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Lucerne Genetics and Breeding

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Introduction

I am deeply honoured to receive this Award. I am also very humbled, following in the footsteps of so many other researchers who have worked to better Australian agriculture through the application of science and technology. The work of William Farrer focussed on wheat, an industry that today has a farm gate value in excess of $5 billion and is one of Australia's major export earners. Tonight I wish to address the topic of lucerne genetics and breeding. This may seem a long way from wheat, but lucerne may well hold the key to the sustainability of the Australian wheat and pastoral industries.

Lucerne is Australia's most important perennial forage and ley pasture legume, with over 200,000 ha grown for hay production, and a further 3.5 m ha used in ley farming operations (Pearson et al. 1997). The largest areas of lucerne production are in New South Wales, and modelling indicates that an additional 81 mn ha could be sown to lucerne in Australia, mostly in New South Wales (31 m ha) and Queensland (20 m ha) (Hill 1996). The utilisation of lucerne could be increased if a number of traits were able to be bred into commercial cultivars: drought tolerance, grazing tolerance, acid soil tolerance, big seededness, longevity, yield (both summer and winter) and non-bloating (Irwin et al. 2001).

The oration encompasses the following major themes which have arisen from lucerne research that I have been involved in over the past 35 years:

- biology of lucerne
- brief history of lucerne breeding in Australia
- classical genetics of resistance to *Phytophthora medicaginis, Colletotrichum trifolii* and *Stagonospora meliloti* in lucerne
- molecular genetics and mapping in autotetraploid lucerne
- future directions for lucerne research.

Biology 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 basic chromosome number for *Medicago* is x = 8, except for some annual species where x = 7 (Quiros and Bauchan 1988). Lucerne (alfalfa in North America) is part of the M. *sativa* complex, which includes autotetraploid (2n = 4x = 32) and diploid (2n = 2x = 16) forms, and is essentially a hybrid swarm between ssp. *sativa* (purple flowered) and ssp. *falcata* (yellow flowered) (Lesins and Gillies 1972). All cultivated lucerne is autotetraploid (Stanford 1951), which introduces a high degree of complexity to lucerne genetic studies where the gamete is diploid, and a tetra-allelic heterozygote is possible versus di-allelic in a diploid. Segregation distortion is commonly observed in lucerne genetic research (Yu and Pauls 1992), and autotetraploidy contributes substantially to this. Lucerne is an outbreeding species and subject to inbreeding depression; a consequence of this is that it is commercialised as synthetic cultivars. Consequently, a lucerne cultivar consists of a heterogeneous mixture of heterozygous plants. Although genetic and cytoplasmic male and female sterility have been identified in lucerne, large scale hybrid seed production is still not economically viable (Viands et al. 1988).

Brief history of lucerne breeding in Australia

Until 1977, Hunter River was the predominant lucerne cultivar grown in Australia, occupying over 95% of the total lucerne area (Cameron 1973). Hunter River's origin remains unclear, although it is generally accepted that it derived from a Flemish background (Rogers 1967). It is most likely that a range of introductions were assembled into the one background. Hunter River is semi-dormant (dormancy group 5). As a cadet with the Queensland Department of Primary Industries in 1972, my first job was to determine the role of plant disease as a factor contributing to poor persistence of lucerne in Queensland. It soon became apparent that two acute diseases, Colletotrichum crown rot, and Phytophthora root rot, were the major causes of lucerne's declining productivity (Irwin 1974(a), Irwin 1974(b), Irwin 1977), and that these same diseases were also major issues in New South Wales (Rogers et al. 1978, Stovold and Francis 1988). More recently, Stagonospora crown rot has been established as a major disease leading to stand decline in southern New South Wales (Irwin et al. 2004).

Phytophthora and Colletotrichum resistant lines were developed by recurrent selection within Hunter River and Siro Peruvian (Bray and Irwin 1978, Irwin et al. 1980), and yield increases of up to 300% were recorded. The commercial release of this material was prevented by the sudden appearance of lucerne aphids (spotted and blue green) in Australia in 1977, with Hunter River being highly susceptible to both aphids. The winter active US variety,

CUF101, had high levels of resistance to the aphids, and it was extensively used as a parent in the development of resistant varieties (Irwin et al. 2001).

The first Australian-bred cultivars to be commercialised with resistance to *Phytophthora medicaginis* (Pm), *Colletotrichum trifolii* (Ct), spotted and blue green aphids were Trifecta (semi-dormant) and Sequel (winter active). Both were developed by recurrent selection within Hunter River and Siro Peruvian respectively, with introgression of aphid resistance from CUF101 (Clements et al. 1984). After 20 years, Sequel is the most widely grown lucerne in Queensland, and still consistently outperforms all other commercial cultivars in Queensland Department of Primary Industries and Fisheries trials at Gatton Research Station (Lowe et al. 2000).

Currently, there are about 40 public and proprietary cultivars of lucerne on the market in Australia, including Hunter River. Several more are currently progressing through the Plant Breeders Rights process. Only three (including Hunter River) of these 40 cultivars on the market are listed as susceptible to *Colletotrichum* and *Phytophthora*. While large increases in persistence and productivity have been obtained (estimated 20% yield increase on average over the 200,000 ha irrigated lucerne) through the use of multiple disease resistant varieties, in more recent years lucerne yields have plateaued, both in Australia and the US (Lowe et al. 2000, Brummer 1999). Indications in the US are that there have been no advances made in lucerne yield per se, over the last 50 years.

Classical genetics of resistance to Phytophthora, Colletotrichum and Stagonospora in lucerne

Phytophthora medicaginis

Our breeding activities indicate two genetic mechanisms conditioning resistance to *Phytophthora* in lucerne; an incompletely recessive mechanism where in an intercross of a resistant (R) and a susceptible (S) clone < 50% of the progeny are resistant, and an incompletely dominant system where > 50% of the intercross progeny are resistant (Irwin et al. 1981). In-depth inheritance studies were conducted on 4x clone M193 which exhibited high levels of resistance to Phytophthora. In an R x S intercross, this clone gave 87% resistant progeny, and the observed segregations in S_1, F_1 and F_2 populations could be explained on the basis of two incompletely dominant complementary genes, Pm_1 and Pm_2 (Irwin et al. 1981). This clone was scaled down to 2x level through a triploid bridging cross, and inheritance studies were conducted on the derived diploid plants (Havey et al. 1987).

Two of the derived diploids segregated for two independent complementary loci designated Pm_1 and Pm_2 (as described in the 4x parent M193) while the remaining two derived diploids segregated for two independent dominant genes, which were also independent of Pm_1 and Pm_2, and designated Pm_5 and Pm_6. In addition to these genes, Havey and Maxwell (1987) identified Pm_3 and Pm_4 in 2x *M. sativa* ssp. *falcata*. These resistant *falcata* clones were crossed with the derived diploids from M193, to generate 2x plants carrying the genes Pm_1, Pm_2, Pm_3, Pm_4, Pm_5 and Pm_6. From 2x x 4x crosses, using the 2x as the female, and through unreduced gametes, a 4x Phytophthora-resistant population was developed, and registered as WAPRS-4 (Havey et al. 1989). This material has contributed substantially to Australian agriculture. Aquarius was bred by New South Wales Department of Primary Industries by crossing S1 plants of M193 to CUF101 (Oram 1990), and Aquarius has the highest resistance levels (> 70%) to *Phytophthora* of any lucerne cultivar in the world. Hallmark and UQL-1 were bred from crosses of WAPRS-4 with elite clones from Trifecta, Sequel and Aquarius (Irwin et al. 2001).

Colletotrichum trifolii

Three pathotypes (pathogenic races) of *C. trifolii* have been identified worldwide (Mackie et al. 2003), and all three are present in Australia. In the US, resistance to race 1 in cultivar Arc was reported to be conditioned by a completely dominant gene, An_1, and resistance to race 2 by another independent dominant gene An_2 (Elgin and Ostazeski 1985). Plants resistant to race 2 were also resistant to race 1, indicating An_2 conferred resistance to races 1 and 2. Research on clones from Trifecta (W126) and Sequel (W116) have indicated a more complex inheritance (Mackie and Irwin 1998, Mackie et al. 2003). In clone W126, resistance to races 1 and 4 appeared to be linked, and was incompletely dominant, whereas resistance to race 2 was incompletely recessive. The resistance in clone W116 to race 1 was incompletely recessive, with only 7% of an R x S intercross progeny being resistant (Irwin et al. 2006). These findings have major implications for lucerne breeding programs aimed at incorporating Colletotrichum resistance.

Stagonospora meliloti

Resistance to *S. meliloti* (Sm) has been studied in clones W126 and W116. Again, it was not possible to fit the observed segregation to a single completely dominant tetrasomic gene. Resistance was however incompletely dominant, indicating good progress could be made through recurrent selection for *Stagonospora* resistance (Irwin et al. 2004).

Molecular genetics and mapping in autotetraploid lucerne

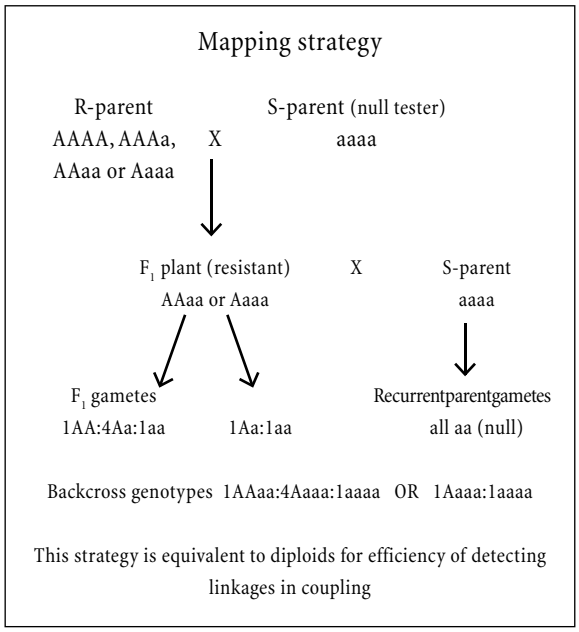
If improvements are going to be made in lucerne productivity and adaptation, then a different approach to the current breeding methodologies, based on production of broadly based synthetics, will be required. With the synthetic approach, it is difficult to accumulate favourable dominant genes in the frequency required to achieve significant gains (Bingham et al. 1994).

Breeding strategies are required to capture the early (syn 1) generation heterosis that can be achieved with lucerne as demonstrated by Mackie et al. (2005). This can be achieved to some extent without male sterility, by identifying heterotic groups, separately improving these by recurrent selection, and only converging the two gene pools in the final increase, to give rise to commercial seed (Brummer 1999).

The process described above, particularly the selection of So parents, can be greatly facilitated by the application of DNA marker technology (Musial et al. 2005). Markers for chromosome regions which contribute to yield heterosis, as well as markers for disease and pest resistances, would facilitate the selection of individual clones which carry multiple favourable traits. We have initiated research to map resistances to Pm, Ct, Sm, as well as yield per se, with the goal of developing robust DNA markers for use in identifying individual clones carrying multiple favourable traits. Another major goal of this research is to link this autotetraploid map to other 4x lucerne maps (Brouwer and Osborn 1999; Julier et al. 2003) and to the map of the model legume, the diploid *Medicago truncatula* (Thoquet et al. 2002).

Mapping strategy

In generation of the 4x lucerne map, single-dose restriction fragments (SDRFs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeat (SSR) were mapped in backcross (BC) populations segregating for the traits described above (Musial et al. 2005, Musial et al. 2006). Two BC mapping populations have been generated, using the mapping strategy outlined below:



The above strategy allowed development of a coupling phase map of the resistant F1 plant's gametes, giving 16 possible linkage groups. Homologous linkage groups were joined using duplex markers linked in repulsion (Brouwer and Osborn 1999; Musial et al. 2005).

Two separate maps have been generated, as described below.

clone W116 map (Phytophthora resistance)

This mapping population comprised 120 individuals, which were segregating for reaction to *Phytophthora medicaginis*. The map contained 18 linkage groups covering 2136.5 cM, with an average distance between markers of 15 cM. Using duplex markers and repulsion phase linkages, the map condensed to 7 homology groups and 2 unassigned linkage groups. Regions located on linkage groups 2, 14 and 18 were identified as associated with Phytophthora root rot resistance, and these quantitative trait loci (QTLs) individually explained from 6-15% of the phenotypic variation at $P<0.01$. These markers are currently undergoing validation testing.

Quantitatively-expressed incompletely recessive resistance to *C. trifolii* was also mapped in the same BC population of plants used to map *Phytophthora* resistance (Irwin et al. 2006). A multi-locus region which appeared to consist of 3 loci located on linkage group 4 of the W116 map, and an unlinked marker, were found to contribute 23% of the total variation of anthracnose reaction. These markers are now being validated.

clone D map (forage yield)

The recurrent and susceptible parent used in generation of the lucerne genetic maps, clone D, is both winter active and very high yielding. We have mapped and identified DNA markers linked to yield in clone D, using the backcross population described above (Musial et al. 2006). Individual markers that accounted for up to 18% of the total yield over 6 harvests at Gatton were identified. The same marker, AC/TT8, was consistently identified at each individual harvest, and in individual harvests accounted for up to 26% of the phenotypic variation for yield. This marker was located in linkage group 2 of the D map, and several other markers positively associated with yield were consistently identified in this linkage group. Clone W116, while resistant to Pm and Ct, was relatively low yielding, and markers negatively associated with yield were consistently identified in the W116 map. Validation of these markers is required before they can be routinely used in breeding programs. Also, the D and W116 maps require linking to other published 4x lucerne maps, and to the *M. truncatula* map, to make these findings more accessible to other researchers.

W126 map (resistance to *C. trifolii* races 1, 2 and 4, and resistance to *S. meliloti*)

Through collaboration with Professor Richard Oliver's group at Murdoch University in Western Australia, the W126 map, generated using the same strategies as the W116 map, has been linked to the *M. truncatula* map.

The molecular analysis confirmed the findings of the classical phenotypic genetic studies, that resistance to Ct races 1 and 4 was tightly linked (2.3 cM). DNA marker CCTA 17 showed linkage to major QTLs that explained about 40% of the phenotypic variation in reaction to the two races. One of these loci could be An1, as reported in the US as conferring resistance to race 1 (Elgin and Ostazeski 1985). These genes are located on the end of a chromosome that is homoeologous to *M. truncatula* chromosome 8.

Resistance to race 2 was inherited independently of resistance to races 1 and 4, and a major QTL which explained about 30% of the phenotypic variation to race 2 was marked by DNA marker CATG 12. This gene is most likely located on a chromosome homoeologous to *M. truncatula* chromosome 6, and could be the An_2 gene reported by Elgin and Ostazeski (1985). These markers would appear to have great potential for identifying plants such as W126 which carry resistance to all known Ct races. Several other QTLs which explained up to 10% of the phenotypic variation, and were independent of the QTLs described above, were also identified.

Resistance to *S. meliloti* was also successfully mapped in the same W126 BC population used to map Ct resistance. Three QTLs which collectively identified 38% of the phenotypic variation were mapped to chromsomes homoeologous to *M. truncatula* chromosomes 2 and 7, with one QTL remaining unlinked.

While much work remains to be done to validate these markers, the research to date has exposed 4x lucerne to the extensive genomics initiatives underway in *M. truncatula.*

Future directions for lucerne research

The following future research directions for lucerne breeding in Australia, and northern Australia in particular, are advocated:

- **linking existing maps to other 4x lucerne maps in existence, and to the *M. truncatula* map.** This will greatly enhance the utility of our maps, and expose 4x lucerne to the gene discovery research being done in *M. truncatula.*
- validating and testing the utility of the QTL markers identified for yield, and for resistance to Phytophthora, Colletotrichum, and Stagonospora. Once this has been done, markers for other traits such as aphid resistance, tolerance to acid soils (Al^{+++} tolerance), and others can be developed.
- **overcoming yield stagnation through introducing new genes into the lucerne gene pool currently being utilised in Australia, through conventional breeding is a high priority for all applications of lucerne.** Our DNA marker research has identified *M. sativa* ssp. falcata as an outgroup to *M. sativa* ssp. sativa, and a potential source of new genes (Musial et al. 2002). Substantial heterosis for yield has been observed by us in ssp. *sativa* x ssp. *falcata* crosses, in a subtropical environment (Mackie et al. 2005). *M. sativa* ssp. *falcata* has not been widely used up to now in Australian lucerne breeding programs, but we are now actively introgressing it into our adapted material, and promising results from sward evaluation of this material at Gatton have been forthcoming.

  Increased winter activity is a trait which has the potential to significantly lift lucerne productivity across much of Australia. Germplasm tracing to oases in Saudi Arabia and Oman has been introduced, and introgressed into our elite Sequel clones. Early sward trials with this material at Gatton have demonstrated up to 40% increased winter yields over Sequel. Over the course of a year, these same lines had a 2% higher cumulative yield than Sequel. This work indicates the untapped potential in Australia of lucerne germplasm from the Arabian peninsula.

  Dr E.T. Bingham from the University of Wisconsin-Madison, has generated hybrids between *M. sativa* and *M. arborea*. *M. arborea* is a very winter-active, long-lived and drought-resistant forage, naturalised to the Mediterranean sea coast. The sativa x arborea hybrids have been crossed to Sequel clones, and yield increases up to 40% over the best lucerne synthetics have been observed both by our team in Australia and by Dr Bingham in Wisconsin. We have also generated sativa x arborea hybrids in Brisbane using male sterile *M. sativa* clones as the female parent, and we are currently studying levels of genome introgression with AFLP markers. This research may have a lot of potential for extending the adaptation of lucerne.
- **lucerne yields have plateaued over the last 20 years in the US and Australia, and changes to current lucerne breeding methodologies, based on synthetics, are needed.** It is now recognised that the early generation heterosis, that has been long observed in synthetics, is due to favourable dominant linked genes, linked in repulsion (Bingham et al. 1994). Breeding methodologies that capture of early generation heterosis hold the key to future lucerne improvement. Instead of introgressing new germplasm into an increasingly diverse gene pool, as has been the case in the past, we should be identifying heterotic groups, keeping them separate while improving them by recurrent selection, then converging them to produce the commercial product. This is achievable at present, and until some of the current difficulties with commercial utilisation of male sterility are overcome, this "semi-hybrid" approach offers a way forward.

Through application of all of the research outlined above, and integrating findings into breeding programs, I am confident that lucerne adaptation, productivity and utilisation can be increased to the benefit of Australian agriculture.

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