean (Glycine max) has resulted in the production of a series of lupin-based gluten-free foods including pasta and meat replacement products for the vegetarian and vegan markets. Furthermore, specific lupin seed proteins have been demonstrated to reduce glycaemia to comparable levels as achieved with the predominantly used hypoglycaemic drug metformin (Lee et al., 2006) and to have additional nutraceutical properties, for example, improving inflammatory-related diseases, as well as anti-microbial properties (reviewed in Jimenez-Lopez, 2020).

While NLL is emerging as a human health food, toxic specialised metabolites of the quinolizidine alkaloids class are required to remain below a 0.02% threshold in the grain for it to be used for food or feed purposes (Frick et al., 2017). A picture is emerging in which environmental stresses cause an increase in alkaloids in the NLL leaves and grain (Frick et al., 2018; Frick et al., 2019), but independent studies have identified different candidate genes for the key alkaloid regulatory locus iucundus (Kroc et al., 2019b; Wang et al., 2021).

All lupins belong to the genus Lupinus in the Genistoid clade of legumes, which diverged early in the evolution of papilionoid legumes (Nevado et al., 2016). There are over 267 species of lupin, some of which are ecological pioneers in impoverished conditions. The natural distribution of the genus is around the Mediterranean region (the ‘Old World’ lupins) and North America (‘New World’ lupins), where the Andean lupin species have shown speciation rates not seen elsewhere in the plant kingdom (Nevado et al., 2016). The genus contains both annual and perennial species that occur in a range of different habitats (Nevado et al., 2016), and thus possesses a wealth of information for adaptive traits useful in different agricultural and climatic zones. These may be used to develop a sustainable profitable lupin crop through the development of new lupin crop species or, in the case of NLL, through genetic base broadening (Berger et al., 2012; Berger et al., 2013). To this end a range of genetic and genomic resources have been developed for NLL in recent years. These include the generation of bacterial artificial chromosome (BAC) libraries (Gao et al., 2011; Kasprzak et al., 2006), transcriptome datasets (reviewed in Kamphuis et al., 2020), cytogenetic maps (Bielski et al., 2020) and various genetic maps. In addition, a survey genome sequence was generated in 2013 for NLL (Yang et al., 2013), followed by the first comprehensive draft genome sequence coupled with a dense reference genetic map for the species (Hane et al., 2017). More recently, an improved reference genome was released for NLL (Wang et al., 2021) and two independent reference genomes were released for its close relative white lupin (Hufnagel et al., 2020; Xu et al., 2020).

In the last decade, there has been a move to generate pan-genomes for plant species to capture the genomic diversity for a species and allow genes core to all genomes and those that are variable to be identified (Bayer et al., 2020). Variable genes are often associated with processes involved in biotic and abiotic stress and could thus be important breeding targets in crop breeding programmes. To date, two predominant approaches have been used to assemble a pan-genome. The first approach assembles a de novo genome for each accession, after which alignment of the genomes allows one to identify dispensable genomic regions. The second approach aligns all sequence reads from multiple accessions to a high-quality chromosome-length reference genome and subsequently assembles the unaligned reads into novel contigs. Following either or both approaches a pan-genome graph can be constructed, the variants can be called, and core and variable genes can be identified. The first approach has recently been used to generate a pan-genome for white lupin using 39 accessions, including 11 varieties, one landrace and two wild accessions (Hufnagel et al., 2021). This study identified 32,068 core and 14,822 variable genes and discovered candidate genes for alkaloid biosynthesis.

Here we report on the fourth iteration of a reference genome for NLL and the first pan-genome for NLL, which represents the second pan-genome for a genistoid legume (2n = 40). We use these NLL resources to perform a survey of the gene content and its conserved and variable gene sets. Furthermore, we demonstrate the absence of unique mycorrhizal associated genes in all NLL lines, confirm the identity of the iucundus locus for alkaloid biosynthesis and demonstrate that the NLL pan-genome is underrepresented in classical disease resistance genes compared to other sequenced legume species.

RESULTS

Development of a chromosome-length L. angustifolius reference genome

We previously generated a draft reference assembly by combining short-read Illumina sequencing reads with
insert sizes from 170 bp to 40 kb and BAC-end sequencing data (Hane et al., 2017; Table S1). To improve this assembly, we generated approximately 98.5× coverage PacBio long-read sequence data across 19 single-molecule real-time (SMRT) cells on a PacBio Sequel as well as approximately 50× coverage in situ Hi-C data generated across two lanes on an Illumina HiSeq2500. The draft genome was assembled using CANU (v1.8; Koren et al., 2017) and scaffolded using Hi-C. The Hi-C reads were used to anchor, order, orient and correct misjoins in the draft genome assembly created above using the 3D de novo assembly (3D-DNA) pipeline (Dudchenko et al., 2017). The resulting assembly was then polished using the Juicebox Assembly Tools (Dudchenko et al., 2018) and the contact maps were visualised using Juicebox visualisation software (Durand et al., 2016). Following the integration of Hi-C sequencing data, the new reference assembly contained 2349 scaffolds assigned to 20 chromosomes with a scaffold N50 length of 30.7 Mbp and the longest scaffold being 45.7 Mbp (Table 1, Figure 1).

Using RepeatMasker we determined that the updated reference assembly has a high repetitive sequence content (57.7% of the genome; Tables S2 and S3), of which 54.4% are known transposable elements (TEs). These TEs were mostly long terminal repeat retrotransposons (Table S2). Following repeat masking, gene annotation was performed and identified 38 545 genes (Data S1 and S2) with an average gene length of 3497 bp in the 2017 assembly gene length of 3665 bp, compared to 33 076 genes and identified 38 545 genes (DataS1 and S2) with an average gene length of 3497 bp, compared to 33 076 genes.

Following repeat masking, gene annotation was performed and identified 38 545 genes (Data S1 and S2) with an average gene length of 3497 bp in the 2017 assembly gene length of 3665 bp, compared to 33 076 genes and identified 38 545 genes (DataS1 and S2) with an average gene length of 3497 bp, compared to 33 076 genes.

To identify distinct groupings within the species, clustering using STRUCTURE analysis on DarTseq data was conducted for 194 NLL accessions (154 wild accessions, 26 Australian varieties and 17 European varieties) (Mousavi-Derazmahalleh et al., 2018b). Clustering resulted in three groups: Cultivated varieties from Australia and Europe (n = 46), wild accessions from southern Europe (n = 43) and wild accessions from the Iberian Peninsula and North Africa (n = 59). There remained some wild mixed accessions (n = 58) that were less clearly defined and which

| Narrow-leaved lupin reference genomes | White lupin reference genomes | NLL pan-genome |
|--------------------------------------|--------------------------------|----------------|
| Hane et al., 2017                     | Wang et al., 2021               | This publication, 2022, Hufnagel et al., 2020, Xu et al., 2020, This publication, 2022 |
| Illumina                              | ✘                              | ✘              | ✘              | ✘              |
| PacBio                                | –                              | ✘              | ✘              | ✘              |
| Hi-C                                  | ✘                              | ✘              | ✘              | ✘              |
| BioNano optical map                   | –                              | ✘              | ✘              | ✘              |
| Genome size                           | 609 Mb                         | 616 Mb         | 653 Mb         | 451 Mb         | 559 Mb         | 975 Mb         |
| N50 length                            | 11                             | 9              | 9              | 12             | 14             | 15             |
| GC content (%)                        | 33.46                          | 33.27          | 33.46          | 33.79          | 36.82          | 38.06          |
| Annotated protein-coding sequences    | 33 076                         | 33 097         | 38 545         | 38 258         | 47 603         | 39 339         |

© 2022 The Authors.
The Plant Journal published by Society for Experimental Biology and John Wiley & Sons Ltd., The Plant Journal, (2022), 111, 1252–1266
originated across the whole geographic range of NLL. These showed evidence of admixture characterised by high levels of heterozygosity (5.5%), compared to the main groups (1.3%, 0.4% and 3.2%, respectively), and with heterozygosity as high as 20% in some lines. For re-sequencing to generate the pan-genome, lines had a heterozygosity level of <2%, except Mandelup. Mandelup (2.5% heterozygosity) was selected because of its commercial importance as a variety, its role as a parental line to increase genetic diversity from the wild in breeding efforts in Australia and its role as an important experimental line in NLL pre-breeding research.

We selected a total of 55 accessions, including Tanjil, to construct the NLL pan-genome with representatives from each of the major three groupings identified in the STRUCTURE analysis (Mousavi-Derazmahalleh et al., 2018b). Of these, four accessions from each of three main NLL groupings were sequenced at high coverage (approximately 55× or more) and the remaining accessions were sequenced at a lower coverage (approximately 10–30× for most accessions; Table S4), using short-read sequencing. k-Mer clustering analysis of short-read sequencing data for these 55 accessions also resulted in the formation of three major clades (Figure 2), where a cluster of all cultivated

---

Table 2 BUSCO analysis of sequenced reference legume genomes identified two Plantae BUSCOs that are absent in most legume species sequenced to date

| Plantae BUSCO statistics | Complete | Fragmented | Missing | 31855at33090 | 40630at33090 | Reference |
|--------------------------|----------|------------|---------|--------------|--------------|-----------|
| Adzuki bean (Vigna angularis) | 417 | 5 | 3 | Absent | Absent | Yang et al., 2015 |
| Alfalfa (Medicago sativa) | 420 | 2 | 3 | Absent | Absent | Shen et al., 2020 |
| Barrel medic (Medicago truncatula) | 420 | 1 | 4 | Absent | Absent | Young et al., 2011 |
| Chickpea (Cicer arietinum) (desi) | 414 | 6 | 5 | Present | Absent | Parween et al., 2015 |
| Chickpea (Cicer arietinum) (kabuli) | 419 | 2 | 4 | Present | Absent | Varshney et al., 2013 |
| Common bean (Phaseolus vulgaris) | 420 | 3 | 2 | Absent | Absent | Schmutz et al., 2014 |
| Cowpea (Vigna unguiculata) | 420 | 2 | 3 | Absent | Absent | Lonardi et al., 2019 |
| Lotus (Lotus japonicus) | 386 | 19 | 20 | Absent | Present | Sato et al., 2008 |
| Mungbean (Vigna radiata) | 421 | 2 | 2 | Absent | Absent | Kang et al., 2014 |
| Pea (Pisum sativum) | 409 | 11 | 5 | Absent | Absent | Kreplak et al., 2019 |
| Peanut (Arachis hypogaea) | 418 | 3 | 4 | Absent | Absent | Bertioli et al., 2019 |
| Pigeonpea (Cajanus cajan) | 414 | 6 | 5 | Absent | Absent | Varshney et al., 2012 |
| Soybean (Glycine max) | 420 | 1 | 4 | Absent | Absent | Schmutz et al., 2010 |
| Subclover (Trifolium subterraneum) | 417 | 5 | 3 | Absent | Absent | Hirakawa et al., 2016 |
| Narrow-leafed lupin (Lupinus angustifolius) | 421 | 1 | 3 | Absent | Absent | This manuscript |
| White lupin (Lupinus albus) | 418 | 3 | 4 | Absent | Absent | Hufnagel et al., 2020 |

---

We selected a total of 55 accessions, including Tanjil, to construct the NLL pan-genome with representatives from each of the major three groupings identified in the STRUCTURE analysis (Mousavi-Derazmahalleh et al., 2018b). Of these, four accessions from each of three main NLL groupings were sequenced at high coverage (approximately 55× or more) and the remaining accessions were sequenced at a lower coverage (approximately 10–30× for most accessions; Table S4), using short-read sequencing. k-Mer clustering analysis of short-read sequencing data for these 55 accessions also resulted in the formation of three major clades (Figure 2), where a cluster of all cultivated...
Australian and European accessions grouped together, a second cluster contained two branches with Iberian and North African accessions and a third cluster contained most of the Southern European accessions.

**NLL pan-genome assembly and validation**

The NLL pan-genome was built using an iterative mapping and assembly approach described by Hu et al. (2020), using the improved reference assembly described herein as a starting point. The assembled pan-genome is approximately 975 Mb in size and includes scaffolds longer than 500 bp with an L50 value of 24.8 Mb. The pan-genome contains 39,339 gene models, compared with the Tanjil reference assembly of 653 Mb and 38,545 gene models (Table 1). The 975 Mb is very close to the estimated genome size for the species of 951 Mb (Hane et al., 2017). A BUSCO analysis (Simão et al., 2015) was conducted as described earlier, where no additional missing BUSCOs from the reference assembly were identified. Paired-end data for all accessions were mapped back to the pan-genome, with a range of 91.1–99.6% (Table S4). These data were used for gene presence/absence variation analysis and single nucleotide polymorphism (SNP) discovery. The number of retro elements and other repeats increased in

![k-mer-based phylogeny of narrow-leafed lupin (Lupinus angustifolius) pan-genome accessions.](image-url)

**Figure 2.** k-mer-based phylogeny of narrow-leafed lupin (Lupinus angustifolius) pan-genome accessions.
the pan-genome relative to the reference genome (Tables S2 and S5).

Gene presence/absence discovery and characterisation

A total of 39,339 genes were predicted in the pan-genome, which is an additional 794 genes compared to the reference assembly (Table 1; Data S3 and S4). The majority (95.2%, 37,459 genes) of the pan-genome is composed of core genes present in all lines, while 4.8% (1880 genes) of the genes are variable, including 30 genes present in one line only (Figure 3a). The pan-genome size and annotated gene set expands with each added NLL line up to 39,339 genes (Figure 3b). The size of the core genome diminishes with every added NLL line to 37,459 genes. Of the 1880 variable genes, 1192 (63.4%) were variable across the domesticated (24 lines/cultivars) and wild material (31 accessions), such that they were missing in at least one domesticated and one wild line. Of the remaining variable genes, 491 (26.1%) were present in all domesticated lines, but absent in at least one wild accession, with 37 of these absent in all wild accessions. Conversely, 197 variable genes were present in all wild accessions and absent in at least one domesticated line with 127 absent in all domesticated lines.

In total, 20,285,108 SNPs were identified in the pan-genome, creating an overall SNP density of 22.03 SNPs/kb. In contrast, when identifying SNPs in breeding lines and cultivars only we found a total of 7,165,473 SNPs, resulting in a density of 7.78 SNPs/kb. Thus, the high SNP density in the pan-genome was predominantly contributed by the presence of many wild lines and was consistent with previous observations of high SNP density in a wild NLL line (P27255) when mapped to the Tanjil assembly (Hane et al., 2017). Private SNPs, which are present in only one sample but absent in others, are much more abundant in wild compared to domesticated lines (Figure 3c), with the maximum number of private SNPs found in P26170 (602,327 SNPs).

Figure 3. Lupinus angustifolius pan-genome statistics. (a) Number of absent variable genes across different narrow-leafed lupin (NLL; Lupinus angustifolius) lines. Here, 30 variable genes are absent in 54 lines and present in only one line. (b) Model describing the sizes of the core (green) and pan-genome of NLL (blue). (c) The number of private single nucleotide polymorphisms (SNPs) for each NLL pan-genome accession.

© 2022 The Authors.
The Plant Journal published by Society for Experimental Biology and John Wiley & Sons Ltd., The Plant Journal, (2022), 111, 1252–1266
Functional annotation of variable genes

Functional annotation of the 39 339 genes revealed that 73% of the core genes (28 721 gene models) had a functional annotation, whereas 26% (489 gene models) of the variable genes had a functional annotation. Among the most abundant protein domains found in the variable genes were reverse transcriptase, integrase, MYB-like DNA binding and pentatricopeptide repeat domains (Table S6). The most represented Pfam domains in the core genes also included pentatricopeptidase, reverse transcriptase and MYB-like DNA binding domains, whereas other highly abundant core Pfam domains also encoded leucine-rich repeat (LRR) and protein kinase domains (Table S6). Subsequent analysis around gene content focused on three areas, including genes associated with symbiosis, alkaloid regulation and plant disease resistance.

Pan-genome gene content with industry-relevant phenotypes

Symbiosis genes. Most legumes partake in symbiotic relationships with mycorrhizal fungi and/or rhizobia. Some of the plant genes required for a successful association are shared by both types of symbioses. It has been suggested that the evolutionary younger rhizobium–legume symbiosis recruited part of the genetic programme from the more ancient arbuscular mycorrhizal symbiosis (Parniske, 2008). Interestingly, NLL does not form symbiosis with mycorrhizal fungi, but does so with rhizobia. To expand our previous findings using the reference genome of (Hane et al., 2017), we compared the list of genes associated with arbuscular mycorrhizal and rhizobial associations identified in the Medicago truncatula genome with the core and variable gene set (TableS7). None were found in the variable gene set. The identified genes were all confirmed to group with the M. truncatula genes in the gene family trees on the Legume Information System genomic data portal (Dash et al., 2016; Gonzales et al., 2004; https://legumeinfo.org/). These included all genes currently known to be involved in rhizobium symbiosis. However, NLL lacked nearly all genes examined that are required specifically for arbuscular mycorrhizal symbiosis (in italics in Table S7). Two exceptions were PP2AB’1 (Lupan_009748) and KIN2 (Lupan_004177) homologues, which encode a serine/threonine protein phosphatase and a protein kinase, respectively.

Analysis of the alkaloid regulatory locus iucundus. Quinolizidine alkaloids are specialised metabolites toxic to animals and humans and are a significant industry issue for the production and use of NLL crops. In NLL a major locus controlling alkaloid grain content termed iucundus has been mapped to a small region in the genome (746 kb) between molecular markers LaSSR_025 and LaSNP_509 (Hane et al., 2017). This region contains several candidate genes, including a dihydrodipicolinate synthase-like gene (LaDHDPs-like; Lupan_013751), an APETALA2/ethylene response factor-like gene (LaRAP2-7; Lupan_013780) and an LRR receptor-like serine/threonine protein kinase (LaRSTK; Lupan_013738) gene. Two independent publications have proposed different candidates as the causal gene underlying the iucundus low alkaloid phenotype. Kroc and associates (2019b) determined that LaDHDPs was closely linked to the iucundus locus, whereas LaRAP2-7 co-segregated with the iucundus locus. Subsequently, a molecular marker in the coding sequence of LaRAP2-7 was developed for marker-assisted selection in breeding programmes and demonstrated to correlate perfectly for 199 of the 202 NLL accessions tested and it was speculated that the alkaloid content of the remaining three accessions was under a different regulatory mechanism (Kroc et al., 2019a). However, independent analysis by Wang et al. (2021) suggested that LaRAP2-7 is not located in the iucundus region and these authors proposed that LaRSTK is the key regulatory gene for alkaloid biosynthesis and that this gene activates LaRAP2-7 gene expression.

We used the pan-genome resource to further explore the iucundus locus and resolve the identity of the gene underlying low alkaloid phenotypes. In total, 54 accessions constituting the NLL pan-genome for which we had seed were evaluated for grain alkaloid content using the Drakenhoff test. This is a semi-quantitative test whereby alkaloids, if present, react with the Dragendorff reagent to produce an orange to orange-red pigment. All cultivars and breeding lines except for the Polish cultivar Krasnolistny have a ‘sweet’ alkaloid phenotype as they did not react with the reagent (Table 3). In contrast, all wild accessions displayed a ‘bitter’ phenotype with the Dragendorff reagent producing the orange to orange-pink reactions on Whatman filter paper. These phenotypic data were subsequently correlated with the proposed causal SNP mutations for LaRAP2-7 and LaRSTK. The comparison clearly demonstrated a perfect correlation of the SNP genotype data for the 54 accessions with the grain alkaloid content phenotype for LaRAP2-7 and not LaRSTK. Our phenotype data combined with the NLL pan-genome resource therefore provide additional evidence to support the studies of Kroc et al. (2019a) and Kroc et al. (2019b) and the hypothesis that iucundus encodes an ethylene response factor that regulates grain alkaloid content in NLL.

Classical disease resistance genes are underrepresented in the NLL pan-genome. Classical disease resistance genes of the nucleotide binding site (NBS)-LRR (NLR) family are key genes at the frontline of plant defence to numerous pathogens and pests. NLR genes were identified in the
reference genome using two approaches. Firstly, conserved consensus sequences from plant extended NBS domains using the sequences described in Ameline-Torregrosa et al. (2008) were used to identify genes in the pan-genome annotation. Secondly, genes containing the Pfam domain PF00931 (NB-ARC) identified using Interproscan were identified as classical NLR genes. This approach identified a total of 67 NLRs in the NLL pan-genome, a surprisingly low number compared to other legume crops. Thirdly, RGAugury (Li et al., 2016) was used, which identified 67 NLRs and 48 receptor-like proteins.

We compared the NLR domains identified above with 32 NLR domain sequences that we derived from PCR amplification of the NLL genome using degenerate primers derived from *M. truncatula* conserved NBS/NB-ARC sequences. All 32 experimentally verified genes were

---

Table 3

| Accession ID | Cultivar/alternate name | Origins | Line Information | Dragenoff test | Alkaloid phenotype | RAPD-7** | RS2** |
|--------------|-------------------------|---------|------------------|----------------|-------------------|----------|-------|
|             | Consupas                 | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
|             | Jindelie                | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
|             | Kreamissner              | POL Cultivar | Sweet           | Bitter        | C                 | A        | T     |
|             | Döker                   | POL Cultivar | Sweet           | Bitter        | C                 | A        | T     |
|             | PBA Berlock             | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
|             | PBA Juneha              | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P20670       | Univite                 | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P20671       | Univite                 | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P24118       | Manil                   | POL Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P24141       | Chittick                | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P25795       | Enthur                  | POL Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P26970       | Dacev                   | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P26973       | Gemburg                 | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P26975       | Yousil                  | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28137       | Myallie                 | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28317       | Sonnet = MTD1889       | POL Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28324       | Kelsue                  | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28364       | Quinimol                | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28578       | Tanjil                  | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28596       | Bellaro                 | POL Cultivar | Sweet           | Bitter        | C                 | A        | T     |
| P28646       | Mandarupa               | AUS Cultivar | Sweet           | Bitter        | C                 | A        | T     |

**Alkaloid content in the seeds were determined using the Dragenoff test, where bitter seeds interacts with the Dragenoff reagent and produces orange/pink pigments, whereas sweet seeds do not react. Causal SNP according to Kroc et al 2019 Genes 4, 428; Scaffold coordinate with causal A/C SNP: scaffold_8: 10,935,826. **Causal SNP according to Wang et al 2021 The Plant Journal 5, 1192-1210; Scaffold coordinate with causal T/G SNP: scaffold_8: 8,743,239.

© 2022 The Authors. *The Plant Journal* published by Society for Experimental Biology and John Wiley & Sons Ltd., *The Plant Journal* (2022), 111, 1252–1266.
contained within the set of 68 predicted NLR genes and no additional NLR homologues were identified.

In the pan-genome contigs only one additional NLR was identified. Of the 68 NLRs identified in the pan-genome, 62 NLRs were found in all 55 NLL accessions, whereas six were found to be members of the variable gene set. Only one variable NLR gene came from a new pan-genome contig (Lupan_039653), with the remainder present in the Tanjil reference genome. All but two NLRs (Lupan_039653 and Lupan_026540) were anchored in pseudochromosomes and were distributed across all pseudochromosomes except NLL-14 and NLL-16. Clusters of three or more NLR homologues on a pseudochromosome were identified on NLL-02 and NLL-06, which also harboured the largest numbers of NLRs with 13 and 10 genes, respectively (Figure 4; Table S8).

DISCUSSION

Comparison of different lupin reference genomes

As long-read sequencing technologies have become more affordable, the number of more complete and updated plant genome assemblies has increased. For NLL, the first draft assembly of 523 Mb was published in 2013 (Yang et al., 2013), followed by the first reference assembly of 609 Mb in 2017 (Hane et al., 2017). Both assemblies used short-read sequencing data and were fragmented with scaffold N50 values of 7319 and 232, respectively. More recently, Wang et al. (2021) published an improved assembly incorporating PacBio data, which resulted in a 616-Mb assembly and N50 and L50 values of 9 and 30.8 Mb, respectively, which are comparable to the 653-Mb assembly with N50 and L50 values of 9 and 30.7 presented herein (Table 1). The mean coding sequence length for the new assembly compared to the Hane et al. (2017) assembly increased from 1289 to 1337 bp, while the GC content of all the various NLL assemblies was between 33.27 and 33.46%. The latter is similar to the GC content observed in the white lupin genome assembly of Hufnagel et al. (2020) of 33.79%, whereas another white lupin genome assembly had a GC content of 36.82% (Xu et al., 2020).

Compared to previous assemblies, the number of annotated protein-coding sequences increased from 33,076 and 33,097 in the Hane et al. (2017) and Wang et al. (2021) assemblies, respectively, to 38,545 in the current assembly. The gene content of NLL identified here is like that

Figure 4. Schematic presentation of the 20 Lupinus angustifolius chromosomes and the physical location of classical disease resistance genes of the nucleotide binding site–leucine-rich repeat (NLR) class.
reported for white lupin by Hufnagel et al. (2020), who predicted 38,258 genes, whereas the white lupin assembly by Xu et al. (2020) was predicted to contain 47,603 genes. To validate the completeness of gene-coding content we conducted BUSCO analysis of the different NLL and white lupin genomes (Table 1). Comparing the different NLL assemblies in both the Embryophyta and Plantae BUSCO analyses, the improved assembly presented herein had the most complete and least fragmented BUSCOs. Alignment of RNA sequencing reads from different tissues further corroborated that 89.1-99.0% mapped back to the improved assembly, indicating it has captured most of the genome-rich space. Differences in completeness of the gene content have also been observed between the different white lupin assemblies (Table 1), with the Hufnagel assembly having a similar number of missing and fragmented BUSCOs. Two of the missing Plantae BUSCOs (31855at33090 and 40630at33090) were absent in both lupin species and were subsequently found to also be absent in other legume genomes except for 31855at33090 in chickpea and 40630at33090 in lotus (Table 2). It is thus possible that some conserved Plantae genes have been lost in Fabaceae species and can therefore affect the completeness scores for legumes. This is supported by the re-sequencing of the additional 54 NLL accessions herein, which did not uncover any of the missing BUSCOs (Table 1).

Features of the NLL pan-genome

To capture as much of the genetic diversity in the NLL pan-genome, wild and domestic lines from distinct clades identified in a previous diversity study were selected as representatives for each clade (Mousavi-Derazmahalleh et al., 2018b). To assemble the NLL pan-genome an iterative read mapping approach to the reference was used where-after the unaligned reads were de novo assembled. This added 300,705 contigs to the reference assembly and produced a pan-genome size of 975 Mb. This assembly size is slightly larger than the estimated diploid genome size for cultivar Tanjil of 951 Mb by k-mer analysis or 924 Mb by flow cytometry (Hane et al., 2017). The k-mer-based phylogenetic tree also showed a similar relationship as the previous analyses using molecular markers (Mousavi-Derazmahalleh et al., 2018a; Mousavi-Derazmahalleh et al., 2018b), with three clear groupings of (i) domesticated varieties, (ii) wild accessions from the Iberian Peninsula and North Africa and (iii) wild accessions from Southern Europe.

In terms of gene content, 39,339 genes were predicted, with the 794 additional genes identified in the new pan-genome contigs having a shorter average gene length than those genes identified in the reference genome. This could be due to small contigs that might contain partial/fragmented genes or due to expanding/evolving gene families. Of the variable genes, 37 were absent from all wild accessions, but present in all domesticated material, while 127 were present in all wild accessions and absent in all domesticated lines. Of the 127 genes only found in wild accessions, only five had a Pfam domain, while 29 of the 37 only found in domesticated lines had a Pfam domain, with no obvious overrepresentation of specific Pfam domains.

Comparison of the NLL and white lupin pan-genomes

At this stage it is difficult to conduct a direct comparison of the NLL and white lupin pan-genomes. The first reason for this is because two different assembly approaches have been used, with the white lupin assembly adopting a de novo assembly approach of each of the 39 genomes and aligning these, whereas for NLL we aligned the sequence reads to the reference and de novo assembled the unaligned reads in an iterative approach. Both approaches have their own advantages and disadvantages as described in a recent review (Bayer et al., 2020). For example, we observed that the NLL pan-genome size increased 322 Mb in size relative to the reference genome, whereas the white lupin pan-genome assembly approach added 11.7 Mb. This could be due to the approach taken or the second large difference, which is the ratio of wild versus domesticated accessions used in the pan-genome assemblies. The NLL pan-genome utilised many wild accessions (31 out of 55) while the white lupin pan-genome was more focused on domesticated accessions that are closely related, and only used four wild accessions out of a total of 39 accessions. As high-quality long-read sequencing data generation becomes cheaper, a pan-genome graph approach will likely be more common and overcome some of the difficulties observed in the NLL and white lupin pan-genome assembly approaches (Bayer et al., 2020).

Despite these differences an interesting observation was that in both the NLL and white lupin pan-genomes (Hufnagel et al., 2021) relatively low numbers of variable genes were identified (4.8 and 3.1%, respectively) compared to other crop pan-genomes such as Brassica oleracea (20%) (Goliz et al., 2016) and wheat (Triticum aestivum; 35.7%) (Montenegro et al., 2017). Specifically, 1195 variable genes were identified for white lupin (1132 present in the reference assembly and 63 identified from the new pan-genome contigs) and 1880 variable genes were identified for NLL, including 1086 that reside in the reference genome and 794 from contigs produced through the pan-genome assembly. The additional pan-genome contigs relative to the reference assembly are also more sparsely populated with genes (an average of one gene per 16.9 kb versus one gene per 405.5 kb). This suggests that for both lupin species the level of diversity in wild and domesticated material is quite low compared to many other crop species. In both lupins, wild accessions have a higher number of variable genes than domesticated accessions. In both pan-genomes, genes specifically involved in mycorrhizal
pan-genome. The genes were both cases the genes were found to be core genes in the mycorrhizal symbiosis. There were two exceptions, and in nearly all genes that are specifically required for arbuscular Rhizobium action. NLL only interacts with Rhizobium and those that are unique to each inter-

symbiosis were absent and classical disease resistance genes of the NLR type were significantly underrepresented compared to other legume genomes as outlined in more detail below.

**NLL lacks genes specifically associated with arbuscular mycorrhizal symbiosis**

Most legumes undergo beneficial interactions with both rhizobia and mycorrhizal fungi to help fix nitrogen and acquire phosphorus, respectively. The legume genes associated with these interactions are well understood and include genes that overlap between rhizobial and mycorrhizal associations and those that are unique to each inter-

**Analysis of grain alkaloid content of pan-genome lines identifies a candidate for the *iucundus* locus**

Quinolizidine alkaloids in NLL are regulated by a major locus termed *iucundus* (Frick et al., 2017). In the literature two different candidate genes have been proposed (Kroc et al., 2019a; Kroc et al., 2019b) (Wang et al., 2021). Assessment of the relationship between grain alkaloid content (as determined via Dragendorff assays) and the proposed causal SNP mutations in these two candidates showed strong correlation between alkaloid content and causal SNP genotype for LaRAP2-7 as opposed to LaRSTK (Table S7). Our results thus support the previous findings from Kroc et al. (2019a); Kroc et al. (2019b), that LaRAP2-7 is a strong candidate for regulation of grain alkaloid content. However, final validation through cloning and complementation or targeted gene-editing studies is required to conclusively prove that RAP2-7 is the causal gene.

**Classical disease resistance genes of the NLR class are underrepresented in the lupin genome**

Analysis of NLR genes in the previous reference Tanjil genome assembly (Hane et al., 2017) and the updated version herein was conducted, and only 67 classical disease resistance genes of the NLR class were revealed, which is a relatively low number compared to other crops that typically contain several hundred of such genes. We were therefore interested to see if other domesticated and wild accessions had additional NLRs that were not present in the reference assembly. However, examination of the pan-genome only identified a total of 68 NLR genes, of which 67 were in common with Tanjil, demonstrating both a surprisingly low number and high conservation in the NLR gene repertoire of NLL among both wild and domesticated accessions. NLRs can have a high sequence similarity and it is thus possible that additional NLRs were not identified in the pan-genome assembly as their reads aligned to other NLRs in the assembly as a short-read sequencing approach was used.

We also mined the reference genome of white lupin (Hugnagel et al., 2020) and identified 44 NLRs. The 67 and 44 NLRs in the reference NLL and white lupin genomes, respectively, are considerably less than those observed in other closely related legumes, such as barrel medic (764), soybean (506), pigeonpea (406) and chickpea (187), or other key crops, such as wheat, which contains 604 NB-ARC genes (Chandra et al., 2017). In the other sequenced legume species, NLRs are often found in clusters, where members of these clusters have a high sequence similarity as they have duplicated and diverged. Surprisingly, we only found two such clusters (on chromosomes NLL-02 and NLL-06), while other NLRs were distributed as single loci across the NLL genome. The reasons for this relatively low number of NLRs in the two examined lupin species remain unclear, but the inherent high alkaloid levels in wild lupin accessions which provide protection from pathogens and pests could be a possible explanation.

**CONCLUSION**

Legumes, including domesticated lupin species, are an important part for future sustainable agricultural production systems, especially with the growing demand for plant-based protein for not only animal feed but also human consumption. Two lupin species (NLL and white lupin) now have well-advanced genomic resources including transcriptome datasets and reference/pan-genomes. This will likely be further augmented by ongoing efforts to generate similar resources for the other two domesticated lupin species (yellow and pearl lupin) and genomic data-
ssets for other New World lupin species. The combination of these resources will allow a better understanding of lupin biology, diversity and evolution and help accelerate lupin crop improvement. For example, the NLL pan-genome resources have contributed to the generation of a 30k multispecies pulse SNP chip (Kaur et al., 2020), which includes 5425 evenly distributed SNPs across the 20 chromosomes of NLL. In addition, the SNPs identified in the pan-genome can help identify candidate genes for important agronomic traits such as the alkaloid gene identified herein and a novel flowering time gene (Taylor
et al., 2021). The improvement in yield and other agronomic traits of interest to lupin breeding programmes will help provide viable lupin crops for substantial parts of Mediterranean grain growing regions around the globe.

**EXPERIMENTAL PROCEDURES**

**Sequencing of NLL lines**

NLL seed was provided by the Department of Primary Industries and Regional Development of Western Australia. Seeds were ver- nalised in Jiffy pellets for 2 weeks at 4°C in temperature-controlled growth cabinets in the dark before being planted in pots in temperature-controlled glasshouses to collect tissue for DNA extraction and seed for future experiments. DNA was isolated from frozen leaf material that was ground to a fine powder under a stream of liquid nitrogen using the CTAB method as described by Kamphuis et al. (2008). DNA quality was assessed on an Illumina HiSeq.

High-coverage lines (56.0 bp size of 951 Mb for NLL assembly (Hane et al., 2017). High-yielded 91 Gb (approximately 98 coverage).– high-coverage lines (56.0 bp size of 951 Mb for NLL assembly (Hane et al., 2017). High-yielded 91 Gb (approximately 98 coverage).– high-coverage lines (56.0 bp size of 951 Mb for NLL assembly (Hane et al., 2017). High-yielded 91 Gb (approximately 98 coverage).– high-coverage lines (56.0 bp size of 951 Mb for NLL assembly (Hane et al., 2017). High-yielded 91 Gb (approximately 98 coverage).– high-coverage lines (56.0 bp size of 951 Mb for NLL assembly (Hane et al., 2017). High-yielded 91 Gb (approximately 98 coverage).

The reference genome and pan-genome annotation

Repetitive DNA regions were first predicted in both the reference and pan-genome for both TEs and tandem repeats as described previously by Hane et al. (2017). Briefly, annotation of TEs was conducted using RepeatMasker v4.1.0 (Smit et al., 2013-2015) to identify known repeat sequences in Repbase v26.10 (Jurka et al., 2005). Non-coding RNA was predicted using Rfam 14.6 (July 2021, 4070 families) and Infernal v 1.1.3 (Nawrocki & Eddy, 2013).

Following repeat masking the reference genome and newly assembled pan-genome contigs greater than 1 kb in length were annotated using BRAKER version 2.1.2 (Hoff et al., 2019). De novo gene prediction was conducted using Genemark-ES (Lomsadze et al., 2005) and Augustus (Stanke et al., 2004). Proteins from previously published lupin and soybean genomes (Hane et al., 2017; Hufnagel et al., 2020; Schmutz et al., 2010) were used for external validation. Annotations were filtered for incomplete models and longest isoforms using AGAT version 0.5.1 (Daïnai et al., 2021). The pan-genome was functionally annotated using the Blast2GO CSIRO server (Conesa et al., 2005). To identify protein domains and map protein families, Interproscan v 5.24-63.0 (Quevillon et al., 2005) was used. Both programmes were run separately for core and variable genes.

Gene presence/absence variation analysis and SNP discovery

Gene presence/absence variation was characterised using the SGSGeneLoss package (Golicz et al., 2015) using default parameters. Reads from all the NLL lines were mapped to the pan-genome using Bowtie2 v2.3.4 (-end-to-end --sensitive -X 1000) (Langmead & Salzberg, 2012). Based on presence/absence variation, gene models were divided into core and variable genes. A gene was considered core if it was present in all lines and variable if it was absent in at least one line. Curves describing pan-genome size, core genome size and new gene number for individual genes were drawn using the PanGP programme (Algorithm: Distance guide; Sample size: 1000; Sample repeat: 100; Amplification coefficient: 50) (Zhao et al., 2014). Following the read mapping using Bowtie2 as described above, duplicate sequences were marked using Picard toolkit (2019). MarkDuplicates (https://broadinstitute.github.io/picard/) and SNPs were discovered and filtered for low quality using SAMtools v1.10., mpileup (Li et al., 2009) and BCFTools v1.3 (Danecek et al., 2021).

**Symbiosis analysis**

In total, 57 M. truncatula genes associated with mycorrhizal association that were previously used to identify homologues in the L. angustifolius genome (Hane et al., 2017) were used to identify annotated homologous genes in the pan-genome by BLASTP (Altschul et al., 1997). Best hits were subsequently used to identify the old reference gene annotation identifiers. The legume gene family trees on the legume information system portal (https://legumefinfo.org/); Gonzales et al., 2004; Dash et al., 2016) for the M. truncatula and L. angustifolius genes were used to validate that these were true homologues.
Alkaloid analysis

Seed of 54 accessions of the NLL pan-genome were evaluated for grain alkaloid content using the Dragendorff test. Dragendorff reagent was prepared by dissolving 32 g of potassium iodide in 80 ml sterile water and subsequently mixed in a solution containing 3.4 g bismuth nitrate, 40 ml glacial acetic acid and 160 ml of sterile water to make a basal solution. One part basal solution was mixed with one part glacial acetic acid and four parts sterile water to make working solution. Whatman filter papers were soaked in this solution and allowed to dry overnight. Prepared Dragendorff filter papers were stored in the dark. Alkaloid tests were carried out by removing the seed coat and soaking crushed cotyledons overnight in 200 μl of sterile water. Subsequently, 5 μl was placed on the Dragendorff paper. Bitter seeds were identified by an orange/pink reaction with the reagent, while sweet seeds do not react with the reagent. Seeds of each of the accessions were analysed in triplicates.

NLR resistance gene analysis

Candidate genes containing NBS/NB-ARC domains were identified in the NLL pan-genome by blastp similarity (Altschul et al., 1997) with an E-value threshold of 1e−5 to consensus coiled-coil (CC)-NBS-LRR and Toll and mammalian INTERLEUKIN1 receptor-NBS-LRR sequences from plant extended NBS domains using the sequences described in Ameline-Torregrosa et al. (2008). In addition, RGAugury (v1) (Li et al., 2016) was used to identify resistance gene analogues. Furthermore, Interproscan (v 5.24–63.0) (Quevillon et al., 2005) was used to identify genes annotated with the PF00931 (NB-ARC) domain, using the annotated NLL pan-genome protein file (Data S5) and the white lupin genome annotated protein file (GCA_009771035.1) from Hufnagel et al. (2020).

ACCESSION NUMBERS

Genome sequence assembly and annotation data can be found in GenBank under BioProject IDs PRJNA797109, PRJNA512907 and PRJNA299755. In addition, the chromosome-length and pan-genome assemblies and annotated gene and protein sequences can be accessed from the downloads section of our webpage: http://lupinexpress.org/. Furthermore, a JBrowse instance has been put up to browse the genomes and a BLAST server to query the genomes and associated annotated gene/protein sets. The interactive Hi-C contact map for the genome is also available at www.dnazoo.org.

ACKNOWLEDGEMENTS

The authors are grateful to CSIRO, Curtin University, the University of Western Australia and the Grains Research and Development Corporation (GRDC, Project code CSP1806-009RTX) for their financial support. ELA was supported by the Welch Foundation (Q-1986), a McNair Medical Institute Scholar Award, an NIH Encyclopedia of DNA Elements Mapping Center Award (UM1HG009376), a US-Israel Binational Science Foundation Award (2019276), the Behavioural Plasticity Research Institute (NSF DBI-2021795), an NSF Physics Frontiers Center Award (NSF PHY-2019745) and an NIH CEGS (R1HGS011016-01A1). The authors thank Hayley Casarotto, Natalie Fletcher, Nick Pain and Daniel Lim for technical assistance on the project and Drs Jonathan Anderson and Jana Sperschneider for helpful comments on the manuscript. Hi-C data were created in collaboration with the DNA Zoo Consortium (www.dnazoo.org). DNA Zoo is supported by Illumina, Inc., IBM and the Pawsey Supercomputing Centre.

CONFLICT OF INTEREST

The authors declare no conflict of interest or competing interests.

AUTHOR CONTRIBUTIONS

LGK, RF, DE and KBS conceived the project. LGK, CMT and PK isolated the DNA from the NLL accessions for sequencing. PK, CMT and LGK generated the Hi-C library. PK, OD and ELA generated the Hi-C data and Hi-C guided assembly. GG, PB and PK generated the reference and pan-genome assemblies and associated annotations. LGK, LG, KF and RF conducted the NLR, alkaloid and conglutin experiments/analyses. LGK wrote the first draft of the manuscript with input from GG and KBS. All authors reviewed the manuscript and agreed on the final version of the manuscript.

SUPPLEMENTARY INFORMATION

Additional Supporting Information may be found in the online version of this article.

Data S1 Annotated genes in the Lupinus angustifolius reference genome.

Data S2 Protein-coding sequences in the Lupinus angustifolius reference genome.

Data S3 Annotated genes in the Lupinus angustifolius pan-genome.

Data S4 Protein-coding sequences in the Lupinus angustifolius pan-genome.

Data S5 Functional annotations assigned to gene annotations of the Lupinus angustifolius genome.

Data S6 References for Table S7.

Table S1 Summary of the total amount of sequence data generated for the Lupinus angustifolius cv. Tanjil genome assembly and the average coverage per library, assuming an estimated genome size of 951 Mb based on k-mer analysis (Hane et al., 2017).

Table S2. Overview of the repeat content in the fourth generation of the narrow-leaved lupin (Lupinus angustifolius) reference genome assembly of cultivar Tanjil.

Table S3. Summary of non-coding RNA predicted within the narrow-leaved lupin (Lupinus angustifolius) cv. Tanjil genome assembly.

Table S4. Overview of the sequence coverage for the 55 Lupinus angustifolius accessions that were used to assemble the pan-genome.

Table S5. Summary of non-coding RNA predicted within the narrow-leaved lupin (Lupinus angustifolius) pan-genome assembly.

Table S6. Top 10 most represented Pfam protein domains found in variable and core genes of the narrow-leaved lupin (Lupinus angustifolius) pan-genome using Interproscan.

Table S7. Overview of genes associated with arbuscular mycorrhizal and rhizobial associations in the genomes of Medicago truncatula and narrow-leaved lupin (Lupinus angustifolius).

Table S8. Overview of the distribution of NBS-LRR genes across the 20 linkage groups of narrow-leaved lupin (Lupinus angustifolius) cv. Tanjil.
Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergham, N.H. & Phil- lippy, A.M. (2017) Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Research, 27, 722–736.

Kreplak, J., Madoui, M.-A., Capal, P., Novak, P., Labadie, K., Aubert, G. et al. (2019) A reference genome for pea provides insight into legume genome evolution. Nature Genetics, 51, 1411–1422.

Kroc, M., Czepiel, K., Wilczura, P., Mokrzycka, M. & Swiecicki, W. (2019a) Development and validation of a gene-targeted dCAPS marker for marker-assisted selection of low-alcohol content in seeds of narrow-leafed lupin (Lupinus angustifolius L.). Genes, 10, 428.

Kroc, M., Koczky, G., Kamel, K.A., Czepiel, K., Fedorowicz-Stro/C19, Mousavi-Derazmahalleh, M., Nevado, B., Bayer, P.E., Filatov, D.A., Hane, Kroc, M., Koczky, G., Kamel, K.A., Czepiel, K., Fedorowicz-Stro/C19, Montenegro, J.D., Golicz, A.A., Bayer, P.E., Hurgobin, B., Lee, H., Chan, C.-I., 2018 Nature Communications

Li, P., Quan, X., Jia, G., Xiao, J., Cloutier, S. & You, F.M. (2016) RGAugury: a pipeline for genome-wide prediction of resistance gene analogs (RGAs) in plants. BMC Genomics, 17, 852.

Lomsadze, A., Tatusov, R.L., Mian, I.S., Chen, Y. & Borodovsky, M. (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. Nucleic Acids Research, 33, 6494–6506.

Lonardi, S., Muñoz-Amatriain, M., Liang, Q., Shu, S., Wanamaker, S.I., Lo, S. et al. (2019) The genome of cowpea (Vigna unguiculata [L.] Walp.). The Plant Journal, 98, 767–782.

Montenegro, J.D., Golicz, A.A., Bayer, P.E., Hurgobin, B., Lee, H., Chan, C.-I., Kroc, M., Koczky, G., Kamel, K.A., Czepiel, K., Fedorowicz-Stro/C19, Montenegro, J.D., Golicz, A.A., Bayer, P.E., Hurgobin, B., Lee, H., Chan, C.-I., 2018 Nature Communications

Mousavi-Derazmahalleh, M., Bayer, P.E., Nevado, B., Hurgobin, B., Filatov, D., Killian, A. et al. (2018a) Exploring the genetic and adaptive diversity of a pan-Mediterranean crop wild relative: narrow-leafed lupin. Theoretical and Applied Genetics, 131, 897–901.

Mousavi-Derazmahalleh, M., Nevado, B., Bayer, P.E., Filatov, D.A., Hane, J.K., Edwards, D. et al. (2018b) The western Mediterranean region provided the founder population of domesticated narrow-leaved lupin. Theoretical and Applied Genetics, 131, 2543–2554.

Navrotsky, E.P. & Eds, S.R. (2013) Inferral 1.1: 100-fold faster RNA homol- ogy searches. Bioinformatics, 29, 2393–2395.

Nevado, B., Atchison, G.W., Hughes, C.E. & Filatov, D.A. (2016) Widespread adaptive evolution during repeated evolutionary radiations in New World lupins. Nature Communications, 8(12), 384.

Parniske, M. (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Reviews Microbiology, 6, 763–775.

Parveen, S., Nawaz, K., Roy, R., Pole, A.K., Suresh, B.V., Misra, G. et al. (2015) An advanced draft genome assembly of a desi type chickpea (Cicer arietinum L.). Scientific Reports, 5, 1280.

Quevillon, E., Silventoinen, V., Pillal, S., Harte, N., Mulder, N., Apweiler, R. et al. (2005) InterProScan: protein domains identifier. Nucleic Acids Research, 33, W116–W120.

Rao, S.S.R., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T. et al. (2014) A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell, 159, 1665–1680.

Robinson, J.T., Turner, D., Durand, N.C., Thorvaldsdottir, H., Mesirov, J.P. & Lieverman Aiden, E. (2018) Juicebox.js provides a Cloud-Based visualization system for Hi-C data. Cell Systems, 6, 256–258.

Sato, S., Nakamura, Y., Kaneko, T., Asamizu, E., Kato, T., Nakao, M. et al. (2008) Genome structure of the legume, Lotus japonicus. DNA Research, 15, 227–239.

Schmutz, J., Cannon, S.B., Schlueter, J., Ma, J., Mitros, T., Nelson, W. et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature, 463, 193–199.

Schmutz, J., McClean, P.E., Mamidi, S., Wu, G.A., Cannon, S.B., Grimwood, J. et al. (2014) A reference genome for common bean and genome-wide analysis of dual domestications. Nature Genetics, 46, 707–713.

Seurin, M., Kirkegaard, J.A., Peoples, M.B., White, P.F. & French, R.J. (2012) Break-crop benefits to wheat in Western Australia—insights from over three decades of research. Crop and Pasture Science, 63, 1–16.

Shen, C., Du, H., Chen, Z., Lu, H., Zhu, F., Chen, H. et al. (2020) The chromosome-level genome sequence of the autotetraploid alfalfa and resequencing of core germplasms provide genomic resources for alfalfa research. Molecular Plant, 13, 1250–1261.

Simao, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V. & Zdobnov, E.M. (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics, 31, 3210–3212.

Smit, A. F. A., Hubley, R., Green, P. (2013) RepeatMasker Open-4.0. http://www.repeatmasker.org.

Stanke, M., Steinkamp, R., Waack, S. & Morgenstern, B. (2004) AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Research, 32, W309–W312.

Taylor, C.M., Garg, G., Berger, J.D., Ribalta, F.M., Croser, J.S., Singh, K.B. et al. (2021) A Trimethylguanosine Synthase1-like (TGS1) homologue is implicated in vernalisation and flowering time control. Theoretical and Applied Genetics, 134, 3411–3426.

Varshney, R.K., Chen, W., Bharti, A.K., Saxena, R.K., Schlueter, J.A. et al. (2012) Draft genome sequence of pigeonpea (Cajanus cajan), an orphan legume crop of resource-poor farmers. Nature biotechnology, 30, 83–89.

Varshney, R.K., Song, C., Saxena, R.K., Azam, S., Yu, S., Sharpe, A.G. et al. (2013) Draft genome sequence of chickpea (Cicer arietinum) provides a resource for trait improvement. Nature Biotechnology, 31, 240–246.

Wang, P., Zhou, G., Jian, J., Yang, H., Renshaw, D., Aubert, M. et al. (2021) Whole-genome assembly and resequencing reveal genomic imprint and key genes of rapid domestication in narrow-leaved lupin. The Plant Journal, 115, 1192–1210.

White, C.L., Staines, V.E. & Staines, M.H. (2007) A review of the nutritional value of lupins for dairy cows. Australian Journal of Agricultural Research, 58, 185–202.

Wu, X., Zhang, G., Yuan, W., Xu, F., Muhammad Aslam, M., Miao, R. et al. (2020) The genome evolution and low-phosphorus adaptation in white lupin. Nature Communications, 11, 1069.

Yang, H., Tao, Y., Zheng, Z., Zhang, Q., Zhou, G., Sweetingham, M.W. et al. (2013) Draft genome sequence, and a sequence-defined genetic linkage map of the legume crop species Lupinus angustifolius L. PLoS one, 8, e64799.

Yang, K., Tian, Z., Chen, C., Luo, L., Zhao, B., Wang, Z. et al. (2019) Genome sequencing of adzuki bean (Vigna angularis) provides insight into high starch and low fat accumulation and domestication. Proceedings of the National Academy of Science, United States of America, 112, 13 213–13 218.

Young, N., Debelle, F., Oldroyd, G., Geurts, R., Cannon, S.B., Udvardi, M.K. et al. (2011) The Medicago genome provides insights into the evolution of rhizobial symbioses. Nature, 480, 520–524.

Zhao, Y., Jia, X., Yang, J., Ling, Y., Zhang, Z., Yu, J. et al. (2014) PanGP: a tool for quickly analyzing bacterial pan-genome profile. Bioinformatics, 30, 1297–1299.

Zimin, A.V., Marcias, G., Puiu, D., Roberts, M., Salzberg, S.L. & Yorke, J.A. (2013) The MaSuRCA genome assembler. Bioinformatics, 29, 2669–2677.