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Lucerne biology and genetic improvement — an analysis of past activities and future goals in Australia*

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Abstract. Breeding methodologies for cultivated lucerne (*Medicago sativa* L.), an autotetraploid, have changed little over the last 50 years, with reliance on polycross methods and recurrent phenotypic selection. There has been, however, an increase in our understanding of lucerne biology, in particular the genetic relationships between members of the *M. sativa* complex, as deduced by DNA analysis. Also, the differences in breeding behaviour and vigour of diploids versus autotetraploids, and the underlying genetic causes, are discussed in relation to lucerne improvement. *Medicago falcata*, a member of the *M. sativa* complex, has contributed substantially to lucerne improvement in North America, and its diverse genetics would appear to have been under-utilised in Australian programs over the last two decades, despite the reduced need for tolerance to freezing injury in Australian environments.

Breeding of lucerne in Australia only commenced on a large scale in 1977, driven by an urgent need to introgress aphid resistance into adapted backgrounds. The release in the early 1980s of lucernes with multiple pest and disease resistance (aphids, *Phytophthora*, *Colletotrichum*) had a significant effect on increasing lucerne productivity and persistence in eastern Australia, with yield increases under high disease pressure of up to 300% being recorded over the predominant Australian cultivar, up to 1977, Hunter River. Since that period, irrigated lucerne yields have plateaued, highlighting the need to identify breeding objectives, technologies, and the germplasm that will create new opportunities for increasing performance. This review discusses major goals for lucerne improvement programs in Australia, and provides indications of the germplasm sources and technologies that are likely to deliver the desired outcomes.

Additional keywords: alfalfa, breeding, autotetraploid.

Introduction

Lucerne (*Medicago sativa* L.) is the oldest known cultivated forage plant, with historical records of its use dating to 1300 BC in Turkey and 800 BC in Babylonia (Hendry 1923). Its value as a forage was readily recognised by the Romans 2000 years ago, and during the period of the Roman Empire, lucerne was intentionally established in all of their provinces (Ahlgren 1949). Lucerne was introduced into South America in the 16th Century (Klinkowski 1933), and to Australia soon after white settlement in the late 18th Century (McMaster and Walker 1970). World lucerne areas in the 1980s were estimated at 32 million ha, of which 70% were located in the USA, USSR, and Argentina collectively (Michaud *et al.* 1988). Australian areas in 1999 comprised 192 000 ha (Table 1) grown exclusively for hay (Australian Bureau of Statistics), and an estimated 3.5 million ha of pastures containing lucerne used for grazing in dryland farming operations (Pearson *et al.* 1997). It is estimated that up to 86 Mha could be planted to lucerne under dryland conditions in eastern Australia and a further 9 Mha in Western Australia, leaving considerable scope for expansion of current production (Hill 1996). These estimates were based on climate (rainfall and temperature), and critical thresholds for growth and persistence. The greatest opportunities for expansion were in New South Wales (NSW) and Queensland (Hill 1996). For example, in Queensland in 1999, the area sown to pure lucerne stands for hay and grazing was 32 300 ha (Australian Bureau of Statistics) compared with a potential of 8.75 Mha (Weston *et al.* 1984).

This review provides an analysis of lucerne biology, breeding goals, and achievements up to the present, and indicates future activities likely to lead to greater and more productive exploitation of lucerne in Australia. Because of

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Table 1. Australian irrigated lucerne areas and production in 1999 (1982 areas in parentheses)

	New South Wales	Victoria	Queensland	South Australia	Western Australia	Tasmania	Northern Territory	ACT	Total
Lucerne hay areas (ha)	92000 (58200)	42000 (20300)	23000 (18600)	26000 (14400)	2000 –	2000 –	4000 –	–	192000 (111500)
Lucerne hay production (t)	406000	199000	196000	86000	16000	11000	19000	1000	933000

its wide adaptation, tolerance to abiotic physiological stresses, and deep taproot, use of lucerne has considerable potential to improve the sustainability of Australian broadacre agriculture through improving soil properties including soil nitrogen levels, lowering the watertable and hence salinity, and improving soil water penetration.

Lucerne biology

Origins of lucerne

The genus *Medicago* comprises more than 60 species, which grow over a wide area stretching from China to Spain and from Sweden to North Africa (Lesins and Lesins 1979). The primary centre for the genus is in the Caucasus, north-western Iran and north-eastern Turkey (Ivanov 1977). The basic chromosome number for *Medicago* is $x = 8$, except for the annual species *M. constricta*, *M. praecox*, *M. polymorpha*, *M. rigidula*, and *M. murex* where $x = 7$ (Quiros and Bauchan 1988). Three ploidy levels are found in the genus: $2n = 2x = 14$ and $2n = 2x = 16$, $2n = 4x = 32$, and $2n = 6x = 48$. It is considered that the basic evolution of the genus has taken place at the $2x$ level, and that $4x$ (autotetraploid) species arose through unreduced gametes, giving rise to heterozygous individuals that were aggressive enough to colonise new habitats and to increase the distribution range of the diploid (Gillies 1972). Annual species are autogamous, whereas the perennials are allogamous, with different degrees of self sterility (Quiros and Bauchan 1988).

Lucerne, or alfalfa as it is known in North America, is part of the *M. sativa* complex that belongs to the section Falcago, subsection Falcatae, which includes $4x$ and $2x$ forms of *M. sativa* ssp. *sativa*, *M. sativa* ssp. *falcate*, and *M. sativa* ssp. *glutinosa* (Lesins and Gillies 1972). Members of the above taxa all intercross readily, and share the same karyotype. In this review, the term lucerne is used to encompass this *M. sativa* complex. The main barrier to gene exchange between representatives is ploidy, which is overcome by diploids producing unreduced gametes. Diploid forms of purple-flowered *M. sativa* are named *M. sativa* ssp. *coerulea*, whereas the purple-flowered tetraploid forms are *M. sativa* ssp. *sativa* (Quiros and Bauchan 1988). In both cases, the purple-flowered forms are characterised by coiled pods. This contrasts with *M. sativa* ssp. *falcata*, which has straight to sickle-shaped pods and yellow flowers. *M. sativa* ssp. *falcata* is also characterised by $2x$ and $4x$ forms. The third subspecies of the *M. sativa* complex, ssp. *glutinosa*, is $4x$ with bright yellow flowers and coiled pods.

Additional members of the *M. sativa* complex that hybridise readily with lucerne include ssp. × *varia*, ssp. × *hemicycla* (hybrids between ssp. *sativa* or ssp. *coerulea* and ssp. *falcata*), ssp. × *polychroa* (hybrids between ssp. *sativa* and ssp. *glutinosa*), and ssp. × *tunetana* (hybrids between ssp. *sativa* or ssp. *coerulea* and *M. glomerata*). *M. glomerata* is a species related to the *M. sativa* complex, characterised by yellow flowers and coiled pods covered in glandular hairs (Lesins and Lesins 1979).

Genetics and evolution of cultivated lucerne

Cultivated lucerne is always autotetraploid, and is distinguished from diploid forms by the larger size of its flowers, pods, and seeds (Lesins 1970). Forage yield is also higher for $4x$ than $2x$ material. Conclusive evidence for autotetraploidy was provided by Stanford (1951) after demonstrating tetrasomic segregation for flower colour in progenies of diallelic plants. Evidence showing the occurrence of unreduced gametes in diploid *M. sativa* indicates that this might have been a significant event in the origin of the tetraploid subspecies. $2x \times 4x$ or $4x \times 2x$ crosses are possible when the diploid parent has the ability to generate unreduced ($2n$) gametes, giving tetraploid progeny (Vorsa and Bingham 1979). It is unequivocal that the hybridisation of *M. sativa* ssp. *sativa* and *M. sativa* ssp. *falcata* has contributed substantially to the development of cultivated lucerne in temperate regions of the world. This is discussed later in a section on germplasm.

Isozyme analysis has been used to compare variability levels in natural diploids and tetraploids of *M. sativa* and in commercial cultivars (Quiros 1982, 1983; Quiros and Morgan 1981). In general, natural tetraploids were found to be more variable than their diploid counterparts, measured by percentage heterozygosity.

Tetraploid *M. sativa* ssp. *falcata* had the highest values for percentage heterozygosity and number of alleles among all subspecies including $2x$ and $4x$ (Quiros and Morgan 1981). The level of variability of cultivated lucerne was comparable to that of natural populations of *M. sativa* ssp. *sativa*, but far below the values obtained for *M. sativa* ssp. *falcata*. There were twice as many tri- and tetra-allelic plants in natural tetraploids of *M. sativa* ssp. *falcata* as in cultivars (Quiros 1983), lending support to the hypothesis that maximum heterozygosity, reflected in the tri- and tetra-allelic plants, plays an important adaptive role, at least in wild populations of autotetraploids (Bingham 1980). In both of the above

populations, <1.5% of plants were tetra-allelic, whereas 19% of the *M. falcata* plants were tri-allelic *v.* 9% for the cultivars.

Isozyme data have also shown that *M. falcata* is present in many cultivated lucernes of different geographic origins, thought to have only ssp. *sativa* genes (Quiros 1983). This was the case for cultivars of Flemish and Chilean origins, previously thought only to contain the *M. sativa* genetic background (Barnes *et al.* 1977).

When DNA markers were used to assess diversity in 2*x* and 4*x* lucernes, similar findings were made to those with isozymes (Kidwell *et al.* 1994*a*, 1994*b*). RFLP analysis showed that herbage yield in 4*x* populations was more responsive to genetic diversity than in their corresponding isogenic diploid populations, this genetic diversity being manifested in greater opportunity for complementary gene interactions in the 4*x v.* 2*x* populations (Bingham *et al.* 1994). Whereas differences in yield and certain other traits can be attributed to increased cell and organ size associated with polyploidy (Arbi *et al.* 1978), differences in 4*x* and 2*x* breeding behaviour and combining ability require a genetic explanation (Bingham *et al.* 1994).

Autotetraploid genetics and breeding behaviour

The difference between 2*x* and 4*x* populations in the way heterosis is manifested is important in population development. Maximum heterozygosity and resultant heterosis is reached in diploids in one generation (the single cross), whereas heterozygosity and heterosis is progressive in tetraploid alfalfa and is not maximised until the double cross or later generation, depending on the level of inbreeding in the parents (Bingham 1980). Maximum heterozygosity and resultant heterosis in tetraploid lucerne could be due to either multiple allelic interactions at a single locus (over-dominance) or chromosome segments with complementary alleles (termed linkats), or a combination of both (Bingham *et al.* 1994). Demarly (1972) and Dunbier and Bingham (1975) acknowledged that multiple alleles at a single locus cannot be distinguished from linkats in most cases, and breeding methods for maximising heterozygosity are the same in either case.

Bingham *et al.* (1994), working in 2-allele populations derived by doubling diploids and thus eliminating the possibility of tri- and tetra-allelic loci, have suggested that the progressive heterosis phenomenon in 4*x* lucerne could be due to a progressive increase in complementary gene interactions involving favourable alleles with additive effects in linkage blocks (linkats). Complementary gene interaction occurs when dominant alleles at heterozygous loci, which affect the same trait, complement each other by masking recessive deleterious alleles at the respective loci. This state is also termed epistasis. There are greater opportunities for complementary gene interactions in 4*x* than in 2*x*, since tetrasomic segregations of linkage blocks containing favourable dominant alleles in repulsion linkages produce complementary gene interactions not possible in diploids. The rapid loss of complementary gene interactions upon inbreeding 4*x* lucerne may explain the severe inbreeding depression experienced upon selfing or sib mating. The greater complementary gene interactions in 4*x* lucerne help to explain DNA marker research indicating that yield in tetraploids is more responsive to genetic diversity than in diploids (Bingham *et al.* 1994).

In breeding lucerne, to maximise yield, every effort should be made to minimise inbreeding depression, and to maximise heterozygosity and resultant heterosis (Bingham 1980; Brummer 1999). Recurrent half-sib family selection following polycrossing of at least 100 unrelated parents (individual S_0 plants, each a different genotype) has been the most commonly employed breeding process since first described for use in alfalfa by Tysdal *et al.* (1942). All lucerne cultivars in commercial use today are synthetics, which may be defined as open-pollinated cultivars produced by random mating of many parents (clones or genotypes) (Busbice 1969). Synthetics can be reconstituted from the original selected parents, and this differentiates a synthetic from an open-pollinated cultivar.

Recognised world germplasm sources of lucerne and traits associated with these sources

Barnes *et al.* (1977) identified and provided characteristics for 9 distinct lucerne germplasm sources that had been introduced into the US between 1850 and 1947, and which had contributed to lucerne development in North America. These sources (groups) are: 1, *M. falcata*; 2, Ladak (contains a lot of *M. falcata*); 3, *M. varia* (naturally occurring hybrids of *M. falcata* and *M. sativa*); 4, Turkistan (primarily *M. sativa*); 5, Flemish (primarily *M. sativa*); 6, Chilean (primarily *M. sativa*); 7, Peruvian (only *M. sativa*); 8, Indian (only *M. sativa*); 9, African (only *M. sativa*). Groups 6–9 are all non-dormant types, whereas Groups 1– 5 have a high to moderate level of winter dormancy. Sources 6 and 7 derive from Spanish lucernes. Source 4 (Turkistan) is generally susceptible to leaf diseases, but has resistance to insects and root and crown diseases, and for this reason has been extensively used in breeding. The Flemish lucernes, moderately winter-hardy, are generally resistant to foliar diseases and susceptible to root and crown diseases. The 9 germplasm sources correspond with the generally accepted fall dormancy classes (1, very dormant; 9, non-dormant), but Fairey *et al.* (1996) found that there was a lack of exact equivalence between fall dormancy class and plant height of the fall regrowth. They indicated 2 clear dormancy classes only: dormant with a large falcata/ladak infusion, and non-dormant comprised of Indian and African material.

Root morphology has a critical role in influencing persistence and productivity traits of lucerne (Johnson *et al.* 1998). Using ecotypic correlations among plant introductions, these authors found that tap root diameter and

lateral root diameter were positively correlated with fall dormancy; and that lateral root number, lateral root position, and fibrous root mass were negatively correlated. However, for cultivars released since 1980, only fibrous root mass was correlated with fall dormancy, indicating that plant breeding strategies could be effectively used to modify root architecture. Also, the wide variability observed for the above traits, particularly in plant introductions, suggested that there was a lot of scope for breeding cultivars with a specific root architecture; however, the ideotype for specific environments remains undefined.

Application of molecular mapping and molecular marker technology to lucerne improvement

Advances in molecular biology over the last decade have provided DNA-based markers, including restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD) markers, which are not affected by environmental or developmental factors (Tanksley *et al.* 1989; Williams *et al.* 1990). A molecular linkage map can be readily constructed using these markers in segregating populations, and such maps when generated can simplify genetic analyses that are applicable to plant improvement, including lucerne (Brummer *et al.* 1991). Several such genetic linkage maps have been created for lucerne using diploid populations, to avoid the complicated tetrasomic inheritance and linkage relationships of tetraploids (Brummer *et al.* 1993; Kiss *et al.* 1993; Echt *et al.* 1994; Tavoletti *et al.* 1996). Three of the 4 maps generated from this research have identified 8 linkage groups corresponding to the basic chromosome number of $x = 8$. These maps have been used to locate genes controlling flower colour, dwarfness, and sticky leaves (Kiss *et al.* 1993), seed proteins and nodulation factors (Kiss *et al.* 1997), a unifoliate leaf, cauliflower head mutation (Brouwer and Osborn 1997), and winter hardiness (Brouwer *et al.* 2000).

Yu and Pauls (1993) used RAPD markers in tetraploid lucerne to analyse segregation patterns, and the results indicated that random chromosome segregation was the predominant, but not exclusive, mode of inheritance in the tetraploid clones studied. These workers also discussed the relative merits of various strategies for molecular mapping in the tetraploid lucerne genome. Brouwer and Osborn (1999) used single dose restriction fragments (Wu *et al.* 1992) to generate a linkage map in 2 backcross populations of tetraploid lucerne. The molecular probes used had also been used in generating diploid maps, allowing a direct comparison of diploid and tetraploid maps. Generally, the locus map orders and distances were in concordance across the 2 ploidy levels, eliminating the need for ploidy level manipulations, as conducted by Havey *et al.* (1987) when studying inheritance of *Phytophthora* resistance in tetraploids. This latter finding is significant, since general combining abilities for forage yield and fertility are not correlated when studied in isogenic diploid and tetraploid lucerne, suggesting that genes affecting these quantitative traits may have different effects at the 2 ploidy levels (Groose *et al.* 1988). It can thus be concluded that mapping at the tetraploid level may be more informative for quantitative traits such as yield and fertility, and would eliminate the need for ploidy manipulations. The populations studied by Brouwer and Obsorn (1999) and Brouwer *et al.* (2000) were also segregating for winter hardiness, autumn dormancy, and freezing tolerance.

Molecular markers would appear to have significant application in the mapping of traits important to lucerne improvement in Australia, e.g. resistance to *Phytophthora*, *Colletotrichum*, and spotted, blue-green, and pea aphids; and particularly in the identification of individual plants and clones possessing multiple resistances to these pests and disease. Because of the population breeding approach that has to be adopted for lucerne, simply inherited, dominant sources of resistance, as have been identified for *Phytophthora* resistance (Irwin *et al.* 1981*a*, 1981*b*) and *Colletotrichum* resistance (Mackie and Irwin 1998*b*), are more likely to lead to more rapid population improvement than those sources which are of more complex inheritance (Mackie and Irwin 1998*a*, 1998*b*). Molecular markers would also facilitate mapping of quantitative trait loci (QTLs). A QTL has been identified in diploid lucerne controlling variation in aluminium tolerance (Sledge *et al.* 1996), and more mapping of QTLs influencing forage yield and other quantitative traits in tetraploid material will greatly facilitate breeding of improved lucernes for Australia, once the required specific traits other than yield and persistence have been identified.

Kidwell *et al.* (1994*c*) used RFLPs to assess genetic diversity between the *Medicago* sources of accessions representing the 9 original germplasm sources for North American cultivars. The results suggested high levels of genetic diversity between individuals within accessions, and compared with the diversity between accessions, most of the germplasm sources were not very distinct genetically. The exceptions were *M. falcata* and Peruvian, which formed distinct clusters from the remaining 7 accessions, which clustered together. Tools such as RFLPs have potential to be used to assess the relative contributions of different germplasm sources to cultivar development, although the study of Kidwell *et al.* (1994*c*) detected very few accession-specific polymorphisms. Use of additional probes that may be more closely linked to the ecologically significant genes that distinguish the groups may assist in resolving this issue.

Clearly, molecular marker technologies have much to offer to lucerne improvement in Australia. The challenge will be to identify specific traits needed for lucerne improvement over the range of climates, soil types, and management conditions to which lucerne is subjected in Australia. A molecular analysis of Australian cultivars, using

the technology of Kidwell *et al.* (1994*c*) and later workers, would provide valuable information on genetic diversity levels in Australian cultivars, and may provide indications for future improvement using the maximum heterozygosity concept of Bingham (1980), once the alleles or linkats conditioning important traits have been identified. Yield increases that accompany increased heterozygosity may be associated with the interaction of multiple alleles at individual loci, or chromosome segments containing linked favourable dominant alleles, or a combination of the two (Bingham 1980; Groose *et al.* 1989). Use of molecular marker technology on appropriate populations will help elucidate these gaps in our knowledge and contribute in a practical way to lucerne improvement.

Role of genetic engineering in lucerne improvement

Genetic engineering bypasses the constraints of sexual hybridisation, and provides for the exchange of genetic material between widely different organisms. This has been achieved for lucerne chiefly using *Agrobacterium tumefaciens* (Deak *et al.* 1986) or microinjection (Reich *et al.* 1986). Since these early studies, lucerne has been transformed for a variety of single-gene traits, including herbicide resistance (D'Hallium *et al.* 1990), virus resistance (Hill *et al.* 1991), and an insect proteinase inhibitor (Thomas *et al.* 1994). Thomas *et al.* (1994) described a transformation system using *Agrobacterium tumefaciens* that was highly efficient (10% of all explants exposed to *Agrobacterium*). High levels of expression of the proteinase inhibitor (0.125% of total protein) were obtained using the Cauliflower Mosaic Virus 35S promoter-proteinase inhibitor fusion. *Agrobacterium* transformation has been further improved in *Medicago truncatula* using *in planta* infiltration methods (Trieu *et al.* 2000), and this technology will have application in lucerne.

This research indicates that it is practical to transform lucerne with foreign genes. The challenge is to identify single genes that will effect better results than using conventional methods. Antimicrobial genes which will provide improved management of pathogens for which there is no naturally occurring resistance, e.g. *Rhizoctonia solani*, the causal agent of rhizoctonia root canker, would appear to offer promise. An excellent example of the application of genetic engineering to effect improved traits in lucerne concerns bloat management. A major disadvantage of grazing ruminants on lucerne is bloat, an abnormal distension of the rumino-reticulum caused by excessive retention of microbial fermentation gases within the ruminal cavity. Instead of forming pockets of free gas above the rumen contents that can be eliminated by eructation, the gas bubbles remain dispersed throughout the rumen contents, producing an abnormal increase in the volume of the contents, distending the rumen to the point where breathing and circulation are impaired, sometimes resulting in rapid death (Reid *et al.* 1975). The characteristic frothiness is caused by inadequate coalescence of gas bubbles, and identification of the substances in lucerne responsible for frothy rumen contents is still not resolved, although total soluble leaf proteins are thought to play a major role (Majak *et al.* 1995).

It has been known for many years that some leguminous forages such as sainfoin (*Onobrychis viciifolia*) do not cause bloat (Tanner *et al.* 1997). Sainfoin produces condensed tannins, which bind, via hydrogen bonding, to the soluble leaf proteins. There is sufficient tannin in the leaves of sainfoin to precipitate the soluble leaf protein in the rumen, thus preventing the formation of a stable protein foam. Lucerne and other bloat-inducing legumes do not produce condensed tannins. Tanner *et al.* (1997) have isolated a key tannin biosynthetic gene from sainfoin that synthesises leaf tannins. Work is in progress through genetic engineering approaches to transfer this sainfoin gene into lucerne, to switch on tannin biosynthesis. Single gene manipulations such as described above hold great promise for addressing issues such as bloat, and for introducing novel disease resistances into lucerne that are effective against pests and diseases for which strongly expressed naturally occurring resistances have not been identified.

Lucerne improvement in Australia

Performance of lucerne lines and germplasm in Australia up to 1977

Until 1977, Hunter River was the predominant cultivar grown in Australia, occupying over 95% of the total lucerne area (Cameron 1973; Rogers *et al.* 1978). Hunter River's origin remains unclear. Daday *et al.* (1961) indicated that Hunter River was assumed to have originated from the Mediterranean region (Williams 1950), whereas Rogers (1967) suggested that Hunter River derived from the French variety Provence, and had a Flemish background (Group 5). It is probable that a range of introductions was assimilated into the one variety, contributing, together with over 100 years of natural selection, to its wide adaptability. It has been shown that there was considerable genotypic variation within Hunter River, with seed derived from the Tamworth region of NSW considered the best in NSW in the early 1900s (Cameron 1973). However, when selections from Queensland and NSW were compared with commercial seed derived from South Australia, Gramshaw (1978) found no consistent yield or persistence differences.

Although Hunter River held a virtual monopoly in Australia up to the mid 1970s, Rogers (1961) presented evidence from the Riverina showing that Hairy Peruvian (*syn*. Siro Peruvian) (Group 7), Indian (Group 8), and African (Group 9) all had higher production in the first 3 years of the stand than Hunter River, but lacked Hunter River's genetic base for persistence *per se*. These studies and those of Daday *et al.* (1961) demonstrated the importance of

Table 2. Lucerne cultivars listed in the Register of Australian herbage plant cultivars (Oram 1990) or described in the *Australian Plant Varieties Journal*, their year of first certification or registration or grant of PBR in Australia, and their origins

Cultivar	Year	Geographic or varietal origin of parental germplasm	Sources of parental germplasm[A]
Hunter River	1962–1963	French or Mediterranean-type, Provence	5[A]
African	1962–1963	Egyptian	9
Du Puits	1963–1964	French	5
Siro Peruvian	1964–1965	Chilean	8
Cancreep	1968	Rambler, African, Siro Peruvian, Hunter River	1, 5, 7, 9
Paravivo	1971	Egyptian	9
Demnat	1972	Algerian	9
Falkiner	1976	cv. Lahontan (Turkistan)	4
Walkabout	1977	Hunter River, Indian, Saladina, Hairy Peruvian Pampa	1, 5, 7, 8
Nova	1979	cv. Lahontan	4
CUF101	1979[B]	UC Cargo, UC Salton, UC 76, 1972 Breeding Mixture and Niagara N71 Brand (refer *Crop Science* **23**, 398)	2, 3, 4, 5, 6, 7, 8, 9
Siriver	1980	cv. Hunter River, cv. CUF 101 and Turkistan	2, 3, 4, 5, 6, 7, 8, 9
Sirotasman	1980	Du Puits, Saranac, Washoe, CUF 101, UC 110 and UC 112	2, 3, 4, 5, 6, 7, 8, 9
Springfield	1980	CUF 101 and NZ bacterial wilt resistant clones (cf. Sirotasman)	2, 3, 4, 5, 6, 7, 8, 9
Wakefield	1980	Afganistan and Hunter River, Paravivo, Demnat, African and CPIs	4, 5, 9
Sheffield	1980	Afganistan and Spanish CPIs, plus Wakefield	4, 6, 9
Maxidor 2	1983	WL 318, WL 512, CUF 101, African and Turkistan	2, 3, 4, 5, 6, 7, 8, 9
Validor	1983	WL 318	–
Hunterfield	1983	Hunter River	5
Trifecta	1983	Hunter River, CUF-101 and various other clones (Siro Peruvian, Lahontan, UC 76)	2, 3, 4, 5, 6, 7, 8, 9
Sequel	1985	Siro Peruvian, CUF 101	2, 3, 4, 5, 6, 7, 8, 9
Aurora	1986	Falkiner, Siriver and WL 318	2, 3, 4, 5, 6, 7, 8, 9
Quadrella	1991	Trifecta	2, 3, 4, 5, 6, 7, 8, 9
Prime	1992	–	–
L69	1995	–	–
L34.HQ	1996	Apollo, NCMP-1, Saranac AR, Anchor, Atra 55, 532, 521, 531, 520, 530. Du Puits, Vernal, Narragansett, Culver, Maryland, Dawson, Iroquois, MSA	1, 2, 3, 4
Aquarius	1997	CUF 101, M193 (refer *Crop Science* **29**, 833)	1, 2, 3, 4, 5, 6, 7, 8, 9
Genesis	1997	Hely 7 × Hely 11, M193	1, 2, 3, 4, 5, 6
Sceptre	1997	–	–
Sequel HR	1998	Siro Peruvian, CUF 101	2, 3, 4, 5, 6, 7, 8, 9
Jindera	1998	–	–
Eureka	1998	–	–
Flairdale	1998	Hunter River, Wakefield, Springfield, Pioneer 581, CUF 101	2, 3, 4, 5, 6, 7, 8, 9
Grasslands Kaituna	1999	Experimental lines AG3E and 83 + 34	–
Hallmark	1999	Trifecta, Sequel, M193	1, 2, 3, 4, 5, 6, 7, 8, 9
Salado	2001	Mesa Sirsa, AZ-Gern Salt II, AZ90NDC-ST	8 (predominately)
Rapide	2001	Hassawi, Pioneer 5929, WL 605	9 (predominately)
UQL-1	2001	Hallmark, M193, highly winter dormant clones (refer *Plant Disease* **64**, 396–397; *Crop Science* **29**, 833), Aquarius	1, 2, 3, 4, 5, 6, 7, 8, 9

–, Could not be ascertained from published descriptions.
[A] The number refers to the parental germplasm source: 1, *M. falcata*; 2, Ladak; 3, *M. varia*; 4, Turkistan; 5, Flemish; 6, Chilean; 7, Peruvian; 8, Indian; 9, African (refer Barnes *et al.* 1977).
[B] Year of first commercial use in Australia.

G × E interactions in breeding programs through the superior performance of Du Puits (Group 5) in cooler tableland environments in New South Wales compared particularly with the better performance of Hairy Peruvian in the warmer environments of the Riverina, the New South Wales south coast (Daday 1965), the north-western plains of New South Wales (Launders 1970), and the Darling Downs in Queensland (Fletcher 1970).

Cameron (1973) found that Hairy Peruvian, Indian, and African were initially superior to Hunter River in the warmer and more humid environment of Biloela in Queensland but the advantage there lasted only 1 year. Most of the extra production occurred during the cooler months. In these studies, Du Puits lacked the winter production of African and Hairy Peruvian, and all were less productive and persistent than Hunter River (Cameron 1968; Cameron and Mullaly 1972).

Also, in the above period, Leach (1969, 1970) tested over 100 lines of diverse lucernes for persistence and productivity at the Waite Research Institute in South Australia. No line was found to consistently yield more than Hunter River, but some erect lines from Mediterranean regions (in particular Spain and Portugal) yielded more in winter. Hunter River was also one of the most persistent lines, as were prostrate lines from Spain and Portugal, whereas the erect Mediterranean lines were poorly persistent. In further studies in the upper south-east of South Australia, Leach (1971*b*) found that Portuguese lines were initially superior to Hunter River and suggested that the group be widely tested in order to release them commercially. This did not occur, as the lines were less persistent than Hunter River and their relative production declined in the longer term.

Gramshaw (1978) reviewed the performance of genotype evaluation in the central Queensland environment and concluded that there was no production advantage over Hunter River or Siro Peruvian from selected genotypes from USA, South America, or the Mediterranean.

In summary, up to the mid 1970s, Hunter River was clearly the dominant lucerne variety grown in Australia, occupying >95% of the area sown. However, introductions of winter-active lucernes of Peruvian and African origins had shown yield increases over Hunter River, but were less persistent.

Lucerne breeding efforts in Australia up to 1972

Hunter River was first certified in Australia in 1962–63 (Oram 1990) (Table 2), and around that time Du Puits and African were also certified. These were direct introductions without breeding, their potential value being established in the trial work described above. Siro Peruvian and Paravivo were selected directly from Hairy Peruvian and African, respectively, and Demnat was an introduction of Algerian origin. All of these lucernes were highly winter active, but less persistent than Hunter River.

It was clearly recognised by the 1960s that lucerne was not particularly persistent when heavily grazed. Two significant CSIRO breeding programs aimed to develop rhizomatous and creeping rooted lucernes that were both productive and persistent. The temperate program in Canberra produced Cancreep from a diverse mixture of dormant and creeping rooted material tracing to Rambler, Hunter River, and Siro Peruvian (Oram 1990). Cancreep showed potential in cooler environments where heritability for persistence was high (Daday 1968) but was quite unproductive in warmer and drier environments (Daday *et al.* 1968). The expression of creeping rootedness in the Mediterranean climate of South Australia was low (Leach 1971*a*) and the cultivar was not sown widely. In the subtropical environment of Queensland, the second breeding program produced germplasm registered as Walkabout (Oram 1990) that was never released commercially. Persistence of this creeping rooted line was less than Hunter River in its target area, the brigalow clay soils of southern inland Queensland as, without a robust taproot, it seemed more susceptible to drought, which is a feature of this environment (Bray and Leach 1981).

During this period, emphasis was placed on productivity and persistence as the main selection criteria, but without attempts to identify underlying contributing factors.

Identification of factors contributing to poor lucerne productivity and persistence in Australia (1970–77)

Rogers (1961) considered attributes desirable for lucerne both under irrigation (hay production) and under dryland grazing conditions. She identified high production at all seasons of the year, resistance to grazing damage, and persistence for at least 3–4 years as desirable characteristics. At that time, the causes of the generally poor persistence of lucerne through Australia were unknown.

During the 1970s, it became apparent that plant diseases were major factors contributing to poor lucerne persistence and productivity throughout eastern Australia. Phytophthora root rot, caused by *Phytophthora medicaginis*, was found to be a major cause of poor lucerne persistence and productivity, particularly on clay soils, from Biloela in central Queensland (Irwin 1977) to Deniliquin in south-western NSW (Rogers *et al.* 1978). Hunter River was very susceptible, as were all of the cultivars that had been released up to that time (Irwin 1974*a*). The disease is always associated with wet soil conditions. Another major disease that is widely distributed throughout eastern Australia is anthracnose, caused by *Colletotrichum trifolii*. This fungus is dispersed by raindrop splash, causing stem lesions (anthracnose) which spread into the crown, causing rapid plant death (Irwin 1974*b*). Again, all of the material released in Australia up to 1972 was highly susceptible to this disease, in particular the winter-active cultivars Siro Peruvian, African, Paravivo, and Demnat (Irwin 1974*b*). Hunter River is very susceptible to the stem lesion phase of the disease, but is less susceptible to crown rot than the above cultivars. It was discovered that Hunter River possessed a small (<1%) proportion of plants with resistance to both diseases, and recurrent selection within Hunter River provided a substantial component of the base genetic material for Trifecta (Bray and Irwin 1978; Irwin *et al.* 1980) and Hunterfield (Oram 1990), both released in 1983. In 1977, the spotted alfalfa aphid (SAA) and blue-green aphid (BGA) appeared in Australia for the first time (Passlow 1977*a*, 1977*b*), followed by the pea aphid in 1979 (Rogers 1981). Hunter River and all other cultivars used in Australia at that time were highly susceptible to these aphids and commercial lucerne stands throughout most parts of eastern Australia were devastated by them. This presented a specific set of objectives for lucerne breeding, with emphasis on disease and pest resistance, and the potential to substantially increase yield and persistence in the presence of these pests and

diseases. Prior to the initial aphid invasion in 1977, there had been selection to improve the disease resistance of the two main Australian cultivars, Hunter River and Siro Peruvian (Bray and Irwin 1978; Rogers *et al.* 1978; Irwin *et al.* 1980) and this provided a basis on which to develop multiple pest resistant cultivars.

Breeding objectives post 1972

Lucerne cultivars bred in Australia, or bred elsewhere and released in Australia, over the last 38 years are set out in Table 2. The list includes public and Plant Breeders Rights (PBR) protected cultivars. The first PBR lucerne cultivar released in Australia was Quadrella in 1991, derived by selection within Trifecta for resistance to *Stemphylium* leaf spot (Bray and Irwin 1989). Since that time, all cultivars on the list are PBR protected. Almost without exception, major selection criteria used by breeders over this period have been resistance to aphids, or the acute diseases caused by *Phytophthora* or *Colletotrichum*, or all three. The first cultivar to achieve at least moderate (>20% of plants) levels of resistance to aphids, *Phytophthora*, and *Colletotrichum* was Trifecta, which was released in 1983 (Oram 1990). It is worth noting that in a variety with 20% of plants resistant to either *Phytophthora* or *Colletotrichum*, and on the basis of independent inheritance, only 4% of individual plants could be expected to be resistant to both diseases. When more traits are added, the percentage decreases geometrically.

The incorporation of resistance to *Phytophthora*, *Colletotrichum*, and aphids into adapted genetic backgrounds has lead to substantial yield increases in the presence of these parasites. For example, Trifecta outyielded Hunter River 3-fold over a 16-month period at Gatton Research Station; Trifecta's advantage over the aphid-resistant, disease-susceptible cultivars, CUF 101 and Siriver, was 19% and 24%, respectively, over the same period (Clements *et al.* 1984). In the same trial, the *Phytophthora*-resistant Hunter River selection and the *Colletotrichum*-resistant Hunter River selection, which were used as germplasm in the breeding of Trifecta, outyielded Hunter River by 195% and 156%, respectively. In NSW, the cultivar. Aquarius was released with a very high level of resistance to *Phytophthora* (Williams and Young 1992), and two further elite cultivars with resistance to the diseases and aphids, Genesis and Venus (Y8622), have since been released from that program (Williams 1998).

It is noteworthy that there is genetic variability in the disease reaction of lucerne lines to the chronic foliar pathogens *Leptosphaerulina trifolii*, *Pseudopeziza medicaginis*, and *Cercospora medicaginis* (Inch *et al.* 1993). These have not been considered as major priorities in any breeding program.

The US bred cultivar CUF 101 (Lehman *et al.* 1983) has been used extensively as a parent in Australian lucerne breeding programs as a donor source of spotted and blue-green aphid resistance (refer Table 2). In ongoing Australian lucerne breeding programs, resistances to aphids, *Phytophthora*, and *Colletotrichum* constitute major breeding objectives, particularly for varieties being grown for hay production under irrigation. It is this latter category upon which most of the emphasis has been placed in lucerne improvement in Australia.

The creeping-rooted varieties Cancreep and Walkabout in the Dormancy Class 5–6 have been bred with grazing tolerance as a principal objective. These have been discussed previously. Breeding for grazing tolerance has been a priority between 1972 and 2000 in South Australia, with the cultivars Wakefield and Sheffield being selected under continuous grazing pressure from sheep, and possessing high levels of aphid resistance (Oram 1990).

Performances of cultivars imported directly from the USA and Australian-bred cultivars have been measured under irrigated conditions at Gatton Research Station since 1977. Up to 1983, there was a substantial increase in the general mean of the 3-year accumulated lucerne yields of breeders' lines and released cultivars, relative to that of Hunter River and reaching a maximum of 167%. Since then, it has remained static at around 130% indicating that there has been little further improvement in yield potential for the humid, subtropical environment (Lowe *et al.* 2000). Cultivars in these evaluations included Trifecta, Sequel, Aurora, Aquarius, Quadrella, Sequel HR, Sceptre, Eureka, Pioneer L69, Hallmark, and UQL–1. All have been selected for resistance to aphids, *Phytophthora*, and *Colletotrichum*, and all will generally outyield Hunter River by up to 50% in this environment. Similar results were obtained at all irrigated sites in Queensland, NSW, and Victoria. These results prove the effectiveness of selection for multiple disease and insect resistance for developing high yielding lucerne for irrigated environments.

Where possible, the germplasm sources, as identified by Barnes *et al.* (1977), have been listed in Table 2. It is worth noting that generally, *M. falcata* has not been widely used in the breeding of widely grown Australian material, probably due to the reduced need in Australia for varieties with tolerance to freezing conditions. A recent exception is UQL–1, which has 17% variegated flowers (Anon. 2000), indicating substantial introgression of *M. falcata* genetic material.

Performance of lucerne lines and germplasm in Australia since 1977

Rogers (1981) was evaluating a range of *Phytophthora*-resistant cultivars from the USA and Australian selections at Deniliquin, NSW, when aphids arrived in Australia. The Australian cultivar Falkiner (based on Lahontan) and a selection, C3 Composite (based on Siro Peruvian), were high-yielding and highly resistant to *Phytophthora*, although both suffered significant damage from aphids. Although

neither cultivar had a major influence commercially, they contributed substantially to future breeding programs. Concurrently, selections were being made in Queensland for both *Phytophthora* and *Colletotrichum* in Hunter River and Siro Peruvian (Bray and Irwin 1978; Irwin *et al.*1980). These also had no direct commercial impact but contributed to future breeding programs, including Siriver (Oram 1990), which is a high seed producer and which is used almost exclusively for sprouting and for export to the Gulf States as a uniform winter-active cultivar for hay production.

Following the devastation of stands of Hunter River by lucerne aphids in 1977, State Departments of Agriculture and commercial seed companies commenced evaluation of cultivars from the USA to identify adapted and productive germplasm. In these there was a range of resistance to the lucerne aphids SAA and BGA (Lloyd *et al.* 1980*a*, 1980*b*; Ridland and Berg 1981; Turner *et al.* 1981), with CUF 101, an important progenitor of future lucerne cultivars (Table 2), expressing high levels of resistance to both. This confirmed the resistance of CUF 101 described by Nielson and Lehman (1974) and Lehman *et al.* (1977) to Australian populations of SAA and BGA.

Generally, it was shown that there was a wide range in the dry matter production of these imported cultivars under Australian conditions. Winter-active cultivars produced higher yields than semi-dormant and dormant cultivars but were less persistent (in NSW, Rogers 1981; Lodge 1985, 1986; in Queensland, Gramshaw *et al.* 1985; Lloyd *et al.* 1985; Lowe *et al.* 1985*a*). The superior imported cultivars became significant in commercial sowings before the release of elite Australian-bred cultivars.

In understanding the hitherto unknown basis for the performance of these new lines in Queensland and NSW, it was found that lucerne yield was correlated with winter activity level and persistence. Persistence was directly related to disease resistance and initial stand density (Lowe *et al.* 1985*a*, 1987). It was established that different defoliation systems were needed to manage cultivars from different dormancy classes and these differed from those previously utilised for Hunter River (Lowe *et al.* 1985*b*; Lodge 1986; Gramshaw *et al.* 1993). Experimentation in Queensland since 1993 has evaluated newly released cultivars and breeding lines from the 3 Australian public breeding programs and commercial companies. In earlier evaluation trials conducted with irrigation, the production and persistence of these lines varied widely, but since 1993, most lines and cultivars showed little difference in yield or persistence (Lloyd and Lowe 2000; Lowe *et al.* 2000). In semi-arid environments where lucerne is less well adapted and is used in mixed farming systems for grazing, the production and persistence of commercial cultivars has been exceeded by a number of breeder's lines in evaluation trials (Lloyd *et al.* 2001). There is clearly scope to release better cultivars for these environments.

Future objectives in Australian lucerne breeding (2000 onwards)

The choice of breeding objectives for lucerne is complex, since the species has a number of uses, particularly growth under dryland or irrigated conditions and use for hay or grazing. Increasingly, grazing lucerne is being used in conjunction with cereals for its capacity to increase soil nitrogen levels, improve water retention properties of soil, to reduce dryland salinity through lowering of the watertable, and to limit the deep drainage of water from soil profiles into river systems. The more widespread use of lucerne under dryland conditions in the cropping zone calls for cultivars with much wider adaptation than those currently available. Two of the main types required by farmers are a lucerne for short-term rotations that grows and fixes N more quickly than current cultivars, and a long-term, more persistent lucerne for sowing with grass. The third farmer requirement is for bloat-safe lucernes, particularly where cattle are the predominant grazing animals.

Thus, attributes required for lucerne in the cropping zones include ease of establishment, the capacity to utilise deep subsoil moisture, drought tolerance, tolerance to acid soils, and tolerance to high temperature and root damage from cracking clay soils. Williams and Boschma (1996) have identified plant morphological attributes that correlate with long-term persistence. Where lucerne is being used for broad-scale planting in the cropping areas, ease of seed production is critical to the provision of low cost seed supplies. Belloti *et al.* (1998) suggested that for lucerne to better fulfil a role in the development of more sustainable farming systems, there should be a shift in breeding objectives. Until now, breeders have focused on disease and pest resistance, herbage production, and persistence. Belloti *et al.* (*ibid*.) suggest that there should be a shift to attributes that enhance the ability of the lucerne plant to perform its sustainability function (i.e. high water use, rapid root elongation, waterlogging tolerance, tolerance of soil acidity, and salt tolerance).

Naturally occurring genetic variation is known for some of these traits. Tolerance to saline conditions at germination and during forage growth has been reported (Rumbaugh and Pendery 1990; Smith *et al.* 1994) as has tolerance to continuous grazing (Smith and Bouton 1993), tolerance to acid soil and aluminium toxicity (Dall'Agnol *et al.* 1996), and tolerance to drought (Quiros and Bauchan 1988). The challenge is to develop breeding technologies that will allow recombination of the genetic factors conditioning these traits and others, e.g. disease and pest resistance, forage quality, and others, into new varieties with improved adaptation to the environment.

It would appear that only a small component of the lucerne (*M. sativa* complex) gene pool has been used in Australian lucerne breeding efforts up to now and some

genetic resources such as CUF 101 have been extensively used. This has lead to a predominance of winter-active to highly winter-active cultivars being released (see Oram 1990). Smith *et al.* (1995) conducted a detailed morphological and agronomic examination in Arizona of lucerne accessions collected from oases in the Middle East and which up to then had not been extensively used in lucerne breeding in North America. Some accessions were very winter-active, with fast regrowth following cutting, whereas others from low elevation oases in Yemen were very sensitive to frost damage. Warburton and Smith (1993) also further characterised diversity for agronomic traits in lucerne from India and the Middle East. Vastly different responses were obtained, with the western Indian material being the least persistent, and the Arabian selections exhibited very rapid regrowth after harvest.

Crown structure/morphology of Australian cultivars has changed significantly with the inclusion of winter-active germplasm into breeding programs. The cultivar Hunter River is a semi-dormant cultivar with a low and densely branched crown supporting fine stems and leaves. It had a reputation as an excellent producer of high quality hay. Almost all of the new Australian cultivars have lost these morphological traits and have a higher, less branched crown supporting fewer, larger stems with large leaves. Although these cultivars usually make hay of acceptable quality, none consistently made hay of as high quality as Hunter River (Lowe *et al.* 1988). Commercial haymakers believe that there should be a return to the 'Hunter River' crown morphology to make Australian cultivars more suitable for hay production.

There is also a belief that a low and broad crown morphology confers greater grazing tolerance. This is probably true in intensive sheep grazing systems but is less effective in cattle grazing systems, particularly in the subtropics where biotic factors seem to be more important in plant persistence. Lodge (1985) found that the persistence of lucerne from different dormancy classes (and implied crown morphologies) was similar when grazed leniently with sheep in the subtropics. However, grazing management best practice is required with sheep to achieve this outcome.

In the US, Johnson *et al.* (1998) showed that a wide contrast existed in the root architecture of lucernes from the 8 recognised world germplasm sources. *M. falcata* and *M. varia* (a hybrid of *sativa* × *falcata*) were characterised by having a large number of lateral roots, and a substantial fibrous root mass, in contrast to the non-dormant Indian and African material with fewer lateral roots and a low fibrous root mass. Through breeding, it is possible to break these linkages between architecture and fall dormancy. The challenge is to identify particular root phenotypes that best confer attributes required in Australian lucernes. Drought resistance, and the capacity to produce winter growth following moderate rainfall events, are attributes that would be beneficial over much of the Australian cropping belt. Drought resistance might be best conferred through a deep taproot, whereas the latter attribute could come from a plant with many shallow lateral roots and an extensive fibrous root mass. Physical blending of seed of cultivars possessing these different characteristics is a viable option, since both attributes described above would often be required at a single site.

Climate matching procedures, as outlined by Smith *et al.* (1994), should be adopted to identify geographic regions, from which lucerne accessions could be obtained and screened for the desired traits. It would appear that winter-active material would have the greatest potential for further expansion of lucerne in dryland farming systems. It is currently thought that this material is better able to exploit the stop–start pattern of rainfall and soil water availability throughout the year. Winter activity is also a most useful trait when lucerne is used in the dairy industry where the provision of high quality forage in the cool season is of high priority.

Breeding methodologies, based on recurrent selection and polycrossing to produce synthetic varieties, have changed little over the last 50 years. Molecular marker technology could improve lucerne breeding practices. Difficulties associated with distinguishing genetically diverse individuals to be used as parents have forced breeders to include large numbers of clones in their synthetic cultivars to minimise inbreeding and to conserve genetic variability for other traits (Hill *et al.* 1988). Kidwell *et al.* (1994*a*, 1994*b*) used molecular markers to identify genetically diverse parents for producing highly heterozygous and high-yielding single-cross hybrid progenies. Although genetic and cytoplasmic male sterility, and female sterility have been identified in lucerne, large-scale production of hybrid seed remains not economically viable (Viands *et al.* 1988). Because of this, Kidwell *et al.* (1999) have commenced assessing the application of molecular markers to identify more than two genetically diverse individuals to produce synthetic populations with a higher number of different alleles and more heterozygosity than unselected populations, using the maximum heterozygosity concept for alfalfa breeding expounded by Bingham (1980). This approach is likely to be most successful if the marker technology is targeted to specific genomic regions influencing forage yield. Such quantitative trait loci can be located through mapping in populations segregating for desired traits (Brouwer and Osborn 1999). Mapping in Australian-derived material (clones from Trifecta and Sequel) is under way in the Cooperative Research Centre for Tropical Plant Protection using single dose restriction fragments employed in constructing the map of Brouwer and Osborn (1999) (J. A. G. Irwin and J. Musial, unpubl. data).

Genetic engineering lucerne for single-gene-controlled traits such as disease and insect resistance, where naturally occurring resistance in the *M. sativa* complex is not available or only weakly expressed, also has application to lucerne improvement in Australia. Resistance in the *M. sativa*

complex to many of the fungal leaf spot pathogens is only partial, and expression of novel resistances, such as the antifungal peptides identified from *Macadamia integrifolia* (Marcus *et al.* 1997), may provide new opportunities for improving lucerne productivity through improving resistance to chronic diseases.

In summary, future improvement programs in Australia would be enhanced by greater exploitation of the *M. sativa* complex germplasm pool to identify breeding material possessing traits likely to increase the adaptation and usefulness of lucerne to Australian agriculture. An analysis of Australian-bred cultivars released over the last 20 years indicates that there is considerable opportunity for broadening the genetic base. *M. falcata*, although widely exploited in North America, has not been extensively used in Australia, even though Quiros and Morgan (1981) have demonstrated its high inter- and intra-locus variability compared with *M. sativa*. This is important in the light of the results of Bingham (1980) and Bingham *et al.* (1994) demonstrating that maximising heterozygosity in tetraploid lucerne maximises yield. Molecular marker technology will have an increasingly important role to play in the process of maximising heterozygosity, particularly by selection for chromosomal regions known to influence productivity traits and against segments containing genes for poor adaptation to Australian conditions. However, only a few cross-over events occur in each set of homologous chromosomes in each pollen and egg mother cell, and tetrasomic inheritance slows down the recovery of the required recombinants. Therefore, several generations of marker-assisted selection will be required to fully exploit the opportunities created by broadening the gene pool.

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