

# NARROW-LEAFED LUPIN BREEDING IN AUSTRALIA - WHERE TO FROM HERE?

B.J. Buirchell

Department of Agriculture and Food, Western Australia, Locked Bag 4, Bentley Delivery Centre 6983 Western Australia

email: bbuirchell@agric.wa.gov.au

## ABSTRACT

**Over the last 30 years the lupin industry in Australia has come from nothing to an essential part of the agricultural system. Breeding has contributed to this rise through increasing yield, overcoming major diseases and contributing to the agronomic success of the crop. The lupin industry now faces new challenges within the farming system and in the market place. On farm challenges such as herbicide resistant weeds, new diseases, low economic return, inconsistent yields, low yields, harvest height, drought and water logging. Market challenges include the lack of differentiation from other sources of protein, low protein levels, allergenicity, lack of penetration into human consumption markets, etc. Questions addressed in the current paper include: how is breeding going to contribute to the future success of the lupin industry? What solutions can be found through breeding?**

## KEYWORDS

*L. angustifolius*, breeding, yield, disease resistance, quality

## INTRODUCTION

The Australian breeding program commenced in the 1960s (Gladstones, 1994), and since then has made steady increases in yield (1.5-2.5% per annum) with current cultivars now yielding double that of the first released variety, Unicrop. There have been a number of diseases, which have put pressure on the lupin industry including phomopsis stem blight (*Diaporthe toxica* Williamson) which produces a toxin causing lupinosis in sheep (Gardiner, 1975). Early efforts to breed resistance to phomopsis were successful (Allen *et al.* 1985) and this continues to be a breeding objective. Anthracnose (*Colletotricum lupini* (Bondar) Nirenberg, Feiler and Hagedorn) appeared in commercial crops in Australia in 1996 and resistance was identified rapidly and has since been routinely incorporated into all varieties. A molecular marker was developed (Yang *et al.* 2004) and is used routinely to achieve this breeding objective. Damage caused by aphid colonisation in the eastern wheatbelt and the transmission of Cucumber Mosaic Virus (CMV) by aphids in the high rainfall regions has resulted in efforts to breed resistance to both these biotic problems. There

are now varieties that have good resistance to aphids and sources of low levels of CMV seed transmission have been introgressed into the breeding program (Jones and Cowling, 1995). Screening and elimination of high CMV transmitting breeding lines is now routine.

Lupins continue to be used widely in the stock feeding industry, both domestically and internationally, and the breeding program has maintained low alkaloid levels and protein above 30% to ensure that lupins fit into the market requirements in this area. The price is dependent upon the soybean meal price (approximately 70%) or the domestic market demands, especially in drought years. The long term average price of lupins has only slowly increased and has not kept up with increases in costs of production. The changing rotations, low prices for lupin grain and several years of drought have resulted in a large reduction in the production area. Most of the reduction has occurred in the low rainfall regions and on soils not suitable for lupins where the economics of growing lupins was marginal.

There are many challenges still facing the industry and ones that need to be overcome before the industry could be considered a mature crop like soybeans. It is with this background in mind that I would like to present some of the challenges and future directions that face lupins and to highlight where breeding will play a role.

## THE YIELD PROGRESS CHALLENGE

The progress in breeding for higher yields in lupins, while impressive in comparison to other crops, has resulted in a narrowing of the germplasm base. In the Australian breeding program most of the genetic gain can be traced back to crosses made in the late 1970s between cv. Illyarrie and four wild types from Spain and one from Morocco; plus crosses to two accessions from Italy and one from Israel. All the modern cultivars have been developed from inter crossing the best lines that have come out of those wild crosses. In other crop species, like rice, a similar story is told where the domestication process and modern breeding has narrowed the genetic base of the breeding programs (Tanksley and McCouch, 1997)

There are three sources of new germplasm that can be introduced to a breeding program:

- wild types or exotic germplasm from genebanks;

- advanced breeding lines and varieties from other breeding programs;
- old varieties within the same breeding program.

#### Alleles from exotic germplasm

Tanskey and McCouch (1997) highlighted the usefulness of the genetic potential from wild germplasm in terms of gains in yield and in other traits. They hypothesised that all breeding programs could gain significantly by introducing wild alleles and to explore alleles that may have been lost due to the selection process and genetic drift (Kovach and McCouch, 2008).

While only eight wild accessions have appeared in the pedigrees of modern varieties from the Australian lupin breeding program, it does not suggest a lack of wide crossing with other wild material. A search of the parents used in the crossing program between 1982 and 1998 showed that 112 wild types were used. Only two of those accessions appear in lines that are now in advanced stages of testing. Why has the introgression of wild alleles into the lupin breeding program failed?

There are two main reasons for this, firstly, only primary crosses (elite x wild parents) were made with subsequent selection for all seven-domestication genes before the remaining lines were tested for yield. In doing this many of the 'good alleles' accumulated in the advanced domesticated material were compromised by wild alleles thus no high yielding lines were ever produced. Secondly, the majority of crosses were made with Greek accessions which were difficult to keep clean of CMV and therefore eventually abandoned. Recently it was shown that the Greek accessions, from a core collection of lupins, have very high rates of CMV seed transmission (average 23.3%, range 4.7-59.5%) compared to accessions from Spain (average 12.7%, range 2.3-35.6%), Portugal (average 11.8%, range 2.5-33.1%) and Morocco (average 9% , range 0-22.4%).

Since 2001 the strategy has been to introgress wild material into the core breeding program through a Bc2 strategy so that we can sample the wild alleles in combination with the 'good alleles' already assembled through the breeding program. The paper by Cowling (2008 this volume) covers the theory and complexity of the integration strategy. The Greek material will especially benefit from this approach as we can eliminate the high CMV seed transmission issues associated with the wild parents. The 2001 crosses entered yield trials in 2008.

The Australian Lupin Collection (ALC) list 1252 accessions under the wild type/landrace category so there is still over 99% of the genetic variation available for exploration.

#### Alleles from other breeding programs

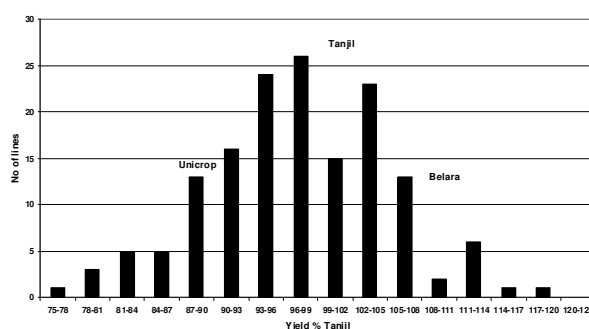
The Australian breeding program has not exploited breeding material from other programs for a number of reasons. Firstly, not all the European material has both non-shattering genes (*tardus* and *lentus*) so those lines

have been difficult to evaluate for yield under our growing conditions due to harvest losses. Secondly, given that they have been developed for a different environment and therefore have accumulated a set of 'yield and adaptation alleles' suited to that environment, primary crosses with Australian material is likely to be disruptive to the 'yield and adaptation alleles' accumulated here. As a result, similar to the wide crosses with wild germplasm, primary crosses tend to deliver unadapted germplasm, and therefore a different backcrossing strategy is required to progress the material.

Other breeding programs have probably tapped into a different set of exotic germplasm and it would be interesting to be able to identify which part of the exotic germplasm each of the international breeding programs have used. Certainly this would be an easier way of sourcing wild alleles rather than going back to the wild types themselves.

#### Alleles left behind in old varieties

A third source of alleles is older varieties within the breeding program. As selection is made within a cross genes or alleles or gene combinations are left behind which if combined with the accumulated 'yield and adaptation alleles' in modern varieties may add to the advancement in yield that is needed in the future. We have tested this with a cross between Unicrop (released in 1972) and Tanjil (released in 1998) and found transgressive segregation for yield within the recombinant inbred lines (Fig. 1).



**Fig. 1.** Frequency of progeny with range of yield (%) Tanjil) for Recombinant Inbred Lines from the Tanjil/Unicrop cross and control varieties grown at three sites (Merredin, Badgingarra, Wongan Hills) in Western Australia in 2006.

We were rather surprised to find progeny significantly higher yielding than Tanjil, some of which were as productive as Belara. While the yields were not competitive with Mandelup it does demonstrate that new gene combinations can be gained from older varieties. Further crosses will be made using the backcrossing strategy to recapture any alleles from older varieties into the breeding program.

## DISEASE CHALLENGES

### Anthracnose resistance in pods

Resistance to Anthracnose is a priority in the breeding program and there is a range of resistance to suit the various growing environments within Australia. Cvs Tanjil and Wonga have the highest level of resistance and this gives good protection to the plant stems, petioles and leaves, however the resistance does not protect the flowering tissue and the early pods. As a result yield losses can still occur when growing the resistant varieties. Rain splash and wind spreads Anthracnose and if a favourable weather event occurs when flowers are exposed then they can be lost. Buds further up the inflorescence will not be affected and may go onto produce pods if further disease-inducing weather events are absent. This also applies to the flowers on the lateral branches and it is not unusual for Tanjil, for instance, to have most of its yield on the lateral branches due to the mainstem pods being affected in the manner outlined above.

Finding resistance in flowering tissue would have a two fold benefit. Firstly, allowing pods to be set and thus protecting yield and secondly reducing seed transmission of Anthracnose which reduces the incidence of disease in the following crop. In Australia the major infection times are late winter and early spring where the growing plants themselves and the early orders of flower are most vulnerable. In Europe Anthracnose infections are observed later in the growing cycle and it is the late flowers and pods that are most vulnerable.

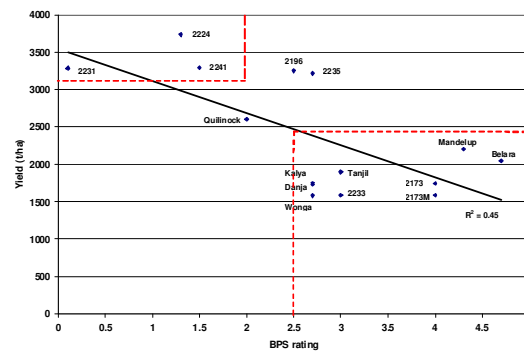
Screening for resistance to Anthracnose on the flower tissue has been difficult. In our disease nursery we have assessed all the material we grow for pod set at the end of the year and have identified several lines that seemed to be promising in terms of pod retention. However, re-assessment in the following year yielded variable results, and suggest that the apparent resistance was disease escape associated with late phenology. Therefore we are yet to identify any material with robust resistance. Nevertheless, Ruge-Wehling *et al.* (2008) reported that they have a line that shows good resistance on pods.

### Black pod syndrome

Black Pod Syndrome (BPS) has been known to occur for a number of years on the south coastal regions of Western Australia. In south coastal regions yield potential for lupins can be high (4 t/ha). Seymour (1994) observed that in some lupin crops the number of pods present close to maturity indicated very high potential yields while actual harvested yields were often disappointing (2 t/ha). The yield loss is associated with poor development of seeds within pods leading to a degeneration of those pods. The pods become blackened, flattened and/or twisted and contain only small seeds.

It is estimated that 40% of the yield potential can be lost. The symptoms are very similar to that of Bean yellow Mosaic Virus infection and maybe the result of late infection with BYMV.

In trials at Esperance and Katanning, in the south of Western Australia rating for BPS were made and there was a reasonable correlation between the scores at the two sites. When yield at Esperance was graphed against the BPS score there was a strong inverse relationship between yield and BPS (Fig. 2).



**Fig. 2.** Yield (tonnes/hectare) and black pod syndrome rating at Esperance in 2005. BPS score 0 = no black pods, 5 = high percentage of black pods.

We have been looking at this syndrome over the last three years to try to ascertain its origin or causal agent but we still have not proven beyond doubt that it is BYMV rather than something else. With such a dramatic effect on yield it is important to identify the cause and to be able to screen for resistance. We do know that there are lines less affected by BPS and cv. Jenabillup, released in 2007, is one of the best lines. We also know that BPS has a genetic basis as nearly all the lines with resistance to BPS have Quilnock as one of the parents. If it does turn out to be BYMV then we have identified a source of moderate resistance that will allow expansion of the lupin industry into the southern areas where BYMV is harboured in pasture species.

### Exotic diseases like lupin rust and fusarium wilt

Australia is a very isolated country with strict quarantine barriers to protect its agricultural industry from exotic pests and diseases. However it is inevitable that exotic diseases and pests will eventually land on our shores, as did Anthracnose. Two exotic diseases of lupins that Australia does not have are Fusarium wilt and lupin rust. The lupin industry in Europe was nearly completely wiped out by Fusarium wilt and it is only since resistance was found and introgressed into varieties that the industry is returning. While Australia does not have this disease we are fortunate in being able to source resistance from European lines and some of the resistance genes, although not characterised or identified, are already in the breeding program through crossing with European material. It would be

advantageous to the future of the breeding program if molecular markers were available for these genes, especially as there are races of fusarium wilt that require different genes for resistance.

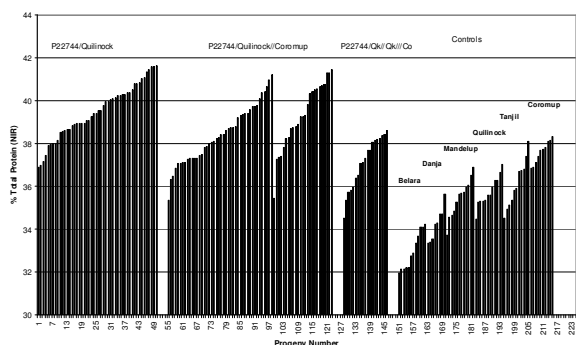
A similar case could be made for rust resistant genes although as yet there has not been any record of yield loss due to rust infections either in Europe or elsewhere. Rust in winter grown *L. albus* has been a problem in the United Kingdom but has declined as crop areas diminished. Sweetingham *et al.* (2006) reported that Australian cultivars Jindalee and Tallerack were resistant to lupin rust (*Uromyces lupinicolus*) found on *L. albus* in the United Kingdom. As a consequence we have sources of resistance if required.

## PRICE SENSITIVE CHALLENGES

### Protein content

The cost of production of lupins continues to rise much faster than the farm gate price for the grain. If lupins are to attract better prices then they either need to be differentiated from other grain legumes based on their 'special' attributes or fall into markets that are 'protein sensitive'. There are industries, which require higher protein feed, like aquaculture, and kernel protein content of NLL can fit into this market if the whole seed protein content can be increased above 35%. Gladstones and Crosbie (1978) have reported the natural variation in protein content in wild accessions as just under 32%, dry matter basis (db), to almost 43%. Currently the breeding program maintains protein content above 32% db, which leaves considerable scope for increasing protein levels.

The breeding program started breeding for higher protein content in 2001 with some initial crosses of wild accessions with current varieties. The F2 progeny showed an even distribution of protein content indicating that protein is quantitatively inherited. We now have a composite set of material including lines selected from the initial crosses and those selected from Bc1 and Bc2. All this material displays a range of protein content within crosses and the protein content reduces as backcrossing increases, as expected with a quantitative character (Fig. 3).



**Fig. 3.** Total protein content (%), as measured by NIR, of lines derived from primary cross with P22744/Quilinoack and backcrosses to Quilinoack and Coromup. Control values of varieties grown in the same trial are included for comparison.

There are some lines that now have protein levels considerably higher than Belara (32% – the lowest protein content) and even higher than Coromup (35% – the highest protein content). The inverse relationship between yield and protein content, which is apparent in other legumes, has not been demonstrated in lupins. This set of lines, with a larger range of protein content than previous lines, may assist in demonstrating if that relationship exists in lupins.

So far the breeding for higher protein content have been encouraging and we have already released Coromup in an attempt to stimulate interest in supplying high protein lupins into the aquaculture market. Further increases in protein content will add to the impetus.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge generous research funding support from the Australian Grains Research and Development Corporation (GRDC), the Department of Agriculture and Food, Western Australia (DAFWA).

## REFERENCES

- Allen, J.G., McR. Wood, K.P. Croker, W.A. Cowling and D.N. Sawkins. 1985. The prevention of lupinosis in sheep. *IN* Seawright, A.A. *et al.* (ed) Plant Toxicology. Qld. Poisonous Plants Committee, Queensland Dpt. Primary Industries, Animal Res Inst. Yeerongpilly, Queensland, Australia.
- Berger J.D., J. Speijers, D. Shrestha, C. Ludwig, J.A. Palta and B. Buirchell. 2008. Ecogeography of the old world lupins: 2) Characterizing the Habitat Range. *IN* 'Proceedings of the 12th International Lupin Conference, Lupins for Health and Wealth'. Fremantle, Western Australia. (Eds J.A. Palta and J.D. Berger).
- Cowling, W.A. 2008. Efficient introgression of valuable new alleles from lupin gene banks into elite lupin breeding programs. *IN* 'Proceedings of the 12th International Lupin Conference, Lupins for Health and Wealth'. Fremantle, Western Australia. (Eds J.A. Palta and J.D. Berger).
- Gardiner, M.R. 1975. Lupinosis. *Western Australian Journal of Agriculture*, Fourth series 16: 2-6.
- Gladstones, J.S. 1994. An historical review of lupins in Australia. pp. 1-38 *IN* '1st Lupin Technical Symposium'. Perth, WA (Department of Agriculture).
- Gladstones, J.S. and G.B. Crosbie. 1978. Lupin wild types introduced into Western Australia to 1973. *Western Australian Dept of Agriculture*, Bulletin 43.
- Jones, R.A.C. and W.A. Cowling. 1995. Resistance to seed transmission of cucumber mosaic virus in narrow-leaved lupins (*Lupinus angustifolius*). *Australian Journal Agricultural Research* 46; 1339-1352.
- Kovach, M.J. and S.R. McCouch. 2008. Leveraging natural diversity: back through the bottleneck. *Current Opinions in Plant Biology* 11: 193-200.
- Ruge-Wehling, B., R. Dietrick, C. Thiele, F. Eickmeyer and P. Wehling. 2008. Resistance to Anthracnose in narrow-leaved lupin (*L. angustifolius* L.): sources of resistance and development of molecular markers. *IN* 'Proceedings of the 12th International Lupin Conference, Lupins for Health and Wealth'. Fremantle, Western Australia (Eds J.A. Palta and J.D. Berger).

- Seymour, M. 1994. Lupin management in high rainfall wheat/lupin farming systems. GRDC Final Report on project DAW15W.
- Sweetingham, M.W., H. Yang, B.J. Buirchell, G. Shea and I. Shield. 2006. Resistance to rust in narrow-leafed lupin and development of molecular markers. pp. 14-16. *IN* E. van Santen and G.H. Hill (eds.). Proceedings of the 11th International Lupin Conference, Mexico, where old and new world lupins meet'. Guadalajara, Lalisco, Mexico.
- Tanskey, S.D. and S.R. McCouch. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277: 1063-1066.
- Yang, H., J.G. Boersma, M. You, B.J. Buirchell and M.W. Sweetingham. 2004. Development and implementation of a sequence-specific PCR marker linked to a gene conferring resistance to Anthracnose disease in narrow-leafed lupin (*Lupinus angustifolius* L.) *Molecular Breeding* 14: 145-151.