Improving lupin tolerance to metribuzin and developing germplasm with tolerance to the new herbicides, Balance and Affinity

Summary
Effective weed control is critical to profitable lupin production. The emergence of herbicide resistant weeds and poor metribuzin tolerance of the anthracnose resistant varieties (Tanjil and Wonga) have become major obstacles for many growers. The project addressed the need to breed lupins with 1) improved tolerance to metribuzin by understanding the genetic basis of triazine tolerance; and 2) novel tolerance by mutation to isoxaflutole and carfentrazone-ethyl, with potential to control herbicide-resistant weeds. Tolerant breeding lines were developed and provided to the National Lupin Breeding Program (NLBP), along with efficient screening procedures to facilitate future breeding.

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Conclusions

1. Two Tanjilmutant lines induced from this project showed high levels of tolerance to metribuzin and are likely to have high levels of resistance to anthracnose. They have the potential to be fast-tracked for direct variety release to lupin growers, particularly in the high rainfall Northern Agricultural Region of Western Australia (WA).

2. New germplasm with tolerance to new herbicides of either isoxaflutole or carfentrazone-ethyl was developed through mutation, with potential to be incorporated into the breeding program for development of new varieties to control Group B resistant wild radish.

3. Reliable glasshouse assays for metribuzin tolerance were developed and adopted by the lupin-breeding program to select for tolerance.

4. Improved tolerance to metribuzin in WALAN 2173M which is scheduled for release was an unexpected outcome of this project.

5. Differential responses to metribuzin were characterised among the currently grown lupin varieties including Mandelup.

6. Inheritance of metribuzin tolerance is controlled by one gene with semi-dominance effects with narrow-sense heritability of 78% that allow selection for tolerance at early segregating generations. Tolerant progenies are available for the breeding program to adopt.

7. An implementable molecular marker for metribuzin tolerance should be identifiable and will assist rapid progress of breeding.

Recommendations

Tanjilmutants with enhanced metribuzin tolerance already provided to the Department of Agriculture Western Australia (DAWA) should be fast-tracked into its evaluation program as a possible variety in its own right for the high rainfall Northern Agricultural Region of WA.

Although it is relatively simple to spray large numbers of breeding plots under field conditions with metribuzin to assess for tolerance, the glasshouse test should be applied to early generation material to remove it from the program earlier. Metribuzin application in the field can give misleading results where rainfall subsequent to application can reduce damage.

The lupin industry should consider how to best utilise and protect the levels of metribuzin tolerance available in Mandelup.
WALAN 2173M and potentially even more tolerant lines without accelerating the onset of triazine tolerance in radish and ryegrass.

It is evident that some degree of tolerance to isoxaflutole (Balance) and carfentrazone-ethyl (Affinity) already exists in certain lupin varieties, such as Mandelup, and that higher levels of tolerance are achievable in future lupin varieties through the mutant material being carried forward into the new GRDC project. Research is required to examine Affinity and Balance in mixtures with other herbicides to enhance their radish killing efficacy while maintaining lupin safety. Preliminary work by Dr Harmohinder Dhammu (DAWA) on mixtures of Brodal, Bounty and Eclipse should be encouraged. Affinity and Balance should also be evaluated on the other lupin species, Lupinus albus, L. luteus and L. mutabilis, which at present have fewer herbicide options.

Progress made in the project suggests a similar approach could be taken with the other lupin species and pulse crops, particularly chickpeas where broadleaved weed control is difficult. The longer term industry need is for improved tolerance in pulses to a wider range of herbicide chemistry than is currently in use in Australia.

Outcomes

This research will contribute to the improved profitability of lupin production and the sustainability of grain cropping systems, particularly in the western region. Enhanced lupin germplasm, breeding knowledge and screening tools will lead to improved herbicide tolerance in future lupin varieties. Grower adoption of these varieties will improve their ability to manage annual ryegrass and in particular wild radish populations, as well as doublegee, capeweed and other grass weeds. This will improve the yield of lupins and may reduce total herbicide costs. Industry analysis suggests it will assist in regaining the dramatic decline in area sown to lupin since 1999 in the western region. Regaining lupin production to 1999 levels will underpin wheat production and protein levels on about four million hectares of sandplain soils in WA alone.

This project developed a reliable glasshouse bioassay to differentiate lupin genotypes with tolerance or susceptibility to metribuzin in the seedling stage. The lupin breeding program at DAWA adopted this method in 2003 and screened more than one thousand advanced breeding lines to select elite lines with a high level of metribuzin tolerance. Genetic studies show that metribuzin tolerance in lupins is under single gene control. This knowledge enabled the DAWA program to develop appropriate breeding and selection strategies for rapidly improving and maintaining tolerance to metribuzin. This knowledge was used further by GRDC Project DAW00102 to develop a microsatellite-anchored fragment length polymorphism (MFLP) marker that should further improve the efficiency of breeding. Notably, screening identified that advanced breeding line WALAN 2173 (high protein, anthracnose resistant and good yield) was not a pure line and contained about 25% metribuzin susceptible individuals. With this information, the DAWA breeding program made a metribuzin tolerant reselection (WALAN 2173M) assigned for release in 2006.

A large amount of new germplasm was developed from this project either through crossing between tolerant and susceptible genotypes or from induced mutation. Tolerant progenies (F4) from the study of inheritance for metribuzin tolerance are available for adoption by breeding programs. Germplasm with tolerance to the newer herbicides isoxaflutole and carfentrazone-ethyl was developed through induced mutation with chemical mutagens, ethyl methane sulphonate (EMS) and azide. These mutant lines (30 tolerant to isoxaflutole and 16 to carfentrazone-ethyl) have been selected for tolerance in both M2 and M3. They are being seed-increased in 2005 and will be characterised for tolerance in a new GRDC project. Tanjil mutants with very high tolerance to metribuzin (better than Mandelup) were also identified. These mutants are being fast tracked as potential high anthracnose resistant and metribuzin tolerant varieties.

Achievements/Benefits

Background and Importance:

Weed management has emerged as the major agronomic concern for most lupin growers with herbicide costs typically accounting for 40-50% of the variable cost of lupin production. Annual ryegrass with herbicide resistance to Groups A (fops and dims) and B (sulphonylureas) and wild radish with emerging resistance to Group B and Group C (triazines) are increasing the complexity of management. Doublegee and brome grass are the next most important weeds.

The widespread practice of dry sowing lupins with acceptable results in the 1980s has now compounded the problem through the missed opportunity for a mechanical and chemical pre-emergent knockdown and the often sub-optimal performance from simazine. Weed control has become particularly difficult where lupins have been grown on less suitable
soil types. Under these circumstances the crop grows less vigorously and competes poorly with weeds. In addition, the first generation anthracnose resistant varieties (WongaP and TanjilP) had high sensitivity to the post-emergent herbicide metribuzin#, commonly used alone or in mixes with simazine and Brodal®# herbicides, in the Northern Agricultural Region of WA.

In response to a more difficult weed control scenario, growers have begun to apply a wider spectrum of weed control strategies. However, many growers now sacrifice lupin yield to set up a paddock for the following wheat crop by delaying sowing the lupins to achieve a chemical and mechanical knockdown and by ‘crop-topping’ with a non-selective herbicide to kill weed seed set.

The project aimed to assist weed management by genetically improving the tolerance of lupin varieties to three herbicides. Two strategies were employed within this project. One was to improve lupin tolerance level to a registered herbicide of metribuzin (Lexone®# Group C) through crossing to known sources of high tolerance. The other was to improve lupin tolerance to two herbicides of isoxaflutole# (Balance®#, Group F) and carfentrazone-ethyl# (Affinity®# Group G) through induced chemical mutation. These herbicide groups were chosen because they are not widely used in the wheat-lupin rotation at present and are active against Group B resistant wild radish.

Major achievements:
The project made significant steps towards improving lupin tolerance to herbicides during its three year duration. Details of the achievements and benefits to industry are described as follows.

1. Improving tolerance to a registered herbicide metribuzin
   1.1 Bioassay for metribuzin tolerance adopted by the breeding program

A reliable glasshouse bioassay was developed to differentiate lupin genotypes with tolerance to metribuzin under controlled temperature conditions that can be applied to seedlings (Si et al., 2004). The metribuzin tolerance of the new variety MandelupP# was characterised in comparison with other important varieties. Both Tanjil and Mandelup are important parents in the lupin breeding program. Under bioassay conditions, Mandelup survived metribuzin at the equivalent of 1,600g/ha while Tanjil seedlings reached 100% mortality at 800g/ha. Ranking of tolerance between a large number of advanced breeding lines and varieties was consistent across controlled environment and normal winter field conditions (Si et al., 2006). It was also found that tolerance is independent of early vigour, which differs from the situation with triazine tolerant (TT) canola. Therefore, it is feasible to breed for tolerance with vigorous early growth. The lupin breeding program at the DAWA used this method in 2003 and screened more than one thousand advanced breeding lines to ensure elite breeding lines carried high metribuzin tolerance.

1.2 Improved metribuzin tolerance in WALAN 2173M

An important finding developed from a closer analysis of the data on the breeding line WALAN 2173 in the above study that showed intermediate tolerance to metribuzin across two environments. WALAN 2173 is the highest protein narrow-leafed lupin line thus far identified and is keenly sought by the market. It also has good anthracnose resistance. This line was, in fact, made up of a mixture of individual plants either tolerant (75%) or susceptible (25%). Other genotypes or breeding lines were uniform in their response to metribuzin. This information was used by the breeding program that applied a strong metribuzin selection pressure to enable the re-selection of a tolerant line (WALAN 2173M). This new line has been bulked up and scheduled for release in 2006.

1.3 Tolerance controlled by one gene

The project made several crosses between known susceptible and tolerant parents to investigate the genetic basis and inheritance of metribuzin tolerance from F1, F2 and F3 phenotyping. This knowledge is required to optimise the future breeding approach. Results have shown that inheritance of metribuzin tolerance is controlled by one gene showing semi-dominance in a F2 population of a cross between the tolerant Merrit and the susceptible Tanjil. This type of inheritance has been confirmed in 200 F3 families of the same cross. Evaluation of response to selection at F2 was carried out. Highly tolerant F2 plants remained tolerant at F3 families and the narrow-sense heritability was 78%. This indicates that early generation selection for tolerance is feasible. Inheritance of tolerance between the tolerant Mandelup and Tanjil was also investigated. Results from the F2 population indicated that metribuzin tolerance in Mandelup was controlled by one dominant gene. Segregation in F2 of Gungurru (tolerant) x Danja (susceptible) and performance of F1 showed tolerance in Gungurru over Danja was controlled by one dominant gene.
1.4 Molecular marker for tolerance identified

It was anticipated at the time of writing the project that understanding the genetics of tolerance would lay the foundation for future molecular marker development. However, during the course of this project, there was an opportunity to involve molecular geneticist Dr Yang (DAWA) who had a marker population of a cross between a susceptible (Unicrop) and tolerant line (75A:258). From phenotyping this population, a MFLP marker was identified linked to tolerance but was subsequently found not to work in crosses with Tanjil. Further work is planned to develop markers from F3 families of a Mandelup x Tanjil in 2006.

1.5 Tanjil mutants highly tolerant to metribuzin

This project identified several mutants highly tolerant to metribuzin although this work was not a specified objective of the project. Tanjil populations were mutated in June 2002 using chemical mutagens of either EMS or sodium azide. They were screened at a very high rate of metribuzin at Wongan Hills alongside the other mutant populations specified in the project. Only 180 M2 seedlings survived the spray regime and this represented at most 0.22% seedling survival in the M2 population in the field. The surviving mutants were further tested in subsequent generations for tolerance. Of the original mutants, only eight M4 lines had little leaf damage among 40 seedlings with metribuzin at 600g/ha at 20/12°C in a phytotron while unmutated Tanjil seedlings reached 100% mortality. Two showed better tolerance than the tolerant variety of Mandelup in a dose response study. These two tolerant mutant lines screened positive for a molecular marker specific for anthracnose resistance, the same as Tanjil. It is therefore highly likely that they are tolerant to both metribuzin and anthracnose. These two valuable mutants and the other eight mutants have been passed onto the breeding program to evaluate their yield and grain quality. They have been used as parents in a lupin-crossing program in 2005. A new variety with both good resistance to anthracnose and high tolerance to metribuzin will have a high adoption rate by growers in the high rainfall Northern Agricultural Region of WA.

2. Improving tolerance to new herbicides isoxaflutole and carfentrazone-ethyl

The other strategy of this project was to improve lupin tolerance to herbicides currently not used on lupin, but capable of controlling Group B resistant wild radish. The two herbicides selected, isoxaflutole (Group F) and carfentrazone-ethyl (Group G), have the potential to broaden the chemical base of products useable on lupins. Identification of tolerance was investigated by both screening of diverse lupin germplasm and induced chemical mutation.

2.1 Improving tolerance to carfentrazone-ethyl

2.1.1 Screening germplasm for tolerance

Screening of germplasm for carfentrazone-ethyl tolerance was carried out in a subset of the narrow-leafed collection held by the Genetic Resource Centre of DAWA in South Perth. This subset of wild types, landraces, breeding lines and varieties represents the full diversity of geographical origin and morphological differences available from the full collection of 1,800 accessions in Australia. There was a wide range of susceptibility in response to the herbicide. Two accessions from Spain and Israel showed reasonable tolerance. Some varieties and WALAN 2173M appeared to have good tolerance although they had some leaf damage after exposure to the herbicide.

Differential responses between lupins and canola to carfentrazone-ethyl in a dose study revealed that lupins had a reasonable level of tolerance to carfentrazone-ethyl and canola as pseudo-weed of wild radish had total mortality at three days after spray suggesting the likely effectiveness of carfentrazone-ethyl in controlling wild radish. Tanjil and Mandelup showed no differences in their tolerance. It is highly possible to improve lupin tolerance to carfentrazone-ethyl via induced mutation so that this herbicide can be used in lupins to control Group B resistant wild radish.

2.1.2 Mutants tolerant to carfentrazone-ethyl

Sixteen mutants tolerant to carfentrazone-ethyl were identified and a large quantity of Mandelup, Tanjil and Wodjil seed was treated with either EMS or azide in June 2002. Very large M2 mutant populations (consisting of more than 20,000 individual plants per population) were screened in 2003 in the field at Wongan Hills for tolerance to carfentrazone-ethyl at 400g/ha (eight times the rate recommended on wheat). A total number of 400 M2 tolerant plants (200 of Tanjil origin and 200 of Wodjil origin) were harvested individually in November 2003. These plants were threshed individually and the seed became M3 lines. Sixteen out of 125 M3 mutant lines of Tanjil origin were selected as the most tolerant in 2004 under glasshouse
conditions. They were transplanted individually into a screen house for seed production. Multiplication of seed of these selections was carried out further over summer in field plots in 2004 and again in a screen house in 2005 as one metre rows to provide sufficient seed for further characterisation. Characterisation of these mutants is one component of the new GRDC project and will be performed in 2005.

2.2. Improving tolerance to isoxaflutole

The herbicide isoxaflutole is registered in chickpeas. Improving lupin tolerance to isoxaflutole would not jeopardise its sustainable use in chickpeas because they grow at different soil types. Differential responses among 23 lupin varieties revealed that Mandelup was the most tolerant variety although it was not as good as chickpea Sona.

Mutation was induced in seed of Mandelup and Tanjil in June 2002. Very large M2 mutant populations were screened in the field at Wongan Hills in 2003 for tolerance to isoxaflutole at 300g/ha (3RR). A total number of 250 M2 tolerant plants (200 of Mandelup origin and 50 of Tanjil origin) were harvested individually in November 2003. In 2004, 30 out of 200 M3 mutant lines were selected as most tolerant and were transplanted individually into a screen house for seed production. In fact, a couple of mutant lines from Mandelup showed as good tolerance to isoxaflutole as chickpeas at seedling stage. These selected 30 mutant lines were all of Mandelup origin and will be carried over for further characterisation in the new GRDC project. It is highly likely that a couple of mutants with tolerance equal to chickpea Sona will emerge. Then new varieties will be tolerant to isoxaflutole and the herbicide will be registered for use in lupins to control Group B resistant wild radish.

3. Communications

During the project period, results were communicated to the lupin industry and wider communities on a regular basis as well as to lupin breeders and geneticists. The project team contributed to the Crop Updates 2004 and 2005 and delivered two seminars at the Centre for Legumes in Mediterranean Agriculture (CLIMA) in 2003 and 2005 and a seminar at the Western Australian Herbicide Resistance Initiative (WAHRI) in 2005. Results were presented at a number of workshops concerning issues of pulses organised by CLIMA/DAWA. A poster on genotypic variation in lupins to metribuzin tolerance was presented at the 5th European Conference on Grain Legumes. A scientific paper was published in the Australian Journal of Experimental Agriculture.

Other research

A new proposal was submitted in 2004 to expand research into improving herbicide tolerance of other pulse species including chickpeas, field peas, faba beans and lentils. This proposal seeks to identify germplasm with tolerance to four new herbicides that could be deployed to allow the prevention and/or management of herbicide resistant weed populations in grain cropping systems. This new project is being funded by GRDC for three years with national benefits but is based in WA and collaborates closely with the National Pulse Improvement Program as well as the Narrow-leafed Lupin Improvement Program in WA.

In the new project, testing the germplasm and mutation populations of pulse species with four new herbicides with different chemistries rarely used in current cropping rotations is proposed. The four herbicides include mesotrione® (Group F), isoxaflutole® (Group F), carfentrazone-ethyl® (Group G) and isoxaben® (Group K). These herbicides have the ability to control current herbicide resistant wild radish, but are currently not registered for use in pulses except for isoxaflutole in chickpeas.

A number of issues emerged from the lupin project that raise the need for further investigation through a new PhD project or a separate research project.

1. It was found that lupin tolerance to foliar uptake of post emergent metribuzin® may not necessarily correlate with tolerance to root uptake of simazine® applied pre-seeding even though the chemicals are both triazines®. Tanjil® is a case in point where tolerance to simazine may be better than tolerance to metribuzin. Bioassay of post-emergent herbicides is relatively easier to use than pre-emergent and pre-seeding herbicides. If a correlation of tolerance between post-emergent metribuzin and pre-seeding simazine is established, post-emergent bioassay would be applied to improve tolerance in the breeding program. This would improve breeding efficiency. Such a correlation is also applicable to metribuzin in chickpeas, field peas and lentils where metribuzin is used pre-emergent to control weeds.

2. In dose response studies, Danja is consistently more susceptible to metribuzin than Tanjil. These varieties may have two
different alleles on the same locus, if not two different genes. This may explain why the molecular marker developed via Unicrop (which has a Danja susceptibility phenotype) is not working for Tanjil. This project produced crosses that could be used to test this hypothesis.

3. Tanjil mutants highly tolerant to metribuzin raise questions as to what possible tolerance mechanisms are involved and at what genetic level the mutation was induced. Comparison between Tanjil and mutants would answer these questions. This knowledge could have practical implications as chemical induced mutation is applied into the new project funded by GRDC.

**Intellectual property summary**

Intellectual Property (IP) developed from this project is either knowledge of the herbicide tolerance profiles of existing lupin germplasm or new germplasm (through mutagenesis or hybridisation). Knowledge of tolerance profiles of existing varieties should continue to be freely provided to all interested sectors of the grains and chemical industries. This may encourage new herbicide registrations or label modifications. Germplasm derived by this project is owned jointly by CLIMA and GRDC. It would be expected that this germplasm would be commercialised through the DAWA Lupin Breeding Program or as otherwise mutually agreed. There is limited opportunity to capitalise on this germplasm internationally, but this can be explored in Europe, South Africa and Chile.

**Additional information**


