# POTENTIAL AND PROBLEMS OF *LUPINUS POLYPHYLLUS* LINDL. DOMESTICATION

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## ABSTRACT

**We are investigating the agricultural potential of the perennial large-leaved lupin (*Lupinus polyphyllus* Lindl.) and its hybrids in Finland and Russia. Naturalised populations of this lupin grow widely in both countries and some workers characterise it as a noxious weed. We, however, recognise its potential as an arable crop. Reduced-alkaloid breeding lines have been isolated. Interspecific hybridisation with *L. mutabilis* Sweet has provided hardy perennial materials with high seed oil content, suitable for further development into crops when other limitations are overcome.**

## KEYWORDS

alkaloids, oilseed, perennial, biomass, interspecific hybridisation

## INTRODUCTION

American lupin species of subgenus *Platycarpos* (Wats.) Kurl. offer great opportunity for breeding enhancement (Kurlovich and Stankevich, 2002). Large-leaved lupin (*Lupinus polyphyllus* Lindl.) was selected for breeding because it is well adapted to Finnish and North-West Russian conditions. Finnish latitude and climate do not permit the growth of the majority of annual lupins except for the earliest forms of *L. angustifolius*. Maturation of perennial large-leaved lupin is highly reliable in Finland and in the North-West of Russia. Now forms with reduced alkaloid content have been developed and this species has achieved potential as a high-grade fodder crop as a result. Large-leaved lupin is 'difficult' in breeding terms, because of its perennial developmental cycle, cross-pollination and indeterminate mode of growth.

## MATERIALS AND METHODS

Research material was obtained from the lupin collection of the N.I. Vavilov Institute, St Petersburg, Russia. Many years (1973-1997) of observing the collection sown in various regions of many countries and the use of methods applicable to lupin (Kurlovich *et al.* 1990), have enabled us to identify and develop new materials of *L. polyphyllus* with economically beneficial characteristics. Mechanisms of pollination, pod set and seed productivity in *L. polyphyllus* were investigated with the purpose of artificial creation of highly productive populations. Four basic pollination methods were used in our germplasm development: 1. Open-pollinated control; 2. Spontaneous self-pollination of plants isolated under gauze; 3. Enhanced self-pollination by shaking of plants isolated under gauze; 4. Open self-pollination of individual plants in isolation plots. Rapid field tests for the presence or absence of alkaloids were carried out by means of paper saturated with alkaloid-sensitive Dragendorff reagent. The quantity of protein, oil, and alkaloids and the composition of the alkaloids (both in seeds and in green mass) were determined in the biochemical laboratory of N.I. Vavilov Institute (St Petersburg, Russia) following standard methods (Mironenko, 1975; Wink, 1992). Two lines of perennial large-leaved *L. polyphyllus*, bitter cv. '*Pushkinsky*' and semisweet cv. '*Pervenec*', along with two of annual *L. mutabilis*, bitter *k-2770* selected in Belarus and sweet cv. '*Inti*' were used as parents for interspecific crosses in 1990-2007.

## RESULTS AND DISCUSSION

Each lupin sample comprises a complex population consisting of several biotypes varying in rates of growth and development, dimensions and quantity of podding branches, and lifespan. Individual plants started flowering and fruiting already in the first year (annual forms), others not until the second, third, fourth or even the fifth year, and some plants died without reproducing. Productivity and quality of a sample population depends on the interrelation of the biotypes.

Pod set ranged from 21.5 to 70.5% in open-pollinated control (treatment 1) (Table 1), with an average of 44.3% across five years. Spontaneous self-pollination of plants under gauze (treatment 2) gave only 0.0-5.2% seed set. Enhanced self-pollination following shaking of plants isolated under gauze (treatment 3) provided seed set of 2.5-56.2%. The success of artificial self-pollination depended also on the time of pollination, with maximal podding (50.3-56.2%) obtained after pollination on the third day of anthesis. Pod setting following open self-pollination of individual plants in isolation plots (treatment 4) was only slightly less than in treatment 1, and much greater than in treatments 2 and 3 (17.5-58.3%). The absence in

**Table 1.** Variability in pod set of *L. polyphyllus* in 5 years following 4 different modes of pollination.

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| Pollination method | Pod set, % (mean ± SE) | | | | | |
|---|---|---|---|---|---|---|
| | 1999 | 2000 | 2001 | 2002 | 2003 | Average |
| Open-pollinated control | 21.5 ± 1.3 | 40.5 ± 2.6 | 23.8 ± 0.8 | 70.5 ± 4.2 | 65.1 ± 4.4 | 44.3 |
| Spontaneous self-pollination of plants isolated under gauze | 0.0 | 3.8 ± 0.1 | 0.2 ± 0.03 | 5,2 ± 0.7 | 4.6 ± 0.8 | 2.76 |
| Enhanced self-pollination by shaking plants isolated under gauze | 2.5 ± 1.9 | 26.2 ± 6.1 | 6.5 ± 2.5 | 56.2 ± 12.1 | 51.8 ± 9.2 | 28.6 |
| Open self-pollination of individual plants in isolation plots | 17.5 ± 1.2 | 38.6 ± 2.3 | 18.9 ± 0.8 | 58.3 ± 1.2 | 55.1 ± 3,1 | 37.7 |

separate years (1999) of self-pollination was attributable partly to protandry and partly to herkogamy (Vishnyakova and Kurlovich, 1995).

Insects are the primary pollen vectors for cross-pollination. Papillae and hollows in the epidermis on different organs of the flower have been identified as another component of adaptation to cross-pollination (Vishnyakova and Kurlovich, 1995). Furthermore, the stigma of the pistil in large-leaved lupin is set much higher than the anthers and is protected by a collar of hairs (Fig. 1A). Similar hairs are found in *L. mutabilis*, whereas in *L. angustifolius* (Fig. 1B), they are few and short so they do not significantly obstruct self-pollination.

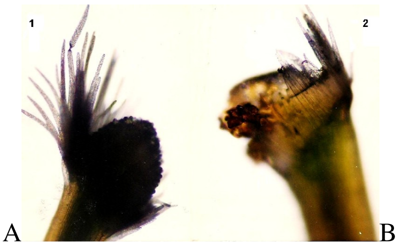

**Fig. 1.** The stigmas of (A) *L. polyphyllus* and (B) *L. angustifolius*

Tripping of individual flowers and shaking of the whole plant has proved a reasonable method for enhancing self-pollination, resulting in a good production of self-pollinated seeds. The level of seed set, however, depends on the level of self-incompatibility of individual plants and on environmental conditions. Pollination treatments 3 and 4 appeared to be the most productive for practical breeding.

As a result of over 30 years of self-pollination, numerous presumed recessive mutants have been isolated and fixed. Among these are: reduced alkaloid content; large seeded, suitable for cultivation as a pulse; non-shattering pods; water-permeable seed coat (non-dormant); annual instead of perennial; non-vernalising (spring) instead of vernalising (winter): alternative flower colours; alternative seed colours. The reduced-alkaloid mutant (0.2%) was identified as a single plant in 1990 among self-pollinated inbred lines, by use of the rapid Dragendorff test for the presence of alkaloids. The attribute of reduced alkaloid level was fixed during the next few years of the breeding program. Other medium and low-alkaloid mutants were received from other researchers in Belarus, Russia and Germany. Intercrossing of all of these reduced-alkaloid mutants consistently produced reduced-alkaloid progeny, demonstrating that the mutations were in a common gene. The inheritance of the trait was further studied in reciprocal interspecific crosses between reduced-alkaloid *L. polyphyllus* and high-alkaloid (wild-type) *L. mutabilis* plants (Table 2). The alkaloid level in the $F_1$ generation was generally high while the $F_2$ segregation ratio was consistent with a single recessive gene.

Since the gene conditioning reduced alkaloid content of 0.2 ± 0.02% came to light repeatedly in accessions of different origin and was sufficiently stable, we gave it the symbol *redalkpol* and name *reducedalkpolyphyllus*. Normal alkaloid content in this material is about 3.2%. Lupanine group compounds accounted for 96.5% of total alkaloid content, the angustifoline group 2.6%, the sparteine 0.6% and gramine 0.1%, and this distribution was similar in both the low-alkaloid and normal-alkaloid material.

We aspire to achieve a further decrease in the alkaloid content (to 0.01-0.02%) by carrying out research in the following two directions. First, we continue to investigate whether this new allele is fully recessive. Current investigations indicate that this question is valid, as not only bitter plants but also plants with lower content of alkaloids in vegetative organs (< 0.02%) appear from time to time. We have had three plants showing a consistently low alkaloid content (< 0.02%) in green mass for two years in our experimental field, but they have not yet flowered. It gives us hope to identify more viable sweet plants in the future.

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**Table 2.** Summary of segregation in $F_2$ families derived from crossing reduced-alkaloid plants with high alkaloid plants (Classification by Dragendorff reagent).

| $F_2$ families | Year | Number of plants | | $\chi^2_{3:1}$ |
|---|---|---|---|---|
| | | Bitter | Sweet | |
| *L. polyphyllus* cv. Pervenec x *L. mutabilis* line k-2770 | 2003 | 19 | 5 | 0.22 |
| *L. mutabilis* line k-2770 x*L. polyphyllus* cv. Pervenec | 2004 | 15 | 6 | 0.14 |
| *L. polyphyllus* cv. Pervenec x *L. mutabilis* line k-2770 | 2005 | 88 | 20 | 2.41 |

**Table 3.** Characteristics of parents and the best interspecific hybrids from the cross *L. mutabilis* x *L. polyphyllus*. (Average for 2005-2006).

| Identity | Days from plantlets to maturity | 1000 seed mass, g | Seed protein content, % | Seed oil content, % | Yield per plant, g | |
|---|---|---|---|---|---|---|
| | | | | | Green mass | Seed |
| *L. polyphyllus* cv. Pervenec (1st and 2nd years) | 85-120 | 21- 25 | 43.9 ± 2.4 | 4.2 ± 0.61 | 2420 ± 79 | 150 ± 2 |
| *L. mutabilis* line k-2770 | 120-140 | 160-165 | 46.5 ± 1.8 | 14.0 ± 1.12 | 2117 ± 25 | 296 ± 9 |
| Perennial hybrids | 80-120 | 26- 58 | 45.8 ± 2.8 | 7.5 ± 1.36 | 1820 ± 58 | 178 ± 10 |
| Annual hybrids | 95-125 | 24-105 | 46.1 ± 7.6 | 13.2 ± 3.23 | 1640 ± 93 | 201 ± 8 |

Second, we have crossed sweet *L. mutabilis* with our reduced-alkaloid *L. polyphyllus*, so we can explore the possibility of combining some of the desirable traits of each species in the presence of stable low alkaloid content. The first results have been encouraging, because the alkaloid level in the $F_1$ generation appeared very high (up to 3.5%). This suggests that the low-alkaloid mutations in the two species are at different parts of the synthetic pathway. If so, and if the meiosis in these interspecific hybrids behaves in a reasonable manner, we may expect to find transgressive segregants in the $F_2$ and subsequent generations with even lower alkaloid content. Earlier efforts to produce interspecific hybrids between bitter *L. mutabilis* and *L. polyphyllus* in Russia (1990-1995) demonstrated the feasibility and efficiency of crossing these species, which have identical chromosome numbers, for investigations of the inheritance of some traits and to derive interesting segregants for further study and practical use. It is not difficult to cross these species and to obtain a wide diversity of hybrids, though the percent of success in fertilisation is lower than in intraspecific crosses. The most typical deflections of development in hybrids are non-uniform allocation of pods on inflorescences flower brushes (rice) and not identical (frequently lowered) quantity of seeds in pods. Cytogenetic analysis of the $F_1$ interspecific hybrid revealed sometimes abnormal chromosome pairing at metaphase I with a high frequency of univalents in the hybrids in comparison with the parents (Siamasonta, 1996). Because of the widespread naturalised populations of *L. polyphyllus*, it is necessary to quantify the frequency of crossing between derivatives of the interspecific hybrids and the natural populations. Significant inflow of bitter genes, among other undesirable traits, may significantly impede the development of this crop.

Some 10-15% of the obtained hybrids had individual traits beneficial to the future breeding: larger seed, perenniality, and in annuals a shorter season than *L. mutabilis* (Table 3), along with also good seed composition, shattering resistance, frost resistance and high vigour.

It was not difficult to isolate each of these characters separately. However, because of the perennial nature of the species and its dependence on cross-pollination, considerable time will be required to recombine some of these attributes into a single genotype. Dominant in Russian and Finnish conditions are: perennial developmental cycle, spring growth habit, shattering of pods, dark blue coloration of flowers, dirty-brown pattern colour of seed coat and bitterness.

In addition, by crossing highly inbred lines of *L. polyphyllus* (obtained from pollination treatments 3 and 4), we have obtained a wide range of different flower and seed colours, without using *L. arboreus* as was done in the development of the Russell lupins.

Our major breeding objectives for large-leaved lupin and its hybrid with *L. mutabilis* in Finland since 1996 have been stable low alkaloid content (< 0.02%), different types of pollination (either cross- or self-pollinating), non-dehiscent pods, winter hardiness and frost tolerance. Improved cultivars should also have green mass yield potential in the order of 40-50 Mg fresh matter $ha^{-1}$. The first commercial perennial *L. polyphyllus* cultivars with reduced alkaloid content were bred in Russia (cv. Pervenec). Tests and breeding of the best forms of *L. polyphyllus* with reduced alkaloid content for the conditions of Finland is now in progress. They are multiplied by farmers near Tampere. Perennial lupin with reduced content of alkaloids is used as green mass for preparation of silage. Farmers value this, as the productivity of their animals has increased and acceptability of the silage was good, while no detriment to animal health (cows and horses) was noted.

## CONCLUSIONS

*L. polyphyllus* is not just a weed, and has great potential in the Nordic region as a cultivated plant. Now that reduced-alkaloid forms have been identified, future use of this species for fodder production, green manure, and silage looks possible. Enforced self-pollination for several generations has allowed the isolation of self pollinated forms with valuable attributes. Crossing of these inbred lines, in turn, frequently provides highly heterotic in progeny with new combinations of traits. Enhancing nature's gift of lupins is possible with effective utilisation of other closely related species with the same number of chromosomes (48), particularly *L. mutabilis*, although this process is time-consuming on account of the cross-pollinating nature and perenniality of the species.

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