

# IMPROVEMENT OF YELLOW AND NARROW LEAF LUPINS FOR THE UNITED KINGDOM

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## ABSTRACT

**As part of an integrated approach to developing the potential of *L. luteus* and *L. angustifolius* in the UK, we have initiated a germplasm improvement program based initially mainly on field selection. Progress has been made with respect to yield and on going studies, including experiments carried out in flowing solution culture, are focusing on enhancing growth under alkaline soil conditions. This work is being combined with the development of new mapping families and the use of molecular markers.**

## KEYWORDS

*Lupinus luteus*, *Lupinus angustifolius*, breeding, yield, pH

## INTRODUCTION

Within Europe, the need to increase home grown sources of high quality protein for reasons of traceability, environmental protection and self sufficiency has been recognised for a number of years (European Parliament, 2002). In 2004, a new project was established to develop an integrated approach to the development of the spring grown lupin crop in the UK. This project (Lupins in Sustainable Agriculture, LISA) centres on yellow and narrow leaf lupins (*Lupinus luteus* and *L. angustifolius*) and involves studies on agronomy, ruminant and non ruminant nutrition, contribution to rotations and genetic improvement (Biddle, 2008; Marley *et al.* 2008, Fychan *et al.* 2008).

The main focus of this paper is on the last aspect: development of germplasm and molecular markers to underpin breeding of new varieties adapted to UK conditions. In particular the major objectives are:

- Increased yield and yield stability.
- Earlier maturity.
- Enhanced adaptation to alkaline conditions.

Initial results are presented with respect to (i) and (iii) and other aspects of the work being carried out are referred to briefly.

## MATERIALS AND METHODS

### FIELD EXPERIMENTS

All field trials over the four years were carried out on two fields of similar silt clay loam (Denbigh series) at the Institute of Grassland and Environmental Research, Aberystwyth. The areas were prepared in the same manner each time. The area was initially treated with 4-5 L/ha Glyphogan (360 g/L glyphosate) or Gramoxone herbicide prior to ploughing and power-harrowing. Fertiliser P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (0:24:24) was distributed according to soil indices and harrowed / rolled in prior to planting.

Three types of field experiments were carried out: individual spaced plants, drilled rows and plots.

In 2004, *Lupinus luteus* cv. Wodjil and *L. angustifolius* cvs Prima and Bora were grown as individual plants (*L. luteus*, n = 540; *L. angustifolius*, n = 280 each). The ground was prepared as for plots below but marked out in 1 m<sup>2</sup> grid pattern with 3 seed of each variety sown at each station/intersection (thinned to single seedling, randomly, following germination). Spaced plants were all harvested individually and measured for: number of branches, branch length, plant height to tip of main spike, number of branches with pods, dry weight of branches and dry weight of stem (biomass d.wt), dry weight of pods, number of pods, main spike length, main spike number of pods, main spike number of seed and weight, main spike dry weight, main spike dry weight of pods, and harvestability (based on a visual score of pods together with plant and stem die back below pods).

In 2005, genotype selections made from spaced plants in 2004 were assessed as individual drills. The experimental area was prepared as for the plots and seed from individual mother plants hand sown in drills; 12 seed per drill spaced at 10 cm within drills and 50 cm between drills. All seeds were inoculated with *Bradyrhizobium*. The layout included guard plants at either end of each drill and guard drills at the end of each row. 84 drills each of *L. angustifolius* (Prima and Bora) and 150 drills of *L. luteus* (Wodjil) per replicate in randomised block layout with 3 replicates (954 drills in total).

Commercial seed of the respective varieties were used as controls. Drills were assessed at maturity (height and visual score for plant architecture and uniformity). Drills were harvested by removing the guard plant at each end and saving the seed. The remaining plants in each drill were then harvested by hand and threshed using a static Wintersteiger plot combine. Both seed and straw were collected and weighed fresh. Material was then dried (forced air oven at 80°C for 7 days) and dry weight recorded. Individual moisture contents were calculated and all results calibrated to standard units at 15% moisture content. Thousand grain weight (TGW) was also measured and calibrated to standard moisture content.

Further selection from drills assessed in 2005 provided F<sub>2</sub> seed for assessment as drills in 2006. The experimental area and lay out was as for drills in 2005. 52 drills each of *L. angustifolius* and 78 drills of *L. luteus* per replicate in randomised block layout with 3 replicates (546 drills in total). Assessment and harvest was as described for 2005.

*L. angustifolius* and *L. luteus* were also assessed in plots. Plot size was 11 x 2 m in 2004, then 6 x 2 m for 2005, 2006 and 2007. Plots were marked out and commercial variety seed inoculated with *Bradyrhizobium* ('Hi-Stick', Becker Underwood) was drilled with a coulter drill (98, 82 and 71 Kg/ha for *L. angustifolius* (Prima and Bora) and *L. luteus* (Wodjil) respectively in 2004 and 2005, then 150, 90 and 90 kg/ha for 2006 and 2007). Pre-emergence herbicide 2.3 L/ha 'Opoguard' (terbuthylazine 150 g/L; terbutryn 350 g/L) or Stomp (3.3 L/ha) was applied 2-4 days post-sowing and 'Huron' slug pellets (5 Kg/ha) applied 4-6 days post-sowing. Growth was monitored throughout the season and 20 randomly selected plants per plot were measured for height at harvest. Plots were harvested by cutting a strip (1.6 m wide) through the centre of each plot using a Wintersteiger nursery master plot combine. Growing period was assessed as number of days post-sowing to harvest date. Both grain and straw (plant biomass) was collected and weighed fresh. A subsample was removed from each plot to determine moisture content by weighing fresh and after drying (forced air oven; 80°C for 7 days). TGW was measured and all figures calibrated to standard units at 15% moisture content. Plant final populations were assessed with six 0.25 m<sup>2</sup> quadrats per plot. Soil samples were taken from all plots before sowing and again post harvest.

#### EXPERIMENTS IN FLOWING SOLUTION CULTURE (FSC)

All work was carried out in a FSC system (Clement *et al.* 1974) in a greenhouse, enabling plants to be grown from seed to flowering at a range of rhizosphere pH values. Three plant culture units were used, each containing 200 dm<sup>3</sup> of nutrient solution and 24 individual 1 dm<sup>3</sup> culture vessels, through which the solution was continuously circulated (Clement *et al.* 1974). The initial composition of the nutrient solution in

each culture unit was (mmol m<sup>-3</sup>): NO<sub>3</sub><sup>-</sup>, 250; K<sup>+</sup>, 250; Ca<sup>2+</sup>, 322; So<sub>4</sub><sup>2-</sup>, 424; Mg<sup>2+</sup>, 100; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 5; Fe<sup>2+</sup>, 5.4; with micronutrients as in Clement *et al.* 1978. This was allowed to equilibrate overnight, prior to adjusting pH. Preliminary studies indicated that stability was difficult to achieve using Ca(OH)<sub>2</sub> to raise pH from an initial range of 4.8-5.4 to the desired treatment values. However, 0.5M KOH was found to be more effective in achieving stable pH values > 6. The use of KOH instead of Ca(OH)<sub>2</sub> also avoided the potential compounding effect of excess Calcium in the system affecting plant growth (Birchall *et al.* 1995). Based on preliminary calibration experiments 0.5M KOH was continuously delivered by means of a peristaltic pump (Watson Marlow, H-R Flow Inducer) to the header tank of each plant culture unit and mixed into the bulk nutrient solution before circulating through the plant culture vessels. The three culture units were set (+/- 0.2) to pH 6, pH 7 and pH 8, respectively, and allowed to equilibrate for one week prior to planting seeds. The pH of each tank was checked daily and adjustments made as necessary to compensate for any minor fluctuations.

Pre-germinated seed was sown singly into the individual vessels according to the randomisation schedule (randomised complete block), with two replicates of each selection (including control – unselected commercial variety seed) at each pH. Initial weights of planting caps and seed were recorded. Seedlings were allowed to develop and grow and a non-destructive assessment of plant weight and root length was completed at 12, 19, 26 and 33 days post sowing. Supplementary lighting was provided by a 400W SON-T (Phillips) lamp over each unit with 12 h photoperiod.

#### SELECTIONS FOR IMPROVED GROWTH ON ALKALINE SOILS

Genotype selections were made from both field and FSC (hydroponics) system. Field plots at Biggleswade (a field site of The Arable Group (TAG)), Denver and Boston (a field site of Processor and Growers Research Organisation (PGRO)) U.K. with pH 8.3, 7.9 and 8.2 respectively were utilised. Individual surviving genotypes were identified and seed harvested along with soil cores adjacent to each plant. Soil cores were analysed for pH, N, P, K and trace elements (Na, Mg, Ca) and seed from each genotype sown in controlled environment facility for seed multiplication.

Selections were also made from genotypes used in the preliminary tests of the FSC system. Plants removed and potted into five inch pots of standard 'humax' compost and grown to maturity when pods harvested and seed collected.

#### MOLECULAR MARKER AND MAPPING FAMILY DEVELOPMENT

DNA samples extracted from 60 genotypes of each variety (Prima, Bora, Wodjil) were tested with white lupin (*L. alba*) simple sequence repeats in collaboration with the University of Reading. Significant polymorphisms were present within each variety and a

range of markers identified to use on mapping families currently under development. A number of F<sub>2</sub> families have been developed from crosses between genotypes selected for a range of traits including yield, plant

architecture, tolerance of alkaline soils and earliness of maturity. Future generations from these lines will be used in QTL mapping of these traits.

## RESULTS AND DISCUSSION

### FIELD EXPERIMENTS

**Table 1.** Improvement in selected genotypes of *L. angustifolius* and *L. luteus*. 2006 data from field drills is shown.

Comparison of means of control and improved genotype selections in *L. angustifolius* (determinate and indeterminate) and *L. luteus*. Controls: commercial varieties (Prima, Bora, Wodjil), Selections: determinate (P 126/251, P 101, P 263, P XXV/229 ) indeterminate (B XI/190, B XIX/190, B 267/198, B 248/192 ) and *L. luteus* (W 36/366, W XI, W XI/321, W XI). Yield: mean threshed grain weight per plant (adjusted to standard 15% moisture content), Biomass: mean standardised dry weight of plant at harvest, Height: mean height of plants in cm measured to tip of main spike, TGW: mean thousand grain weight of threshed seed (adjusted to standard 15% moisture content). s.d. = standard deviation.

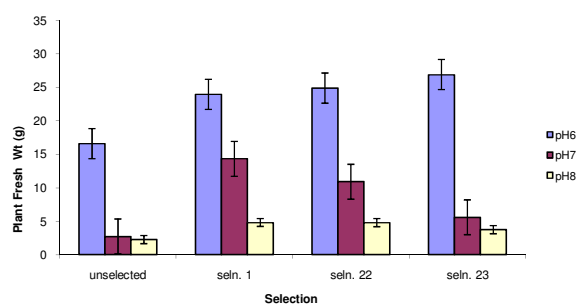
	Yield/plant (g)	Yield/plant (g)	Plant biomass (g)	Plant biomass (g)	Plant height (cm)	Plant height (cm)	TGW seed (g)	TGW seed (g)
	Control	Selection	Control	Selection	Control	Selection	Control	Selection
<i>L. angustifolius</i> (determinate)	13.2	18.4	9.9	18.1	54	59	141.4	148.6
s.d.		2.32		3.03		4.17		7.63
<i>L. angustifolius</i> (indeterminate)	11.5	20.1	12.6	26	58	68	109.7	156.5
s.d.		3.21		6.4		6.75		18.12
<i>L. luteus</i> (semi-determinate)	10.7	13.9	16.6	25.7	57	69	132.4	163.9
s.d.		1.86		3.05		6.3		17.27

Differences were observed between genotypes after only a single cycle of selection. This variation was used in the selection of material for subsequent field experiments and Table 1 shows data from field drills in 2006. Differences in plant architecture, particularly height of both spike and branches was observed in both determinate and indeterminate selections of *L. angustifolius* derived from the varieties Prima and Bora and in *L. luteus* selections derived from the variety Wodjil. Although fresh weight of plant biomass was significantly different in selections derived from all varieties, the same differences were not observed in percentage dry matter. This suggests that the biggest difference was in moisture content and leaf retention on the plant. Differences in grain yield in selections derived from Prima were not significant although quality of the grain produced (TGW) was, along with moisture content (maturity). In this experiment, percentage moisture content of the grain was used as a potential quantitative indicator of grain and subsequently plant maturity.

### EXPERIMENTS IN FLOWING SOLUTION CULTURE

Following optimisation of the protocol to allow differences between genotypes at pH > 7 to be quantified, indeterminate *L. angustifolius* plant lines derived both from field experiments carried out on

alkaline soils and previous FSC experiments were analysed. Fig. 1 shows that significant differences were observed between selected lines and the control variety.



**Fig. 1.** Comparison of selected genotypes in Flowing Solution Culture (FSC). Data shown are from an FSC experiment carried out in 2008 with *L. angustifolius* (indeterminate) in a replicated randomised complete block trial, 33 days post-sowing (3 treatments, 2 reps). Control is commercial variety Bora, Selections: FSC.1 (from earlier FSC experiment), pH 22 and pH 23 (field selections from alkaline soil (pH > 8) Boston UK).

However, yields at higher pH were low for all selections. Given the nature and complexity of this trait (Liu and Tang, 1999) it seems clear that a combined

approach to identifying heritable improvement will be required. In addition to those studies outlined here we are also carrying out ongoing field experiments under different liming regimes.

### CONCLUSIONS

This work is in its early stages. However, it seems clear that relatively rapid progress can be made with respect to improvements in yield and yield components, including plant architecture. The attainment of significant benefits with respect to growth and satisfactory yields on alkaline soils is likely to be more difficult, due in part to incomplete understanding of underlying mechanisms.

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