COGGO Council of Grain Grower Organisations Limited ACN 091 122 039	Final Report COGGO Research Fund for 2019 projects **A project completion report covering the project. The acceptance of a satisfactory report against the objectives of the project, and agreement on the sharing of any commercial returns and/or IP will trigger payment within 4 weeks, by COGGO for any outstanding payments.**

This Final Report should be completed with reference to the Research and Intellectual Property Agreement (the Research Agreement) signed between the proponent and COGGO Pty Ltd.

1. Project information

Project title	Ground-truthing field expression and value of new flowering-time genes in lupins for Western Australia
Commencement Date	31st March 2019
Completion Date	7th May 2021

Name of Proponent	The University of Western Australia
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Project Number	
Date Received	

2. Project results	This section provides a final report against the Project Aim and the Planned Outputs for the Project.

Achievement of the Project Aim	Brief statement of achievement in relation to the aim of the project

The two major aims of this project were (i) to determine the potential value of new genes for flowering time in narrow-leafed lupin in WA farming systems; and (ii) to develop molecular markers for these genes so that lupin breeders will be able to easily transfer the genes to future lupin varieties. The project was run by UWA Research Officers Dr Renu Saradadevi in 2019 and Dr Candy Taylor (both 0.4 FTE) in 2020-21. The project was based on results of PhD research of Dr Candy Taylor at UWA, who discovered two new genes for flowering time, *LanFTc1-Jul* in eastern European variety Krasnolistny and a new gene *LanFTc1-P22660* from wild lupin P22660.

The project was built on the work of a group of collaborating researchers at UWA, Department of Primary Industries and Regional Development (DPIRD), CSIRO and Royal Botanic Gardens Kew (UK). In her previous PhD research, supported by a GRDC project subcontracted through DPIRD, UWA's Dr Candy Taylor "unmasked" new genetic variation for flowering time and discovered a new gene *LanFTc1-P22660*, which is normally hidden by the dominant *LanFTc1-Ku* gene. DPIRD researcher Dr Darshan Sharma (Manager Genetic Improvement) contributed to the success of the project through the provision of advice and services for field trials and methods for genetic evaluation of lupins. CSIRO researchers Dr Matthew Nelson (previously at Royal Botanic Gardens Kew, UK), Drs Lars Kamphuis and Jens Berger contributed through their experience in phenology and genomics.

The project achieved its goals. In the first year (2019), the two new genes for flowering time *LanFTc1-Jul* and *LanFTc1-P22660* were evaluated in a field trial at UWA Shenton Park Field Station which was hand-sown and managed by Dr Saradadevi. The new genes were segregating in the F_3 progeny of four biparental populations, derived from crosses between parent plants with the two new genes and Australian lupin varieties Tanjil (early flowering, *LanFTc1-Ku*) and Geebung (late flowering, *LanFTc1-ku*). Dr Saradadevu identified single plants with new flowering dates in the "mid-season" range between Tanjil and Geebung. She also selected plants with low alkaloid content and water-permeable seeds for subsequent sowing in 2020. She took leaf samples for subsequent molecular marker analysis.

In the second year (2020), a field trial was hand-sown by Dr Taylor with F_4 seed harvested in the 2019 field trial. This field trial was located at a lupin breeding field site managed by AGT Pty Ltd near Mumberkine (north of Toodyay, WA) in late May 2020. New genotypes which flowered in the mid-season range were associated with *LanFTc1-P22660*, and new genotypes in the early-flowering range were associated with *LanFTc1-Jul*. UWA MSc student Mr Julian van der Zanden contributed to the project by collecting leaf samples and using DNA isolations from these leaf samples for molecular marker analysis based on a new multiplex molecular marker, developed by Dr Taylor. Dr Sharma (DPIRD) provided advice and help to Dr Taylor in selection of plants in the field trial in 2020.

In 2020 and 2021, the new multiplex molecular marker was refined and accurately identified the four major alleles of the *LanFTc1* flowering time gene. The new marker system was able to distinguish loci that were heterozygous or homozygous for these alleles. In 2020, we confirmed that some genotypes were homozygous for *LanFTc1-P22660* and *LanFTc1-Jul*. The new marker system will hasten the breeding process by allowing the breeder to identify the new genes and to select homozygous progeny early in the breeding process. We anticipate that breeders will cross and reselect the new genes *LanFTc1-P22660* and *LanFTc1-Jul* in several cycles of recurrent selection, and that the new multiplex molecular marker will be essential for speed of recovery of target genotypes and for rapid commercialization of progeny with the new genes.

In the F_4 trial in 2020, Dr Taylor identified genotypes with unique flowering dates which were "sweet" and "soft-seeded" as required in crop lupin varieties. We favoured selection of non-shattering progeny, but we have not yet confirmed that these selections contain both non-shattering genes *lentus* and *tardus*. We were also not able to measure biomass in the 2020 field trial due to insufficient seed and lack of replication. However, we did measure grain yield of single plants harvested in both 2019 and 2020 field trials, and identified genotypes with high grain yield and high potential for further breeding.

An important result from this project was confirmation that the mid-season flowering gene *LanFTc1-P22660* is highly heritable across years and results in F_4 genotypes with a wide range of mid-season flowering times under field conditions. This project has opened up new possibilities for adaptation in new lupin varieties. Lupin breeders can now access flexible flowering dates to match future lupin environments in southern Australia; this opportunity was not available to lupin breeders before this project.

Project Outputs	Please provide a report on the achievement, or otherwise, of the project outputs as per the planned outputs provided in the Project Proposal.

1	-	**Output 1 (from Project proposal)** Valuable new genes in narrow-leafed lupin will be measured for their impact on flowering time, biomass and grain yield in field environments
		Comment: We successfully measured flowering time and grain yield in plants containing two new flowering genes *LanFTc1-P22660* and *LanFTc1-Jul* in narrow-leafed lupin in field trials in Western Australia at Shenton Park in 2019 and Mumberkine in 2020. These measurements were made on individual plants which were confirmed by molecular markers to contain the new genes. We were not able to measure plot biomass or plot grain yield due to insufficient seed quantities for replicated multi-row plots. This should occur in future lupin trials in relevant cropping regions once sufficient seed of selected lines has been bulked.
2	-	**Output 2 (from Project proposal)** Gene-specific markers for valuable new genes for flowering time in narrow-leafed lupin will be identified
		Comment: We successfully designed a multiplex PCR-based genetic marker system to simultaneously assay four flowering time genes, including the two new flowering time genes *LanFTc1-P22660* and *LanFTc1-Jul*. The genetic marker has 100% accuracy and reliably detected heterozygous (i.e. hybrid) and homozygous (i.e. pure) genotypes in individual F_3 and F_4 plants.
3	-	**Output 3 (from Project proposal)** We will promote our research to commercial breeders and encourage them to use new germplasm and markers to breed lupins with new flowering time genes
		Comment: We engaged with Australian Grain Technologies (AGT) plant breeders Dr Matthew Aubert (lupins) and Dr Dion Bennett (wheat) throughout this project. Dr Aubert and Dr Bennett attended our 2019 field trial at Shenton Park where we first showcased the flowering time variation made possible with the two new flowering time genes. AGT subsequently became partners for the 2020 field trial, which was hosted at the AGT lupin breeding site in Mumberkine, WA, and the AGT breeders again attended our field day in 2020. Valuable pre-breeding lines were identified for future breeding activities during the 2020 trial in consultation with Dr Aubert and Dr Bennett. Currently, UWA is negotiating legal agreements with AGT and COGGO to continue this research in 2021 and beyond.

Project results	Please provide brief statements on the results of the Project

This section should cover aspects identified in *Section 7.3* of the Research Agreement

- the results of the Project, including discoveries made and other achievements (including any Project IP and Project Confidential Information);
- the potential application of the outputs of the Project to the Western Australian grains industry and broader community;
- the actual or potential economic benefits flowing to the Western Australian grains industry and broader community from the Project;
- the difficulties encountered;
- the conclusions reached;
- the Researcher's recommendations for any further research;
- a list of scientific papers or publications resulting from the Project; and
- attach copies of any photos, diagrams or other artworks (including, if requested by COGGO, negatives, bromides or the like) which the Researcher has and which may be of assistance to COGGO in the dissemination of information concerning the Project to COGGO's stakeholders.

Design and application of a new molecular marker for narrow-leafed lupin flowering time genes

A new *LanFTc1* multiplex PCR molecular marker (Tables 1 – 3) was successfully designed to genotype four flowering time genes in narrow-leafed lupin, including:

1. *LanFTc1-ku*: late flowering gene observed in most wild narrow-leafed lupins
2. *LanFTc1-Ku*: early flowering gene present in modern Australian varieties
3. *LanFTc1-Jul:* early flowering gene derived from European varieties
4. *LanFTc1-P22660*: mid-season flowering gene derived from wild Israeli accession P22660

Table 1: PCR primers used in the *LanFTc1* multiplex PCR marker to genotype four flowering time genes in narrow-leafed lupin.

Flowering time gene	Primer name	Direction	Sequence (5'–3')	Length (bp)	T_m (°C)	Amplicon length (bp)
LanFTc1-ku	F1*	Forward	AGCATGCGAGAAAACAACG	19	57.2	1,301
	R1	Reverse	GGAAATCTTGCATTTCTCCTCACT	24	59.3	
LanFTc1-P22660	F1*	Forward	AGCATGCGAGAAAACAACG	19	57.2	1,069
	R2*	Reverse	CAGCTTACTCCATAGTTCAAAGCA	24	59.1	
LanFTc1-Ku	F1*	Forward	AGCATGCGAGAAAACAACG	19	57.2	854
	R2*	Reverse	CAGCTTACTCCATAGTTCAAAGCA	24	59.1	
LanFTc1-Julius	F2	Forward	ATGCGGTCTGATGCAGTTCA	20	60.0	594
	R3	Reverse	TCAAGGGATGCAACTTCAGCT	21	59.9	

* Primer designed by Nelson et al. (2017).

Table 2: Chemical reaction components for the *LanFTc1* multiplex PCR marker to genotype four flowering time genes in narrow-leafed lupin.

PCR reaction component	**Final concentration/volume**
10x DreamTaq™ Buffer*	1x
dNTP mix	0.2 mM
F1 primer	0.60 µM
F2 primer	0.05 µM
R1 primer	0.40 µM
R2 primer	0.60 µM
R3 primer	0.05 µM
DreamTaq™ Hot Start DNA Polymerase*	0.024 U/µL
DNA template	50.0 ng
Nuclease-free water	to 25 µL

* Included within DreamTaq™ Hot Start DNA Polymerase (Catalog Number: EP1702) kit from Thermo Scientific™

Table 3: Chemical reaction cycling conditions* for the *LanFTc1* multiplex PCR marker to genotype four flowering time genes in narrow-leafed lupin.

Cycling stages	**Temperature (°C)**	**Duration**	**Number of cycles**
Initial denaturation	95	2 min	1
Denaturation	95	30 sec	35
Annealing	55	60 sec	
Extension	72	60 sec	
Final extension	72	10 min	1
Hold	4	∞	1

* Cycling conditions were optimized using a VeritiTM 96-Well Thermal Cycler (Applied Biosystems®) machine.

This new *LanFTc1* multiplex PCR molecular marker offers four significant benefits, including:

1. Time and cost savings:
   The molecular marker can simultaneously assay all four genes within a single chemical reaction. This offers considerable financial and labor cost savings, as previously two separate molecular markers (Nelson et al. 2017; Taylor et al. 2019) were required to genotype these four flowering time genes.

2. Elimination of false negative results:
   Previously, when two separate molecular markers were required to genotype narrow-leafed lupin flowering time genes (Nelson et al. 2017; Taylor et al. 2019), it was difficult to differentiate between chemical reactions that failed for technical reasons and chemical reactions that failed because a particular flowering time gene was absent from a plant (i.e. false negative results). The ability to simultaneously genotype all four flowering time genes within a single chemical reaction now means there is a very clear distinction between these two possible outcomes, as all successful chemical reactions produce unique positive results (Figure 1).

3. Codominance:
   The new molecular marker is codominant and can therefore determine if plants are homozygous (i.e. have one gene) or heterozygous (i.e. have two genes) for any of the four flowering time genes. This makes the marker vastly more appropriate for use in breeding programs and research compared to previous markers, which were unreliable when genotyping heterozygous plants due to bias in PCR which favoured one gene over another. This issue often caused heterozygous plants to be identified mistakenly as homozygous.

4. Accuracy:
   The molecular marker targets regions of DNA within the four flowering time genes that directly regulate flowering time. The marker is therefore "perfect" for genotyping and is 100% predictive of flowering time. This represents a significant achievement as molecular markers used for breeding are typically strongly, rather than perfectly, associated with traits.

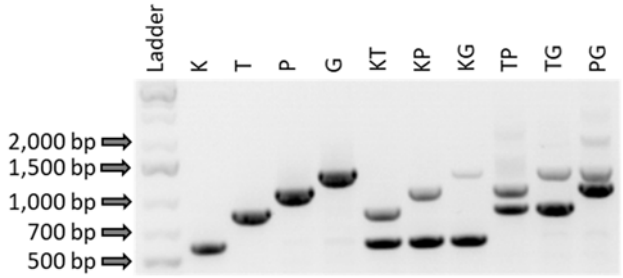

Figure 1: The new *LanFTc1* multiplex PCR molecular marker identifies each possible combination of four flowering time genes in narrow-leafed lupin. Narrow-leafed lupins genotyped include varieties Krasnolistny (K; *LanFTc1-Jul* gene), Tanjil (T; *LanFTc1-Ku* gene), P22660 (P; *LanFTc1-P22660* gene) and Geebung (G; *LanFTc1-ku* gene), plus six hybrids of these varieties (KT, KP, KG, TP, TG and PG).

The value of the new *LanFTc1* multiplex marker for breeding was demonstrated during this project. Firstly, the marker was able to identify homozygous and heterozygous plants in the F_3 and F_4, which hastened the selection process of pure lines. Also, the molecular marker provided evidence of rare out-crossing in lupins. Generally, narrow-leafed lupin is assumed to be a mostly self-pollinating species. However, several F_4 individuals in the 2020 field trial had flowering times that were inconsistent with their siblings in F_4 families and contained genes that were not present in the F_3 parent plant (Table 4). The new molecular marker revealed unexpected genotypes in each of the affected individuals (Table 4). Human error was eliminated as a possible cause of contamination, as two of the genotypes (i.e. *LanFTc1-Jul* / *LanFTc1-P22660* and *LanFTc1-Ku* / *LanFTc1-ku*) identified could only be achieved through crosses which were not made at any point during development of the four populations examined in this project. This indicated that that the F_4 individuals were the products of cross pollination of F_3 plants during the 2019 field trial at Shenton Park. This outcome highlights the value and utility of the marker for maintaining pure breeding lines during variety development.

Table 4: Examples of genotypes identified by application of a new *LanFTc1* multiple molecular marker to F_4 families during the 2020 field trial at Mumberkine. F_4 individuals derived through cross-pollination events are highlighted in grey.

F_3 parent gene(s)	F_4 individual	F_4 individual gene(s)	DTF
LanFTc1-P22660	PG007-F3A08-F4A31	*LanFTc1-P22660*	99
	PG007-F3A08-F4A32	*LanFTc1-P22660*	97
	PG007-F3A08-F4A33	*LanFTc1-P22660*	100
	PG007-F3A08-F4A34	*LanFTc1-P22660*	97
	PG007-F3A08-F4A35	*LanFTc1-P22660*	95
	PG007-F3A08-F4A36	*LanFTc1-P22660*	96
	PG007-F3A08-F4A37	*LanFTc1-P22660*	97
	PG007-F3A08-F4A38	*LanFTc1-P22660*	100
	PG007-F3A08-F4A39	*LanFTc1-Jul* / *LanFTc1-P22660*	90

	PG007-F3A08-F4A40	*LanFTc1-Jul* / *LanFTc1-P22660*	90
	PG007-F3A08-F4A41	*LanFTc1-P22660*	101
	PG007-F3A08-F4A42	*LanFTc1-P22660*	96
LanFTc1-P22660 / *LanFTc1-ku*	PG046-F3A11-F4A31	*LanFTc1-P22660* / *LanFTc1-ku*	95
	PG046-F3A11-F4A32	*LanFTc1-P22660* / *LanFTc1-ku*	96
	PG046-F3A11-F4A33	*LanFTc1-P22660* / *LanFTc1-ku*	99
	PG046-F3A11-F4A34	*LanFTc1-ku*	102
	PG046-F3A11-F4A35	*LanFTc1-P22660*	94
	PG046-F3A11-F4A36	*LanFTc1-P22660*	96
	PG046-F3A11-F4A37	*LanFTc1-ku*	103
	PG046-F3A11-F4A38	*LanFTc1-P22660* / *LanFTc1-ku*	99
	PG046-F3A11-F4A39	*LanFTc1-P22660* / *LanFTc1-ku*	98
	PG046-F3A11-F4A40	*LanFTc1-Ku* / *LanFTc1-ku*	85
	PG046-F3A11-F4A41	*LanFTc1-P22660* / *LanFTc1-ku*	95
	PG046-F3A11-F4A42	*LanFTc1-Jul* / *LanFTc1-ku*	85
LanFTc1-P22660	PG049-F3A09-F4A31	*LanFTc1-Jul* / *LanFTc1-P22660*	83
	PG049-F3A09-F4A32	*LanFTc1-Jul* / *LanFTc1-P22660*	82
	PG049-F3A09-F4A33	*LanFTc1-P22660*	98
	PG049-F3A09-F4A34	*LanFTc1-P22660*	91
	PG049-F3A09-F4A35	*LanFTc1-P22660*	95
	PG049-F3A09-F4A36	*LanFTc1-Ku* / *LanFTc1-P22660*	85
	PG049-F3A09-F4A37	*LanFTc1-P22660*	93
	PG049-F3A09-F4A38	*LanFTc1-Jul* / *LanFTc1-P22660*	82
	PG049-F3A09-F4A39	*LanFTc1-P22660*	94
	PG049-F3A09-F4A40	*LanFTc1-P22660*	90
	PG049-F3A09-F4A41	*LanFTc1-P22660*	100
	PG049-F3A09-F4A42	*LanFTc1-P22660*	95

Field-based evaluation of new flowering time genes

In the 2019 and 2020 field trials, we evaluated the field performance of plants with the new *LanFTc1-Jul* and *LanFTc1-Ku* flowering time genes. The main findings concerning their effects on flowering time and the practical implications of these effects are summarised below.

Flowering in parental varieties

Krasnolistny (*LanFTc1-Jul*) was consistently the earliest flowering variety in this project and was followed closely, although significantly later, by Tanjil (*LanFTc1-Ku*) (Table 2). Krasnolistny (*LanFTc1-Jul*) was 4.2 days earlier than Tanjil (*LanFTc1-Ku*) in 2020 at Mumberkine, but only 1.5 days earlier at Shenton Park in 2019. Geebung (*LanFTc1-ku*) was 24.7 days later than Tanjil in 2020 at Mumberkine, but only 20.9 days later in 2019 at Shenton Park, which may reflect the warmer average winter temperatures at Shenton Park compared with Mumberkine. Interestingly, P22660 (with the new gene *LanFTc1-P22660*) had similar delay of flowering 12 to 13 days after Tanjil in both years/locations. *LanFTc1-P22660* was discovered in genotype P22660 by Dr Taylor in her previous PhD project at UWA, but this is the first time this genotype has been tested in the field.

Table 2: Flowering times produced by four narrow-leafed lupin flowering time genes in field trials at Shenton Park and Mumberkine, WA.

Year and field site climate data (from sowing to end of flowering)	Plant variety and flowering time gene	Average days to flowering ± SE	Days to flowering relative to Tanjil (*Ku*)
2019 - Shenton Park (31°56'59.8"S, 115°47'37.2"E) Rain: 369 mm Av temp range: 10.8 – 19.7 °C	Geebung (*LanFTc1-ku*)	93.2 ± 0.7	+ 20.9 ($P < 0.05$)
	P22660 (*LanFTc1-P22660*)	84.3 ± 0.7	+ 12.0 ($P < 0.05$)
	Tanjil (*LanFTc1-Ku*)	72.3 ± 0.7	0.0
	Krasnolistny (*LanFTc1-Julius*)	70.8 ± 0.7	- 1.5 ($P < 0.05$)
2020 - Mumberkine (31°20'47.5"S, 116°38'45.2"E) Rain: 137 mm Av temp range: 8.1 – 20.3 °C	Geebung (*LanFTc1-ku*)	108.7 ± 0.3	+ 24.7 ($P < 0.05$)
	P22660 (*LanFTc1-P22660*)	96.8 ± 0.3	+ 12.8 ($P < 0.05$)
	Tanjil (*LanFTc1-Ku*)	84.0 ± 0.4	0.0
	Krasnolistny (*LanFTc1-Julius*)	79.8 ± 0.4	- 4.2 ($P < 0.05$)

Flowering in cross progeny

The F_2 and F_3 progeny and parents of the cross Krasnolistny (new gene *LanFTc1-Jul*) × Tanjil (*LanFTc1-Ku*) had similar flowering times with little evidence of genetic variation among the progeny (Figure 2b). This suggests that the new gene *LanFTc1-Jul* has a similar impact on flowering as *LanFTc1-Ku* to achieve early, vernalisation-insensitive flowering time in narrow-leafed lupin breeding programs. Therefore, new European varieties with *LanFTc1-Jul* may be used as parents in Australian lupin breeding programs to introduce desirable traits without disrupting the early flowering phenotype.

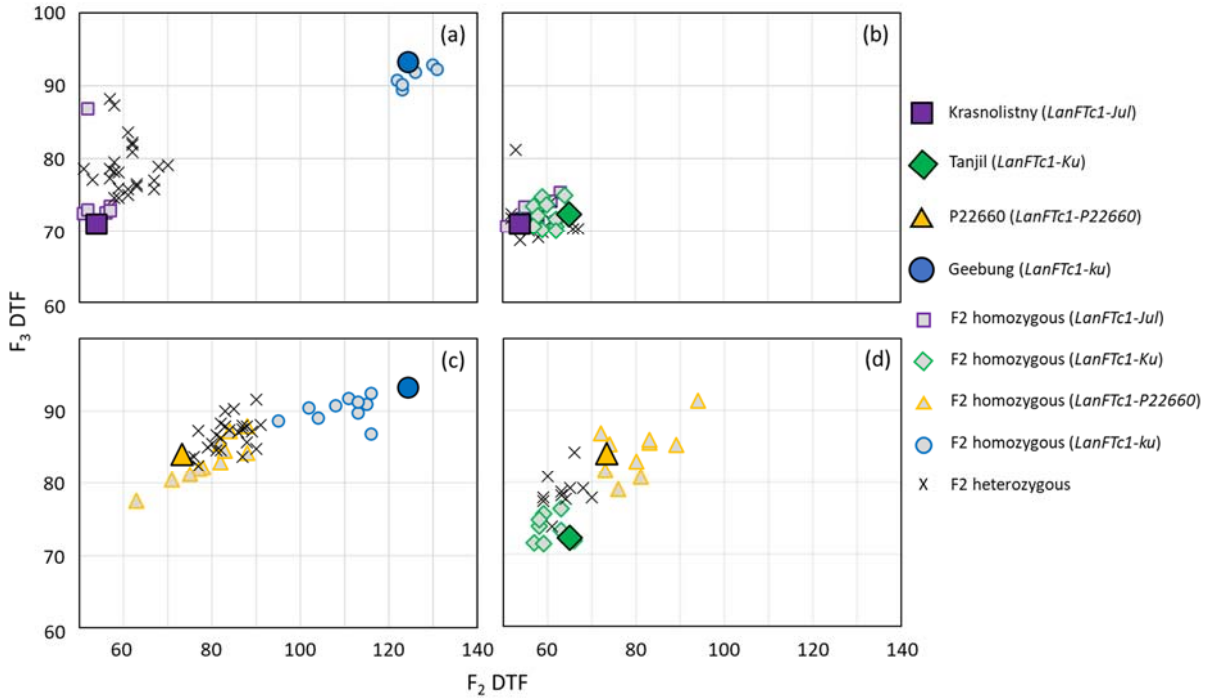

Figure 2: Segregation of flowering times and genes in F_2 and F_3 progeny derived from crosses between (a) Krasnolistny (*LanFTc1-Jul*) x Geebung (*LanFTc1-ku*), (b) Krasnolistny x Tanjil (*LanFTc1-Ku*), (c) P22660 (*LanFTc1-P22660*) x Geebung, and (d) P22660 x Tanjil. The F_2 generations were evaluated under glasshouse conditions prior to this COGGO-funded project, while the F_3 generation were evaluated in 2019 at Shenton Park.

The new gene *LanFTc1-P22660* showed very interesting genetic segregation in the F_2 and F_3 generations of crosses of P22660 × Tanjil and P22660 × Geebung. In both crosses, substantial genetic diversity was revealed in the F_2 and F_3 (Figs. 2c and 2d). This confirms that a wide range of mid-season flowering types can be achieved in progeny of crosses with the *LanFTc1-P22660* gene.

LanFTc1-P22660 is an important new mid-season flowering gene for Australian narrow-leafed lupin breeding. Until now, mid-season flowering has only been achieved in two Australia varieties (Chittick and Wandoo) through a mutation known as *efl*. However, *efl* has not been widely adopted due to its association with cucumber mosaic virus susceptibility, which caused the withdrawal of Wandoo from commercial production.

There are potential economic and agronomic benefits to be gained from the adoption of *LanFTc1-P22660* in lupin breeding. Recent modelling by Chen et al. (2017) indicates that delays of 14 to 22 days in flowering time relative to variety Mandelup (*LanFTc1-Ku*) would result in average annual grain yields increasing by 13% to 16% (390 to 480 kg/ha) in high-rainfall environments of the southern WA wheatbelt. In addition, *LanFTc1-P22660* may also be advantageous for early sowing of lupin crops in lower rainfall environments. Early sowing is becoming a popular practice in WA to accommodate the increasing scale and size of cropping systems and to capitalise on increasingly frequent summer/autumn rainfall events. The mid-season flowering time of *LanFTc1-P22660* would potentially capture a yield benefit from early sowing. Lastly, this new gene may be important for navigating agronomic challenges linked to climate change. Farmers are very concerned that current early flowering lupin varieties are too short in height at harvest. This problem is exacerbated by the responsiveness of *LanFTc1-Ku* to warmer winter temperatures in southern Australia as a result of climate change (BOM 2015). The new gene *LanFTc1-P22660* may increase height at harvest due to its delay in flowering.

Selection of pre-breeding lines

Identifying pre-breeding lines that are homozygous for new flowering time genes, and also have domestication traits necessary for commercialization, was the third and final objective of this project.

A total of 2,360 individual F_3 plants in 178 F_3 families were evaluated in the 2019 field trial at Shenton Park. Flowering time and alkaloid content were carefully characterised in each of these plants. In addition, seed coat permeability was screened in the F_4 seed harvested from these plants. These tests identified 151 F_4 pre-breeding lines that were low in alkaloids and had water permeable seeds, and these lines were promoted to the 2020 field trial.

In the 2020 field trial at Mumberkine, we evaluated 101 sweet, water permeable F_4 pre-breeding lines in single rows. Twelve individual plants were tagged and recorded for flowering time in key F_4 plots with the new genes *LanFTc1-P22660* and *LanFTc1-Jul*. F_5 seed was harvested only from F_4 plants which were classified as homozygous for these genes based on the *LanFTc1* multiplex marker, and which had little or no pod-shattering in the field at maturity. A total of 57 F_5 pre-breeding lines were ultimately harvested based on these selection criteria (please refer to Supporting Information).

The 57 F_5 pre-breeding lines selected from this project will serve as useful parents to introduce the *LanFTc1-Jul* and *LanFTc1-P22660* into future commercial lupin varieties. All 57 pre-breeding lines are homozygous for the two new flowering time genes, which is critical. However, they also contain several domestication genes that will accelerate the breeding process. We did not have the time to confirm if these progeny had the pod shatter resistance genes (*lentus* and *tardus*). Therefore, more work is required to cross and reselect fully domesticated progeny from future crosses.

3. Project resources

This section describes use of the funding listed in the initial plan and any refunds due to COGGO

Expenditure of funds requested from COGGO	$ Total funds budgeted	$ Total funds expended (actual)	$ Total funds requested from COGGO*	$ Total COGGO funds expended	$ Refund due to COGGO of any unexpended COGGO funds
Salary/Contractors	146,000		66,500	80,408	
Operating costs	44,000		21,000	19,549	
Capital	NA	NA	NA	NA	NA
TOTAL	**190,00**		**87,500**	**99,957**	**0.00**

*Funding provided by COGGO.

IMPORTANT: Return of unused funds to COGGO is required as per *Clause 3.3* of the Research Agreement.

4. Commercialisation

Insert details of the proposed commercialisation process,as applicable, with reference back to the planned commercialisation plan in the project proposal) for any outputs from the project.

This should include recommendations for the commercialisation of the results of the project and the registration or other protection of Project IP and Project Confidential Information as per the Research Agreement.

A plan for publication and protection of Project IP and Project Confidential Information has been agreed upon between UWA and COGGO, and drafting of Material Transfer Agreements is underway at UWA. The plan has two parts:

(i) development of a Material Transfer Agreement between UWA and potential licensees that considers the share in Project IP of all potential IP owners (COGGO, UWA, GRDC and DPIRD) and proposes a royalty scheme for potential licensees for commercialisation of the Project IP.

(ii) publication of multiplex molecular marker system in a scientific journal, with draft title "A multiplex marker distinguishes between multiple alleles of the *LanFTc1* flowering time gene in narrow-leafed lupin (*Lupinus angustifolius* L.)"

It is understood that this may require further discussion and agreement with COGGO via its' agent GIWA, as per the undertakings given and terms agreed, in the project proposal. This can be the subject of an appended letter and attachments. In all cases such discussion and subsequent agreements need to be governed by *Section 8 Project IP, Improvements and Project Confidential information* of the Research Agreement.

5. Communication/ Extension	Insert details of how the communication and extension of the project outcomes has been achieved to date and recommendations for future activities to disseminate and promote adoption of the results of the Project.

Communication to date

The research in this project has been communicated to academic, industry and general public audiences on several occasions in 2019 and 2020.

In both years, we hosted annual field site visits where the trials were showcased to colleagues from academia (UWA, CSIRO, Curtin, Murdoch) and industry (DPIRD, AGT) with interests in phenology and/or lupins (please refer to attached images and Supporting Information). These field site visits enabled discussions about the progress and future potential directions for the project.

The 2020/2021 research officer (Dr Candy Taylor) and Masters student (Mr Julian van der Zanden) associated with this project attended the AGT-GIWA Lupin Industry Field Day event (28th August 2020) in Northam. At this event, Dr Taylor and Mr van der Zanden engaged in informal discussions with several lupin farmers and representatives from grains industry organisations regarding the 2020 field trial and value of the project to the Australian lupin breeding program. A strong desire to see the new genes used in the breeding program was expressed during these discussions.

Mr van der Zanden also presented his research via oral presentations on several occasions in fulfilment of his UWA Masters course requirements and those of his UWA Agribusiness Connect Masters Research Project Scholarship (funded by Royalties for Regions). These presentations were attended by the broader agricultural science community at UWA and collaborating organisations (e.g. CSIRO, Curtin).

Finally, a manuscript providing a comprehensive scientific report of this research project is currently in preparation. The manuscript will outline all aspects of genetic population development, phenotypic evaluation of the two new flowering time genes, and genetic marker development. The manuscript will be submitted to a peer-reviewed genetics and breeding journal, such as "Molecular Breeding" or "Theoretical and Applied Genetics" (TAG). These journals are "open-access", meaning that the published article will be freely and publicly available. COGGO will be acknowledged in this manuscript. Permissions from COGGO to publish the manuscript were requested by Prof Cowling in January 2021.

Future extension

Genetic material containing the new flowering genes *LanFTc1-Jul* and *LanFTc1-P22660* developed in this research is of interest to the national lupin breeding program operated by AGT in Northam, WA. However, the genetic material retains a number of undesirable traits that limit its immediate value for commercial release, especially cross progeny with wild lupin P22660 which contain 50% wild alleles which inevitably will reduce agronomic performance. Further crossbreeding and selection is necessary to introduce the two new flowering time genes into more favourable genetic backgrounds with all other domestication and agronomically superior traits.

Our recommendation is to undertake rapid backcrossing of progeny selected at harvest in 2020 to current commercial lupin varieties, and to reselect the new flowering genes *LanFTc1-Jul* and

LanFTc1-P22660 with the aid of our new multiplex PCR marker system. UWA has access to rapid generation cycling platforms that would enable this work to be completed within a period of approximately 18 months. This is a significant improvement upon the timeframe this work would require through conventional breeding, which would require 3 to 5 years as a minimum. Funding is now being sought to complete this recommended work.

Note: As per *Clause 7.3 (b) (ii)* of the Research Agreement COGGO may require the Researcher to produce an edition of the Final Report in a form suitable for general distribution. If so required by COGGO, the Researcher must produce a non-confidential version of the Final Report within 28 days of receiving a request to that effect from COGGO.

6. Certification

The Project Supervisor and the Research Organisation certify that all information contained in, and forming part of, this final project report is complete and accurate. The project supervisor and research organisation further warrant that the project complied with all the relevant guidelines affecting the conduct of research, for example in relation to ethics, bio-safety, environmental legislation, GMAC or National Health and Medical Research Council Codes.

Project Supervisor's signature 

Name (in Capitals) Wallace A. Cowling

Date: 4 August 2021 (revised version)

Research Organisation signature 

Name and title of authorised signatory (in Capitals)

Robert Roche, Manager Research Grants

Date: 6 August 2021

Completed Final Project reports

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COGGO representative

For the purpose of this Project agreement contract, COGGO will be represented by Grains Industry Association of Western Australia (GIWA), or such other representative that is nominated by COGGO as authorised to operate on behalf of COGGO.

PROJECT SYNOPSIS SUITABLE FOR GENERAL PUBLICITY AND COGGO WEBSITE

Ground-truthing field expression and value of new flowering-time genes in lupins for Western Australia

COGGO Research Fund project based at The University of Western Australia, 2019-2021.

Narrow-leafed lupin has been a valued component of crop rotations in Australian farming systems since the 1960's. Over the past two decades, however, narrow-leafed lupin production has been decreasing as more competitive break crops (including canola and chickpea) gain favour. The transition away from lupins is particularly noticeable in WA, where land allocations for this crop have been contracting at an average rate of 43,500 ha per year since peak production in 1999/2000 (ABARE 2000 & 2018). Genetic improvement of crop adaptation traits and yield is necessary to address this decline.

Flowering time is one of the most important traits for adaptation of crops to diverse agricultural environments. Australian lupin breeders have heavily relied on a single gene, known as *Ku*, to adapt lupin crops to southern Australia for the past 50 years. *Ku* achieves this by ensuring crops consistently flower early because they do not require vernalization (cold temperatures which stimulate flowering). Recent genomic research by Dr Candy Taylor at The University of Western Australia (UWA) revealed that *Ku* is a mutation of the *LanFTc1* flowering time gene, and identified two other new mutations (referred to as *LanFTc1-Jul* and *LanFTc1-P22660*) that may potentially enhance crop adaptation and production.

The aims of this COGGO project at UWA were to (i) determine the potential effect of these new *LanFTc1* gene variations on narrow-leafed lupin flowering time adaption in WA field environments and (ii) design a molecular marker to assist efficient adoption of these genes in future lupin varieties.

We evaluated the two new *LanFTc1* flowering time genes in representative high- and mid-rainfall environments at UWA Shenton Park Field Station (369 mm sowing to flowering) in 2019 and Mumberkine (137 mm sowing to flowering) in 2020. The two new *LanFTc1* genes were characterized in Krasnolistny (a European variety with *LanFTc1-Jul*) and P22660 (a wild lupin with *LanFTc1-P22660*), in addition to the F_2, F_3 and F_4 progeny derived from crosses between these two lupins with local varieties Tanjil (*LanFTc1-Ku*, early flowering) and Geebung (*LanFTc1-ku*, late flowering). There was little evidence of flowering time variation in F_2 and F_3 progeny in the Krasnolistny x Tanjil population, which suggests that *LanFTc1-Jul* behaves similarly to *LanFTc1-Ku* in terms of producing early, vernalization-insensitive flowering times. Therefore, new European varieties with *LanFTc1-Jul* may be used as parents in Australian lupin breeding programs to introduce desirable traits without disrupting the early flowering phenotype. However, substantial diversity of mid-season flowering times was observed in the progeny of P22660, which consistently flowered about 12 days later than Tanjil and 12 days earlier than Geebung. This delay in flowering time associated with *LanFTc1-P22660* is estimated to increase average annual grain yields by 13% to 16% (390 to 480 kg/ha) in high-rainfall environments in southern Australia according to recent modelling (Chen et al. 2017). The mid-season flowering time of *LanFTc1-P22660* would potentially also capture a yield benefit from early sowing, which is becoming a more prominent agronomic practice to accommodate the growing scale of WA farming systems and take advantage of increasingly frequent summer/autumn rainfall events. Both new genes will therefore help breeders to now develop future lupin varieties with greater adaptation to a range of environments in southern Australia.

A new "multiplex" molecular marker was successfully designed to identify all possible combinations of the four of the *LanFTc1* gene variations within a single reaction. The molecular marker was designed by Dr Taylor and test conditions developed by UWA MSc student Mr Julian van der Zanden. The molecular marker was able to reliably identify homozygous (i.e. contain only one gene) and heterozygous (i.e. contain two genes) plants in the F_2, F_3 and F_4 progeny. In addition, it enabled detection of rare cross-pollination events in 2019, which caused unexpected segregation of genotypes and flowering times among F_4 siblings in 2020. The new multiplex marker will enable the *LanFTc1-Jul* and *LanFTc1-P22660* genes to be efficiently incorporated during breeding and fast-track the development of new valuable narrow-leafed lupin varieties that are better adapted to targeted environments.

Photographs taken during COGGO project:

Ground-truthing field expression and value of new flowering-time genes in lupins for Western Australia

Dr Renu Saradadevi in lupin field trial at UWA Shenton Park Field Station in 1999

Lupins in field trial at UWA Shenton Park Field Station in 1999

Lupin field trial at Mumberkine in 2020. From left to right: Prof Wallace Cowling (UWA), Dr Candy Taylor (UWA, research officer on this project), Mr Julian van der Zanden (UWA MSc student), and Dr Matthew Aubert (AGT lupin breeding).

Lupin field trial at Mumberkine, WA, in 2020.