# MONITORING IMPROVEMENTS IN SEED PRODUCTION AND SEEDLING VIGOUR OF GREATER LOTUS (*LOTUS PEDUNCULATUS* Cav.)

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*Summary.* Late flowering and poor seed production are negative characteristics of the otherwise valuable *Lotus pedunculatus* cv. Grasslands Maku in northern NSW. A selected earlier-flowering population and a population unselected for flowering, both derived from crosses between New Zealand and Portuguese accessions, were compared at northern and southern sites in NSW to monitor selection progress. The selected population was 6-10 days earlier to first flower than the unselected population and produced more seed than the parent populations and cv. Grasslands Maku at the northern site. Narrow-sense heritability for early flowering, based on the response to selection, was 0.65 and 0.60 at the southern and northern sites respectively. Mean seedling vigour of the two populations was lower than that of cv. Grasslands Maku but sufficient variability was present to indicate that improvements in seedling vigour could be made. Free-seeding types of *L. pedunculatus* should be more persistent in northern NSW and also be valuable in the seed production phase of cultivar development.

## INTRODUCTION

*Lotus pedunculatus* (greater lotus) is a forage legume with natural adaptability to acid and waterlogged soils in coastal and wet tableland regions of south-eastern Australia (1). The increasing use of this species has been based on the success of the tetraploid cultivar Grasslands Maku (4). However, this cultivar has poor seed production in the northern parts of its range (2) and is also slow to establish from seed in autumn sowings. Sources of germplasm with better seed production and large seeds have been identified and used in crosses with diploid accessions to produce populations in which selection has been practised for early flowering and seed set (5). To monitor selection progress and to estimate heritability for flowering time, a selected early flowering population and a population unselected for flowering were grown at northern and southern sites in NSW. During the establishment phase of these experiments the seedling vigour of the two populations was compared with that of cv. Grasslands Maku.

## MATERIALS AND METHODS

A series of single crosses between the New Zealand breeding lines, G4703 and G4704, and the Portuguese accessions, CPI 67676, CPI 67677 and CPI 67678, was made in August/September 1991. A bulked F1 population was grown in the glasshouse and interpollinated. The combined F2 seed was used to grow a spaced plant population at Canberra during summer 1992/3. Two populations were selected from the F2 plants:

• a population selected only on the basis of early flowering.

• a population selected on the basis of good agronomic type but without regard to early flowering.

Open-pollinated seed of the selections in each group was bulked and F3 populations were established in jiffy cups in the glasshouse in August 1993, together with cv. Grasslands Maku and the five parent populations. Fifty seeds of cv. Grasslands Maku and of the five parents and 200 seeds each of the two F3 populations were sown in trays at the same time. Forty-seven days after sowing the seedlings were harvested from the trays and the number of crown shoots recorded on each plant. The seedlings were dried at 70$^{o}$C and seedling dryweight recorded.

The seedlings in jiffy cups were transplanted to field sites at Canberra, ACT, and Grafton, NSW in October 1993. The plants were layed out in plots of 5 plants in a single row, 0.5m between plants and 1.0m between rows There were 4 replicates, each containing one plot of each of the parents and cv.

Grasslands Maku (20 plants), and 4 plots each of the two F3 populations (80 plants). Individual plants were monitored three times per week to record the date of appearance of the first open flower and the date of the first mature pod set. At 50% mature pod set the seed of individual plants was harvested and weighed.

The estimate of heritability for flowering time was made separately at each site from the response to selection equation $R = i\ h^2\ \sigma_p$ (3), where R is the mean difference in flowering time between the F2 and F3 populations, i is the standardised selection differential (a measure of the proportion of plants selected from the F2 generation), $h^2$ is the heritability and $\sigma_p$ is the phenotypic standard deviation for flowering time in the F3 population. It was assumed that the mean flowering time of the unselected F3 population was a close approximation of that of the F2 in the previous generation.

## RESULTS AND DISCUSSION

### *Seedling dryweights and number of crown shoots*

The mean seedling dryweights of the F3 populations and the parents were significantly lower than that of the cv. Grasslands Maku (Table 1). However, the mean dryweight of the unselected F3 population (NZPOF3-2) was greater than the best parent population (t=1.36;P=0.09), and 10% of the seedlings in this population had dryweights greater than one standard deviation above the mean of the cv. Grasslands Maku distribution.

Table 1. Seedling dry weight and number of crown shoots in seven populations of *Lotus pedunculatus.*

| Population | Seedling dry weight (mg) | | | | No. of crown shoots | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean | s[c] | t[a] | r[b] | Mean | s | t |
| G. Maku | 31 | 61.7 | 22.4 | 0.34 | 4.2 | 1.01 | | |
| G4703 | 49 | 39.2 | 21.1 | 4.53** | 0.31 | 3.7 | 1.12 | 2.09** |
| G4704 | 47 | 38.4 | 15.1 | 5.49** | 0.29 | 3.6 | 1.03 | 2.43** |
| CPI 67676 | 38 | 48.0 | 18.6 | 2.77** | 0.46 | 2.9 | 0.95 | 5.64** |
| CPI 67678 | 44 | 47.7 | 19.2 | 2.90** | 0.31 | 2.6 | 0.85 | 7.54** |
| NZPOF3-1 | 232 | 49.7 | 21.2 | 2.92** | 0.65 | 2.9 | 1.16 | 5.96** |
| NZPOF3-2 | 227 | 52.6 | 22.4 | 2.08* | 0.70 | 3.1 | 1.15 | 5.31** |

[a] t-test of mean difference between Grasslands Maku and each of the other populations.

[b] correlation coefficient between seedling dryweight and number of crown shoots.

[c] standard deviation.

*, **: significant at P<0.05 and P<0.01, respectively.

The mean number of crown shoots present 47 days after sowing in cv. Grasslands Maku was also significantly greater than that of the other populations (Table 1). Crown shoot number and seedling

dryweight were significantly correlated in all populations, with the best correlations present in the larger F3 populations.

A contributing factor to the superior seedling vigour of cv. Grasslands Maku is its increased seed size which was induced by tetraploidy. Polyploidy could be induced in the F3 populations of the diploid crosses to improve seedling vigour. The seedling weight variation present in these populations also suggests that selection could be used in the diploid or derived tetraploids to improve seedling vigour. Selection for higher crown shoot number in seedlings would be a simpler, non-destructive criterion than dryweight for the improvement of seedling vigour. It would also be wise to consider other characters that influence seedling vigour, such as rate of germination, particularly at low temperatures.

*Flowering time and podset*

There was no significant difference in the time from transplanting to first flower between the Canberra and Grafton sites (P=0.81), but at each site the Portuguese accessions and the selected early-flowering population were 8-31 days earlier to first flower than the New Zealand populations (Table 2). The NZPOF3-1 population was 6-10 days earlier to first flower than the unselected F3 population. Based on this response to selection, the heritability for flowering time was 0.65 at Canberra and 0.60 at Grafton.

Table 2. Reproductive development and seed yield of spaced plant populations of *Lotus pedunculatus* at Canberra and Grafton.

| Population | Days to first flower[a] | | Days to mature pods[b] | | Seed wt. per plant (g) | |
|---|---|---|---|---|---|---|
| | Canberra | Grafton | Canberra | Grafton | Canberra | Grafton |
| Grasslands Maku | 76 | 87 | - | - | 0.0 | 0.0 |
| G4703 | 82 | 93 | 112 | 110 | 5.4 | 0.6 |
| G4704 | 75 | 78 | 110 | 104 | 7.3 | 0.7 |
| CPI 67676 | 67 | 64 | 104 | 90 | 8.0 | 0.8 |
| CPI 67677 | 64 | 65 | 104 | 97 | 7.2 | 0.9 |
| CPI 67678 | 64 | 54 | 103 | 89 | 8.9 | 1.4 |
| NZPOF3-1 | 64 | 61 | 103 | 90 | 6.7 | 1.2 |
| NZPOF3-2 | 70 | 75 | 109 | 99 | 6.3 | 1.2 |
| Standard error | 2.9 | 4.4 | 2.6 | 5.0 | 1.7 | 0.4 |

[a] the mean number of days from transplanting to flowering.

[b] the mean number of days from transplanting to mature pod set.

In contrast to flowering time, the time of first mature podset was 2-14 days earlier at Grafton than at Canberra ($P<0.001$), most likely reflecting the faster maturation of pods under the higher summer temperatures at Grafton. Mature podset was earliest in the Portuguese accessions and NZPOF3-2 and was 7-21 days earlier than the New Zealand populations.

The seed yield per plant was much higher at Canberra than at Grafton, the yield at Grafton being adversely affected by the particularly dry summer (Table 2). The Portuguese accession CPI 67678 yielded best at both localities. The seed yield per plant of the F3 populations was lower than the parental populations at Canberra but higher than all populations except CPI 67678 at Grafton. The cv. Grasslands Maku did not set mature pods at either locality. The poor seed production of cv. Grasslands Maku in its northern range has been documented previously by Blumenthal and Harris (2), who found a significant positive correlation between seed bank size and latitude over 57 sites in NSW. The diploid populations examined in this study do not appear to have this limitation. This free-seeding capability will be advantageous for the persistence of the species following loss of stands through drought or flooding and will give wider scope for the choice of seed production sites in the development of cultivars.

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