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Heterosis in lucerne testcrosses with *Medicago arborea* introgressions and Omani landraces and their performance in synthetics

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Abstract. Testcrosses were made with novel sources of lucerne germplasm. These were evaluated in the field in a subtropical environment to identify the lines which produced the highest yielding hybrids as a guide to future breeding efforts. The novel sources were derivatives of *Medicago sativa* × *M. arborea* partial (asymmetric) hybrids (termed **sac**) and very highly winter-active Omani landraces of *M. sativa*. As testers, 2 lines were used; a *Colletotrichum trifolii* race 2 resistant selection from the group 9 Australian-bred and adapted cultivar PacL 901 (selection hereafter termed 901) and the Omani landrace, Oman 2, collected at 17°N latitude, from Salalah, Oman. In the row experiment, substantial and significantly positive tester parent heterosis for overall yield (sum of 13 harvests) was observed in all of the **sac** × Oman 2 testcrosses, with the mean performance of the 11 testcrosses (1839 g/m row) significantly ($P < 0.05$) exceeding the mean performance of the **sac** × 901 testcrosses (1703 g/m row) evaluated. Where 901 was used as the tester, heterosis values relative to the tester for the same **sac** lines were negative for all testcrosses with 8 of the testcrosses being significantly negative. For the Omani landrace × 901 testcrosses, positive and negative heterosis values for total yield relative to the tester were observed, but none were significantly different from zero. The 901 tester yielded significantly ($P < 0.05$) more *per se* than the Oman 2 tester (1956 *v.* 1470 g/m row), although in an adjacent sward experiment Oman 2 yielded comparably to most of the standard commercial cultivars.

The potential of the novel germplasm in the subtropics was verified in sward experiments with synthetics and/or strain crosses with yield increases of up to 42% over the benchmark synthetic Sequel. Further improvements can be expected following selection for disease and pest resistance within the lines and in the case of Oman 2 and **sac**, converging to maximise complementary gene action.

Additional keywords: alfalfa, arborea × sativa, heterosis, Omani landraces.

Introduction

Lucerne (*Medicago sativa* L.) is one of the world's most important cultivated forage legumes with over 32 million ha sown worldwide, of which 3.5 million ha are in Australia (Michaud *et al*. 1988; Pearson *et al*. 1997). The wide use of lucerne is driven by its possessing valuable agronomic traits including high feeding value, its perenniality and its ability to fix atmospheric nitrogen. Lucerne is autotetraploid (Stanford 1951), as well as being allogamous and highly subject to inbreeding depression. These biological characteristics have dictated that most lucerne cultivars are bred by recurrent phenotypic selection usually within half-sib families and commercialised as synthetic cultivars after 3–4 generations of panmictic reproduction of varying numbers of S_0 parental clones (Tysdal *et al*. 1942).

Effectively, lucerne breeding methodologies have not changed over several decades, and this has been a contributing factor to yield stagnation, which has developed in lucerne in both North America (Lamb *et al*. 2006) and Australia (Lowe *et al*. 2010). In breeding lucerne, 2 subspecies have been extensively used; purple-flowered *M. sativa* subsp. *sativa* and yellow-flowered *M. sativa* subsp. *falcata*. Falcata has been used to introduce traits into the lucerne gene pool, including winter hardiness (Lesins and Lesins 1979) and disease resistance (Havey *et al*. 1987). Inter-mating between multiple lucerne germplasms has been extensively practised over the period since 1950 in an attempt to ingress multiple disease and pest resistances, and this practice is thought to have reduced opportunities to exploit complementary gene interactions which are important to maximising lucerne yields (Bingham *et al*. 1994; Bhandari *et al*. 2007). Brummer (1999) proposed a semi-hybrid model for lucerne breeding, which involved separately improving, by recurrent selection, lucerne populations that are known to exhibit heterosis, and by converging these at commercialisation

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to produce a semi-hybrid. This convergence provides maximum opportunities for complementary gene interactions and exploitation of heterosis. The effectiveness of this approach is contingent upon identifying populations which exhibit heterosis in material from the 9 recognised germplasm sources of lucerne (Barnes *et al*. 1977). Heterosis between falcata and sativa has been demonstrated (Mackie *et al*. 2005; Riday and Brummer 2005), and the potential of non-dormant Peruvian germplasm as a heterosis source with semi-dormant lucerne has been suggested (Maureira *et al*. 2004). Irwin *et al*. (2008) demonstrated that general combining ability effects were more important than specific combining ability effects (SCA) in crosses between clones representing a wide range of dormancies from improved populations, whereas SCA effects were more important in crosses between clones from improved and unimproved populations. These results are consistent with those reported by Segovia-Lerma *et al*. (2004) who found SCA effects were not detected when highly recombined parents were used to generate the hybrids.

While there is considerable genetic diversity in the lucerne gene pool, there are germplasm sources which have not been extensively studied for their potential to express heterosis. One such source is from the Arabian Peninsula where lines have extreme winter activity and rapid regrowth following harvest (Smith *et al*. 1995). Little research has been done on landraces from the Arabian Peninsula, compared with the 4 recognised sources of non-dormant germplasm, namely Peruvian, Chilean, African and Indian, of which Peruvian is the most genetically distinct (Maureira *et al*. 2004). Another possible source of traits not well developed in the lucerne gene pool is *Medicago arborea*, a long-lived (up to 30 years) shrub, which is used as forage in its place of origin, the Mediterranean Sea coast and islands (Small and Jomphe 1988). *M. arborea* has seeds 400% larger than lucerne, a reputation for drought resistance (Lefi *et al*. 2004) and absence of winter dormancy (Nenz *et al*. 1996).

Partial (asymmetric) hybrids have been made between sativa and arborea by crossing with male sterile sativa clones (Bingham 2005; Armour *et al*. 2008; Bingham *et al*. 2009). The hybridisation barrier between arborea and sativa is post-zygotic failure of endosperm/embryo development (Fridriksson and Bolton 1963); however, in certain male sterile lucerne genotypes this barrier appears to be weakened, allowing fertilisation or genetic exchange by other means to occur. The hybrids carry predominately the sativa genotype except for introgressions of only small parts of arborea chromosomes confirmed by DNA analysis and are near tetraploid (Armour *et al*. 2008). A similar phenomenon was reported by de Wet *et al*. (1984) for teosinte/maize partial hybrids and for introgression hybrids between *Brassica napus* and *Orychophragmus violaceus* (Cheng *et al*. 2002) among others. Bingham (2005, 2009) provides a description of 10 sativa × arborea (**sac**) hybrids generated at the University of Wisconsin (UW). Each hybrid shows different arborea traits, indicating that in each **sac** plant, different parts of the arborea genome have been introgressed. The **sac** hybrids show up to 50% of normal female fertility when crossed with lucerne but most produce only small quantities of pollen. Limited quantities of seed have been produced by inter-crossing **sac** 1 – **sac** 10 in Madison, Wisconsin and the seed of these **sac** derivatives was sent to Australia for evaluation in a subtropical environment.

This paper describes the results of testcrosses made between the **sac** derivatives (Table 1) and each of a selection from PacL 901 [Australian group 9 cultivar commercially marketed as Titan 9 (Anon. 2007); the selection hereafter designated 901] and a highly winter-active (at least group 10) landrace from Oman (Arabian Peninsula), designated Oman 2. Similarly, testcrosses were made between 11 Omani landraces and 901. The testcrosses were agronomically characterised over 1 year in a row experiment in the field in a subtropical environment at Gatton, Queensland. The purpose of this work was to determine which **sac** derivatives and Omani landraces produced the highest yielding hybrids, to guide future breeding efforts. Heterosis, relative to the testcross parent for forage yield and its seasonality, winter dormancy and persistence, was estimated. The performance in swards over the same period of **sac**-derived experimental synthetics (UQL 10 and 12), synthetics derived from the Omani material (UQL 14 and 16), synthetics bred from progeny-tested adapted clones (UQL 11) and male sterile maintained lines (UQL 13) (Irwin *et al*. 2008) is also reported along with that of a range of current Australian commercial cultivars and US-bred elite experimental lines.

Materials and methods

*Generation of testcrosses of **sac** derivatives with 901 and Oman 2 for field evaluation*

Information on the derivation and morphology of the *M. sativa* × *M. arborea* crosses (**sac** 1–10) generated at UW-Madison can be found in Bingham (2005). Both 2-way and 3-way crosses were made between these **sac** 1–10 plants and the resultant inter-cross (F_2) seed was sent to the University of Queensland (UQ) St Lucia, Brisbane, and planted in the post-entry quarantine glasshouses. Plants which grew from this seed (Table 1) were individually crossed to populations of tester plants of both 901 (40 race 2 anthracnose-resistant clones selected from 901) and Oman 2 (15 clones grown from imported seed) to generate the testcross seed. Oman 2 was chosen because of its extreme winter activity, very erect growth habit and large seed size (220 seeds/g) (J. A. G. Irwin and D. J. Armour, unpubl. data). Pollen was bulked from either 901 or Oman 2 and the **sac**-derived clones were used as females due to their low self-fertility. In the generation of the testcross material for field testing, it was generally necessary to bulk seed within a family to allow adequate replication. This was not the case for WA3044, WA3047 and WA3048, which are individual **sac** F_3 derivatives (from **sac** segregate 06/2, Table 1) and which produced relatively high levels of testcross seed.

Determination of M. arborea *introgression in **sac** partial hybrids and their derivatives*

The determination of arborea-specific alleles in **sac** hybrids, **sac** derivatives and **sac** testcross material from the field was performed with simple sequence repeat (SSR) markers using methods described elsewhere (Mackie *et al*. 2007). A total of 46 previously described SSR markers of known *M. sativa* genomic location, and covering all 8 linkage groups (Julier *et al*. 2003; Mun *et al*. 2006; Mackie *et al*. 2007) that also produced clear,

Table 1. Origins of genetic material used in the agronomic evaluations

Clone or line used as female parent in testcrosses	Parentage and/or geographic origin	Field evaluation experiment 2008 row	2008 sward	2007 sward
WA3044	Individual **sac**[A] segregate 06/2 clone (cross of **sac** 3 with a *Medicago sativa* × *M. falcata* clone)	+	–	–
WA3047	As above	+	–	–
WA3048	As above	+	–	–
sac seg 06/1	Cross of **sac** 3 with *M. sativa* clone; 10 full-sib clones used in testcrosses to generate a bulk	+	–	–
sac seg 06/2	Refer WA3044 for origin; 7 full-sib clones used in testcrosses, including WA3044, WA3047 and WA3048, to generate a bulk	+	–	–
sac 4⊗	18 S_1 clones of **sac** 4, used in testcrosses to generate a bulk	+	–	–
sac(7 × 2)	29 clones of **sac**(7 × 2), used in testcrosses to generate a bulk	+	–	–
sac(8 × 2) × 1	11 clones derived from **sac**(8 × 2 × 1), used in testcrosses to generate a bulk	+	–	–
sac(10 × 1)	10 clones derived from **sac**(10 × 1), used in testcrosses to generate a bulk	+	–	–
sac(9 × 7)	11 clones derived from **sac**(9 × 7), used in testcrosses to generate a bulk	+	–	–
sac(9 × 2)	11 clones derived from **sac**(9 × 2), used in testcrosses to generate a bulk	+	–	–
Oman 1	Landrace from Hamra, central inland Oman	+	–	–
Oman 2	Landrace from Salalah, southern coastal Oman	+	+	–
Oman 3	Landrace from Manah, central inland Oman	+	–	–
Oman 5	Landrace from Sur, southern coastal Oman	+	–	–
Oman 6	Landrace from Bahla, central inland Oman	+	–	–
Oman 7	Landrace from Dhank, NW inland Oman	+	–	–
Oman 8	Landrace from Mudhylir, southern inland Oman	+	–	–
Oman 9	Landrace from Kamil, southern inland Oman	+	–	–
Oman 10	Landrace from Ibri, NW inland Oman	–	–	–
Oman 11	Landrace from Nizwa, central inland Oman	+	–	–
Oman 13	Landrace from Yangul, NW inland Oman	+	–	–
Lines used as males in testcrosses				
Oman 2	Refer above, 15 clones used in testcrosses with all **sac** lines	+	+	–
901	40 clones resistant to race 2 of *Colletotrichum trifolii* were selected from PacL 901 (refer *Plant Varieties Journal* Vol. **20**, issue 1) and used in testcrosses with all **sac** and Omani lines	+	–	–
WISFAL	*M. sativa* subsp. *falcata*, refer Bingham (1993), and crossed with Oman 2, Oman 5 and **sac**(7 × 2) as females	+	–	–
Experimental control line		–	–	–
MBX	Cross of 2 plants, one an S_1 of clone MB and the other deriving from MB × P, where P was a purple-flowered dormant maintainer of male sterility. MB was the male sterile dormant (group 4) *M. sativa* maternal parent of **sac** (refer Bingham 2005)	+	–	–
Synthetic cultivars or breeding lines tested				
Hunter River	Australian public cultivar (refer Oram 1990)	+	+	–
Trifecta	As above	+	+	–
Sequel	As above	+	+	+
Super Sequel	Australian proprietary cultivar (refer IP Australia website: www.ipaustralia.gov.au/pbr/index.shtml)	+	+	–
Hallmark	As above	+	+	–
UQL 1	As above	+	+	–
SARDI Five	As above	+	+	–
SARDI Seven	As above	+	+	–
SARDI Ten	As above	+	+	–
Silverado	As above	+	+	–
54Q53	As above	+	+	–
UQL 10	*M. arborea* × *M. sativa* hybrids (**sac** 3, 9 and 10) were crossed with Sequel clones in Madison, and 10 plants of each family tracing to each of **sac** 3, 9 and 10 were polycrossed to derive UQL 10	–	+	+
UQL 11	*M. sativa* synthetic derived by polycrossing the 8 highest yielding S_0 clones identified in a partial diallel yield test at Gatton (clones WA272, WA335, WA332, WA258, WA334, WA381, W116 and D; Irwin *et al*. 2008)	+	–	+

(*continued on next page*)

Table 1. (*continued*)

Clone or line used as female parent in testcrosses	Parentage and/or geographic origin	Field evaluation experiment 2008 row	2008 sward	2007 sward
UQL 12	UQL 10 (refer above) was mass selected for resistance to *C. trifolii* race 2, and 5 clones were selected. They were used as females and polycrossed with 40 *C. trifolii* race 2 resistant clones selected from PacL 901	+	–	+
UQL 13	Clone WA1103, a cytoplasmic male sterile plant tracing to 6–4 ms (Bingham 2005), and shown to have good GCA was used to generate a male sterile line in 2 backcrosses with the clones identified in UQL 11 (used as males)	+	–	+
UQL 14	Polycross of maintained clones from UQL 13 used as females with 15 clones from Oman 2 (same 15 clones as used in testcrosses) used as males	–	+	–
UQL 16	Oman 2 clones (same as used in UQL 14) used as females and polycrossed with 40 901 clones with *C. trifolii* race 2 resistance	+	+	–
Third-party experimental lines[B]				
Experimental 1	Leading North American-bred non-dormant cultivars or breeding lines	+	–	–
Experimental 2	As above	+	–	–
Experimental 3	As above	+	–	–
Experimental 4	As above	+	–	–
Experimental 5	As above	+	–	–
Experimental 6	As above	+	–	–

[A]**sac** = *M. sativa* × *M. arborea* hybrids. Ten **sac** partial hybrids (**sac** 1–**sac** 10) were generated by E. T. Bingham, University of Wisconsin – Madison (Bingham 2005).
[B]These cultivars and/or lines were bred in North America and their evaluation was paid for by a third party under a secrecy agreement.

reproducible bands was used. An average of 5.75 markers per linkage group (range 4–8) was scored. Markers were scored for the presence and absence of each allele in the maternal male sterile *M. sativa* parent MB (Table 1), *M. arborea* pollen donors, and putative **sac** hybrids using DNA samples from individual plants. Markers specific to *M. arborea* introgression were used to track the transmission of the *M. arborea* specific alleles into the **sac** derivatives and the testcross material for field evaluation, using DNA bulks. These bulks were generated by pooling equal quantities of DNA from individual plants. Where the number of plants to be bulked exceeded 6, multiple bulks were made; for example **sac** 4⊗ constituted 18 S_1 clones (Table 1) and was analysed as 3 bulks to confirm marker transmission (see Table 3).

Generation of testcrosses of Omani landraces for field evaluation

Testcross seed was generated between Omani landraces (sourced directly from Dr Abdullah Al-Sa'di of Sultan Qaboos University, Al-Khod 123, Oman and processed through post-entry quarantine in Australia) and 901. The relationship between these Omani lines and those reported up by Smith *et al*. (1995) is not known. Fifteen plants (grown from imported seed) of each of the lines Oman 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, and 13 were used as females and pollinated without emasculation using bulk pollen from the same 40 901 clones described above in generating the **sac** testcrosses. The geographic origins of the landraces in Oman are shown in Table 1. For field evaluation, seed was bulked in equal quantities from each plant within a landrace.

Generation of seed of experimental synthetics for sward testing

Experimental synthetics and/or strain crosses utilising **sac**, Omani and male sterile material (Table 1) were developed for sward testing at Gatton on the same soil type and location as for the row experiment. Polycrossing was conducted by hand in the glasshouse at UQ, St Lucia, without emasculation.

Experimental design and agronomic measurements for field experiments

Row experiment

The experiment was sown in June 2008 at Gatton Research Station (27°34′S, 152°20′E, elevation 95 m). The soil type was an alluvial black clay (Ug 5.12, Northcote 1971). Because the purpose of the work was to assess genetic potential for yield *per se*, a spraying schedule using a combination of benlate and mancozeb, applied fortnightly in alternate applications, was implemented to manage endemic diseases. Insect damage from leaf rollers and aphids was monitored and applications of insecticides were used when deemed necessary. There were 11 **sac** derivatives testcrossed with both 901 and Oman 2; 11 Omani landraces (including Oman 2) testcrossed with 901; tester parents 901 and Oman 2; MBX (a derivative of MB, the male sterile maternal parent of the **sac** hybrids); crosses of Oman 5 × WISFAL, Oman 2 × WISFAL and **sac**(7 × 2) × WISFAL; 11 commercial cultivars (Hunter River, Trifecta, Sequel, Super Sequel, Hallmark, UQL 1, SARDI Five, SARDI Seven and SARDI Ten, Silverado and 54Q53); UQL 11, 12 and 13; and 6 other elite experimental winter-active lines bred in North America and tested under secrecy (Table 1). The testcrosses with WISFAL (*M. sativa* subsp. *falcata*, Bingham 1993), which has previously demonstrated heterosis with Australian-adapted material at Gatton (Mackie *et al*. 2005) were used as dormant checks. Each plot consisted of a 1-m row into which 1 g (~300 seeds) were sown, with a 50-cm spacing between plots, and an inter-row spacing of 1 m. The field layout consisted of 59 rows and 4 columns. Within the columns, lines were allocated at random to the plots. While all 59 lines were planted in the first

column, there was only sufficient seed for 52, 42 and 32 lines to be planted in the remaining columns. The other plots in columns 2–4, were planted with Sequel. Once rows were established, plots received 50 mm of irrigation every 2 weeks using a fixed, solid set layout with overhead sprinklers. Irrigation schedules were maintained unless more than 25 mm of rainfall was received in the week before irrigation.

Yield was assessed 13 times over the period August 2008–August 2009, with a 4-week cutting interval except in winter when the interval was extended to 6 weeks. The entire row of each entry was defoliated to 2.5 cm with hand shears, oven-dried, then weighed. Analyses were conducted on dry matter (DM) yield (g/plot). Height was measured according to accepted UPOV practice (UPOV 2005), at the autumn and spring solstices. Seasonal yields were calculated by summing the sampling periods that fell within the following: autumn, 1 March to 31 May; winter, 1 June to 31 August; spring, 1 September to 31 November; and summer, 1 December to 28 February. A sampling period was deemed to fall into a season if more than half of the growth period occurred in that season. Persistence was assessed by measuring the width of the gaps in each row and expressing this as a percentage (i.e. a total gap of 5 cm would give 95% persistence).

Sward experiments

Two sward experiments were conducted (sown 2007 and 2008). The material evaluated in each experiment is shown in Table 1 and included synthetics derived from **sac**, Omani and male sterile material. Experiments were laid out as randomised block designs with 3 replicates. Seed, at 15 kg/ha, was sown by hand into weed-free seedbeds in winter, rolled and followed by 12.5 mm irrigation, applied weekly for 4 weeks. Plot size was 5×2 m. All experimental seed was hand scarified and it was assumed that mechanical harvesting provided sufficient scarification for commercially produced seed. Irrigation management was similar to that used in the row experiment. Fungicides and insecticides were applied as described above for the row experiment in the 2007 planted sward experiment; in the 2008 sown experiment only insecticides were used, to simulate commercial management practices.

Harvesting of each experiment commenced around 10 weeks after sowing. Regrowth was assessed every 4 weeks except in winter when the interval was extended to 6 weeks. Dry matter yield was measured by cutting, to 5 cm above ground level, a 5.85-m^2 quadrat from the centre of each plot with a reciprocating mower. Samples were dried in a force-draught oven at 80°C for 24 h. The remaining foliage on each plot was removed using a forage harvester. Seasonal yields and heights were assessed as for the row experiment.

Statistical analyses

Row experiment

The total yield over 13 harvests, seasonal yields, autumn and spring heights and density were analysed by fitting general linear mixed models with rows, columns and hybrid combinations and lines within hybrid combinations as random effects using residual maximum likelihood methods in GENSTAT (Payne *et al*. 2007). At 2 harvests in the autumn there was minor damage to some plots caused by hares. This effect was removed by including whether or not there was damage as a covariate. Means were only adjusted if the covariate was significant. The model was determined to be adequate using sample variograms and plots of the residuals.

As parental performance was only available for Oman 2 and 901, tester (testcross) parent heterosis was defined as the deviation of a line's or genotype's testcross performance from the tester's performance. Whether this was significantly different from the tester (either positive or negative heterosis) was determined by *t*-test.

Sward experiments

Data for DM yields, plant heights and persistence were subjected to a general ANOVA using the statistical package GENSTAT (Payne *et al*. 2007).

Results

Determination of M. arborea *introgression in the* **sac** *hybrids and their derivatives*

Analysis of the marker profiles generated by the 46 SSR primers across the male sterile *M. sativa* parent MB, the putative **sac** hybrids 2, 3, 4, 7, 8, 9 and 10 and the *M. arborea* pollen donor indicated regions of introgression from *M. arborea* chromosomes 1, 6 and 7 (Table 2). The profiles generated by markers from chromosomes 2, 3, 4, 5 and 8 did not indicate *M. arborea* introgression. Hybrids **sac** 4 and **sac** 10 possessed a marker allele showing one region of *M. arborea* introgression each (chromosomes 1 and 6, respectively). **Sac** 2 and **sac** 7 each had 2 alleles from the same chromosome showing *M. arborea* introgression (chromosomes 1 and 6, respectively). Hybrids **sac** 1 and **sac** 3 possessed marker alleles indicating regions of introgression from chromosomes 1 and 6, and **sac** hybrids 8

Table 2. Establishment of *Medicago arborea*-specific simple sequence repeat alleles in *M. sativa* × *M. arborea* (sac) hybrids generated at the University of Wisconsin and used to generate the sac derivatives used in testcrosses and synthetics

Linkage group	Marker	**sac** 1	**sac** 2	**sac** 3	**sac** 4	**sac** 7	**sac** 8	**sac** 9	**sac** 10
1	003D10	–	+	–	–	–	–	–	–
1	MtB160	–	–	+	+	–	–	–	–
1	4F06	+[A]	+	+	–	–	+	+	–
6	MTIC134	–	–	+	–	+	+	+	+
6	MTIC153_1	+	–	+	–	–	+	+	–
6	MTIC153_2	–	–	–	–	+	–	–	–
7	MTIC417	–	–	–	–	–	+	+	–

[A] + = *M. arborea* specific allele present in **sac** partial hybrid and absent from MB (the maternal male sterile *M. sativa* parent of the sac hybrids).

and 9 possessed markers indicating regions of introgression from chromosomes 1, 6 and 7.

M. arborea-specific SSR alleles were transferred from both the maternal and paternal **sac** hybrids into their derivatives and from the **sac** hybrids into the **sac** × Sequel crosses, which were subsequently polycrossed to derive UQL 10 (Table 3). **Sac** derivative 9 × 7 has an arborea-specific allele from the male parent (marker MTIC153_2 from **sac** 7) and an arborea-specific allele (MTIC417) from the female parent **sac** 9, but lacks the other 3 arborea-specific alleles present in the parents. Derivative **sac**(10 × 1) contains the arborea-specific allele (MTIC153_1) from the male parent **sac** 1 only, while the remaining derivatives possess arborea alleles present in one or more of the parents (Table 3).

Transmission of arborea-specific alleles from the **sac** hybrids and derivatives into the testcross material evaluated in the row experiment was observed. The arborea-specific allele from marker MTIC471 was transferred from **sac**(9 × 2) into the testcrosses with both Oman 2 and 901 (Fig. 1). The same allele from **sac**(9 × 7) was transferred into the testcrosses with 901, but not the Oman 2 testcross. Allele 2 from marker MTIC153 was transferred from **sac**(7 × 2) into testcrosses with 901 (testcross bulks 1 and 3) and Oman 2; however, an allele of the same molecular weight is present in the Oman 2 testcross parent bulks (Fig. 1). Similarly, transmission of alleles from MTIC153 in **sac**(10 × 1) (allele 2) and **sac**(8 × 2) × 1 (allele 1) into the testcrosses with 901 was observed; however, alleles of the same molecular weight were present in the above **sac** derivatives testcrossed to Oman 2 and the Oman 2 testcross parents themselves making transmission into the testcrosses equivocal. Arborea-specific alleles identified in the **sac** hybrids and **sac** derivatives with markers MtB160, MTIC134, 003D10 and 004F06 were present in the corresponding testcrosses; however, alleles of the same molecular weight were also present in the Oman 2 and 901 tester parents.

Agronomic determinations

Yield (row experiment)

The line MBX represents a baseline for the **sac** derivatives, as it contains ~75% of the genome of the *M. sativa* male sterile clone (MB) used to generate MBX (Table 1). This line gave a total yield of 1084 g/m of row (Table 4), which was the second lowest yielding of the 59 entries evaluated in the experiment, the lowest being **sac**(7 × 2) × WISFAL (*M. falcata*). MBX yielded significantly ($P < 0.05$) lower than the means of all other lines except the WISFAL testcrosses (1300 g/m of row). Both MB and WISFAL are dormancy group 4 lines, and group 4 lines are known to yield very poorly in the subtropical environment (Mackie *et al*. 2005; Irwin *et al*. 2008). WISFAL yielded only 62% of non-dormant cultivated lucerne at Gatton in previous studies (Mackie *et al*. 2005). However, crosses of Oman 2 × WISFAL gave a total yield of 1448 g/m of row in this work, which was in the range of Oman 2 (1470 g/m of row). The highest yielding entry was Oman 5 × 901 (2089 g/m of row), followed by UQL 12 (2082 g/m of row), a narrow-based synthetic derived from **sac** × Sequel crosses, and which yielded 15% higher than Sequel. The synthetics UQL 11 (2073 g/m of row) and UQL 13 (2066 g/m of row) yielded not significantly different to UQL 12 and gave higher total yields than all of the commercial synthetics tested, and all of the North American elite experimental non-dormants. The highest cultivated and adapted line tested (1956 g/m of row) was the race 2 *Colletotrichum trifolii*-resistant selection (901) from 901; the 40 parents of 901 were used as testcross parents. The other testcross parent, Oman 2, yielded significantly ($P < 0.05$) lower than 901 (1470 *v.* 1956 g/m of row). However, the testcross mean for **sac** × Oman 2 (1839 g/m of row) was significantly ($P < 0.05$) higher than the testcross means for **sac** × 901 (1703 g/m of row) and not significantly different to the means of the commercial synthetics or 901. The best performing testcrosses were the Omani lines × 901, with a mean of 1941 g/m of row, which was significantly ($P < 0.05$) better than all other lines except 901 and the third-party experimentals, where the differences were not significant. The UQL synthetic experimental lines had mean yields (2074 g/m of row) significantly better than all other line means except 901.

The groups of lines which showed the highest summer yield were the UQL experimental synthetics (1101 g/m of row) and the Oman lines × 901 testcrosses (1021 g/m of row), and they both had the highest autumn yields (275 and 267 g/m of row, respectively). The highest yielding group in spring was the **sac** × Oman 2 testcrosses, with a mean yield of 512 g/m of row. The highest yielding material in winter was the UQL experimental lines (185 g/m of row), followed by the third-party elite experimentals (178 g/m of row). By far the highest seasonal contributor to total yield for all lines was summer, followed in decreasing order by spring, autumn and winter, with the spring yield about twice the autumn yield, and winter

Table 3. Transmission of *Medicago arborea*-specific simple sequence repeat alleles in progeny derived from 2- and 3-way crosses of the sac hybrids, and in sac × Sequel crosses used in generation of UQL 10

Linkage group	Marker	**sac** (8 × 2) × 1	**sac** 4⊗	**sac** (10 × 1)	**sac** (9 × 2)	**sac** (9 × 7)	**sac** (7 × 2)	**sac** seg 06/1	**sac** seg 06/2	UQL 10 parents **sac** 3 × Sequel	**sac** 9 × Sequel	**sac** 10 × Sequel
1	003D10	–	–	–	+	–	+	–	–	–	–	–
1	MtB160	–	+	–	–	–	–	–	+	+	–	–
1	4F06	+[A]	–	–	+	–	–	–	–	–	–	–
6	MTIC134	–	–	–	–	–	–	+	+	–	+	+
6	MTIC153_1	+	–	+	+	–	–	–	–	–	+	–
6	MTIC153_2	–	–	–	–	+	+	–	–	–	–	–
7	MTIC417	–	–	–	+	+	–	–	–	–	+	–

[A] + = *M. arborea*-specific allele present in **sac** derivatives.

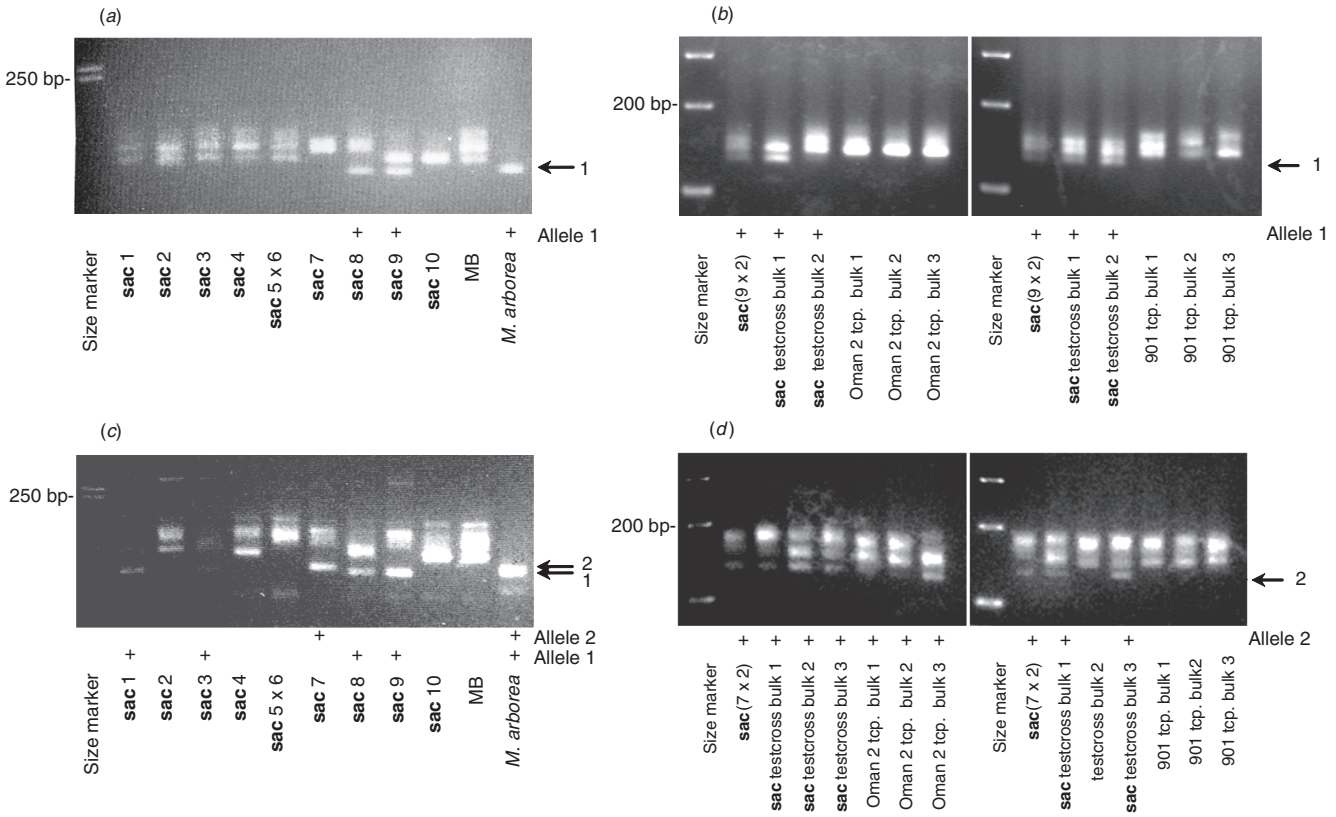

Fig. 1. (*a*) Gel image of marker MTIC471 showing introgression of an allele from *Medicago arborea* into **sac** hybrids 8 and 9. (*b*) Transmission of the allele from **sac** hybrid 9 into derivative **sac**(9 × 2), and testcross bulks with both Oman 2 and 901. (*c*) Gel image of marker MTIC153 showing introgression of two separate alleles from *M. arborea* into **sac** hybrids 1, 3, 8 and 9 (allele 1) and **sac** 7 (allele 2). (*d*) Transmission of allele 2 from **sac** 7 into derivative **sac**(7 × 2), and testcross bulks. An allele of the same molecular weight as allele 2 from arborea is observed in the Oman 2 bulks, but is absent from the 901 bulks, where transmission of allele 2 from **sac**(7 × 2) into testcross bulks 1 and 3 can be seen. tcp., testcross parent; MB, male sterile maternal *M. sativa* parent of **sac.**

yield ~60% of autumn yields. Additional information on individual and group line means for total yield is lodged as Accessory Publication Tables 1 and 2, available on the journal's website.

Yield (sward experiments)

The total yields from the 2 sward experiments sown in 2007 and 2008 are shown in Tables 5 and 6, respectively. The best performing line in the 2007 experiment where fungicides were applied was UQL 13, the male sterile maintained line whose parents had been selected as a result of progeny testing (Table 1). After 2 years, UQL 13 had produced 42% more DM than the benchmark standard cultivar Sequel (Oram 1990). UQL 10, a polycross of plants from direct crosses between **sac** clones and Sequel performed comparably to Sequel when yield tested with fungicides applied regularly. UQL 12 (derived from UQL 10), in the same experiment was also significantly higher ($P < 0.05$) yielding than Sequel, indicating the possible potential of *M. arborea* to enhance lucerne productivity. In the 2008 sown experiment, the outstanding line was UQL 16, which outyielded Sequel by 13%. UQL 16 was the only 1 of 14 synthetics tested to significantly outperform Sequel. UQL 10, in contrast to the 2007 sown experiment, was the lowest performing line in the absence of fungicidal applications, producing only 80% of Sequel. This was also the only line to show high susceptibility to downy mildew (*Peronospora trifoliorum*). Oman 2, although yielding 5% less than Sequel, was not significantly ($P > 0.05$) different in the 2008 sown experiment (Table 6). The superiority of UQL 16 was largely manifested over winter, when it significantly ($P < 0.05$) outyielded all other synthetics; it was also the highest yielding line over summer, followed by Oman 2.

Autumn and spring heights (row and sward experiments)

Apart from some of the testcrosses, the most non-dormant line tested in the row experiment was Oman 2, based on both autumn (53.9 cm) and spring (52.2 cm) heights (Table 4). The most dormant line was **sac**(7 × 2) × WISFAL (24.7 and 27.9 cm, autumn and spring harvests, respectively). MBX, a derivative of the maternal parent of the **sac** lines, was also dormant (27.9 cm autumn height), but was more active in the spring (41.3 cm). The testcrosses of Oman 2 and Oman 5 with WISFAL were non-dormant with autumn heights of 40.9 and 39.7 cm, respectively, which were not significantly different ($P < 0.05$) to Sequel (38.0 cm). All of the **sac** × 901 testcrosses were relatively non-dormant, with spring and autumn heights comparable to those expressed by the commercial cultivars in the group 7–9 range. The Omani lines × 901 testcrosses were all significantly ($P < 0.05$)

Table 4. Total and seasonal yield (g/m of row), autumn and spring heights (cm), and persistence (%) of testcrosses of sac and Omani lines and commercial and experimental synthetics grown in rows at Gatton over a 16-month period

Line/cultivar	Yield										Autumn height	s.e.	Spring height	s.e.	Persistence	s.e.
	Total	s.e.	Spring	s.e.	Summer	s.e.	Autumn	s.e.	Winter	s.e.						
901 polycross[A]	1956	92.0	497	19.5	1026	56.8	239	21.9	171	16.33	47.7	2.8	46.9	2.2	91.5	5.0
***sac* × *901 testcrosses* (*means*)**	1703	38.9	499	10.5	882	24.3	188	8.3	122	6.2	37.3	1.3	41.4	1.0	90.6	2.1
sac(9 × 2)	1607	99.9	483	18.3	806	66.5	186	17.5	124	15.6	35.9	3.2	40.4	2.6	91.9	5.8
sac(9 × 7)	1650	85.3	491	16.7	836	56.1	188	16.0	118	13.9	33.8	2.7	39.7	2.2	93.3	5.0
sac(10 × 1)	1656	100.0	494	18.3	845	66.6	183	17.5	124	15.6	37.1	3.2	37.6	2.6	88.7	5.8
WA3048	1658	76.9	506	15.7	880	49.8	172	15.2	104	12.8	37.1	2.5	45.2	2.0	86.9	4.5
sac(7 × 2)	1659	78.2	500	15.9	837	49.9	184	15.4	129	13.0	35.6	2.5	40.2	2.0	93.8	4.5
WA3044	1676	87.0	491	16.9	893	56.3	184	16.3	113	14.1	36.7	2.8	37.5	2.3	76.1	5.0
sac(8 × 2) × 1	1683	76.3	506	15.7	853	49.7	188	15.0	124	12.7	36.2	2.4	40.4	2.0	95.8	4.5
sac seg 06/1	1723	70.2	508	14.9	881	45.1	189	14.3	121	11.9	36.6	2.2	41.9	1.8	93.4	4.1
sac 4⊗	1790	70.0	494	14.9	934	45.1	204	14.2	131	11.8	42.8	2.2	42.9	1.8	95.1	4.1
WA3047	1807	70.9	503	15.0	959	45.1	194	14.3	135	11.9	39.8	2.3	45.6	1.8	92.6	4.1
sac seg 06/2	1826	71.2	514	15.0	978	45.8	195	14.4	122	12.0	39.1	2.3	44.5	1.9	89.3	4.1
Oman lines × *901 testcrosses* (*means*)	1941	39.6	475	10.6	1021	24.3	267	8.4	156	6.3	53.6	1.3	50.6	1.0	80.6	2.1
Oman 8	1748	102.6	461	18.6	900	66.7	251	17.8	142	15.9	44.1	3.3	47.6	2.7	79.0	5.8
Oman 2	1848	76.9	480	15.7	961	49.7	250	15.1	146	12.8	55.2	2.5	53.7	2.0	81.1	4.5
Oman 3	1910	70.1	480	14.9	1054	45.1	249	14.2	125	11.8	55.6	2.2	49.5	1.8	73.7	4.1
Oman 1	1935	69.5	458	14.8	1024	45.1	274	14.1	158	11.8	57.3	2.2	51.6	1.8	83.6	4.1
Oman 7	1950	88.7	475	17.1	1050	56.4	262	16.6	152	14.4	50.9	2.8	49.2	2.3	81.1	5.0
Oman 10	1951	99.7	472	18.3	978	66.4	278	17.5	170	15.6	56.3	3.2	51.3	2.6	85.5	5.8
Oman 9	1964	77.3	478	15.8	1037	49.8	268	15.3	157	12.9	53.3	2.5	51.1	2.0	84.0	4.5
Oman 11	1971	86.9	470	16.9	1037	56.3	282	16.4	156	14.1	58.8	2.8	49.1	2.2	76.1	5.0
Oman 6	1987	71.2	462	15.0	1054	45.1	279	14.4	171	12.0	53.2	2.3	50.3	1.8	80.3	4.1
Oman 13	1998	76.5	484	15.7	1027	49.8	276	15.1	172	12.8	51.9	2.4	50.7	2.0	89.9	4.5
Oman 5	2089	70.1	505	14.9	1114	45.1	271	14.3	163	11.9	53.0	2.2	52.8	1.8	72.1	4.1
Oman 2 polycross[B]	1470	83.8	403	18.0	794	51.2	166	20.1	107	14.9	53.9	2.6	52.2	2.1	71.7	4.5
***sac* × *Oman 2 testcrosses* (*means*)**	1839	42.8	512	11.1	960	26.2	212	9.3	136	7.0	50.0	1.4	46.9	1.1	76.8	2.3
sac(9 × 7)	1739	102.6	507	18.7	836	66.9	215	18.2	147	16.1	52.3	3.3	47.6	2.6	71.7	5.8
sac(7 × 2)	1753	77.8	518	15.9	915	49.9	201	15.5	117	13.0	47.4	2.5	43.5	2.0	77.6	4.5
WA3047	1808	94.5	516	17.8	928	58.4	210	17.5	136	15.0	49.9	3.1	47.0	2.5	80.4	5.0
sac(9 × 2)	1818	101.9	518	18.6	926	66.8	215	18.0	135	16.0	48.3	3.3	44.9	2.6	78.2	5.8
sac 4⊗	1848	69.8	504	14.9	992	45.2	208	14.4	133	11.9	54.7	2.2	49.0	1.8	68.4	4.1
sac seg 06/1	1867	69.8	509	14.9	996	45.2	207	14.3	133	11.8	50.2	2.2	49.4	1.8	77.4	4.1
sac(8 × 2) × 1	1871	87.1	517	17.0	941	56.4	226	16.6	146	14.2	50.6	2.8	43.5	2.2	84.1	5.0
WA3044	1875	85.8	521	16.9	1019	56.3	206	16.3	125	14.0	50.1	2.7	48.1	2.2	76.8	5.0
WA3048	1876	85.8	513	16.9	1008	56.3	204	16.3	142	14.0	49.0	2.7	49.2	2.2	74.3	5.0
sac seg 06/2	1886	78.6	508	16.0	1016	49.9	216	15.6	139	13.1	47.9	2.5	46.8	2.0	75.6	4.5
sac(10 × 1)	1891	100.3	503	18.5	984	66.7	221	17.8	147	15.8	49.7	3.2	47.0	2.6	79.8	5.8
WISFAL testcrosses (*means*)	1300	59.1	449	13.8	613	36.6	143	13.9	90	10.4	35.1	1.9	34.1	1.5	77.5	3.3
sac(7 × 2)	1024	80.9	418	16.9	413	52.0	128	17.4	77	14.0	24.7	2.5	27.9	2.1	91.1	4.7
Oman 5	1429	93.4	468	18.5	716	59.7	150	19.2	87	15.7	39.7	3.0	36.8	2.4	64.1	5.3
Oman 2	1448	75.8	462	16.1	709	47.6	150	16.7	107	13.1	40.9	2.4	37.6	1.9	77.2	4.2
Experimental control line																
MBX	1084	113.5	359	23.1	486	68.3	153	26.9	107	19.5	27.9	3.4	41.3	2.7	89.9	5.8
Synthetic cultivars (*means*)	1791	34.4	457	9.9	946	21.2	219	7.1	143	5.2	39.4	1.2	43.2	0.9	94.8	1.8
54Q53	1608	69.4	443	14.7	843	45.0	202	14.0	120	11.7	37.4	2.2	41.8	1.8	97.3	4.1
UQL 1	1667	70.1	439	14.8	864	45.0	207	14.1	137	11.8	36.8	2.2	41.3	1.8	95.6	4.1
SARDI Five	1676	70.0	443	14.8	880	45.1	210	14.1	126	11.8	37.7	2.2	40.3	1.8	90.7	4.1
Hunter River	1720	71.7	444	15.0	907	45.7	213	14.3	138	12.0	40.3	2.3	42.1	1.9	98.1	4.1
Sequel	1816	71.0	461	14.9	941	45.1	223	14.2	158	11.9	38.0	2.3	42.9	1.8	93.1	4.1

(*continued on next page*)

Table 4. (*continued*)

Line/cultivar	Yield										Autumn height	s.e.	Spring height	s.e.	Persistence	s.e.
	Total	s.e.	Spring	s.e.	Summer	s.e.	Autumn	s.e.	Winter	s.e.						
Silverado	1818	69.1	471	14.7	964	44.9	213	13.9	139	11.6	39.6	2.2	43.1	1.8	93.1	4.1
Trifecta	1848	70.0	455	14.8	968	45.0	230	14.0	148	11.8	38.4	2.2	42.1	1.8	96.4	4.1
SARDI Ten	1872	69.9	462	14.8	1004	45.1	221	14.0	157	11.7	41.5	2.2	45.9	1.8	92.3	4.1
SARDI Seven	1884	69.8	474	14.8	1008	45.0	223	14.0	149	11.7	40.6	2.2	46.4	1.8	93.1	4.1
Hallmark	1885	71.8	468	15.0	999	45.7	232	14.3	149	12.0	41.4	2.3	44.4	1.9	97.3	4.1
Super Sequel	1905	69.9	462	14.8	1027	45.0	230	14.0	157	11.7	41.5	2.2	44.9	1.8	95.6	4.1
Synthetic experimental lines (*means*)	2074	51.7	479	12.6	1101	31.9	275	12.0	185	9.0	45.4	1.7	47.8	1.3	95.1	2.8
UQL 13	2066	73.0	482	15.6	1078	46.6	277	15.8	188	12.6	45.0	2.3	49.5	1.9	95.4	4.2
UQL 11	2073	75.2	470	15.9	1103	47.4	279	16.2	190	12.9	47.2	2.4	47.7	1.9	94.6	4.2
UQL 12	2082	73.7	484	15.8	1123	46.5	269	15.9	178	12.8	44.1	2.3	46.2	1.9	95.4	4.2
Third-party experimental lines (*means*)	1957	39.4	484	10.7	1008	24.9	257	8.6	178	6.4	43.3	1.3	45.6	1.0	95.8	2.2
Experimental 4	1924	70.3	482	15.0	978	45.5	257	14.4	178	11.9	42.8	2.2	44.6	1.8	98.3	4.1
Experimental 1	1930	72.0	483	15.2	981	46.2	256	14.7	175	12.1	44.7	2.3	47.4	1.9	95.0	4.1
Experimental 2	1934	70.1	478	14.9	1028	45.5	244	14.4	174	11.9	43.4	2.2	45.6	1.8	95.0	4.1
Experimental 6	1979	70.0	491	14.9	1001	45.5	266	14.4	178	11.9	43.1	2.2	42.5	1.8	95.0	4.1
Experimental 5	1983	71.7	488	15.1	1028	45.5	255	14.6	186	12.1	41.1	2.3	45.3	1.8	96.6	4.1
Experimental 3	1995	70.1	484	14.9	1032	45.5	268	14.4	178	11.9	44.9	2.2	48.0	1.8	95.0	4.1

[A]Polycross of the 40 clones used as testcross parents.
[B]Polycross of the 15 clones used as testcross parents.

Table 5. Total and average seasonal yield (kg/ha), initial plant density (plants/m^2) and persistence (%) of elite experimental lucerne synthetics, some with *Medicago arborea* introgressed and the standard control cultivar Sequel, grown in swards at Gatton over the period August 2007 to November 2009, with regular fungicide applications

Cultivar	Total yield	Average seasonal yield				Initial density	% persistence
		Summer	Autumn	Winter	Spring		
UQL 13	34 337	4975	2692	2302	6560	48	36
UQL 12	29 267	4810	2050	1995	5353	21	68
UQL 10	26 984	3948	2228	1844	4980	53	21
Sequel	24 258	3538	2066	1689	4209	25	34
UQL 11	24 023	3768	2061	1465	4282	31	35
l.s.d. ($P = 0.05$)	4770	786	354	536	1090	24	25

Table 6. Total and seasonal yield (kg/ha), autumn and spring heights (cm), initial plant density (plants/m^2) and persistence (%) of elite experimental lucerne synthetics, some with *Medicago arborea* introgressed, and standard cultivars including Sequel, grown in swards at Gatton over the period June 2008 until October 2009, without fungicidal applications

Cultivar	Total yield	Seasonal yield				Plant height		Initial density	Final persistence (%)
		Spring 08	Summer 08–09	Autumn 09	Winter 09	Autumn	Spring		
UQL 16	32 559	6639	13 607	4891	4490	60.0	58.3	132.3	31.0
Hallmark	29 839	6955	12 738	3799	3101	35.6	46.1	150.7	25.9
Super Sequel	29 623	7149	12 280	3804	3159	41.1	45.7	156.3	19.6
Sequel	28 800	7263	12 077	3572	2987	40.9	45.7	117.3	36.0
SARDI Five	28 158	7311	12 493	3578	2027	38.5	43.7	129.3	25.9
SARDI Ten	27 819	6364	11 937	3712	2951	40.5	48.8	107.3	33.4
UQL 1	27 795	6460	11 838	3521	2719	39.8	45.3	135.7	27.7
Oman 2	27 338	6023	12 986	3348	2763	51.4	55.2	100.6	31.0
Trifecta	27 093	6812	11 881	3227	2462	37.3	42.8	115.7	32.8
Silverado	26 983	6484	12 039	3025	2693	35.9	46.3	103.3	27.9
UQL 14	26 779	7130	11 425	3044	2131	39.5	43.1	143.7	23.9
Hunter River	26 155	6468	11 112	3014	2373	33.7	42.6	146.7	26.7
SARDI Seven	25 790	6393	11 404	3103	2477	37.7	45.1	92.3	36.4
54Q53	25 221	6457	11 872	2652	1627	31.3	40.7	137.0	24.4
UQL 10	22 908	4536	11 496	2834	1915	61.5	53.9	137.6	19.0
l.s.d. ($P = 0.05$)	2141	584	1344	450	545	5.6	5.3	29.9	8.9

more non-dormant (53.6 cm autumn mean) than the means of all of the other material tested, except Oman 2. This group included the most non-dormant material tested, with Oman 11 × 901 at an autumn height of 58.8 cm. Another very non-dormant testcross was **sac** 4 × Oman 2, at 54.7 cm autumn height. The winter activity of Oman 2 was dominant, with the testcross autumn height mean for the **sac** × Oman 2 lines (50 cm) and for Oman 2 (53.9 cm) being not significantly different ($P < 0.05$). In the 2008 sward experiment, very high levels of winter activity (as measured by autumn height) were recorded by UQL 10 (61.5 cm), UQL 16 (Oman 2 × 901) (60 cm) and Oman 2 (51.4 cm). All of these lines were significantly more non-dormant than the other material tested, including SARDI Ten (41.5 cm autumn height, 48.8 cm spring height). The most non-dormant line, UQL 10, results from a polycross of **sac** × Sequel plants. This very high level of winter activity must have come from the introgression of the *M. arborea* genome, as the male sterile used to generate the **sac** hybrids was dormant, as evidenced by the performance of MBX in these experiments, and the only other *M. sativa* used in their development was Sequel (dormancy group 9, autumn height 38.0 cm). Additional information on individual and group line means for autumn height in the row experiment is lodged as Accessory Publication (Tables 3 and 4).

Persistence

In the row experiment, the plots were regularly treated with fungicide to manage anthracnose (*C. trifolii*). Generally, persistence was relatively high (>70%), with **sac** × 901 mean persistence (90.6%) being significantly ($P < 0.05$) higher than Oman 2 (71.7%), **sac** × Oman 2 (76.8%), the WISFAL testcrosses (77.5%) and Oman 2 × 901 (80.6%). In the 2007 planted sward experiment, protective fungicidal treatments were applied and after 2.5 years, UQL 12 was the most persistent line (68%). In the 2008 sward experiment where fungicides were not applied, the least persistent line was UQL 10 which was significantly less ($P < 0.05$) persistent than several lines including Sequel, the standard check cultivar. High levels of anthracnose were observed in the Oman 2 plots in this sward experiment, reflecting the inherent susceptibility of the material to this disease. UQL 10 was the only line in the 2008 experiment to show high levels of susceptibility to downy mildew, with systemic infection leading to symptoms manifesting themselves from winter to late spring 2008 and appearing again from autumn until the final harvest in 2009.

Testcross parent heterosis (row experiment)

Yield, height and persistence

The testcross parent which showed the highest positive levels of testcross parent heterosis for yield was Oman 2. All of the **sac** derivatives demonstrated positive levels of testcross parent (Oman 2) heterosis for total yield, which were significantly ($P < 0.05$) different from zero (Table 7). Most of this could be attributed to higher yields over summer, where 7 out of the 11 lines tested showed positive heterosis significantly different to zero. This result contrasted markedly with those obtained when 901 was the testcross parent with either the Omani or **sac** lines. Here, only significantly ($P < 0.05$) negative testcross heterosis values for total yield were observed with the **sac** lines, except for **sac** seg 06/2, **sac** 4 and WA3047, which were also negative, but not significant. With the Omani lines, for total yield, both negative and positive values, none significantly ($P < 0.05$) different from zero were obtained, with the highest positive value being for Oman 5 × 901 (133 g/plot). With 901 as the testcross parent, 6 of the 11 **sac** derivatives gave positive [but not significantly ($P > 0.05$) different from zero] testcross heterosis for yield over spring. This contrasted with the Omani lines, when crossed with 901 where all, except Oman 5, returned negative values over spring which were not, however, significantly different from zero. This result for the Oman lines × 901 also contrasted to the autumn, where all testcrosses returned positive values, albeit not significantly different from zero. For the **sac** lines × 901 testcrosses, most gave negative heterosis values over autumn, winter and summer, several of which were significantly different from zero.

When Oman 2 was the testcross parent, no autumn height testcross heterosis values were significantly ($P < 0.05$) different from zero for the **sac** lines except **sac**(7 × 2), and all except one (**sac** 4) were negative. In the spring, the trend was the same, although more pronounced with 3 of the 11 crosses being significantly ($P < 0.05$) less than zero. Similar results were obtained for the **sac** lines × 901 testcrosses, with 10 out of the 11 being significantly ($P < 0.05$) less than zero in the autumn and 5 out of the 11 being significantly ($P < 0.05$) less than zero in the spring. This contrasted with the Omani lines × 901 testcrosses, where all were positive (except Oman 8) in autumn, and 5 being significantly ($P < 0.05$) greater than zero.

Only 4 testcrosses (Oman 5 × 901, Oman 11 × 901, Oman 3 × 901 and WA3044 × 901) were significantly ($P < 0.05$) negative for persistence. Generally, the **sac** × Oman 2 and **sac** × 901 testcrosses were positive for persistence.

Discussion

The utilisation of lucerne in both Australia and North America at least, has been hampered by lack of progress in improving forage yield *per se*, with annual yield increments estimated to be from 0.15 to 0.30% (Hill *et al*. 1988; Holland and Bingham 1994), which compare unfavourably with the 2% annual increases obtained for corn and other crops in the US (Duvick and Cassman 1999). Irwin *et al*. (2001) made an analysis of the Australian-bred synthetic cultivars released in the preceding 20 years. They established that there was considerable opportunity for broadening the genetic base as there had been a heavy reliance over that period on cultivars such as CUF101, introduced from North America and used for introgression of aphid resistance. More recent efforts in Australia and the US have been directed towards identifying populations from diverse sources, which when crossed, express heterosis for yield (Bhandari *et al*. 2007; Sakiroglu and Brummer 2007; Irwin *et al*. 2008) and in developing breeding methodologies to commercially capture heterosis (Brummer 1999). In this paper, we showed the breeding potential in the subtropics of two previously untested germplasms, one containing introgressions into lucerne from *M. arborea*, and termed **sac**, and the other a range of highly winter-active Omani landraces. With further appropriate selection, both sources were demonstrated to have potential to increase lucerne productivity in the subtropics. Strain

Table 7. Testcross parent heterosis (g/plot) for total and seasonal forage dry matter yield (g/m of row), autumn and spring height (cm) and final persistence (%) of testcrosses derived from Omani landraces and sac lines crossed with the testers Oman 2 and 901

*Testcross parent heterosis (deviation of a line's testcross performance from the tester's performance) significantly different from zero at $P = 0.05$

Line	Relative to Oman 2								Relative to 901							
	Total	Spring	Yield Summer	Autumn	Winter	Autumn height	Spring height	Persistence	Total	Spring	Yield Summer	Autumn	Winter	Autumn height	Spring height	Persistence
sac(10 × 1)	421*	99.7*	189.9*	55.5	40.1	–4.2	–5.27	8.14	–300*	–2.7	–180.5*	–55.2*	–47.3*	–10.68*	–9.25*	–2.86
sac seg 06/2	416*	104.3*	221.3*	50.5*	32.0	–6.03	–5.46	3.95	–130	17.0	–47.5	–43.8	–49.6*	–8.64*	–2.33	–2.22
WA3048	406*	109.6*	213.7*	38.4	34.9	–4.93	–3.00	2.63	–298*	9.2	–145.4*	–66.5*	–67.0*	–10.63*	–1.67	–4.63
WA3044	405*	117.4*	224.7*	40.8	18.3	–3.88	–4.16	5.08	–280*	–5.3	–132.2	–55.1*	–58.4*	–11.05*	–9.33*	–15.46*
sac(8 × 2) × 1	401*	113.8*	146.3*	60.1	39.3*	–3.38	–8.72*	12.45	–273*	9.8	–172.9*	–51.1*	–47.6*	–11.54*	–6.5*	4.24
sac seg 06/1	397*	105.7*	202*	41.9	25.6	–3.77	–2.87	5.75	–233*	10.9	–144.9*	–50.0	–50.7*	–11.14*	–5.01	1.9
sac 4⊗	378*	100.8*	197.9*	42.4	25.8	0.79	–3.21	–3.31	–166	–2.9	–91.2	–34.3	–40.4*	–4.96	–3.98	3.55
sac(9 × 2)	348*	114.4*	132.0	49.1	28.1	–5.69	–7.31*	6.51	–349*	–3.5	–219.4*	–52.4	–47.3*	–11.87*	–6.46	0.40
WA3047	338*	112.4*	133.5	44.2	29.0	–4.04	–5.2	8.77	–149	6.0	–66.4	–45.1	–36.1	–7.93*	–1.22	1.07
sac(7 × 2)	283*	114.6*	120.4	35.7	10.1	–6.51*	–8.69*	5.92	–297*	3.1	–188.3*	–54.8*	–42.6*	–12.09*	–6.63*	2.27
sac(9 × 7)	269*	104.1*	42.2	49.9	39.6	–1.6	–4.68	0	–306*	–5.3	–190*	–50.9	–52.9*	–13.93*	–7.19*	1.73
Oman 5	–	–	–	–	–	–	–	–	133	8.2	88.8	32.7	–7.9	5.3	5.97*	–19.47*
Oman 13	–	–	–	–	–	–	–	–	42	–12.3	1.4	37.1	0.5	4.13	3.87	–1.62
Oman 6	–	–	–	–	–	–	–	–	31	–35.0	28.5	40.3	–0.2	5.46	3.40	–11.24
Oman 11	–	–	–	–	–	–	–	–	15	–26.5	11.6	43.4	–15.1	11.06*	2.2	–15.4*
Oman 9	–	–	–	–	–	–	–	–	8	–18.7	11.5	29.3	–14.5	5.58	4.22	–7.54
Oman 10	–	–	–	–	–	–	–	–	–5	–24.6	–47.6	39.3	–0.8	8.61*	4.46	–6.03
Oman 7	–	–	–	–	–	–	–	–	–6	–21.8	24.0	23.3	–19.7	3.13	2.32	–10.48
Oman 1	–	–	–	–	–	–	–	–	–21	–38.8	–1.6	35.3	–12.8	9.55*	4.72	–7.94
Oman 3	–	–	–	–	–	–	–	–	–46	–16.4	28.4	10.5	–46.0*	7.91*	2.59	–17.82*
Oman 2	0	0	0	0	0	0	0	0	–108	–17.1	–64.3	11.5	–24.8	7.49*	6.86*	–10.49
Oman 8	–	–	–	–	–	–	–	–	–208	–35.6	–125.9	12.3	–29.3	–3.68	0.77	–12.54

crosses or synthetics generated between Oman 5, Oman 2 and the **sac** material with the adapted material outyielded all commercial and experimental synthetics when tested in swards, with yields up to 13% (UQL 16) and 20% (UQL 12) better than Sequel, the established adapted cultivar in subtropical Australia (Lowe *et al*. 2000, 2010) after 16 and 27 months, respectively.

Using Oman 2 and 901 as testers, testcrosses were made with the **sac** derivatives and the Omani lines. Substantial positive testcross parent heterosis for yield was obtained with testcrosses between Oman 2 and the **sac** lines, which was chiefly manifested over the spring–summer period. This contrasted to the testcrosses with 901, where neither significantly positive nor significantly negative testcross parent heterosis for yield was observed for the Omani lines; for most of the **sac** lines, the testcross parent heterosis was significantly negative. The testcross results emphasise the importance of identifying populations each containing unique favourable alleles which, when crossed, lead to complementary gene action and positive heterosis for yield (Bingham *et al*. 1994). This appears to be the case for the landrace Oman 2 and the **sac** lines, but not for the adapted 901. This difference is most likely due to Oman 2 being relative genetically uniform, having been grown in isolation at Salalah oasis, Oman (Abdullah Al-Sadi, pers. comm.), and 901 being selected from a very broad-based synthetic, synthesised from a range of cultivated winter-active Australian cultivars, including Sequel and Sequel HR (Anon. 2007). Thus, the prerequisite causes of heterosis, namely directional dominance and divergent allele frequencies (Sakiroglu and Brummer 2007) are not likely to occur reliably in broadly based populations such as 901 *v.* the narrow genetically based Oman 2. Oman 2 plants were observed to be very morphologically uniform, as were all of the Omani lines compared to the 901 and all of the standard cultivars (J. A. G. Irwin and D. J. Armour, unpubl. data). Thus, the failure of the adapted 901 to express significant positive testcross heterosis in crosses with either the **sac** or Omani lines was not unexpected.

The difficulty of producing seed on the **sac** derivatives themselves prevented us field testing them, but it would have been expected that their yields would have been considerably lower than those of 901 and Oman 2. Indirect evidence of this is shown by MBX, which contains at least 75% of the genome of MB, the male sterile used to generate the **sac** hybrids. MBX yielded only 55% of 901 and 74% of Oman 2 in this work and was very autumn dormant. Deoxyribonucleic acid analysis indicates that only a small percentage of the *M. arborea* genome has been transferred to the Wisconsin-generated **sac** hybrids, which was also the case for a *M. sativa* × *M. arborea* hybrid generated in Australia using the MB source of male sterility (Armour *et al*. 2008). On our results, the performance of the **sac** derivatives in testcrosses with 901 and Oman 2 demonstrates that this material can be further developed, with the individual lines **sac**(10 × 1) and **sac** seg 06/2 (bred from **sac** 3) and a single clone from it, WA3047 having the greatest yielding potential in testcrosses with 901; **sac**(10 × 1) and the aforementioned **sac** seg 06/2 showed the best yield when testcrossed to Oman 2. To a lesser degree, Oman 2 also showed complementary gene action with WISFAL, a subsp. *falcata* line previously demonstrated to have potential in the Gatton environment in crosses with cultivated and adapted subsp. *sativa* (Mackie *et al*. 2005). The winter activity of Oman 2 combined with the low-branching crown of WISFAL provides scope for breeding a subtropical grazing lucerne.

While Oman 2 was relatively low yielding in the row experiment, it demonstrated substantial testcross heterosis with the **sac** lines, with the mean (1839 g/m row) being higher than the mean of the **sac** crosses × 901 (1703 g/m row) and the mean for the 11 commercial cultivars tested (1791 g/m row). This indicates that strong complementary gene action (Bingham *et al*. 1994) of favourable alleles conditioning yield has occurred between the **sac** lines and Oman 2. The very high levels of winter activity demonstrated by Oman 2 indicate it has unique alleles conditioning this trait, and there is dominance in their expression. Complementary gene action also appears to have occurred between Oman 2 and the **sac** lines for winter activity, with their testcross mean for autumn (50.0 cm) and spring heights (46.9 cm) being larger than SARDI Ten (41.5 and 45.9 cm, respectively), the most non-dormant standard cultivar tested. Irwin *et al*. (2008) demonstrated that in the subtropics, increasing the winter activity levels led to increasing forage yield, and these results reinforce the importance of winter activity in this environment. Although it was not possible to test the **sac** lines *per se* for reasons described above, observations in a non-air conditioned glasshouse in St Lucia indicated that only **sac** 4⊗ and **sac** seg 06/1 and **sac** seg 06/2 plants demonstrated substantial levels of winter activity and at levels comparable to Sequel (group 9) (J. A. G. Irwin and D. J. Armour, unpubl. data). This indicates introgression of winter activity genes from *M. arborea*, and interestingly both **sac** 3 (a parent of **sac** seg 06/1 and **sac** seg 06/2) and **sac** 4 contain the same SSR allele specific to *M. arborea* (MtB160). This allele was not present in any of the other **sac** hybrids suggesting it is linked on chromosome 1 to other alleles in *M. arborea* controlling winter activity.

The sward experiments demonstrated the potential of both the Omani and **sac** material to enhance lucerne productivity in the subtropics. UQL 16 (Oman 2 × 901) outyielded the best performing synthetic Hallmark by 9% and Sequel by 13% after 16 months. Although 901, which has Sequel and Sequel HR in its pedigree, was not tested in either of the sward experiments planted in 2007 or 2008 due to seed shortage, the parent PacL 901 has been tested in earlier experiments planted at Gatton, where it outyielded Sequel over 3 years by up to 5% (K. F. Lowe, unpubl. data). Thus, there are grounds for expecting the selection from it, 901, to have yielded comparably to Sequel or slightly higher (1–5%) if it had been included in the 2007 and 2008 sown experiments. In the row experiment, the cross Oman 2 × 901 only outyielded Sequel by 2% and underperformed 901 by 6%. The line Oman 2 also performed better in the sward experiment than in the row experiment, indicating differences in canopy architecture may have contributed to the difference in performance of the lines in the row and sward experiments. The polycross of the **sac** (3, 9 and 10) × Sequel, UQL 10, was the lowest yielding in the same sward experiment mentioned above, yielding only 80% of Sequel. This was, however, the most non-dormant line in the autumn, just ahead of UQL 16 with the positions reversed between the 2 lines in the spring, but not significantly different. The only source of this high (substantially greater than Sequel) winter activity in UQL 10 can be *M. arborea* introgression into the **sac** hybrids 3, 9 and 10 used to cross with

Sequel to generate UQL 10. UQL 10 did perform better in the 2007 sown experiment, where after 27 months it yielded 11% more than Sequel under a spraying regime to prevent anthracnose and downy mildew. The very high winter activity derived from *M. arborea* introgression represents potentially new alleles to those in the Omani lines, and may have further application for improvement of subtropical lucerne. The line UQL 12, which contains 50% of its genome from UQL 10, demonstrated its yield potential, both in the row experiment where it outyielded its progenitors 901 by 6% and Sequel by 15%, and in the 2007 sown sward experiment where it outyielded Sequel by 21%. This yield improvement in UQL 12 may trace to heterosis blocks transferred from *M. arborea* through the partial **sac** hybrids 3, 9 and 10.

The significantly positive testcross heterosis for yield identified in the **sac** × Oman 2 lines has provided a direction for future strategic research aimed at improving lucerne productivity in a subtropical environment. The work has identified **sac** derivatives tracing to **sac** 3 and **sac**(10 × 1) as having the greatest yield potential. It would appear that these **sac** derivatives and Oman 2 complement each other for favourable alleles conditioning yield, and also for winter activity (autumn height). These unadapted × unadapted crosses have already yielded at least comparably to the best commercial synthetics, and additional advances could be expected with further selection within each of these lines for disease and pest resistance and then converging them in development of the final commercial product (Brummer 1999). It is possible that a cultivar so developed may provide a substantial yield improvement over current cultivars. Similarly because of the differences we identified in seasonality of yield, with Omani lines × 901 having the highest summer and autumn yields, and **sac** × Oman 2 having highest spring yield, recurrent selection within each family for these traits, followed by converging in development of the final product may also provide greater overall productivity. Male sterility has a role in achieving this, and a male sterile maintained line (UQL 13) bred by us from progeny-tested materials we previously reported on (Irwin *et al*. 2008), was one of the leading yielders in both row and sward evaluations. Strain crosses of Oman 2 with Australian-adapted material, for example 901, produced a line (UQL 16), which significantly outperformed for total yield after 13 harvests the best adapted commercial synthetics, demonstrating the feasibility of making significant increases in lucerne productivity in the subtropics. A similar methodology has been proposed by Carelli *et al*. (2009) using landraces from Siwa oasis (Egypt), which display similar bio-agronomic traits (particularly winter activity) to the Oman material, as a heterotic group in diallelic crossing with European material. The work also demonstrates the potential of the Omani material to represent a fifth (after Peruvian, Chilean, African and Indian) recognised non-dormant germplasm source which is likely to form a heterosis group with other independent populations.

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