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Potential to increase yield in lucerne (*Medicago sativa* subsp. *sativa*) through introgression of *Medicago sativa* subsp. *falcata* into Australian adapted material

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Abstract. The effect of interspecific heterosis in crosses between *Medicago sativa* subsp. *sativa* and *M. sativa* subsp. *falcata* was assessed. Three sativa and 3 falcata plants were crossed in a diallel design. Progeny dry matter yield and natural plant height were assessed in a replicated field experiment at Gatton, Queensland. Yield data were analysed using the method of residual maximum likelihood (REML) and Griffing's model 1. There were significant differences between the reciprocal, general combining ability (GCA), and specific combining ability (SCA) effects. As expected, S_1 populations were lower yielding than their respective intraspecific cross and falcata × falcata crosses were significantly lower yielding than sativa × sativa crosses. Some of the interspecific crosses indicated substantial SCA effects, yielding at least as well as the best sativa × sativa crosses. We have demonstrated the potential usefulness of unselected *M. sativa* subsp. *falcata* as a heterotic group in the improvement of yield in northern Australian adapted lucerne material, and discuss how it could be incorporated into future breeding to overcome the yield stagnation currently being experienced in Australian programs.

Additional keywords: alfalfa, plant breeding, winter-active.

Introduction

Lucerne is the world's most important perennial forage legume (Michaud *et al*. 1988). In Australia, areas include approximately 200 000 ha grown exclusively for hay, and an estimated 3.5 M ha of pastures containing lucerne used for grazing in dryland farming operations (Pearson *et al*. 1997). There is room for considerable expansion of lucerne areas in Australia, with Hill (1996) estimating that up to 86 M ha and 9 M ha in eastern and Western Australia, respectively, could potentially be sown to lucerne. As well as the established uses for lucerne in hay or grazing operations, it is being used increasingly in conjunction with cereals for its capacity to increase soil nitrogen levels, improve water retention properties of soil, reduce dryland salinity through lowering of the watertable, and limit the deep drainage of water from soil profiles into river systems (Irwin *et al*. 2001).

All cultivated lucerne is autotetraploid ($2n = 4x = 32$), derived from either *Medicago sativa* subsp. *sativa* (hereafter 'sativa'), or sativa introgressed with *M. sativa* subsp. *falcata* (hereafter 'falcata'). Taxogenetic characteristics of sativa and falcata are discussed by Lesins and Lesins (1979). Most lucerne cultivars are genetically broad-based synthetics developed by randomly intermating elite S_0 clones (genotypes) and advancing through several generations by open pollination (Busbice 1969). The number of S_0 clones used to initiate the synthetic has varied from 4 to >100 (Hill *et al*. 1988). Lucerne is subject to severe inbreeding depression, this being why the use of larger numbers of S_0 clones is favoured (Hill *et al*. 1988). Bingham (1980) and Brummer (1999) have both emphasised that to maximise yield in lucerne, every effort should be made to minimise inbreeding depression, and to maximise heterozygosity and resultant heterosis through accumulating favourable dominant alleles linked in repulsion.

A prominent trend, both in North America and Australia, has been for lucerne yields to plateau since the mid 1980s (Lowe *et al*. 2000; Riday and Brummer 2002*a*). This has been attributed to the way in which synthetics have been bred, where new germplasm is introgressed into an increasingly diverse current breeding population undergoing recurrent selection (Brummer 1999). This breeding scheme

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does not maximise the frequency of dominant favourable alleles in repulsion, which can be achieved by separately improving diverse populations by recurrent selection and converging them in final production of the synthetic, or semi-hybrid (Brummer 1999). Essential to this scheme is the finding of genetically diverse populations that will express heterosis. Both Kidwell *et al*. (1994) and Musial *et al*. (2002) have demonstrated that falcata is genetically distinct from sativa, based on studies using restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPDs) respectively. In both of these studies, the tetraploid falcata accession WISFAL (Bingham 1993) was used as the falcata source. Heterosis for yield has been previously reported as early as 1920 in sativa × falcata crosses in North America (Waldron 1920), with the most recent study showing the interspecific crosses yielding 18% more than the average of the intraspecific crosses (Riday and Brummer 2002*a*).

Interspecies heterosis has not been widely explored in lucerne in Australia, but it may have huge potential benefits. Although highly winter-dormant, falcata presents a relatively untapped source of new alleles for lucerne genetic improvement in Australia. This paper reports studies made to assess the potential of falcata to contribute positively to lucerne productivity in northern Australia. The previous study of Musial *et al.* (2002) using RAPD analysis was used to select the 2 most closely related clones from within each of sativa and falcata, and the clones most genetically diverse to each of the former from within the 2 species. There was no *a priori* information on the yield potential of the clones, or their disease or pest resistance status. We used this approach not to test the relationship between random genetic diversity and heterosis, which has been previously shown not to exist (Kidwell *et al.* 1999), but because it at least provided some known benchmark information about the clones, rather than selecting them randomly or arbitrarily. A partial diallel was made between the 6 clones, and yield was measured in the field over a 6-month period in subtropical south-eastern Queensland, in the absence of disease and pest selection pressures. The overall objective was to measure yield heterosis in the populations, with the ultimate aim of assessing the potential of using falcata introgressions in sativa to overcome yield stagnation in the Australian environment.

Materials and methods

Genetic material used and generation of populations

The sativa and falcata clones used in the study are listed in Table 1, along with their genetic similarity estimates (GSEs) computed using RAPD analysis and previously published in Musial *et al.* (2002). These clones represent the most closely related (WA221 and WA222) and divergent (WA227) from within the sativa sample of 171 clones from 9 cultivars, and the most closely related (WA225 and WA226) and divergent (WA228) from within the falcata (WISFAL) sample of 19 clones, based on RAPD analysis of their genomes (Musial *et al.* 2002).

These 6 clones were intercrossed in a glasshouse in a diallel mating design. Vacuum emasculation was used for all crosses, and selfing was also attempted. S_1 (self) seed was obtained for all clones, except falcata WA228, which was relatively self-incompatible and also highly susceptible to powdery mildew (*Erysiphe polygoni*). Even though chemical protectants were used, the extreme susceptibility of this clone precluded us obtaining mature viable seed from it. Due to these issues it was not possible to generate a full diallel set with 36 lines; only 27 lines (5 selfs and 22 crosses) could be made and evaluated. This comprised a full diallel for 4 clones (WA221, WA222, WA225, and WA226), a half diallel with selfs for 5 clones with WA228 excluded, and a half diallel without selfs for 5 clones with WA227 excluded, due to WA227 being a poor pollen producer.

Table 1. Mean genetic similarity estimates (GSEs), based on Jaccard coefficients, between 3 *Medicago sativa* subsp. *sativa* and 3 *Medicago sativa* subsp. *falcata* individuals (clones) used to make a diallel

	WA221[A] sativa	WA222[B] sativa	WA227[C] sativa	WA225[D] falcata	WA226[E] falcata	WA228[F] falcata
			M. sativa subsp. *sativa*			
WA221	1.00					
WA222	0.59	1.00				
WA227	0.33	0.31	1.00			
			M. sativa subsp. *falcata*			
WA225	0.25	0.36	0.22	1.00		
WA226	0.29	0.36	0.24	0.78	1.00	
WA228	0.33	0.35	0.24	0.47	0.42	1.00

[A] Clone SEQHR1 from *M. sativa* subsp. *sativa* cv. Sequel HR (refer Musial *et al.* 2002 for details of all clones used).
[B] Clone SEQHR18 from *M. sativa* subsp. *sativa* cv. Sequel HR.
[C] Clone VENUS4 from *M. sativa* subsp. *sativa* cv. Venus.
[D] Clone FALC4 from *M. sativa* subsp. *falcata* cv. WISFAL.
[E] Clone FALC8 from *M. sativa* subsp. *falcata* cv. WISFAL.
[F] Clone FALC19 from *M. sativa* subsp. *falcata* cv. WISFAL.

Evaluation of forage yield and plant height

All seed of each of the 27 crosses and selfs tested was planted into 'Kwikpot 42s' (Yates), filled with UC mix (Baker 1957), and were raised in a glasshouse under natural illumination for 3 weeks before transplanting (July 2003) into a weed-free seedbed at Gatton, in south-eastern Queensland (lat. 27°34′S, long. 152°20′E, altitude 90 m); soil type was an alluvial, black clay (Ug5.12, Northcote 1971). The experiment was planted as a randomised block design with 6 replicates and 5 spaced plants of each cross per plot (50 cm grid). Due to seed numbers and establishment losses, it was not possible to evaluate 30 individuals for each line; the mean number tested per line was 23.

Yield was assessed 5 times (3/9/03, 1/10/03, 26/11/03, 23/12/03, and 21/01/04). A monthly cutting frequency was used based on previous work of Gramshaw *et al*. (1993) who showed that there was little yield advantage in the subtropics of cutting to match bud development, irrespective of the dormancy level of the material studied. The gap between Harvests 2 and 3 was due to a severe hail storm occurring the day before the harvest was due at end of October. This harvest was undertaken; however, the severe damage precluded us using the data. All plants were cut at 5 cm above the ground and the leaf and stem material from each plant was harvested individually, then oven-dried before weighing. Analyses were conducted on dry matter yield (g/plant). All plants were assessed in the field for natural plant height (cm) in spring and autumn according to accepted practice (UPOV 2004).

It was expected that at least some plants in these populations could have been susceptible to *Colletotrichum trifolii*, and all plants were protected in the field with bi-weekly sprays with fungicide (Benlate, DuPont), and insecticide as required, starting 3 weeks after transplanting. Plots were managed and kept weed-free by manual chipping.

Analysis of the data

Data for natural plant height in spring and autumn, the 5 individual harvest yields, and total yield (sum of yield from all harvests) were analysed using the method of residual maximum likelihood (REML, Patterson and Thompson 1971) in GenStat (2004). The 27 lines (selfs and crosses) were treated as fixed effects and the blocks, plots within blocks, and plants within plots and blocks, as random effects. The components of variance of blocks, and block × plots from the REML analysis of total yield approximated zero. The variance model thus reduced to Griffing's model 1, in which experimental material is regarded as the population, about which inferences can be made (Griffing 1956).

Diallel analysis

Least square means of total yield, from the REML analysis, were initially partitioned to give: a complete diallel analysis for 2 sativa (WA221 and WA222) and 2 falcata (WA225 and WA226) clones; a half diallel analysis with selfs for 3 sativa (WA221, WA222, and WA227) and 2 falcata (WA225 and WA226) clones; and a half diallel analysis without selfs for 2 sativa (WA221 and WA222) and 3 falcata (WA225, WA226, and WA228) clones.

The data were analysed to estimate general combining ability (GCA), specific combining ability (SCA), and reciprocal effects (where applicable). The statistical diallel analyses for model 1 were adjusted for unequal number of plants, using the variance matrix to estimate the variance of differences between general combining ability effects, specific combining ability effects, and reciprocal effects. The differences within combining abilities and within reciprocal effects (where applicable) were tested for significance. In general, the estimates of combining abilities of the same cross were similar for the 3 diallel analyses (data not shown), although differences in SCA existed where there were reciprocal effects. These estimates and their variance/covariance matrices were then recalculated using a GenStat procedure, based on Griffing's definitions of combining abilities, to give a single value for each.

Results

Agronomic determinations

Yield for 5 harvests, and mean total yield (g/plant) are shown in Fig. 1 and Table 2, respectively. Generally, lines that gave the highest total yield also yielded highest at the individual harvests. In the last 2 harvests, in late December and late January, yields for all lines tended to decline (Fig. 1). This could have been due to shortening daylength and other environmental effects. The lowest yielding lines were always the S_1 populations of falcata, which yielded on average 71% of the total yield of the falcata × falcata lines, whereas the S_1 populations of sativa yielded on average 76.5% of the total yield of the sativa × sativa lines. The S_1 lines of sativa and the sativa × sativa lines produced a mean total yield of 115.5 g/plant and 151 g/plant, respectively, compared with 66 and 93 g/plant for the S_1 of falcata and the falcata × falcata lines, respectively (Table 2). These results indicated lower yields for the S_1 populations than for their respective intraspecific crosses. Sativa crosses produced, on average, 62.4% more total biomass than falcata crosses. There was significant evidence of maternal or reciprocal effects for yield, with the sativa × falcata and falcata × sativa lines having total yields of 135.9 g/plant and 125.4 g/plant, respectively. The highest total yielding individual cross was sativa (WA222) × falcata (WA226) (179.8 g/plant), followed by sativa (WA227) × sativa (WA222) (177.2 g/plant), and sativa (WA227) × falcata (WA226) (169.4 g/plant). These results indicate substantial heterotic effects in the hybrid crosses, particularly those involving falcata clone WA226 and the sativa clones WA222 and WA227 (Table 2), given the significantly lower yields of the falcata × falcata crosses compared with the sativa × sativa crosses.

Natural plant height in the spring was greatest for the sativa × sativa crosses (mean = 42.4 cm), followed by the S_1 of sativa (37.2 cm), sativa × falcata (28.3 cm), falcata × sativa (27.7 cm), falcata × falcata (17.7 cm) and the S_1 of falcata (14.5 cm) (Table 2). These results confirm a more prostrate growth habit for falcata *v.* sativa, with the hybrids showing an intermediate growth habit to either parent. In the autumn, generally similar trends were evident, with sativa × sativa being the highest (22.0 cm), followed by sativa × falcata (18.1 cm), falcata × sativa (18.0 cm), sativa selfs (15.5 cm), falcata × falcata (13.7 cm), and falcata selfs (13.7 cm). The sativa selfs appeared more dormant in the autumn than the spring, but this may also be a reflection of the effects of inbreeding depression being manifested more in the autumn.

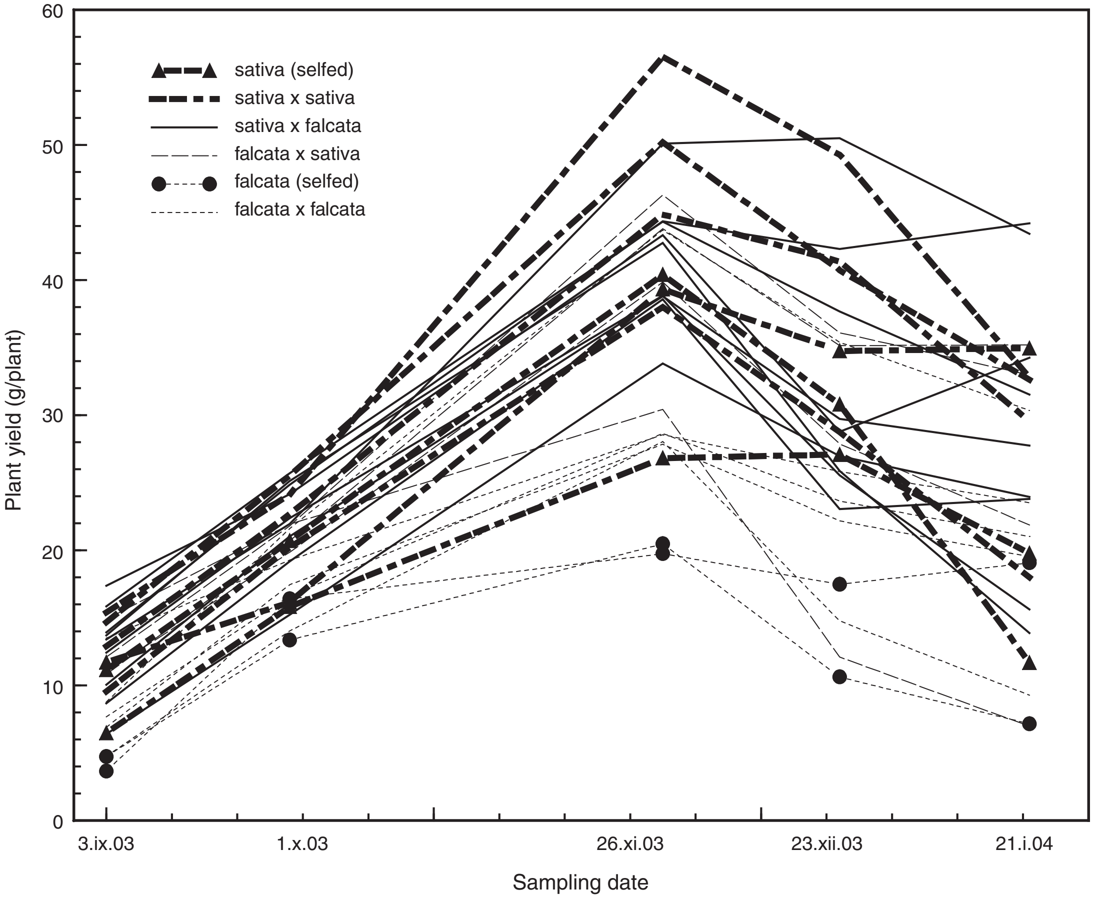

Fig. 1. Dry matter yield (g/plant) for each of 5 harvests over spring and summer for sativa S_1, falcata S_1, sativa × sativa, falcata × falcata, sativa × falcata and falcata × sativa. The average l.s.d., generated from residual maximum likelihood (REML) analysis, for each harvest is as follows: 3/9/03, 6.2; 1/10/03, 5.8; 26/11/03, 11.1; 23/12/03, 12.6; and 21/1/04, 13.7.

Combining ability analysis

There were very highly significant differences ($P < 0.01$) between the effects for both GCA (Table 3) and SCA (Table 4) from the diallel analysis conducted for total yield.

The sativa clone WA227 had a significantly higher GCA than all other clones except sativa clone WA222. The sativa clones WA222 and WA221 were significantly higher than the falcata clones WA225 and WA228, which showed negative GCA effects. These negative GCA effects arise principally due to the lower yields of the S_1 populations of falcata and of the falcata × falcata crosses, compared with the respective sativa material (Table 2).

The highest positive SCA effects (Table 4) were those between all 3 sativa clones that were tested and the falcata clone WA226. The SCA for the cross between sativa (WA222) and falcata (WA226) was significantly greater than those between sativa clones (WA221 and WA227) and falcata (WA225), and the sativa (WA221) crossed with falcata (WA228). The superiority of the crosses WA222 × WA226, WA221 × WA226 and WA227 × WA226 is demonstrated in Fig. 2.

Discussion

Lucerne responds well to selection for improved yield, but it is difficult to capture heterosis in a conventional broad-based synthetic (Daday 1965). Numerous papers have been published that indicate the theoretical value of the use of hybrids in lucerne (Bingham 1980; Brummer 1999) and, although sources of cytoplasmic male sterility were first identified in lucerne prior to 1967 (Davis and Greenblatt 1967), poor seed production has hampered commercial adoption of lucerne hybrids (Viands *et al*. 1988). Interspecific hybrids between winter-dormant or moderately winter-dormant sativa and falcata have been experimentally assessed in the USA (Riday and Brummer 2002*a*, 2002*b*), as a potential source of heterosis to be captured as synthetics, semi-hybrids, or hybrids. This is the first report, however, of assessment of

Table 2. Mean total yield (g/plant) over 5 harvests and natural plant height (cm) in spring and autumn, 28 days after harvest, for selfs and crosses between and within 3 *Medicago sativa* subsp. *sativa* and 3 *Medicago sativa* subsp. *falcata* clones

Significant differences are generated from the residual maximum likelihood analysis (REML), and are influenced by the number of plants available for testing, thus an average l.s.d. is given in the table. Within columns, means followed by the same letter are not significantly different at $P = 0.05$

	Mean total yield	Spring height 1/10/03	Autumn height 5/04/04
	sativa self		
WA221 × WA221	114.7abcd	38.1ab	10.0abcd
WA222 × WA222	96.2abcm	40.1a	24.3eghi
WA227 × WA227	135.7adhq	33.4bei	12.2cd
	sativa × *sativa*		
WA221 × WA222	114.5abcd	41.4ac	18.8abcef
WA222 × WA221	151.4efghil	42.1ac	23.5eghi
WA222 × WA227	148.1efghijl	45.9c	24.7ehi
WA227 × WA221	163.7fghi	41.3ac	19.4afhj
WA227 × WA222	177.2fhi	41.5ac	23.4fi
	falcata self		
WA225 × WA225	56.3n	15.9jkl	13.8bcdj
WA226 × WA226	75.8mnop	13.1kl	13.7cdj
	falcata × *falcata*		
WA225 × WA226	93.8bop	20.1jm	15.0cdj
WA225 × WA228	70.8mnop	17.3jkl	15.5bcdj
WA226 × WA225	109.7acdlp	20.1jkmn	10.3d
WA226 × WA228	97.5coq	13.3l	13.9cd
	sativa × *falcata*		
WA221 × WA225	114.7abcd	32.7de	16.1abcd
WA221 × WA226	154.0efghi	32.1def	17.1abcdf
WA221 × WA228	121.3acdjk	31.8defg	20.7aef
WA222 × WA225	103.4abcdegmn	33.3abdfgh	21.0abcef
WA222 × WA226	179.8f	27.8fghi	20.3abef
WA222 × WA228	127.3ade	28.1ghi	21.0aef
WA227 × WA225	113.6abcd	25.6hno	15.7bcdj
WA227 × WA226	169.4fh	22.2mo	15.0cdj
WA227 × WA228	139.8di	20.7jm	15.6bcdj
	falcata × *sativa*		
WA225 × WA221	86.3mnop	26.8h	16.9abcdfg
WA225 × WA222	120.4abcdjk	28.1fghi	19.5abefg
WA226 × WA221	145.6eghikl	26.9h	17.8abcfg
WA226 × WA222	149.2efghil	28.9deh	17.8abcfg
Average l.s.d.	34.6	5.1	6.7

Table 3. Estimates of general combining ability (GCA) (±standard errors) for total yield for crosses between and within 3 *Medicago sativa* subsp. *sativa* and 3 *Medicago sativa* subsp. *falcata* clones

Effects followed by the same letter are not significantly different at $P = 0.05$

	GCA
M. sativa subsp. *sativa*	
WA221	6.8 ± 3.19b
WA222	9.7 ± 3.91ab
WA227	21.8 ± 4.38a
M. sativa subsp. *falcata*	
WA225	−28.7 ± 4.08d
WA226	3.8 ± 3.89b
WA228[A]	−13.5 ± 4.36c

[A] Value for WA228 calculated from use of this clone as a pollen donor only.

heterosis for yield between falcata and adapted winter-active Australian sativa in northern Australia. Even in unselected and lower yielding falcata material, highly significant SCA effects were observed in specific sativa × falcata crosses in our study, where the highest yielding individual cross was sativa × falcata. This indicates that unselected falcata does have application for improving lucerne yields, even in winter-active backgrounds and in a subtropical environment, as were used in this study.

The identification of potential heterotic yield groups in *M. sativa* germplasm has been studied, with Kidwell *et al.* (1994), Riday and Brummer (2002*a*), and Segovia-Lerma *et al.* (2003) all identifying falcata as a genetically distinct group, likely to lead to heterosis for yield in crosses with sativa. The 6 parental clones we used were chosen from a study of random genetic diversity, based on RAPD analysis of their genomes (Musial *et al.* 2002). Sativa clone WA227, the most diverse clone used in our studies, also gave the highest GCA effects and positive SCA effects in all crosses except one. This clone was obtained from cv. Venus, which is substantially derived from Turkistan germplasm (Anon. 2003). The relatively small number of clones we studied, however, does not allow us to draw any conclusive inferences about the correlation between SCA and random genetic distance as assessed with RAPDs, and other studies would indicate that there is no such relationship. Riday *et al.* (2003) studied the genetic and morphological distance of plants that they used in creating interspecific hybrids upon yield heterosis. They found that genetic distance between parental genotypes did not correlate with specific combining ability or mid-parent heterosis, but that heterosis was correlated with subspecies, indicating that genetic diversity alone did not ensure yield heterosis (Riday and Brummer 2005). Kidwell *et al.* (1999) explored neutral DNA markers for selection of genetically diverse and non-diverse parents from which to generate synthetics. Their studies indicated a lack of significant differences in forage yield between synthetics selected for random genetic dissimilarity or similarity, this being attributed to the inability to target diversity to specific genomic regions affecting yield.

In our study we showed highly significant reciprocal or maternal effects, with higher yields occurring where sativa was used as the female parent. Reciprocal effects have previously been shown for seed yield in lucerne (Bolaños-Aguilar *et al.* 2001). Inheritance of chloroplasts in lucerne has been shown to be biparental with a strong paternal bias (Keys *et al.* 1995), whereas mitochondria are maternally inherited

Table 4. Estimates of specific combining ability (SCA) (±standard errors) for total yield from diallel analysis of 3 *Medicago sativa* subsp. *sativa* and 3 *Medicago sativa* subsp. *falcata* clones

Effects followed by the same letter are not significantly different at $P = 0.05$; a, b refer to differences between specific combining ability effects of selfs; x, y, z refer to differences between specific combining ability effects of hybrid

	WA221 sativa	WA222 sativa	WA227 sativa	WA225 falcata	WA226 falcata
			M. sativa subsp. *sativa*		
WA221	−21.1 ± 7.88a				
WA222	−5.7 ± 6.40yz	−45.4 ± 11.33ab			
WA227	12.9 ± 9.31xy	9.9 ± 8.62xy	−30.1 ± 11.61ab		
			M. sativa subsp. *falcata*		
WA225	0.32 ± 6.30yz	8.8 ± 11.70xyz	−1.6 ± 9.76yz	−8.4 ± 11.69a	
WA226	17.1 ± 6.28xy	28.8 ± 7.30x	21.7 ± 9.72xy	4.6 ± 8.46yz	−53.9 ± 11.58b
WA228	5.8 ± 8.35yz	8.9 ± 8.57xyz	9.3 ± 8.70xyz	−9.1 ± 9.13yz	−14.9 ± 8.59z

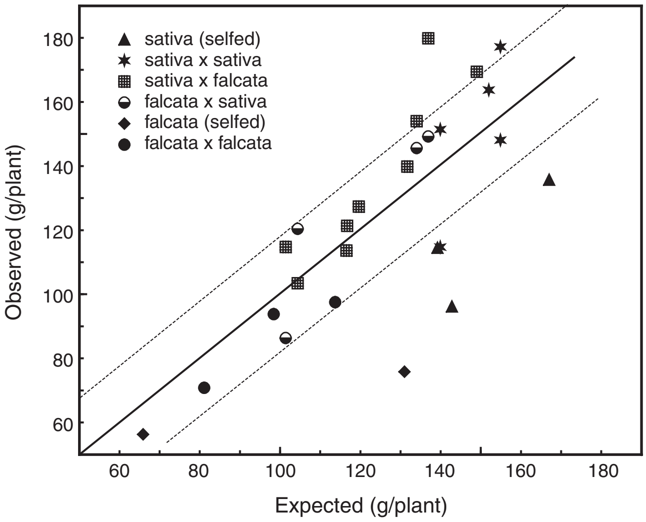

Fig. 2. Observed *v.* expected mean total dry matter yields (g/plant) and 95% confidence limits for sativa S_1, falcata S_1, sativa × sativa, falcata × falcata, sativa × falcata and falcata × sativa from diallel analysis. Expected mean and confidence limits derived from parental general combining abilities (GCAs).

(Forsthoefel *et al.* 1992). Although our results were obtained from a relatively small number of clones, they indicate that in developing lucerne hybrids or semi-hybrids, sativa should be used as the female parent if forage yield is to be maximised.

The current study assessed yield over 6 months and thus only looked at early vigour afforded by heterosis. The effect of heterosis on persistence was not assessed. In the current study, very highly significant yield effects for both SCA and GCA were shown. The clear-cut SCA effects we observed were determined from crosses between individual genotypes, and not strain crosses. Observed SCA effects are diluted when seed is bulked over multiple genotypes within each cross as was done and observed by Segovia-Lerma *et al.* (2004) in a population-based diallel analysis among the 9 historically recognised lucerne germplasms, including falcata. The relatively high levels of heterosis we observed were also due in part to the use of S_1 populations for the parental evaluations, rather than using cuttings, also termed clonal propagules. Both of the methods for parental analysis have associated limitations, as outlined by Brummer *et al.* (2000). Also, biomass yield of lucerne is known to differ between space-planted and sward plots. Riday and Brummer (2004) have considered the effect of sward planting *v.* space-planted trials for the determination of heterosis. They found that heterosis expression is greater in space-planted trials for progeny of both sativa and falcata genotypes, probably due to the lower competition in space-planted trials. Lower competition allows more shoots/plant, which is a component of yield (Volenec *et al.* 1987). Spaced-plant designs do not provide information on the agronomic potential of material under a dense canopy (Crochemore *et al.* 1998); however, when seed is limited, as in our study, they do provide a first step in evaluation of potential benefits of introgressing falcata germplasm.

Intraspecies and interspecies heterosis has not been widely explored in lucerne in Australia, and mostly, the easily obtained sativa × falcata crosses have been tested in other parts of the world. More recently, Dr E. T. Bingham of the University of Wisconsin, Madison, has produced a small number of hybrids between tetraploid *M. arborea* (a drought-resistant, non-dormant, woody perennial) and sativa, using male sterile sativa as the female parent (Bingham 2005). This interspecific hybrid may show improvement over sativa × falcata crosses for drought resistance and salinity resistance, both important attributes in the Australian environment.

Although sativa × falcata crosses are relatively easy to make, the value of falcata, purely for heterotic effects on yield in a warm temperate to subtropical environment, needs to be more fully assessed on the basis of the promising results reported here. Falcata had a strong negative GCA effect in our studies due to the relatively low yields produced by the parental genotypes. WISFAL, the source of the falcata clones used in the study, has never undergone selection for yield or disease and pest resistance, and is a highly winter-dormant, vegetatively robust population, that is fertile in intra-strain or inter-strain crosses with cultivated lucerne (Bingham 1993). Our studies indicate the potential of falcata to contribute new alleles that may improve the yield *per se* of synthetics being developed for use in northern Australia, provided selection is done for disease and pest resistance in the introgressed populations. Even further progress may be made in improving yield in lucerne in Australia, if falcata populations are improved separately before then converging them with improved sativa populations in the production of semi-hybrids (Brummer 1999). Our studies indicate that it may be worthwhile improving falcata through selection for a range of traits, including yield, under Australian conditions. Our results with unselected falcata are in agreement with those of Segovia-Lerma *et al.* (2004) who reported that important allelic combinations influencing yield could be identified from the use of unadapted and unselected material.

The historic germplasm source, Peruvian, has also recently been identified as an additional unselected source of heterosis for lucerne improvement (Segovia-Lerma *et al.* 2004). The highly successful cv. Sequel, which was bred for use in northern Australia (Clements *et al.* 1984), contains up to 50% Peruvian germplasm that has undergone extensive selection for traits important to the Australian environment. The sativa clones WA221 and WA222 were both obtained from cv. Sequel HR (derived from Sequel) and hence are likely to contain large proportions of Peruvian germplasm. Semi-hybrid cultivars developed by converging separately improved falcata and Sequel populations would appear to have potential for improving lucerne yields in northern Australia.

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