final report

Project code: B.PBE.0028

Prepared by: Phillip Nichols

Department of Agriculture and Food Western Australia

Date published: June 2014

ISBN: 9781740362191

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Development of R&D pre-breeding plans for annual legumes

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

An annual pasture legume pre-breeding R & D plan to increase the profitability of red meat production has been developed, following recommendations from a workshop held to determine priority traits and species and subsequent industry consultation. The project will deliver a measure of the phenotypic diversity of at least 20 key agronomic traits (from this and other related projects), their potential for genetic improvement through plant breeding, and identification of potential parents for crossing in a range of annual legume species. Molecular markers associated with genes for these traits will be developed in subterranean clover and for genes associated with seedling growth and boron tolerance in annual medics. The project will also sequence the genome of subterranean clover and develop a robust protocol to triple generation turnover rates. Technical outputs will be made freely available to private and public breeding organisations.

Executive summary

The aim of this project was to develop a comprehensive pre-breeding R & D plan for annual legumes over the period 2013-2017 (with scope to consider a further five year investment) to improve the profitability and sustainability of red meat production in southern Australia. A multi-agency, public and private sector team was assembled to develop the pre-breeding R&D plan. This was led by the Department of Agriculture and Food Western Australia (DAFWA), with collaborating agencies being the South Australian Research and Development Institute (SARDI), New South Wales Department of Primary Industry (NSW DPI) and the Centre for Legumes in Mediterranean Agriculture (CLIMA) from the University of Western Australia (UWA), while private industry interests were represented by Seed Force Pty. Ltd. and Pristine Forage Technologies.

A workshop was held to identify the most important annual legume traits for meat production and the species best able to deliver them. This consisted of an invited panel of 28 industry experts comprised of leading animal specialists, farm consultants, economists, pasture breeders, pre-breeders, agronomists and pathologists and representatives from seed companies with an interest in annual legumes. Discussions were then held with agencies and breeding companies about technologies currently being used and their technical capacity and ability to conduct pre-breeding activities. This information was used to determine the project activities and the project team to implement them. A response was also sought from the private sector on how they would use the outputs from this project.

The workshop recommended the pre-breeding project should focus on traits to increase persistence and autumn-winter biomass production in regenerating stands. This was supported by economic modelling data which showed this would have the biggest positive impact on pasture productivity and profitability. A high legume density early in the growing season leads to greater competitiveness against weeds, resulting in pastures with greater legume dominance and higher feeding value into spring. The workshop recommended similar emphases should be given to mixed farming and permanent pasture systems and a high emphasis should be placed on subterranean clover, with some emphasis on annual medics and other species currently used. The workshop findings and subsequent consultation formed the basis for the proposed annual legume pre-breeding R & D plan.

Four organisations will implement the proposed project: DAFWA, SARDI, CLIMA and NSW DPI. It will also collaborate with Kazusa DNA Institute and Murdoch University. The project comprises the following 10 component activities:

- *In vitro* tools to reduce cultivar development time in subterranean clover (CLIMA);
- Molecular markers for key morphological traits in subterranean clover (CLIMA);
- Identification of genes for persistence in subterranean clover (CLIMA);
- Diversity for red legged earth mite (RLEM) resistance in subterranean clover, annual medics, balansa, bladder and Persian clovers, biserrula, yellow and French serradella and cultivars of other species (DAFWA);
- Genes for RLEM resistance in gland clover (DAFWA);
- Improved false break tolerance and seed dormancy traits in subterranean clover (DAFWA);
- Diversity of agro-morphological traits within subterranean clover (DAFWA);
- Increased growth at low temperatures in annual medics and subterranean clover (SARDI);
- Boron tolerance in annual medics (SARDI); and
- Project communication

A PhD/Masters student will be sought for the project topic: *Yellow serradella genetics and diversity*. Other students will be brought in to the project if possible.

Project outputs will include:

- A measure of the phenotypic diversity of at least 20 key agronomic traits in subterranean clover and annual medics (from this and other related projects), their potential for genetic improvement through plant breeding, and identification of potential parents for crossing;
- Molecular markers in subterranean clover associated with genes for at least 20 key agronomic traits (from this and other related projects) and molecular markers in annual medics associated with genes for seedling growth and boron tolerance;
- A framework to generate molecular markers for future new traits in subterranean clover and other annual legumes;
- A robust protocol to triple generation turnover rates for subterranean clover that can be incorporated into current breeding methodology;
- The genome sequence of subterranean clover, which will also have international applications for the breeding of white clover and red clover; and
- Training of junior scientists in important aspects of annual legume pre-breeding.

Technical outputs will be made freely available to private and public breeding organisations in a technical bulletin (likely to be web-based), listing the values of at least 20 key agronomic traits and the molecular markers closely associated with them, for diverse germplasm of subterranean clover, annual medics and other annual pasture legumes. Scientific outputs will be published in high impact, peer-reviewed journals; at least six publications are anticipated.

Resistance to important root rot pathogens is recognised as a major trait affecting seedling establishment and autumn-winter biomass. However, it was not considered in this project due to budget constraints and because of concurrent investments by MLA into the impacts of root rots on pasture productivity in other areas of the Feedbase Investment Plan. This trait is flagged here for future investment by MLA into pre-breeding activities.

Table of Contents

1. Background

1.1. MLA Feedbase Investment Plan

MLA is seeking to build a comprehensive pipeline of research, development and extension (R, D & E) for the southern Australian feedbase through the implementation of the Feedbase Investment Plan (FIP), which has been developed as a flow-on from the release of the National R, D & E strategies for the Beef and Sheep meat industries. The FIP was developed from 576 responses from within the feedbase industry to a major survey initiated by MLA. Its aim is for pasture improvement to add $25m per year on-farm value by 2020, with meat production (kg/ha) increasing by 2.5% and no decline in sustainability indicators.

The FIP comprises five ‘pillars’

- Pasture breeding and evaluation
- Productive and sustainable pastures
- Grazing management and production systems
- Weeds and biodiversity
- Decision tools

This project is in the Pasture Breeding and Evaluation Pillar, as part of a pasture improvement theme.

In August 2012 MLA called for Expressions of Interest to develop plans for investment over the period 2013-2017 into pre-breeding of:

- the perennial grasses, phalaris and cocksfoot; and
- the annual legumes, subterranean clover, annual medics and other legumes.

These priorities are the result of extensive consultation and recognise that investment by private sector plant breeding companies is limited for these species, resulting in market failure. The focus was to be on pre-breeding, rather than breeding itself. The Department of Agriculture and Food Western Australia (DAFWA) was awarded the contract to lead the development of an investment plan for annual legumes, while the University of Melbourne was to develop a plan for the perennial grasses.

1.2. Definition and scope of pre-breeding

Pre-breeding can be considered as all activities designed to identify desirable characteristics and/or genes for breeders to use in producing new varieties (FAO 2012). It does not involve development of a new cultivar itself. It can include the following activities:

- Screening accessions or populations for new traits;
- Developing core collections to better utilise plant collections;
- Identifying genes or Quantitative Trait Loci (QTLs) for traits of interest;
- Identifying molecular markers associated with traits of interest;
- Developing genetic maps;
- Developing rapid generation technologies; and
- Developing germplasm and breeding lines for use as parents in crossing and selection programs

The brief from an MLA perspective was that the pre-breeding project should involve technologies to increase the rate of genetic improvement in pasture plant breeding, through the development of new tools and processes to aid plant breeding and utilisation of all available resources.

1.3. Annual pasture legumes in southern Australia

1.3.1. Role in Australian farming systems

Pasture legumes are highly valued in the farming systems of southern Australia. In combination with the use of superphosphate, pasture legumes have led to greater animal production (Doyle *et al.* 1993; Thomas *et al.* 2010) and to increased crop yields when grown in rotation (Puckridge and French, 1983; Zhang and Evans 2004). This is particularly important in southern Australia, where the majority of soils have inherently low N status (McDonald 1989; Peoples and Baldock 2001; Howieson *et al.* 2008; Angus 2012). The ability of pasture legumes to fix atmospheric N and increase soil fertility benefits growth of both non-leguminous species in the pasture and of subsequent crops grown in rotation. Howieson *et al.* (2000) list other benefits for incorporating pasture legumes into farming systems, including their ability to improve soil structure and to break disease and pest life cycles of crops when grown in rotation, while Loi *et al.* (2005) has demonstrated the use of pasture legumes for weed control in crop rotations.

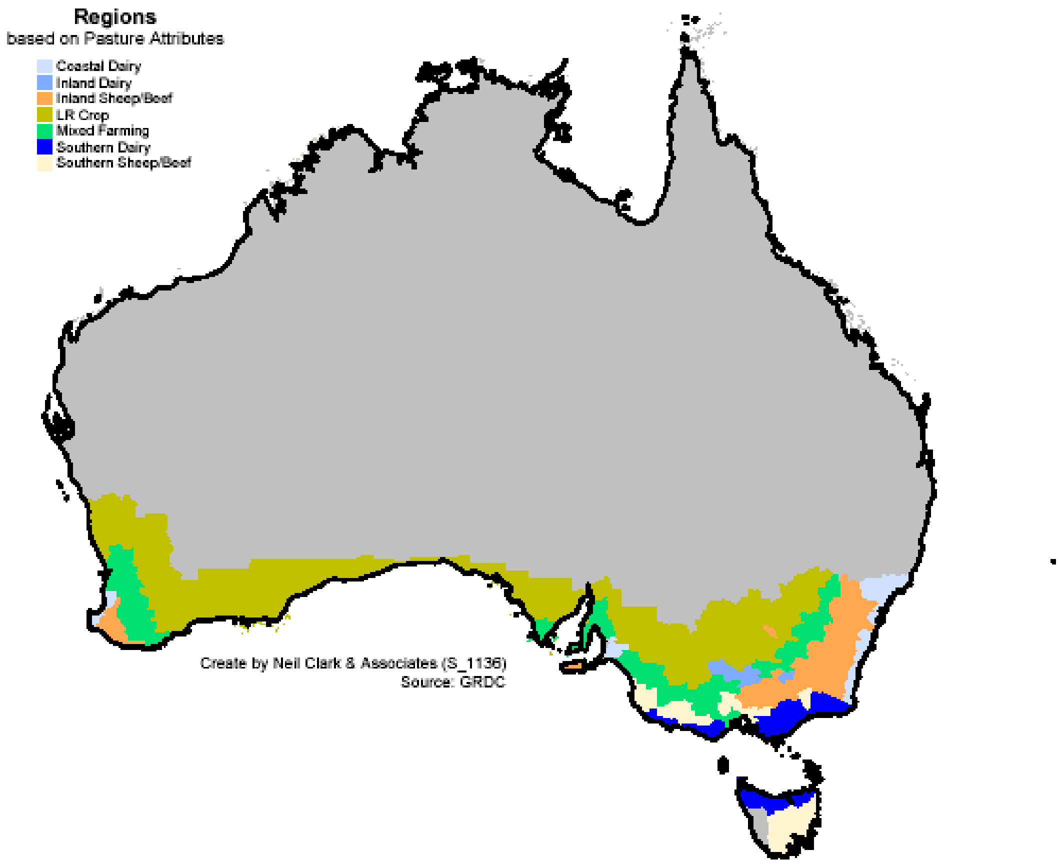


Figure 1. The agricultural regions of southern Australia, based on pasture attributes.

Australia's pasture legumes have been developed to fit the diversity of climates, farming systems and soil types across southern Australia. Figure 1 shows the agricultural regions of southern Australia based on pasture attributes. The agricultural regions of Western Australia (WA) and South Australia (SA) have mediterranean climates, characterised by winter-dominant rainfall, while quasi-mediterranean climates occur in western Victoria and south-western New South Wales (NSW). Annual average rainfall (AAR) varies from about 250 to 1200 mm, with corresponding growing season lengths of 3–10 months. South-western Australia has the most mediterranean climate, with up to 65% of rain falling in the winter months. Rainfall tends to become more evenly distributed throughout the year from west to east, so that eastern Victoria, Tasmania and central and southern NSW have more temperate climates. In eastern Australia the incidence of summer rainfall also increases with decreasing latitude, so that climates of northern NSW and southern Queensland (the northern-most limit of annual pasture legumes) are more subtropical, with > 50% of rainfall falling in the summer months. Pastures tend to be permanent or semi-permanent in irrigated and high rainfall areas and are usually comprised of a mixture of annual and perennial legumes with perennial grasses. Cropping, particularly wheat, is the major agricultural industry in low and medium rainfall areas, where annual legumes are commonly grown in rotation with crops.

1.3.2. Pasture legumes used in southern Australia

Australia has no native legumes suited to the farming systems of southern Australia and many are toxic (Cocks 2001). The pasture legumes used in southern Australia have, therefore, been introduced from other countries, particularly from the Mediterranean basin and surrounding areas (Nichols *et al.* 2012). Most noted among these are the annual legumes, subterranean clover (*Trifolium subterraneum*) and annual medics (*Medicago* species), which are well adapted to the winter-dominant rainfall climates of southern Australia. A wider range of annual legume species has been developed since the mid-1960s, particularly over the past two decades, for soil types and farming systems not suited to annual subterranean clover and annual medics (Nichols *et al.* 2007; 2012). Table 1 is taken from Nichols *et al.* (2012) and summarises the annual legume species with cultivars registered in Australia, their extent of utilisation and their main use.

Subterranean clover
Subterranean clover, first marketed in 1907, is the most widely sown annual pasture legume in Australia, having been sown over an estimated 29.3 M ha (Hill and Donald 1998). It consists of 3 subspecies: (i) ssp. *subterraneum*, adapted to well-drained, acidic soils; (ii) ssp. *yanninicum*, adapted to poorly drained, acidic soils, and (iii) ssp. *brachycalycinum*, adapted to neutral-alkaline cracking or stony soils (Katznelson and Morley 1965). Subterranean clover has two distinctive features contributing to its success as a pasture plant. Firstly, it is highly tolerant of regular, close grazing (particularly set-stocking by sheep), attributable to its prostrate growth habit and growing points close to the ground. Secondly, its ability to bury its burrs protects its seeds from predation by stock. Another factor contributing to its widespread use is a range of cultivars which differ in flowering time, enabling it to be grown in environments with AAR ranging from 250–1200 mm (corresponding to winter-spring growing season lengths from 4–10 months). The 45 cultivars registered in Australia include 15 naturalised strains, 11 introductions collected from the wild, 18 cross-breds and one induced mutant.

Annual medics
Nine annual medic species, with 40 registered cultivars, have been commercialised since 1938 (Table 1), having been sown over an estimated 24.6 M ha (Hill and Donald 1998). These consist of the most widely sown species, barrel medic (*Medicago truncatula*), strand medic (*M. littoralis*) and burr medic (*M. polymorpha*), and the lesser important species, disc medic (*M. tornata*), snail medic (*M. scutellata*), sphere medic (*M. sphaerocarpos*), gama medic (*M. rugosa*), murex medic (*M. murex*) and button medic (*M. orbicularis*). Hybrids between disc and strand medics (cv. Toreador) (PBR database 2013) and between barrel and strand medics (cvv. Harbinger AR (Lake *et al.* 1989), Mogul (Lake 1993), Herald (Lake *et al.* 1997) Lynx and Cheetah) (PBR database 2013)) have also been released. The range of species enables them to be used on different soil types to subterranean clover, particularly alkaline soils. Similar to subterranean clover, their principal success lies in their ability to regenerate naturally without the need for re-sowing. However, they have higher

hard seed levels than subterranean clover, making them well suited to rotation with crops (Cocks *et al.* 1980; Crawford *et al.* 1999).

Alternative annual pasture legumes

Until the mid-1980s annual pasture legume options in southern Australia were largely confined to subterranean clover and six annual medic species (Table 1). Persian clover (var. *majus* types), berseem clover (*T. alexandrinum*), crimson clover (*T. incarnatum*) and arrowleaf clover (*T. vesiculosum*) seeds were imported into Australia for fodder production, but they were not adapted to self-regeneration under Australian conditions. Yellow serradella, first commercialised in the late 1950s, was found to perform well in WA and northern NSW on deep acidic, infertile sands, where subterranean clover failed to persist (Bolland and Gladstones 1987; Oram 1990), but adoption of the initial cultivars was low, due to high seed costs associated with harvesting and wastage, caused by difficulties extracting seed from their pods (Loi *et al.* 2005). Release of the cultivars Yelbini, Charano and Santorini in the late 1990s, in conjunction with improved seed processing technology, have resulted in lower seed costs and greater adoption (Loi *et al.* 2005; Nichols *et al.* 2007). In the 1960s and early 1970s CSIRO released cultivars of rose and cupped clovers, the University of Sydney released woolly pod vetch and SARDI released purple clover (Oram 1990).

A major change in the direction of annual pasture legume breeding and selection in southern Australia has occurred since the mid-1980s, as a result of sustainability and economic challenges to existing farming systems. Initial intentions were to develop new annual legumes to overcome shortcomings in traditional species (Ewing 1989; Loi *et al.* 2005; Nichols *et al.* 2007), while a more recent interest has been the search for deeper-rooted species to reduce groundwater recharge and the expansion of dryland salinity (Dear and Ewing 2008). The first releases in this era were balansa clover and Persian clover (*T. resupinatum* var. *resupinatum*) for waterlogged acid and neutral–alkaline soils, respectively (Table 1). The var. *resupinatum* type of Persian clover differed from the var. *majus* type in having a degree of hard seeds. In 1988 murex medic (*Medicago murex*) was released as a medic for ley farming systems on moderately acid soils, along with slender serradella (*Ornithopus pinnatus*), a species adapted to sandy soils prone to waterlogging (Table 1).

Since 1992, 14 annual pasture and fodder legumes new to Australian agriculture have been released including seven species new to world agriculture (Table 1). Their development and roles are discussed in detail in Loi *et al.* (2005) and Nichols *et al.* (2007; 2012) and the most important of these are summarised here.

i. French serradella, an alternative species to yellow serradella for deep, acidic, sandy soils with much greater ease of seed harvesting and processing (Nutt *et al.* 2009);
ii. biserrula, a more hardseeded, deeper-rooted and more persistent pasture legume than subterranean clover for ley farming systems on acid soils (Loi *et al.* 2010);
iii. bladder clover (*T. spumosum*), a semi-erect, aerial seeding alternative to subterranean clover able to set seed on hard setting soils, where subterranean clover is unable to bury its burrs (Loi *et al.* 2012);
iv. gland clover (*T. glanduliferum*), an aerial seeding, easy to harvest species with resistance to RLEM and aphids (Loi *et al.* 2005; Nutt and Loi 2002);
v. arrowleaf clover (*T. vesiculosum*), an erect, deep-rooted, aerial-seeding legume suited to grazing and fodder production (Hall and Smith 2005; Snowball and Revell 2011); eastern star clover (*Trifolium dasyurum*), selected as an erect, aerial-seeding fodder legume for low–medium rainfall areas with delayed germination, allowing control of crop weeds following the break of season by non-selective herbicides or cultivation prior to its germination (Loi *et al.* 2007);

vi. crimson clover (*T. incarnatum*), an erect, aerial-seeding legume suited to grazing and fodder production in short-term phase pastures (Carr and Paterson 1998);
vii. berseem clover (*T. alexandrinum*), a fodder legume suited to fertile, medium to heavy textured soils of mildly acidic to neutral pH (Hackney *et al.* 2007); and
viii. white melilot (*Melilotus albus*) an erect, late flowering, deep-rooted legume for moderately saline, well-drained soils (Evans and Kearney 2003).

Table 1. Annual legume species with cultivars registered in Australia, their extent of utilisation and main use. Cultivars are either listed in the Registrar of Australian Herbage Plant Cultivars (Oram 1990), the Australian Plant Breeders Rights database (PBR database 2013) or The Australian Seeds Authority (2013). Taken from Nichols *et al.* (2012).

Species	Common name	No. cultivars	First registered cultivar	First year commercial	Current utilisation[h]	Main use[i]
Astragalus hamosus	Milk vetch	1	Ioman	1977[b]	6	–
Biserrula pelecinus	Biserrula	2	Casbah	1997[c]	2	L
Medicago littoralis	Strand medic	5	Harbinger	1959[b]	1	L
M. murex	Murex medic	1	Zodiac	1988[b]	5	L
M. orbicularis	Button medic	1	Bindaroo	2010[d]	*	L, PP
M. polymorpha	Spineless burr medic	5	Serena	1976[b]	2	L
M. rugosa	Gama medic	3	Paragosa	1966[b]	4	L, F
M. scutellata	Snail medic	4	Robinson	1977[b]	4	L, Ph, F
M. sphaerocarpos	Sphere medic	1	Orion	1993[c]	4	L
M. tornata	Disc medic	4	Tornafield	1969[b]	4	L
M. tornata x *M. littoralis*	Hybrid disc medic	1	Toreador	2001[c]	5	L
M. truncatula	Barrel medic	16[a]	Hannaford	1938[e]	1	L
Melilotus albus	White melilot	2	Jota	2006[c]	4	F, PP
Ornithopus compressus	Yellow serradella	11	Pitman	1958[e]	2	L
O. pinnatus	Slender serradella	1	Jebala	1988[b]	5	PP
O. sativus	French serradella	5	Grasslands Koha	1988[d]	1	Ph, F, L[j]
Trifolium alexandrinum	Berseem clover	2	Elite II	1996[d,f]	4	F
T. cheleri	Cupped clover	3	Yamina	1963[e]	5	L
T. dasyurum	Eastern star clover	1	AGWEST Sothis	2007[c]	5	Ph, L, F
T. glanduliferum	Gland clover	1	Prima	2001[c]	4	L, Ph
T. hirtum	Rose clover	5	Kondinin	1965[e]	4	L, Ph
T. incarnatum	Crimson clover	2	Caprera	1998[c,f]	4	F, Ph
T. michelianum	Balansa clover	6	Paradana	1984[b]	1	PP, F
T. purpureum	Purple clover	2	Paratta	1971[b]	5	F, Ph
T. resupinatum var. *majus*	Persian clover	6	Laser	1995[d,g]	2	F
T. resupinatum var. *resupinatum*	Persian clover	4	Kyambro	1988[b]	2	Ph, F, PP
T. spumosum	Bladder clover	1	AGWEST Bartolo	2008[c]	3	L, Ph
T. subterraneum ssp. *brachycalycinum*	Subterranean clover	5	Clare	1950[e]	1	Ph, PP, L
T. subterraneum ssp. *subterraneum*	Subterranean clover	33	Mt Barker	1900[e]	1	Ph, PP, L
T. subterraneum ssp. *yanninicum*	Subterranean clover	7	Yarloop	1939[e]	1	PP, Ph, F
T. vesiculosum	Arrowleaf clover	2	Arrotas	1997[c,g]	4	F, Ph

[a]Includes backcrossed hybrids to *M. littoralis*
[b]Date registered with the Registrar of Australian Herbage Plant Cultivars (Oram 1990)
[c]Details in Nichols *et al.* (2007)
[d]Date first registered with the Plant Breeders Rights Office (PBR database 2012)
[e]First seed sold (Oram 1990)
[f]Seed of common (unregistered) varieties first imported in early 1900s (Oram 1990)
[g]Seed of common (unregistered) varieties first imported before 1970 (Oram 1990)
[h]Utilisation status, where: 1= widely utilised; 2 =moderately popular; 3 = increasing in importance; 4 = special purpose or local use; 5 = rarely used or seed difficult to source; 6 = no or limited commercial seed ever produced; and * = too soon after release to determine status
[i]Primary use on-farm, where: L = ley farming; F = fodder (hay and silage); Ph = phase farming; and PP = permanent pasture

[j]Harseeded cultivars Erica, Margarita and Elisa suited to ley farming, while soft seeded cvv. Cadiz and Grasslands Koha are unsuited

Messina (*Melilotus siculus*) is also being developed as a new annual pasture legume for saline soils prone to winter waterlogging through the Future Farm Industries Cooperative Research Centre (FFI CRC) (Nichols *et al.* 2007, 2012; Bonython *et al.* 2011)***.***

1.3.3. Seed production and sales

Accurate figures are not available for total pasture legume seed crop production and sales for individual species or cultivars. Of the available data, seed produced under seed certification schemes is the most accurate. Table 2 shows data from the Australian Seeds Authority (2013) for certified seed production of annual pasture legume species in Australia from 2007/08 to 2011/12. This data shows that subterranean clover was the dominant species certified, with total seed production of 7,069 tonnes, or 65.6% of all pasture legumes. This compares with 1,312 tonnes (12.2%) for Persian clover, 1,000 tonnes (9.3%) for balansa clover, 835 tonnes for all annual medics (7.8%) and 556 tonnes (5.2%) for all other annual legume species.

However, certified seed production only represents a proportion of all seed marketed. Much seed of older public varieties is sold as uncertified seed and accurate records of these sales are not available. For example, Holland (2012) estimated the production and sale of uncertified subterranean clover seed in WA during the 2003/04 season was well over 2000 tonnes, representing 90% of the State's production. Seed of some proprietary varieties is sold following 'in-house' quality testing, which also does not appear on seed certification statistics. Seed production of some of the newer aerial seeding species is also markedly under-estimated, due to farmers harvesting their own seed crops for sowing in other paddocks on their properties. Nevertheless, given the constraints of available seed production and sales data, it is readily apparent that the annual pasture legume seed market is dominated by subterranean clover. Holland (2012) estimates the annual value of the subterranean clover seed industry at $12-24 million.

Table 2. Certified seed production (tonnes) of annual pasture legume species in Australia from 2007/08 to 2011/12. Data from the Australian Seeds Authority (2013).

	Seed production year					Total	% of all
Species	2007/08	2008/09	2009/10	2010/11	2011/12	(5 years)	species
Biserrula	0	6	0	2	7	15	0.14
Barrel medic	20	18	84	114	99	335	3.11
Burr medic	1	15	33	183	183	415	3.85
Snail medic	19	3	14	32	17	85	0.79
Strand medic	0	0	0	0	0	0	0.00
French serradella	28	4	18	6	64	120	1.11
Yellow serradella	13	4	0	0	6	23	0.21
Arrowleaf clover	8	24	35	138	17	222	2.06
Balansa clover	346	196	209	191	58	1,000	9.28
Berseem clover	20	6	5	34	0	65	0.60
Gland clover	1	4	0	8	0	13	0.12
Persian clover	411	95	322	243	241	1,312	12.18
Rose clover	47	18	0	0	24	89	0.83
Subterranean clover	1,461	872	1,127	1,619	1,990	7,069	65.62
Other *Trifolium* species	11	11	78	14	15	129	1.20
Total	2,358	1,272	1,907	2,578	2,657	10,772	100.00

Table 3. Estimated sheep and cattle numbers in 2011 in different NRM regions on land best suited to subterranean clover, annual medics or alternative annual legumes. Sheep and cattle numbers in each region obtained from MLA (2012a, 2012b). Proportions grazing the different pasture types based on Donald (2012).

NRM region	Sheep numbers			Cattle numbers		
	Sub clover	Annual medics	Alternative legumes	Sub clover	Annual medics	Alternative legumes
Western Australia						
Northern Agricultural	731,425	365,713	731,425	41,905	20,953	41,905
Swan	318,009	-	35,334	64,352	-	7,150
Avon	2,094,737	837,895	1,256,842	25,065	10,026	15,039
South West	3,868,939	-	429,882	423,713	-	47,079
South Coast	2,075,262	296,466	592,932	217,036	31,005	62,010
Total	*9,088,371*	*1,500,073*	*3,046,416*	*772,071*	*61,984*	*173,184*
South Australia						
Eyre Peninsular	153,912	1,385,204	-	2,268	20,408	-
Northern and Yorke	409,107	1,534,153	102,277	12,275	46,030	3,069
Kangaroo Island	502,750	-	55,861	18,573	-	2,064
Adelaide/Mt Lofty Ranges	269,057	-	29,895	81,785	-	9,087
SA Murray Darling Basin	184,249	1,566,113	92,124	16,144	137,221	8,072
South East	2,516,436	359,491	718,982	461,368	65,910	131,819
Total	*4,035,510*	*4,844,961*	*999,139*	*592,412*	*269,569*	*154,111*
Victoria						
Mallee	-	493,416	-	-	13,141	-
Wimmera	685,972	1,486,272	114,329	14,644	31,728	2,441
Glenelg Hopkins	4,937,224	-	548,580	835,418	-	92,824
North Central	2,096,948	559,186	139,797	233,693	62,318	15,580
Coragamite	1,310,111	-	145,568	410,099	-	45,567
Port Phillip/Westernport	187,838	-	20,871	278,676	-	30,964
West Gippsland	354,922	-	39,436	702,605	-	78,067
Goulburn Broken	1,368,756	-	152,084	532,597	-	59,177
East Gippsland	195,290	-	21,699	112,532	-	12,504
North East	254,677	-	28,297	291,806	-	32,423
Total	*11,391,738*	*2,538,875*	*1,210,661*	*3,412,069*	*107,187*	*369,546*
Tasmania						
North	1,085,377	-	120,597	262,598	-	29,178
South	955,632	-	106,181	50,906	-	5,656
North West	13,803	-	1,534	61,357	-	6,817
Total	*2,054,811*	*-*	*228,312*	*374,861*	*-*	*41,651*
New South Wales						
Murray	1,840,148	490,706	122,677	314,229	83,794	20,949
Lower Murray Darling	-	40,997	-	-	1,559	-
ACT	51,387	-	2,705	8,367	-	440
Murrumbidgee	3,586,172	956,313	239,078	432,581	115,355	28,839
Southern Rivers	146,472	-	7,709	44,240	-	2,328
Lachlan	4,616,675	1,231,113	307,778	367,121	97,899	24,475
Central West	3,836,546	1,023,079	255,770	583,169	155,512	38,878
Hunter-Central Rivers	41,343	-	4,594	102,443	-	11,383
Namoi	630,027	290,782	48,464	428,422	197,733	32,956
Border Rivers-Gwydir	637,921	877,141	79,740	261,117	359,036	32,640
Northern Rivers	202,079	-	10,636	178,852	-	9,413
Total	*15,588,771*	*4,910,131*	*1,079,150*	*2,720,540*	*1,010,889*	*202,300*
Queensland						
South East	1,360	-	-	42,599	-	-
Condamine	42,804	28,536	-	264,531	176,354	-
Border Rivers Maranoa Balonne	18,185	163,663	-	24,607	221,461	-
Total	*62,348*	*192,199*	*-*	*331,737*	*397,815*	*-*
Grand total	42,221,550	13,986,239	6,563,677	8,203,690	1,847,444	940,792
% of nation's flock/herd	57.8	19.1	9.0	28.8	6.5	3.3

1.3.4. Animal production on annual pasture legume species

Accurate animal production estimates attributable to different pasture types are very difficult to obtain. However, an attempt to rank the relative importance of the main annual pasture legume species is made in Table 3. Sheep and cattle numbers at June 2011 in each of the different Natural Resource Management (NRM) regions across Australia were obtained from MLA (2012a, 2012b). This was matched up with data based on the MLA Feed-base audit for the different pasture types in the agricultural Statistical Local Areas (SLAs) across Australia. Data is presented for sheep and cattle numbers on land best suited to subterranean clover, annual medics or alternative annual legume species. This very crude analysis suggests that approximately 58% of the Australia's sheep and 29% of cattle are produced on land best suited to subterranean clover, 19% of sheep and 7% of cattle are produced on land best suited to annual medics and 9% of sheep and 3% of cattle are produced on land best suited to alternative annual legume species. The remaining sheep (14%) and cattle (61%) are produced in areas not suited to annual legumes.

1.4. Inventory of current annual legume breeding activities

1.4.1 Industry structure

The pasture seed industry supply chain is shown in Figure 2. This is discussed in detail in a policy document by the Department of Primary Industries Victoria (DPI Victoria 2005) and a report commissioned by MLA (2011). Pre-breeding activities cover the first four categories:

- New knowledge and enabling technologies, which concerns aspects including genetic and genomic studies, genetic mapping and the development of molecular markers;
- Germplasm collection, conservation and maintenance, which are important for providing the sources of genetic variation necessary for plant breeding;
- Technology discovery and development, which concerns strategic R & D that develops tools to support and enhance plant breeding. Examples include development of molecular markers associated with traits of interest, development of screening methodologies to enable selection of desirable traits and technologies to increase breeding efficiency; and
- Germplasm development (strategic plant breeding), which relates to the identification and screening of germplasm for new traits, such as disease or pest resistance, tolerance of abiotic stresses and enhanced quality parameters.

Cultivar development is concerned with applied plant breeding, which is aimed at developing new cultivars for commercial release and concerns breeding and selection and field evaluation of advanced lines in target environments. The final step in the supply chain deals with commercial production and distribution of seed, marketing and royalty collection.

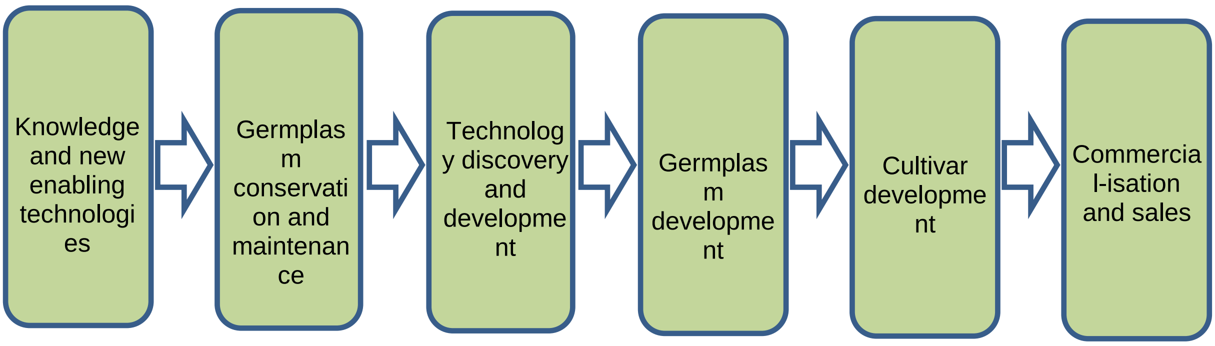


Figure 2. The pasture seed industry supply chain (source: DPI Victoria (2005)).

To date all pasture legume pre-breeding activities in Australia have been conducted by public PISC (Primary Industries Standing Committee) agencies and the Universities. This was also the case, until recently, for the applied breeding, selection and field evaluation activities of the cultivar development phase. However, private industry has become increasingly involved in these activities over the past decade or so, particularly for the more important pasture legume species. Nichols *et al.* (2012) suggest there are two main reasons for this. Firstly, the advent of Plant Breeders Rights (PBR) in 1987 (initially Plant Variety Rights) has allowed the protection of new cultivars as intellectual property. Secondly, public sector funding into agricultural research has been reduced in general, with pasture plant improvement affected specifically (Kingwell and Pannell 2005; Wolfe 2009). Three main models for pasture plant breeding currently operate for annual legumes: (i) public agency alone; (ii) public agency-private company partnerships; and (iii) private industry alone.

Public agency breeding

Public agency breeding and selection programs currently operate for alternative annual legume species (those other than subterranean clover and annual medics), with varying degrees of activity. In general, these programs have mainly been concerned with screening germplasm from Genetic Resource Centres, with limited hybridisation or population selection. Examples include:

(i) DAFWA – yellow serradella, French serradella, biserrula, bladder clover, Eastern star clover, Persian clover, arrowleaf clover, gland clover, crimson clover, purple clover; and
(ii) Future Farm Industries CRC – messina
(iii) SARDI – balansa clover, Persian clover

Public agency-private company partnerships

Partnerships with public agency pasture breeding programs have been formed by some Australian seed companies, particularly for the more important species. Under such arrangements, public agencies provide the technical skills and infra-structure for pre-breeding and plant breeding, with a shared responsibility for field evaluation of advanced lines. The private companies then make commercialisation decisions on their analyses of market potential and conduct their own marketing and extension activities. Initial seed multiplication, to provide small quantities of breeders or pre-basic seed, is often conducted by the breeding agency, while basic and certified seed multiplication is conducted by the seed companies.

Nichols *et al.* (2012) suggest such partnerships have had a positive two-way effect. It has provided additional funding for pasture breeding, in the face of declining investment by the public purse, and has resulted in closer alignment between the visions of pasture breeders and the commercial realities of the seed market. The greatest interest has been shown by seed companies in the more traditional, widely-sown species, as there is less risk attached to them than new species and less investment required to educate farmers and the supply chain on their merits.

Formal partnerships currently exist between Australian pasture seed companies and public breeding programs for annual legumes. Examples include:

- SARDI, which has relationships with a range of seed companies for annual medics, subterranean clover and other legume species;
- DAFWA and Seed Force for subterranean clover;
- TIA and Tasglobal Seeds for other alternative legumes (including perennials); and
- PGG Wrightsons and AgResearch in New Zealand for subterranean clover.

Private industry breeding
There is little breeding and selection of annual legumes conducted by private industry. Pristine Forage Technologies is a small private company which has developed its own annual medic and balansa clover cultivars and also markets lucerne cultivars. Seed Distributors market a range of their own annual legume brands, but their status as improved cultivars is in many cases unclear, with them not being listed on the National list of Plant Varieties Eligible for Seed Certification in Australia (Australian Seeds Authority 2013) nor having published breeding records.

1.4.2 Issues of market failure in annual pasture legume R & D

Market failure is the situation where the private sector is unable, or unwilling, to invest to the extent required by society, which consequently justifies public investment (DPI Victoria 2005). A policy document by DPI Victoria (2005) and a report commissioned by MLA (2011) discuss the types of market failure that can occur in plant breeding and cultivar development. Market failure occurs in both the pre-breeding and applied breeding stages of annual pasture legume improvement.

Pre-breeding market failure
Pre-breeding activities are largely knowledge-based and hence, tend to be subject to market failure. The outputs are public goods, freely available to applied plant breeders, unless they are patentable. Traditionally these activities have been publicly funded, as there is little incentive for investment by private industry, unless the developed technologies can be patented. Pre-breeding activities are likely to remain in the public domain, as this lack of incentive for private industry investment (in what is public good research) will continue.

Market failure in cultivar development
Market failure exists in annual legume cultivar development. It occurs largely because of inadequate market size, even for the most important species. Market failure can also occur when seed companies restrict promising new cultivars from reaching their full market potential. Unlike globally important pasture species, such as lucerne, white clover, perennial ryegrass and tall fescue, the annual legumes are largely restricted to domestic sales and pasture re-sowing rates are sub-optimal. The advent of public organisation-private industry partnerships has reduced reliance on the public purse to fund all breeding activities. The decision by private companies to increase their investment in annual legume R & D will depend on their business models and estimated returns on investment.

1.4.3 Funding of annual pasture legume breeding and evaluation programs

The FIP indicates the meat industry is moving towards the investment decision process used in the grains industry (Figure 3). In this model Grains Research and Development Corporation (GRDC) levies of the major crops (e.g. bread wheat) are concentrated on the scientific discovery and pre-breeding stages of the R & D pipeline, where there is market failure and little incentive for private industry investment. Plant breeding and evaluation is now conducted by private companies for the major crops. However, GRDC invests heavily into breeding programs for crops with market failure. One example is Pulse Breeding Australia, in which GRDC has formed an unincorporated joint venture with several PISC agencies to breed cultivars of chickpeas, field peas, faba beans, lentils and lupins, which are sown over much smaller areas than bread wheat. Even within the wheat industry, GRDC invests in breeding of durum and dual purpose wheats, where market failure exists. In 2011-12 GRDC invested $17.7 M in breeding programs, which is one-third of their total pre-breeding/breeding budget (GRDC 2012).

In the case of annual legumes, the GRDC model for funding of breeding activities in Pulse Breeding Australia seems highly applicable (Figure 3). The more important species (subterranean clover and annual medics) have co-investment by private industry, but support is still required by public agencies due to market failure. Cultivar development of alternative legumes is likely to be highly reliant on public agency support.

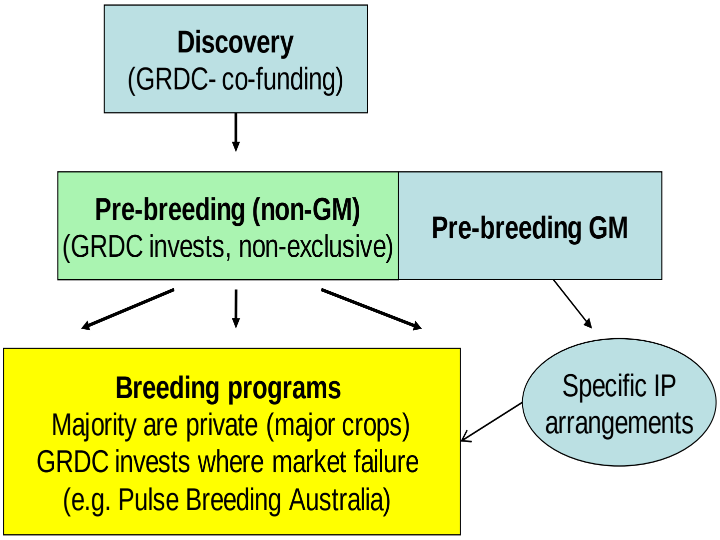


Figure 3. The GRDC investment model for pre-breeding and breeding. GRDC invests in pre-breeding activities and the breeding of crops with market failure.

1.4.4 Breeding methods in annual legumes

The annual pasture legumes used in southern Australia are derived from three main sources (Nichols *et al.* 2012): (i) naturalised populations, in which seeds have been accidentally brought in from other countries, leading to colonisation and the development of locally adapted ecotypes; (ii) deliberate introduction of germplasm collected from their native habitats; and (iii) breeding programs, involving crossing and selection among progeny for desirable traits. The original subterranean clover and annual medic cultivars were derived from naturalised populations. Cultivars of alternative species and new germplasm of the mainstream species all originate directly from introductions collected in the wild. Breeding programs have been implemented for subterranean clover, barrel medic, strand medic, burr medic, disc medic, balansa clover and French serradella.

Most of the annual pasture legumes in commerce are predominantly self-pollinated (inbreeding), similar to wheat, barley, oats and peas. This means that cultivars are true breeding, in which all plants in the population are effectively genetically identical. This contrasts with out-crossing species, such as the ryegrasses, lucerne and white clover, which consist of highly heterogeneous populations. Inbreeding annual legumes, therefore, require different breeding methodologies to out-crossing species. Not all annual legumes are inbreeding, with balansa, Persian and gland clovers being exceptions. The following section focuses on breeding methodology for species that are predominantly inbreeding.

Selection in the segregating generations

For inbreeding species breeding methodology is generally aimed at incorporating genes for new traits into varieties with good adaptation. It involves making a cross between two parent lines, in which pollen from flowers of one parent is added to flowers of the other parent (which has previously had the anthers removed to prevent self-pollination). Self fertilisation is left to occur in subsequent generations. Selection for traits of interest generally commences among spaced plants in the F_2 generation, with each plant being genetically different from all other progeny. Selection continues from the F_3 generation, often to the F_6 generation, on either spaced plants or small plots. Effective selection during the segregating generations is only possible for traits of high heritability. With each generation the proportion of heterozygotes at each genetic locus halves, so that final selection in the F_6 generation is among plants that are essentially homozygous and true breeding at all loci. Seed multiplication of elite lines is then conducted for field evaluation trials.

There are several variations on this methodology. Back-crossing is sometimes conducted to incorporate traits with simple inheritance (from a donor variety) into an adapted (recipient) cultivar. In this method, the F_1 hybrid, resulting from an initial cross, is back-crossed to the recipient parent. This process is repeated for several generations, with selection conducted among progeny in each generation for the trait of interest. The outcome is effectively homozygous varieties that contain the majority of the recipient parent genes, with the added desirable genes for the trait of interest from the donor parent. This method has been successfully used to breed aphid resistance into annual medics. Other variations that have been widely used in subterranean clover are crossing between two F_1 hybrids (4-way cross) or between an F1 hybrid and another variety (3-way cross) to incorporate traits from more than one parent.

One of the biggest constraints in breeding is the time taken to progress from the F_1 to F_6 generations, leading to a bottleneck in cultivar development. This arises because generally only a single generation per year is possible, due to issues such as embryo dormancy, vernalisation requirements for over-summer field seed increase and a lack of glasshouse space. Rapid generation technologies to reduce the time taken to develop homozygous F_6 lines would have a major impact on the time taken to develop cultivars.

Field evaluation of elite lines
Elite breeding lines are sown in field trials in order to assess their adaptation under 3-4 years of farming conditions. Such lines may be direct introductions or the result of cross-breeding. In this process candidate varieties are sown in replicated, multi-location trials in target environments, where their performance is compared with the best current varieties under typical grazing conditions. Cropping can also be imposed for material aimed at crop rotations. Pasture measurements include biomass production in autumn, winter and spring, seedling regeneration densities, seed yields, seed bank reserves and reaction to any local pests, diseases and abiotic stresses that may impact on trials.

2. Project objectives

The aim of this project was to develop a comprehensive pre-breeding R & D plan for annual legumes over the period 2013-2017 (with scope to consider a further five year investment).

The R & D plan was to include:

1. A description of the organisations to implement the R & D plans including their roles and responsibilities (i.e. lead organisation, participating, etc)
2. An audit of the capacity and ability of the team members to implement the R&D plans (this to include both public and private organisations). This needs to include a cost-effective approach to delivery of the tasks identified within the plan and may include the involvement of organisations that are not already included in the team developing the plan.
3. An inventory of existing breeding technologies currently in use by public and private sector plant breeders for the nominated species. This may include the use of these technologies in other species and countries.
4. The R & D activities, outputs and budgets (cash and in-kind) for each species group in pre-breeding technology, traits and improvement for the period 2013-2016 – as per MLA full application form.

5. A response from public and private sector plant breeders on how they will use the outputs to facilitate an increase in genetic gain for the proposed species, including a communications and extension plan to ensure the adoption of the developed technologies by private sector plant breeders.
6. A definition of the potential benefits of the proposed research, including case studies which include an analysis of how the proposed outputs have increased the rate of genetic gain in other species, areas or another field.
7. How the proposed activities will assist in meeting the goal of the program, i.e. to add $25m per year on-farm value by 2020, with the kg meat produced per ha increasing by 2.5% with no decline in sustainability indicators.
8. Recommendations on project IP management and ownership that ensures the project IP will be freely available to all public and private sector breeders for the benefit of Australia's livestock industries.
9. Identification of synergies regarding technology development with the team that is currently developing the R & D plan for pre-breeding for annual legumes.
10. A draft milestone table.

Draft plans were to be submitted to MLA, for distribution and comment as part of a consultation process. MLA was to collate responses for inclusion into the final version of the R & D plan. Revised plans, that included responses to received comments, were to be submitted to MLA.

3. Methodology

3.1 Project development team

A multi-agency, public and private sector team was assembled to develop the pre-breeding R & D plan. This was led by the Department of Agriculture and Food Western Australia (DAFWA), with collaborating agencies being the South Australian Research and Development Institute (SARDI), New South Wales Department of Primary Industry (NSW DPI) and the Centre for Legumes in Mediterranean Agriculture (CLIMA) from the University of Western Australia (UWA), while private industry interests were represented by Seed Force Pty. Ltd. and Pristine Forage Technologies.

The following personnel were on the team to develop the R & D plan:

- **Dr Phillip Nichols** (DAFWA) was the Project leader, who coordinated delivery of the R&D plan to MLA and provided expertise on traits of importance to subterranean clover and on screening for disease and pest resistance;
- **Professor William Erskine** (CLIMA), who provided expertise on legume breeding and pre-breeding technologies and their implementation;
- **Dr Bradley Nutt** (DAFWA), who provided expertise on traits of importance to a broad range of annual legumes;
- **Dr Clinton Revell** (DAFWA), who provided expertise on the integration of new annual legume traits into farming systems;
- **Dr Daniel Real** (DAFWA), who provided expertise on plant breeding methodology and the analysis and applications of pre-breeding to legumes;
- **Dr Steven Gherardi** (DAFWA), who provided expertise on pasture traits for increased animal production;
- **Mr Graeme Sandral** (NSW DPI), who provided expertise on traits of importance to annual legumes in the mixed farming and permanent pasture zones of eastern Australia;
- **Mr Jake Howie** (SARDI), who provided expertise on annual medic agronomy and variety evaluation;

- **Mr David Peck** (SARDI), who provided expertise on annual medic and annual legume breeding and selection;
- **Mr Michael Gout** (Seed Force Pty Ltd), who provided commercial guidance and an understanding of the seed market for annual legumes; and
- **Mr Andrew Lake** (Pristine Forage), who is a private breeder with expertise in annual legume breeding and an understanding of the seed market for annual legumes.

3.2 Guiding principles

The R & D plan was developed along the four following principles:

- Project outcomes were to directly benefit meat producers, through increasing production or decreasing costs;
- Project outputs were to be of commercial relevance to the seed industry;
- The plan was to be achievable and project outputs deliverable to new cultivars within an 8-10 year time-frame; and
- The planning process was to be demonstrably open, fair and accountable to MLA and its stakeholders.

The objective was for the plan to be attractive, achievable and accountable to MLA and its stakeholders and deliver on the MLA goal of increasing production or decreasing costs by 15% by 2020. It was to be driven from the pasture priorities of beef and sheep meat producers and generate material that will be used by breeding programs with traits and in species that will be strongly promoted and marketed by private industry. Delivery of new traits to industry, in the form of new cultivars, should occur within an 8-10 year time-frame.

3.3 Annual Legume Traits and Species Pre-breeding Workshop

Clearly, one R & D project can only deliver a limited number of high impact traits in a few key species. It was, therefore, critical that the most important traits and species in the annual legumes were identified to have the biggest impact on improving the profitability and sustainability of red meat production across southern Australia. This resulted in an Annual Legume Traits and Species Pre-breeding Workshop being held in Melbourne in November 2012 where trait and species priorities were discussed. The outcomes from the workshop provided a key platform for development of the R & D investment plan.

The workshop had three main objectives:

- To identify the most important annual legume traits to improve the profitability and sustainability of red meat production in southern Australia;
- To identify the annual legume species to best deliver the top priority traits; and
- To determine key factors that need to be in the annual legume pre-breeding project for its success.

Workshop attendees consisted of an invited panel of 28 industry experts (Table 4), in addition to Mr Warren Mason, the meeting facilitator. The participants consisted of leading animal specialists, farm consultants, economists, pasture breeders, pre-breeders, agronomists and pathologists and representatives from most of the seed companies with an interest in annual legumes. Representatives also covered each of the main agro-ecological zones in southern Australia. This mix was designed to ensure the identified target traits and technologies are of the greatest importance to meat production, are deliverable from a plant improvement perspective and are of commercial relevance.

The meeting was held over two days. The first day (November 22) consisted of the Annual Legume Traits and Species Pre-breeding Workshop, in which all 28 participants took part. The second day (November 23) involved only the project team, which synthesised the previous day's findings and developed some preliminary recommendations for further consideration.

Table 4. List of participants at the Annual Legume Traits and Species Pre-breeding Workshop in Melbourne on 22 November 2012

Participant	Affiliation	State	Expertise
Andrew Bates	Bates Agricultural Consulting	SA	Farm consultant
Andrew Craig	SARDI	SA	Pasture agronomist/breeder
Andrew Lake	Pristine Forage Technologies	SA	Private breeder/Seed industry
Belinda Hackney	NSW DPI	NSW	Pasture agronomist
Bob Reid	Tasglobal Seeds	Tasmania	Private breeder/Seed industry
Bradley Nutt	DAFWA	WA	Pasture agronomist/breeder
Clinton Revell	DAFWA	WA	Pasture agronomist
David Peck	SARDI	SA	Pasture breeder
Eric Hall	TIAR	Tasmania	Pasture agronomist/breeder
Graeme Sandral	NSW DPI	NSW	Pasture agronomist
Hayley Norman	CSIRO	WA	Animal production
Jake Howie	SARDI	SA	Pasture agronomist
James Sewell	PGG Wrightsons	Victoria	Seed industry
John Francis	Holmes Sackett	NSW	Farm consultant
John Milton	UWA	WA	Animal production
John Webb Ware	Melbourne University	Victoria	Animal production
John Young	Farming Systems Analysis	WA	Economist
Kevin Smith	Melbourne University	Victoria	Pasture breeder
Linda Hygate	MLA	Queensland	Animal production
Martin Barbetti	UWA	WA	Pasture pathologist
Mike Gout	Seed Force	NSW	Seed industry
Pedro Evans	DLF Trifolium	New Zealand	Private breeder/Seed industry
Phillip Nichols	DAFWA	WA	Pasture breeder
Richard Prusa	Heritage Seeds	SA	Seed industry
Richard Simpson	CSIRO	ACT	Pasture agronomist
Rowan Smith	TIAR	Tasmania	Pasture breeder
Sue Boschma	NSW DPI	NSW	Pasture agronomist
William Erskine	CLIMA	WA	Legume pre-breeder

Table 5. Annual Legume Traits and Species Pre-breeding Workshop program

Time	Topic	Speaker
8.30	*Traits for annual pasture legumes* (10 mins plus 2 mins for clarification)	
	Introduction and meeting objectives (including contexting prior workshops and reports)	Phil Nichols
	MLA plans for investment in pre-breeding and the Feedbase Investment Plan	Linda Hygate
	Constraints and opportunities to increase sheep meat production from annual pastures	John Milton
	Constraints and opportunities to increase beef production from annual pastures	John Webb Ware
	Critical points in the economics of meat production systems	John Young
	Seed industry considerations for annual legume cultivars	Mike Gout
	Annual legume constraints and opportunities for mixed farming systems	Brad Nutt/ Jake Howie
	Annual legume constraints and opportunities for permanent pasture systems	Graeme Sandral
	Pasture disease issues of annual legumes	Martin Barbetti
	Presentation of pre-workshop trait survey results	Clinton Revell
10.40	*Morning tea*	
11.00	*Group session 1: Identification of traits*	
	3 groups (Pasture scientists, Seed industry, Animal scientists)	
	Each group to identify the 10 most important annual legume traits (in priority order) for: (i) mixed farming; and (ii) permanent pasture systems	
11.40	Report back to meeting	
12.00	*Current annual legume breeding and technology* (10 mins plus 2 mins for clarification)	
	Subterranean clover	Phil Nichols
	Annual medics	Jake Howie/ David Peck
	Low rainfall alternative legumes	Brad Nutt
	High rainfall alternative legumes	Eric Hall
12.50	*Lunch*	
1.30	*Group session 2: Matching species to traits*	
	4 mixed groups to identify priority species for the top priority traits. Two groups for mixed farming and 2 groups for permanent pasture systems	
2.00	Report back to meeting	
	Pre-breeding technologies (10 mins plus 2 mins for clarification)	
2.20	New technology opportunities for legume pre-breeding	Willie Erskine
	MLA perennial grass pre-breeding project - priorities and technologies	Kevin Smith
2.45	What does the seed industry want and can utilise from this pre-breeding project?	Andrew Lake
3.00	*Afternoon tea*	
3.25	*Group session 3: What key factors have to be in the annual legumes pre-breeding project for success?*	
	4 mixed groups to identify key factors to ensure project success	
3.50	Report back to meeting	
4.10	Discussion of outcomes	
4.30	*Meeting close*	

3.3.1 Pre-workshop preparation

Prior to the workshop, participants were asked to give a 1-10 rating of importance to a list of traits that could be incorporated into new pasture legumes to have the greatest impact on improving profitability and sustainability of the sheep meat and beef industries in southern Australia and to provide comments on the annual legume species to which these traits apply. The list of traits was developed from a similar survey conducted internally among the DAFWA Animal Industries and Pasture Science groups 12 months previously and was refined by the project team. The aim was for participants to put some thought into the main issues affecting animal production from annual legumes prior to the workshop and there was the opportunity to add additional traits that had not been considered. The completed forms were emailed back and the preliminary results compiled prior to the workshop for initial consideration.

3.3.2 Workshop structure

The workshop program is shown in Table 5 and consisted of 16 short scene-setting presentations from industry experts and three group sessions. Presentations, consisting of 10 minute talks with 2 minutes for questions, were made in four main topic areas:

- Introduction to the project;
- Traits for annual pasture legumes;
- Current annual legume breeding and technology activities; and
- Pre-breeding technologies

The main purpose of these presentations was to provide background information to workshop participants on a range of topics of relevance to pre-breeding annual legumes. This was an important process, due to the diverse backgrounds and expertise of the workshop participants.

The first two group sessions were held to distil the additional information presented in the presentations to fine-tune priorities for traits and species of most importance to livestock production. The third group session considered the key factors needed in the annual legumes pre-breeding project for it to be successful. Groups reported their findings back to all participants at the end of each group session.

3.3.3 Group session 1: identification of traits

Following the presentations related to traits of annual pasture legumes, workshop participants were divided into four equal groups according to areas of expertise (Animal production, Seed industry and two Pasture science groups). Such a grouping enabled comparisons to be made between the priorities of the different areas of expertise. Membership of the two Pasture Science groups ensured State and agro-ecological zone interests were spread evenly between them. The aim of this Group session was to examine animal production needs at the trait level, independent of annual legume species.

Two lists of traits were provided to each group – one for the mixed farming zone and one for permanent pastures. The traits were the same as those initially provided prior to the workshop, with the addition of other traits suggested by participants. For each farming zone, groups were asked to rank the top 7 traits that should be incorporated into new pasture legumes to have the greatest impact on improving profitability and sustainability of the sheep meat and beef industries in southern Australia (compared to current options) in priority order (7 = most important, 1 = least important). Groups were also asked to suggest a percentage allocation of funds to each farming zone.

3.3.4 Group session 2: Matching species to traits

Following the presentations on current annual legume breeding activities, workshop participants were divided into four groups, with each containing a mixture of industry expertise and representation. Two groups contained members most familiar with the permanent pasture zone and two contained members most familiar with mixed farming systems.

The groups were provided with lists containing the traits that had been scored as having the most importance (i.e a score of 1-7) by at least one group in Group session 1 for each farming zone. This comprised 19 traits for the mixed farming zone (Table 8) and 16 traits for the permanent pasture zone (Table 9), with many of these traits being common to both. The total scores for each trait were also provided (from Tables 6 and 7). Groups were asked to allocate the scores for each trait across what they considered to be the most important species. In this way a species x trait matrix was generated (Tables 8 and 9), showing relative weightings for each combination. Where specific allocations were not made for each species, they were amalgamated into the 'All species' category for that trait.

The scores from both farming systems were then averaged to give an overall summary of relative importance of traits for the different annual legume species across both mixed farming and permanent pasture systems (Table 10). We were able to do this by giving equal weighting to both farming systems, which approximated the relative priorities for funding suggested in Group session 1.

3.3.5 Group session 3: Key factors for project success

Following the presentations on pre-breeding technologies, workshop participants were divided into the same four groups as for Group session 2. The groups were asked to address the question "What key factors have to be in the annual legumes pre-breeding project for success? The groups then reported their findings to the main group.

3.4 Further consultation

The project team met the day after the Annual Legume Traits and Species Pre-breeding Workshop to synthesise the previous day's findings and developed some preliminary project activity ideas, based on the trait and species priorities identified in the workshop. Discussions were then held with agencies and breeding companies about technologies currently being used for annual legumes and other pasture species and their technical capacity and ability to conduct pre-breeding activities. This information was used to determine the project activities and the project team to implement them. A response was also sought from the private sector on how they would use the outputs from this project.

4. Results

4.1 Workshop outputs and recommendations

4.1.1 Outcomes from Group session 1: Identification of traits

The summary ratings of each group are shown in Table 6 for the mixed farming zone and Table 4 for permanent pastures. All groups gave increased feed supply in autumn-winter as the highest rating trait for both farming systems. High seed production was also rated highly for both farming systems in each group. Resistance to below-ground diseases (root rots and nematodes) was rated highly in each group for permanent pastures and for three of the groups for mixed farming systems (not the Animal production group).

There was less consistency with the other traits across groups. Increased nitrogen fixation ranked in the top 7 priorities for three of the groups in the mixed farming zone (not the Animal production group), but was only considered important by the Seeds group for permanent pastures. Increased P- and K-use efficiencies were ranked in the top 7 priorities for three of the groups in the permanent pasture zone, while it was only considered important in the mixed farming zone by the Animals group.

Increased resistance to redlegged earth mites (RLEM) and blue oat mites (BOM) were ranked in the top 7 priorities for both farming systems by the Seeds and one of the Pastures groups. Reducing seedling losses from false breaks and increased seedling vigour/early root growth were also in the top 7 priorities of two groups for the mixed farming zone, while increased seed dormancy and increased reliability of legume regeneration between seasons were in the top 7 priorities of two groups for the permanent pasture zone. Increased tolerance of broad-leaved herbicides also received a score of 1 in two groups for the permanent pasture zone. Twelve other traits were in the top 7 rankings of one group only for the mixed farming zone and 9 traits for the permanent pasture zone.

Of note was the score of 7 given for increased persistence *per se* by one of the Pastures groups for both farming systems (this was on the list as a heading and not intended to be scored as an individual item).

Among the groups, the Animal production group placed a higher emphasis on feed quality issues, the Seeds group had a higher emphasis on increased herbicide tolerance, while the Pastures groups had a higher emphasis on increased reliability of legume regeneration.

Three of the groups suggested a percentage allocation of funds to the two farming system types. Overall it was suggested close to 50% funding should be for the mixed farming zone and 50% for the permanent pasture zone.

4.1.2 Outcomes from Group session 2: Matching species to traits

The mixed farming zone groups suggested a similar weighting be given to subterranean clover (20%) and annual medics (19%), with a combined weighting of 15% for serradellas, biserrula and balansa clover (Table 8). However, the groups suggested 44% of funding in this zone should go across all annual legume species currently used in the mixed farming zone. There was little need seen for new species development.

In the permanent pasture zone, subterranean clover was clearly seen as the species to which most effort should be applied, with a 58% weighting (Table 9). The groups suggested a 9% combined allocation to balansa clover, arrowleaf clover, Persian clover and serradellas, and a 32% weighting across all annual legume species currently used in the permanent pasture zone.

Table 6. Annual legume trait priorities for the mixed farming zone (ranked 1-7, where 7 = most important, 1 = least important of the highest ranked traits) of four groups (Animal production, Seed industry and two Pasture science groups), along with a suggested percentage allocation of funds to the zone.

Trait	Animals	Seeds	Pastures 1	Pastures 2	Total
Feed supply					
Reduced seedling losses from false breaks		3	5		8
Increased seedling vigour/early root growth		4		6	10
Increased feed in autumn-early winter	7	7	7	7	28
Increased feed in winter					
Increased feed in spring					
Extended period of green feed into late spring/summer	3				3
Ability to produce fodder (specialist hay or silage)					
Feed value					
Increased nutritive value of green feed	5				5
Increased nutritive value of dry feed over summer					
Increased animal preference (palatability)					
Decreased animal preference (palatability)					
Lack of anti-nutritional or toxic chemicals					
Lack of chemicals affecting reproduction					
Anthelmintic (anti-worming) properties					
Anti-bloating properties				3	3
Reduced greenhouse gas emissions from grazing					
Capacity to improve meat quality or flavour					
Pasture sowing and establishment					
Low costs of establishment (seed and sowing costs)					
Ease (reliability) of establishment					
Increased tolerance of broad-leaf herbicides		5			5
Persistence			7		7
Increased seed production	6	7	4	7	24
Increased reliability of regeneration between years			6		6
Adaptation to increasing climate variability					
Adaptation to increased CO_2 levels (climate change)					
Persistence over seasons (reduced need to re-sow)					
Increased seed dormancy		3			3
Ability to compete with weeds			3		3
Ability to compete with other pasture species					
Drought tolerance					
Grazing tolerance					
Frost tolerance					
Pests and diseases					
Resistance to leaf and stem fungal diseases	2				2
Resistance to virus diseases					
Resistance to below-ground diseases (root rots, nematodes)		6	2	1	9
Resistance to aphids					
Resistance to redlegged earth mites/ blue oat mites		1		2	3
Resistance to lucerne flea					
Resistance to other pests or diseases - specify					
Soil constraints					
Salt tolerance (for saline areas)					
Waterlogging tolerance					
Acid soil tolerance					

Trait	Animals	Seeds	Pastures 1	Pastures 2	Total
Increased P & K efficiency (growth with less P & K)	4				4
Adaptation to sandy soils					
Adaptation to heavy clays					
Tolerance of soil toxicities (boron, heavy metals etc)					
Adaptation to soils with a high free lime content					
Integration with cropping					
Increased legume regeneration after cropping					
Increased nitrogen fixation		2	1	5	8
Increased weed management options				4	4
Increased tolerance of spray-topping herbicides					
Reduced susceptibility to cropping herbicide residues				4	4
Others					
Improved seed harvesting characteristics					
% allocation of funding to the mixed farming zone	50	60	-	50	

The combined scores from both farming systems provide an overall summary of the relative importance of traits for the different annual legume species across both mixed farming and permanent pasture systems (Table 10). The workshop participants suggested the overall funding weight should be 39% specifically for subterranean clover, 10% specifically for annual medics, 10% specifically for serradellas, biserrula and balansa clover combined and 38% generally across all annual legumes used in southern Australia.

The trait judged to be the most important to meat production across southern Australia was increased autumn-winter feed production (21%), followed by increased seed production (18%) (Table 10). Traits related to increased persistence (persistence *per se*, persistence over seasons and increased reliability of regeneration) comprised 12%, resistance to diseases and pests totalled 15%, (with more than 9 % due to below-ground diseases), traits related to seed dormancy (hardseededness and increased tolerance of false breaks) scored more than 5% and increased seedling vigour and root growth scored 3.6%.

Of other traits, it is interesting to note that increased P- and K-use efficiency was considered of some importance (5% of the total allocation), with all of this in the permanent pasture zone, justifying MLA's investment in this area (Project B.PUE.0104). This project is seen as even more important if the suggested allocation to root traits (both seedling and adult) is also considered (almost 5%). Weed-related traits comprised 8% of the suggested funding allocation, consisting of ability to compete with weeds, increased weed management options and tolerance to applied broadleaf herbicides and residues from in-crop herbicides. Traits related to nutritive and feeding value (nutritive value of green feed, extended period of green feed into late spring/summer and anti-bloating properties) comprised 7% of the suggested funding allocation. Increased nitrogen fixation was also scored 4%, with greater importance in the mixed farming zone.

A range of traits are related to seedling regeneration and early season growth. Together these traits comprised 57% of the suggested total funding allocation across all legumes and both mixed farming and permanent pasture systems (Table 11).

Table 7. Annual legume trait priorities for the permanent pasture zone (ranked 1-7, where 7 = most important, 1 = least important of the highest ranked traits) of four groups (Animal production, Seed industry and two Pasture science groups), along with a suggested percentage allocation of funds to the zone.

Trait	Animals	Seeds	Pastures 1	Pastures 2	Total
Feed supply					
Reduced seedling losses from false breaks					
Increased seedling vigour/early root growth	1				1
Increased feed in autumn-early winter	7	7	7	7	28
Increased feed in winter					
Increased feed in spring					
Extended period of green feed into late spring/summer	3				3
Ability to produce fodder (specialist hay or silage)					
Feed value					
Increased nutritive value of green feed	5				5
Increased nutritive value of dry feed over summer					
Increased animal preference (palatability)					
Decreased animal preference (palatability)					
Lack of anti-nutritional or toxic chemicals					
Lack of chemicals affecting reproduction					
Anthelmintic (anti-worming) properties					
Anti-bloating properties					
Reduced greenhouse gas emissions from grazing					
Capacity to improve meat quality or flavour					
Pasture sowing and establishment					
Low costs of establishment (low seed and sowing costs)					
Ease (reliability) of establishment					
Increased tolerance of broad-leaf herbicides		1		1	2
Persistence			7		7
Increased seed production	6	7	4	7	24
Increased reliability of regeneration between years			2	2	4
Increased seed dormancy		2		6	8
Adaptation to increasing climate variability					
Adaptation to increased CO_2 levels (climate change)					
Persistence over seasons (reduced need to re-sow)			6		6
Ability to compete with weeds			5		5
Ability to compete with perennial grasses			1		1
Drought tolerance					
Deeper root systems				3	3
Grazing tolerance					
Frost tolerance					
Pests and diseases					
Resistance to leaf and stem fungal diseases					
Resistance to virus diseases					
Resistance to below-ground diseases (root rots, nematodes)	2	6	3	5	16
Resistance to aphids					
Resistance to redlegged earth mites/ blue oat mites		5		4	9
Resistance to lucerne flea					
Resistance to other pests or diseases - specify					
Soil constraints					
Salt tolerance (for saline areas)					
Waterlogging tolerance					

Trait	Animals	Seeds	Pastures 1	Pastures 2	Total
Acid soil tolerance					
Increased P & K efficiency (growth with less P & K)	4	4		1	9
Adaptation to sandy soils					
Adaptation to heavy clays					
Tolerance of soil toxicities (boron, heavy metals etc)					
Adaptation to soils with a high free lime content					
Integration with cropping					
Increased legume regeneration after cropping					
Increased nitrogen fixation		3			3
Increased tolerance of spray-topping herbicides					
Reduced susceptibility to cropping herbicide residues					
Others					
Improved seed harvesting characteristics					
% allocation of funds to permanent pastures	50	40	-	50	

Table 8. Suggested % expenditure on important traits for different annual legume species for the mixed farming zone - mean ratings of two groups.

Trait	All	Sub clover	Annual medics	Serradellas	Biserrula	Balansa clover	Persian clover	Bladder clover	Arrowleaf clover	New species	Mean % trait scores
Increased feed in autumn-early winter		3.6	5.0	4.3	4.3	1.4				1.4	20.1
Increased seed production	8.6	4.3	4.3								17.3
Seedling vigour / early root growth	3.6	1.1	1.4			1.1					7.2
Resistance to below-ground diseases (root rots, nematodes)		2.9	2.2	0.4	0.4	0.4		0.4			6.5
Reduced seedling losses from false breaks	1.4	2.5	1.1	0.4		0.4					5.8
Increased nitrogen fixation		1.8	2.2		1.1	0.7					5.8
Increased persistence	2.9	1.1	1.1								5.0
Increased reliability of regeneration between years	4.3										4.3
Increased nutritive value of green feed	3.6										3.6
Increased tolerance of broad-leaf herbicides	3.6										3.6
Increased P & K efficiency (need for less P & K fertiliser)	1.8		1.1								2.9
Reduced susceptibility to crop herbicide residues	2.9										2.9
Weed management	2.9										2.9
Extended period of green feed into late spring/summer	1.1	0.4	0.4						0.4		2.2
Anti-bloating properties	2.2										2.2
Ability to compete with weeds	1.1	0.5	0.5								2.2
Resistance to redlegged earth mites/ blue oat mites	2.2										2.2
Increased seed dormancy		1.4				0.4		0.4			2.2
Resistance to leaf and stem fungal diseases	1.4										1.4
Species totals	43.5	19.6	19.2	5.0	5.8	4.3	0.0	0.7	0.4	1.4	100.0

Table 9. Suggested % expenditure on important traits for different annual legume species for the permanent pasture zone - mean ratings of two mixed groups in Group session 2.

Trait	All	Sub clover	Annual medics	Serradellas	Biserrula	Balansa clover	Persian clover	Arrowleaf clover	New species	Mean % trait scores
Increased feed in autumn-early winter		13.8	0.5	1.7		2.5	1.7	1.3		21.5
Increased seed production	1.6	16.8								18.4
Resistance to below-ground diseases (root rots, nematodes)	6.3	6.0								12.3
Resistance to redlegged earth mites/ blue oat mites	3.5	2.6				0.4		0.4		6.9
Increased P & K efficiency (need for less P & K fertiliser)	3.5	3.4								6.9
Increased persistence	2.7	3.0								5.7
Persistence over seasons (reduced need to re-sow)	2.3	2.6								5.0
Increased nutritive value of green feed	2.0	1.5		0.2		0.2	0.2	0.2		4.2
Ability to compete with weeds	3.8									3.8
Increased reliability of regeneration between years	1.6	1.9								3.4
Increased seed dormancy	0.2	2.5								2.7
Extended period of green feed into late spring/summer	1.2	0.9						0.2		2.3
Increased nitrogen fixation	2.3									2.3
Deep root systems		2.3								2.3
Increased tolerance of broad-leaf herbicides	0.9	0.6								1.5
Ability to compete with perennial grasses	0.5	0.3								0.8
Species totals	32.3	58.3	0.5	1.9	0.0	3.0	1.9	2.1	0.0	100.0

Table 10. Suggested overall % expenditure on important traits for different annual legume species across both mixed farming and permanent pasture systems (both systems considered of equal importance).

Trait	All	Sub clover	Annual medics	Serradellas	Biserrula	Balansa clover	Persian clover	Bladder clover	Arrowleaf clover	New species	Mean % trait scores
Increased feed in autumn-early winter		8.7	2.8	3.0	2.2	2.0	0.8		0.7	0.7	20.8
Increased seed production	5.1	10.6	2.2								17.8
Resistance to below-ground diseases (root rots, nematodes)	3.1	4.4	1.1	0.2	0.2	0.2		0.2			9.4
Increased persistence	2.8	2.0	0.5								5.4
Increased P & K efficiency (need for less P & K)	2.7	1.7	0.5								4.9
Resistance to redlegged earth mites/ blue oat mites	2.8	1.3				0.2			0.2		4.5
Increased nitrogen fixation	1.1	0.9	1.1		0.5	0.4					4.0
Increased reliability of regeneration between years	2.9	0.9									3.9
Increased nutritive value of green feed	2.8	0.8		0.1		0.1	0.1		0.1		3.9
Seedling vigour / early root growth	1.8	0.5	0.7			0.5					3.6
Ability to compete with weeds	2.5	0.3	0.3								3.0
Reduced seedling losses from false breaks	0.7	1.3	0.5	0.2		0.2					2.9
Persistence over seasons (reduced need to re-sow pastures)	1.2	1.3									2.5
Increased tolerance of broad-leaf herbicides	2.3	0.3									2.6
Increased seed dormancy	0.1	2.0				0.2		0.2			2.4
Extended period of green feed into late spring (or summer)	1.1	0.6	0.2						0.3		2.2
Reduced susceptibility to herbicide residues from cropping	1.4										1.4
Weed management	1.4										1.4
Anti-bloating properties	1.1										1.1
Deep root systems		1.1									1.1
Resistance to leaf and stem fungal diseases	0.7										0.7
Ability to compete with perennial grasses	0.2	0.2									0.4
Species totals	37.9	39.0	9.9	3.5	2.9	3.7	0.9	0.4	1.2	0.7	100.0

Table 11. Summary of suggested overall % expenditure on traits of importance related to persistence and early season production in regenerating stands of annual legumes across both mixed farming and permanent pasture systems in southern Australia

Trait	Mean trait score (% of total)
Increased feed in autumn-early winter	20.8
Resistance to below-ground diseases (root rots, nematodes)	9.4
Increased persistence *per se*	5.4
Resistance to redlegged earth mites/ blue oat mites	4.5
Increased reliability of regeneration between years	3.9
Seedling vigour / early root growth	3.6
Reduced seedling losses from false breaks	2.9
Persistence over seasons (reduced need to re-sow pastures)	2.5
Increased seed dormancy	2.4
Reduced susceptibility to herbicide residues from cropping	1.4
Total	56.8

4.1.3 Outcomes from Group session 3: Key factors for project success

The issues raised by each group are shown below.

Group 1

- Project outputs need to be defined by industry needs and the largest economic gains for meat production - the focus should be on targeting feed gaps.
- For the project to be achievable it needs to be based on good science, taking into account Genotype x Environment factors that relate to meat production.
- There needs to be good communication, involving a two-way flow of information between industry and trait specialists.
- The Genetic Resource Centres will need to be maintained and supported, to enable ready access to germplasm and use of core collections.
- An audit of technical capacities is needed.

Group 2

- There will need to be maintenance and ready access to key genetic resources – access should also require obligations to ‘inform” the GRCs about traits discovered.
- “High level genetic tools” need to be developed that improve access to materials in the GRCs (e.g. the subterranean clover core collection being used to develop genetic maps and molecular markers for traits of importance).
- There needs to be strong links with public and private breeding programs.
- Cast-iron agreements need to be in place early in the project about how ‘tools’ and ‘genes’ are to be made available to industry breeding/marketing companies. This is important as there are likely to be inevitable spin-offs from trait identification and tool development that may lead to early identification of elite varieties.

Group 3

- The organisations running the project need to have the technical capability and infrastructure to do the work.
- Junior scientists should be employed on the project, as part of succession planning in pasture science.

- There needs to be an economic basis for valuing individual traits, although the knowledge of underlying causative factors is often not well understood to accurately do this (e.g. powdery mildew in medics).
- There needs to be a strong plant pathology component that can undertake disease resistance screening for national issues.
- There needs to be access to genetic diversity in the GRCs, which need to be adequately funded, and good core collections need to be developed.
- There needs to be good internal project communication between collaborators, involving regular teleconferences and an annual meeting.
- There should be financial recognition of the pre-breeding effort when a cultivar is eventually developed.
- There needs to be absolute assurance of no 're-badging' of cultivars developed with new traits developed by the project.
- Given the constraints of funding, the project needs a specific focus and consideration needs to be given as to how thinly the money is to be spread among species.

Group 4

- The traits to be researched need to have high economic value to industry. Indices of economic value should be determined for each trait.
- The project needs to be technically feasible, with an honest analysis of risks vs. rewards.
- There should be an analysis of options – pasture management vs. improved genetics (e.g. for redlegged earth mites, the comparison between spraying and resistant cultivars).
- The value proposition should be a package that considers the flow of pre-breeding to breeding to evaluation to commercialisation.
- Pre-breeding and subsequent breeding needs to be strongly related to potential cultivar seed sales – this is a commercial imperative for future breeders.
- The project team needs to have the technical capacity to deliver planned project outputs.
- There should be mixed sward research, involving evaluation in combination with grasses.

5. Discussion

5.1 Recommendations from the Annual Legume Pre-breeding Workshop

The Annual Legume Traits and Species Pre-breeding Workshop was highly valuable in helping determine the key traits in annual legumes to increase the profitability and sustainability of red meat production, along with the species that should be the main focus of work. While the identified priorities should not be considered as prescriptive for the R & D to be conducted, they can, however, be taken as an indicative guide as to how MLA funding should be allocated.

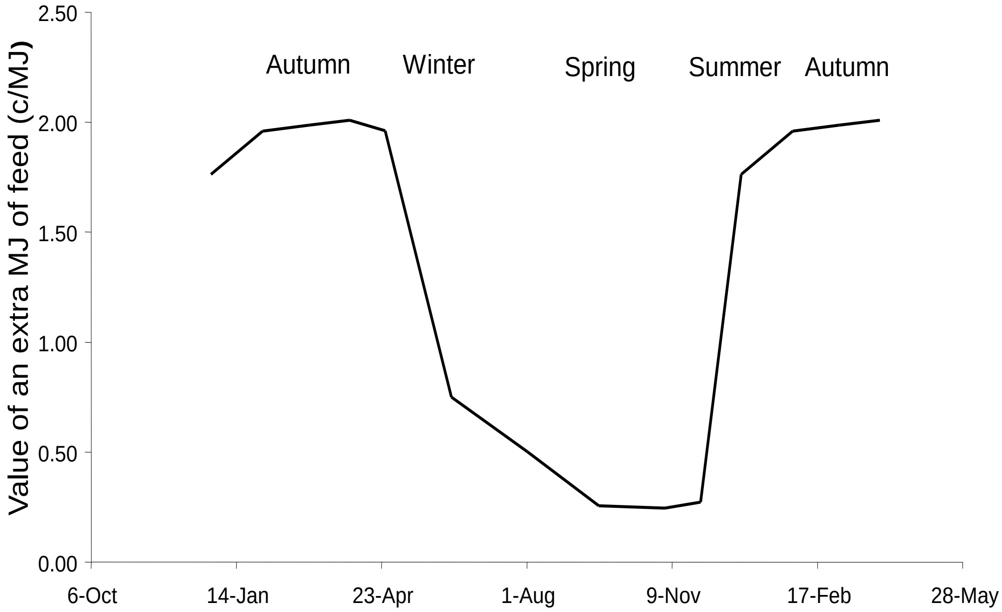
The following recommendations arose from the workshop:

- The pre-breeding project should place a large emphasis on traits to increase persistence and autumn-winter biomass production in regenerating stands. This was supported by economic modelling data from John Young (Farming Systems Analysis Service), which demonstrated that the value of an extra MJ of feed from annual pastures in autumn-early winter is worth around ten times its value in spring, when feed is usually abundant (Figure 4). The strong relationship between early season pasture production and seedling density, first shown by Donald (1951), indicates that the primary focus should be on traits that increase seedling establishment and survival in regenerating swards.

- Traits that increase seedling germination and establishment survival in regenerating swards include seed production, the degree of hardseededness and the timing of its breakdown (ability to tolerate false breaks) and resistance to root rot pathogens and redlegged earth mites (RLEM). Focusing on improvement in these traits will lead to greater persistence and increased autumn-winter biomass production in regenerating stands. Increased seedling vigour and root growth is another tactic to increase autumn-winter biomass. Improvement in these traits was strongly supported by the workshop, collectively accounting for 57% of all suggested expenditure on traits that could be improved (Table 11).

Figure 4. Value of extra feed from annuals (without summer active species). Data from J. Young (Farming Systems Analysis Service).

.



- Similar emphases should be given to mixed farming and permanent pasture systems.

- A high emphasis should be placed on subterranean clover, with some emphasis on annual medics and that work on some traits should be conducted on a broad cross-section of the species used in southern Australia. There is little need to develop new annual legume species at this stage.

Five key areas were identified for the project to be successful.

1. *Economics*: The project needs to focus on the most economically important traits and species for the meat industry and deliver outputs able to be incorporated into cultivars for the seed market.

2. *Technical capacity*: The project team will need to have the technical skills and capacity to conduct the work and a risk analysis will need to be conducted. There were also some comments related to building technical capacity, particularly the training of junior scientists for succession planning.
3. *Communication*: There needs to be good communication between the project team and the meat and seed industries. Good internal project communication is also essential to ensure the project remains on track.
4. *Genetic Resource Centres*: The Genetic Resource Centres need to be supported and germplasm and core collections need to be readily accessible.
5. *Commercialisation of project outputs*: Agreements need to be reached about how 'tools' and 'genes' are to be made available to industry breeding/marketing companies and to how the pre-breeding project should be recognised in any cultivars developed using project outputs.

5.2 Determination of project directions and outputs

In the day following the Annual Legume Traits and Species Pre-breeding Workshop, the project team deliberated on the workshop findings and used them to develop an outline of the essential directions and outputs for the R & D plan. These were deemed to include:

- A measure of the genetic diversity within important annual legume species for the traits of interest and the potential for genetic gains through plant breeding;
- Identification of potential parents for cross-breeding;
- The development of molecular markers associated with traits of interest to enable future marker-assisted selection;
- The development of rapid generation technologies to increase the rate of cultivar development
- Peer-reviewed scientific publications;
- A summary of project results for distribution to private and public breeding organisations; and
- A list and description of any germplasm and breeding lines developed from the project, which will be made available for variety development by private and public breeding organisations.

An outline of the species and actual traits to be examined were also formulated by the project team, on the basis of the workshop findings. Potential team members to implement the project were also suggested by the project team, on the basis of their familiarity, experience and capabilities with the species and technologies. Subsequent consultation by email and telephone with the agencies and seed companies refined as the project and confirmed the team membership.

5.3 The annual legume pre-breeding R & D plan

5.3.1 Project outline

A comprehensive R & D plan has been developed with the aim of delivering pre-breeding technologies in annual pasture legumes that will enable the development of cultivars that result in greater red meat production to be bred more rapidly. It combines the disciplines of genetic resources, molecular biology, cell biology, entomology, plant physiology, plant chemistry and genetics and will capitalise on prior and current R & D investments in cutting edge technology to deliver world-class, pre-breeding outputs available for use by public and private breeding organisations. This project will transform annual pasture legume pre-breeding and breeding programs and lay the foundation for future investments by MLA and other industry stakeholders in the development of improved pasture legume cultivars. New cultivars, based on the technologies developed in this project, should be available for farmers within 10 years.

The project will run over 3.5 years and focus predominantly on traits associated with increased persistence and autumn-winter biomass production in regenerating pastures, which has been identified as having the greatest economic impact on red meat production (Figure 4). These traits include resistance to redlegged earth mites (RLEM), tolerance of false breaks, optimum levels of hard seeds and other seed dormancy traits, genes for increased persistence *per se* and increased seedling growth under cool temperatures. A range of agro-morphological traits will be examined to enable correlations to be conducted between traits associated with persistence and autumn-winter biomass production. Boron tolerance will be examined in annual medics, to capitalise on prior R & D investments. An *in vitro* protocol to reduce cultivar development time will also be delivered. A major emphasis will be on subterranean clover, while important traits will also be examined in annual medics and other pasture legume species. A PhD student will also be sought to conduct supporting research on serradella pre-breeding.

Resistance to important root rot pathogens is recognised as a major trait affecting seedling establishment and autumn-winter biomass. However, it was not considered in this project due to budget constraints and because of concurrent investments by MLA into the impacts of root rots on pasture productivity in other areas of the FIP. This trait is flagged here for future investment by MLA into pre-breeding activities.

Seed production ability also will not be considered in this project, even though it was rated highly as a trait of importance. This is because it is a ‘mega-trait’, composed of many elements, and likely to have low heritability, in a similar manner to grain yield in cereals. Thus, the project team considered it too difficult a trait to make significant genetic progress in the first instance. Conversely, the traits selected for study are likely to have high heritabilities and significant genetic gains can be expected.

Project outputs will include:

- A measure of the phenotypic diversity of at least 20 key agronomic traits in subterranean clover, annual medics and other pasture legumes (from this and other related projects), their potential for genetic improvement through plant breeding, and identification of potential parents for crossing;
- Molecular markers in subterranean clover associated with genes for at least 20 key agronomic traits (from this and other related projects) and molecular markers in annual medics associated with genes for seedling growth and boron tolerance;
- A framework to generate molecular markers for future new traits;
- A robust protocol to triple generation turnover rates for subterranean clover that can be incorporated into current breeding methodology;
- The genome sequence of subterranean clover, which will also have international applications for the breeding of white clover and red clover; and
- Training of junior scientists and postgraduate students in important aspects of annual legume pre-breeding.

A commercialisation plan will form an early milestone to ensure that the seed industry is actively engaged in the technology and ready to adopt when it is available. As a minimum, technical outputs will be made available to private and public breeding organisations in a technical bulletin (likely to be web-based), listing the values of at least 20 key agronomic traits, and the molecular markers closely associated with them, for diverse germplasm of subterranean clover, annual medics and other annual pasture legumes.

Scientific outputs will also be published in high impact, peer-reviewed journals; at least six publications are anticipated.

5.3.2 Capacity, ability and experience of organisations to implement the R & D plan

An audit of the capacity, ability and relevant experience of public and private breeding organisations and personnel to implement key areas of annual pasture legume pre-breeding was conducted, in order to select the team best able to implement the proposed R & D plan. For completion, the areas of annual pasture legume pre-breeding, plant breeding and selection, and field evaluation and commercialisation were assessed. A simple 1-3 rating scale was used, where: 1 = limited capacity/skills/experience; 2 = some capacity/skills/ experience; 3 = high skills/capacity/experience (no rating implies no capacity/skills/ experience). Ratings were compiled on the basis of discussions with individuals, website information and published data. A summary is shown in Figure 12. It is recognised that such ratings are subjective and contentious, but it provides some indication of the organisations best able to implement the R & D plan.

Plant breeding and selection
On the basis of the audit information, it is apparent that DAFWA has significant capability, skills and experience in breeding and selection of subterranean clover, serradellas, biserrula and other clovers, SARDI has particular expertise in breeding annual medics, subterranean, balansa and Persian clovers, while the Tasmanian Institute of Agriculture (TIA) has expertise in arrowleaf clover and other long season annuals. AgResearch has commenced a targeted breeding program in subterranean clover in its joint venture with PGG Wrightsons. Among the other private companies, Pristine Forage has considerable experience and skills in annual medic and balansa clover breeding and selection. Heritage Seeds conducts breeding of some sub-tropical grasses and legumes and have the skills and capacity to conduct breeding of annual legumes, but the preference is for this to be done through the partnership with SARDI.

Field evaluation and commercialisation
DAFWA, SARDI, NSW DPI and TIA have the capacity, skills and experience to conduct field evaluation of elite breeding lines. DPI Victoria has largely lost this capacity, due to their strong focus on pre-breeding. Each of the major seed companies has the capacity and skills to conduct field trials. Seed production usually consists of two phases. The breeding organisations generally produce breeders or pre-breeders seed, with direct involvement by the breeder to ensure purity and trueness of type, although private companies have the ability to do this. Basic and Certified seed production is conducted by the seed companies and there is little need for public agencies to be involved in this process.

Pre-breeding
Several areas of pre-breeding were considered. This is an area where private organisations in Australia have limited capacity, skills or experience, at least for the technologies and traits under consideration. Seed Force and Heritage Seeds do have connections with large international seed companies (RAGT and Royal Barenbrug Group, respectively), which conduct some pre-breeding, but the focus is on perennial species, particularly grasses. Similarly, PGG Wrightsons has a joint venture with AgResearch in New Zealand, which has a major focus on white clover and perennial grasses. Pristine Forage has skills and experience in screening for morphological traits and has field screening capacity for resistance to foliar diseases and aphids.

CLIMA is the only organisation in Australia with skills and experience in rapid generation technologies for legumes. DAFWA has skills and experience with morphological characterisation of subterranean clover, serradellas, biserrula and other clovers, while

SARDI has particular expertise with annual medics and some other species. TIA also has particular experience with characterisation of long season annual legumes. Of other screening technologies under consideration, DAFWA has the most skills and experience in seed dormancy traits and screening for RLEM resistance, while SARDI is the only agency with experience in screening for boron tolerance. Screening for increased seedling vigour and growth is a new area of annual legume research and no organisation has particular skills or experience in this area. DAFWA, SARDI and NSW DPI each have the capacity and skills to screen for tolerance to biotic (pests and diseases) and abiotic stresses in the field.

CLIMA, in partnership with Kazusa DNA Institute in Japan, is the only group world-wide working on the molecular biology of subterranean clover, having published the first genetic map of the species and having laid the groundwork to identify the species genome. CLIMA has also developed molecular markers for biserrula and bladder clover. With barrel medic being a model legume for genomic studies, several laboratories globally are conducting molecular biology research on the species. However, SARDI has considerable skills in the molecular biology of annual medics, and is the only laboratory that has developed molecular markers for use in a breeding program. Several other organisations have general molecular biology skills, which could be used for annual legumes. DPI Victoria and AgResearch have considerable skills and experience in the molecular biology of white clover. UWA and DAFWA have developed molecular markers for lupins, tedera and chickpeas, while DPIV has been the lead agency for the GRDC-funded pulse molecular marker program since 2009, which has generated large scale genomic resources and molecular markers for lentils, chick peas, field peas and faba bean. NSW DPI, CSIRO and several universities have molecular biology skills in other crops.

5.3.3 Organisations to implement the annual legume pre-breeding R & D plan

On the basis of the audit of capacity, ability and relevant experience, four organisations were selected to implement the proposed project. These are: DAFWA, SARDI, CLIMA and NSW DPI. The project will also collaborate with Kazusa DNA Institute and Murdoch University.

The proposed project comprises the following 10 component activities and delivery agencies:

- *In vitro* tools to reduce cultivar development time in subterranean clover (CLIMA);
- Molecular markers for key morphological traits in subterranean clover (CLIMA);
- Identification of genes for persistence in subterranean clover (CLIMA);
- Diversity for red legged earth mite (RLEM) resistance in subterranean clover, annual medics, balansa, bladder and Persian clovers, biserrula, yellow and French serradella and cultivars of other species (DAFWA);
- Genes for RLEM resistance in gland clover (DAFWA);
- Improved false break tolerance and seed dormancy traits in subterranean clover (DAFWA);
- Diversity of agro-morphological traits within subterranean clover (DAFWA);
- Increased growth at low temperatures in annual medics and subterranean clover (SARDI);
- Boron tolerance in annual medics (SARDI); and
- Project communication

A PhD/Masters student will be sought for the project topic *Yellow serradella genetics and diversity*. Other students will also be sought to work on other project areas.

The Department of Agriculture and Food Western Australia (DAFWA)
DAFWA will lead the project. It has an internationally recognised, world-class pasture legume breeding, selection and agronomy program and a proud history of delivering

improved pasture cultivars and farming systems for the benefit of southern Australian farmers and the environment. It has the world's most successful subterranean clover breeding program, having been involved in subterranean clover breeding and selection since 1967 and developed 27 cultivars. DAFWA has also had a program over the past 25 years to develop cultivars of new species for soils and farming systems to which subterranean clover and the traditional annual medic species are poorly adapted. DAFWA, in conjunction with CLIMA, was an intellectual driver of innovation for the highly successful *National Annual Pasture Legume Improvement Program (NAPLIP)*, which resulted in development of new annual legumes that underwent field testing across southern Australia. DAFWA has developed cultivars of French, yellow and slender serradellas, biserrula, burr, murex and sphere medics, bladder, gland, crimson, arrowleaf, eastern star, Persian and purple clovers and the short-lived perennial species, sulla. The DAFWA Pasture Science group has worked with all the pasture legume species used in southern Australia and has a strong understanding of their role in agricultural systems. This includes seed collection trips to their natural habitat, which has provided a strong foundation in understanding their ecology.

In conjunction with CLIMA, DAFWA has developed core collections of subterranean clover, bladder clover and biserrula, which have captured the vast majority of the genetic diversity of the whole collections within small and more manageable core groups of accessions. DAFWA also has experimental, genetically diverse populations of subterranean clover, which will be exploited to develop an understanding of the genetics of important traits, leading to the identification of Quantitative Trait Loci (QTLs) and molecular markers for breeding.

DAFWA has the field and glasshouse facilities needed for crossing, screening and field selection programs. It hosted the annual legume pest and disease screening sub-program in the NAPLIP project and has expertise in screening for resistance to redlegged earth mite and to clover scorch disease. The DAFWA pasture science group has a strong understanding of annual legume seed and seedling dynamics and seed dormancy traits for reliable establishment and pasture persistence in different environments and farming systems. The development of screening systems for hardseededness and seed softening patterns has been used to select several cultivars with levels appropriate to their use. DAFWA also has experience in the application of molecular markers for legume breeding and currently uses them to screen for disease resistance in its lupin breeding program.

The Centre for Legumes in Mediterranean Agriculture (CLIMA)

CLIMA has expertise in the use of molecular markers for legume breeding and genomics, the development of core collections in pasture legumes and an increasing skill base in bio-informatics (Dr Parwinder Kaur), tissue culture and embryo rescue (Dr Janine Croser), wide-crossing (Dr John Clements), selection for herbicide tolerance (Dr Ping Si), and legume transformation (Dr Susan Barker and Professor Willie Erskine).

CLIMA has developed molecular markers for subterranean clover, identified several QTLs for important traits and published the first genetic linkage maps of subterranean clover. A CLIMA ARC Linkage project developed the core collection of subterranean clover. This is now being used in a further ARC Linkage project to investigate genetic diversity within subterranean clover and identify differences in their methanogenic potential. The subterranean core collection is also being used to identify differences in phosphorous use efficiency in the MLA/AWI-funded project *Phosphorous efficient pasture systems* (MLA Project No. B. PUE. 0104), operated by CSIRO Plant Industry, UWA School of Plant Biology, DAFWA and NSW DPI. The close linkage between CLIMA and DAFWA will be used to develop screening methods for new traits, particularly those requiring biotechnology skills.

The South Australian Research and Development institute (SARDI)

SARDI is a leading research and development institute and the Livestock Feed and Forage program area has a strong history of pre-breeding and the development of annual legumes and has released over 20 cultivars of annual medics, subterranean clovers, and alternative annual legume species (e.g. balansa and Persian clovers). The SARDI project team has a thorough understanding of genetic diversity in annual pasture legumes and how they can be used in pre-breeding programs and is host to the world's largest collection of annual pasture legume germplasm. Pre-breeding traits that have been developed by SARDI and later developed into annual medic cultivars include bluegreen aphid resistance, spotted alfalfa aphid resistance, suitable levels of hardseededness and tolerance to SU herbicide residues.

Table 12. Rating of public and private breeding organisation capacities, abilities and experience in the areas of annual pasture legume pre-breeding, plant breeding and selection, and field evaluation and commercialisation. Rating scale of 1-3, where: 1 = limited capacity/skills/experience; 2 = some capacity/skills/ experience; 3 = high skills/capacity/experience (no rating implies no capacity/skills/experience).

Activity	Public organisations										Private organisations						
	DAFWA	SARDI	NSW DPI	DPI Vic	TIA	CLIMA	UWA	Ag Research	CSIRO	Others	Seed Force	PGG Wrightsons	Heritage	Seed Distributors	Pristine Forage	Tas-global	Others
Pre-breeding																	
In vitro tools for rapid generation turn-over						3											
Subterranean clover morphological characterisation	3	3	1		1	1											
Annual medic morphological characterisation	3	3	1			1									3		
Other legume species morphological characterisation	3	3	3	3	3	3									3		
Subterranean clover molecular biology	1	1	1	2		3	2	1	1	1	1		1				
Annual medic molecular biology	1	3	1	2		2	2	2	3	2	1		1				
Other legume species molecular biology	3	3	1	3		3	3	3	2	2	1		1				
Disease screening - root rots	1	2	1				3		1								
Disease screening - foliar diseases	1	2					3								1		
RLEM screening	3	1															
Seed dormancy	3	2															
Seedling growth at low temperatures	2	2	2	2		2	2	2	2	2					1		
Boron tolerance		3															
Field screening for biotic and abiotic stresses	3	3	3												2		
Plant breeding and selection																	
Subterranean clover	3	3						2				1					
Annual medics	2	3													3		
Serradellas	3																
Biserrula	3																
Balansa clover	2	3													3		
Persian clover	2	2															
Bladder clover	3																
Arrowleaf clover	2				2												
Other annual species	3				3												
Field evaluation and commercialisation																	
Field evaluation of advanced lines	3	3	3	1	3						2	2	3	2	1	2	2
Production of breeders/pre-basic seed	3	3			3						2	2	2	2	3	2	
Seed production of new cultivars	1	1			2						3	3	3	3	3	3	1

SARDI has developed successful crossbred cultivars from inter-specific crosses. One example is Herald strand medic (*M. littoralis*) in which aphid resistance was successfully transferred from an aphid resistant barrel medic (*M. truncatula*) accession into a "Harbinger strand medic" genetic background (backcross 3). Herald was extensively field evaluated across multiple states within the National Annual Medic Improvement Program before commercialisation. As a successful cultivar in its own right, it has been the benchmark medic cultivar for over 15 years in low rainfall regions with neutral/alkaline light-textured soils. In addition it has also been used as the platform for further cultivar development via mutagenesis, resulting in cvs. Angel and Jaguar strand medic. More recently, SARDI has transferred tolerance to SU herbicide residues from the strand medic cv. Angel into barrel medic (Peck and Howie 2012). Short-listed lines are currently being seed increased for cultivar release and they have similar performance as current barrel medic cultivars in the absence of SU herbicide residues and up to ten times the dry matter of current cultivars when SU herbicides are present.

The molecular capabilities of the SARDI Gene Function group cover traditional marker analyses (e.g. SSRs), high-throughput SNP genotyping and the application of genotyping by sequencing for high resolution marker analysis. SARDI has developed molecular markers for important traits in annual medics (e.g. diagnostic marker for SU herbicide tolerance, closely linked marker for boron tolerance in barrel medic, QTL's for dry matter production and also tolerance to root lesion nematode in strand medic. Genetic studies are also carried out in close linkage with trait specialists and breeders addressing biotic stress, abiotic stress, quality traits (e.g. anti-nutritional factors) and herbicide tolerance

New South Wales Department of Primary Industries (NSW DPI)
NSW DPI has a long history of strong and successful collaboration with DAFWA and CLIMA to deliver improved pasture legume cultivars to the marketplace. This dates back to 1983, with formation of the *National Subterranean Clover Improvement Program*, and continued into the *NAPLIP* project. NSW is the largest consumer of subterranean clover seeds and has great potential to expand the area of many alternative legumes. NSW DPI will provide a link to the field activities conducted as part of the MLA project *Phosphorous efficient pasture systems* (Project No. B. PUE. 0104).

Kazusa DNA Institute (KDRI)
The project will collaborate with Kazusa DNA Research Institute (KDRI) in Japan, which is a world leader in plant and human DNA research and genome analysis. KDRI has state of the art new generation genome sequencing, bio-informatics and supercomputing facilities. The project will utilise the close relationship developed between CLIMA and KDRI since 2009, particularly with renowned scientist Dr Sachiko Isobe. Dr Isobe's laboratory has a particular interest in legume genomics and is widely published in high impact journals (see reference list). These are the only two laboratories globally that are conducting molecular research into subterranean clover and the collaboration has been highly productive.

Murdoch University
The project will also collaborate with the Centre for Comparative Genomics at Murdoch University, notably with Professor Rudi Appels. This will build on the existing relationship developed between CLIMA and Professor Appels, who has been instrumental in helping develop the first linkage maps of subterranean clover and development of the subterranean clover core collection.

CLIMA has collaborated closely with KDRI and Murdoch University in two ARC Linkage projects. It has lead to publication of the world's first linkage map for subterranean clover and to development of the subterranean clover core collection. Under this collaboration *de novo* sequencing of subterranean clover has been undertaken, resulting in a genome scaffold and SNP discovery, to generate a very high resolution QTL map. This proposed project will build on this relationship and skills-base. It will use the high quality molecular datasets to sequence the whole subterranean clover genome, develop a core collection HapMap, identify molecular markers associated with important traits, and conduct KASP genotyping on bulk-hybrid populations to identify genes for persistence.

KDRI have a particular interest in subterranean clover as a model *Trifolium* species for molecular and genomic analyses. This is because the internationally important *Trifolium* species, white clover and red clover, are genetically more complex and difficult to conduct such studies on. Consequently, KDRI will provide considerable in-kind to this project, including costs for operation and maintenance for use of their next generation sequencer (NGS) and Bio-informatics and supercomputing support to handle the huge molecular data-sets generated. This makes this arrangement for sequencing highly cost-effective compared to alternative Australian sequencing service providers. In Australia, CLIMA will analyse the sequence data with a remote log-in to the KDRI super-computer facility, working closely with their bio-informatics team and will also undertake subsequent marker development.

5.3.4 Project team

Potential team members to implement the project were first suggested by the project planning team following the Annual Legumes Traits and Species Workshop. Recommendations were made on the basis of their recognised familiarity, experience and capabilities with the species and technologies. Team membership was confirmed following subsequent consultation by email and telephone with the agencies and seed companies.

There were very few options to choose from for the morphological and phenotyping studies. DAFWA and SARDI are the only agencies with extensive experience in breeding and pre-breeding of annual legumes, with DAFWA having particular expertise in subterranean clover and alternative legumes for acid soils and SARDI having particular expertise in annual medics and balansa and Persian clovers. TIA has experience in characterisation of legume species at the accession level, rather than breeding, and their main interest has been in perennial legumes and alternative legumes, rather than mainstream species. Of the private companies, Pristine Forage has the experience and skills to do the work in medics and balansa clover, but they have a main focus on breeding, rather than pre-breeding.

For the molecular biology work in subterranean clover, the CLIMA/KDRI partnership had several advantages, from both productivity and cost perspectives. KDRI has world-class facilities and a very strong research output. The team already has an established track record of successful molecular research outputs in subterranean clover and is familiar with the species, the relevant germplasm and molecular markers and research techniques. Conversely, it would take a new collaborator several months to gain such familiarity, resulting in a much slower rate of progress. KDRI also brings considerable in-kind to this project by means of the operating and maintenance costs of their next generation sequencers (NGS) and provision of bio-informatics and supercomputing support to handle the very large molecular data-sets generated. This makes this arrangement for sequencing highly cost-effective compared to alternative Australian sequencing service providers.

For the molecular biology work in annual medics, SARDI has an excellent track record, having developed molecular markers for a range of traits, including herbicide tolerance, boron tolerance and for tolerance to root lesion nematodes. The SARDI team is familiar with the species, the relevant germplasm and molecular markers, and the appropriate research techniques.

There are several other laboratories in Australia with capacity and skills in molecular biology. DPI Victoria, for example, has world-class facilities and capabilities. Discussions were held with DPIV as a potential project partner, but it became apparent that annual legumes were not among the mandated species for their research and that their main focus would be on the perennial grass pre-breeding project. Other laboratories were not considered, as it was not apparent they could offer significant advantages.

On the basis of these deliberations, the following project team will deliver the R & D plan to MLA:

- **Dr Phillip Nichols** (DAFWA) will be Project leader, and provide expertise on traits of importance to subterranean clover and on screening for RLEM resistance;
- **Dr Clinton Revell** (DAFWA) will provide expertise on seed dormancy traits and other traits of importance to annual legumes and their integration into farming systems;
- **Dr Daniel Real** (DAFWA) will provide expertise on plant breeding methodology and the analysis and applications of pre-breeding to legumes;
- **Mr Richard Snowball** (DAFWA) will provide expertise on characterisation of morphological traits;
- **Professor William Erskine** (CLIMA) will provide expertise on molecular biology and rapid generation technologies;
- **Dr Parwinder Kaur** (CLIMA) will provide expertise on molecular biology of subterranean clover;
- **Dr Janine Croser** (CLIMA) will provide expertise on *in vitro* techniques for rapid generation turn-over
- **Dr Klaus Oldach** (SARDI) will provide expertise on molecular biology of annual medics;
- **Mr Jake Howie** (SARDI) will provide expertise on annual medic agronomy and variety evaluation;
- **Mr David Peck** (SARDI) will provide expertise on annual medic and annual legume breeding and selection; and
- **Mr Graeme Sandral** (NSW DPI) will provide expertise on traits of importance to annual legumes in the mixed farming and permanent pasture zones of eastern Australia.

  Dr Sachiko Isobe (Kazusa DNA Institute) and **Professor Rudi Appels** (Murdoch University) will be associates to this project, through the related ARC Linkage project *Exploiting subterranean clover genetic variation for methane mitigation & ruminant health challenges to the Australian livestock industries*. Dr Isobe will provide expertise on the molecular biology of subterranean clover and provide access to the sequencing, bio-informatics and super-computing facilities of KDRI. Professor Appels will provide expertise on molecular biology technologies and the analysis and interpretation of molecular data.

5.3.5 Details of Personnel

Dr Phillip Nichols

Qualifications:	PhD (UWA) 2005, B.Ag.Sci. (1st Class Honours) (Adelaide University) 1984
Current institution:	Department of Agriculture and Food Western Australia
Current position title:	Senior Research Officer (Pasture ecology, agronomy and breeding)
Prior positions (10 years):	Manager (DAFWA Pasture Improvement Program) - July 2004-June 2007

Contribution to this field

Dr Nichols is a Senior Pasture Scientist with 29 years of pasture legume breeding and pre-breeding experience. He has released 13 subterranean clover cultivars (Denmark, Goulburn, Leura, York, Riverina, Napier, Urana, Coolamon, Izmir, Mintaro, Bindoon, Rosabrook, Narrikup) and contributed to the development of Gosse. Annual certified seed sales across Australia of these cultivars have been in the order of 800 tonnes over the past four years. He is also a breeder of two lucerne cultivars (Aurora and Aquarius) during his tenure with the New South Wales Department of Agriculture (1984-86). Dr Nichols, in collaboration with SARDI (through the FFI CRC), has also just developed a cultivar of messina (*Melilotus siculus*) as a new annual pasture legume for saline, waterlogged soils.

Dr Nichols has considerable pre-breeding experience with annual pasture legumes. This includes operating screening programs for a range of annual legume traits, including resistance to clover scorch disease (*Kabatiella caulivora*) and to redlegged earth mites, levels of oestrogenic isoflavones, hardseededness and timing of its breakdown and salinity tolerance. He has a good understanding of the applications of molecular biology for plant breeding through his role as a Partner Investigator (PI) on two ARC Linkage projects that have used molecular techniques to develop a core collection of subterranean clover, along with molecular markers for important traits and publication of the first linkage maps for the species.

Dr Nichols has prior experience leading a national, multi-agency project, having been leader of the FFI CRC project *Reliable establishment of non-traditional perennial legumes* from 2006-10, which delivered establishment packages for sub-tropical perennial grasses and saltbush and new recruitment strategies for native grasses that are being widely adopted by industry. He was the national breeder of subterranean clover in the National Pasture Legume Improvement Program (NAPLIP) and the National Subterranean Clover Improvement Program (NSCIP) before that. He has also been involved in several other national projects involving interstate collaboration through the FFI CRC, the CRC for Plant-based Management of Dryland Salinity (Salinity CRC) and the Sustainable Grazing of Saline Lands project.

Dr Nichols has written or co-authored 165 scientific, technical and extension publications, including senior authorship of three recent reviews on pasture legumes and is an adjunct Senior Lecturer with the School of Plant Biology at UWA. He is an accredited Qualified Person for the Plant Breeders Rights Office in the area of pasture legumes and developed the technical document for subterranean clover for international Plant Breeders Rights (UPOV).

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications and book chapters:

1. ***Nichols PGH,** Foster KJ, Piano E, Kaur P, Ghamkhar K, Pecetti L, Collins WJ (2013) Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop and Pasture Science* (submitted)
2. * **Nichols PGH** (2013) *Trifolium subterraneum* var. *subterraneum* subterranean clover. 'Narrikup'. *Plant Varieties Journal* **25** (4), 149, 423-426.
3. ***Nichols PGH**, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia – their history, current use and future prospects. *Crop and Pasture Science* **63**, 691–725.
4. ***Nichols PGH**, Snowball R, D'Antuono MF, Barbetti MJ (2010) Resistance to clover scorch disease (*Kabatiella caulivora*) among accessions of purple clover (*Trifolium purpureum*) and its relationship to the eco-geography of collection sites. *Crop and Pasture Science* **61,** 44-49.

5. ***Nichols PGH**, Loi A, Nutt BJ, Snowball R, Revell CK (2010) Domestication of new Mediterranean annual pasture legumes. In 'Sustainable use of genetic diversity in forage and turf breeding' (Ed C Huyghe) pp 137-141 (Springer: Dordrecht)
6. ***Nichols PGH**, Cocks PS, Francis CM (2010) Using bulk-hybrids for breeding adapted genotypes of subterranean clover. In 'Sustainable use of genetic diversity in forage and turf breeding' (Ed C Huyghe) pp 557-562 (Springer: Dordrecht)
7. ***Nichols PGH**, Cocks PS, Francis CM (2009) Evolution over 16 years in a bulk-hybrid population of subterranean clover (*Trifolium subterraneum* L.) at two contrasting sites in south-western Australia. *Euphytica* **169,** 31-48.
8. ***Nichols PGH**, Malik AI, Stockdale M, Colmer TD (2009) Salt tolerance and avoidance mechanisms at germination of annual pasture legumes and their importance for adaptation to saline environments. *Plant and Soil* **315**, 241–255.
9. ***Nichols PGH** (2009) *Trifolium subterraneum* var. *subterraneum* subterranean clover. 'Bindoon'. *Plant Varieties Journal* **22** (4), 304-308.
10. ***Nichols PGH** (2009) *Trifolium subterraneum* var. *subterraneum* subterranean clover. 'Rosabrook'. *Plant Varieties Journal* **22** (4), 309-312.
11. ***Nichols PGH**, Craig AD, Rogers ME, Albertsen TO, Miller S, McClements DR, Hughes SJ, D'Antuono MF, Dear BS (2008) Production and persistence of annual legumes at five saline sites in southern Australia. *Australian Journal of Experimental Agriculture* **48**, 518-535.
12. **Nichols PGH**, Rogers ME, Craig AD, Albertsen TO, Miller S, McClements DR, Hughes SJ, D'Antuono MF, Dear BS (2008) Production and persistence of temperate perennial legumes and grasses at five saline sites in southern Australia. *Australian Journal of Experimental Agriculture* **48**, 536-552.
13. ***Nichols PGH**, You MP, Barbetti MJ (2008) Resistance to race 1 of *Kabatiella caulivora* in subterranean clover (*Trifolium subterraneum* L.) cultivars and breeding lines. *Australian Journal of Agricultural Research* **59**, 561-566.
14. ***Nichols PGH**, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* **104**, 10-23.
15. ***Nichols PGH**, Barbetti MJ, Sandral GA, Dear BS, de Koning CT, Lloyd DL, Evans PM, Craig AD, Si P, You MP (2007) Coolamon subterranean clover (*Trifolium subterraneum* L. *var. subterraneum* (Katz. et Morley) Zohary and Heller). *Australian Journal of Experimental Agriculture* **47**, 223-225.
16. ***Nichols PGH**, Sandral GA, Dear BS, de Koning CT, Lloyd DL, Evans PM, Craig AD, Barbetti MJ, Si P, You MP (2007) Izmir subterranean clover (*Trifolium subterraneum* L. *var. subterraneum* (Katz. et Morley) Zohary and Heller). *Australian Journal of Experimental Agriculture* **47**, 226-229.

Conference proceedings:

17. ***Nichols PGH**, Teakle NL, Bonython AL, Ballard RA, Charman N, Craig A (2012) Messina (*Melilotus siculus*) – a new annual pasture legume for Mediterranean-type climates with high tolerance of salinity and waterlogging. In '*Options Méditerranéennes Series A* No. 102 - New Approaches for Grassland Research in a Context of Climatic and Socio-Economic Changes', Proceedings of the 14th Meeting of the FAO-CIHEAM sub-network on Mediterranean pastures and fodder crops, Samsun, Turkey (Eds Z Acar, A López-Francos, C Porqueddu) pp. 155-160.

18. **Nichols PGH**, Ewing MA (2012) Mediterranean grasslands research - priorities and future challenges in the Australian context. In 'Proceedings of the 14th Meeting of the FAO-CIHEAM sub-network on Mediterranean pastures and fodder crops', Samsun, Turkey (Eds Z Acar, A López-Francos, C Porqueddu).

19. ***Nichols PGH**, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA , Ghamkhar K, Harris CA (2012) Temperate pasture legumes – past, present and future. In: Proceedings of the 16th Australian Nitrogen Fixation Conference, Manly, NSW, 24-27 June 2012.

20. ***Nichols PGH**, Teakle NL, Bonython AL, Ballard RA, Charman N, Craig AD (2012) Messina (*Melilotus siculus*) – a new salt and waterlogging tolerant annual pasture legume for southern Australia. In: Proceedings of the Australian Legume Symposium, Australian Grasslands Association Research Series no 1, Melbourne 2012 (Ed C Harris) pp. 73-76 (The Australian Grasslands Association: Tooborac)

21. ***Nichols PGH**, Dear BS, Hackney BF, Craig AD, de Koning CT and Evans PM (2010) Bindoon and Rosabrook - two new subterranean clovers with improved seedling resistance to redlegged earth mites. In: Proceedings of the Pastures Australia Update, Attwood, Australia, 23 March 2010.

22. ***Nichols PGH**, Dear BS, Hackney BF, Craig AD, de Koning CT and Evans PM (2010) Bindoon and Rosabrook - two new subterranean clovers with reduced cotyledon susceptibility to redlegged earth mites. In: Proceedings of the Pastures Australia Update, Wagga Wagga, Australia, 18 February 2010.

23. ***Nichols PGH**, Dear BS, Hackney BF, Craig AD, Evans PM, de Koning CT, Foster KJ, Barbetti MJ, You MP and Micic S (2009) New subterranean clovers with reduced cotyledon susceptibility to redlegged earth mites. In: Proceedings of the 14th Australasian Plant Breeding Conference and 11th Society for the Advancement of Plant Breeding in Asia and Oceania (SABRAO) Congress 2009, Cairns, 10-14 August 2009.

24. ***Nichols PGH**, Craig AD, Bonython AL, Rogers ME, Colmer TD, Ballard RA, Charman N McClements DR and Barrett-Lennard EG (2009) Development of *Melilotus siculus* – a new salt and waterlogging-tolerant annual pasture legume species. In: Proceedings of the 14th Australasian Plant Breeding Conference and 11th Society for the Advancement of Plant Breeding in Asia and Oceania (SABRAO) Congress 2009, Cairns, 10-14 August 2009.

25. ***Nichols P**, Craig A, Bonython A, Rogers M, Colmer T, Ballard R, Charman N and Barrett-Lennard E (2009) Development of *Melilotus siculus* – a new salt and waterlogging-tolerant annual fodder legume species for Mediterranean-type climates. In: 'Proceedings of the XVIIIth Meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section: Sustainable use of genetic diversity in forage crops and turf breeding', La Rochelle, France, 11-14 May, 2009

26. ***Nichols P**, Cocks P and Francis C (2009) Using bulk-hybrids for breeding adapted genotypes of subterranean clover. In: 'Proceedings of the XVIIIth Meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section: Sustainable use of genetic diversity in forage crops and turf breeding', La Rochelle, France, 11-14 May, 2009

27. ***Nichols P**, Loi A, Nutt B, Snowball R and Revell, C (2009) Domestication of new Mediterranean annual pasture legumes. In: 'Proceedings of the XVIIIth Meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section: Sustainable use of genetic diversity in forage crops and turf breeding', La Rochelle, France, 11-14 May, 2009

28. ***Nichols PGH**, Stockdale M, Malik AI, Colmer TD (2008) Salt tolerance in germinating seedlings of annual pasture legumes. In 'Proceedings of the 2nd International Salinity Forum: Salinity, Water and Society - Global Issues, Local Action', Adelaide, Australia. (Department of Agriculture, Fisheries and Forestry: Adelaide) Available at http://www.internationalsalinityforum.org/14_final.html [Verified 8 March 2009]

29. ***Nichols PGH**, Loi A, Nutt BJ, Snowball R, Revell CK (2008) Domestication of mediterranean annual pasture legumes for Australian farming systems. In 'Proceedings of the XXI International Grasslands Congress/International Rangelands Congress', Hohhot, China. (International Grassland Society: Hohhot)

30. ***Nichols PGH**, McClements DR, Albertsen TO (2008) Annual pasture legumes for increased productivity of saline soils in south-western Australia. In 'Proceedings of the XXI International Grasslands Congress/International Rangelands Congress', Hohhot, China. (International Grassland Society: Hohhot)

Technical reports and extension publications:

31. **Nichols PGH**, Yates RJ, Loo C, Stevens JC, Titterington JW, Wintle BJ, Moore GA, Dixon KW, Barrett-Lennard EG (2013) Direct seeding of chenopod shrubs for saltland and rangeland environments, Technical Report 10, Future Farm Industries CRC Perth, Western Australia (in press)

32. **Nichols PGH**, Yates RJ, Loo C, Wintle BJ, Titterington JW, Barrett-Lennard EG, Steven JC, Dixon KW, Moore GA (2012) Establishment of sub-tropical perennial grasses in south-western Australia, Technical Report 9, Future Farm Industries CRC, Perth, Western Australia. Available at: http://www.futurefarmonline.com.au/publications/Technical%20Reports/Technical_Reports

33. **Nichols PGH**, Yates RA, Barrett-Lennard EG (2011) Direct seeding of old man saltbush. Department of Agriculture and Food Western Australia, Farmnote No 485.

34. ***Nichols PGH** (2011) Narrikup – a vigorous high rainfall RLEM resistant subterranean clover. Department of Agriculture and Food Western Australia, Farmnote No 481.

35. ***Nichols PGH** (2010) Rosabrook – a subterranean clover with RLEM resistance for high rainfall pastures. Department of Agriculture and Food Western Australia, Farmnote No 454.

36. **Nichols PGH**, Yates R, Moore GA (2010) Establishing sub-tropical perennial grasses. Department of Agriculture and Food Western Australia, Farmnote No 443.

37. **Nichols PGH**, Moore GA (2010) Pasture rehabilitation after mining on the Fawcett property at Boddington. Report prepared for BHP Billiton Worsley Alumina Pty Ltd.

38. ***Nichols PGH**, Barrett-Lennard EG, Bennett SJ (2010) Pasture legumes and grasses for saltland. Department of Agriculture and Food Western Australia, Farmnote No 432.

39. ***Nichols PGH** (2010) Bindoon – a midseason subterranean clover with RLEM resistance. Department of Agriculture and Food Western Australia, Farmnote No 420.

40. ***Nichols PGH** (2010) Dalkeith subterranean clover. Department of Agriculture and Food Western Australia, Farmnote No 424.

41. ***Nichols P**, Bonython A (2009) Ecology and collection of seed and rhizobia from *Melilotus siculus* and other pasture legumes in salt marsh areas of southern Spain, Report on Project 110909, Estación Biológica de Doñana, Spain.

Ten career best publications (those related to this work are marked with an asterisk)

1. ***Nichols PGH**, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia – their history, current use and future prospects. *Crop and Pasture Science* **63**, 691–725.
2. ***Nichols PGH**, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* **104**, 10-23.
3. ***Nichols PGH**, Foster KJ, Piano E, Kaur P, Ghamkhar K, Pecetti L, Collins WJ (2013) Genetic improvement of subterranean clover (Trifolium subterraneum L.). 1. Germplasm, traits and future prospects. *Crop and Pasture Science* (submitted)
4. *Ghamkhar K, Isobe S, **Nichols PGH**, Faithfull T, Ryan MH, Snowball R, Sato S, Appels R (2012) The first genetic maps for subterranean clover (*Trifolium subterraneum* L.) and comparative genomics with T. pratense L. and *Medicago truncatula* Gaertn. to identify new molecular markers for breeding. *Molecular Breeding* **30**, 213–226.
5. ***Nichols PGH**, Malik AI, Stockdale M, Colmer TD (2009) Salt tolerance and avoidance mechanisms at germination of annual pasture legumes and their importance for adaptation to saline environments. *Plant and Soil* **315**, 241–255.
6. ***Nichols PGH**, Cocks PS, Francis CM (2009) Evolution over 16 years in a bulk-hybrid population of subterranean clover (Trifolium subterraneum L.) at two contrasting sites in south-western Australia. *Euphytica* **169,** 31-48.
7. Rogers ME, Craig AD, Munns R, Colmer TD, **Nichols PGH**, Malcolm CV, Brown AJ, Semple WS, Evans PM, Cowley K, Hughes SJ, Snowball RS, Bennett SJ, Sweeney GC, Dear BS, Ewing ME (2005) The development of fodder plants for the salt-affected areas of southern and eastern Australia: an overview. *Australian Journal of Experimental Agriculture* **45**, 301- 329.
8. ***Nichols PGH**, Snowball R, D'Antuono MF, Barbetti MJ (2010) Resistance to clover scorch disease (*Kabatiella caulivora*) among accessions of purple clover (*Trifolium purpureum*) and its relationship to the eco-geography of collection sites. *Crop and Pasture Science* **61,** 44-49.
9. *Barbetti MJ, Si P, **Nichols PGH** (2005) Genetic basis for and inheritance of resistance to Race 1 and Race 2 of *Kabatiella caulivora* in *Trifolium subterraneum* ssp. *subterraneum* and ssp. *yanninicum*. *Euphytica* **144**, 237-246.
10. ***Nichols PGH**, Craig AD, Rogers ME, Albertsen TO, Miller S, McClements DR, Hughes SJ, D'Antuono MF, Dear BS (2008) Production and persistence of annual legumes at five saline sites in southern Australia. *Australian Journal of Experimental Agriculture* **48**, 518-535.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
#Development of R & D pre-breeding plans for annual legumes (MLA)	P Nichols (20) C Revell (5) B Nutt (5) D Real (5) S Gherardi (5) W Erskine (5) G Sandral (5) M Gout (5) D Peck (5) J Howie (5) A Lake (5)	77,590	2012-13	2 months
Transforming saline landscapes through the development of messina and its rhizobia (FFI CRC)	P Nichols (25) A Craig (25) A Bonython (50) R Ballard (10) N Charman (40)	540,000	2011-14	On time
#Unincorporated joint venture to develop subterranean clover cultivars (Seed Force)	P Nichols (25)	Commercial in Confidence	2011-ongoing	On time
Reliable establishment of non traditional perennial pasture species (MLA, AWI, Land Water & Wool, Salinity CRC/ FFI CRC)	P Nichols (20) C Loo (100) R Yates (80) E Barrett-Lennard (15) G Moore (15) K Dixon (10) M Mitchell (10) D Kemp (15) G Lodge (5) M Ryan (5)	850,000	2006-10	On time
#Commercialisation of the first subterranean clovers with cotyledon resistance to redlegged earth mites (Pastures Australia)	P Nichols (40)	116,000	2007-08	On time

#Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Dr Clinton Revell

Qualifications: PhD (UWA), B.Sc. (Agriculture) (UWA)

Current institution: Department of Agriculture and Food Western Australia

Current position title: Manager (Pastures for livestock) since 2003, Executive Director (Research Delivery) Future Farm Industries CRC since 2013

Prior positions (10 years): Senior Research Officer (Pasture Science) since 1984

Contribution to this field

Dr Revell is a Senior Pasture Scientist with over 28 years experience focused on the agronomy and genetic improvement of pastures in southern Australia, with particular expertise on the contribution of legumes to mixed cereal and livestock farming systems. He has considerable experience in annual medic, serradella and sulla plant selection and agronomy and also has expertise on seed dormancy and germination requirements of annual legumes, the topic of his PhD studies. Dr Revell manages the Pastures for Livestock project at DAFWA. He is also currently Executive Director (Research Delivery) for the Future Farm Industries CRC (FFI CRC), following four years as a Program Leader with the FFI CRC. He is also an adjunct Associate Professor with CLIMA.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications:

1. ***Revell CK**, Ewing MA, Nutt BJ (2012) Breeding and farming system opportunities for pasture legumes facing increasing climate variability in the southwest of Western Australia *Crop and Pasture Science* **63**, 840–847.
2. ***Revell CK** Revell DK (2007) Meeting 'duty of care' obligations when developing new pasture species. *Field Crops Research* **104**, 95-102.

Conference Papers:

3. ***Revell** CK, Ewing MA, Real D, Nichols PGH and Sandral GA (2013) Commercialisation and impacts of pasture legumes in southern Australia – lessons learnt. In Proceedings of XXII International Grassland Congress September 2013, Sydney, Australia (accepted)

Ten career best publications (those related to this work are marked with an asterisk).

1. ***Revell CK**, Ewing MA, Nutt BJ (2012) Breeding and farming system opportunities for pasture legumes facing increasing climate variability in the southwest of Western Australia *Crop and Pasture Science* **63**, 840–847.
2. *Nichols PGH, **Revell CK**, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia—their history, current use, and future prospects. *Crop and Pasture Science* **63**, 691-725.
3. *Ghamkhar K, **Revell C,** Erskine W (2012) *Biserrula pelecinus* L. – genetic diversity in a promising pasture legume for the future. *Crop and Pasture Science* **63**, 833-839.
4. Bathgate A, **Revell CK,** Kingwell R (2009) Identifying the value of pasture improvement using whole farm modelling. *Agricultural Systems* **102**, 48–57.
5. ***Revell CK,** Revell DK (2007) Meeting 'duty of care' obligations when developing new pasture species. *Field Crops Research* **104**, 95-102.
6. *Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, **Revell CK**, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* **104**, 10-23.
7. *Taylor GB, **Revell CK** (2002) Seed softening, imbibition time and seedling establishment in yellow serradella. *Australian Journal Agricultural Research* **53,** 1011-18.
8. ***Revell CK**, Taylor GB, Cocks PS (1999) Effect of length of growing season on development of hard seeds in yellow serradella and their subsequent softening at various depths of burial. *Australian Journal Agricultural Research* **50,** 1211-23.
9. *Taylor GB, **Revell CK** (1999) The effect of pod burial, light and temperature on seed softening in yellow serradella. *Australian Journal Agricultural Research* **50,** 1203-9.
10. ***Revell CK**, Taylor GB, Cocks PS (1998) Long term softening of surface and buried hard seeds of yellow serradella grown in a range of environments. *Australian Journal Agricultural Research* **49**, 673 - 85.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
#Soft seeded sulla – a novel approach for lowering seed costs (RIRDC)	C. Revell (5) K Foster (30) R Yates (5)	187,000	2006-13	On time
#Drought tolerant perennial legumes (FFICRC)	C Revell (10) D Real (65) R Nair (40) R Snowball (20) A Albertsen (20) R Yates (10) A Humphries (5) M Ryan (10) G Sandral (25) S Clark (5)	595,000	2008-11	On time
Working with growers to overcome the constraints to adoption of the new annual legume pastures in the medium and low rainfall mixed farming zone of southern Australia (Pastures Australia)	C Revell (20) B Nutt (20) A Loi (80) J Howieson (10) D Ferris (30)	1,263,000	2007-10	On time
Improving weed management with biserrula in the pasture phase of WA cropping systems (GRDC)	C Revell (15)	356,058	2003-07	On time

#Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Dr Daniel Real

Qualifications: PhD (Massey University)
Current institution: Department of Agriculture and Food Western Australia
Current position title: Senior Plant breeder (Pasture legumes) since 2008
Prior positions (10 years): Senior Research Scientist (UWA)

Contribution to this field

Dr Real is a Senior Plant Breeder with DAFWA and the Future Farm Industries CRC and has 21 years experience in breeding both annual and perennial legumes for temperate, subtropical and Mediterranean climates. He has released 8 cultivars of 5 forage legume species and has published 30 peer-reviewed journal articles. Most recently he led a forage breeding program to develop cultivars of lotus (*Lotus corniculatus*) better adapted to Australian environments and is now leading an international program to domesticate the drought-tolerant perennial legume, tedera. Dr Real has considerable expertise on plant breeding methodology and biometrical analysis and the use of molecular markers for pasture legume breeding and selection.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications

1. ***Real, D**, Sandral GA, Rebuffo M, Hughes SJ, Kelman WM, Mieres JM, Dods K, Crossa J (2012) Breeding of an early-flowering and drought-tolerant *Lotus corniculatus* L. variety for the high-rainfall zone of southern Australia. *Crop and Pasture Science* **63**, 848-857.
2. ***Real D**, Sandral GA, Rebuffo M, Hughes SJ, Kelman WM, Mieres JM, Dods K, Crossa J (2012) Breeding of an early-flowering and drought-tolerant *Lotus*

corniculatus L. variety for the high-rainfall zone of southern Australia. *Crop and Pasture Science* **63**, 848-857.

3. ***Real D**, Li GD, Clark S, Albertsen TO, Hayes RC, Denton MD, D'Antuono MF Dear BS (2011) Evaluation of perennial forage legumes and herbs in six Mediterranean environments. *Chilean Journal of Agricultural Research* **71**, 357-369.
4. ***Real D**, Warden J, Sandral GA, Colmer TD (2008) Waterlogging tolerance and recovery of 10 *Lotus* species. *Australian Journal of Experimental Agriculture* **48**, 480-487.

Conference papers

5. ***Real D**, Verbyla AP (2010) Maximizing genetic gains using a "plant" model in the Tedera (*Bituminaria bituminosa* var. *albomarginata* and var. *crassiuscula*) breeding program in Australia. In 'Options Méditerranéennes'. (Eds C Porqueddu, S Ríos Ruiz) pp. 87-96. (CIHEAM: Alicante, Spain).
6. **Real D**, Albertsen T, Snowball R, Howieson J, Revell C, Ewing M, Correal E, Mendez P, Rios S (2008) *Bituminaria bituminosa* var. *albomarginata* (Lancelot trefoil), a novel perennial forage legume for low-rainfall Mediterranean environments in Western Australia. In: XXI International Grassland Congress and VIII International Rangeland Congress, Hohhot, China. Vol II - 452.
7. ***Real D**, Foster K, Correal E, Kidd D, Mendez P (2008) Techniques for artificial hybridization in the self-pollinated forage legume *Bituminaria bituminosa* and the cross-pollinated forage legume *Hedysarum coronarium*. In: XXI International Grassland Congress and VIII International Rangeland Congress, Hohhot, China. Vol II – 347.

Ten career best publications (those related to this work are marked with an asterisk).

1. ***Real, D**, Sandral GA, Rebuffo M, Hughes SJ, Kelman WM, Mieres JM, Dods K, Crossa J (2012) Breeding of an early-flowering and drought-tolerant *Lotus corniculatus* L. variety for the high-rainfall zone of southern Australia. *Crop and Pasture Science* **63**, 848-857.
2. *Pazos-Navarro M, Dabauza M, Correal E, Hanson K, Teakle N, **Real D**, Nelson M (2011) Next generation DNA sequencing technology delivers valuable genetic markers for the genomic orphan legume species, *Bituminaria bituminosa*. *BMC Genetics*, **12**:104.
3. ***Real D**, Dalla Rizza M, Reyno R, Quesenberry K (2007) Breeding system of the aerial flowers in an amphicarpic clover species: *Trifolium polymorphum*. *Crop Science* **47**, 1401-1406.
4. **Real D**, Lamandera CA, Howieson JG (2005) Performance of temperate and subtropical forage legumes when over-seeding native pastures in the basaltic region of Uruguay. (Special issue: Application of rhizobial inoculants to Australian agriculture.). *Australian Journal of Experimental Agriculture* **45**, 279-287.
5. ***Real D**, Altier N (2005) Breeding for disease resistance, forage, and seed production in *Lotononis bainesii* Baker. *New Zealand Journal of Agricultural Research* **48**, 93-100.
6. ***Real D**, Dalla Rizza M, Quesenberry KH, Echenique M (2004) Reproductive and molecular evidence for allogamy in *Lotononis bainesii* Baker. *Crop Science* **44**, 394-400.
7. *Dalla Rizza M, **Real D**, Quesenberry KH, Albertini E (2004) Plant reproductive system determination under field conditions based on co-dominant markers. *Journal of Genetics and Breeding* **1**, 47-57.

8. **Real D**, Gordon IL, Hodgson J (2001) Statistical modelling of grazing preference of sheep when presented with a range of plant types. *Journal of Agricultural Science*, **136**, 111-117.

9. ***Real D**, Gordon IL, Hodgson J (2000) Genetic advance estimates for red clover (Trifolium pratense) grown under spaced plant and sward conditions. *Journal of Agricultural Science* **135**, 11-17.

10. ***Real D**, Gordon IL, (1999) Standard errors of heritabilities for forage breeding nurseries. *Biometrics* **55**, 891-895.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
Tedera seed increase (MLA)	D Real (15) C Revell (5)	120,000	2011-13	On time
#Tedera breeding (FFI CRC)	D Real (70) M Nelson (10) J Croser (10) M Castello (80) G Smith (10) A Verbyla (5)	699,000	2011-14	On time
Tedera seed technology (RIRDC)	D Real (15) C Revell (10) R Snowball (10) M Ryan (10)	247,000	2009-14	On time
#Drought tolerant perennial legumes (FFICRC)	D Real (65) C Revell (10) R Nair (40) R Snowball (20) A Albertsen (20) R Yates (10) A Humphries (5) M Ryan (10) G Sandral (25) S Clark (5)	595,000	2008-11	On time
Lotus commercialisation and evaluation (FFI CRC)	D Real G Sandral S Hughes	240,000	2007- 11	On time
#Developing new and innovative perennial lotus species for grazing systems of southern Australia (AWI)	D Real G Sandral	1,200,000	2002-07	On time
Developing innovative seed production technologies to ensure the commercial success of new perennial lotus species targeted at reducing recharge in the mid-catchment regions of Australia (RIRDC)	D Real G Sandral	586,258	2002-07	On time

#Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Mr Richard Snowball

Qualifications: B.Sci.Agric. (University of Western Australia)
Current institution: Department of Agriculture and Food Western Australia
Current position title: Research officer (Pasture science) since 2013

Contribution to this field

Mr Snowball has 29 years experience as a pasture scientist with DAFWA. He has been curator of the Australian Trifolium Genetic Resource Centre since 1995, which has involved managing germplasm acquisition and collection, agro-morphological characterisation, conservation and distribution. Since 1998 he has contributed directly to the collection of new germplasm of pasture legumes from countries including the Aegean Islands of Greece, central Italy and Sicily, the Azores, Morocco, south-eastern Spain, Canary Islands, Eritrea, and Israel. I have managed the growing and characterisation of these collections predominantly for use in Australian plant improvement programs.

Between 2003 and 2007 he supervised a GRDC-funded project, managed through CLIMA, aimed at developing a Core collection of bladder clover (*Trifolium spumosum*). He was a major participant in a subsequent ACC Linkage project from 2007-2010 that resulted in development of a Core Collection of subterranean clover *Trifolium subterraneum* in an ARC Linkage project. In both projects he was responsible for both plant morphological and eco-geographic data from original collecting sites, which was used, together with molecular data, to develop the Core Collections. These genetically diverse collections will be utilised in this proposed project and in future plant breeding activities.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal and conference publications

1. ***Snowball R**, Mahdere A, Tesfay E, Aberra M, Carr RM, D' Antuono MF (2013) Exploring the wider potential of forage legumes collected from the highlands of Eritrea. *Plant Genetic Resources* (in press) DOI: http://dx.doi.org/10.1017/S1479262112000494
2. ***Snowball R**, D'Antuono M, Cohen B, Gajda K, Bennett R (2010). The value of germplasm nurseries in selecting species for field evaluation. *Crop and Pasture Science* **61**, 957-969.
3. **Snowball R**, Albertsen T, Gajda K, Real D, Yates R, Cohen B (2008) Preliminary evaluation of *Lespedeza juncea* var. *sericea* in south-western Australia. Proceedings XXI International Grasslands Congress, Hohhot China.
4. ***Snowball R**, Hadas R, Galili S, Ur Y, Nichols P, Kigel J (2008) Collecting pasture legumes in Israel with a focus on species of importance to southern Australia. *Plant Genetic Resources Newsletter* **155**, 15-24.

Ten career best publications (those related to this work are marked with an asterisk).

1. ***Snowball R**, Mahdere A, Tesfay E, Aberra M, Carr RM, D' Antuono MF (2013) Exploring the wider potential of forage legumes collected from the highlands of Eritrea. *Plant Genetic Resources* (in press) DOI: http://dx.doi.org/10.1017/S1479262112000494
2. ***Snowball R**, D'Antuono M, Cohen B, Gajda K, Bennett R (2010). The value of germplasm nurseries in selecting species for field evaluation. *Crop and Pasture Science* **61**, 957-969.
3. *Rogers, ME, Colmer TD, Frost K, Henry D, Cornwall D, Hulm E, Hughes S, **Snowball R**, Nichols PGH, Craig AD (2010) The influence of NaCl salinity and hypoxia on aspects of growth in *Trifolium* species. *Crop and Pasture Science* **61**, 1049-1050.
4. *Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, **Snowball R**, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* **104**, 10-23.
5. *Nichols PGH, **Snowball R**, D'Antuono MF, Barbetti MJ (2009) Resistance to clover scorch disease (*Kabatiella caulivora*) among accessions of purple clover (*Trifolium purpureum*) and its relationship to the eco-geography of collection sites. *Crop and Pasture Science* **61**, 44-49.

6. *Hughes SJ, **Snowball R**, Reed KFM, Cohen B, Gajda K, Williams AR, Groeneweg SL (2008) The systematic collection and characterisation of herbaceous forage species for recharge and discharge environments in southern Australia. *Australian Journal of Experimental Agriculture* **48** 397–408.
7. *Ghamkhar K, Isobe S, Nichols PGH, Faithfull T, Ryan MH, **Snowball R**, Sato S, Appels R (2012) The first genetic maps for subterranean clover (*Trifolium subterraneum* L.) and comparative genomics with T. pratense L. and *Medicago truncatula* Gaertn. to identify new molecular markers for breeding. *Molecular Breeding* **30**, 213–226.
8. ***Snowball R**, Hadas R, Galili S, Ur Y, Nichols P, Kigel J (2008) Collecting pasture legumes in Israel with a focus on species of importance to southern Australia. *Plant Genetic Resources Newsletter* **155**, 15-24.
9. *Ghamkhar K, **Snowball R**, Wintle BJ, Brown AHD (2008) Strategies for developing a core collection of bladder clover (*Trifolium spumosum* L.) using ecological and agro-morphological data. *Australian Journal of Agricultural Research* **59**, 1103-1112.
10. *Ghamkhar K, **Snowball R**, Bennett SJ (2007) Ecogeographical studies reveal genetic diversity and the gaps in the germplasm collection of bladder clover (*Trifolium spumosum* L.). *Australian Journal of Agricultural Research* **58**, 728-38.

Professor William Erskine

Qualifications:	Ph.D (Cambridge) 1979
Current institution:	The University of Western Australia (since 2008)
Current position title:	Director of Centre for Legumes in Mediterranean Agriculture, Director of International Centre for Plant Breeding Education and Research (ICPBER)
Prior positions (10 years):	Assistant Director General (Research) - International Center for Agricultural Research in the Dry Areas (ICARDA), Syria (2000 – 2007)

Contribution to this field

Professor Erskine is Director of the Centre for Legumes in Mediterranean Agriculture (CLIMA) and Director of the International Centre for Plant Breeding Education and Research (ICPBER) at UWA. He has over 35 years experience in legume pre-breeding and leads a team of legume scientists with skills in molecular marker technology, tissue culture, wide-crossing, embryo rescue, selection for herbicide tolerance, development of core collections, legume transformation and bio-informatics. Prior to his appointment to CLIMA, Professor Erskine worked for 26 years at the International Centre for Agricultural Research in the Dry Areas (ICARDA) in Syria, initially as a Lentil Breeder, then Leader of the Germplasm Improvement Program and from 2000-2007 as Assistant Director General of Research. During his tenure as a Legume Breeder at ICARDA, 68 cultivars of lentil were released in a range of countries.

Since 1995 Professor Erskine has been involved in legume mapping and genomics. This was initially with lentil (see publications below) and more recently with subterranean clover, in his role as leader of the ARC Linkage project *Exploiting subterranean clover genetic variation for methane mitigation and ruminant health challenges to the Australian livestock industries*. He has published 193 articles in refereed journals and edited book chapters, 99 conference papers and miscellaneous scientific publications and edited 3 books on legume breeding and pre-breeding.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications and books

1. *__Erskine__ W, Sarker A, Ashraf M (2011) Re-constructing an ancient bottleneck of the movement of the lentil (*Lens culinaris* ssp. *culinaris*) into South Asia. *Genetic Resources and Crop Evolution* **58**, 373-381.
2. **Erskine** W, Sarker A, Kumar S (2011) Crops that feed the World 3. Investing in lentil improvement toward a food secure world. *Food Security* **3**, 127-139.
3. **Erskine** W, Muehlbauer FJ, Sarker A, Sharma B (eds.) (2009) The Lentil: Botany, Production and Uses. CABI, Wallingford, UK. 468 pp. ISBN 9781845934873.
4. **Erskine** W, Nesbitt H (2009) How can agriculture research make a difference in countries emerging from conflict? *Experimental Agriculture* **45**, 313-321.

Ten career best publications (those related to this work are marked with an asterisk).

1. Summerfield RJ, Roberts EH, **Erskine W**, Ellis RH (1985) Effects of temperature and photoperiod on flowering in lentils (*Lens culinaris* Medic.). *Annals of Botany* **56,** 659-671.
2. Ahmed S, Akem C, Bayaa B, **Erskine W** (2002) Integrating host resistance with planting date and fungicide seed treatment to manage *Fusarium* wilt and so increase yields. *International Journal of Pest Management* **48,** 121-125.
3. Sarker A, **Erskine W**, Singh M (2003) Regression models for lentil seed and straw yields in the Near East. *Agricultural and Forest Meteorology* **116,** 61-72.
4. *__Erskine W__, Muehlbauer FJ (1991) Allozyme and morphological variability, out crossing rate and core collection formation in lentil germplasm. *Theoretical and Applied Genetics* **83,** 119-125.
5. **Erskine W**, Hussain A, Tahir M, Baksh A, Ellis RH, Summerfield RJ, Roberts EH (1994) Field evaluation of a model of photothermal flowering responses in a world lentil collection. *Theoretical and Applied Genetics* **88,** 423-428.
6. *Eujayl I, Baum M, Powell W, **Erskine W**, Pehu E (1998) A genetic linkage map of *Lens* species based on RAPD and AFLP markers using recombinant inbred lines. *Theoretical and Applied Genetics* **97,** 83-89.
7. Keatinge JDH, Aiming Q, Küsmenoglu I, Ellis RH, Summerfield RJ, **Erskine W**, Beniwal SPS (1995) Defining critical weather events to predict the phenological development of lentil adapted to winter-sowing in the highlands of West Asia. *Agricultural and Forest Meteorology* **74,** 251-263.
8. Keatinge JDH, Aiming Q, Küsmenoglu I, Ellis RH, Summerfield RJ, **Erskine W**, Beniwal SPS (1996) Using genotypic variation in flowering responses to temperature and photoperiod to select lentil for the West Asian Highlands. *Journal of Agricultural and Forest Meteorology* **78,** 53-65.
9. Eujayl I, **Erskine W**, Bayaa B, Baum M, Pehu E (1998) Fusarium vascular wilt in lentil: Inheritance and identification of DNA markers for resistance. *Plant Breeding* **117,** 497-499.
10. *Eujayl I, **Erskine W**, Baum M, Pehu E (1999) Inheritance and linkage analysis of frost injury in a lentil population of recombinant inbred lines. *Crop Science* **39,** 639-642.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE)	$	Years	Reports delivery[$]
[#] Exploiting subterranean clover genetic variation for methane mitigation & ruminant health challenges to the Australian livestock industries (ARC Linkage)	W Erskine (10) P Kaur (50) P Nichols (10) C Revell (5) R Appels (10) K Ghamkhar (5) S Isobe (10)	310,000	2011-15	On time
[#] Biotechnology tools to accelerate lupin and lentil improvement (GRDC)	W Erskine (10) J Croser (40)	540,000	2010-13	On time
Improved herbicide tolerance for break crops (GRDC)	W Erskine (15) P Si (100)	602,995	2008-11	On time
Generation of genetically-modified herbicide tolerant narrow-leaf lupin (GRDC)	W Erskine (15)	2,797,169	2009-14	On time
Seeds of Life (SOL3), East Timor (ACIAR/AusAID)	W Erskine (5)	25,000,478	2011-16	On time
Introduction of short duration pulses into rice-based cropping systems in western Bangladesh (ACIAR)	W Erskine (15)	1,987,013	2011-16	On time

[#]Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); [$]Delivered on time or months late

Dr Parwinder Kaur

Qualifications:	Ph.D in Molecular Biology and Plant Pathology (University of Western Australia)
Current institution:	The Centre for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia
Current position title:	Research Associate
Prior positions (10 years):	PhD student (UWA) 2007 - 2011 Senior Research Fellow (Punjab Agricultural University) 2006

Contribution to this field

Dr Kaur has expertise in molecular biology through her PhD studies at UWA. Since 2011 Dr Kaur has been the research scientist on the ARC-linkage project *Exploiting subterranean clover genetic variation for methane mitigation & ruminant health challenges to the Australian livestock industries*. During this time Dr Kaur has strengthened the collaborative relationship between CLIMA and KDRI and has produced a genome scaffold and SNP discovery to generate a very high resolution QTL map of subterranean clover. This is in readiness to sequence the whole subterranean clover genome and develop a core collection HapMap to identify molecular markers associated with important traits.

In her relatively short career Dr Kaur's skills and outputs have been widely acclaimed. She is widely published and was the winner of the 2013 Science and Innovation Award for Young People in Agriculture, Fisheries and Forestry, sponsored by MLA and DAFF.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications and book chapters:

1. **Kaur P** (2013) Agronomic challenges from novel pathotypes of *Albugo candida* to the emerging *Brassica juncea* industry in Western Australia. *Phytopathologia mediterranea* (accepted)

2. **Kaur P**, Li H, Sivasithamparam K, Barbetti MJ (2012) Pre-Inoculation with *Hyaloperanospora parasitica* reduces incubation time and increases severity of disease caused by *Albugo candida* in a *Brassica juncea* variety resistant to Downy mildew. *Journal of General Plant Pathology* **77**, 101-106.

3. **Kaur P**, Jost R, Barbetti MJ, Sivasithamparam K (2011) Proteome analysis of *Albugo candida-Brassica juncea* pathosystem reveals how timing of the expression of the defence related genes plays a crucial role in pathogenesis. *Journal of Experimental Botany* **62**, 1285-1298.

4. **Kaur P**, Barbetti MJ, Sivasithamparam K (2011) Host range and phylogenetic relationships of *Albugo candida* from cruciferous hosts in Western Australia, with special reference to *Brassica juncea*. *Plant Disease* **95**, 712-718.

5. **Kaur P**, Barbetti MJ, Sivasithamparam K (2011) Site of inoculation and stage of plant development determine symptom type and expression in *Brassica juncea* following infection with *Albugo candida*. *Journal of Plant Pathology* **93**, 383-388.

6. **Kaur P**, Barbetti MJ, Sivasithamparam K (2008) Pathogenic behaviour of strains of *Albugo candida* from *Brassica juncea* and *Raphanus raphanistrum* (Wild Radish) in Western Australia. *Australasian Plant Pathology* **37**, 353-356.

7. **Kaur P**, Li CX, Barbetti MJ, You MP, Li H, Sivasithamparam K (2008) First Report of Powdery Mildew Caused by *Erysiphe cruciferarum* Opiz ex Junnel, on *Brassica juncea* (L.) Czern & Coss in Australia. *Plant Disease* **92**, 650.

8. **Kaur P**, Maninder S (2008) Biology of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on *Parthenium hysterophorus* (L.) in Punjab. *Journal of Insect Science* **21**, 139-145.

9. **Kaur P**, Maninder S (2008) Feeding potential of *Zygogramma bicolorata* (Coleoptera: *Chrysomelidae*) on *Parthenium hysterophorus* (Linnaeus). *Journal of Insect Science* **21**, 403-404.

10. **Kaur P**, Maninder S (2006) Seasonal abundance of *Zygogramma bicolorata* (Coleoptera: *Chrysomelidae*) on *Parthenium hysterophorus* (Linnaeus) in Punjab. *Journal of Insect Science* **19**, 129-133.

11. **Kaur P**, Maninder S (2006) New Report of Maconellicoccus hirsutus G. (Homoptera: *Pseudococcidae*) on *Parthenium hysterophorus* (L.) in Punjab. Journal *of Insect Science* **19**, 140-141.

12. **Kaur P**, Maninder S (2006) Record of *Zygogramma bicolorata* Pallister (Coleoptera: *Chrysomelidae*) on plants other than *Parthenium hysterophorus* (L.) in Punjab. *Journal of Insect Science* **19**, 142-144.

Conference papers

13. *Kaur P, Erskine W, Appels R, Durmic Z, Nichols P, Isobe S (2013) Comparative genomics in pasture species – a powerful new approach to assist in methane mitigation strategies for Australian livestock industries. Proceedings of Plant and Animal Genome XXI conference, San-Diego, USA
14. *Kaur P, Appels R, Banik B, Durmic Z, Ghamkhar K, Isobe S, Nichols P, Erskine (2012) Methanogenic potential in the rumen and quantitative trait locus analysis of subterranean clover. In Proceedings of the Molecular Breeding of Forage & Turf Meeting, Salt Lake City, USA
15. **Kaur P**, Barbetti MJ, Sivasithamparam K (2010) Pathogen vs plant – How the war is won or lost. Proceedings: Combined Biological Sciences Meeting, Perth
16. **Kaur P**, Barbetti MJ, Sivasithamparam K (2010) Host-pathogen interactions in the Mustard-White rust pathosystem: Protein expression profiling. Proceedings of the Australia Pathology Society Annual Meeting, Phytopathology 100:S60
17. **Kaur P**, Barbetti MJ, Sivasithamparam K (2009) Racial status of *Albugo candida* in Australia and India. Proceedings: 5th International Congress on "Plant Pathology in the Globalized Era"
18. **Kaur P**, Barbetti MJ, Sivasithamparam K (2008) Response of *Brassica juncea* genotypes to races of *Albugo candida* in Western Australia. Proceedings of the 9th International Congress of Plant Pathology (ICPP 2008)

Ten career best publications (those related to this work are marked with an asterisk).

1. **Kaur P**, Jost R, Barbetti MJ, Sivasithamparam K (2011) Proteome analysis of *Albugo candida-Brassica juncea* pathosystem reveals how timing of the expression of the defence related genes plays a crucial role in pathogenesis. *Journal of Experimental Botany* **62**, 1285-1298.
2. ***Kaur P**, Erskine W, Appels R, Durmic Z, Nichols P, Isobe S (2013) Comparative genomics in pasture species – A powerful new approach to assist in methane mitigation strategies for Australian livestock industries. *Plant and Animal Genome XXI*, San-Diego, CA, USA
3. ***Kaur P**, Appels R, Banik B, Durmic Z, Ghamkhar K, Isobe S, Nichols P, Erskine W (2012) Methanogenic potential in the rumen and quantitative trait locus analysis of subterranean clover. *Molecular Breeding of Forage & Turf Meeting*, Salt Lake City, UT, USA
4. **Kaur P**, Barbetti MJ, Sivasithamparam K (2011) Host range and phylogenetic relationships of *Albugo candida* from cruciferous hosts in Western Australia, with special reference to *Brassica juncea*. *Plant Disease* **95**, 712-718.
5. **Kaur P**, Li H, Sivasithamparam K, Barbetti MJ (2012) Pre-Inoculation with *Hyaloperanospora parasitica* reduces incubation time and increases severity of disease caused by *Albugo candida* in a *Brassica juncea* variety resistant to Downy mildew. *Journal of General Plant Pathology* **77**, 101-106.
6. **Kaur P**, Barbetti MJ, Sivasithamparam K (2011) Site of inoculation and stage of plant development determine symptom type and expression in *Brassica juncea* following infection with *Albugo candida*. *Journal of Plant Pathology* **93**, 383-388.
7. Virk JS, Kaur R, **Kaur P** (2009) Performance of Eri silkworm, *Samia cynthia* ricini Boisduval in different seasons of Punjab. *Indian Journal of Sericulture* **48**, 78-80

8. **Kaur P**, Barbetti MJ, Sivasithamparam K (2008) Pathogenic behaviour of strains of *Albugo candida* from *Brassica juncea* and *Raphanus raphanistrum* (Wild Radish) in Western Australia. *Australasian Plant Pathology* **37**, 353-356.

9. **Kaur P**, Li CX, Barbetti MJ, You MP, Li H, Sivasithamparam K (2008) First Report of Powdery Mildew Caused by *Erysiphe cruciferarum* Opiz ex Junnel*,* on *Brassica juncea* (L.) Czern & Coss in Australia. *Plant Disease* **92**, 650.

10. **Kaur P**, Maninder S (2008) Biology of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on *Parthenium hysterophorus* (L.) in Punjab. *Journal of Insect Science* **21**, 139-145.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE)	$	Years	Reports delivery[$]
[#] Exploiting subterranean clover genetic variation for methane mitigation & ruminant health challenges to the Australian livestock industries (ARC Linkage)	P Kaur (50) W Erskine (10) P Nichols (10) C Revell (5) R Appels (10) K Ghamkhar (5) S Isobe (10)	310,000	2011-15	On time
Pathogenic behaviour of *Albugo candida* on *Brassica juncea* and mechanisms of host resistance (PhD project)	P Kaur (100)	International Postgraduate Research Fellowship	2007-11	On time

[#]Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); [$]Delivered on time or months late.

Dr Janine Croser

Qualifications: Ph.D in cell biology (The University of Melbourne)

Current institution: The Centre for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia

Current position title: Research Assistant Professor

Prior positions (10 years): Research Fellow (UWA)

Contribution to this field

Dr Croser has been at the forefront of developing *in vitro* technologies for increasing the rate of cultivar development in legume improvement. She has worked predominantly on doubled haploidy (DH) in chickpea, field pea, lentil and lupin. She was a co-author of the first chickpea DH protocol and has co-authored papers on fundamental pea DH work. In the last 3 years Dr Croser has pioneered the use of *in vitro* techniques for accelerating generation turnover in lupin and lentil, resulting in technologies that enable up to 6 generations per year to be obtained. She has undertaken a CLIMA and DAFWA funded pilot project to test this technology in pasture legumes tedera, subterranean clover and French serradella. This has led to her involvement as a chief investigator on the Tedera breeding project with Dr Daniel Real and to the current MLA proposal for undertaking rapid generation turnover research in subterranean clover. Dr Croser maintains close linkages with legume *in vitro* research groups at INRA France, CDC Canada and IMIDA, Spain.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications and reports:

1. **Croser JS**, Lulsdorf MM, Grewal RK, Usher KM, Siddique KHM (2011) Isolated microspore culture of chickpea (*Cicer arietinum* L.): induction of androgensis and cytological analysis of early haploid divisions. *In Vitro Cellular and Developmental Biology Plants* **47**, 357-368.
2. ***Croser J**, Siddique KHM, Kuo J, Khan T (2010) ARC Linkage project 0562111 Final Report: Accelerating the genetic improvement of grain legumes by developing doubled haploid technology for field pea and chickpea. Australian Research Council.

Ten career best publications (those related to this work are marked with an asterisk).

1. **Croser JS**, Lulsdorf MM, Davies PJ, Clarke HJ, Bayliss KL, Mallikarjuna N, Siddique KHM (2006) Toward doubled haploid production in the Fabaceae: Progress, constraints and opportunities. *Critical Reviews in Plant Sciences* **25,** 139-159.
2. **Croser JS**, Clarke HJ, Siddique KHM, Khan TN (2003) Low temperature stress: Implications for chickpea (*Cicer arietinum* L.) improvement. *Critical Reviews in Plant Sciences* **22,** 185-219.
3. **Croser JS**, Ahmad F, Clarke HJ, Siddique KHM (2003) Utilisation of wild *Cicer* in chickpea improvement – progress, constraints and prospects. *Australian Journal of Agricultural Research* **54**, 429-444.
4. *Ribalta FM, **Croser JS**, Erskine W, Finnegan P, Ochatt SJ (2013) The antigibberellin Flurprimidol permits *in vitro* flowering and seed-set across a range of pea (*Pisum sativum* L.) genotypes by reducing internode length. *Biologia Plantarum* (in press).
5. *Ghamhkar K, **Croser J**, Aryamanesh N, Campbell MC, Kon'kova N, Francis CM (2010) Camelina (*Camelina sativa* (L.) Crantz) as an alternative oilseed: molecular and ecogeographic analyses. *Genome* **53**, 558-567.
6. *Ribalta FM, Croser JS, **Ochatt SJ** (2012) Flow cytometry enables identification of sporophytic eliciting stress treatments in gametic cells. *Journal of Plant Physiology* **169**, 104-110.
7. Lulsdorf MM, **Croser JS**, Ochatt SJ (2011) Androgenesis and Doubled Haploid Production in Food Legumes. In: A. Pratap (Ed.) Biology and Breeding of Food Legumes. CABI International, UK. pp. 159-177.
8. **Croser JS**, Lulsdorf MM, Grewal RK, Usher KM, Siddique KHM (2011) Isolated microspore culture of chickpea (*Cicer arietinum* L.): induction of androgensis and cytological analysis of early haploid divisions. *In Vitro Cellular and Developmental Biology Plants* **47**, 357-368.
9. Grewal R, Lulsdorf M, **Croser J**, Ochatt S, Vandenberg A, Warkentin T (2009) Doubled-haploid production in chickpea (*Cicer arietinum* L.): role of stress treatments. *Plant Cell Reporter* **28**, 1289-1299.
10. Khan TN, **Croser JS** (2004) Pea/ Overview. In 'Encyclopedia of Grain Science'. (Wrigley C, Corke H, Walker C, Eds.) Elsevier Science Ltd. London, pp. 418-427.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator Name (FTE)	$	Years	Reports delivery[$]
[#]*In vitro* tools for accelerated breeding and screening for abiotic stress in grain legumes (GRDC)	J Croser (30) W Erskine (10)	750,000	2013-16	Project beginning June 30
[#]Biotechnology tools to accelerate lupin and lentil improvement (GRDC)	J Croser (40)	450,000	2010-13	On Time
[#]Breeding new cultivars of the perennial forage legume tedera (FFI CRC)	J Croser (10) D Real (70) M Nelson (10) M Castello (80) G Smith (10) A Verbyla (5)	699,000	2011-14	Project Underway
Accelerating the genetic improvement of grain legumes for Australia by developing doubled haploid technology for field pea and chickpea (ARC Linkage)	J Croser (80) K Siddique (10) J Kuo (10) T Khan (10)	950,000	2006-10	On Time

[#]Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); [$]Delivered on time or months late.

Dr Klaus Oldach

Qualifications: Ph.D
Current institution: South Australian Research and Development Institute (SARDI)
Current position title: Science Program Leader, Crop Improvement since 2011
Group Leader, Gene Function since 2006
Prior positions (10 years): ARC Postdoctoral Fellow at the Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide 2003-2006

Contribution to this field

Dr Oldach is leader of the Gene Function group and the Crop Improvement program in SARDI and assures that the latest technologies are made available to pre-breeders and breeders. With 17 years of experience in molecular research in crop plants, his research aims to complement and extend classical crop research activities, such as pathology, physiology, biochemistry and agronomy through collaborations. For example, breeding programs that historically hadn't have access to using molecular tools (oats, pasture legumes) can now use these facilitating techniques and benefit from the information that molecular biology contributes to conventional approaches.
Regarding legumes, Dr Oldach has developed molecular markers for a range of traits and species, including:

- diagnostic markers for herbicide tolerances in annual medics, lucerne, lentils and faba beans;
- diagnostic markers for Boron tolerance in *Medicago truncatula*; and
- closely linked markers for tolerance to root lesion nematodes in *M. littoralis*.

Besides the genetic analysis of agronomically relevant traits in crops, Dr Oldach uses mutagenesis techniques to extend the genetic diversity of crop species (wheat, barley, oats, pasture legumes and grain legumes) to generate genetic resources that serve the pre-breeding and breeding community.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications and book chapters:

1. ***Oldach KH** (2011) Mutagenesis in Food Legumes (Ed.) A. Pratap and J. Kumar, Biology and Breeding of Food Legumes. CABI (Oxfordshire, UK), ISBN 978-1-84593-766-9, 418 pages.
2. ***Oldach KH**, Peck DM, Cheong J, Williams KJ, Nair RM (2008) Identification of a chemically induced point mutation mediating herbicide tolerance in annual medics (*Medicago* spp.) *Annals of Botany* **101**, 997-1005.

Ten career best publications (those related to this work are marked with an asterisk).

1. ***Oldach KH**, Peck DM, Cheong J, Williams KJ, Nair RM (2008) Identification of a chemically induced point mutation mediating herbicide tolerance in annual medics (*Medicago* spp.) *Annals of Botany* **101**, 997-1005.
2. *Bogacki P, Peck DM, Nair RM, Howie J, **Oldach KH** (2013) Genetic analysis of tolerance to Boron toxicity in the legume *Medicago truncatula*. *BMC Plant Biology* **2013**, 13-54 http://www.biomedcentral.com/1471-2229/13/54
3. ***Oldach KH** (2011) Mutagenesis in Food Legumes (Ed.) A. Pratap and J. Kumar, Biology and Breeding of Food Legumes. CABI (Oxfordshire, UK), ISBN 978-1-84593-766-9, 418 pages.
4. *****Genc Y, Oldach **K**, Gogel B, Wallwork H, McDonald GK, Smith AB (2013) Quantitative trait loci for agronomic and physiological traits for a bread wheat population grown in environments with a range of salinity levels. *Molecular Breeding* (in press).
5. *Trethowan RM, Mahmood T, Ali Z, **Oldach K**, Garcia AG (2012) Genetic control of wheat adaptation to conservation agriculture. *Field Crops Research* **132**, 76–83.
6. Francki MG, Crawford AC, **Oldach K** (2011) Transcriptomics, Proteomics and Metabolomics, Integration of Latest Technologies for Improving Wheat Productivity in Future (Ed.) N. Benkeblia, Advances in Agro-Ecology. CRC Press (FL, USA), ISBN 978-1-4398-2504-4, 533 pages.
7. Genc Y, **Oldach K**, Verbyla A, Lott G, Hassan M, Tester M, Wallwork H, McDonald G (2010) Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. *Theoretical and Applied Genetics* **121**, 877-894.
8. *Humphries AW, Peck DM, Robinson SS, Rowe T, **Oldach K** (2012) A new biotype of bluegreen aphid (*Acyrthosiphon kondoi* Shinji) found in south-eastern Australia overcomes resistance in a broad range of pasture legumes. *Crop and Pasture Science* **63**, 893-901.
9. *Mahmood H, **Oldach K**, Baumann U, Langridge P, Sutton T (2010).Genes mapping to boron tolerance QTL in barley identified by suppression subtractive hybridization. *Plant, Cell and Environment* **33**, 188–198.
10. Linsell KJ, Davies K, Riley I, Wallwork H, Oldach K**.** (2009) Biological and genetic characterisation of resistance to root lesion nematode *Pratylenchus* spp. in wheat. *Journal of Nematology* **41**, 349

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
#Map-based cloning of LMA tolerance genes in wheat (GRDC)	Oldach 10% Mares 50%	$276,000	2011-15	
#Development of a cost-effective molecular selection tool for high beta-glucan content in oat grain (SAGIT).	Oldach 15%	$237,000	2012-15	
Epigenetic control of stress tolerance in wheat (SAGIT).	Oldach 10% Sadras 10%	$299,000	2011-14	
##Improving weed management option in pulses (mutagenesis and marker development in faba beans and lentils to generate new herbicide tolerances) (GRDC)	Oldach 5% McMurray 10%	$300,000	2010-13	On time
##Genetic analysis of tolerance to *Pratylenchus neglectus* in pasture legumes (RIRDC)	Oldach 10%	$234,000	2011-13	On time
#Development of molecular markers in oat (*Avena sativa*) (RIRDC)	Oldach 10%	$170,000	2011-13	On time
Identification of cereal cultivars that can suppress *Rhizoctonia solani* as basis for the development of molecular markers (SAGIT)	Oldach 10%	$60,000	2010-12	On time
#Development of molecular markers for CCN resistance in oats (SAGIT/GRDC)	Oldach 10% FTE Zwer 5% FTE	$245,000	2008-12	On time
#Map-based cloning of the scald resistance gene Rrs1 in barley (GRDC)	Oldach 15%	$400,000	2009-12	On time
##Molecular characterisation of Boron toxicity tolerance in *Medicago* spp. (RIRDC/Seedmark)	Oldach 10% Peck 5%	$159,000	2009-11	On time
Brassica Development for New Markets and Climate Change (DFEEST)	Oldach 10%	$262,000	2008-11	On time
#Genetic and physiological characterisation of resistance to root lesion nematodes in wheat	Oldach 10% Linsell (PhD thesis)	$105,000	2009-11	On time
#Fine mapping of rust resistance, yield and maturity loci in wheat (GRDC)	Oldach 15%	$207,000	2008-10	On time
#Molecular analysis of defence responses in barley (MPBCRC/SAGIT)	Oldach 10%	$276,000	2007-09	On time
#Molecular marker – trait linkage analysis in wheat and barley (Molecular Plant Breeding CRC)	Oldach 30%	$328,000	2006 - 2008	On time

#Projects that share the molecular approaches that will also be used in this project; ##Projects where these molecular technologies were applied on pasture or grain legumes *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Two other major projects both to be led by Dr Oldach, are currently being negotiated with GRDC:

- Pre-breeding in oats: Improving biotic and abioitic stress tolerance via molecular and phenotypic tool development. This is a 5-year collaborative project between SARDI and the University of Sydney; $1,500,000.
- Development of a family-based chickpea mutant population. This is to develop a genetically diverse germplasm available to the pulse pre-breeding community in PBA (Pulse Breeding Australia); $490,000 over 5 years.

Mr Jake Howie

Qualifications:	B.Ag.Sci. (The University of Adelaide)
Current institution:	South Australian Research and Development Institute (SARDI)
Current position title:	Senior Research Officer (Feed and forage science)

Contribution to this field

Mr Howie has 30 years experience in the field evaluation and agronomy of annual pasture legumes, particularly annual medics, but also alternative pasture legumes through the National Annual Pasture Legume Improvement Program (NAPLIP). During that time he has been closely involved in the development and commercialisation of nine annual medic cultivars, including Sephi, Caliph, Mogul, Herald, Jester, Toreador, Cavalier Scimitar and Angel. The most recent release, Angel strand medic, is the world's first annual pasture legume with tolerance to sulfonylurea (SU) herbicide residues. He currently has a South Australian Grains Industry Trust (SAGIT) project to commercialise a new strand medic cultivar from a short-list of Breeders lines which have combined SU tolerance, powdery mildew resistance and improved agronomic performance. Mr Howie is an accredited Qualified Person with the Plant Breeders Rights Office and assisted in the IP protection of these cultivars. He has also assisted in the commercialisation of Prima gland clover, Kelson snail medic, Bindaroo button medic and Monti subclover through the provision of supporting agronomic data. Other recent work includes published research into abiotic stresses of *Medicago* species, particularly boron tolerance (Howie 2012, Bogacki et al 2013) and Group B herbicide tolerance. This will lead to the incorporation of improved germplasm in farming systems.

Research experience and roles

- From 2005 – current Mr Howie has had the following key responsibilities:
    - Leader, Annual Pasture Legume Improvement Unit, SARDI Feed & Forage Group
    - Supervisor of South Australian Grains Industry Trust and Australian Pastures Alliance pasture improvement projects
    - Development of annual pasture cultivars
    - Cultivar commercialisation (PBR, patents, liaison with industry partners)
    - Management of Pasture Group royalties
    - "Qualified Person", Plant Breeders Rights Office, IP Australia

Prior to this (1996-2005) Mr Howie was a Zone Leader for the NAPLIP project, with responsibility for coordinating the evaluation of annual pasture legumes in the low rainfall, alkaline soil zones of Australia. This included sourcing and dissemination of germplasm to collaborators in WA, Qld, NSW, Vic and SA for evaluation, coordinating analysis of results, supervision of local staff for the conduct of evaluation trials in South Australia and extensive liaison with industry, commercial partners and scientific collaborators.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal publications, book chapters and conference proceedings

1. ***Howie, JH** (2012) Boron tolerance in annual medics. *Crop and Pasture Science* **63**, 886-892.
2. ***Howie JH** (2010) *The strategic use of chlorsulfuron in the seed production of Medicago littoralis cv. Angel*. In H Dove and RA Culvenor (Eds). “Food Security from Sustainable Agriculture”. Proceedings of the 15th Australian Agronomy Conference, 15-18 November November 2010, Lincoln, New Zealand.
3. ***Howie, J.H.,** Dyson, C. & Preston, C. (2008). *Tolerance of annual strand medic cv. ‘Angel’ to ALS-inhibiting herbicides.* Proceedings of the joint XXI International Grassland Congress & VIII International Rangeland Congress, Huhhot, China, 29 June – 5 July 2008, Vol II, p.420.

Research reports and extension publications

4. ***Howie J**, Ballard R, Peck D (2013) Powdery mildew resistant medics for the Mallee and Eyre Peninsula. Mallee Sustainable Farming Results compendium, 2012, https://www.msfp.org.au/docs/research_111.pdf
5. ***Howie J**, Ballard R, Peck D (2013) Powdery mildew resistant medics for the Mallee and Eyre Peninsula. Eyre Peninsula Farming Systems Summary, 2012, pp. XX-XX.
6. ***Howie J**, Ballard R, Peck D (2013) Powdery mildew resistant medics for the EP and Mallee. Upper North Farming Systems, 2012 Harvest Report, pp. 23-26.
7. ***Howie J**, Ballard R, Peck D (2012) Better medics for the Mallee – update. Mallee Sustainable Farming Results compendium, 2011, http://www.msfp.org.au/docs/research_67.pdf
8. ***Howie J**, Ballard R, Peck D (2012) Better medics update. Eyre Peninsula Farming Systems Summary, 2011, pp. 68-70.
9. ***Howie J**, Ballard R, Peck D, Hill J (2012) Multi-trait medics for the Mallee. Karoonda Field Day Information Booklet 2012. Mallee Sustainable Farming Inc., pp. 27-30.
10. ***Howie J**, Ballard R, Peck D, Latta R (2012) Multi-trait medics for the Mallee. 2012 Minnipa Field Day, 12th September, pp. 28-30.
11. ***Howie J**, Ballard R, Peck D (2011) New strand medics for the Eyre Peninsula and the Murray Mallee – early results. Mallee Sustainable Farming results compendium, 2010, pp. 70-72.
12. ***Howie J**, Ballard R, Peck D (2011) New strand medics for the Eyre Peninsula and the Murray Mallee – early results. Eyre Peninsula Farming Systems Summary, 2010, pp. 61-62.
13. ***Howie J**, Ballard R, Peck D (2011) Powdery mildew in annual medics. Eyre Peninsula Farming Systems newsletter, Issue 29, Winter 2011, p. 3.
14. ***Howie J**, Ballard R Peck D, Hill J (2011) Multi-trait medics for the Mallee. 2011 Karoonda Trial Site Information Booklet. Mallee Sustainable Farming Inc., pp. 32-35.
15. ***Howie J** (2011) Pasture Varieties. Hart 2011 Official Field Day Guide, pp. 40-42.
16. ***Howie J**, Ballard R, Peck D, Hill J, Latta R, Richter I (2010) Multi-trait medics for the Mallee. 2010 Karoonda Trial Site Walk field book. Mallee Sustainable Farming Inc., pp. 38-41.

17. ***Howie J,** Lloyd D (2009) Strand medic, *Medicago littoralis*, Pastures Australia Variety Fact Sheet, January 2009. http://keys.lucidcentral.org/keys/v3/pastures/Html/Strand_medic.htm

18. ***Howie J,** Lloyd D (2009) Hybrid disc medic, *Medicago tornata x littoralis*, Pastures Australia Variety Fact Sheet, http://keys.lucidcentral.org/keys/v3/pastures/Html/Hybrid_disc_medic.htm

19. ***Howie J** (2009) Disc medic, *Medicago tornata*, Pastures Australia Variety Fact Sheet, http://keys.lucidcentral.org/keys/v3/pastures/Html/Disc_medic.htm

20. ***Howie J** (2009) Gama medic, *Medicago rugosa*, Pastures Australia Variety Fact Sheet, http://keys.lucidcentral.org/keys/v3/pastures/Html/Gama_medic.htm

21. ***Howie J,** de Koning C (2009) *Pastures Update.* Hart Field Site Group Inc., 15th September 2009, pp. 43-45.

22. ***Howie J** (2009) Twin sowing - what is it and can it work in SA? In Crop Science Society of SA Newsletter No 256 June 2009

23. ***Howie J,** Dyson C (2008) *Tolerance of 'Angel' to Group B herbicides: Part I – simulated residues. In* Eyre Peninsula Farming Systems 2007 Summary, March 2008, pp. 69 – 70.

24. ***Howie J** (2008) *Tolerance of 'Angel' to Group B herbicides: Part II – Foliar Applied.* In Eyre Peninsula Farming Systems 2007 Summary, March 2008, pp. 71 - 72.

25. **Howie J** (2008) Travel Final Report submitted to Grains Research & Development Corporation to attend The XXI International Grassland Congress, Hohhot, China, 29 June – 5 July 2008 (DAS00085).

26. ***Howie J** (2008) Final Report submitted to South Australian Grains Industry Trust for project S0207R – Angel strand medic – increasing the benefits for SA farmers.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
#Development of a strand medic cultivar resistant to powdery mildew (SAGIT)	J Howie (30) R Ballard (5)	297,000	2013-16	New
#Field evaluation and development of a advanced multi-trait strand medic lines (SAGIT)	J Howie (25) D Peck (10) R Ballard (10)	234,000	2010-13	On time
#Field evaluation and development of subterranean clover and SU tolerant barrel medics (Seedmark)	J Howie (50) D Peck (50) C de Koning (50) A Craig (30)	Commercial in Confidence	2006-ongoing	On time
#Field evaluation and development of subterranean clover and SU tolerant barrel medics (DAFF/Seedmak)	J Howie (50) D Peck (50) C de Koning (50) A Craig (30)	750,000	2009-11	On time
#Angel strand medic – increasing the benefits for SA farmers	J Howie (25)	76,000	2007-09	On time

#Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Mr David Peck

Qualifications: B.Ag.Sci. (The University of Queensland)

Current institution: South Australian Research and Development Institute (SARDI)

Current position title: Senior Research Officer (Feed and forage science)
Prior positions (10 years): Research Officer (SARDI)

Contribution to this field

For the last ten years Mr Peck has conducted pasture legume improvement research for SARDI. He has extensive knowledge and experience in pre-breeding and breeding annual legumes, including annual medics (barrel medic, burr medic, strand medic, disc medic), subterranean clover and balansa clover. Mr Peck has developed lines with an extensive range of biotic (e.g. aphid resistance, root lesion nematode tolerance, *Rhizobium* promiscuity, powdery mildew resistance) and abiotic traits (e.g. tolerance to SU herbicide residues, boron tolerance), and has developed populations which have been successfully used to develop molecular markers (e.g. SU herbicide tolerance, B tolerance, root-lesion nematode tolerance). Mr Peck has made inter-specific crosses, in order to transfer SU herbicide tolerance from strand medic into barrel medics (two cultivars are expected in the next 1-3 years). He has bred strand medic lines which have delivered a 30% increase in dry matter production and seed yield over three years of field evaluation (including increased early growth). Mr Peck has also had significant input into several new cultivars that SARDI expect to release over the next 1-3 years. These expected new cultivars have resulted from pre-breeding (e.g inter-specific crosses) and breeding work conducted by him. Mr Peck has the extensive laboratory, glasshouse and field skills and experience with annual legumes required for this pre-breeding project.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journals and conference papers

1. ***Peck DM**, Habili N, Nair RM, Randles JW, De Koning CT, Auricht GC (2012) Bean leafroll virus is widespread in subterranean clover (Trifolium subterraneum L.) seed crops and can be persistently transmitted by bluegreen aphid (*Acyrthosiphon kondoi* Shinji). *Crop and Pasture Science* **63**, 902-908.
2. ***Peck DM**, Howie JH (2012) Development of an early season barrel medic (*Medicago truncatula* Gaertn.) with tolerance to sulfonylurea herbicide residues. *Crop and Pasture Science* **63**, 866-874.
3. ***Peck DM**, Howie JH, Hill JR, Nair RM (2009) Progress in the development of barrel medics (*Medicago truncatula*) tolerant to sulfonylurea herbicide residues. 14th Australasian Plant Breeding (APB) conference. In 'Proceedings of Joint 14th Australasian Plant Breeding Conference and 11th SABRO Congress'. Cairns, Qld, 10-14 August 2009. SABRAO Journal of Breeding and Genetics 41(Special Suppl), August 2009. www.sabrao.org/apbc14/papers/apb09Final00153.pdf

Extension publications

4. ***Peck D**, Habili N, Randles J, Auricht G, Nair R Control of red-leaf disease in subterranean clover seed crops. Fact sheet http://www.sardi.sa.gov.au/pastures/annual_pastures/clover_red_leaf_disease

Ten career best publications (those related to this work are marked with an asterisk).

1. *Bogacki P, **Peck DM**, Nair RM, Howie J, Oldach KH (2013) Genetic analysis of tolerance to boron toxicity in the legume *Medicago truncatula*. *BMC Plant Biology* **2013**, 13-54 doi:10.1186/1471-2229-13-54

2. *Oldach KH, **Peck DM**, Cheong J, Williams KJ, Nair RM (2008) Identification of a chemically induced point mutation mediating herbicide tolerance in annual medics (*Medicago* spp.) *Annals of Botany* **101**, 997-1005.

3. ***Peck DM**, Howie JH (2012) Development of an early season barrel medic (*Medicago truncatula* Gaertn.) with tolerance to sulfonylurea herbicide residues. *Crop and Pasture Science* **63**, 866-874.

4. ***Peck DM**, Habili N, Nair RM, Randles JW, De Koning CT, Auricht GC (2012) Bean leafroll virus is widespread in subterranean clover (*Trifolium subterraneum* L.) seed crops and can be persistently transmitted by bluegreen aphid (*Acyrthosiphon kondoi* Shinji). *Crop and Pasture Science* **63**, 902-908.

5. *Humphries AW, **Peck D M**, Robinson, S. S, Rowe, T, Oldach, K. (2012) A new biotype of bluegreen aphid (*Acyrthosiphon kondoi* Shinji) found in south-eastern Australia overcomes resistance in a broad range of pasture legumes. *Crop and Pasture Science* **63**, 896-901.

6. McDonald GK, **Peck D** (2009) Effects of crop rotation, residue retention and sowing time on the incidence and survival of ascochyta blight and its effect on grain yield of field peas (Pisum sativum L.) *Field Crops Research* **111**, 11-21.

7. *Nair RM, **Peck DM**, Dundas IS, Samac DA, Moore A, Randles JW (2008) Morphological characterisation and genetic analysis of a bi-pistil mutant (*bip*) in *Medicago truncatula* Gaertn. *Sexual Plant Reproduction* **21**, 133-141.

8. ***Peck DM**, Howie JH, Hill JR, Nair RM (2009) Progress in the development of barrel medics (*Medicago truncatula*) tolerant to sulfonylurea herbicide residues. 14th Australasian Plant Breeding (APB) conference. In 'Proceedings of Joint 14th Australasian Plant Breeding Conference and 11th SABRO Congress'. Cairns, Qld, 10-14 August 2009. SABRAO Journal of Breeding and Genetics 41(Special Suppl), August 2009. www.sabrao.org/apbc14/papers/apb09Final00153.pdf

9. *Ballard RA, **Peck DM**, Lloyd DL, Howie JH, Hughes SJ, Hutton RE, Morgan BA (2012) Susceptibility of annual medics (*Medicago* spp.) to powdery mildew (*Erysiphe trifolii*). 16th Australian Agronomy Conference http://www.regional.org.au/au/asa/2012/pests/8226_ballard.htm#TopOfPage

10. *Humphries A, **Peck D**, Robinson S, Oldach,K, Glatz R, Howie J (2010) A new highly virulent bluegreen aphid causes severe damage in previously tolerant pasture and grain legumes. Australian Agronomy Conference http://www.regional.org.au/au/asa/2010/crop-production/legumes/6945_humphries.htm

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
#Field evaluation and development of subterranean clover and SU tolerant barrel medics (Seedmark)	D Peck (50) J Howie (50) C de Koning (50) A Craig (30)	Commercial in Confidence	2006-ongoing	On time
#Field evaluation and development of subterranean clover and SU tolerant barrel medics (DAFF/Seedmark)	D Peck (50) J Howie (50) C de Koning (50) A Craig (30)	750,000	2009-11	On time
Diagnostic tools for subterranean clover red leaf disease (RIRDC)	Peck (10) Habili (10) Nair (5) De koning (5) Randles (5)	375,000	2008-11	On time

#Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Mr Graeme Sandral

Qualifications: M.App.Sci. (Charles Sturt University), B.App.Sci (CSU)
Current institution: New South Wales Department of Primary Industries
Current position title: Senior Research Scientist (Pastures)

Contribution to this field

Mr Sandral is a Senior Pasture Scientist with over 25 years experience in pasture plant selection, agronomy and ecology in NSW. He has extensive experience in evaluation of subterranean clover, annual medics and alternative legumes in a wide variety of environments in NSW, including his work on the NAPLIP project and prior projects to it. Mr Sandral has further broadened his pasture legume expertise in his PhD project on the ecology of *Lotus* species. He is the NSW DPI field collaborator on the MLA FIP project *Phosphorus-efficient legume pasture systems* (Project No. B. PUE. 0104), which will have strong links to this pre-breeding project.

Plant Breeding: Mr Sandral has been involved in lucerne breeding in NSW, annual legume breeding in the NAPLIP project and perennial legume breeding in the Salinity CRC and FFI CRC. Through each of these programs he has also been involved in rhizobial research. Mr Sandral has made major contributions to the release of nine subterranean clover cultivars, two balansa clover cultivars, two burr medic cultivars, one gland clover cultivar, one biserrula, two French serradella cultivars, one lucerne cultivar and five *Lotus* cultivars.

Farming Systems: Mr Sandral has been involved in several farming systems projects which have examined a range of perennial pasture species, including their production and value in pasture and cropping systems. This research highlighted the value of perennial pastures in cropping systems and the importance of removal time of the perennial plants prior to cropping to maximise crop yield. He has also examined the use of perennial pastures to overcome induced sub-soil constraints following cropping.

Mr Sandral has worked in a range of pasture and farming system settings in NSW (NSW DPI) and WA (University of Western Australia) and has been involved in the co-ordination of national projects in Victoria and SA. He has collaborated internationally with groups of plant ecologists interested in the preservation and potential use of legumes native to the

Mediterranean. For example, two RIRDC-funded and GRDC-funded projects were aimed at the collection of legumes in low rainfall environments of the Mediterranean and involved Dr. Angelo Loi from DAFWA, Dr Luigi Russi from the University of Perugia, Dr Luigi Sringi from the University of Palermo (Sicily) and Dr Gus Gintzburger from CIRAD France. The purpose was to collect, conserve and test the adaptation of exotic species in southern Australian farming and grazing systems.

Mr Sandral has collaborated nationally with scientists from the NAPLIP and Lotus breeding programs, which have collectively produced new annual legume cultivars for ley, phase and forage farming systems for Australian agriculture, with a special emphasis on farming systems fit. In addition the Lotus breeding project focused on permanent pasture systems with an international focus. These projects involved close collaboration with State Departments in Western Australia, South Australian, Victoria and Queensland and international agencies such as INIA Uruguay. He has also collaborated closely with CSIRO on the project *Optimising crop performance through innovative phase farming systems – new approaches to improve the nitrogen supply and management of the hydrological balance for crops in wet environments*. This was a joint project between NSW DPI and CSIRO aimed at improving the hydrology of heavy clay soils and subsequent crop returns through the innovative use of pasture species.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journals and conference papers

1. **Sandral GA**, Degtjareva GV, Kramina TE, Sokoloff DD, Samigullin TH, Hughes S, Valiejo-Roman CM (2013) Are *Lotus creticus* and *Lotus cytisoides* (Leguminosae) closely related species? Evidence from nuclear ribosomal ITS sequence data. *Genetic Resources and Crop Evolution* (submitted).

Ten career best publications (those related to this work are marked with an asterisk).

1. *****Nichols PGH, Revell CK, Humphries AW, Howie JH, Hall EJ, **Sandral GA**, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia – their history, current use and future prospects. *Crop and Pasture Science* **63**, 691–725.
2. Dear BS, **Sandral GA**, Coombes NE (1995) Differential tolerance of *Trifolium subterraneum* L. (subterranean clover) cultivars to broadleaf herbicides. 1. Herbage yield. *Australian Journal of Experimental Agriculture* **35**, 467-74.
3. **Sandral GA**, Dear BS, Coombes NE (1995) Differential tolerance of *Trifolium subterraneum* L. (subterranean clover) cultivars to broadleaf herbicides. 2. Seed yield and quality. *Australian Journal of Experimental Agriculture* **35**, 475-82.
4. **Sandral GA**, Dear BS, Virgona JM, Swan AD, Orchard BA (2006) Changes in soil water content under annual- and perennial-based pasture systems in the wheatbelt of southern New South Wales. (Special Issue: Changed water use to fit farming and community needs) *Australian Journal of Agricultural Research* **57**, 321-333.
5. **Sandral GA**, Dear BS, Pratley JE, Cullis BR (1997) Herbicide dose response curves in subterranean clover determined by a bioassay. *Australian Journal of Experimental Agriculture* **37**, 67-74.
6. Peoples MB, Gault RR, Scammell GJ, Dear BS, Virgona JM, **Sandral GA**, Paul J, Wolfe EC, Angus JF (1998) The effect of pasture management on the contributions of fixed N to the N economy of ley-farming systems. *Australian Journal of Agricultural Research* **49**, 459-74.
7. *Real D, **Sandral GA**, Rebuffo M, Hughes SJ, Kelman WM, Mieres JM, Dods K, Crossa J (2012) Breeding of an early-flowering and drought-tolerant *Lotus*

corniculatus L. variety for the high-rainfall zone of southern Australia. *Crop and Pasture Science* **63**, 848-857.

8. *Real D, Warden J, **Sandral GA**, Colmer TD (2008) Waterlogging tolerance and recovery of 10 Lotus species. (Sustainable pastures in marginal environments.) *Australian Journal of Experimental Agriculture* **48**, 480-487.
9. *****Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, **Sandral GA**, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research* **104**, 10-23.
10. **Sandral GA**, Remizowa MV, Sokoloff DD (2006) A taxonomic survey of Lotus Pedrosia (Leguminosae, Lotae). *Wulfenia* **13**, 97-192.

Current research projects and projects/grants completed over the last 5 years (principle investigator only)

Project Title	Investigator names (% FTE*)	$	Years	Reports delivery$
Developing feedbase management systems for *Lotus corniculatus* (FFI CRC)	G Sanrdal R Hayes G Li E Hall	453,960	2011-14	
#Phosphorus Efficient Legume Pasture Systems (MLA/AWI)	G Sandral (30) R Simpson (30) M Ryan (30) P Nichols (5) R Hayes (10) G Li (10)	2,100,000	2011-15	
Lotus commercialisation and evaluation (FFI CRC)	G Sandral S Hughes D Real	240,000	2007- 11	
#Developing new and innovative perennial lotus species for grazing systems of southern Australia (AWI)	G Sandral D Real	1,200,000	2002-07	
Developing innovative seed production technologies to ensure the commercial success of new perennial lotus species targeted at reducing recharge in the mid-catchment regions of Australia (RIRDC)	G Sandral D Real	586,258	2002-07	

#Same research area as proposal; *Full time equivalent (proportion of each investigator's time contribution to each project); $Delivered on time or months late

Dr Sachiko Isobe (project associate)
Qualifications: Ph.D in Molecular breeding
Current institution: Kazusa DNA Institute
Current position title: Laboratory Head

Contribution to this field

Dr Isobe will be an associate of the project. She is a highly acclaimed molecular biologist, with a particular interest in legumes, notably white clover and red clover and more recently subterranean clover. She has been involved in numerous genomic and molecular marker studies and is widely published in high impact factor journals (see reference list).

Dr Isobe has collaborated closely with CLIMA in two ARC Linkage projects. This has lead to publication of the world's first linkage map for subterranean clover and to development of the subterranean clover core collection. Under this collaboration *de novo* sequencing of subterranean clover has been undertaken, resulting in a genome scaffold and SNP discovery, to generate a very high resolution QTL map. This proposed project will build on this relationship and skills-base. Dr Isobe will contribute considerable resources from KDRI to this project, including the operation and maintenance costs of their next generation sequencers and bio-informatics and supercomputing support to handle the huge molecular data-sets generated.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

Journal papers and book chapters

1. ***Isobe SN**, Hisano H, Sato S, Hirakawa H, Okumura K, Shirasawa K, Sasamoto S, Watanabe A, Wada T, Kishida Y, Tsuruoka H, Fujishiro T, Yamada M, Kohara M, Tabata S (2012) Comparative genetic mapping and discovery of Linkage Disequilibrium across linkage groups in white clover (*Trifolium repens* L.). *G3 (Bethesda)* **2**, 607-617.
2. **Isobe SN**, Hirakawa H, Sato S, Maeda F, Ishikawa M, Mori T, Yamamoto Y, Shirasawa K, Kimura M, Fukami M, Hashizume F, Tsuji T, Sasamoto S, Kato M, Nanri K, Tsuruoka H, Minami C, Takahashi C, Wada T, Ono A, Kawashima K, Nakazaki N, Kishida Y, Kohara M, Nakayama S, Yamada M, Fujishiro T, Watanabe A, Tabata S (2012) Construction of an Integrated High Density Simple Sequence Repeat Linkage Map in cultivated strawberry (*Fragaria* x *ananassa*) and its applicability. *DNA Research* **20**, 79-92.
3. ***Isobe S**, Boller B , Klimenko I, Kölliker S, Rana JC, Sharma TR,Shirasawa K,Hirakawa H, Sato S, Tabata S (2012) Genome-wide SNP marker development and QTL identification for genomic selection in red clover. In: Breeding strategies for sustainable forage and turf grass improvement. (Eds.Barth S, Milbourne D) (Springer, Dordrecht) DOI 10.1007/978-94-007-4555-1
4. ***Isobe S**, Tabata S (2010) Molecular genetic linkage map of soybean. Genetics, genomics and breeding of soybean. (Eds K Bilyeu, MB Rathaparkhe, C Kole) (CRC Press: New York) ISBN 978-1-57808-681-8. Pp 71-91.
5. ***Isobe S**, Kolliker R, Hisano H, Sasamoto S, Wada T, Klimenko I, Okumura K, Tabata S. (2009) Construction of a consensus linkage map for red clover (*Trifolium pratense* L.). *BMC Plant Biology* **9**, 57.
6. ***Isobe S**, Nakaya A, Tabata S (2007) Genotype matrix mapping: searching for quantitative trait Loci interactions in genetic variation in complex traits. *DNA Research* **14**, 217-225.

Ten career best publications (those related to this work are marked with an asterisk).

1. *Shirasawa K, Bertioli DJ, Varshney RK, Moretzsohn MC, Leal-Bertioli SCM, Thudi M, Pandey MK, Rami JF, Fonceka D, Gowda MVC, Qin H, Guo B, Hong Y, Liang X, Hirakawa H, Tabata S, **Isobe S** (2013) Integrated consensus map of cultivated peanut and wild relatives reveals structures of the A and B genomes of *Arachis* and divergence of the legume genomes. *DNA Research* **20**, 173-184.
2. ***Isobe SN**, Hisano H, Sato S, Hirakawa H, Okumura K, Shirasawa K, Sasamoto S, Watanabe A, Wada T, Kishida Y, Tsuruoka H, Fujishiro T, Yamada M, Kohara M, Tabata S (2012) Comparative genetic mapping and discovery of Linkage Disequilibrium across linkage groups in white clover (*Trifolium repens* L.). *G3 (Bethesda)* **2**, 607-617.
3. *Nakaya A, **Isobe S** (2012) Will genomic selection be a practical method for plant breeding? *Annals of Botany* (in press) doi:10.1093/aob/mcs109.
4. *The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **485**, 635-641.
5. *Shirasawa K, Koilkonda P, Aoki K, Hirakawa H, Tabata S, Watanabe M, Hasegawa M, Kiyoshima H, Suzuki S, Kuwata C,Naito Y, Kuboyama T, Nakaya A, Sasamoto S, Watanabe A, Kato M, Kawashima K, Kishida Y, Kohara M, Kurabayashi A,Takahashi C, Tsuruoka H, Wada T, **Isobe S** (2012) *In silico* polymorphism analysis for the development of simple sequence repeat and transposon markers and construction of linkage map in cultivated peanut. *BMC Plant Biology* **12**, 80.
6. *Ghamkhar K, **Isobe S**, Nichols PGH, Faithfull T, Ryan MH, Snowball R, Sato S, Appels R (2011) The first genetic maps for subterranean clover (*Trifolium subterraneum* L.) and comparative genomics with *T. pratense* L. and *Medicago truncatula* Gaertn. to identify new molecular markers for breeding. *Molecular Breeding* **5**, 1-14.
7. *Sato S, **Isobe S**, and Tabata S (2010) Structural analyses of the genomes in legumes. *Current Opinions in Plant Biology* **13**, 146-152.
8. *Klimenko I, Razgulayeva N, Gau M, Okumura K, Nakaya A, Tabata S, Kozlov N.N., and **Isobe S** (2010) Mapping candidate QTLs related to plant persistency in red clover. *Theoretical and Applied Genetics* **120,** 1253-1263.
9. ***Isobe S**, Tabata S (2010) Molecular genetic linkage map of soybean. Genetics, genomics and breeding of soybean. (Eds K Bilyeu, MB Rathaparkhe, C Kole) (CRC Press: New York) ISBN 978-1-57808-681-8. Pp 71-91.
10. ***Isobe S**, Kolliker R, Hisano H, Sasamoto S, Wada T, Klimenko I, Okumura K, Tabata S (2009) Construction of a consensus linkage map for red clover (*Trifolium pratense* L.). *BMC Plant Biology* **9**, 57.

Professor Rudi Appels (project associate)

Qualifications:	Ph.D in Biochemistry (The University of Adelaide)
Current institution:	Centre for Comparative Genomics, Murdoch University
Current position title:	Professor of Comparative Genomics

Contribution to this field

Professor Rudi Appels is an internationally recognised scientist with expertise in molecular plant breeding, genome sequencing, DNA-based diagnostics and technologies and comparative genomics, particularly in cereals. He is a co-chair of the International Wheat Genome Sequencing Consortium, an international effort focused on identifying wheat genes that contribute to the survival of wheat across a broad range of global environments, and is Director of the new Australia-China Centre for Wheat Quality. Professor Appels is also leader of the GRDC National Wheat Marker Program, which compiles molecular genetic/QTL analyses in wheat. His previous work has included leading a group that discovered the major factors underpinning the molecular genetics and cereal chemistry of wheat flour quality attributes at the level of new proteins and starch biosynthesis. He established the molecular marker-biotechnology laboratory in the State Agricultural Biotechnology Centre/DAFWA facility at Murdoch University. Professor Appels led the first DNA sequence level analysis of wheat and rye chromosomes, and was a co-author of a paper in *Science* (Paux *et al.* 2008) reporting the first large-scale assembly of wheat chromosome 3B. Subsequent work has reported the structure and function of 18 Mb of the wheat genome. He is on the GMO advisory committee for WA Government and the Program Advisory Committee (PAC) for GRDC. He is the Chief Editor of the Springer journal *Functional and Integrative Genomics* and has been on the editorial boards of the international journals *Genome*, *Chromosoma*, *Cell Science* and *Plant Systematics and Evolution*. Professor Appels has published 225 refereed papers and a text book and supervised 31 PhD students. He was awarded the Gottschalk medal by the Australian Academy of Sciences for distinguished research in biological sciences.

Professor Appels has collaborated closely with CLIMA in two ARC Linkage projects and made a major contribution to publication of the world's first linkage map for subterranean clover and to development of the subterranean clover core collection. This project will utilise Professor Appel's expertise on molecular biology technologies to ensure the most appropriate methods and analysis of molecular data are used.

Publications, reports and extension material (first authorship only) during the last 5 years (those related to this work are marked with an asterisk)

1. *__Appels R__, Barrerro R, Keeble G, Bellgard M (2010) Advances in genome studies: 2010 Plant and Animal Genome Conference. *Functional and Integrative Genomics* **10**, 1-9.
2. *__Appels R__ (2009) Diversity of genome research at the 2009 Plant and Animal Genome Conference: Editorial. *Functional and Integrative Genomics* **9**,1-6.
3. **Appels R** (2007) The annotation of gene *HgAffx.13168.1.S1_at*. *Functional and Integrative Genomics* **7**, 303.

Ten career best publications (those related to this work are marked with an asterisk).

1. *Ghamkhar K, Isobe S, Nichols PGH, Faithfull T, Ryan MH, Snowball R, Sato S, **Appels R** (2012) The first genetic maps for subterranean clover (*Trifolium subterraneum* L.) and comparative genomics with T. pratense L. and *Medicago truncatula* Gaertn. to identify new molecular markers for breeding. *Molecular Breeding* **30**, 213–226.
2. *Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Berges H, Eversole K, **Appels R**, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C (2008) A Physical Map of the 1-Gigabase Bread Wheat Chromosome 3B. *Science* **322**, 101–104.
3. *Zhang J, Huang S, Fosu-Nyarko J, Dell B, McNeil M, Waters I, Moolhuijzen P, Conocono E, **Appels R** (2008) The genome structure of the *1-FEH* genes in wheat (*Triticum aestivum* L): New markers to track stem carbohydrates and grain filling QTLs in breeding. *Molecular Breeding* **22**, 339-351.

4. *Li D, Barclay I, Jose K, Stefanova K, **Appels R** (2008) A mutation at the *Ala122* position of acetohydroxyacid synthase (AHAS) located on chromosome 6D of wheat: improved resistance to imidazolinone and a faster assay for marker assisted selection. *Molecular Breeding* **22**, 217 – 225.
5. Verbyla AP, Saint-Pierre C, Peterson CJ, Ross AS, **Appels R** (2007) Fourier based analysis and interpretation of wheat flour Mixograph data. *Journal of Cereal Science* **46**, 11-21.
6. *Lagudah ES, Moullet O, **Appels R** (1997) Map based cloning of a gene sequence encoding a nucleotide binding domain and a leucine rich region at the *Cre3* nematode resistance locus of wheat. *Genome* **40**, 659-665.
7. Kellogg EA, **Appels R** (1995) Intraspecific and interspecific variation in 5S RNA genes are decoupled in diploid wheat relatives. *Genetics* **140**, 325-343.
8. Hilliker AJ, **Appels R** (1982) Pleiotropic effects associated with the deletion of heterochromatin surrounding rDNA on the X chromosome of Drosophila. *Chromosoma* **86**, 469-490.
9. **Appels R**, Dvorak J (1982) The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. *Theoretical and Applied Genetics* **63**, 337-348.
10. **Appels R**, Wells JRE (1972) Synthesis and turnover of DNA-bound histone during maturation of avian red blood cells. *Journal of Molecular Biology* **70**, 425-434.

5.3.6 Inventory of pre-breeding technologies currently in use for annual legume species

In vitro tools to reduce cultivar development time

CLIMA has widespread experience in tissue culture, embryo rescue and *in vitro* rapid generation technologies for legumes. Projects led by Dr Janine Croser have successfully developed *in vitro*-assisted rapid generation technologies for lupin, lentil and field pea (Ribalta *et al.* 2013). This technique is also being used on a Future Farm Industries CRC-funded project for the promising pasture legume species tedera (*Bituminaria bituminosa*).

CLIMA has developed close international linkages with legume *in vitro* research groups in France (Dr Sergio Ochatt, INRA, Dijon) and Canada (Dr Monika Lulsdorf, Crop Development Centre, University of Saskatchewan). Dr Ochatt and Dr Lulsdorf are collaborating with UWA to develop *in vitro* techniques for accelerating generation turnover in crop legume species. Dr Ochatt has already published a protocol for *in vitro* rapid generation cycling in *Arabidopsis thaliana* (Ochatt and Sangwan 2008) and protein pea (Ochatt *et al.* 2000, 2002).

A pilot study has shown a full lifecycle (from germination to seed maturation) of subterranean clover can be grown *in vitro* on culture medium and the time to flowering and seed set can be markedly reduced by optimising light, temperature and medium regimes (Croser *et al.* unpublished data; Figure 3). Preliminary results suggest three generations per year can be achieved in subterranean clover, effectively tripling the current generation turnover.

Genetic material for phenotypic diversity studies

A wide range of germplasm is available to measure phenotypic diversity for traits of agronomic importance within the key annual legumes, determine their potential for genetic improvement through plant breeding, and identify potential parents for crossing. The following range of germplasm can be exploited to undertake these diversity studies.

A large *genetic resource*, totalling around 59,000 accessions collected throughout their native habitat in the Mediterranean basin and surrounding areas (Nichols *et al.* 2012), is available for the important annual legume species in the DAFWA-operated Australian Trifolium Genetic Resource Centre and the SARDI-operated Medicago Genetic Resource Centre. Included in this are over 9,000 subterranean clover accessions (Nichols *et al.* 2013) and over 25,000 annual medics.

Core collections, which are designed to contain a high proportion of the total diversity of a species within a much smaller and more manageable group (Frankel and Brown 1984), can be utilised to examine phenotypic diversity of key traits. Such core collections have been developed by CLIMA and DAFWA for subterranean clover (Nichols *et al.* 2013), bladder clover (Ghamkhar *et al.* 2007) and biserrula (Ghamkhar *et al.* 2012a), while SARDI holds core collections of barrel, strand and burr medics (Skinner *et al.* 1999; Ellwood *et al.* 2006). Core collections are ideal for a systematic examination of diversity within a species, as they contain accessions selected on the basis of eco-geographical, agro-morphological and molecular marker diversity. The subterranean clover core collection is currently being used to examine trait diversity in two projects: (i) phosphorus use-efficiency and root traits in the MLA-funded project *Phosphorus-efficient legume pasture systems* (B. PUE. 0104); and (ii) oestrogenic isoflavone (formononetin, genistein and biochanin A) levels and *in vitro* fermentability parameters (gas pressure and methane production) from the Australian Research Council (ARC)-funded project *Exploiting subterranean clover genetic variation for methane mitigation and ruminant health challenges to the Australian livestock industries*.

Genetic mapping populations, involving progeny derived from parents differing widely in particular phenotypic traits, have been developed by CLIMA for subterranean clover (Ghamkhar *et al.* 2012b) and by SARDI for barrel and strand medics. These can be utilised to develop genetic maps, which show the genetic linkage distances on the chromosomes between DNA molecular markers and between molecular markers and quantitative trait loci (QTLs) or genes for phenotypic traits. These can provide an understanding of the genetics of key traits (for which the parents differ) and enable identification of molecular markers associated with genes controlling them. The subterranean clover mapping populations have recently been used to map eight phenotypic traits and for comparison with the red and white clover genomes (Ghamkhar *et al.* 2012b).

Bulk-hybrid populations, involving a mixture of genetically segregating seed derived from many crosses and sown in different target environments, have been developed in subterranean clover. Seeds from these populations have been harvested over a number of generations at each site and allow an examination of traits important for long-term persistence. Two sets of bulk-hybrid populations are available. One population, described by Nichols *et al.* (2009) involves a mixture of genetically segregating seed derived from 256 crosses sown at two environments in Western Australia (WA) (one low rainfall and the other high rainfall). Seeds from these populations were harvested over 16 years at both sites. Nichols *et al.* (2009) grew out these populations and compared the divergence after 16 years of a range of agro-morphological characters from the original mixture (which had been kept in cold storage). Another bulk-hybrid population, derived from F_2 seed from 300 crosses, was sown at 6 sites and seed samples were harvested after 7 years. These genetically diverse populations consist of genotypes that have clearly persisted and offer a unique opportunity to examine genetic differences at the genomic level that are associated with persistence.

Commercially available cultivars of a range of annual pasture legumes are available for inclusion in phenotypic studies to provide practical information to industry on traits of importance to graziers among current species and cultivars.

Development of DNA-based molecular markers

DNA-based molecular marker technology has a range of potential applications for pasture legume improvement programs. It can be used for marker-assisted selection (MAS) to select among cross-bred progeny or genetically variable populations for closely linked target traits, where conventional phenotyping methods are difficult or unreliable. It allows rapid introgression of simply inherited traits and can be used to identify and estimate the genetic effects of economically important Quantitative Trait Loci (QTLs), which are DNA regions associated with particular phenotypic traits. Polymorphic DNA markers can also be used to develop genetic maps, in which QTLs and individual gene loci are mapped into linkage groups and ultimately onto chromosomes. Such maps allow identification of candidate genes for traits of interest. Comparative mapping studies can be used to measure the degree of synteny between legume species and identify conserved chromosome regions containing genes or QTLs of interest. Molecular markers can also be used to authenticate plant cultivars and identify hybrids following crossing.

Major reductions in the cost of genome sequencing and high throughput technologies have heralded 'genomics-assisted breeding', in which genome sequence comparisons of many genotypes can provide a precise prediction of phenotypic performance on the basis of genotype, particularly for traits related to resistance or tolerance to biotic or abiotic stresses. This, coupled with DNA micro-array and RNA sequencing techniques, has led to the development of other fields of molecular biology with the potential to aid future pasture legume improvement, including: functional genomics, which aims to understand the gene function and protein products of particular DNA sequences; transcriptomics, which investigates differences in gene expression between Single Nucleotide Polymorphisms (SNPs), leading to discovery of the active genes; and proteomics, which examines the structure, function and synthesis of proteins. A further development is the field of phenomics, which is the study of the full set of phenotypes of an organism, in response to environmental influences. Automated, high resolution, whole-plant phenotyping facilities can accelerate the study of phenomics, providing a platform for discovery of new genes for incorporation into future cultivars.

No pasture legume cultivars have been released to date globally as a result of using MAS. The technology has been used to a limited extent in Australian pasture breeding programs, but its application is likely to increase as more molecular markers for specific traits are developed and they become cheaper to use. Barrel medic (*Medicago truncatula*) has been designated as a model legume for the more genetically complex related species, lucerne, and is the subject of many genomic studies world-wide. Molecular markers have been used to develop genetic maps for barrel medic (Thorquet et al. 2002; Choi et al. 2004; Mun et al. 2006; Ané et al. 2008) and have been applied by SARDI to confirm sulfonylurea herbicide tolerance (Oldach *et al.* 2008) and boron tolerance (Howie 2012; Bogacki *et al.* 2013). The genome of *M. truncatula* was DNA sequenced in the United States (Young et al. 2011), which will greatly facilitate marker development for other important QTLs.

CLIMA, in partnership with Dr Sachiko Isobe of the Kazusa DNA Research Institute (KDRI) in Japan and Professor Rudi Appels of Murdoch University, has developed a genome scaffold and a high resolution QTL map with 172 Simple Sequence Repeat (SSR) markers and 21,060 Single Nucleotide Polymorphism (SNP) markers (sites in the DNA sequence where individuals differ at a single DNA base) (Kaur *et al.* 2013). This work has been funded by the Australian Research Council (ARC) and also by MLA, through co-sponsorship of a 2013 DAFF Science and Innovation Award for Young People in Agriculture. Additional funding in this project will allow sequencing of the subterranean clover genome to be completed. Access to a fully sequenced genome will have major implications for the pre-breeding and molecular marker development of subterranean clover, in addition to the internationally important and closely related species, white clover and red clover, which are genetically much more complex and difficult to conduct genomic research with. No other laboratories are conducting genomic work with subterranean clover.

Screening for cotyledon resistance to redlegged earth mite
RLEM are found throughout the winter growing-season areas of southern Australia (Allen 1987; Ridsdill-Smith 1997). These mites feed on the upper surface of cotyledons and leaves, causing high mortality of germinating seedlings, loss of herbage production in winter and spring and a reduction in seed yield. Insecticides have been the main form of control, but some populations have been recently found with resistance to some pyrethroid pesticides (Umina 2007).

DAFWA has developed expertise, using controlled conditions in a phytotron, in screening seedlings for RLEM cotyledon resistance. Genetic variation for resistance has been found in subterranean clover and breeding for resistance has been an important breeding objective, culminating in release of the cultivars Bindoon, Rosabrook and Narrikup with increased RLEM cotyledon resistance (Nichols *et al.* 2009b). However, resistance in these cultivars is not absolute and high RLEM densities are able to overcome it. If new genes can be identified for resistance, it may be possible to pyramid them to enhance the levels of resistance.

Major species differences occur in their susceptibility to RLEM. Annual medics and many of the other clovers tend to be very susceptible, but a thorough screening may identify less susceptible types. Gland clover, on the other hand, is highly resistant (Nutt and Loi 2002) and the potential exists to identify and isolate the gene(s) controlling this resistance, if a RLEM susceptible gland clover can be found to conduct genetic analyses. One method of doing this is by mutagenesis. Ethyl methyl sulfonate (EMS) is a mutagen that has been widely used to generate mutant plants in several species. CLIMA has used this method to develop lupin mutants highly tolerant of metribuzin herbicide (Si *et al.* 2011). The University of Adelaide used this method to develop an induced mutant of the strand medic cv. Herald with tolerance to soil residues of sulfonylurea (SU) herbicides, which was subsequently developed and released by SARDI as cv. Angel (Heap 2000; Howie *et al.* 2002).

Screening for seed dormancy traits
The DAFWA pasture science group has developed considerable expertise in annual legume seed and seedling dynamics and seed dormancy traits for reliable establishment and pasture persistence in different environments and farming systems. Several seed dormancy traits act to influence persistence, the timing of germination and the survival and growth of annual legume seedlings. Improvements in these traits can increase the reliability of seedling regeneration between seasons and have a major impact on legume density and pasture productivity. DAFWA has used this understanding to develop screening systems for hardseededness and seed softening patterns, which has led to selection of a range of annual legume cultivars with seed dormancy traits tailored to their use.

<u>Increased persistence between seasons</u>
Hardseededness, in which an impermeable seed coat prevents imbibition by water, is the most important mechanism for regulating the germination of annual legume seeds (Quinlivan 1971b; Ehrman and Cocks 1996). Newly ripened seeds of most annual legumes contain a high proportion (usually >90%) of hard seeds (Taylor 2005). Some of these hard seeds *soften* (become permeable to water) over the summer-autumn period under the influence of high and fluctuating soil surface temperatures, the proportion being species and genotype dependent (Taylor 2005; Loi *et al.* 2005). These soft seeds are available for germination to re-establish the pasture, while the residual hard seeds form a seed bank and soften over subsequent summers.

Hardseededness has an ecologically significant role for pasture legumes by spreading germination of the seed pool over several seasons, allowing the species to persist when seed production is curtailed, due to severe drought, flooding, insect attack or disease infection (Russi *et al.* 1992; Taylor 2005). In mixed farming systems, high levels of hardseededness are also needed to ensure pasture regeneration after a cropping phase (Taylor *et al.* 1991, 2005). Selection of appropriate levels for different environments and farming systems has been a major objective of annual legume breeding and selection (Loi *et al.* 2005; Nichols *et al.* 2007; 2012).

Subterranean clover is among the least hardseeded of the annual legumes, which results in sub-optimal persistence in low and medium rainfall environments, particularly where there is a high emphasis on cropping. Higher levels of hardseededness have been sought for varieties aimed at these environments, while lower levels are required in late flowering varieties aimed at more favourable high rainfall environments, which generally contain permanent or semi-permanent pastures (Nichols *et al.* 1994, 1996, 2007, 2013). Breeding has been successful in developing more hardseeded varieties, but even the most hardseeded genotypes of subterranean clover are relatively soft-seeded compared to annual medics, yellow serradellas, biserrula and most other annual legumes (Russi *et al.* 1992; Norman *et al.* 2002; Loi *et al.* 2005; Nichols *et al.* 2007, 2012).

A laboratory procedure for measuring residual hardseed levels in subterranean clover was originally developed by DAFWA, using a diurnally fluctuating 60/15˚C temperature regime for 16 weeks, designed to simulate the summer soil temperatures experienced on bare soil Quinlivan (1961). However, different species respond to different temperature stimuli, requiring experimentation to determine the appropriate settings.

<u>Increased tolerance to false breaks</u>
A major weakness of subterranean clover, barrel medics, balansa clover and several other species is their susceptibility for germination following *false breaks* to the season, defined as a germination-inducing rainfall event (in the summer-autumn period), followed by a period of drought which leads to widespread seedling death. For example, seeds of most subterranean clovers will germinate following rains from early March onwards (Chapman and Asseng 2001; Loi *et al.* 2005; Norman *et al.* 2006). Chapman and Asseng (2001) have shown false breaks occur in 61-72% of years in the WA cropping zone, with the period of greatest risk being early autumn. False breaks, leading to seedling losses are also a regular occurrence in eastern Australia, with the consequence being weedy, unproductive pastures when the true break of season occurs.

Recent work by DAFWA has provided a greater understanding of the germination processes of annual legumes and shown that the timing of hard seed softening within the summer-autumn period, in relation to germination-inducing rainfall events, is crucial in determining the chances of successful plant establishment. Species differ in the timing when most of their hard seeds soften, with the major softening event in some species (including gland clover burr medics, bladder clover and messina) occurring a few weeks later than subterranean clover, balansa clover and barrel medics (Smith *et al.* 1996; Norman *et al.* 1998, 2006; Loi *et al.* 2005; Nichols *et al.* 2007, 2009a). Such pasture legumes suffer fewer losses following false breaks to the season, and can produce a legume-dominant stand when the true break of season occurs.

Genotypic differences in the timing of hard seed softening are known in subterranean clover, in which the main seed softening event occurs at least 3 weeks later than others (Smith *et al.* 1996; Norman *et al.* 2006; P.F. Smith, unpublished data) and it is possible that further delay in softening exists in some accessions. Selection for delayed seed softening would confer increased protection against seedling losses from false breaks in early-mid autumn, by deferring the majority of germination until mid-late autumn, when follow up rainfall is more likely. Although it has been recognised as an important trait for environments with unreliable autumn rainfall, the lack of a rapid screening technique and information on its genetics has prevented it from being considered as a breeding objective to date. On the other hand, in more favourable environments, where false break frequency is low and there is a high probability of reliable follow up rainfall (Evans and Smith 1999), such a germination mechanism is less important.

DAFWA has developed a methodology for plotting the pattern of hard seed softening under field conditions over the summer-autumn period (Loi *et al.* 2005; Norman *et al.* 2006; Nichols *et al.* 2009b). In this process, freshly harvested seeds are divided into equal seed lots, which are placed in mesh envelopes on the soil surface. Seed lots are removed from the field at regular intervals until early winter and subjected to germination tests in the laboratory to count the proportions of hard and soft seeds.

Measuring diversity of agro-morphological traits
DAFWA and SARDI are experienced at measuring morphological traits in annual pasture legumes. Much of this work has been conducted by the Genetic Resource Centres as part of their characterisation of new germplasm. DAFWA has particular expertise in clovers, serradellas and biserrula, while SARDI has particular expertise in the annual medics. Such traits include mean seed weight, flowering time, plant diameter, leaflet size, petiole length, petiole diameter, stem diameter, internode length, peduncle length, peduncle diameter and plant diameter at maturity. Other qualitative and semi-quantitative traits relating to plant morphology (e.g. leaflet markings and degree of indentation, anthocyanin flecking and flush patterns, hairiness of leaves, petioles, stems and peduncles) can also be recorded.

Annual legumes with increased growth at low temperatures
Increased seedling growth, particularly at low temperatures is considered important if the break of season does not occur until late autumn or early winter, when low ambient and soil temperatures reduce plant growth and increase the time to establish an optimal leaf area index. With climate change predictions for less autumn rainfall across southern Australia, late breaks to the season are likely to become more common and increased seedling growth is likely to become more important.

Little work has been done in this area with annual legumes. However, when compared to other plant systems, it is likely that plants with increased early shoot growth will also have increased root growth, which, in turn, will help seedling survival and minimise the effect of root diseases. An example of this approach is the development of wheat with high vigour (Rebetzke and Richards 1999), where early vigour correlates well with total biomass production. Wheats with higher vigour make more efficient use of soil water early in the season and increase total dry matter production and seed yield. The high vigour wheats were selected for increased early shoot dry matter, but subsequent studies showed they also have high root vigour, which increases their ability to uptake water and nutrients (Liao *et al*. 2004 & 2006, Palta *et al.* 2011). These results suggest that focusing selection in annual pasture legumes on increased seedling shoot dry matter production may also result in selection for improved root growth.

Annual medic accessions with exceptionally high seedling vigour and winter production have previously been observed. For example, Crawford *et al.* (1989) reported that SARDI's genetic resource centre hosts barrel medic accessions with up to 1.5 times the seedling vigour of current cultivars and up to 3.0 times the winter production. This indicates that pre-breeding for improved seedling vigour and winter production in the main annual pasture legume species is highly feasible. SARDI also has an existing QTL for high seedling dry matter production in a strand medic population. The mapping population used to isolate this QTL can be screened under cool conditions to see if it is still linked to dry matter production.

Boron tolerance in annual medics
High levels of boron (B) are widespread on neutral to alkaline soils, which are widely distributed in the temperate slopes and plains of southern Australia. Cereal and pulse breeders have initiated the development of B tolerant cultivars for these soils with the aid of diagnostic molecular markers. Annual medics are the best performing annual pasture legumes on neutral to alkaline soils and, similarly to cereals and pulse crops, B tolerance is correlated with increased yields.

SARDI has used a candidate gene approach to identify a B tolerance molecular marker for the B tolerant barrel medic cultivars, Caliph and Paraggio. All current burr medic cultivars are susceptible to high B levels and the lack of B tolerance restricts their wider use (Howie 2012). Current strand medic cultivars were rated as tolerant by Howie (2012), but moderately susceptible by Paull *et al*. (1992). The B tolerance molecular marker in barrel medics enables this trait to be readily selected for in the breeding of new barrel medic cultivars. However, no molecular markers for B tolerance exist at this stage for strand and burr medics. Given the close phylogenetic relationships between the annual medics, it is likely that molecular markers closely linked to genes for B tolerance in strand and burr medics can be readily developed.

Genetic diversity for B tolerance has been described by Paull *et al*. (1992) and three burr medic accessions and five strand medic accessions with B tolerance have been identified. SARDI has an established phenotyping protocol and F_2 plants from a cross between tolerant and intolerant cultivars/accessions could be used to verify the usefulness of the existing B tolerance molecular marker (from barrel medic) for use in the breeding of strand and burr medics with this trait.

5.3.7 Project activities

A description of the proposed activities follows, while a draft operating plan of proposed R & D activities and outputs for the project is shown in Table 13. This will be refined by the project team each year at an annual review and project planning meeting.

In vitro tools to reduce cultivar development time in subterranean clover
Part of the brief from an MLA perspective was that the pre-breeding project should lead to tools and traits to increase the rate of genetic gain. To help meet this objective an *in vitro* protocol to accelerate cultivar development of subterranean clover will be developed in this project. The aim is to develop three generations per year, effectively tripling the current generation turnover. This will deliver to breeding organisations the ability to breed cultivars more rapidly, and provides an important strategy for rapid cultivar release to counter new industry threats, such as a devastating new pest or disease. It can also be used for research purposes in the rapid production of Recombinant Inbred Lines (RILs), which can be used in genetic mapping studies.

The method involves growing plants *in vitro* on culture medium and reducing the time to flowering and seed set by optimising light, temperature and culture media regimes. Following seed set, the immature embryos are dissected, germinated *in vitro* and the process repeated. This procedure can be readily coupled to a plant breeding program through use of a single seed descent methodology. The *in vitro* induction of flowering and seed set has been successfully used to accelerate generation turn-over in other legume species including field pea, in which up to nine generations per year have been grown, and peanut (Ochatt *et al.* 2000; 2002; 2008; Kone *et al.* 2007).

A pilot study has shown a full lifecycle (from germination to seed maturation) of subterranean clover can be grown *in vitro* on culture medium and the time to flowering and seed set can be markedly reduced by optimising light, temperature and medium regimes (Croser *et al.*

unpublished data; Figure 5). Preliminary results suggest three generations per year can be achieved in subterranean clover, effectively tripling the current generation turnover. This project will extend this protocol to a wide array of genotypes that cover a broad flowering time range (from early to late flowering) across the three subspecies of subterranean clover. It will also investigate ways to integrate the protocol into a breeding program in an efficient and cost-effective way. The principles are likely to be applicable across other pasture legumes, but each species requires individual growth conditions to optimise flowering times.

a. Germinated plant **b. *In vitro* flowering and burr formation**

Figure 5. Subterranean clover *in vitro* growth and flowering on an agar medium. Preliminary results suggest 3 generations per year are possible using this system.

Molecular markers for key morphological traits in subterranean clover
This activity will integrate all the phenotypic trait information on the subterranean clover core collection from this and related projects (at least 20 traits) and develop molecular markers for them in a ready-to-use form as per a commercialisation plan (to be developed) for use by public and private breeding organisations.

Traits will include:

- Key agro-morphological traits (flowering time and leaf, stem, seed and plant growth characters);
- Cotyledon resistance ratings to redlegged earth mite;
- Hardseededness, false break tolerance and other seed dormancy traits; and
- Seedling growth parameters under cool temperatures
- Root and phosphorus (P)-use traits (carboxylate composition and concentration, colonisation by arbuscular mycorrhizal fungi (AMF), WinRhizo root length and diameter classes, shoot DW, root DW, root:shoot dry weight ratios, root P levels, levels of P and a range of other elements in shoots from the MLA-funded project *Phosphorus-efficient legume pasture systems* (B. PUE. 0104); and
- Oestrogenic isoflavone (formononetin, genistein and biochanin A) levels and *in vitro* fermentability parameters (gas pressure and methane production) from the Australian Research Council (ARC)-funded project *Exploiting subterranean clover genetic variation for methane mitigation and ruminant health challenges to the Australian livestock industries*.

The project will build on prior and current work funded by the ARC and also by MLA (through co-sponsorship of a 2013 DAFF Science and Innovation Award for Young People in Agriculture). Recent work, in partnership with Dr Sachiko Isobe of the Kazusa DNA

Research Institute (KDRI) in Japan, has used a next generation sequencer (Illumina HiSeq 1000) to develop a genome scaffold and a high resolution QTL map with 172 Simple Sequence Repeat (SSR) markers and 21,060 Single Nucleotide Polymorphism (SNP) markers (sites in the DNA sequence where individuals differ at a single DNA base) (Kaur *et al.* 2013). This project will allow further collaboration with KDRI to sequence the whole genome of subterranean clover, using their world-class next-generation sequencing facilities and to conduct the bio-informatics associated with project. Having a fully sequenced genome will have profound implications for the pre-breeding and molecular marker development of subterranean clover, in addition to the internationally important and closely related species, white clover and red clover, which are genetically much more complex and difficult to conduct genomic research with.

Elucidation of the subterranean clover genome will make it possible to develop a *haplotype map* (HapMap), which is a powerful tool that will enable the project team to find genes and genetic variations for important phenotypic traits. This technique has been recently developed for human genomics (International HapMap Consortium 2007) and is based on the principle that the vast majority of the DNA sequence of any two individuals is identical, but the variations, however, may greatly affect an individual's disease risk or physical appearance. In this project the 97 subterranean clover core collection lines and 28 diverse cultivars will undergo association mapping to develop a HapMap that identifies SNPs that differ from the reference genome sequence for the traits of interest. This will allow molecular markers to be designed for marker-assisted selection to enable rapid screening of germplasm for the traits of interest and identification of candidate genes. These SNP markers will subsequently be modified to Polymerase Chain Reaction (PCR) markers for key phenotypic traits, which are in a ready-to-use form for use by public and private breeding organisations.

The development of the HapMap in this project, with its dense molecular marker coverage of the genome, will have longer-term benefits for subterranean clover improvement, as it will provide the framework for development of molecular markers for any new traits. All that will be required is for the core collection to be phenotyped for the trait of interest and conducting association mapping to identify molecular markers associated with it. This process is analogous to adding new columns to a spreadsheet.

Identification of genes for persistence in subterranean clover
Can we identify genes for persistence in subterranean clover? Two sets of bulk-hybrid populations are available to examine this question. One population, described by Nichols *et al.* (2009) involves a mixture of genetically segregating seed derived from 256 crosses sown at two environments in Western Australia (WA) (one low rainfall and the other high rainfall). Seeds from these populations were harvested over 16 years at both sites. Nichols *et al.* (2009) grew out these populations and compared the divergence after 16 years of a range of agro-morphological characters from the original mixture (which had been kept in cold storage). Another bulk-hybrid population, derived from F_2 seed from 300 crosses, was sown at 6 sites and seed samples were harvested after 7 years. These genetically diverse populations consist of genotypes that have clearly persisted and offer a unique opportunity to examine genetic differences at the genomic level that are associated with persistence.

The project team will collaborate with KDRI to use a genotyping assay on the bulk-hybrid populations, termed KASP **(**K-Bioscience Competitive Allele Specific PCR), which enables accurate bi-allelic discrimination of SNPs and nucleotide insertions or deletions. This method requires no labelling of the target-specific primers/probes, making it less expensive than other PCR-based genotyping assays, and has the ability to genotype large populations. Around 1000 individuals will be screened from the long-term populations, to give a broad assay of types that have proved persistent in the field, which will be compared with the original sown populations. In this way SNPs and nucleotide insertions or deletions can be identified that are associated with persistence, with the prospect of developing markers for screening in breeding programs. The KASP assay method has recently been used for SNP identification and discrimination of *Citrus* species and cultivars (Bernardi *et al.* 2013).

Diversity for cotyledon resistance to red legged earth mite (RLEM)
This activity will provide a measure of the phenotypic diversity for RLEM cotyledon resistance, its potential for further genetic improvement and identify potential parents for crossing among subterranean clover, annual medics, balansa, bladder and Persian clovers, biserrula, yellow and French serradella and cultivars of other species. Molecular markers for genes associated with RLEM cotyledon resistance will also be developed for subterranean clover.

A screening method, using controlled conditions in a phytotron, has been developed to screen seedlings for RLEM cotyledon resistance (Gillespie 1991, 1993; Nichols *et al.* 2009b). To date, only a limited proportion of accessions in the ATGRC has been screened and this has been done in an *ad hoc* fashion. This project will use the subterranean core collection, and those of other species, to search for new sources of resistance in a systematic way. It will provide a measure of the phenotypic diversity for RLEM cotyledon resistance, its potential for further genetic improvement and identify potential parents for crossing, among the important annual pasture legume species.

Molecular markers for genes associated with RLEM cotyledon resistance will also be developed for subterranean clover. This will enable marker-assisted selection in segregating breeding populations and provide gains in breeding efficiency, as screening for resistance to RLEM is also technically challenging and limited to a short period of the year when RLEM can be collected from the field.

Identification of genes for RLEM resistance in gland clover
Gland clover cv. Prima has very strong resistance to RLEM in both seedlings and adult plants (Nutt and Loi 2002). Screening of a range of gland clover accessions showed they also had strong resistance. The potential exists to identify and isolate the gene(s) controlling this resistance, for subsequent transfer into other legume species. However, in order to do this a RLEM susceptible gland clover is required in order to conduct genetic analyses and isolate the gene(s) responsible.

This project will attempt to generate RLEM susceptible plants of cv. Prima gland clover through mutagenesis with ethyl methyl sulfonate (EMS). Following mutagenesis, the M_1 plants will be seed increased. Seedlings of the M_2 generation will be challenged by RLEM in a growth room and any showing susceptibility will be rescued and transplanted into pots for seed production. Repeat screening will be conducted in the following year to confirm susceptibility. Molecular profiles of confirmed susceptible plants can then be compared to cv. Prima to identify SNPs that may be associated with resistance (this is likely to be undertaken beyond the time-frame of this project).

This activity is more ‘blue-sky’ than the other project components, but if novel genes for RLEM resistance can be identified, the pay-off could be very large, as it will pave the way to incorporate strong RLEM resistance into other legumes (pasture and crop), in addition to canola, which is also susceptible to RLEM at the seedling stage.

Annual legumes with false break tolerance and seed dormancy traits for increased persistence
This activity will provide a measure of the phenotypic diversity for different seed dormancy traits that influence persistence, the timing of germination, and seedling survival and growth in subterranean clover and annual medics. Other important annual legumes will be examined if time and resources permit. Improvements in these traits will increase the

reliability of seedling regeneration between seasons and have a major impact on legume density and pasture productivity. Molecular markers for genes associated with these traits will be developed for subterranean clover.

Subterranean clover is among the least hardseeded of the annual legumes, which results in sub-optimal persistence in low and medium rainfall environments, particularly where there is a high emphasis on cropping. Another major weakness of subterranean clover, along with barrel medics, balansa clover and several other species, is their susceptibility for germination following *false breaks* to the season, resulting in major seedling losses, with the consequence being weedy, unproductive pastures when the true break of season occurs. Work by DAFWA has shown that the timing of hard seed softening within the summer-autumn period, in relation to germination-inducing rainfall events, is crucial in determining the chances of successful plant establishment. Species differ in the timing when most of their hard seeds soften, with the major softening event in some species (including gland clover burr medics, bladder clover and messina) occurring a few weeks later than subterranean clover, balansa clover and barrel medics (Smith *et al.* 1996; Norman *et al.* 1998, 2006; Loi *et al.* 2005; Nichols *et al.* 2007, 2009a). Such pasture legumes suffer fewer losses following false breaks to the season, and can produce a legume-dominant stand when the true break of season occurs.

Genotypic differences in the timing of hard seed softening are known in subterranean clover, in which the main seed softening event occurs 2-3 weeks later than others (Smith *et al.* 1996; Norman *et al.* 2006; P.F. Smith, unpublished data) and it is possible that further delay in softening exists in some accessions. Selection for delayed seed softening would confer increased protection against seedling losses from false breaks in early-mid autumn, by deferring the majority of germination until mid-late autumn, when follow up rainfall is more likely. Although it has been recognised as an important trait for environments with unreliable autumn rainfall, the lack of a rapid screening technique and information on its genetics has prevented it from being considered as a breeding objective to date. On the other hand, in more favourable environments, where false break frequency is low and there is a high probability of reliable follow up rainfall (Evans and Smith 1999), such a germination mechanism is less important.

This project will examine diversity for the timing of seed softening. It will focus on the subterranean core collection, but also include other important species. Initial phenotyping will be conducted using samples softened in the field, using the methods of Taylor (2005), Loi *et al.* (2005), Norman *et al.* (2006) and Nichols *et al.* (2009b). In this process, freshly harvested seeds are divided into equal seed lots, which are placed in mesh envelopes on the soil surface. Seed lots are removed from the field at regular intervals until early winter and subjected to germination tests in the laboratory to count the proportions of hard and soft seeds. This is a time consuming and labour-intensive process and a laboratory screening method will be developed to streamline the process. This will be based on the standard procedure for measuring residual hardseed levels in a diurnally fluctuating 60/15°C temperature regime for 16 weeks, designed to simulate the summer soil temperatures experienced on bare soil Quinlivan (1961). Funding for a controlled environment (temperature and humidity) cabinet will be sought as part of this project to enable us to do this work.

Molecular markers for genes associated with hardseededness and seed softening parameters will be developed for subterranean clover. This will enable marker-assisted selection for these traits in segregating breeding populations. This should to lead to major gains in breeding efficiency, as screening for these traits is technically challenging, subject to environmental influences and is labour intensive.

Diversity of agro-morphological traits

This activity will measure the phenotypic diversity of a range of agro-morphological traits among subterranean clover. Annual medics and other legumes used in southern Australia will also be examined, time and resources permitting. Molecular markers for genes associated with important agro-ecological traits will be developed in subterranean clover.

Measurements will be conducted on spaced plants grown in the field. The subterranean core collection and those of other species will be examined. Traits will include: mean seed weight, flowering time, plant diameter, leaf size, petiole length, petiole diameter as seedlings and at the time of first flowering, stem diameter, internode length, peduncle length, peduncle diameter, angle of burr burial at first flowering and plant diameter at maturity. Other qualitative and semi-quantitative traits relating to plant morphology (e.g. leaflet markings and degree of indentation, anthocyanin flecking and flush patterns, hairiness of leaves, petioles, stems and peduncles) will also be recorded. Measurement techniques will be based on those described by Nichols *et al.* (2009a)

Molecular markers for genes associated with measured traits will be developed in subterranean clover. Correlation analyses will also be conducted between morphological traits and other agronomic traits to determine whether selection for difficult traits can be made using morphological markers, instead of (or as well as) molecular markers.

Annual legumes with increased growth at low temperatures
This activity will focus on identifying diversity within subterranean clover and annual medics for increased seedling growth, particularly at low temperatures. Other annual legume species will be considered, time and resources permitting. It has four main aims:

1. Screen a diverse range of germplasm of important annual legume species (particularly annual medics and subterranean clover) to identify accessions with high seedling vigour and dry matter production;
2. Cross high and low early vigour accessions to develop populations for molecular mapping;
3. Generation of genetic maps to identify the genetic control of seedling growth; and
4. Development of molecular markers for high seedling growth.

High vigour lines will be crossed with low vigour lines to develop mapping populations to understand the underlying genetic control of the traits and to develop molecular markers. Closely linked molecular markers will be derived from genome-wide SNP analysis in each mapping population. Outputs of this activity will be molecular selection tools and germplasm that will be handed over to annual legume breeding programs.

Core collections of important annual legume species will be screened for high seedling vigour at relatively cool temperatures. Additional accessions in the genetic resource centres that have previously been observed as having high seedling vigour and winter production will also be included. This work will be carried out in controlled environment rooms where the effect of performance under cool temperatures can be more precisely controlled. The accessions with the best seedling growth in the controlled environment room will have early production validated in the field. Accessions with the best seedling growth and winter dry matter production will be crossed with low vigour accessions. Molecular markers will then be developed for the traits that correlate with high seedling growth. Annual pasture legume breeders will be able to use these markers as selection tools to combine the genes controlling early vigour with other traits required in cultivars.

SARDI has an existing QTL for high seedling dry matter production in a strand medic population. The mapping population used to isolate this QTL will be screened under cool conditions to see if it is still linked to dry matter production. If it is, further work will be conducted to define this QTL more precisely. A QTL for increased seedling dry matter in strand medic will be an early output from this research.

The proposed research program will consist of the following phenotypic and genotypic elements:

- A literature search will be conducted to ensure the most relevant traits for increased early vigour are identified – this will include crop species that have been bred for improved early vigour and will not be restricted to annual legumes;
- Development of a seedling vigour screen to obtain preliminary data for cultivars and highest priority accessions;
- Phenotyping of a Recombinant Inbred Line (RIL) strand medic population for early seedling vigour under cool conditions (SARDI has a QTL for dry matter production);
- Testing will be conducted to see if the QTL for dry matter production in the strand medic RIL population also correlates to early vigour under cool conditions; if it does, the marker density will be increased;
- Germplasm will be sourced from core collections plus accessions that the Australian Pasture Genetic Resource Centres reports as having high seedling vigour and winter production;
- Initial screens will be conducted in controlled environment rooms with cool temperatures (i.e. typical of late autumn);
- Accessions with the best performance in initial screens will be validated in follow up field based screens;
- The best accessions will be crossed with accessions with low early vigour to develop populations for molecular marker work. These will be genotyped using a SNP platform such as Restriction site Associated DNA (RAD) sequencing' providing a high resolution genotype of each individual plant;
- Molecular markers closely linked to each trait will be tested on genetically diverse germplasm (core collections and wider gene pool of genetic resource centres) to confirm that the markers are suitable for trait selection in material that is unrelated to the population parents they were derived from; and
- Apart from close linkage to the trait, low cost per data point is an important criterion for the uptake of the molecular tools developed in this project. Therefore we will adapt the best markers to a high-throughput platform (Biomark system) to minimise costs for routine genotyping.

This activity will develop the following outputs:

1. A QTL for seedling dry matter production in strand medic;
2. Accessions with high early vigour;
3. Phenotypic screening methods;
4. Genetic maps of annual medics and subterranean clover populations; and
5. Molecular markers closely linked to phenotype for high seedling vigour.

Boron tolerance in annual medics

High levels of boron (B) are widespread on neutral to alkaline soils, which are widely distributed in the temperate slopes and plains of Australia (Figure 6). No precise figure is available for the area of land directly affected by B toxicity. However, B toxicity occurs widely on sodic soils, which cover approximately 30% of Australia's agricultural areas (Rengasamy and Olsson 1991). Figures are available for some individual States. In SA alone 4.9 Mha (31%) of the agricultural zone is at risk from B toxicity (SA Soil and Land Program 2007), while in WA, 15% of agricultural soils are considered to be at risk of B toxicity (Lacey and Davies 2009). No figures for areas at risk of B toxicity are available for the other States. However, in Victoria alkaline, sodic soils account for around 19 Mha agricultural land (Ford *et al* 1993).

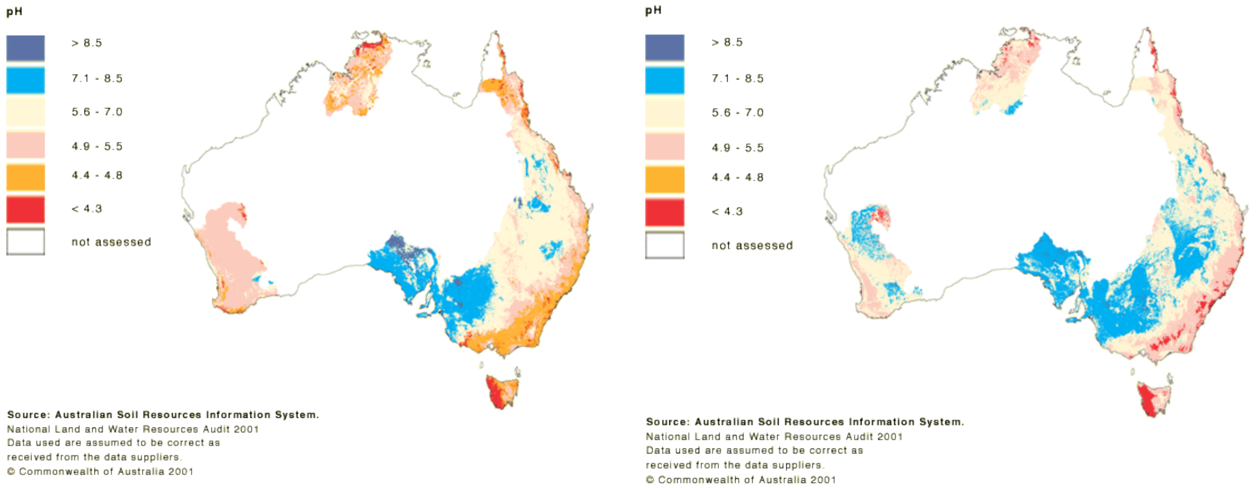


Figure 6. Distribution of pH of the topsoil (left) and subsoil (right) in the agricultural areas of Australia. Data from Australian Natural Resources Atlas (2001).

B tolerance is correlated with increased yields in both cereals (McDonald 2013) and pulses (Paull *et al.* 1992; Yau and Erskine 2000; Hobson *et al.* 2005; Yau and Ryan 2008). Cereal breeders have developed B tolerant cultivars for these soils, with the aid of diagnostic molecular markers (Sutton *et al.* 2007; Reid 2007; Schnurbusch *et al.* 2008, 2010a, 2010b). B tolerant germplasm has been identified in pulse germplasm (Paull *et al.* 1992, Hobson *et al.* 2003, Hobson et al 2005). B tolerance has also been identified as a target trait to improve lentils and field peas and active pre-breeding and breeding efforts are also being undertaken as part of Pulse Breeding Australia (PBA).

Annual medics are the best performing annual pasture legumes on neutral to alkaline soils. As with cereals and pulse crops, medic biomass and seed production is correlated with B tolerance on soils with the high B levels commonly found on many neutral to alkaline soils (Paull *et al.* 1992; Bogacki *et al.* 2013). SARDI has identified a B molecular marker for the B tolerant barrel medic cultivars, Caliph and Paraggio. The molecular marker will make it easy for barrel medic breeders to conserve this trait when breeding new cultivars with other traits. Current burr medic cultivars are all susceptible to high B levels and the lack of B tolerance restricts their wider use (Howie 2012). Current strand medic cultivars were rated as tolerant by Howie (2012), but moderately susceptible by Paull *et al*. (1992). No molecular markers exist at this stage for strand medic cultivars. However, the annual medic species show great genetic similarity and developing a closely linked marker to genes for B tolerance in strand medics will be readily achievable.

Genetic diversity has been described by Paull *et al*. (1992) and three burr medic accessions and five strand medic accessions with B tolerance have been identified. SARDI has an established phenotyping protocol and F_2 plants from a cross between tolerant and intolerant cultivars/accessions could be used to quickly verify the usefulness of the existing B molecular marker for use by annual medic breeders. The successful identification of B tolerant markers within burr medic would be of particular importance, as a breeding program could use the marker to combine B tolerance with the salt tolerance of cultivars Scimitar and Cavalier, resulting in a large improvement in burr medic production and persistence. For example, Nuttal *et al*. (2010) demonstrated that combining B and salinity tolerance in lentils resulted in 1.4 times higher dry matter production and 2.5 times higher grain yield when grown in a hostile subsoil. Seed yield is also very important in annual medics, as it is the single largest driver of persistence and dry matter production in following years.

The proposed research program will consist of the following elements:

- Use the established screening method to phenotype three putative B tolerant burr medic accessions and five putative B tolerant strand medic accessions for B tolerance, and test for gene expression. This will determine the best B tolerant burr and strand medic accessions to use as B tolerant parents;
- Generate F_2 populations by crossing the best B tolerant burr and strand medic parents with susceptible cultivars/accessions;
- Phenotype F_2 populations for B tolerance and test the B molecular marker from barrel medics; and
- Adapt the B marker to the gene sequences in burr and stand medics and convert for future high-throughput analysis.

The outputs from this activity will be accessions with B tolerance and molecular markers closely linked to genes for B tolerance.

PhD/Masters projects
A PhD/Masters student will be sought to for the topic *Yellow serradella genetics and diversity*. The project will develop a core collection of serradellas from the ATGRC collection of more than 1,500 accessions, which can then be used to examine phenotypic and genotypic diversity within the genus and relationships to their eco-geographic origins. This will have a molecular biology component, as polymorphic primers will need to be identified – these molecular markers can be used to map phenotypic traits in the future.

Other students will be sought to work on other areas related to the project. This will expand the capacity of the project and provide training of junior scientists in aspects of pasture legume pre-breeding.

5.3.8 Project outputs

Project outputs will include:

- A measure of the phenotypic diversity of at least 20 key agronomic traits (from this and other related projects), their potential for genetic improvement through plant breeding, and identification of potential parents for crossing;
- Molecular markers in subterranean clover associated with genes for at least 20 key agronomic traits (from this and other related projects) and molecular markers in annual medics associated with genes for seedling growth and boron tolerance;
- A framework to develop molecular markers for new traits in the future;
- A robust protocol to triple generation turnover rates for subterranean clover that can be incorporated into current breeding methodology;
- The first sequenced genome of a *Trifolium* species, which will also have international applications for the breeding of white clover and red clover;
- Publication of at least six scientific papers in high impact, peer-reviewed journals;
- A technical bulletin (likely to be web-based), for distribution to private and public breeding organisations, summarising project results and listing the values of agronomic and morphological traits for diverse germplasm of subterranean clover, annual medics and other annual pasture legumes and the molecular markers closely associated with them; and
- A list and description of any germplasm and breeding lines developed from the project, which will be made available for variety development by private and public breeding organisations;

5.3.9 Linkage to other projects

The subterranean clover core collection is currently being used to examine trait diversity in two other projects:

(i) the MLA-funded project *Phosphorus-efficient legume pasture systems* (B. PUE. 0104). A range of phosphorus use-efficiency and root traits are being examined, including carboxylate composition and concentration, colonisation by arbuscular mycorrhizal fungi (AMF), WinRhizo root length and diameter classes, shoot dry weight, root dry weight, root:shoot dry weight ratios, root P levels, and the shoot levels of P and a range of other elements; and

(ii) the Australian Research Council (ARC)-funded project *Exploiting subterranean clover genetic variation for methane mitigation and ruminant health challenges to the Australian livestock industries*. Traits being examined in this project include levels of the oestrogenic isoflavones (formononetin, genistein and biochanin A) and *in vitro* fermentability parameters (gas pressure and methane production).

Traits measured in these two projects will be incorporated into the molecular marker analyses in this proposed project.

This project is also linked to the MLA-funded project *Managing Soil-borne Root Disease in Sub-clover Pastures* (B.PSP.0005), which is aimed at quantifying productivity losses and better understanding the factors influencing disease expression of pastures in southern Australian and developing practical measures to reduce the impact of soil borne pathogens.

5.3.10 Synergies with the phalaris/cocksfoot pre-breeding program

After discussion with Professor Kevin Smith, Leader of the MLA phalaris/cocksfoot pre-breeding project, it is apparent there are few technical synergies between the two projects. The factors important for persistence and production (particularly in the autumn-winter) of annual legumes are quite different to the perennial grasses. This is dictated largely by their different life cycles; persistence in annual legumes is governed to a large extent by seed and seedling dynamics, whereas persistence (and biomass production in later years) in perennial grasses is largely governed by plant density. The two groups of plants also tend to have different breeding systems, which require different breeding strategies. Phalaris and cocksfoot are out-crossing, and have some commonality with animal breeding. Most of the annual legumes (e.g. subterranean clover, annual medics, serradellas, biserrula), however, are in-breeding and require similar breeding strategies to the winter cereals.

There may be some synergies between the two projects in IP management and commercialisation strategies.

The two project leaders will have the opportunity to liaise and examine the progress of each project through the Pillar Advisory Group established by MLA.

5.3.11 Communications plan

The project will require good external communication between the project team and the meat and seed industries to ensure the project remains relevant, in addition to good internal project communication to ensure it remains on track.

A commercialisation plan will be developed in the first six months of the project to ensure the seed industry is actively engaged in the technology and ready to adopt when it is available. This will be developed in consultation with the ASF and individual seed companies.

The red meat and pasture seed industries will be provided with regular updates on project findings and the implications for their industries. Articles will be prepared for the MLA

Feedback and/or Prograzier magazines to inform graziers about the project. An article is proposed at the commencement of the project to inform graziers about its aims and objectives and annual updates will be prepared. Presentations to the Australian Seeds Federation will be made at some of their Plant Breeders and Proprietary Marketers meetings to inform the pasture seed industry about the project and to obtain feedback. Articles will also be written for their newsletter.

A technical bulletin (likely to be web-based) will be published for use by public and private breeding organisations, summarising project results and listing the values of agronomic and morphological traits for diverse germplasm of subterranean clover, annual medics and other annual pasture legumes and the molecular markers closely associated with them. At least six scientific papers will be published in high impact, peer-reviewed journals.

Internal project communications will involve an annual technical update and planning meeting, at which project results will be discussed and the following year's operational plan developed. A draft operational plan is shown in Table 10; this will be updated and refined at the annual meetings. These meetings will involve project staff, MLA representatives and other participants as required. Meeting reports and the annual operational plan will be presented to MLA for ratification. Quarterly teleconferences of the project team will be held in between these planning meetings.

5.3.12 Project outcomes and benefits

The main project outcome will be the ability of annual pasture legume breeding programs to increase the rate of genetic gain for increased productivity, sustainability and profitability of red meat production. This will be achieved by a greater understanding of the diversity of important agronomic and morphological traits, the development of molecular markers associated with them and rapid generation cycling technology. These technologies will be directly applicable to subterranean clover and annual medics, as prior R & D into these species can now be capitalised upon. The technologies will also be available for use in other legumes in the future.

The identification of molecular markers for a range of traits will provide pasture breeders with the potential to conduct marker-assisted selection (MAS) for each of these traits. This makes the technology very powerful, as screening can be conducted for each of these traits simultaneously. Even if only moderate improvements can be made in individual traits, the additive effects will lead to large genetic gains overall. The value of molecular markers is increased when they are associated with traits that are technically difficult or costly to phenotype. This is certainly the case with most of the traits under consideration in this project. Development of this technology for breeding is now readily achievable in annual medics and subterranean clover, due to recent major developments in defining the genomes of these species and in generating a high density of molecular markers. Further molecular work in other annual legumes will also allow these technologies to be used on them in the future.

The development of rapid generation turn-over technology will deliver to breeding organisations the ability to breed cultivars more rapidly. This will overcome one of the biggest constraints in breeding self-pollinated species - the time taken to progress from the F_1 to F_6 generations, leading to a bottleneck in cultivar development. This arises because generally only a single generation per year is possible, due to issues such as embryo dormancy, vernalisation requirements for over-summer field seed increase and a lack of glasshouse space. Rapid generation technologies will reduce the time taken to develop homozygous F_6 lines and have a major impact on the time taken to develop cultivars. Having access to such technology provides an important strategy for rapid cultivar release to counter new industry threats, such as a devastating new pest or disease. It can also be used for research purposes in the rapid production of Recombinant Inbred Lines (RILs), which can be used in genetic mapping studies.

A further project outcome will be the training and further development of junior scientists in important aspects of annual legume pre-breeding. This succession planning is important to the red meat industry, as it will ensure the necessary skills are present in the future to enable it to further increase pasture productivity and to respond to any pasture production threats.

Technology use in other species
No pasture legume cultivars have been released to date globally as a result of using MAS. This is largely because the most important species internationally (lucerne, white clover and red clover) are genetically complex, making molecular biology techniques difficult to apply. Considerable molecular biology research is being conducted globally on barrel medic (*M. truncatula*), due to its acceptance as a model legume. However, much of this is fundamental research, with little or no connection to applied plant breeding programs. Molecular marker technology has been used in barrel medic by SARDI to confirm sulfonylurea herbicide tolerance (Oldach *et al.* 2008) and boron tolerance (Howie 2012; Bogacki *et al.* 2013). The techniques are too new in subterranean clover and other pasture legumes to have been implemented. The recent DNA sequencing of the *M. truncatula* genome (Young et al. 2011) and the pending sequencing (subject to funding) of subterranean clover will greatly facilitate marker development for important QTLs.

Use of MAS is increasingly being used in crop improvement programs. For example, the DAFWA lupin breeding program is using molecular markers routinely to select for resistance to anthracnose disease (*Colletotrichum gloeosporioides*) and there are numerous other examples.

5.3.13 Meeting MLA Feedbase Investment Program goals

The MLA goal for the FIP is for pasture improvement to add $25m per year on-farm value by 2020, with meat production (kg/ha) increasing by 2.5% and no decline in sustainability indicators. This project will make a major contribution towards this goal. It will focus on annual legume traits that can increase persistence and autumn-winter biomass production in regenerating stands, which has been shown to have the biggest positive impact on pasture productivity and profitability (Figure 3). The high nutritive value of legumes means that increased legume content increases overall feeding value of the pasture. Thus, a high legume density early in the growing season has a positive flow-on effect during the season from better competitiveness against weeds, resulting in greater legume dominance into spring. The development of new plant breeding tools means that improved pasture legume genetics can be delivered more rapidly to graziers and improvement can be made in several traits simultaneously. It is anticipated that new cultivars resulting from technologies developed in this project could be developed for commercialisation by 2020 with seed being available to farmers 2-3 years afterwards.

The support of the private seed companies in this project will result in greater awareness of project outputs and their uses and benefits. Providing all sectors of the pasture seed industry supply chain with access to the outputs of this project will potentially result in greater competition in cultivar development, with the prospect of increased genetic gains. If clear advantages can be demonstrated to farmers of cultivars bred using the technologies developed in this project, it is likely to result in higher re-sowing rates.

Improved annual pasture legume genetics will be part of the overall package delivered by the FIP. This will be coupled with the development of improved perennial grasses, new pasture and grazing management packages and outputs from other projects delivered through the FIP.

5.3.14 Response from the public and private sectors

Initial response from private companies
Telephone discussions with Seed Force, Heritage Seeds, PGG Wrightsons and Pristine Forage Technologies all indicated strong support for this proposed project. The following comments were received.

"This is good public good research and something MLA should be involved with."
"The focus on improving seedling regeneration and early season growth makes sense, as this is the time when farmers most need feed."
"The project is attractive because the outputs will be freely available to all sectors of the market."
"Anything that speeds up genetic gain is good for the seed industry and for farmers."
"If costs of using this technology are reasonable, it will make sense to use it for cultivar improvement."
"We will want to ensure our breeding partners are aware of the information that will come from this project".
"The project will lead to better utilisation of material in the Genetic Resource Centres."
"We want to stay engaged with MLA and are happy to back public good research such as this."

As discussed previously pre-breeding and plant breeding of annual legumes is conducted predominantly by the public sector, often in partnership with private seed companies, who are involved in the field evaluation and commercialisation stages. Pristine Forage Technologies is the only private company currently conducting breeding in annual legumes (annual medics and balansa clover). Seed Force, Heritage Seeds and PGG Wrightsons indicated they would use this technology through their public agency partners, although the potential exists for this work to be done 'in-house' by Seed Force and Heritage Seeds through their international backing companies. Each of the private companies indicated that use of the technologies developed in this project, however, would be business decisions, based on potential returns on investment. This would depend on the costs of using the technologies and the perceived benefits.

Subsequent written response from Pristine Forage Technologies
A response to written comments by Pristine Forage Technologies is included at the end of the report.

Response from public sector breeders
The main public sector breeding agencies for annual pasture legumes are DAFWA and SARDI, with some activity by TIA in alternative legumes for long growing seasons. Each of these agencies have a partnership with a private company, with the public organisation providing the skills and infra-structure for breeding and pre-breeding. Both DAFWA and SARDI have indicated a strong interest in using the outputs from this project. With the cost of molecular technologies continuing to decline, their application is becoming increasingly affordable. The identification of molecular markers for a range of traits will enable screening to be conducted for each of these traits simultaneously. SARDI already uses marker-assisted selection for some traits in barrel medics and their application is likely to increase. DAFWA currently uses molecular markers, developed in partnership with CLIMA, in its lupin breeding program. The relationship with CLIMA will also lead to use of the molecular technologies, developed in this project, for subterranean clover improvement. Further molecular work in other annual legumes will also allow these technologies to be used on them in the future.

The development of rapid generation turn-over technology will have a major impact on the time taken to develop cultivars. This will be particularly important to combat any devastating new pest, disease or some other threat to the feedbase.

It is important to note that these technologies will provide tools to enhance plant breeding, and not replace it. Breeding lines that have been selected using these technologies will require validation in the field to ensure the traits of interest have been improved and that they are adapted to intended target environments and farming systems. However, this can be conducted on a smaller and more focussed group of material than is currently possible.

5.3.15 Project IP management and ownership

This project will essentially conduct public good research, with the main intellectual property (IP) developed being new knowledge. This will take the form of:

- Phenotypic values of measured traits of core collection lines, genetic mapping populations and commercial cultivars;
- Molecular markers associated with traits of interest; and
- Methodologies associated with *in vitro* rapid generation turn-over, molecular marker and trait screening technologies.

This new knowledge will be published in the form of a technical bulletin, which will be freely available to all public and private sector breeders for the ultimate benefit of Australia's livestock industries. It will also be published for a scientific audience in high impact, peer-reviewed journals.

This project is unlikely to develop breeding lines with traits of interest in adapted germplasm that can be directly used by breeding organisations for variety development; this is more likely to occur in a subsequent project. If such germplasm was to be developed in this project, the IP implications would need to be dealt with on a case by case basis.

5.3.16 Potential risk factors to project outcomes

Staffing risks
There is some risk arising from potential loss of key competencies. A key strength of this project is the experience and international standing of the key personnel. Although not particularly likely, large changes in the personnel on the project could inhibit progress. This is thought to be unlikely over the life of the project.
Another risk is the inability to attract suitable candidates for the Research Officer essential for the conduct of this project or the proposed PhD/Masters project.

Technical risks
There is a risk that the envisaged technologies and methodologies will not work on the selected species and germplasm. This risk is low, as considerable preliminary R & D has been conducted to date to give confidence that they will work.
There is a low-medium risk that priority traits will not show sufficient variation for plant breeding to make a major difference to plant improvement.

IP and seed access risks
There will be no restriction on use of cultivars in this project, even if they are trade-marked or under PBR protection. There will be ready access to the core collections and other germplasm held in Australian genetic resource centres The agreement to fund the national pasture genetic resource centre provides free access to material for projects funded by the Rural Industry Research Corporations (including MLA).

The background IP in molecular biology relevant to this project is in the public domain and the project has freedom to use it.

Adoption risks

There is a risk that pasture breeding programs will fail to adopt the new technology developed in this project. This could arise because the technology costs are too high or too complex for commercial use. This risk will be minimised by the development of a commercialisation plan to ensure the seed industry is actively engaged in the technology and ready to adopt it when it is available.

No risks are associated with human, material or financial resources if full funding is provided.

Table 10. Draft operating plan of proposed R & D activities and project outputs.

Activity	2013/14	2014/15	2015/16	2016/17	Outputs
1. Molecular markers for key phenotypic traits in subterranean clover (CLIMA)	Grow core collection and control cultivars for DNA collection	Conduct bio-informatics to develop HapMaps	Convert SNP markers to PCR markers for industry use	Prepare manuscripts for peer-reviewed papers	*The first sequenced genome of a Trifolium species*
	Commence HapMap development	Develop database for association mapping	Conduct association mapping of all phenotypic traits (from this and related projects) to molecular markers	Conduct correlation analyses between key phenotypic traits	*Publication of PCR markers for at least 20 phenotypic traits (from this and other related projects) of subterranean clover, for use by public and private breeding organisations*
	Conduct whole genome sequencing				*An understanding of the relationships between key traits of subterranean clover*
2. Can we identify genes for persistence in subterranean clover? (CLIMA)			Conduct KSAP genotyping of bulk-hybrid populations	Identify molecular markers associated with persistence in subterranean clover	*Molecular markers for genes associated with persistence in subterranean clover*
3. In vitro tools to reduce cultivar development time in subterranean clover (CLIMA)	Develop protocols for rapid generation turn-over on a representative sample of subterranean clovers	Refine protocols using an expanded range of subterranean clovers to cover the 3 subspecies and different flowering times			*A robust protocol to triple generation turnover rates for subterranean clover that can be incorporated into current breeding methodology*
4. Diversity for RLEM cotyledon resistance in key pasture legume species (DAFWA)	Refine screening methodologies	Screen core collections, parents of genetically characterised lines, and cultivars of subterranean clover, barrel medic, strand medic and burr medic and gland clover with RLEM	Screen core collections and cultivars of biserrula, bladder clover and lines of balansa clover, arrowleaf clover and other species with RLEM	Screen core collections and cultivars of French serradella and yellow serradella with RLEM	*A measure of the phenotypic diversity for RLEM cotyledon resistance, its potential for genetic improvement through plant breeding, and identification of potential parents for crossing, among the important annual pasture legume species used in southern Australia*
			Screen F2 mapping population Denmark x DGI007 with RLEM to identify genes associated with RLEM resistance		*Molecular markers for genes associated with RLEM cotyledon resistance in subterranean clover*

Activity	2013/14	2014/15	2015/16	2016/17	Outputs
			Conduct association mapping to identify molecular markers and genes associated with RLEM cotyledon resistance (in conjunction with CLIMA biotechnologist)		
5. Identification of genes for RLEM resistance in gland clover (DAFWA)	Apply mutagenic agents to Prima gland clover to induce RLEM susceptible mutants	Grow out M_1 seed and harvest M_2 seed	Screen M_2 seeds with RLEM, identify, rescue and produce seed of susceptible plants	Re-screen susceptible lines with RLEM, to confirm susceptibility and produce seed from them	*An RLEM susceptible gland clover plant ready to cross with Prima gland clover, to enable identification of genes for RLEM resistance for future incorporation into a range of pasture (and grain) legume species*
6. Diversity of agro-morphological traits within subterranean clover (DAFWA)		Conduct measurements on at least 15 key agro-morphological traits of the subterranean clover core collection and cultivars	Conduct association mapping to identify molecular markers and genes associated with key agro-morphological traits in subterranean clover (in conjunction with CLIMA biotechnologist)		*A measure of the phenotypic diversity of key agro-morphological traits in subterranean clover*
7. Annual legumes with false-break tolerance and seed dormancy traits for increased persistence (DAFWA)	Appoint Junior RO	Grow subterranean clover core collection and cultivars for seed production	Grow barrel medic core collection, parents of genetically characterised lines, and cultivars for seed production		*A scientist trained in annual legume physiology, agronomy and genetic resources*
	Grow subterranean clover core collection and cultivars for seed production	Determine hard seed softening patterns of early flowering subterranean clover core collection lines and cultivars over the summer-autumn period	Determine hard seed softening patterns of barrel medic core collection lines, parents of genetically characterised lines, and cultivars over the summer-autumn period		*A measure of the phenotypic diversity of seed dormancy traits among subterranean clover, annual medics and other important annual legumes*

Activity	2013/14	2014/15	2015/16	2016/17	Outputs
	Determine hard seed softening patterns of early flowering subterranean clover core collection lines and cultivars over the summer-autumn period	Refine temperature cabinet screening protocols	Conduct association mapping to identify molecular markers and genes associated with false break tolerance, hardseededness and other dormancy traits in subterranean clover (in conjunction with CLIMA biotechnologist)		*A technique for rapid screening of annual legumes for false break tolerance and other seed dormancy traits*
	Develop temperature cabinet protocols for screening annual legumes for hard seed softening patterns over the summer-autumn period				*Molecular markers for genes associated with false break tolerance, hardseededness and other seed dormancy traits in subterranean clover*
8. Annual legumes with increased seedling growth at cool temperatures (SARDI)	Conduct literature review	Conduct genetic analyses to validate high dry matter QTL for strand medic under cool temperatures	Screen vigour	Complete genetic analyses to identify molecular markers associated with seedling biomass production in annual medics	*A measure of the phenotypic diversity for seedling growth parameters, the potential for genetic improvement through plant breeding, and identification of potential parents for crossing, among annual medics and subterranean clover*
	Develop seedling biomass screening protocols in a controlled environment phytotron	Complete screening of annual medics and subterranean clover for seedling growth under cool temperatures	Screen F2 medic mapping populations for seedling growth parameters		*Molecular markers for genes associated with increased seedling biomass in annual medics and subterranean clover*
	Measure growth parameters under cool conditions of a strand medic inbred line mapping population with a QTL for high biomass production	Confirm phytotron screening results in the field	Commence search for molecular markers for seedling biomass production		
	Commence screening the core collections, parents of genetically characterised lines, and cultivars of subterranean clover, barrel medic, burr	Initiate crosses between lines with high and low seedling growth to develop genetic mapping populations	Conduct association mapping to identify molecular markers and genes associated with seedling vigour in subterranean clover (in conjunction with CLIMA		

Activity	2013/14	2014/15	2015/16	2016/17	Outputs
	medic and strand medic for seedling growth under cool temperatures		biotechnologist)		
9. Boron tolerance in burr and strand medics (SARDI)	Confirm suitability of B tolerant and susceptible parent lines of burr medic and strand medic Initiate crosses between B tolerant and B sensitive parents to develop genetic mapping populations	Screen F2 mapping populations for B tolerance Commence genetic analysis of B tolerance Confirm the B tolerance genetic marker from barrel medic can be applied to burr and strand medics	Adapt the B marker to the gene sequences in burr and strand medics and convert for future high throughput analysis		Identification of burr and strand medic accessions with boron tolerance Molecular markers associated with B tolerance in burr and strand medics
10. Project communication	Development of a commercialisation plan to ensure the seed industry is actively engaged in the technology and ready to adopt when it is available Quarterly teleconferences involving project staff, MLA and other participants as required Project meeting (Feb 2014) to review progress and develop operational plan for 2014/15 Presentation to the Australian Seeds Federation on the aims and objectives of the project	Quarterly teleconferences involving project staff, MLA and other participants as required Project meeting (Feb 2015) to review progress and develop operational plan for 2015/16	Quarterly teleconferences involving project staff, MLA and other participants as required Project meeting (Feb 2016) to review progress and develop operational plan for 2016/17	Quarterly teleconferences involving project staff, MLA and other participants as required Presentation to the Australian Seeds Federation on the main project outcomes and their implications for the pasture seed industry	*A final report to MLA outlining the project outcomes and their implications to the red meat and pasture seed industries* *A technical bulletin (likely to be web-based) for use by public and private breeding organisations, listing the values at least 20 key agronomic traits, and molecular markers closely associated with them, for subterranean clover, annual medics and other annual pasture legumes*

Activity	2013/14	2014/15	2015/16	2016/17	Outputs
	Article written for the MLA Feedback or Groundcover magazine to inform graziers about the aims and objectives of the project	Article written for the MLA Feedback or Groundcover magazine on important findings from the project	Article written for the MLA Feedback or Groundcover magazine on important findings from the project	Article written for the MLA Feedback or Groundcover magazine to inform graziers about the project outcomes and their implications for the red meat industry	*Publication of at least six peer-reviewed scientific papers*

5.3.17 Budget Summary

	2013/14	2014/15	2015/16	2016/17	TOTAL
MLA	**457,787**	**400,175**	**452,629**	**192,275**	**1,502,865**
DAFWA	289,982	319,371	346,785	183,795	1,139,933
SARDI	41,741	42,810	59,442	29,431	173,424
CLIMA	184,816	192,612	203,167	74,791	655,386
NSW DPI	17,746	18,824	19,961	21,159	77,690
Total Research Organisation contributions	**534,285**	**573,617**	**629,355**	**309,176**	**2,046,433**

5.3.18 Interest (IP proportions)

MLA	42.3%
Research Organisations	57.7%

5.4 Draft Milestone table

Milestone Number	Achievement criteria	Due date
1	Project contract signed with MLA	31/07/13
2	Project staff appointed	31/09/13
3	Development of a commercialisation plan to ensure the seed industry is actively engaged in the technology and ready to adopt when it is available	31/12/13
4	Article on the aims and objectives of the project submitted for publication in the MLA Feedback or Prograzier magazine	31/12/13
5	Genome sequence of subterranean clover described A report on a project update and planning meeting with an outline of the operational plan for 2014/15	31/04/14
6	A robust *in vitro* protocol to enable at least three generations per year in subterranean clover A report on the diversity of at least 15 agro-morphological traits in subterranean clover Complete development of F_2 burr medic and strand medic mapping populations segregating for boron tolerance Article on project findings submitted for publication in the MLA Feedback or Prograzier magazine	31/12/14
7	A report on a project update and planning meeting with an outline of the operational plan for 2015/16	31/04/15
8	Complete HapMap development of subterranean clover core collection lines Complete screening of annual medics and subterranean clover for seedling growth under cool temperatures Complete genetic analyses of high dry matter QTL in strand medic under cool temperatures A report on the diversity of seed dormancy traits in subterranean clover	31/07/15
9	Identification of molecular markers associated with genes for at least 20 agronomic traits in subterranean clover Boron tolerance molecular marker from barrel medic adapted for high throughput screening of burr and strand medic populations A report on the diversity for RLEM cotyledon resistance in subterranean clover Initial screening completed to identify gland clover seedlings susceptible to RLEM Article on project findings submitted for publication in the MLA Feedback or Prograzier magazine	31/12/15

Milestone Number	Achievement criteria	Due date
10	A report on a project update and planning meeting with an outline of the operational plan for 2016/17	31/04/16
11	PCR markers of key phenotypic traits of subterranean clover available for industry use A report on the diversity of seed dormancy traits in other annual pasture legume species	31/07/16
12	Molecular markers for genes associated with persistence of subterranean clover identified Molecular markers for genes associated with seedling vigour in annual medics identified A report on the diversity for RLEM cotyledon resistance among other annual pasture legume species A report on the development of RLEM susceptible gland clover plants to enable identification of genes for RLEM resistance	31/12/16
13	Final project report submitted to MLA Publication of a technical bulletin summarising project results on key traits and the molecular markers closely associated with them, for use by public and private breeders At least six scientific peer-reviewed papers submitted for publication Article on project findings submitted for publication in the MLA Feedback or Prograzier magazine	31/03/17

6. Response to comments following stakeholder consultation

Differences in the perceived capacity of organisations to deliver on particular outcomes within this plan

Public and private breeding organisations were consulted to discuss their capacities, abilities and experience in the areas of annual pasture legume pre-breeding, plant breeding and selection, and field evaluation and commercialisation. These ratings were provided in Table 9. In the main, it appears the majority of organisations were satisfied with the ratings given. However, it is acknowledged that DPI Vic has a high capacity and ability to conduct morphological and molecular characterisation of plant species. It is also recognised that Pristine Forage has high skills and experience in annual medic and balansa clover morphological characterisation, breeding and selection. These have been adjusted in the revised version of Table 12.

Justification why much of the proposed expenditure will be directed towards subterranean clover at the expense of medic species

The decision to direct much of the proposed expenditure to subterranean clover, at the expense of annual medics, has been carefully considered and can be readily justified. A section in the Background of this report has been added to explain the roles of subterranean clover, annual medics and alternative species in Australia's farming systems. Also included is a table showing certified seed sales of the different pasture legumes (Table 2) and a table that estimates sheep and cattle numbers on land best suited to subterranean clover, annual medics or alternative legumes (Table 3).

The reasons for the focus on subterranean clover in this project are:

- Subterranean clover is the most widely sown pasture legume species in southern Australia (Hill and Donald 1998), grown on suitable soils in rainfall zones ranging from 300-1200 mm annual average rainfall (AAR). Annual medics, on the other hand, are only sown in the low and medium rainfall zones (250-450 mm AAR). Clearly subterranean clover has a much wider area of impact and is spread across a wider range of landscapes and farming systems.
- Certified seed sales obtained from the Australian Seeds Authority (2013) shows that subterranean clover has been the dominant pasture legume species in commerce over the past 5 years, with total seed production being 65.6% of all pasture legumes (Table 2). This compares with only 7.8% for all annual medics.
- Animal production estimates attributable to different pasture types suggests that approximately 58% of the Australia's sheep and 29% of cattle are produced on land best suited to subterranean clover, whereas 19% of sheep and 7% of cattle are produced on land best suited to annual medics (Table 3).
- The summary of participant responses at the Annual Legumes Traits and Species Pre-breeding Workshop suggested the overall funding weight should be approximately four times more for subterranean clover than annual medics (Table 10).
- In the case of the molecular biology work, the CLIMA and KDRI laboratories are the only ones globally working on subterranean clover. This compares with barrel medic, which is being researched in many laboratories world-wide, due to its role as a model legume.

It is important to note the project will also focus some areas on alternative legumes, as well as subterranean clover and annual medics. These species include balansa, Persian, bladder and gland clovers, biserrula and yellow and French serradellas.

Why is much of the work being done in WA?

Firstly, the distinction needs to be made between the work location and where the outputs will be delivered. While WA is a long way from the east coast, the outcomes from the project will clearly benefit eastern Australia. An example of this has been the development of pasture legume cultivars. In the case of subterranean clover, the vast majority of cultivars have been developed in WA, whereas most seed is sown in eastern Australia.

In terms of the organisations to do the work, DAFWA, in partnership with UWA, has a proud history of delivering improved pasture cultivars and farming systems for the benefit of southern Australian farmers. It has the world's most successful subterranean clover breeding program, and was the intellectual driver, in conjunction with CLIMA, of the highly successful NAPLIP project. With the funding emphasis on subterranean clover in this project, DAFWA has the most experience, the strongest track record, and the most suitable facilities to do the work.

Justification for the subterranean clover molecular biology work being conducted in collaboration with KDRI, Japan

Kazusa DNA Research Institute (KDRI) in Japan is a world leader in plant and human DNA research and genome analysis. It has state of the art new generation genome sequencing, bio-informatics and supercomputing facilities. The project will utilise the close relationship developed between CLIMA and KDRI since 2009, particularly with renowned scientist Dr Sachiko Isobe. Dr Isobe's laboratory has a particular interest in legume genomics and is widely published in high impact journals (see reference list).

CLIMA has collaborated closely with KDRI in two ARC Linkage projects. It has lead to publication of the world's first linkage map for subterranean clover and contributed to development of the subterranean clover core collection. Under this collaboration *de novo* sequencing of subterranean clover has been undertaken, resulting in a genome scaffold and SNP discovery, to generate a very high resolution QTL map. This proposed project will build on this relationship and skills-base. It will use the high quality molecular datasets to sequence the whole subterranean clover genome, develop a core collection HapMap, identify molecular markers associated with important traits, and conduct genotyping on bulk-hybrid populations to identify genes for persistence. KDRI and CLIMA are the only laboratories globally that are conducting molecular research in subterranean clover.

In Japan the project intends only to undertake gene sequencing for the core collection HapMap and for KASP genotyping on bulk-hybrid populations. In Australia, CLIMA will analyse the sequence data with a remote log-in to the KDRI super-computer facility, working closely with their bio-informatics team and will also undertake subsequent marker development.

Comparison with Australian laboratories
There are several other laboratories in Australia with capacity and skills in molecular biology that could do the work. DPI Victoria, for example, has world-class facilities and capabilities. Preliminary discussions were held with DPIV as a potential project partner, but it became apparent that annual legumes were not among the mandated species for research and that their main focus would be on the perennial grass pre-breeding project.

The CLIMA/KDRI partnership has several advantages, from both productivity and cost perspectives. The team already has an established track record of successful molecular research outputs in subterranean clover. The team is highly familiar with the species, the relevant germplasm and molecular markers and research techniques. Conversely, it would take a new team several months to gain such familiarity, resulting in a much slower rate of progress. KDRI also brings considerable in-kind to this project. They will cover the operation and maintenance costs of their new generation genome sequencers. They will provide considerable bio-informatics and supercomputing support to handle the huge molecular data-sets generated. This makes this arrangement for sequencing highly cost-effective compared to alternative Australian sequencing service providers.

Justification for research into *in vitro* tools to reduce cultivar development time for subterranean clover and the potential benefits relative to existing technology
Part of the brief from an MLA perspective was that the pre-breeding project should lead to an increase in the rate of genetic gain in pasture plant breeding. To help meet this objective it is planned to develop an *in vitro* protocol to accelerate cultivar development of subterranean clover. The aim is to develop three generations per year, effectively tripling the current generation turnover. The routine growing of more than one generation per year has not been done with subterranean clover breeding material before, due to issues such as the long growing season of many clover lines (late flowering lines for high rainfall areas need >170 days to produce viable seed), the need to overcome temperature-induced embryo dormancy, satisfy vernalisation requirements for over-summer field seed increase, a lack of glasshouse space, or the logistics of field sowing in cooler summer climates. Funding to develop new techniques has not been available until now, and it is only recently that a CLIMA pilot study has developed *in vitro* rapid generation technology that can now be exploited to develop a methodology that can be incorporated into a breeding program.

Multiple generations are possible in annual medics without this technology. In general, annual medics have a shorter growing season (less time to develop viable seeds), have lower vernalisation requirements and little or no embryo dormancy. This makes them amenable to growing more than one generation per year.

The development of this technology will deliver to breeding organisations the ability to breed cultivars more rapidly, and provides an important strategy for rapid cultivar release to counter new industry threats, such as a devastating new pest or disease. The principles are likely to be applicable across other pasture legumes, but each species requires individual growth conditions to optimise flowering times.

Justification for funding to identify genes for RLEM resistance
RLEM are the most significant pest of pasture legumes in southern Australia (Allen 1987; Ridsdill-Smith 1997). They cause high mortality of germinating seedlings, loss of herbage production in winter and spring and a reduction in seed yield. Insecticides have been the main form of control, but some populations have been recently found with resistance to some pyrethroid pesticides (Umina 2007). Species differ in their susceptibility to RLEM, with gland clover having a high level of resistance in both seedling and adult plant stages (Nutt and Loi 2002). Annual medics, lucerne and balansa clover, on the other hand tend to be highly susceptible to damage at the seedling stage.

RLEM resistance at the cotyledon stage has been identified in some accessions of subterranean clover and this has been transferred to adapted germplasm, resulting in release of cultivars Bindoon, Rosabrook and Narrikup. However, little is known about the genetics of RLEM cotyledon resistance in subterranean clover and whether how many different genes may be involved. Observations indicate it is quantitatively inherited, involving several genes. The work proposed in this project intends to gain a better understanding of the genes involved and whether it is possible to pyramid complementary genes to give stronger RLEM resistance. Screening for RLEM resistance is quite laborious. If molecular markers can be identified that are associated with genes for resistance, it provides an opportunity to select for resistant types without having to undergo a bio-assay.

Documentation to ensure that the activities considered in this proposal are not currently underway by commercial seed companies
No commercial seed companies are undertaking any of this work. They do not have the capacity to do it.

What is known about the *ph* gene in medics and can it contribute to this project
According to Pristine Forage (2013) the *ph* gene in annual medics is a recessive gene, which stops the maturing medic pod from dropping off the plant, so that it can be harvested from the fully mature plant with a conventional header. This compares with standard (non-ph) medics, in which pods drop off the plant as they approach maturity, requiring seed harvesting with specialist vacuum harvesters, which is a more expensive, time consuming and difficult process. This can result in less expensive seed for the farmer. Pristine Forage (2013) also claim that “seeds of *ph* medics are larger and more fully formed, making resultant plants more productive, vigorous and competitive than ordinary (non-*ph*) medics”. However, no studies have been conducted to show an association between the ph gene and seedling vigour. Furthermore the *ph* gene has patents pending and the project is not free to work with it.

How important is boron toxicity?
High levels of boron (B) are widespread on neutral to alkaline soils, which are widely distributed in the temperate slopes and plains of Australia (Figure 6). No precise figure is available for the area of land directly affected by B toxicity. However, B toxicity occurs widely on sodic soils, which cover approximately 30% of Australia’s agricultural areas (Rengasamy and Olsson 1991). Figures are available for some individual States. In SA alone 4.9 Mha (31%) of the agricultural zone is at risk from B toxicity (SA Soil and Land Program 2007),

while in WA, 15% of agricultural soils are considered to be at risk of B toxicity (Lacey and Davies 2009). No figures for areas at risk of B toxicity are available for the other States. However, in Victoria alkaline, sodic soils account for around 19 Mha agricultural land (Ford *et al* 1993).

What is the justification for the development of a yellow serradella core collection?
A PhD/Masters student will be sought to for the topic *Yellow serradella genetics and diversity*. This will examine phenotypic and genotypic diversity within the serradellas and relationships to their eco-geographic origin and will also deliver a core collection of the species for systematic screening of new traits. Core collections have been developed for subterranean clover, annual medics, bladder clover and biserrula (which will be used in this project). Yellow serradella is an important annual legume (with a specific adaptation to acidic sandy soils) and there are over 1,500 accessions in the collection, which is too large to screen for new traits. A core collection (which represents the vast majority of the species in a much smaller subset) will enable systematic screening of new traits to determine the extent of variation and the extent to which the trait can be improved through plant breeding. This is a much more efficient process than selecting random accessions. Such a project is an ideal topic to train a scientist in important areas of pasture legume pre-breeding, as it will cover topics including genetic resources, genetics, molecular biology, agronomy and plant breeding. Polymorphic primers will be identified as part of the project; these molecular markers can be used to map phenotypic traits.

Other students will be sought to work on other areas related to the project. This will expand the capacity of the project and provide training of junior scientists in aspects of pasture legume pre-breeding.

7. Response to comments consultation

Rationale for proposed activities, traits and project outcomes
The comment is made that the project tries to do too much and lacks focus. On the contrary, the rationale for project activities and outcomes has been developed in a very systematic and well researched way and is focused on annual pasture legume traits that will increase early season feed production and on the most important species for meat production. The focus on early season feed production has been deliberately chosen, as economic modelling shows that increases in the feed profile at this time of year will have the greatest benefit to meat production and profitability. This prioritisation process commenced with an Annual Legume Traits and Species Workshop involving industry specialists from the fields of pasture breeding and agronomy, animal production and the pasture seed industry (including Pristine Forage). Further consultation occurred with the Australian Seeds Federation (ASF) and with individual seed companies that have an interest in annual legumes (including Pristine Forage).

Legumes and traits for areas of greatest need
The comment is made that the project should have more emphasis on legumes and traits suited to the wheat-sheep zone. However, the project is focused on the regions that produce the most meat and on the species most suited to them (see Table 3). The project will place similar emphasis on traits for both the mixed farming and permanent pasture zones (Table 10) and will focus on the species making the biggest contribution to meat production (Table 3).

Can molecular markers for breeding pasture legumes be justified?
Recent technological advances in legume genomics, particularly in annual medics and subterranean clover, mean that use of molecular markers for trait selection in practical

breeding programs is now possible. This has arisen due to the development of genetic maps and identification of a high density of SNPs and other markers across the genome. While use of molecular markers for a single trait may be difficult to justify, the ability to simultaneously select many markers for several traits makes this much more feasible and cost-effective in breeding program. This is particularly so for traits that are difficult to screen in the field or glasshouse or where their expression is strongly influenced by environmental factors. Molecular markers can be used to both select parents for crossing and to screen progeny containing desirable genes and QTLs for the traits of interest. A consideration of the economics of using molecular markers for the traits examined will be an important part of the discussion in the project final report.

Has this work been done before?
This work has not been done before. Many of the technologies that will deliver planned project outputs have only recently been developed.

Are there alternative, non-genetic strategies to pre-breeding and breeding?
MLA have elected to fund a Pasture breeding and evaluation pillar within the Feedbase Investment Plan (FIP). This pre-breeding project lies within this pillar. MLA is investing in other projects that lie within other pillars of the FIP.

Involvement of private industry
Private industry has been and will continue to be involved in project planning and delivery. Representatives from Pristine Forage and Seed Force were part of the project team that developed this annual legume pre-breeding plan. These and four other companies participated in the trait and species prioritisation process at the Annual Legume Traits and Species Workshop. Further consultation occurred at a whole-of-industry level with the ASF and with individual seed companies to refine the plan.
A commercialisation plan will be developed in the first six months of the project to ensure the pasture seed industry is actively engaged in the technology and ready to adopt when it is available. This will be developed in consultation with the ASF and individual seed companies. The seed industry will be provided with regular updates on project findings and the implications for their industries. Presentations to the Australian Seeds Federation will be made at some of their Plant Breeders and Proprietary Marketers meetings to inform the pasture seed industry about the project and to obtain feedback. Articles will also be written for their newsletter.

Access to genetic traits and existing cultivars
The issue was raised about access to traits and cultivars with IP protection (trade-marked or under PBR). However, this was raised in a plant breeding context, which is outside the scope of this project. This project is concerned with identifying phenotypic diversity for important traits and the genes controlling them and there is no intention to conduct hybridisation that leads to cultivar development *per se*. There will be no restriction on cultivar use for screening traits for diversity. Indeed, it will be important that the most recent cultivars are included as benchmarks.

What will be done to ensure new varieties bred from project outputs are better than existing varieties?
It is envisaged that the outputs from this project will enable pasture breeders to make more informed choices about parents for crossing and to more accurately select for progeny containing desirable genes and QTLs for traits of interest. However, the actual breeding of new varieties is beyond the scope of this project.

Input from a professional breeder/geneticist to monitor and advise on project activities

It was suggested that funding be provided for ongoing part-time input by a professional breeder/geneticist to monitor and advise on project work. The project team consists of professional breeders and geneticists who are widely experienced in both practical and fundamental aspects of plant breeding and are in a strong position to plan and implement the project. The project will also be monitored and advised by Professor Kevin Smith, who is a practising and well-renown pasture breeder and geneticist, in his role as Pillar Leader of the Pasture breeding and evaluation pillar of the FIP. This leadership will ensure the project remains focused and delivers outputs that are aligned with industry needs.

Potential risk factors to project outcomes

A section on potential risks to delivering project outputs has been added to the report.

8. Bibliography

Allen PG (1987) Insect pests of pasture in perspective. In: 'Temperate pastures: their production, use and management'. (Eds JL Wheeler, CJ Pearson, GE Robards) pp. 211-225. (Australian Wool Corporation/CSIRO Australia: Melbourne.)

Ané JM, Zhu H, Frugoli J (2008) Recent Advances in *Medicago truncatula* genomics. *International Journal of Plant Genomics*. Available at: http://www.hindawi.com/journals/ijpg/2008/256597/#B29

Angus JF and Peoples MB (2012) Nitrogen from Australian dryland pastures. *Crop and Pasture Science* **63**, 746-759.

Australian Natural Resources Atlas (2001) Appendix 2. Australian Soil Resources Information System - pH (topsoil, subsoil). Available at: http://www.anra.gov.au/topics/soils/pubs/national/agriculture_asris_ph.html

Australian Seeds Authority (2013) National list of Plant Varieties Eligible for Seed Certification in Australia. Available at: http://aseeds.net.au/eligible-varieties

Barbetti MJ (1989) Fungal diseases of pasture legumes in Western Australia. Department of Agriculture, Western Australia, Bulletin No. 4133, pp.21

Barbetti MJ, MacNish GC (1983) Root rots of subterranean clover. *Journal of Agriculture Western Australia* **1**, 9-10.

Barbetti MJ, Sivasithamparam K, Riley IT, You MP (2005) Role and impact of diseases caused by soil-borne plant pathogens in reducing productivity in southern Australian pasture systems. Meat and Livestock Australia, 80pp.

Barbetti MJ, Sivasithamparam K, Wong DH (1986a) Root rot of subterranean clover. *Review of Plant Pathology* **65**, 287–295.

Barbetti MJ, Wong DH, Sivasithamparam K, D'Antuono MF (1986b). Response of subterranean clover cultivars to root rot fungi. *Annals of Applied Biology* **109**, 259-267.

Barbetti MJ, You MP, Li H, Ma X, Sivasithamparam K (2007) Management of root diseases of annual pasture legumes in Mediterranean ecosystems – a case study of subterranean clover root diseases in the south-west of Western Australia. *Phytopathologia Mediterranea* **46**, 239-258.

Bernardi J, Mazza R, Caruso P, Recupero GR, Marocco A, Licciardello C (2013) Use of an expressed sequence tag-based method for single nucleotide polymorphism identification and discrimination of *Citrus* species and cultivars. *Molecular Breeding* **31**, 705-718.

Bogacki P, Peck DM, Nair RM, Howie J, Oldach KH (2013) Genetic analysis of tolerance to Boron toxicity in the legume *Medicago truncatula*. *BMC Plant Biology* **2013**, 13:54 doi:10.1186/1471-2229-13-54.

Bolland MDA, Gladstones JS (1987) Serradella (*Ornithopus* spp.) as a pasture legume in Australia. *Journal of the Australian Institute of Agricultural Science* **53**, 5–10.

Bonython AL, Ballard RA, Charman N, Nichols PGH, Craig AD (2011) New strains of rhizobia that nodulate regenerating messina (*Melilotus siculus*) plants in saline soils. *Crop and Pasture Science* **62**, 427–436.

Brennan RF, Grimm M (1992) Effect of aphids and mites on herbage and seed production of subterranean clover (cv. Daliak) in response to superphosphate and potash. *Australian Journal of Experimental Agriculture* **32**, 39–47.

Carr SJ, Paterson J (1998) Caprera crimson clover – an affordable pasture for high rainfall areas. Farmnote 31/98, Agriculture Western Australia.

Chapman R, Asseng S (2001) An analysis of the frequency and timing of false break events in the Mediterranean region of Western Australia. *Australian Journal of Agricultural Research* **52**, 367-376.

Choi HK, Kim D, Uhm T, Limpens E, Lim H, Mun JH, Kalo P, Penmetsca RV, Seres A, Kulikova O, Roe BA, Bisseling T, Kiss GB, Cook DR (2004) A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa*. *Genetics* **166**, 1463–1502.

Cocks PS (2001) Ecology of herbaceous perennial legumes: a review of characteristics that may provide management options for the control of salinity and waterlogging in dryland cropping systems. *Australian Journal of Agricultural Research* **52**, 137–151.

Cocks PS, Mathison MJ, Crawford EJ (1980). From wild plants to pasture cultivars: annual medics and subterranean clovers in southern Australia. In ‘Advances in Legume Science’. (Eds. RJ Summerfield, AH Bunting,). (Ministry of Agriculture and Fisheries, London)

Crawford EJ, Lake AWH, Boyce KG (1989) Breeding annual Medicago species for sem-iarid conditions in southern Australia. *Advances in Agronomy* **42**, 399–437.

Dear BS, Ewing MA (2008) The search for new pasture plants to achieve more sustainable production systems in Australia. *Australian Journal of Experimental Agriculture* **48**, 387–396.

Donald CM (1951) Competition among pasture plants. 1. Intra-specific competition among annual pasture plants. *Australian Journal of Agricultural Research* **2**, 355-376.

Donald G (2012) Analysis of feed-base audit. Final report to MLA on project B.PAS.0297.

Doyle PT, Grimm M, Thompson AN (1993) Grazing for pasture and sheep management in the annual pasture zone. In ‘Pasture management technology for the 21st century.’ (Eds. DR Kemp, DL Michalk) pp. 71–90. (CSIRO Australia: Melbourne)

DPI Victoria (2005) Plant breeding: policies and principles for investment. Department of Primary Industries Victoria. Available at: http://www.dpi.vic.gov.au/about-us/publications/economics-and-policy-research/2005-publications/plant-breeding-policies-and-principles-for-investment

Ehrman T, Cocks PS (1996) Reproductive patterns in annual legume species on an aridity gradient. *Vegetation* **122**, 47–59.

Ellwood SR, D’Souza NK, Kamphuis LG, Burgess TI, Nair RM, Oliver RP (2006) SSR analysis of the *Medicago truncatula* SARDI core collection reveals substantial diversity and unusual genotype dispersal throughout the Mediterranean basin. *Theoretical and Applied Genetics* **112**, 977–983.

Evans PM, Kearney GA (2006) Alternative perennial legumes for the high rainfall zone of SE Australia. In 'Proceedings of the 13th Australian Agronomy Conference' (Eds. N Turner, T Acuna), Perth, Western Australia

Evans PM, Smith FA (1999) Patterns of seed softening in subterranean clover in a cool, temperate environment. *Agronomy Journal* **91**, 122-127.

Ewing MA (1999) New pasture species. In 'Proceedings of the 11th Australian Plant Breeding Conference'. Glenelg, South Australia (Eds. P Langridge, A Barr, G Auricht, G Collins, A Granger, D Handford, J Paull) pp. 86–90. (CRC for Molecular Plant Breeding, University of Adelaide)

FAO (2012) Pre-breeding for effective use of plant genetic resources - an e-Learning Course. Available at: http://www.fao.org/fileadmin/templates/agphome/documents/PGR/Announcements/PB-elearningcourse_GIPB_en.pdf (Accessed 27/02/2013)

Ford GW, Martin JJ, Rengasamy P, Boucher SC, Ellington A (1993) Soil sodicity in Victoria. *Australian Journal of Soil Research* **31**, 869-909.

Frankel OH, Brown AHD (1984) Plant genetic resources today: a critical appraisal. In 'Crop genetic resources: conservation and evaluation'. (Eds. JHW Holden, JT Williams) pp. 249–257. (Allen and Unwin: Winchester, Massachusetts, USA)

Gillespie DJ (1991) Identification of resistance to redlegged earth mite *Halotydeus destructor* in pasture legumes. *Plant Protection Quarterly* **6**, 170–171.

Gillespie DJ (1993) Redlegged earth mite (*Halotydeus destructor*) resistance in annual pasture legumes. In: Pests of Pastures: Weed, Invertebrate and Disease Pests of Australian Sheep Pastures. pp. 211-213 (CSIRO, Melbourne, Australia).

Ghamkhar K. Banik B, Durmic Z, Revell C, Erskine W (2012*a*) *Biserrula pelecinus* L. – genetic diversity in a promising pasture legume for the future. *Crop and Pasture Science* **63**, 833-839.

Ghamkhar K, Isobe S, Nichols PGH, Faithfull T, Ryan MH, Snowball R, Sato S, Appels R (2012*b*) The first genetic maps for subterranean clover (*Trifolium subterraneum* L.) and comparative genomics with *T. pratense* L. and *Medicago truncatula* Gaertn. to identify new molecular markers for breeding. *Molecular Breeding* **30**, 213–226.

Ghamkhar K, Snowball R, Bennett SJ (2007) Ecogeographical studies identify diversity and potential gaps in the largest germplasm collection of bladder clover (*Trifolium spumosum* L.). *Australian Journal of Agricultural Research* **58**, 728–738.

GRDC (2012) Grains Research and Development Corporation 2011-2012 Annual Report Appendix B.

Greenhalgh FC, Clarke RG (1985) The use of fungicides to study the significance and etiology of root rot of subterranean clover in dryland pastures of Victoria. In: Ecology and Management of Soil-borne Plant Pathogens. (Eds. Parker CA, Moore KJ, Wong PTW, Rovira AD, JF Kollmorgen), pp. 234-236 (St Paul, U.S.A.; American Phytopathological Society).

Hackney B, Dear B, Crocker G (2007) Berseem clover. New South Wales Department of Primary Industries Primefact 388.

Hall E, Smith S (2005) Fact Sheet: Arrowleaf Clover, Tasmanian Institute of Agricultural Research.

Hill MJ, Donald GE (1998) Australian Temperate Pastures Database. National Pasture Improvement Coordinating Committee/CSIRO Division of Animal Production, published as a compact disc.

Hobson K, Armstrong R, Connor D, Nicolas M, Materne M (2003) Genetic variation in tolerance to high concentrations of soil boron exists in lentil germplasm. In: Solutions for a better environment: Proceedings of the 11th Australian Agronomy Conference, Geelong, Victoria, Australia, 2-6 February 2003.

Hobson K, Armstrong R, Nicolas M, Connor D, Materne M (2006) Response of lentil (*Lens culinaris*) germplasm to high concentrations of soil boron. *Euphytica* **151**, 371-382.

Holland M (2012) Establishing a seed scheme for mixed varieties of subterranean clover. Final report to the Rural Industries Research and Development Corporation on project PRJ 000478.

Howie JH (2012) Boron tolerance in annual medics (*Medicago* spp.). *Crop and Pasture Science*, **63**, 886–892.

Howieson JG, Nutt B, Evans P (2000) Estimation of host-strain compatibility for symbiotic N-fixation between *Rhizobium meliloti*, several annual species of *Medicago* and *Medicago sativa*. *Plant and Soil* **219**, 49–55.

Howieson JG, Yates RJ, Foster KJ, Real D, Besier RB (2008) Prospects for the future use of legumes. In 'Nitrogen-fixing leguminous symbioses'. (Eds' MJ Dilworth, EK James, JI Sprent, WE Newton) pp. 363–393. (Springer)

International HapMap Constortium (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* **449**, 851-862.

Jiang Y, Ridsdill-Smith TJ (1996) Examination of the involvement of mechanical strength in antixenotic resistance of subterranean clover cotyledons to the redlegged earth mite *Halotydeus destructor* (Acarina: Penthaleidae). *Bulletin of Entomological Research* **86**, 263–270.

Jiang Y, Ghisalberti EL, Ridsdill-Smith TJ (1996) Correlation of 1-octen-3-one with antixenotic resistance in subterranean clover cotyledons to redlegged earth mite, *Halotydeus destructor* (Acarina: Penthaleidae). *Journal of Chemical Ecology* **22**, 369–382.

Johnstone GR, Barbetti MJ (1987) Impact of fungal and viral diseases on pasture. *In*: Temperate Pastures, their Production, Use and Management. (Eds JL Wheeler, CJ Pearson, GE Robards), pp. 235-248 (Australian Wool Corporation and CSIRO: Melbourne, Australia).

Kaur P, Erskine W, Appels R, Durmic Z, Nichols P, Isobe S (2013) Comparative genomics in pasture species – a powerful new approach to assist in methane mitigation strategies for Australian livestock industries. Proceedings of Plant and Animal Genome XXI conference, San-Diego, USA.

Katznelson J, Morley FHW (1965) A taxonomic revison of sect. *Calycomorphum* of the genus *Trifolium*. I. The geocarpic species. *Israel Journal of Botany* **14**, 112–134.

Kharkwal MC, Roy D (2004) A century of advances in plant breeding methodologies. In 'Plant Breeding – Mendelian to molecular approaches'. (Ed. HK Jain, MC Kharkwal) pp. 17–48. (Kluwer Academic: Dordrecht, The Netherlands)

Kingwell R, Pannell, D (2005) Economic trends and drivers affecting the wheatbelt of Western Australia to 2030. *Australian Journal of Agricultural Research* **56**, 553–561.

Koné M, Patat-Ochatt EM, Conreux C, Sangwan RS and Ochatt SJ (2007) Faster breeding of Bambara groundnut: mutational cum-*in vitro* approaches. *In*: Breeding of neglected and under-utilized crops, herbs and spcies (Eds. SJ Ochatt, SM Jain) pp. 81-94 (Science Press, Plymouth).

Lacey A, Davies S (2009) Boron toxicity in WA soils. WA Department of Agriculture and Food Farmnote, 388/2009. Available at:

http://grains.agric.wa.gov.au/sites/grains/files/fn_boron_toxicity.pdf (Accessed 23/05/2013)

Lake AWH (1993) *Medicago truncatula* Gaertn. (barrel medic) cv. Mogul. *Australian Journal of Experimental Agriculture* **33**, 823–824.

Lake AWH, Howie JH, Drewry RE, Hill JR, Robinson SS, Schutz PR, Hammer A, Heinrich NB (1997) Register of Australian herbage plant cultivars. B. Legumes. 9. Annual medics. (b). *Medicago littoralis* Rhode (strand medic) cv. Herald. *Australian Journal of Experimental Agriculture* **37**, 609–610.

Lake AWH, Mathison MJ (1989) Register of Australian herbage plant cultivars. B. Legumes. 9. Annual Medics (a) *Medicago littoralis* Rhode (strand medic) cv. Harbinger AR. *Australian Journal of Experimental Agriculture* **29**, 755–756.

Liao M, Fillery IRP, Palta JA (2004). Early vigorous growth is a major factor influencing nitrogen uptake in wheat. *Functional Plant Biology* **31**, 121-129.

Liao M, Palta JA, Fillery IRP (2006) Root characteristics of vigorous wheat improve early nitrogen uptake. *Australian Journal of Agricultural Research* **57**, 1097-1107.

Loi A, Hogg N, Revell C, Federenko D (2010) Growing biserrula to improve grain and livestock production. Department of Agriculture and Food Western Australia Bulletin 4805.

Loi A**,** Howieson JG, Nutt BJ, Carr SJ (2005) A second generation of annual pasture legumes and their potential for inclusion in Mediterranean-type farming systems. *Australian Journal of Experimental Agriculture* **45**, 289–299.

Loi A, Nutt BJ, Howieson JG, Yates RJ, Norman HC (2012) Preliminary assessment of bladder clover (*Trifolium spumosum* L.) as an annual legume for ley farming systems in southern Australia. *Crop and Pasture Science* (in press).

Loi A, Nutt BJ, Revell CK, Snowball R (2007) AGWEST Sothis: *Trifolium dasyurum* (eastern star clover). *Australian Journal of Experimental Agriculture* **47**, 1512–1515.

McDonald GK (1989) The contribution of nitrogen fertiliser to the nitrogen nutrition of rainfed wheat crops in Australia: a review. *Australian Journal of Experimental Agriculture* **20**, 455–481.

McDonald GK, Taylor JD, Verbyla A, Kuchel H (2013) Assessing the importance of subsoil constraints to yield of wheat and its implications for yield improvements. *Crop and Pasture Science* **63**, 1043-1065.

MLA (2011) Market failure assessment framework. Report to Meat & Livestock Australia.

MLA (2012*a*) Sheep numbers as at June 2011 by Natural Resource Management Region. Available at: http://www.mla.com.au/About-the-red-meat-industry/Industry-overview/Sheep

MLA (2012*b*) Cattle numbers as at June 2011 by Natural Resource Management Region. Available at: http://www.mla.com.au/About-the-red-meat-industry/Industry-overview/Cattle

Mun J, Kim D, Choi J, Debellé F, Mudge J, Denny R, Endré G, Saurat O, Dudez A, Kiss, GB, Roe B, Young ND, Cook DR (2006) Distribution of microsatellites in the genome of *Medicago truncatula*: a resource of genetic markers that integrate genetic and physical maps. *Genetics* **172**, 2541–2555.

Nichols PGH, Cocks PS, Francis CM (2009a) Evolution over 16 years in a bulk-hybrid population of subterranean clover (*Trifolium subterraneum* L.) at two contrasting sites in south-western Australia. *Euphytica* **169, 31–48.**

Nichols PGH, Collins WJ, Barbetti MJ (1996) Registered cultivars of subterranean clover - their characteristics, origin and identification. Agriculture Western Australia, Bulletin No. 4327, South Perth, Western Australia.

Nichols PGH, Collins WJ, Gillespie DJ, Barbetti MJ (1994) Developing improved varieties of subterranean clover. *Journal of Agriculture Western Australia* **35**, 60–65.

Nichols PGH, Foster KJ, Piano E, Kaur P, Ghamkhar K, Pecetti L, Collins WJ (2013) Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop and Pasture Science* (submitted).

Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007*b*) New annual and short-lived perennial pasture legumes for Australian agriculture-15 years of revolution. *Field Crops Research* **104**, 10–23.

Nichols PGH, Malik AI, Stockdale M, Colmer TD (2009b). Salt tolerance and avoidance mechanisms at germination of annual pasture legumes and their importance for adaptation to saline environments. *Plant and Soil* **315**, 241–255.

Nichols PGH, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia – their history, current use and future prospects. *Crop and Pasture Science* **63**, 691–725.

Nichols PGH, Teakle NL, Bonython AL, Ballard RA, Charman N, Craig A (2012) Messina (*Melilotus siculus*) – a new annual pasture legume for Mediterranean-type climates with high tolerance of salinity and waterlogging. In '*Options Méditerranéennes* Series A No. 102 - New Approaches for Grassland Research in a Context of Climatic and Socio-Economic Changes', Proceedings of the 14th Meeting of the FAO-CIHEAM sub-network on Mediterranean pastures and fodder crops, Samsun, Turkey (Eds Z Acar, A López-Francos, C Porqueddu) pp. 155-160.

Norman HC, Cocks PS, Galwey NW (2002) Hardseededness in annual clovers: variation between populations from wet and dry environments. *Australian Journal of Agricultural Research* **52**, 821–829.

Norman HC, Cocks PS, Smith FP, Nutt BJ (1998) Reproductive strategies in Mediterranean annual clovers: germination and hardseededness. *Australian Journal of Agricultural Research* **49**, 973–982.

Norman HC, Smith FP, Nichols PGH, Si P, Galwey NW (2006) Variation in seed softening patterns and impact of seed production environment on hardseededness in early maturing genotypes of subterranean clover. *Australian Journal of Agricultural Research* **57**, 65-74.

Nutt BJ, Loi A (2002) Prima gland clover. Farmnote no. 4/2002, Department of Agriculture, Western Australia, Perth, Australia

Nutt B, Loi A, Revell C (2009) French serradella: pasture and fodder legumes for neutral and acidic sandy soils. Farmnote 343, Department of Agriculture and Food Western Australia.

Nuttall JG, Hobson KB, Materne M, Moody DB, Munns R, Armstrong RD (2010) Use of genetic tolerance in grain crops to overcome subsoil constraints in alkaline cropping soils. *Australian Journal of Soil Research* **48**, 188-199.

Ochatt SJ, Sangwan RS, Marget P, Assoumou Ndong Y, Rancillac M, Perney P (2002) New approaches towards the shortening of generation cycles for faster breeding of protein legumes. *Plant Breeding* ***121***, 436-440.

Ochatt SJ, Sangwan RS (2008) *In vitro* shortening of generation time in *Arabidopsis thaliana*. *Plant Cell, Tissue and Organ Culture* **93**, 133-137.

Ochatt SJ, Pontécaille, Rancillac M (2000) The growth regulators used for bud regeneration and shoot rooting affect the competence for flowering and seed set in regenerated plants of protein peas. *In Vitro Cellular Developmental Biology - Plant* **36**, 188-193.

Oldach KH, Peck DM, Cheong J, Williams KH, Nair RM. (2008) Identification of a chemically-induced point mutation mediating herbicide tolerance in annual medics (*Medicago* spp.). *Annals of Botany* **101**, 997–991.

Oram RN (1990) 'Register of Australian Herbage Plant Cultivars, 3rd edition'. (CSIRO Publishing: Canberra)

Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer MF, Watt M (2011) Large root systems: are they useful in adapting wheat to dry environments? *Functional Plant Biology* **38**, 347-354.

Paull JG, Nable RO, Lake AWH, Materne MA, Rathjen AJ (1992) Response of annual medics (*Medicago spp*.) and Field Peas (*Pisum sativum*) to High Concentrations of Boron: Genetic Variation and the Mechanism of Tolerance. *Australian Journal of Agricultural Research* **43**, 203-213.

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Berges H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C. (2008) A Physical Map of the 1-Gigabase Bread Wheat Chromosome 3B. *Science* **322**, 101–104.

PBR database (2013) Plant Breeders Rights database. IP Australia, Commonwealth Government of Australia. Available at: http://www.ipaustralia.gov.au/get-the-right-ip/plant-breeders-rights

Peck DM, Howie JH (2012) Development of an early season barrel medic (Medicago truncatula Gaertn.) with tolerance to sulfonylurea herbicide residues. *Crop and Pasture Science* **63**, 866-874.

Peoples MB, Baldock JA (2001) Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. *Australian Journal of Experimental Agriculture* **41**, 327–346.

Pristine Forage Technologies (2013). Available at: http://www.pristineforage.com.au/index.htm

Puckridge DW, French RJ (1983) The annual legume pasture in cereal-ley farming systems of southern Australia: a review. *Agriculture, Ecosystems and Environment* **9**, 229–267.

Quinlivan BJ (1961) The effect of constant and fluctuating temperatures on the permeability of the hard seeds of some legume species. *Australian Journal of Agricultural Research* **12**, 1009-1022.

Quinlivan BJ (1971*a*) The ecological significance of seed impermeability in the annual legume pastures of WA. Western Australian Department of Agriculture, Bulletin No. 11, South Perth, Western Australia.

Quinlivan BJ (1971*b*) Embryo dormancy in subterranean clover seeds. II. Its value relative to impermeability in field germination regulation. *Australian Journal of Agricultural Research* 22, 607-614.

Quinlivan BJ (1971*c*) Seed coat impermeability in legumes. *Journal of the Australian Institute of Agricultural Science* **37**, 283-295.

Rebetzke GJ, Richards RA (1999) Genetic improvement of early vigour in wheat. *Australian Journal of Agricultural Research* **50**, 291-301.

Reid R (2007) Identification of Boron transporter genes likely to be responsible for tolerance to Boron toxicity in wheat and barley. *Plant Cell Physiology* **48**,1673–1678.

Rengasamy P, Olsson KA (1991) Sodicity and soil structure. *Australian Journal of Soil Research* **29**, 935-52.

Ribalta FM, **Croser JS**, Erskine W, Finnegan P, Ochatt SJ (2013) The antigibberellin Flurprimidol permits *in vitro* flowering and seed-set across a range of pea (*Pisum sativum* L.) genotypes by reducing internode length. *Biologia Plantarum* (in press).

Ridsdill-Smith TJ (1995) Responses and feeding damage of redlegged earth mite (Acarina: Penthaleidae) to seedlings of resistant and susceptible subterranean clover varieties. *Australian Journal of Agricultural Research* **46**, 1091–1099.

Ridsdill-Smith TJ (1997) Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae): a review. *Experimental & Applied Acarology* **21**, 193-223.

Ridsdill-Smith TJ, Nichols PGH (1998) Development of pasture legumes resistant to redlegged earth mite. In: Pest Management – Future Challenges, Proceedings of the 6th Australian Applied Entomology Research Conference. (Eds MP Zalucki, RAI Drew, GG White) Volume 2, pp. 382-389 (Horticultural Research & Development Corporation, Brisbane.)

Russi L, Cocks PS, Roberts EH (1992) Coat thickness and hard-seededness in some *Medicago* and *Trifolium* species. *Seed Science Research* **2**, 243-249.

SA Soil and Land Program (2007) Land and soil spatial data for southern SA – GIS format. (CD-ROM) (Government of South Australia)

Schnurbusch T, Hayes J, Hrmova M, Baumann U, Ramesh SA, Tyerman S, Langridge P, Sutton T (2010a) Boron Toxicity Tolerance in Barley through Reduced Expression of the Multifunctional Aquaporin HvNIP2;1. *Plant Physiology* **153**,1706-1715.

Schnurbusch T, Hayes J, Sutton T (2010b) Boron toxicity tolerance in wheat and barley: Australian perspectives. *Breeding Science* **60**, 297-304

Schnurbusch T, Langridge P, Sutton T (2008) The Bo1-specific PCR marker AWW5L7 is predictive of boron tolerance status in a range of exotic durum and bread wheats. *Genome* **51**,963-971.

Si P, Pan G, Sweetingham M (2011) Semi-dominant genes confer additive tolerance to metribuzin in narrow-leafed lupin (*Lupinus angustifolius* L.) mutants. *Euphytica* **177**, 411-418.

Simpson RJ, Richardson AE, Riley IT, McKay AC, McKay SF, Ballard RA, Ophel-Keller K, Hartley D, O'rourke TA, Sivasithamparam K, Li H, Ryan MH, Barbetti MJ (2011) Damage to roots of *Trifolium subterraneum* L. (subterranean clover), failure of seedlings to establish and the presence of root pathogens during autumn-winter. *Grass and Forage Science* **66**, 585-605.

Skinner DZ, Bauchan GR, Auricht G, Hughes S (1999) A method for the efficient management and utilisation of large germplasm collections. *Crop Science* **39**, 1237-1242.

Smith FP, Cocks PS, Ewing MA (1996) Short-term patterns of seed softening in *Trifolium subterraneum*, *T. glomeratum* and *Medicago polymorpha*. *Australian Journal of Agricultural Research* **47**, 775-785.

Snowball R, Revell C (2011) Cefalu arrowleaf clover. Farmnote No 503, Department of Agriculture and Food Western Australia.

Sutton T, Baumann U, Hayes J, Collins NC, Shi B-J, Schnurbusch T, Hay A, Mayo G, Pallotta M, Tester M, Langridge P (2007) Boron-toxicity tolerance in barley arising from efflux transporter amplification. *Science* **318**, 1446–1449.

Taylor GB (1981) Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. *Australian Journal of Plant Physiology* **8**, 547-558.

Taylor GB (1984) Effect of burial on the softening of hard seeds of subterranean clover. *Australian Journal of Agricultural Research* **35**, 201-210.

Taylor GB (2005) Hardessededness in Mediterranean annual legumes in Australia: a review. *Australian Journal of Agricultural Research* **56**, 645–661.

Taylor GB, Ewing MA (1992) Long-term patterns of seed softening in some annual pasture legumes in a low rainfall environment. *Australian Journal of Experimental Agriculture* **32**, 331-337.

Taylor GB, Maller RA, Rossiter RC (1991) A model describing the influence of hardseededness on the persistence of an annual forage legume, in a ley farming system, in a mediterranean-type environment. *Agriculture, Ecosystems and Environment* **37**, 276-301.

Taylor GB, Rossiter RC, Palmer MJ (1984) Long term patterns of seed softening and seedling establishment from single seed crops of subterranean clover. *Australian Journal of Experimental Agriculture and Animal Husbandry* **24**, 200-212.

Taylor PA, Greenhalgh FC (1987) Significance, causes and control of root rots of subterranean clover. In: Temperate Pastures: their Production, Use and Management. (Eds JL Wheeler, CJ Pearson, GE Robards), pp. 249-251 (Australian Wool Corporation and CSIRO: Melbourne, Australia).

Thomas DT, Milton JTB, Revell CK, Ewing MA, Dynes RA, Murray K, Lindsay DR (2010) Preference of sheep among annual legumes is more closely related to plant nutritive characteristics as plants mature. *Animal Production Science* **50**, 114–123.

Thoquet P, Ghérardi M, Journet EP, Kereszt A, Ané JM, Prosperi JM, Huguet T (2002) The molecular genetic linkage map of the model legume *Medicago truncatula*: an essential tool for comparative legume genomics and the isolation of agronomically important genes. *BMC Plant Biology* **2**, 1, Available at: http://www.biomedcentral.com/1471-2229/2/1

Umina PA (2007) Pyrethroid resistance discovered in a major agricultural pest in southern Australian: the redlegged earth mite *Halotydeus destructor* (Acari: Penthaleidae). *Pest Management Science* **63**, 1185–1190.

Varshney, RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for plant breeding. *Trends in Biotechnology* **27**, 522–530.

Wallace MMH, Mahon JA (1963) The effect of insecticide treatment on the yield and botanical composition of sown pastures in Western Australia.*Australian Journal of Experimental Agriculture and Animal Husbandry* **3**, 39-50.

Wallace MMH, Mahon JA (1971) The distribution of *Halotydeus destructor* and *Penthaleus major* (Acari: Eupodidae) in Australia in relation to climate and land use. *Australian Journal of Zoology* **19**, 65–76.

Wolfe EC (2009) Country pasture/forage resource profiles: Australia. Available at: http://www.fao.org/ag/AGP/AGPC/doc/Counprof/Australia/australia.htm

Wong DH, Barbetti MJ, Sivasithamparam K (1985) Fungi associated with root rots of subterranean clover in Western Australia. *Australian Journal of Experimental Agriculture* **25**, 574–579.

Xu Y, Li Z-K, Thomson MJ (2012) Molecular breeding in plants: moving into the mainstream. *Molecular Breeding* **29**, 831–832.

Yau S, Erskine W (2000) Diversity of boron-toxicity tolerance in lentil growth and yield. *Genetic Resources and Crop Evolution* **47**, 55-61.

Yau SK, Ryan J (2008) Boron toxicity tolerance in crops: A viable alternative to soil amelioration. *Crop Science* **48**, 854-865.

You M, Barbetti MJ, Nichols PGH (2005a) New *Trifolium subterraneum* genotypes identified with combined resistance to race 2 of *Kabatiella caulivora* and cross-resistance to fungal root rot pathogens. *Australian Journal of Agricultural Research* **56**, 1111-1114.

You M, Barbetti MJ, Nichols PGH (2005b) New sources of resistance in *Trifolium subterraneum* L. to root rot caused by two races of *Phytophthora clandestina* Taylor, Pascoe and Greenhalgh. *Australian Journal of Agricultural Research* **56**, 271-277.

You M, Barbetti MJ, Nichols PGH (2005c) New sources of resistance identified in *Trifolium subterraneum* breeding lines and cultivars to root rot caused by *Fusarium avenaceum* and *Pythium irregulare* and their relationship to seedling survival. *Australasian Plant Pathology* **34**, 237-244.

Young ND, Debellé F, Oldroyd GE, Geurts R, Cannon SB, Udvardi MK, Benedito VA, Mayer KF, Gouzy J, Schoof H, Van de Peer Y, Proost S, Cook DR, Meyers BC, Spannagl M, Cheung F, De Mita S, Krishnakumar V, Gundlach H, Zhou S, Mudge J, Bharti AK, Murray JD, Naoumkina MA, Rosen B, Silverstein KA, Tang H, Rombauts S, Zhao PX, Zhou P, Barbe V, Bardou P, Bechner M, Bellec A, Berger A, Bergès H, Bidwell S, Bisseling T, Choisne N, Couloux A, Denny R, Deshpande S, Dai X, Doyle JJ, Dudez AM, Farmer AD, Fouteau S, Franken C, Gibelin C, Gish J, Goldstein S, González AJ, Green PJ, Hallab A, Hartog M, Hua A, Humphray SJ, Jeong DH, Jing Y, Jöcker A, Kenton SM, Kim DJ, Klee K, Lai H, Lang C, Lin S, Macmil SL, Magdelenat G, Matthews L, McCorrison J, Monaghan EL, Mun JH, Najar FZ, Nicholson C, Noirot C, O'Bleness M, Paule CR, Poulain J, Prion F, Qin B, Qu C, Retzel EF, Riddle C, Sallet E, Samain S, Samson N, Sanders I, Saurat O, Scarpelli C, Schiex T, Segurens B, Severin AJ, Sherrier DJ, Shi R, Sims S, Singer SR, Sinharoy S, Sterck L, Viollet A, Wang BB, Wang K, Wang M, Wang X, Warfsmann J, Weissenbach J, White DD, White JD, Wiley GB, Wincker P, Xing Y, Yang L, Yao Z, Ying F, Zhai J, Zhou L, Zuber A, Dénarié J, Dixon RA, May GD, Schwartz DC, Rogers J, Quétier F, Town CD, Roe BA (2011) The *Medicago* genome provides insight into the evolution of rhizobial symbioses. *Nature* **480**, 520–524.

Zhang X, Evans PM (2004) Grain yield production in relation to plant growth of wheat and canola following clover pastures in southern Victoria. *Australian Journal of Experimental Agriculture* **44**, 1003–1012.