

# CROP IMPROVEMENT IN *LUPINUS MUTABILIS* FOR AUSTRALIAN AGRICULTURE – PROGRESS AND PROSPECTS

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## ABSTRACT

*Lupinus mutabilis* (Andean or Pearl Lupin) originates from South America where it has been grown for centuries by the indigenous peoples of Ecuador, Peru and Bolivia and is consumed as a traditional food after de-bittering. The species suits annual cropping systems and the seed has the highest whole seed protein and oil of all the domesticated lupins. It therefore provides good prospects as a high value feed and food legume grain. *L. mutabilis* has been domesticated and bred through South American and European programs to a level where low to moderate yields have been achieved, highlighting a number of limitations. In a crop development and pre-breeding program for *L. mutabilis* we have assessed the species for agronomic, adaptation and disease traits. Low alkaloid levels (0.001-0.006%) and good seed protein and oil contents (average 43%, 15% respectively) have been combined with early flowering and maturity. Current breeding lines generally have a lower harvest index and yield than current cultivars of *L. angustifolius* and *L. albus*. *L. mutabilis* nodulates well with standard rhizobia and the crop appears to be suited to better soil types, similar to those for *L. albus*. Breeding lines show moderate to high aphid resistance based on field and glasshouse results. It is highly susceptible to brown spot foliar disease caused by *Pleiochaeta setosa*, but with some genetic variation and moderately susceptible to Anthracnose. Good tolerance to post-emergent metribuzin herbicide has been demonstrated. This paper discusses past and present progress in germplasm evaluation and crop improvement of *L. mutabilis* and outlines future breeding objectives.

## KEYWORDS

Andean lupin, pearl lupin, crop domestication, protein

## INTRODUCTION

*Lupinus mutabilis* Sweet (Andean lupin, pearl lupin) is a New World lupin species cultivated as an indigenous food crop throughout the Andes region of South America. Domesticated independently from Old World crop lupins in the Mediterranean, *L. mutabilis*

populations exist in semi-domesticated form, with non-shattering pods, large, permeable seed, reduced seed coat pigmentation and a more or less annual life cycle. In phylogenetic studies *L. mutabilis* falls within an Andean clade of Western New World species having  $2n = 48$  (Ainouche and Bayer, 1999; Ainouche *et al.* 2004; Hughes and Eastwood, 2006). It is included with the Old World species *L. albus*, *L. angustifolius*, *L. luteus* as among those achieving crop status from among the 200+ lupin species that exist with the majority distributed in the New World (Dunn and Gillett, 1966; Gladstones, 1974). The phenotypic plasticity, presence of both annuals and perennial species in North and South America, ability to adapt to diverse environments and considerable outcrossing has made taxonomic delimitation very difficult, and many so-called taxa are often no more than ecotypes (Planchuelo, 1994). Dunn (1984) demonstrated the heterogeneity of populations noting that for example, the *L. mexicanus*-*L. exaltatus* complex in Mexico contains both annual and perennial species, which are morphologically indistinguishable and interfertile.

Because *L. mutabilis* stands out as having the highest protein and oil content in whole seed compared with the other crop species, it has been the focus of breeding efforts in South America, Europe and more recently in Australia (von Baer and Gross, 1977; Williams *et al.* 1984; Caligari *et al.* 2000; Clements *et al.* 2005a; Sweetingham *et al.* 2005, 2006). This paper summarises the breeding and development of the crop in Western Australia, with reference to research done elsewhere over the past several decades.

## MATERIALS AND METHODS

Crosses and early generation advancement was carried out in conditions suited to lupin in glasshouses and screenhouses at the University of Western Australia with temperatures averaging 22°C day and 13°C night and complete fertiliser applied at approximately 200 g/ha twice during the growing season. Field trials at Mt Barker (rainfall during growing season of 580 mm, 2007) were carried out on sandy loam soils, receiving fertilisers at rates recommended as district practice for lupins, with adequate nodulation by rhizobia. Seed chemical analyses were according to Petterson *et al.*

(1997) and disease incidence using methods described by Yang *et al.* (1996) and Thomas and Sweetingham (2003).

## RESULTS AND DISCUSSION

### DEVELOPMENT OF LOW ALKALOID, EARLY FLOWERING BREEDING LINES IN WESTERN AUSTRALIA AND IDENTIFICATION OF MALE STERILITY

After having made selections in *L. angustifolius* and *L. luteus* in 1928-29 from 1.5 million plants, low alkaloid genotypes of *L. mutabilis* were selected during the 1930s by Von Sengbusch and other workers (Williams *et al.* 1984). Brucher (1971) and Pakendorf (1974) also reported low alkaloid *L. mutabilis* but in all cases lines were apparently not developed or preserved. Through the use of mutation breeding to generate the mutal gene followed by successive selection, lines were developed with total alkaloids of 0.2-0.3% cf. 2.2% in bitter parents (Williams *et al.* 1984). It was suggested that the reselection process recombined additional minor alleles at several loci to lower alkaloid levels. Von Baer and Gross (1977) began with a few seeds from plants identified as natural mutants by Oscar Blanco (Cusco, Peru; Blanco 1974; Blanco 1982), and reselected these over several generations to produce lines with ca 0.4% alkaloids. This process was taken further to produce cv. Inti with an extremely low alkaloid level of 0.0075% (Gross *et al.* 1988). Inheritance of the low alkaloid trait in cv. Inti is recessive but polygenic in nature such that approximately 12% F<sub>2</sub> plants give low alkaloid seeds (von Baer and von Baer, 1988). The major alkaloids in *L. mutabilis* have been reported to be lupanine, sparteine, 4- and 13-hydroxylupanine (Williams *et al.* 1984; Wink *et al.* 1995).

Preliminary crossing in Western Australia during 1999-2002 using standard methods used in the *L. angustifolius* breeding program (J.S. Gladstones, unpublished) provided very few F<sub>1</sub> seeds. Experiments conducted using delayed pollination proved successful and routine crossing to produce high progeny seed numbers was then possible (Clements *et al.* unpublished). A first set of successful crosses combined low alkaloid from cv. Inti (donated by E. von Baer) and P28027 (donated from Ecuador, of Peruvian origin) with early and mid flowering bitter accessions from the UK (donated by W. Williams), mid flowering accessions from South Africa (ex. K.W. Pakendorf) and Ecuador, and a mid-late flowering horticultural accession. In crosses between early flowering and very late flowering lines such as P26961 and cv. Inti, we found early flowering to be recessive. Very late flowering lines such as cv. Inti were found to be responsive to vernalisation, mid-flowering lines such as P27808 were partially responsive while the early flowering P26961 did not respond. *Lupinus mutabilis* has been reported to have a day neutral photoperiod response (Hackbarth, 1936) and generally responsive to vernalisation (Hardy *et al.* 1998). Crosses between cv. Inti (low alkaloid) and P26961 produced F<sub>1</sub> populations

that were entirely bitter and F<sub>2</sub> populations that segregated in proportions consistent with a single recessive gene model for low alkaloid, although proportions of low alkaloid plants have been found to be consistently slightly higher than 1:4 indicating that additional minor alleles are likely to be present as proposed by Williams *et al.* (1984) and von Baer and Ibanez, 1986). By 2004 we had developed a large number of F<sub>3</sub>-derived F<sub>4</sub> lines (referred to as 'Stream 1') using a pedigree method of breeding that combined relatively early flowering with low alkaloid levels, both traits segregating independently. Further crossing cycles ('Streams 2' and above) have involved single plant selection from F<sub>4</sub> bulks. Our work has indicated levels of heterosis of 30% in *L. mutabilis* F<sub>1</sub> populations above best parent mean yield based on small plot trials in screenhouses. Hardy and Huyghe (1997) reported 46% mean heterosis for seed yield and this phenomenon could be exploited within breeding programs.

In 2003, prior to the development of improved crossing methods in *L. mutabilis*, several plants were observed that were not setting pods in proximal positions on main stem racemes. Closer inspection of flowers revealed these to contain pale green anthers resulting in male sterility. These sterile flowers were hand-pollinated using a fully fertile pure line (JC243). The F<sub>1</sub> seed from one of these rescued plants (27033MS4 F<sub>1</sub>) and the subsequent F<sub>2</sub> and BC progenies showed that the sterility was monogenic recessive and that it was cytoplasmically inherited. A range of restorer and maintainer accessions have been identified. Two additional plants were identified in a mutation population of P25954 that were sterile and these sources appear to be single recessive nuclear male sterile lines. Male sterility was used in crossing during 2003-2004 because it greatly improved F<sub>1</sub> seed set. Currently, crossing is now done both with and without male sterility with good success.

### GERMPLASM EVALUATION FOR PLANT AND SEED TRAITS

In the Australian Lupin Collection (ALC) a useful range of plant characters are available in *L. mutabilis* germplasm consisting of 'wild' or landrace accessions (from Peru, Bolivia, Ecuador and Argentina) and breeding lines, selections and genotypes from a mutation program of Pakendorf (1974). A full range of days to flowering and seed size has been found with potentially exploitable variation for other characters such as seed coat and pod wall proportion and seeds per pod (Table 1). Seed shape variability has been observed among different accessions that range from large and almost spherical to round-oval or tear-drop shaped and also flattish.

Flower colours include purple, purple/yellow, purple/white/yellow, blue/yellow, blue/white/yellow, white/yellow, pink/white/yellow, pink and white. Seed colours vary from black, marbled, white with black hilum, crescent or eyebrow, and fully white. Seed coat proportion in *L. mutabilis* is very low relative to other

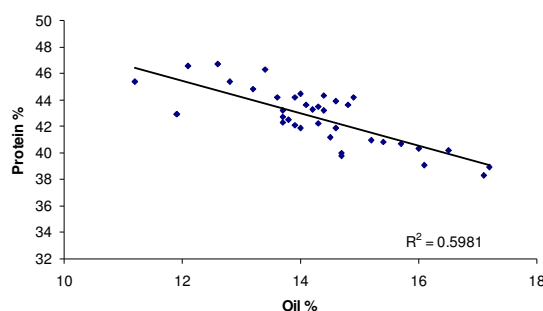
lupin species and is approaching values reported for field pea and soybean (Clements *et al.* 2005b). This makes *L. mutabilis* highly suitable for dehulling or for use as whole seed with relatively low proportions of seed coat fibre. Levels of non-starch polysaccharides (NSPs) found mainly in kernels are around 9% (Brillouet and Riochet, 1983). In contrast, the NSPs contributed by thick cell walls in *L. angustifolius* kernels form approximately 23% of seed weight. Although this NSP is a valuable dietary fibre, with cholesterol-lowering properties (Evans, 1994), it can reduce digestible energy in monogastric diets.

**Table 1.** Variation for plant and seed traits in *L. mutabilis* wild and landrace accessions, breeding lines and mutants in the Australian Lupin Collection.

Traits	n	Min.	Mean	Max.
Wild types and landraces				
Days to flowering	33	65	88	114
Seed weight (mg)	31	68	186	255
Seed coat (%)	36	10.6	12.9	16
Pod wall (%)	17	35.8	45.1	52.6
Seeds per pod	16	2.7	4.4	5.5
Breeding lines, selections, mutants				
Days to flowering	63	65	89	114
Seed weight (mg)	62	103	157	246
Seed coat (%)	63	11.3	12.6	15.6
Pod wall (%)	9	39.4	44.7	53.1
Seeds per pod	9	3.1	5.1	6.8

Seed protein and oil contents in germplasm collections of *L. mutabilis* can vary widely. Results presented in Table 2 show ranges from 37.9 to 46.7% for breeding lines and germplasm with the highest protein found in cv. Inti. Values ranging from 35-50% for protein (mean of 42%) and from 13-24% (mean of 20%) for oil have been demonstrated elsewhere (Römer and Jahn-Deesbach, 1986; Caligari *et al.* 2000). It has been reported that the crop produces more protein but less oil in European conditions (summer grown) relative to South American conditions (September to May growing season (Caligari *et al.* 2000). Wild genotypes were noted as having the highest protein contents by Mujica *et al.* 2004. The protein profile of *L. mutabilis* shows a similar level of sulphur amino acids to that of *L. angustifolius* and soybean while lysine is higher in *L. mutabilis* than *L. angustifolius* and similar to that in soybean. Oil percentages in breeding lines and germplasm have ranged from 11.9 to 17.2% (Table 1). The fatty acid profile has high polyunsaturated fatty acids, with an average profile of oleic (50%), linoleic (26%), palmitic (11%), stearic (8%) and linolenic (4%) with no significant levels of erucic oil. These results were comparable to those found by Williams (1979). We found a negative correlation between protein and oil concentrations in seed ( $r = -0.77$ ,  $r^2 = 0.60$ , Fig. 1),

similar to Perez *et al.* (1984) ( $r = -0.71$  over 217 ecotypes). Correlations between seed yield, oil and protein were much lower:  $r = -0.50$  and  $0.41$ , respectively. A much lower correlation between oil and protein was found in a group of breeding lines indicating that it was possible to combine high levels of both components through selection (Römer and Jahn-Deesbach, 1988). We have found *L. mutabilis* to have low Cd accumulation in seed, in contrast to, for example, *L. luteus*.



**Fig. 1.** Relationship between whole seed protein and oil percentage in *L. mutabilis* low alkaloid breeding lines ( $n = 29$ ) and parental accessions ( $n = 7$ ).

#### DISEASE AND PEST STUDIES

Aphid colonisation of these low alkaloid lines indicates that they were all less susceptible than the yellow lupin cv. Wodjil. Several lines (e.g. P26961) are less susceptible to Anthracnose than *L. albus* cv. Kiev Mutant, but well short of the resistant of *L. angustifolius* cv. Tanjil. Variation exists for brown spot resistance (e.g. P27809 being one of the best, Table 2), but all lines are relatively susceptible. Glasshouse screening has shown the best pearl lupin breeding lines were as tolerant to metribuzin as the best cultivars of narrow-leaved lupin.

#### YIELD AND AGRONOMIC TRIALS

In a field trial in a medium-high rainfall zone (Mt Barker, Western Australia) looking at more detailed agronomic and yield related traits, *L. mutabilis* was found to have generally higher early and final biomass compared to *L. angustifolius* cv. Mandelup but to produce fewer main stem leaves, lower harvest index and most lines producing lower yield (Table 3). Few lines flowered earlier than cv. Mandelup. Traits most highly correlated with seed yield per plant were final plant above ground biomass, number of first order branches, number of first and second order branch pods and total number of pods. The data indicated that final plant height in *L. mutabilis* was associated with total number of pods per plant. There was a low correlation between seed yield and flowering time and the highest yielding *L. mutabilis* accession (P28029, yielding 50% more than cv. Mandelup) flowering 17 days later than cv. Mandelup. Additional field trials have shown Stream 1 breeding lines to yield consistently lower than both *L. angustifolius* and *L. albus* cultivars, typically by 40 and 25% respectively. Some limiting agronomic factors identified in *L. mutabilis* in Europe have been

shown to include low leaf area development early in the growth cycle, a lower conversion of PAR into dry matter and a short duration where LAI was greater than 2.5 resulting in lower yield potential (Hardy *et al.* 1997; Hardy *et al.* 1999). Limited studies in Western Australia indicate that little may be gained for *L. mutabilis* by sowing in wide row spacings. For an environment that was not water limited, it was demonstrated that significantly higher yields could be obtained for some species such as *L. luteus* (Pootalong) and *L. mutabilis* (P26961) from narrow rows (20 cm) compared to wide rows (60 cm; Smith *et al.* 2006).

#### FUTURE WORK

We have found germplasm that overcomes previously reported problems of low biomass production but breeding efforts need to focus on crossing among the highest yielding lines identified to increase pod production, seed yield and harvest index and to attempt to incorporate the best material identified for tolerance to diseases, pests and herbicides. Increasing leaf node number and early leaf area development is recommended. We need to understand the adaptation, genotype by environment interactions and genetic variation available in wider germplasm collections. Characterising collection site climate data could identify collections that may better suit particular growing environments in Australia. It appears that *L. mutabilis* is less able to tolerate moisture stress and therefore, water use studies are required to ascertain the need for drought tolerance. We need to understand the nutritional requirements of *L. mutabilis* in relation to other crop lupin species. There is also the potential to use many of the approximately 80 other closely related lupin species to *L. mutabilis* from the Andean region and Western North American regions, most of which are known or assumed to have the same chromosome number as *L. mutabilis* and be crossable (Clements *et al.* 2008, this conference). Our work has indicated that heterosis exists in *L. mutabilis* F1 populations. Because of variable outcrossing rates both within *L. mutabilis* and among related New World species, it may be possible to exploit this for yield improvement, either through inherent levels of heterozygosity within a breeding line, through release of synthetic lines or through the production of hybrid lines via male sterility. Hypothetically, hybrid lines in *L. mutabilis* may benefit from the introduction of smaller seed size which is available within germplasm of the species, or from interspecific crossing with species such as *L. mexicanus* (*L. hartwegii*). This would allow a higher reproductive ratio which allows a higher F1 seed production for commercial purposes. Further studies will be carried out to determine the potential of pearl lupin for inclusion in fish and pork feed rations. The current research has so far demonstrated that *L. mutabilis* has clear potential as a high value legume crop for production under winter - spring cropping systems in Australia.

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**Table 2.** Agronomic, disease and quality traits for 13 advanced, low alkaloid *L. mutabilis* breeding lines and control cultivars grown at the University Field Station, Shenton Park in 2005-2006. Data for seed quality were for screenhouse-grown seed (2004-2005) and disease ratings were derived from South Perth and Medina plots for brown spot and Anthracnose ratings respectively. Other species identified as follows: \**L. albus*, \*\**L. angustifolius*, \*\*\**L. luteus*.

Genotype	Height (cm)	Flowering (DAS)	Yield (g/m <sup>2</sup> )	Brown spot severity	Anthracnose severity	Oil (%)
ID13	91	85	301	4.0	3.4	14.9
ID18	101	97	359	4.4	4.0	14.6
ID32	105	96	359	3.4	3.6	12.8
ID33	113	98	431	3.2	3.2	13.2
JC243	85	79	290	4.3	3.6	11.9
P27808	100	95	294	3.0	4.4	13.7
P28725	115	97	296	3.3		11.9
Inti	118	120	130	2.7		12.6
P26961	79	81	285	4.2	2.4	17.2
P27809	100	121	135	1.7	3.9	
Andromeda*	98	84	642	1.3	3.8	9.0
Tanjil**	93	81	668	1.4	1.3	6.0
Wodjil***	80	82	397	1.7	-	6.0
LSD(0.05)	9	4	45	0.7	0.4	1.5

Genotype	Protein (%)	Tot. alkaloids (%)	S (ICP) %db	100 seed wt (g)	Seed coat %
ID13	44.2	0.001	0.34	18.2	13.1
ID18	43.9	0.001	0.36	22.4	11.3
ID32	45.4	0.001	0.38	16.5	12.3
ID33	44.8	0.001	0.35	17.7	11.6
JC243	42.9	2.93	0.35	21.1	13.7
P27808	42.3	3.36	0.35	25.5	13.9
P28725	42.9	4.07	0.28	14.3	12.8
Inti	46.7	0.01	0.35	14.4	13.4
P26961	38.9	3.39	0.30	21.9	11.1
P27809				18.7	13.3
Andromeda*	35.0	0.015	0.25a	30.0	15.0
Tanjil**	31.5	0.015	0.23a	14.0	24.0
Wodjil***	38.0	0.005	0.46a	12.7	25.0
LSD (0.05)	2.3	0.001	0.03	1.8	0.8

**Table 3.** Agronomic characteristics for *L. mutabilis* germplasm and breeding lines compared with *L. angustifolius* cv. Mandelup grown at Mt Barker, Western Australia, 2007.

Genotype	Early height (mm)	Early biomass (g/plant)	Mature height (mm)	1 <sup>st</sup> order branches	2 <sup>nd</sup> order branches	Main stem leaves/plant
ID13	128	2.9	827	2.7	1.7	15.3
ID33	123	2.7	823	1.3	0	18
ID8	106	2.5	663	1	0.3	15
JC243	143	2.8	717	2.3	1.7	15.7
P25955	173	2.8	780	2	0.3	15
P26961	151	2.7	727	2	1.3	16.3
P27806	160	2.8	900	3.7	0.7	18
P27808	74	2.8	567	3	2.5	15.5
P28028	133	3	917	2.7	3.3	20.3
P28029	120	2.8	983	3.3	3.3	15.3
P28623	140	2.7	743	2.7	0	15.3
P28725	72	3.4	743	1	0	18
Mandelup*	102	2.6	610	4	1.3	18
LSD (0.05)	23	0.3	274	1.7	3.7	2.1

Genotype	Days to flowering	Mature biomass (g/plant)	Seed yield (g/plant)	Pods/plant	HI	100 seed weight (g)
ID13	97	20	5.9	19.3	0.2	13.9
ID33	109	41	10.4	18	0.23	13.9
ID8	99	10	4.3	11	0.28	13.2
JC243	91	21	6.1	17	0.27	22.1
P25955	84	45	13.3	15	0.26	24.1
P26961	93	12	2.3	13.3	0.33	20.9
P27806	106	48	13.6	22.3	0.22	20.5
P27808	108	58	12.7	17.5	0.22	22
P28028	107	44	8.3	18.3	0.18	24.2
P28029	103	90	17.7	38	0.17	24
P28623	90	19	5.8	13.3	0.29	16.5
P28725	111	31	4.8	20	0.13	17
Mandelup*	90	16	11.2	16	0.42	16.1
LSD (0.05)	4	27	7.9	10.2	0.13	3.7