Implementation of marker assisted selection in lupin breeding

Summary
This project was initially established to provide technical support for projects UMU59 and CLM30. When the support for project UMU59 finished in December 2000, the ongoing work from projects UMU59 and UMU81 on lupin markers and mapping continued under this project.

Overall, this project has been very successful. The work undertaken has led to the following outputs:

- Molecular markers linked at various distances to five phenotypic traits for narrow-leaved lupins.
- Generation of more than 470 amplified fragment length polymorphism (AFLP) and other molecular markers across the lupin genome.
- Development of the most advanced molecular map of the *Lupinus angustifolius* genome currently available worldwide.

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Conclusions

Lupin markers
Markers linked to domestication traits have been found and converted into polymerase chain reaction (PCR)-based tests for these traits. Two traits have associated marker tests - early maturity (Ku), and flower colour, leucospermus. Further work is required to produce routine tests for the other domestication traits. Markers have been found for phomopsis and anthracnose resistance in lupins (CLM30) and these are being implemented in the lupin breeding program using techniques established in this project.

Lupin mapping
The most advanced and highly saturated molecular marker map so far for sweet narrow-leaved lupins (L. angustifolius) has been generated in this project. The map has 368 markers in 61 linkage groups (31 maternal groups, 30 paternal) with a logarithm of the odds (LOD) = 4.0 and maximum distance of 32.9cM. The map provides a valuable resource for researchers working on molecular markers for traits in lupins and associated species. The techniques used to develop molecular markers in lupins are also valuable and applicable to research on genetic diversity in legumes.

Recommendations

It is recommended that:

• GRDC should continue to fund research on molecular mapping and marker research and development (R&D) for lupins - there is a danger that a gap in funding will lead to significant loss of expertise and delayed implementation of markers to assist the breeding program. Compared with investment in R&D in other grain crops, support for mapping in lupins and other pulses is lacking.
• The molecular map produced in this project be used as a tool, in conjunction with other marker techniques, to map the F8 recombinant inbred lines (RILs) subsequently developed from the same population. This will enable comparative mapping to the model legume Medicago truncatula, and assist in the targeting of specific genes for the improvement of the lupin breeding program.
• Full use be made of the tools of comparative genomics using model legume species to speed up advances in lupins R&D, since the same level of support for research solely on lupins cannot be justified readily.
• High throughput, low cost technologies that can be automated should be used to screen germplasm from breeders to ensure the most cost-effective use of funds.

Outcomes

Benefits
Economic Outcomes
The work undertaken in this project provides the most detailed molecular map of the lupin genome now available, and this forms the basis for future refinements and for comparative mapping of target traits. The markers that have been developed are a valuable resource and will be used to map F8 RILs. For the markers, early detection of germplasm with early maturity and low alkaloids that are soft seeded, non-pod shattering and resistant to anthracnose and phomopsis will be valuable contributions to lupin improvement.

Environmental Outcomes
Lupins benefit the environment by contributing nitrogen and binding the topsoil. Plants with improved properties (e.g. disease resistance) may need less inputs in terms of chemical control, with resultant environmental benefits.

Social Outcomes
Improved lupin varieties with higher, more stable yields will benefit growers and the rural community. Lupins provide both a valuable high protein crop, a break between cereal and canola crops, and contribute to nitrogen inputs. The work in this project will help contribute to these benefits, and so to growers’ incomes and support for rural communities.

Achievements/Benefits
From a F2 population of a wild x domestic lupin (L. angustifolius) cross, the F3 families were screened in this project for five characteristics - non-pigmented flowers and seed, low alkaloids, soft seed, pod-shattering and early maturity. The data from the F3 population showed which of the F2 lines were homozygous or heterozygous for the traits tested. The populations that were initially developed under project UMU59 for marker and mapping work are now being maintained under project DAW711. Additional populations have also been developed under DAW711.

A set of new AFLP markers for lupins have been identified in this project. This information has been used to develop and extend the first molecular map for L. angustifolius. There are now markers linked to all five domestication traits from the F2 mapping population of 60 individuals (#97L380). The original cross (#93L377) of 30 individuals was compromised, by inadvertent selection of the F2 plants by technical breeding staff, and so was not appropriate for mapping and marker analysis. However, this population is still useful for screening and marker validation.

To develop AFLP markers, 64 AFLP primer combinations applied to the second cross have been analysed for polymorphisms using the following marker and mapping software - Genescan 2.1, Genotyper 2.5, MapMaker 3.0, Map Manager V13.0 and Qgene 3.0. The first high density linkage map for L. angustifolius has been developed using 470 AFLPs, of which 368 are linked into 61 groups with LOD 4.0 and with the maximum distance of 32.9cM between any two markers. A paper reporting this work, now in preparation, will be submitted in early 2003 (entitled ‘A molecular map of sweet narrow-leafed lupin (L. angustifolius)’). The 61 linkage groups are divided into 31 groups of markers from the maternal parent and 30 groups of markers from the paternal parent. This segregation of the markers is consistent with the coupling and repulsion effects of analysing dominant markers on an F2 population. A limited number of co-dominant markers (e.g. microsatellite or cleaved amplified polymorphic sequences (CAPS) markers) are required to overcome this effect. These were not available for lupins, but initial work has been undertaken to apply information now becoming available using comparative genomics from the model legume M. truncatula to lupins. Selected markers from the F2 map will be used, in conjunction with soybean restriction fragment length polymorphism (RFLP) markers and M. truncatula CAPS markers, in project UWA372 to map the F8 RILs of the mapping population (#97L380). This is working towards comparative mapping between these species and will assist the targeting of specific genes in lupins.

An RIL population of a L. albus cross (Kiev x P27174) is being grown by the Department of Agriculture Western Australia (DAWA) (DAW711). In this project, the F5 progeny were sampled in January 2001, DNA extracted and stored for use in developing markers for anthracnose resistance. Another L. angustifolius cross (Tanjil x Unicrop) has been developed at DAWA (CLM30). F8 progeny are now available. This was the next population to be mapped. It has been characterised for anthracnose resistance, phomopsis resistance and other domestication traits under project CLM30. Support was provided by this project for harvesting, threshing and planting of these lines.

The collaboration between the above projects was co-ordinated by Dr B Buirchell, DAWA.

Several DNA extraction methods have been investigated. Cetyl trimethylammonium bromide (CTAB) extraction was the best method for extracting DNA from lupin tissue. A marker developed by AFLP analysis was found linked to early maturity (Ku) in...
lupins. Under project UMU59, this marker was developed into a co-dominant test for Ku. A fragment is amplified by PCR and then digested using the restriction enzyme Msel. Homozygous plants carrying the dominant allele Ku show a single band of 273bp and homozygous plants carrying the recessive allele Ku (late maturity) show two bands 81bp and 156bp. Heterozygous plants with both alleles show all three bands. This co-dominant marker test for Ku was confirmed on the crosses #97L380 and #93L377.

When this test was subjected to further validation, using 20 different commercial varieties of lupins, all the varieties generated the 273bp fragment, but not all the late varieties showed the presence of the restriction site to produce two bands. In additional AFLP analyses, another marker more closely linked to Ku was identified, a PCR-based test derived from this marker does not produce a polymorphism and further work on this marker is required to produce a test for early maturity. The current mapping work shows markers linked to all five domestication traits. These have been converted into PCR based tests. A co-dominant test for flower colour, leucospermus, based on an SNP has been developed - this involves primer extension and analysis matrix assisted laser desorption/ionization time-of-flight (MALDI-ToF mass spectrometry) - this is the first SNP for lupins analysed in this way. This SNP marker has been tested on the mapping population and the marker segregates with the trait. PCR tests developed for the three other traits do not produce polymorphisms which segregate with the particular trait in question. Further work is required to produce co-dominant tests for these traits. A joint paper (with A/Prof W. Cowling, University of WA (UWAI)) will be submitted early in 2003 combining the phenotypic work with the molecular marker work.

Work on markers for phomopsis resistance in *L. angustifolius* started under project UMU59, in conjunction with DAWA and project CLM30, and was continued under this project. In this project, two markers for susceptibility to phomopsis have been identified. One of these was converted into a co-dominant PCR based test, but when validated on all the available progeny for this population, the test did not segregate with susceptibility to phomopsis.

A Biomek 2000 robotic workstation was used to prepare samples for AFLP-PCR and preliminary experimental work has been undertaken to develop high throughput DNA extraction procedures for lupins, using a matrix mill and 96 well extraction format.

The initial marker for early flowering (Ku) was developed in August 2000 from the two mapping populations (#97L380 and #93L377). As indicated above, the locus was conserved in other genotypes, but the polymorphism linked to late maturity was not present in all late varieties. Another marker which is more closely linked to Ku has been identified and is now being developed as a better marker for this trait. Once tests have been established, they can be used for routine testing in the lupins breeding program. Markers for phomopsis and anthracnose resistance have been developed under CLM30 and implementation of these marker tests has been initiated in the lupins breeding program, using high throughput techniques (Biomek Robotic Workstation and automated gels). This work will continue under CLM30.

**Other research**

Use of comparative genomics to enhance lupin improvement - information from model and other more highly studied legumes can now increasingly be applied to lupins and other pulses, to maintain the lead Australian researchers have on lupin improvement.

**Intellectual property summary**

The work undertaken in this project is to support lupin breeding programs in Australia. The work is therefore essentially in the public domain and the results will be provided to Australian lupin breeders as required. Since Australia is the major centre for narrow-leaved lupin breeding, it is unlikely that commercial intellectual property (IP) will be developed.

**Additional information**

Posters:


In addition, two full refereed publications should result from this work.

**Attachment**

Supplementary data on molecular markers.