Germplasm Description

CSIRO PUBLISHING

Australian Journal of Experimental Agriculture, 2007, **47**, 116–118 www.publish.csiro.au/journals/ajea

Lotus uliginosus (greater lotus) breeding line LUX97: a high seed producing, low condensed tannin population

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Abstract. A *Lotus uliginosus* polycross population (LUX97) was developed from high seed producing Portuguese accessions and locally adapted New Zealand germplasms. Two cycles of selection for early flowering and low condensed tannin content were successful in producing a population with lower condensed tannin content than the Portuguese parents, while maintaining high seed production potential. Seed of this population will be stored at the CSIRO Plant Industry, Canberra.

Introduction

A broader utilisation of *Lotus uliginosus* Schkuhr (greater lotus) in Australia could be achieved by improvements in drought tolerance, seedling vigour and early flowering for uniform seed production (Ayres *et al*. 2006). In addition, concentrations of condensed tannins, polyphenolic compounds in the forage that reduce protein catabolism in ruminants, are inherently high (above 40 g/kg dry weight) in this species and could reduce digestibility and intake (Barry and Duncan 1984; Barry and Manley 1984). The cultivar Grasslands Maku is the only commercial cultivar in use and was estimated to occupy 30 000 ha in high rainfall areas of the north coast of New South Wales (NSW) in 1993 (Harris *et al*. 1993). Grasslands Maku has limited areas suitable for seed production, most likely because of a long daylength requirement for flowering (Ayres *et al*. 2007). A second cultivar, Sharnae, was developed from Portuguese accessions, and showed better seed production and higher spring to summer forage yields than Grasslands Maku in northern NSW (Wilson 1992). However, Sharnae was later withdrawn because of difficulties with commercial seed production (Ayres *et al*. 2006). The Portuguese accessions used in the development of Sharnae also have condensed tannin concentrations that are generally in excess of 40 g/kg dry weight (Kelman *et al*. 1997).

Breeding for wider adaptation to Australian conditions is a longer-term objective for *L. uliginosus* (Ayres *et al*. 2006). Because of the high heritability for condensed tannin concentration (Kelman 1997) and wide variation for flowering time measured in the Portuguese accessions of *L. uliginosus* (Wilson 1992), a more rapid selection progress is expected for these traits. The *L. uliginosus* population described in this paper represents a germplasm with low condensed tannin concentration and high seed yield potential that will be an important source for the development of a more widely adapted cultivar (Ayres *et al*. 2006).

Origin

A germplasm evaluation of 39 accessions of *L. uliginosus* Schkuhr grown at Canberra, Australian Capital Territory (ACT), Moss Vale (NSW) and Nowra (NSW) identified New Zealand and Portuguese accessions as the most promising sources for producing adapted, high seed-producing, low-condensed tannin-containing *L. uliginosus* for the high rainfall (>800 mm annual average rainfall) region of eastern Australia (Kelman *et al*. 1997). In August 1991, population crosses were made by hand in the glasshouse between the Portuguese accessions, CPI 67676, CPI 67677 and CPI 67678, and the New Zealand accessions, G4703 (cv. Sunrise) and G4704. The Portuguese accessions were collected from the Algarve region of southern Portugal in 1974 by A. D. O'Brien. CPI 67677 was selected for uniform flowering in northern NSW and was registered as cv. Sharnae (Wilson 1992). G4703 and G4704 were derived from crosses between naturalised New Zealand ecotypes of *L. uliginosus* and an introduction from Portugal that showed a capacity for winter growth (Barclay 1957).

A F_1 population of 30 plants was grown in the glasshouse and intercrossed by hand. Bulked seed was used to grow 5000 F_2 spaced plants in the field in the summer of 1992–93 at Canberra, ACT. Fifty plants were selected by truncation selection, first on early flowering and then on low condensed tannin concentration, using methods described in Kelman and Tanner (1990). Open-pollinated seeds of the selections were used to grow an 800-plant F_3 population at Canberra in the summer of 1993–94. This seed was also used to grow replicated plots of the F_3 population together with the original New Zealand and Portuguese parental

 10.1071/EA05351 0816-1089/07/010116

accessions and Grasslands Maku to compare flowering time and seed production at Canberra and Grafton, in northern NSW (Kelman and Bowman 1996). Twenty-five selections from the 800-plant population at Canberra were made on the basis of productivity (visually assessed by plant size), early and prolific flowering, and low condensed tannin concentration. Open-pollinated seeds of these selections were grown as half-sib families in 1 by 2 m swards at the Pasture Research Unit at Berry, NSW, and compared over 2 years (1995–97) for seasonal productivity, flowering intensity and condensed tannin concentration. Seedling recruitment was also recorded by counting seedlings in two 1-m^2 quadrats per plot at the end of the trial. Three plants from each of the 12 families (36 plants) were clonally propagated and intercrossed in isolation to produce the breeding line LUX97. Seed of LUX97 will be maintained at CSIRO, Plant Industry, Canberra.

Morphological description

LUX97 is very similar morphologically to Sharnae. LUX97 differs from Grasslands Maku in being less pubescent on leaves and flower buds, less rhizomatous and smaller seeded. Measurements of vegetative characters were made on plants growing in pots at Glen Innes, NSW in May 2000 (Ayres *et al*. 2007). Plant height of LUX97 (14.6 cm) is similar to that of Sharnae (14.8 cm) and slightly taller than Grasslands Maku (12.8 cm). The terminal leaflet of LUX97 is obovate and significantly smaller (leaf area, 67 mm^2) than the obovate-lanceolate leaflet of Sharnae (leaf area, 144 mm^2; $P < 0.05$). The LUX97 shoots are also significantly narrower (diameter, 1.14 mm) than in Sharnae (diameter, 1.48 mm; $P < 0.05$). LUX97 has a similar flowering time to Sharnae, but flowers 12–18 days earlier than Grasslands Maku and Sunrise at Canberra and 28–32 days earlier than these cultivars at Grafton, NSW (Kelman and Bowman 1996). At Glen Innes, NSW, the number of days to peak flowering is similar for LUX97, Sharnae and Grasslands Maku (111–112 days from 1 September; Ayres *et al*. 2007). The acronym, LUX97, indicates *Lotus uliginosus* polycross (X) made in 1997.

Agronomic characteristics

Condensed tannins are an important component of forage quality, conferring bloat protection and improving protein absorption in ruminants (Barry and McNabb 1999). The condensed tannin concentrations measured in LUX97 at three sites were significantly ($P < 0.05$) lower than in Sharnae on four of six occasions (Table 1) and reflect the heritable response to selection that occurred during the development of this population. However, variability for condensed tannin concentrations is still present and further selection for even lower condensed tannin content can be achieved using LUX97 (Kelman 1999). Condensed tannin content is responsive to environmental influences such as moisture content, temperature and soil fertility level (Kelman and Tanner 1990). The condensed tannin content of LUX97 ranged from 18 g/kg dry matter (DM) in winter at a favourable site [Berry: pH_{CaCl}, 4.02; Bray phosphorous (P), 5.71] to 113 g/kg DM in spring at a low fertility site (Moruya: pH_{CaCl}, 4.75; Bray P, 2.1). Thus, on poor fertility sites where growth is limited, concentrations of condensed tannins will accumulate in LUX97 to levels above 40 g/kg dry weight and this may adversely affect palatability and digestibility. However, grazing during the active phase of growth, which stimulates fresh growth during summer, can maintain low condensed tannin concentrations (Blumenthal *et al*. 1994).

High seed yield potential of *L. uliginosus* is critical for seed production phases in cultivar development and subsequent cultivar use; it is also likely to be a major factor in the persistence of this legume by seedling recruitment in permanent pastures (Ayres *et al*. 2006). In the sward trial sown at Berry in autumn 1995, flowering intensity (the mean percentage area of the plot in flower over six replications) was compared among families when Sharnae had reached 50% flowering in mid October. At this time, Grasslands Maku was recorded at 6% flowering. This difference in flowering between the two cultivars was also noted by Wilson (1992) from observations made on the north coast of NSW. The mean flowering intensity of the LUX97 families was 25% in mid October, but 12 families equalled the 50% flowering of Sharnae. LUX97 set a significantly ($P < 0.05$) greater number of umbels/m^2 in swards grown at Canberra than G4703 (Sunrise) and Grasslands Maku (Table 2). Estimated seed yields on the basis of single plant performance (Table 2) indicated a potential seed yield of 268 kg/ha, similar to that of Sharnae and greater than the New Zealand accession G4703 (cv. Sunrise). Seedling recruitment, measured at the end of the 2-year trial at Berry, NSW (1995–97), was higher in plots of LUX97 than in Sharnae,

Table 1. Comparison of condensed tannin concentration (g/kg) in *Lotus uliginosus* cvv. Grasslands Maku, Sharnae and LUX97 at two sampling dates from Moruya and Berry, New South Wales, and Ginninderra Experiment Station (GES), Australian Capital Territory

	Moruya	Berry	GES
August 2001			
LUX97	71	18	39
Grasslands Maku	66	14	29
Sharnae	88	28	64
l.s.d. ($P = 0.05$)	30	8	16
November 2001			
LUX97	113	71	79
Grasslands Maku	115	61	73
Sharnae	102	100	100
l.s.d. ($P = 0.05$)	30	26	20

Table 2. Seed yields and seedling recruitment of the *Lotus uliginosus* breeding population LUX97 and the cultivars Sharnae, G4703 (Sunrise) and Grasslands Maku at Ginninderra Experiment Station, Canberra, Australian Capital Territory

	No. of umbels/m^2	Seed weight per plant (g)	Spaced-plant seed yield (kg/ha)[A]	Seedling recruitment (plants/m^2)
LUX97	401	6.7	268	29
Sharnae	395	7.2	288	12
G4703	162	5.4	216	1
Grasslands Maku	123	0	0	4
l.s.d. ($P = 0.05$)	41	1.7	—	12

[A]Spaced plant seed yield based on 4 plants/m^2.

Grasslands Maku and G4703 (Table 2). This free-seeding habit will favour persistence by seedling recruitment in environments where stands are thinned by periodic drought or inundation. Previous experiences with seed production of *L. uliginosus* in New Zealand and Australia have indicated difficulties caused by the extended period of flowering and pod-shattering in this species (Blumenthal *et al*. 1994). Guidelines for seed production in Australia were based on the growth of Grasslands Maku (McLaughlin and Clarke 1989) and would need modification to the more erect growing Sharnae and LUX97 plant types.

Percentage hardseededness of hand-harvested seed of CPI 67676, CPI 67677 and CPI 67678 ranged from 38 to 66% compared with 84% for Grasslands Maku and 20% for Tamar white clover (*Trifolium repens*) in laboratory tests under alternating 43 to 15°C temperatures for 9 months (Kelman and Blumenthal 1992). Field-harvested seed of Sharnae had 30–45% hard seeds whereas Grasslands Maku averaged 60% (Blumenthal *et al*. 1994). Hardseededness in LUX97 was 47% 2 months after harvest of the seed yield trial summarised in Table 2. Thus, hardseededness of LUX97 is expected to be intermediate between that of Grasslands Maku and Tamar white clover.

LUX97 is a *Lotus uliginosus* germplasm population in which the high seed production potential of Mediterranean Portuguese accessions has been maintained, whereas condensed tannin levels have been significantly reduced. Variation for seed yield and condensed tannin content is also maintained, and will be utilised in the development of cultivars for low latitude conditions in eastern Australia (Ayres *et al*. 2006).

Acknowledgements

Financial support from the former Australian Wool Research and Development Corporation and the Meat Research Corporation in the early phases of this work is acknowledged. The valuable collaboration with the staff at NSW Agriculture, Pasture Research Unit at Berry was much appreciated.

References

Ayres JF, Blumenthal MJ, O'Connor JW, Lane LA, Nicol HI (2006) Birdsfoot trefoil (*Lotus corniculatus*) and greater lotus (*Lotus uliginosus*) in temperate perennial pasture. 1. The effects of grazing management on *Lotus* persistence. *Australian Journal of Experimental Agriculture* **46**, 521–534. doi:10.1071/EA04242

Ayres JF, Kelman WM, McCorkell Lane LA (2007) Characteristics of greater lotus (*Lotus uliginosus*) populations grown under low latitude conditions in eastern Australia. *Australian Journal of Experimental Agriculture* **47**, 17–24.

Barclay PC (1957) Improvement of *L. uliginosus*. *Proceedings of the 19th New Zealand Grasslands Association* **19**, 75–81.

Barry TN, Duncan SJ (1984) The role of condensed tannins in the nutritive value of Lotus pedunculatus for sheep 1. Voluntary intake. *The British Journal of Nutrition* **51**, 485–491. doi:10.1079/BJN19840054

Barry TN, Manley TR (1984) The role of condensed tannins in the nutritive value of *Lotus pedunculatus* for sheep 2. Quantitative digestion of carbohydrates and proteins. *The British Journal of Nutrition* **51**, 493–504. doi:10.1079/BJN19840055

Barry TN, McNabb WC (1999) The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *The British Journal of Nutrition* **81**, 263–272.

Blumenthal MJ, Kelman WM, Lolicato S, Hare MD, Bowman AM (1994) Agronomy and improvement of Lotus: a review. In 'Alternative pasture legumes 1993 Technical Report 219'. (Eds DL Michalk, AD Craig, WJ Collins) pp. 74–85. (Department of Primary Industries South Australia: Adelaide)

Harris CA, Blumenthal MJ, Scott JM (1993) Survey of use and management of *Lotus pedunculatus* cv. Grasslands Maku in eastern Australia. *Australian Journal of Experimental Agriculture* **33**, 41–47. doi:10.1071/EA9930041

Kelman WM (1997) Estimates of heritability and genetic gain from selection for lower condensed tannins in greater lotus (*Lotus uliginosus* Schkuhr). *Journal of Genetics & Breeding* **51**, 235–238.

Kelman WM (1999) Genetics and breeding of low-tannin *Lotus pedunculatus* Cav. In '11th Australian plant breeding conference proceedings, Vol. 2'. (Eds P Langridge, A Barr, G Auricht, G Collins, A Granger, D Handford, J Paull) pp. 43–44. (CRC for Molecular Plant Breeding: Adelaide)

Kelman WM, Blumenthal MJ (1992) Lotus in south-eastern Australia: aspects of forage quality and persistence. In 'Proceedings of the 6th Australian society of agronomy conference, Armidale, NSW'. (Eds KJ Hutchinson, PJ Vickery) pp. 460–463. (Australian Society of Agronomy: Parkville, Vic.)

Kelman WM, Bowman AM (1996) Monitoring improvements in seed production and seedling vigour of greater lotus (*Lotus pedunculatus* Cav.) In 'Proceedings of the 8th Australian society of agronomy conference, Toowoomba, Qld'. (Ed. M Asgar) pp. 341–344. (Australian Society of Agronomy: Parkville, Vic.)

Kelman WM, Tanner GJ (1990) Foliar condensed tannin levels in Lotus species growing on limed and unlimed soils in south-eastern Australia. *Proceedings of the New Zealand Grasslands Association* **52**, 51–54.

Kelman WM, Blumenthal MJ, Harris CA (1997) Genetic variation for seasonal herbage yield, growth habit and condensed tannins in *Lotus pedunculatus* Cav. and *Lotus corniculatus* L. *Australian Journal of Agricultural Research* **48**, 959–968. doi:10.1071/A97024

McLaughlin BD, Clarke JB (1989) Lotus for pasture and seed production. New South Wales Agriculture and Fisheries, Agfact P2.5.30, Sydney.

Wilson G (1992) *Lotus pedunculatus* Cav. (greater lotus) cv. Sharnae. *Australian Journal of Experimental Agriculture* **32**, 794–795. doi:10.1071/EA9920794

Received 20 December 2005, accepted 3 October 2006