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Developing birdsfoot trefoil (*Lotus corniculatus* L.) varieties for permanent pasture applications in low latitude regions of eastern Australia

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Abstract. Birdsfoot trefoil (*Lotus corniculatus* L.) is a potentially important alternative legume for recharge landscapes in the high rainfall zone in eastern Australia. However, in the summer rainfall region in northern New South Wales (NSW) where birdsfoot trefoil has the greatest potential application, flowering and seed set are limited by short daylength. Consequently, existing birdsfoot trefoil cultivars do not set enough seed to develop a seedbank that sustains a productive persistent stand. A breeding program was undertaken to develop birdsfoot trefoil cultivars adapted to short photoperiod to increase the area sown to deep-rooted perennials in the grazing lands in eastern Australia. Three new birdsfoot trefoil experimental varieties, Phoenix, Venture and Matador, were developed through: (1) phenotypic selection within cv. Grasslands Goldie for flowering intensity and pod set, (2) phenotypic selection for these same traits in a broader sample of 49 world-sourced lines, and (3) selection for prostrate growth habit among progeny of pair-crosses between erect and prostrate accessions identified as productive in southern NSW. Following two cycles of selection for flowering prolificacy and pod set, the average number of umbels per stem in the Goldie-derived populations was five times greater than in the commercial Goldie population; this response to selection closely approximated the predicted response based on previous estimates of heritability and phenotypic variance for this trait. In comparison with Goldie, the Syn1 and Syn2 populations of the three experimental varieties consistently expressed earlier flowering maturity and higher seed yield potential in glasshouse and field trials in northern NSW. While germination rate and seedling vigour of the three experimental varieties was slightly less than Goldie, intensive selection pressure on reproductive traits did not compromise seasonal herbage production.

Additional keywords: cultivar development, perennial legume, plant breeding.

Introduction

The need for a broader suite of perennial legumes for temperate regions of eastern Australia has been recognised from at least the 1980s (Hill 1990; Kelman *et al*. 1992; Oram 1994). The potential of tannin-containing *Lotus* species, especially *L. corniculatus* L. (birdsfoot trefoil) and *L. uliginosus* Schk. (greater lotus) to increase the legume content of pastures growing on acidic infertile soils was proposed by Kelman (1991). Whereas the potential of greater lotus is known to be limited by narrow adaptive characteristics to high rainfall coastal districts (Ayres *et al*. 2006*b*), the potential zone of adaptation of birdsfoot trefoil in eastern Australia has been estimated to be comparable with the existing *Trifolium repens* L. (white clover) zone (~8 Mha) (Hill *et al*. 1996). Moreover, birdsfoot trefoil has been shown to have important application for low fertility acidic soils where average annual rainfall (AAR) is 650–1000 mm, especially in the summer rainfall zone of New South Wales (NSW) (Ayres *et al*. 2006*b*).

Birdsfoot trefoil is the most widely distributed of the *Lotus* species, with a primary distribution in the Mediterranean region and north Africa, and secondary spread to temperate grasslands of the United States of America (USA), Canada, South America and parts of Asia (Frame 2005). This wide adaptability is due to tolerance of infertile acidic soils (MacDonald 1946; Seaney and Henson 1970; Blumenthal and McGraw 1999) and tolerance of drought (MacDonald 1946; Hoveland *et al*. 1985) and winter hardiness (MacDonald 1946; Hughes and MacDonald 1951; Beuselinck and Grant 1995). Birdsfoot trefoil is tetraploid ($2n = 4x = 24$) and is cross-pollinated by insect vectors. There is

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substantial variation among ecotypes (Steiner 1999), and this has been exploited in cultivar development, mainly through recurrent phenotypic selection for seasonal herbage growth, seedling vigour and seed yield (Papadopoulos and Kelman 1999). Birdsfoot trefoil is suited to extensive systems of animal production (Marten and Jordan 1979; Hoveland *et al*. 1985), is bloat safe (Seaney and Henson 1970; Beuselinck and Grant 1995) and has high grazing value (Seaney and Henson 1970; Hoveland *et al*. 1985; Frame *et al*. 1998).

Birdsfoot trefoil was introduced to Australia and New Zealand in the early 1900s, but usage and spread were limited because rhizobia specific for birdsfoot trefoil were not present in the soil (Blumenthal *et al*. 1994). However, an effective symbiont was found and a productive cultivar, Grasslands Goldie, was developed in New Zealand (Widdup *et al*. 1988). In New Zealand, in situations where soil fertility is low, birdsfoot trefoil has been found to be the most persistent legume, and often the only surviving productive legume in old pasture trials (Scott and Charlton 1983). In Australia, assessment has been limited to germplasm evaluation on the Northern Tablelands of NSW (FitzGerald and Fogarty 1992; Hill *et al*. 1996), the Southern Tablelands of NSW (Kelman and Oram 1989; Kelman 1996; Kelman *et al*. 1997) and Central Victoria (Lolicato and Rogers 1997). Commercial applications are largely limited to pasture demonstrations with cv. Grasslands Goldie (Ayres *et al*. 2006*b*).

Previous research has shown that the northern limit to the zone of adaptation of birdsfoot trefoil in Australia is determined by photoperiod (Ayres *et al*. 2006*a*, 2006*b*). Although Goldie is a useful cultivar at high latitudes (Widdup *et al*. 1988), neither Goldie nor most other cultivars in use elsewhere in the world flower with sufficient intensity to support the levels of seedbank development and seedling recruitment necessary to sustain regeneration and persistence at the low latitudes (LL) of northern NSW (Ayres *et al*. 2007). Accordingly, it was proposed that there is a requirement for new birdsfoot trefoil varieties adapted to short photoperiod conditions (Ayres *et al*. 2007). Moreover, it was noted that if widespread adoption of new varieties were to be accompanied by increased susceptibility to fungal diseases as experience in the USA indicates (Beuselinck *et al*. 1994), maintenance of population density by seedling recruitment will be essential for long-term persistence of birdsfoot trefoil. Selection for seed production traits and high seedling recruitment is a conventional breeding strategy for achieving persistence in taprooted ‘crown formers’ like birdsfoot trefoil, where exposure to the cumulative stress load of a crown-rot pathogen complex limits the lifespan of individual plants (Beuselinck *et al*. 1994).

Taprooted perennial pasture plants like birdsfoot trefoil have the capacity to reduce deep drainage and recharge of ground water (Singh *et al*. 2003). Moreover, birdsfoot trefoil is relatively tolerant of both waterlogging and high levels of aluminium associated with soil acidity (Cocks 2001). Accordingly, within the research programs of the Cooperative Research Centre for Plant-based Management of Dryland Salinity, birdsfoot trefoil was identified as a potentially valuable species for recharge environments (Real *et al*. 2005). The cultivar development work described in the present study has been undertaken to address dryland salinity by developing birdsfoot trefoil varieties with enhanced flowering and seed production for recharge landscapes in high rainfall temperate regions (Dear and Ewing 2008).

The target environment for the breeding program was the northern upper catchment of the Murray–Darling Basin in the NSW Northern Tablelands and North-West Slopes, where agricultural enterprises are based on beef cattle, sheep and mixed farming. Birdsfoot trefoil has shown particular potential on the North-West Slopes of NSW in a summer dominant rainfall environment (Ayres *et al*. 2006*b*). However, cultivars developed from this program may also have application elsewhere in summer rainfall and winter rainfall regions of the high rainfall temperate perennial pasture zone. This contention is supported by climate modelling, which indicates a potential zone of adaptation of birdsfoot trefoil in Australia of 8–10 Mha (Hill *et al*. 1996). Ayres *et al*. (2006*b*) proposed that a 2.5-Mha birdsfoot trefoil zone where AAR is 650–1000 mm is a realistic industry objective. This would place birdsfoot trefoil usage in Australia on a similar level with homoclimes in South America (1.8 Mha), the USA (1.2 Mha) and eastern Europe (1.5 Mha) (Blumenthal and McGraw 1999).

The target environment for the breeding program is a zone of LL (28–31°S) in northern NSW, Australia, where short photoperiod (~14-h daylength on the summer solstice) inhibits the full expression of flowering, seed set, seedbank development and seedling recruitment that are prerequisites for persistence (Ayres *et al*. 2006*a*). Cultivars of birdsfoot trefoil available in Australia, or in use elsewhere in the world at correspondingly LL, do not flower in northern NSW with sufficient intensity to support the levels of seedling recruitment necessary to sustain regeneration and persistence (Ayres *et al*. 2007). However, the present study showed that a small number of accessions and the breeding line VR5 (Kelman *et al*. 1997; Kelman and Ayres 2004) flowered intensively at LL sites in northern NSW. Moreover, our previous research has shown that there is significant genetic variation and high heritability for flowering prolificacy within these germplasm bases that could be exploited to overcome this limitation through breeding (Kelman and Ayres 2004).

The present paper describes: (1) the breeding processes used to develop three new birdsfoot trefoil experimental varieties (Phoenix, Venture, Matador), (2) reports morphological description and agronomic performance data for the germplasm, and (3) quantifies the response to selection in the key seed yield component ‘umbels per stem’ that was used as a selection criterion to improve seed production in Phoenix and Venture.

Materials and methods

Breeding processes

The three birdsfoot trefoil cultivars (Phoenix, Venture and Matador) were developed for diverse grazing applications in LL environments in eastern Australia. The provenance of the parental germplasm from which the three cultivars were derived is presented in Table 1, and the breeding processes for each experimental variety are depicted in Fig. 1.

Phoenix and Venture were developed concurrently at Glen Innes (29°42′S, 151°42′E, alt. 1052 m) from two germplasm bases: a narrow germplasm base (cv. Grasslands Goldie) and a broad germplasm base (49 lines from LL origins). The selection process within the narrow germplasm base (Fig. 1, strategy 1) comprised two cycles of phenotypic selection. In the first cycle, 310 intensively flowering plants were selected from ~500 000 plants in an old stand of Goldie at Swan Vale, Inverell (29°47′S, 151°05′E, alt. 769 m); this plant number was estimated from population measurements undertaken at this site in a previous study (Ayres *et al*. 2006*b*). These selections were grown as half-sib families in a spaced-plant nursery at Glen Innes. Thirty-six plants were selected with >5 umbels per flowering stem. Twenty-two of these were retained based on growth vigour and morphological uniformity and these were allocated to two morphological groups (‘Goldie erect’ elite selections and ‘Goldie semi-erect’ elite selections). This segregation of selected plants into erect and semi-erect types is in accordance with the historical division of birdsfoot trefoil cultivars into faster-growing/less persistent (erect) and slower growing/more persistent, (semi-erect) types (Frame 2005).

Within the broad germplasm base (Fig. 1, strategy 2), a single round of phenotypic selection for flowering prolificacy (>5 umbels per flowering stem) among 3920 plants from 49 world-sourced, LL lines grown in spaced-plant nurseries at Glen Innes and Armidale (30°31′S, 151°40′E, alt. 1050 m), resulted in 24 selected plants. Seventeen of these plants were retained on the basis of high growth vigour and morphological uniformity, and these were allocated to the erect and semi-erect groups (LL erect, LL semi-erect). The final polycross stage separately recombined erect and semi-erect genotypes from the narrow and broad germplasm sources to produce the nucleus populations $\text{Venture}_{\text{Syn1}}$ and $\text{Phoenix}_{\text{Syn1}}$. Polycrossing was undertaken within morphological groups (as depicted in Fig. 1) in isolation using beehives and isolation cages.

Matador was developed at Canberra (35°06′S, 149°06′E, alt. 600 m) by pair-crossing four erect lines (cvv. Grasslands Goldie, Vega, Quimey and G46) with six prostrate accessions (CPI 123281, CPI 123282, CPI 122153, CPI 122159, CPI 122158 and CPI 115191). The origins of this parental germplasm and the rationale for its use in the development of Matador are provided by Kelman *et al*. (1997). F_1 progeny of these crosses were grown as single rows in the field. Seed of the F_1 was bulk-harvested and a large (~2000 plants) F_2 field nursery was established. Within this population, selection was based on a consistent set of traits (prostrate dense habit, grey-green leaf colour, light yellow flower colour) that characterise the Spanish accessions CPI 122153 and CPI 122158. Open-pollinated seed of 30 selections was bulk-harvested and used to grow an isolation block of 200 plants. Seed of this population constituted breeder seed of Matador ($\text{Matador}_{\text{Syn2}}$, Fig. 1).

Environmental conditions at the study sites

Data for the evaluation of the three birdsfoot trefoil cultivars was derived from two field nurseries (Glen Innes ‘Centre for Perennial Grazing Systems’, Macintyre Station ~40 km north of Inverell), and seeds laboratory and glasshouse facilities at the University of New England, Armidale. Glen Innes and Armidale are located on the Northern Tablelands and Macintyre Station (29°29′S, 151°03′E, alt. 509 m) is located on the adjoining North-West Slopes of NSW. The Northern Tablelands is a high rainfall (775–1000 mm AAR), elevated hilly landscape (alt. 750–1400 m) where photoperiod is low due to the combination of LL, frequent cloud cover and non-uniform

Table 1. Provenance of the parental germplasm used in the development of the birdsfoot trefoil experimental varieties Phoenix, Venture and Matador

Parental selections[A]	Source population[B]	Country of origin[C]
	Phoenix	
GLOC0357	Goldie 190 half-sib	New Zealand
GLOC0358	Goldie 128 half-sib	New Zealand
GLOC0359	Goldie 235 half-sib	New Zealand
GLOC0360	Goldie 1 half-sib	New Zealand
GLOC0361	Goldie 58 half-sib	New Zealand
GLOC0362	Goldie 73 half-sib	New Zealand
GLOC0363	Goldie 13 half-sib	New Zealand
GLOC0364	Goldie 25 half-sib	New Zealand
GLOC0365	PI 251827	Italy
GLOC0366	PI 162456	Uruguay
GLOC0367	PI 304126	Brazil
GLOC0368	Unknown	–
GLOC0369	PI 255305	Italy
GLOC0370	PI 433923	Brazil
GLOC0371	S1 378	Unknown
GLOC0372	cv. Steadfast	USA
GLOC0373	52935	Unknown
GLOC0374	PI 304126	Brazil
GLOC0375	PI 244036	Brazil
	Venture	
GLOC0376	Goldie 55 half-sib	New Zealand
GLOC0377	Goldie 316 half-sib	New Zealand
GLOC0378	Goldie 223 half-sib	New Zealand
GLOC0379	Goldie 294 half-sib	New Zealand
GLOC0386	Goldie 245 half-sib	New Zealand
GLOC0387	Goldie half-sib	New Zealand
GLOC0388	Goldie 252 half-sib	New Zealand
GLOC0389	Goldie 25 half-sib	New Zealand
GLOC0390	Goldie 159 half-sib	New Zealand
GLOC0391	Goldie 137 half-sib	New Zealand
GLOC0392	Goldie 179 half-sib	New Zealand
GLOC0393	Goldie 155 half-sib	New Zealand
GLOC0394	Goldie 170 half-sib	New Zealand
GLOC0395	Goldie 2 half-sib	New Zealand
GLOC0405	PI 251828	Italy
GLOC0406	PI 162456	Uruguay
GLOC0407	PI 300014	Portugal
GLOC0408	PI 418815	Italy
GLOC0409	PI 322555	Brazil
GLOC0410	S1626	Unknown
	Matador	
–	Goldie (G32)	New Zealand
–	G46	New Zealand
–	cv. Vega	USA
–	cv. Quimey	Chile
–	CPI 123281	Russia
–	CPI 123282	Russia
–	CPI 122153	Spain
–	CPI 122159	Italy
–	CPI 122158	Spain
–	CPI 115191	Yugoslavia

[A]Selected parental plants (New South Wales Department of Primary Industries working collection accession number).
[B]Population from which the parental plant was derived.
[C]The geographic origin of the source line.

horizon. The North-West Slopes receives less rainfall (600–775 mm AAR) and the heat factor is greater. A summary of long-term climate data for the two field sites is presented in Table 2. Seedlings were propagated in the glasshouse at Glen Innes for characterisation in the field at Glen Innes and Macintyre. The germination experiments were carried out in a standard germination cabinet, and seedling development work was undertaken in a controlled temperature glasshouse at the University of New England.

Vegetative characteristics and seed yield components

Three generations in the development of Phoenix and Venture were compared with Matador and Goldie for vegetative and reproductive characteristics with Matador and Goldie. These generations were: (i) the parental selections, (ii) the Syn1 populations, and (iii) the Syn2 'experimental varieties'.

Parental selections

The characteristics of the parental selections were determined on the selected plants (19 plants comprising $Phoenix_p$; 20 plants comprising $Venture_p$; 10 plants for each of Matador and Goldie) in 30-cm-diameter pots under glasshouse conditions. The parental selections of Phoenix and Venture were cloned in September 2002 from the selection nurseries at Glen Innes and Armidale. Mature plants of Matador and Goldie were re-potted from 10 cm pots to 30 cm pots at the same time. Morphological characterisation was undertaken on spring primary growth in November 2002 as follows: leaf length and width (mm) was measured on the middle trifoliate leaflet on the terminal leaf of eight representative stems per plant (leaf area was calculated assuming elliptical shape of the leaf); stem length (cm) was measured on the extended stem (eight stems per plant) from the base of the crown to the tip of the growing point;

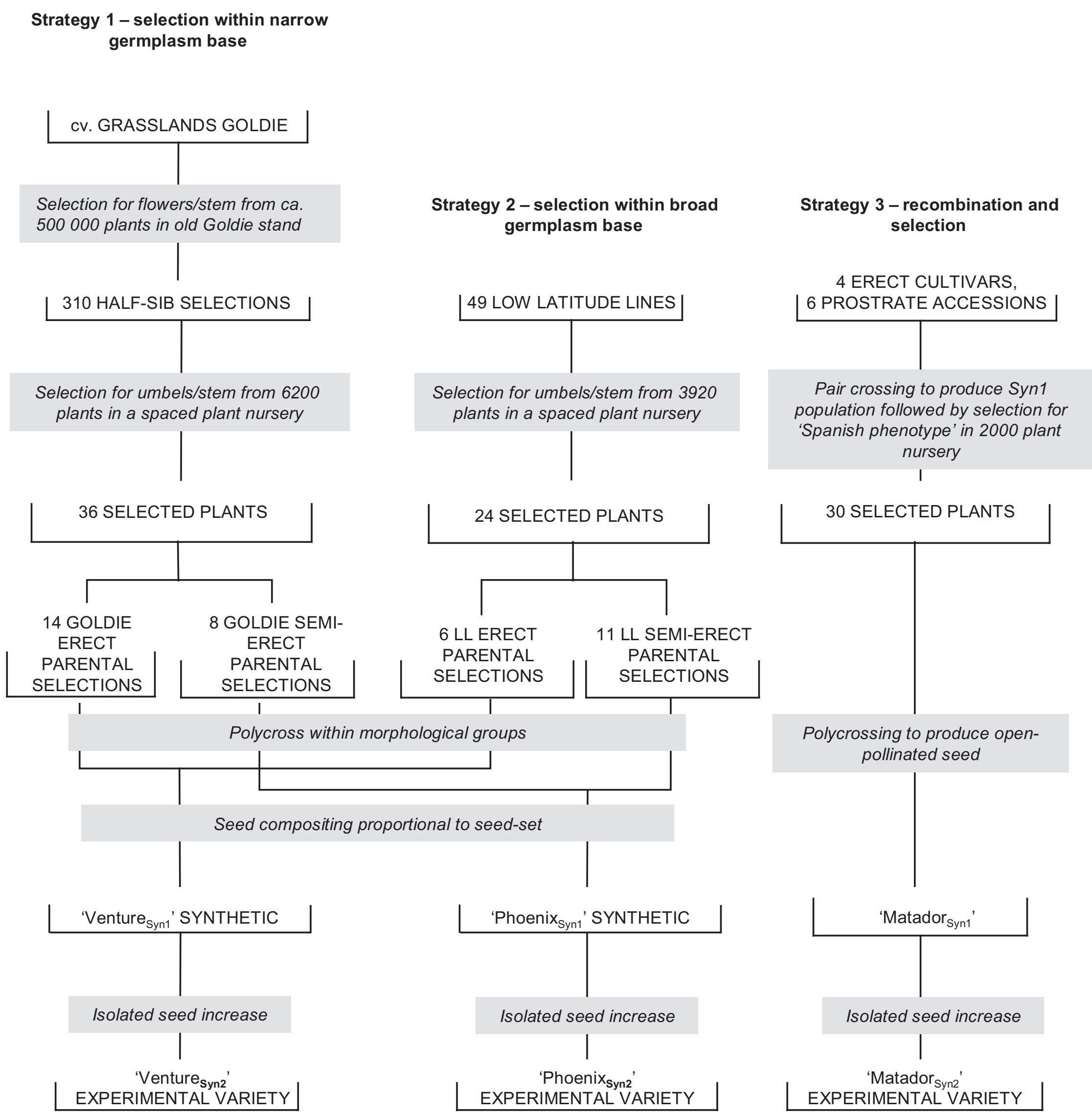

Fig. 1. The breeding processes used to develop the birdsfoot trefoil experimental varieties Phoenix, Venture and Matador for permanent pasture applications in eastern Australia.

stem thickness (mm) was measured as the diameter of the stem between the 3rd and 4th internodal segment (eight stems per plant); internodal length (mm) between the 2nd and 3rd nodes was measured as the distance (mm) between the 2nd and 3rd internodal segment from measurements on eight stems per plant; and, leaf : stem ratio was estimated by shredding leaf from stem (eight stems per plant) and weighing leaf and stem fractions on a dry matter (DM) basis. Reproductive characterisation was undertaken in summer 2002–03 and included the recording of phenological events and measuring the following seed yield components: flowering time was estimated as number of days from 1 September to full bloom; seed yield components (umbels, pods, seed) were measured on eight randomly chosen flowering stems per plant; branching was measured as number of tillers arising from the stem (eight flowering stems per plant); and 1000-seed weight was the mass (g) of 1000 seeds from each of eight flowering stems per plant.

Syn1 populations

Seeds of Venture$_{Syn1}$, Phoenix$_{Syn1}$, Matador$_{Syn1}$ and Goldie were propagated on 20 August 2003 in the glasshouse. The pots were transferred onto tables adjacent to the glasshouse and maintained under pot culture for measurement of early vigour (day 90, 19 November 2003), vegetative characteristics on autumn regrowth (2 April 2004), and reproductive characteristics on mature plants (summer 2004–05). The measurement protocols were the same as those applied to characterisation of the parental selections.

Syn2 'experimental varieties'

The characteristics of the Syn2 experimental varieties were determined on spaced plants in mixed sward culture with tall fescue (*Festuca arundinacea* Schreb. cv. Jessup MaxP) at two field sites (Glen Innes and Macintyre Station) using 2 by 1 m^2 plots and five replications). Seeds of Venture$_{Syn2}$, Phoenix$_{Syn2}$, Matador$_{Syn2}$ and Goldie were propagated in the glasshouse and seedlings were subsequently transplanted (20 birdsfoot trefoil plants per plot) into an established tall fescue sward at the Glen Innes site on 16 September 2005 and the Macintyre Station site on 21 September 2005. Measurements of early vigour (plant height, stem number) were undertaken on day 73 (Glen Innes, 19 November 2005; Macintyre, 2 December 2005) after transplanting; seasonal herbage growth (g DM per plant) was assessed as summer growth and winter growth following 90 days regrowth in the 1st growth cycle; and seed yield potential (number of stems, number of flowering stems, number of umbels per flowering stem) was measured on juvenile plants in their first summer (2005–06) at both sites.

All characterisation data were analysed using generalised linear models (GLM). Vegetative and reproductive characters for the parental selections and Syn1 populations (measured under pot culture at one site) used a simple model consisting of line (fixed) and replication (random) effects. The model for Syn2 populations (evaluated at the two field sites) included line, site and their interaction in the fixed model; and replication, plots within replications and plants within plots in the random model. Data were transformed as required to create uniform variance. Fixed effects were evaluated with Wald statistics ($P \leq 0.05$), and pairwise comparisons were conducted on significant effects using l.s.d. ($P \leq 0.05$).

Response to selection

Goldie-derived populations

The number of umbels per stem was used as a seed yield component to test predictions of response to selection in the breeding strategies that developed the two morphological groups (14 Goldie erect elite selections, eight Goldie semi-erect elite selections, see Fig. 1). In the response to selection equation, $r = ih^2\sigma_P$ (Falconer 1981) selection intensity (i = 6.3), was calculated as the sum of the selection intensities in the two cycles of the breeding process. The narrow sense heritability ($h^2 = 0.48$) was taken from Kelman and Ayres (2004) and the phenotypic standard deviation ($\sigma_P = 0.025$) was estimated from the cv. Grasslands Goldie population grown in 2003 and 2004. Because of non-normality of the data, values were $(x + 10)$ $\log_{10}$-transformed.

Syn1 and Syn2 populations

Response to selection for flowering intensity, based on measurements of umbels per stem was monitored at three stages in the breeding program: at the four morphological group stage (after two cycles of selection for flowering intensity); in the two

Table 2. Climate summary for the field nurseries (Glen Innes Meteorological Station 056013, Pindari Dam Meteorological Station 05410)
Pindari Dam meteorological station is 15 km north-east of the Macintyre station site, New South Wales

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Glen Innes meteorological station													
Mean daily max. temp. (°C)	25.2	24.4	23.1	19.8	16.2	13.1	12.4	13.9	16.8	19.8	22.1	24.4	–
Mean daily min. temp. (°C)	13.5	13.3	11.4	7.9	4.9	1.8	0.7	1.2	4.1	7.1	9.8	12.0	–
Mean monthly rainfall (mm)	106	94	72	41	50	43	57	49	54	78	88	108	848
Mean daily evaporation (mm)	171	134	133	93	62	48	53	75	105	137	153	174	1336
Number of frosts	0	0	1	4	11	17	21	20	12	5	1	1	92
Pindari Dam meteorological station													
Mean daily max. temp. (°C)	31.2	30.5	28.9	25.5	21.5	17.9	17.1	18.8	22.1	25.1	27.5	30.2	–
Mean daily min. temp. (°C)	17.5	17.2	14.8	10.8	7	3.2	2.1	2.9	6.2	10.1	13.5	16.1	–
Mean monthly rainfall (mm)	99	85	66	47	47	33	47	38	47	72	91	88	758
Mean daily evaporation (mm)	233	193	183	129	84	60	65	93	129	171	195	233	1788
Number of frosts	0	0	0	0	4	13	18	15	6	1	0	0	57

Syn1 populations (Venture$_{Syn1}$ and Phoenix$_{Syn1}$) following population intercrossing of the erect and semi-erect lines); and similarly in the two Syn2 populations (Venture$_{Syn2}$ and Phoenix$_{Syn2}$) derived from inter-crossing within the respective Syn1 populations. The number of umbels per stem at the four morphological group stages was recorded in two seasons 2002–03 and 2004–05. A repeated-measure ANOVA in GENSTAT 8.1 on $\log_{10}$- transformed values was used to compare the four morphological group means over the 2 years. Differences between population means in the Syn1 generation were tested using Fisher's l.s.d. following ANOVA of a completely randomised design with three replications at one site (Glen Innes). Differences between population means in the Syn2 generation were tested using Fisher's l.s.d. following ANOVA of a completely randomised design with five replications at the two sites (Glen Innes and Macintyre). Data from the Syn1 and Syn2 generations were $\log_{10}$-transformed before analysis.

Seed quality, germination rate and seedling development

Seed size distribution was assessed by screening seed lots from each of the four lines Phoenix$_{Syn2}$, Venture$_{Syn2}$, Matador$_{Syn2}$ and Goldie through a series of graduated screens providing seed lots with the following dimensions: large seed (>1.4 mm), medium-large seed (1.18–1.4 mm), medium seed (1.0–1.18 mm) and small seed (<1.0 mm). The portions of seed in each category were weighed and results for 'graded seed yield' (% seed >1.00 mm) are presented (Nowak *et al*. 1993). Measurement of 1000-seed weight was undertaken on graded seed (seed >1.00 mm); this was comparable with commercial grading. Eight replicate subsamples (100 seeds per rep) were sampled from graded seed of each line using a grid technique (Ellis *et al*. 1985) and weighed to an accuracy of 0.01 g; the predicted mean values were multiplied by 10 to derive 1000-seed weight. Data were analysed in GENSTAT using ANOVA for 1000-seed weight and log linear model analysis for the seed size distribution data.

A germination test complying with international procedures (Ellis *et al*. 1985) was undertaken on the Syn2 populations and Goldie. Seed was scarified by hand between sheets of fine sandpaper, applying light pressure in a circular motion, and for 30 s at which point dust from abrading the seed coat became apparent. Design included the four populations, four replications per population and 50 seeds per replication using randomised blocks to account for position effects in a germination cabinet that was maintained at 20°C under continuous light for 12 days. Germination rate was calculated using the formula 'number of germinates ÷ days to first count + ….+ number of germinates ÷ days to final count' (Maguire 1962), and data were analysed using repeated-measures ANOVA.

Seedlings of each of the Syn2 populations and Goldie were progressed from the germination test by transplanting germinates into trays when radicle length was 2 mm; this occurred on days 2–3 after germination. Seedlings were inoculated with rhizobium, treated with liquid fertiliser (Aquasol, Mount Druitt, NSW) supplemented with slow release fertiliser (Nutricote blue, Tokyo, Japan), and maintained in a controlled temperature glasshouse (20–28°C) with daily watering to provide non-limiting nutrition. Design comprised the four populations, 21 replications and 294 seedlings per replication in a randomised complete block design. Seedling vigour was assessed as rate of development to different stages of seedling maturity (first leaf, 3-leaf stage, 5-leaf stage and initiation of branching), seedling height and seedling (fresh weight) mass at day 42 after germination. Data were analysed using GLM.

Results

Reproductive characteristics and seed yield components

Parental selections

The parental selections were at least 10 days earlier in flowering maturity, produced a greater number of umbels per stem and pods per stem, and seed was higher in 1000-seed weight than for Goldie (Table 3). Branching of these parental selections and of Matador during reproductive growth was also greater than for Goldie. The progenitors of Phoenix produced more seeds per stem than the progenitors of Venture and matured earlier.

Syn1 generation

The Syn1 generation of the three breeding lines (Phoenix$_{Syn1}$, Venture$_{Syn1}$ and Matador$_{Syn1}$) generally expressed improved seed yield components compared with Goldie (Table 3). Branching of Phoenix$_{Syn1}$ and Venture$_{Syn1}$ during reproductive growth was greater than Goldie and Matador$_{Syn1}$. Phoenix$_{Syn1}$, Venture$_{Syn1}$ and Matador$_{Syn1}$ expressed earlier flowering maturity than Goldie and 1000-seed weight for the three breeding lines was comparable with Goldie.

Syn2 generation

Phoenix$_{Syn2}$ and Venture$_{Syn2}$ produced greater numbers of flowering stems and umbels per flowering stem than Goldie at both field sites (Table 4). Matador$_{Syn2}$ produced more flowering stems than Goldie at both sites, and more umbels per flowering stem than Goldie at the Macintyre site but not at the Glen Innes site. At both sites, Goldie produced a large number of stems but few flowering stems. For example, at Glen Innes, while only 3% of Goldie's stems were flowering stems, flowering stems as a proportion of total stems was 49% for Phoenix$_{Syn2}$, 24% for Venture$_{Syn2}$ and 35% for Matador. While Goldie produced more flowering stems at Macintyre (19%) than at Glen Innes (3%), Phoenix$_{Syn2}$ produced 51%, Venture$_{Syn2}$ produced 62% and Matador produced 43% flowering stems at Macintyre.

Genetic gain in umbels per stem

The predicted response to two cycles of selection for umbels per stem in the parental selections (using the selection response equation $r = ih^2\sigma_P$ on $\log_{10}$-transformed values) was 0.08, which was equal to the realised response measured as the difference between the Goldie erect and semi-erect and Goldie populations (1.10 for the Goldie parental selections compared with 1.02 for cv. Goldie on transformed values, Table 5). Using back-transformed values, this represented a gain of ~2 umbels per stem over the two cycles of selection. The parental LL-derived populations (from the broad germplasm base) had a significantly greater mean number of umbels per stem than the Goldie-derived populations (narrow germplasm base), and both

Table 3. Seed yield components of the parental selections and Syn1 generation used in the development of the birdsfoot trefoil experimental varieties Phoenix, Venture and Matador in comparison with cv. Grasslands Goldie

The sampled fraction for seed yield components was the flowering stem. Within columns, means followed by different letters are significantly different ($P \leq 0.05$)

	Flowering maturity (days)[A]	No. of branches/stem	No. of umbels/stem	No. of pods/stem	No. of seeds/stem	1000-seed weight (g)
			Parental selections[B]			
Goldie	120a	1.2b	0.2b	0.6b	6c	0.72b
Phoenix parents	106b	7.6a	2.9a	8.2a	102a	1.16a
Venture parents	110b	5.5a	1.5a	3.6a	32bc	1.00a
Matador parents	109b	6.9a	2.1a	5.2a	50ab	1.17a
			Syn1 generation[C]			
Goldie	127a	2.0b	0.4c	1.0b	11.2c	0.726
$Phoenix_{Syn1}$	120c	4.4a	2.3b	7.2a	47.7b	0.781
$Venture_{Syn1}$	124ab	4.6a	2.4b	5.9a	52.1a	0.905
$Matador_{Syn1}$	123bc	2.1b	3.1a	5.0a	48.9a	0.885

[A]Day number (from first day of spring) when flowering commenced.
[B]Pot culture; measurements undertaken on mature plants in monoculture; the sampled fraction for seed yield components was the flowering stem.
[C]Field culture; seed yield components measured on mature plants in the second summer (2004–05).

had significantly more umbels per stem than the commercial Goldie population. The two Syn1 populations ($Phoenix_{Syn1}$ and $Venture_{Syn1}$) had similar mean numbers of umbels per stem, and both were significantly greater than Goldie (Table 5). There was no evidence of hybrid vigour for the number of umbels per stem in the Venture and Phoenix Syn1 and Syn2 populations, compared with their respective parental populations. In fact, the number of umbels per stem of Venture and Phoenix in the Syn1 and the Syn2 grown at Glen Innes were much closer to the value for the lower of the two parental populations (i.e. Goldie erect and Goldie semi-erect, respectively). However, this comparison is biased by the fact that the Syn1 and Syn2 values were obtained from plants in their first flowering season and were compared with values from larger, adult plants of the parental populations. It is expected that seed yields of the Syn1 and Syn2 populations in the second season will be at least intermediate between the parent values.

Vegetative characteristics, seedling vigour and seasonal herbage growth

$Phoenix_{Syn1}$ was medium-leafed, fine-stemmed and high yielding relative to Goldie while $Venture_{Syn1}$ was large-leafed, medium in stem thickness, vigorous in warm season growth and intermediate in cool season growth (Table 6). $Matador_{Syn1}$ was small-leafed (Table 6), thick-stemmed with short internodes and low in cool-season growth activity. The characterisation data are consistent for the parental and Syn1 generations, although Matador was inexplicably large leafed in the progenitor population (Table 6). The leaf : stem results for the parental and Syn1 generations indicate that Matador had greater leaf density than Goldie and the other two lines (Table 6).

$Phoenix_{Syn2}$ and $Venture_{Syn2}$ expressed relatively lower germination rate and slower seedling development through early leaf stages than Goldie (Table 7). The time interval to initiation of branching was 31.6 days for Goldie and ~1 day later for

Table 4. Seed yield components of the Syn2 generation of the birdsfoot trefoil experimental varieties Phoenix, Venture and Matador in comparison with cv. Grasslands Goldie

Measurements made on plants propagated from Syn2 seed of Phoenix, Venture and Matador and commercial seed of cv. Grasslands Goldie. Plants were spaced in a mixed sward culture (tall fescue) at two field sites; measurements were undertaken on juvenile plants in their first summer on a common date. Within columns, means with different letters are significantly different ($P \leq 0.05$)

	Glen Innes site			Macintyre site		
	No. of stems[A]	No. of flowering flowering stems[B]	No. of umbels/stem[C]	No. of stems[A]	No. of flowering stems[B]	No. of umbels/stem[C]
Goldie	19.0a	0.6c	0.6c	18.6ab	3.5c	1.9c
$Phoenix_{Syn2}$	11.3b	5.5a	3.0a	13.1c	6.7b	3.1b
$Venture_{Syn2}$	12.1b	2.9b	2.0b	16.4b	10.2a	4.3a
$Matador_{Syn2}$	10.1b	3.5b	1.1c	21.0a	9.0a	3.0b

[A]Number of stems arising from crown. [B]Number of stems with flowers (buds or umbels).
[C]Number of umbels per flowering stem.

Table 5. Means for the number of umbels per stem in four selected populations of birdsfoot trefoil [Goldie erect, low latitude (LL) erect, Goldie semi-erect, LL semi-erect], the Syn1 populations derived from inter-crossing of erect and semi-erect populations from respective Goldie and LL populations, and the Syn2 populations derived from inter-crossing within the respective Syn1 populations at two sites (Glen Innes and Macintyre)

At each stage, populations were compared with unselected plants from cv. Grasslands Goldie. Values in parentheses are $\log_{10}(x + 10)$-transformed values

	Parental selections (morphological groups)					
	Goldie erect[A]	LL erect[B]	Goldie semi-erect[A]	LL-semi-erect[B]	Goldie	l.s.d. (P = 0.05)
Glen Innes 2002–03	1.2	5.7	2.5	4.9	0.6	–
Glen Innes 2004–05	4.3	5.3	2.8	10.0	0.2	–
Mean	2.7 (1.10)	5.5 (1.18)	2.7 (1.10)	7.2 (1.21)	0.4 (1.02)	(0.07)
			Syn1 populations			
	$\text{Venture}_{\text{Syn1}}$	$\text{Phoenix}_{\text{Syn1}}$	Goldie	l.s.d. (P = 0.05)		
Glen Innes	2.4 (1.09)	2.3 (1.08)	0.4 (1.02)	(0.02)		
			Syn2 populations			
	$\text{Venture}_{\text{Syn2}}$	$\text{Phoenix}_{\text{Syn2}}$	Goldie	l.s.d. (P = 0.05)		
Glen Innes	2.0 (1.07)	2.9 (1.11)	0.6 (1.02)	(0.02)		
Macintyre	4.3 (1.15)	3.1 (1.11)	2.0 (1.07)	(0.02)		

[A]Morphological groups derived from narrow (Goldie) germplasm base.
[B]Morphological groups derived from broad (LL lines) germplasm base.

$\text{Phoenix}_{\text{Syn2}}$, $\text{Venture}_{\text{Syn2}}$ and $\text{Matador}_{\text{Syn1}}$. Seedling mass and seedling height of $\text{Phoenix}_{\text{Syn2}}$ and $\text{Venture}_{\text{Syn2}}$ at day 42 were lower than Goldie, and while $\text{Matador}_{\text{Syn1}}$ was least in seedling height, it was comparable in seedling mass with Goldie. However, by day 90 and under field conditions, $\text{Phoenix}_{\text{Syn2}}$ and $\text{Venture}_{\text{Syn2}}$ were taller than Goldie in plant height, but had produced fewer vegetative stems (Table 7).

Discussion

A plant introduction program to investigate the potential of *Lotus* for south-eastern Australia was initiated in 1988 (Kelman 1996), and an Australian *Lotus* breeding program (with birdsfoot trefoil and greater lotus) commenced in 1990 (Kelman 1991). For birdsfoot trefoil, the breeding objectives were: (i) improved tolerance of drought and (ii) increased seedling regeneration (Kelman 1991). This program led to the development of the Matador germplasm developed by pair-crossing erect cultivars with persistent prostrate accessions and selecting for the Spanish phenotype (Kelman *et al*. 1997; Fig. 1, strategy 3). This early breeding project based at Canberra in southern NSW was supplemented subsequently by recurrent selection from both a narrow germplasm base (old stand of cv. Grasslands Goldie) and a broad germplasm base (49 LL lines) in selection nurseries in northern NSW, with intensive selection pressure applied for flowering prolificacy (Fig. 1, strategies 1 and 2). This second phase of the Australian *Lotus* breeding program was previously described by Kelman and Ayres (2004) who reported that significant genetic variation in flowering prolificacy, flowering maturity and growth habit was evident within the narrow germplasm base. Moreover, high heritability estimates for seed yield components (Kelman and Ayres 2004) signalled that selection for umbels per stem should prove effective in developing prolific and persistent birdsfoot trefoil varieties.

Table 6. Vegetative and growth characteristics of the parental selections and Syn1 populations used in the development of the birdsfoot trefoil experimental varieties Phoenix and Venture in comparison with the VR5 germplasm (Matador) and cv. Grasslands Goldie

Within columns, means with different letters are significantly different ($P \leq 0.05$)

	Leaf area (mm^2)	Stem length (cm)	Stem thickness (mm)	Internode length (mm)	Plant height (cm)	Leaf: stem ratio	Summer growth[B]	Winter growth[B]
				Vegetative characteristics of the parental selections				
Goldie	76.3b	23.1a	1.3c	17.0b	12.7b	2.2b	–	–
Phoenix parents	70.7c	18.3c	1.4c	17.0b	6.9c	2.3b	–	–
Venture parents	87.5b	20.2b	1.5b	18.3a	17.9a	2.0b	–	–
Matador parents	95.3a	13.4d	1.8a	18.3a	6.1c	3.3a	–	–
				Vegetative characteristics of the Syn1 populations[A]				
Goldie	97b	–	1.06c	17.2c	–	1.57	9.5b	8.8c
$\text{Phoenix}_{\text{Syn1}}$	88b	–	1.12c	20.5b	–	1.47	14.9a	20.0a
$\text{Venture}_{\text{Syn1}}$	111a	–	1.22b	22.5a	–	1.58	14.7a	14.0b
$\text{Matador}_{\text{Syn1}}$	68c	–	1.35a	13.1d	–	1.68	14.4a	6.6c

[A]Morphological measurements (leaf area, stem thickness and internode length) were undertaken on autumn vegetative growth.
[B]Seasonal herbage growth (g dry matter/plant, 93 days regrowth) undertaken on juvenile plants in first growth cycle.

Table 7. Seedling vigour and herbage growth of the Syn2 populations of the Phoenix, Venture and Matador birdsfoot trefoil experimental varieties in comparison with cv. Grasslands Goldie

Measurements made on plants propagated from Syn2 seed of Phoenix, Venture and Matador and commercial seed of cv. Grasslands Goldie. Within columns, means with different letters are significantly different ($P \leq 0.05$)

	Graded seed yield (%)[A]	1000-seed weight (g)[B]	Germination rate[C]	Developmental pattern of seedling growth: No. of days to first leaf[D]	No. of days to 3 leaf[D]	No. of days to 5 leaf[D]	No. of days to branching[D]	Seedling height (mm)[E]	Seedling mass (mg FW)[E]
Goldie	–	1.26a	22.8a	16.9a	25.6a	35.3a	31.6a	77.5a	444a
$Phoenix_{Syn2}$	82	1.22a	16.1b	17.1b	26.4b	36.1b	32.5b	74.2b	385c
$Venture_{Syn2}$	59	1.14b	14.9bc	17.5bc	26.6b	35.9b	32.5b	74.9ab	393bc
$Matador_{Syn2}$	84	1.05c	13.5c	18.8c	30.9c	38.2c	32.4b	64.8c	430ab

Early vigour and herbage growth[F]

	Plant height[G] (cm) GI	Plant height[G] (cm) Mac.	Stem number[G] GI	Stem number[G] Mac.	Summer growth[H] GI	Summer growth[H] Mac.	Winter growth[H] GI	Winter growth[H] Mac.
Goldie	23.7c	23.8b	13.1a	9.1a	3.0b	7.6a	3.6a	4.5a
$Phoenix_{Syn2}$	30.0ab	28.9a	7.9b	6.0b	5.2a	4.2b	3.5a	1.6b
$Venture_{Syn2}$	30.3a	31.5a	7.8b	7.2ab	3.4b	6.5a	1.6b	4.0a
$Matador_{Syn2}$	27.2b	26.4b	8.7b	10.2a	3.4b	4.1b	0.7c	0.7c

[A]Proportion of seed >1.00-mm seed size.
[B]1000-seed weight of seed >1.00 mm.
[C]Cumulative number of germinations per day.
[D]No. of days to reach each developmental stage.
[E]Height and mass 42 days after germination.
[F]Spaced plants in mixed sward culture with tall fescue at two field sites, Glen Innes (GI) and Macintyre (Mac.).
[G]Early vigour measurements undertaken on day 73 after transplanting.
[H]Herbage growth (g dry matter/plant) undertaken on juvenile plants in first growth cycle.

The results of the breeding program for reproductive characteristics (Tables 3 and 4) show that all three germplasms, Phoenix, Venture and Matador, expressed consistently better seed yield components than Goldie across three consecutive generations. In the Syn2 generation, although only juvenile plants in their first summer, the experimental varieties produced both a greater number of flowering stems and a greater number of umbels per flowering stem than Goldie. It is anticipated that as mature plants, the three experimental varieties will produce even greater seed yield components to overcome the previous limitations in existing birdsfoot trefoil varieties of poor seedling recruitment under LL conditions.

Although data for flowering maturity are not presently available for the Syn2 generation, results for progenitor generations show that the three breeding lines were earlier in flowering maturity than Goldie. For example, Phoenix was 14 days earlier than Goldie at the ‘parental germplasm’ generation, and 7 days earlier than Goldie at the Syn1 generation. Venture and Matador were consistently intermediate between Phoenix and Goldie in flowering maturity.

The close agreement between the predicted and realised response to selection for umbels per stem validates the importance of this trait for improving seed yield components in these populations. Although the parameters of the equation strictly only apply to Goldie and its derived populations, it is likely that selection for this trait will be effective in the germplasm derived from the combination of selections from both the narrow and broad germplasm sources (Fig. 1) in the production of the synthetic cultivars Phoenix and Venture. Previous analysis of seed yield components in North America has also identified ‘the number of umbels setting seed’ (essentially the same trait as number of umbels per stem) as the trait with the greatest influence on seed yield (Albrechtsen *et al.* 1966). Our results support the use of phenotypic selection as an initial strategy for population improvement in seed yield. Further improvements can be expected by utilising progeny testing strategies, as shown by Sanda and Twamley (1973) in cv. Leo birdsfoot trefoil in Canada.

In comparing the Syn1 and Syn2 populations with their respective parent populations, it was notable that in only one instance ($Venture_{Syn2}$ at Macintyre, Table 4) did the mean number of umbels per stem approach that of the higher (LL) parental population (Table 5). This suggests that the combining ability of some of the genotypes with respect to this trait was not high. An analysis of combining ability among the parental types by diallele matings, should uncover more optimal combinations among parents in the make-up of further synthetics from these germplasm sources. A further source of improvement could be to exploit the substantial variation remaining within the Syn2 populations. This variation, measured as the proportion of residual to total sums of squares in ANOVA of the Syn2 populations, was 45% at Glen Innes and 49% at Macintyre. Also, the markedly higher seed production capability (particularly from increase in number of flowering stems) of Phoenix at Macintyre compared with Glen Innes suggests a potential importance for significant genotype × environment interactions between the synthetics and their target environments in eastern Australia.

For the two breeding lines, Phoenix and Venture, which were developed under LL conditions with intensive selection pressure on these reproductive traits, there was no evident negative impact on agronomic performance. In the Syn1 generation, Phoenix and Venture produced both significantly greater warm season herbage growth and cold season herbage growth than Goldie (Table 6). In the Syn2 generation (Table 7), the herbage growth of Phoenix was best at the Northern Tablelands site, while Venture was best at the North-West Slopes site. Matador (derived from Mediterranean germplasm sources and with selection pressure on prostrate dense habit) expressed relatively lower winter growth activity (Tables 6 and 7), reflecting its prostrate growth habit. However, the warm-season growth activity of Matador was generally comparable with Goldie, and its prostrate habit despite lower herbage yield potential may nonetheless confer the advantage of better persistence under close grazing (Kelman 1996).

In terms of seedling vigour, the three breeding lines were lower than Goldie in germination rate and slower in their early developmental pattern to day 42 after germination. However, these effects were small (Table 7) and may reflect different seed size distributions in the seed lots of ungraded breeder seed, rather than intrinsic differences in seed quality or seedling vigour (Wiedemann 2005). For example, while a greater proportion of Phoenix seed was relatively larger in seed size than Venture and Matador (33, 17 and 7% exceeded 1.18-mm seed size for Phoenix, Venture and Matador, respectively), and seedling vigour may be positively associated with seed size (Carleton and Cooper 1972; Beuselinck and McGraw 1983), this association is equivocal (Twamley 1967; McKersie and Tomes 1982). Moreover, in the present study these effects had dissipated under field conditions by day 90 after germination (Table 7), such that all three breeding lines expressed greater plant height than Goldie at both sites.

In conclusion, the breeding program has developed three new birdsfoot trefoil experimental varieties that have provisional Plant Breeders Rights (PBR) protection and are currently being progressed through cultivar development processes (PBR examination, seed multiplication, merit testing) for commercialisation from 2008. The three experimental varieties Phoenix, Venture and Matador are strongly contrasting in growth habit and vegetative morphology to provide for diverse grazing applications in the high rainfall (750–1000 mm AAR) temperate perennial pasture zone. All three experimental varieties express improved seed yield components and favourable agronomic performance at LL in eastern Australia. The magnitude of these components of seed set and seed production capability (early flowering maturity, high intensity of flowering stems, prolific number of umbels per stem) indicates that seed set and seedbank development of all three lines will be adequate to ensure sustained regeneration and population persistence under short daylength conditions. These new varieties should also express high seed production capability especially where seed production occurs in a high latitude locale. High seed production capability should be an incentive for the seeds industry to produce seed to supply adequate quantities of commercial seed at a price conducive for ready adoption of birdsfoot trefoil in pasture development. Where successful, these new cultivars can be expected to contribute a new perennial pasture legume adapted to low fertility acidic soils and highly variable climate conditions, and increase the area of grazing lands based on deep-rooted perennials.

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