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Developing a phalaris pre-breeding plan

Phalaris 2020 vision – better cultivars faster

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Abstract

Phalaris (*Phalaris aquatica* L.) is the most important perennial grass sown in pastures in the medium rainfall regions of southern Australia. Breeders have successfully sought to improve the seasonal growth, grazing tolerance and seed production of phalaris over a number of years. Grazing tolerance and productivity remain the most important traits identified by industry for further improvement. Unfortunately, these traits are controlled by the actions of a large number of genes making them relatively difficult to select for. This project proposes the use of a combination of traditional and molecular breeding technologies to bring the kind of advances that have been seen in animal breeding into phalaris breeding to increase the rate of genetic gain for these important goals.

Executive summary

During the development of the MLA Feedbase Investment Plan there was agreement on the need for investment in perennial grass genetics and breeding with an emphasis on the following attributes (Smith 2012):

- Greatly increased rates of genetic gain for traits of importance to red meat producers
- Description of the value of this gain in economic terms
- Investment aligned with a public:private partnership model whereby the majority of science and development activities were in the public sector and cultivar development activities in the private sector
- That the germplasm and tools developed with MLA co-investment would be utilised in cultivars that would be broadly available to and widely adopted by red meat producers

Prior MLA reviews had demonstrated that progress in pasture plant breeding programs was limited by the low usage of quantitative genetics and economics to develop breeding objectives and that programs generally had a 'trait' emphasis that ignored the need for concurrent selection of a range of traits and the relative importance of traits within the breeding objective.

As a result we MLA contracted the delivery of an investment plan for pre-breeding in phalaris following guidelines set out in the Terms of Reference.

Based on these recommendations and further industry consultation the project team considers that the best approach is to develop a pre-breeding program that embraces quantitative genetics, molecular genetics and genomics to develop genomics assisted breeding values for key target traits in phalaris. Because the key traits are quantitative it is most unlikely that they will be improved greatly with simple marker-assisted breeding strategies and the use of quantitative genetics to develop breeding values is facilitated greatly through the use of genomics to reduce generation intervals and to determine the relationships between individuals. By developing these technologies in phalaris, the breeding systems in this cross-pollinated grass species will align with those used in modern animal breeding programs.

The project to develop a genomics-assisted selection strategy for phalaris will by 2017:

1. Describe genetic diversity in Australian-bred phalaris germplasm to guide the design of future commercial breeding programs with an emphasis on winter-active germplasm pools
2. Develop a methodology of genomic selection in phalaris for Australian commercial breeding programs, in collaboration with private seed companies, to more efficiently select for the quantitative traits which dominate the economic value of phalaris
3. Develop genetic markers for the seed retention trait as a tool to increase the rate of genetic gain in commercial phalaris breeding programs.
4. Use economic analyses to develop robust estimates of the economic importance of genetic gain in phalaris in red meat production systems that can be used to

a. assess of the genetic merit of individual plants and cultivars
b. assess the value of individual sub-traits (such as pest and disease tolerance) that contribute to pasture characteristics such as seasonal yield and persistence
c. calculate the economic benefit of new sowings to producers

These analsyes will address a major deficiency in most forage plant breeding programs, which includes the limited use of quantitative genetics and other tools to develop and implement robust economically-based breeding objectives similar to those which have been used to highly accelerate the rate of genetic gain in sheep and beef cattle breeding, and to prioritise between individual traits in a breeding program.

Due to cost constraints, the project will not address the important areas of livestock toxicity and herbage quality. Both areas are acknowledged to be important, and the technologies developed here will be suitable for application to these traits in future work.

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1. Background

During the development of the MLA Feedbase Investment Plan there was agreement on the need for investment in perennial grass genetics and breeding with an emphasis on the following attributes (Smith 2012):

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- Description of the value of this gain in economic terms
- Investment aligned with a public:private partnership model whereby the majority of science and development activities were in the public sector and cultivar development activities in the private sector
- That the germplasm and tools developed with MLA co-investment would be utilised in cultivars that would be broadly available to and widely adopted by red meat producers

As a result, we have been contracted by MLA to deliver an investment plan for pre-breeding in phalaris that will address the following areas:

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 that has developed 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

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. Identify synergies regarding technology development with the team that is currently developing the R&D plan for pre-breeding of annual legumes

10. Draft plans to be submitted to MLA who will distribute for comment as part of a consultation process. MLA will collate responses for inclusion into final version of the R&D plan. Revised plans (including responses to received comments) to be submitted to MLA.

11. Draft Milestone table

This investment plan includes a description of the importance and history of phalaris in Australian agriculture and a detailed response to the issues raised above.

2. Description of the role and breeding of phalaris in Australia

Since the commercialisation of the 'Australian' cultivar in 1906, phalaris has become arguably the most important sown temperate perennial grass for the inland high rainfall zone and adjacent cropping areas of south-eastern Australia. It combines high productivity with excellent drought survival ability, due to a deep rooting system and partial summer dormancy. It has excellent winter productivity, grows well on a range of soil types, tolerates waterlogging and insect attack, and has very few diseases. The proven ability to survive periods of drought means that phalaris is likely to have increased utility if climate change scenarios of higher incidence of drought for southern Australia eventuate. The importance of phalaris to the grazing industries of southern Australia, relative to other species, is unique on the world scale.

Phalaris is used mainly for the sheep and beef cattle production industries but is also finding increasing use in dairy pastures on lower rainfall dryland dairy farms (Mann 1999). It has been sown in excess of 2.5 M ha in southern Australia (Fig. 1). The area of pasture sown to any species is subject to variability based on estimation technique. However, the area sown to phalaris in 1994 was estimated at 2.67 M ha conservatively (Archer 1995) but possibly up to 5.2 M ha (Hill and Donald 1997) with 61% in NSW, 21% in Victoria, 16% in South Australia and 2% in Tasmania and other states. It is the most widely sown perennial grass in NSW (Archer 1995). Recent data (Donald and Burge 2012) suggests that the current area is around 4.5M ha, showing a consistency with the data of Hill and Donald (1997). Assuming a whole-distribution stocking rate of 10 dry sheep equivalents /ha and $30/dry sheep equivalent gross margin, phalaris-based pastures carry 26.7 M dry sheep equivalents generating $800 M in gross margins.

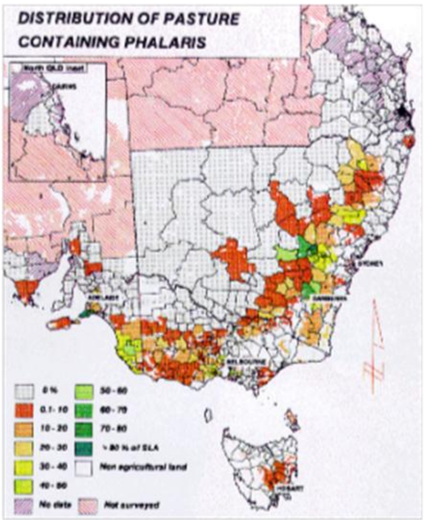

Figure 1. Distribution of phalaris (Hill and Donald 1997)

Phalaris pastures are a proven technology which offer producers:

- Improved profitability – depending on the pasture being replaced and land capability, at least an extra 2.5-5 dry sheep equivalents per hectare* compared with fertilised annual or native grass-based pasture for a planned pasture life of 20 years as the perennial component of a package with legume and P fertiliser. Fertiliser input is an important contributor to this increase but perennials are now viewed as essential components of pasture systems in the HRZ for their sustainability benefits such as ground cover and reduction in leakage of water and nitrate from the profile (Kemp and Dowling 2000).

  *This range represents typical values across a range of situations from a number of sources, butincreases in stocking rate of improved pasture relative to native and volunteer pastures and values can be higher than the range quoted here. For example, Scott *et al.* (2000) assumed a stocking rate of 3 DSE/ha for native pasture compared with 10 DSE/ha for an improved phalaris pasture in northern NSW. Chapman *et al.* (2003) compared a typical volunteer pasture (annual grasses and legume) in western Victoria with a phalaris-based pasture under low and high soil P by set stocking to maintain similar sheep condition. At low P, the phalaris pasture carried an extra 4.3 ewes/ha and at high P an extra 5.6 ewes/ha. These values are similar to those obtained by Saul *et al.* (2011) also in western Victoria, with typical pastures carrying 10.2 DSE/ha compared with upgraded (mostly phalaris) pastures carrying 18.0 DSE/ha averaged across 5 sites and 6 years. Again, extra fertiliser input was the major contributor but high quality perennials are needed to take advantage of this input in a sustainable manner. Saul *et al.* (2009) noted the need for improved grazing tolerance in perennial grasses to best capture the investment in pasture improvement. An example from the Central Tablelands of NSW was provided by Holst *et al.* (2006) who were able to set stock over 3 years a naturalised pasture at 4.65 ewes/ha, a fertilised naturalised pasture at 6.75 ewes/ha and a sown and fertilised pasture which contained phalaris with other perennial grasses at 8.8 ewes/ha. Compared with an alternative sown perennial grass in the Canberra district, Axelsen and Morley (1968) quote a value of 2.2 sheep/ha increase in stocking rate from using Australian phalaris compared with Demeter tall fescue.

- Benefits for sustainability – phalaris provides long-lived ground cover with a deep root system that uses water at depth and reduces nitrate losses. This reduces soil erosion and weed invasion, the risk of high water-tables and the rate at which soils acidify. Phalaris is a viable alternative to or complementary species with lucerne in managing deep drainage and dryland salinity in medium-higher rainfall cropping areas (Sandral *et al.* 2006).

- The highest persistence of currently sown cool-season perennial grasses at the species level – widely recognised by pasture scientists through experience over the last century, e.g. "in summary, phalaris is the most persistent species introduced into Australian agriculture" (Wolfe and Dear 2001), "Phalaris … is the most persistent and productive temperate pasture grass which can be sown in the Tablelands and Slopes regions of New South Wales" (Watson *et al.* 2000). In a survey of sown pastures in southern New South Wales, Virgona and Hildebrand (2006) found that, irrespective of the mixture of pasture species sown, in most cases only phalaris and subterranean clover survived.

Farmers recognise the value of phalaris:

- There has been a 7-fold increase in phalaris sowing increasing from a base 0.35 M ha in 1964 to and area of 2.67 M ha in 1994 (extrapolated from the NSW data of McMaster and Cook 1967 and Archer 1995) and further to an area of 4.547m Ha in 2011 (Donald and Burge, 2012).
- Phalaris seed production is around 400 t/year from 1980 to 1992 (Clark *et al.* 1992), which is sufficient to sow at least 130,000 hectares/year at a sowing rate of 3 kg/ha. Certified seed production for the 7 years 1999 to 2005 averaged 426 t/year (Australian Seed Authority Ltd data). Certified seed production (kg) for the last five years supplied by Australian Seeds Authority Ltd were:

Species	**2007/08**	**2008/09**	**2009/10**	**2010/11**	**2011/12**
Phalaris	155	227	195	314	385
Tall fescue	179	363	259	395	447
Cocksfoot	57	74	15	41	66
Perennial Ryegrass	1282	916	877	1337	1721

  Seed production was affected by drought and insufficient area for seed production during the 2000's but is now recovering. Phalaris is usually sown at one-third or less than the rate used for tall fescue and so has a distinct cost advantage for farmers when resowing or renovating pastures.

- 43% of farmers indicated their desire to sow phalaris in a catchment area of north-central Victoria surveyed by Baird (1993); another 20% were "intenders". Increasing productivity was the dominant reason for sowing of phalaris among adopters.

2.1 Breeding history

Australia

The value of phalaris as a pasture grass is an Australian discovery, and it is in Australia that the most effort in phalaris improvement has been conducted. This has occurred principally in CSIRO which began a program of introduction and breeding in the late 1950’s which continued up to 2008, and which has maintained capability to recommence with industry funding. CSIRO was the sole breeder of phalaris within Australia from the 1950’s to about 2000.

The CSIRO breeding program has emphasized the discovery and exploitation of new domestication, production and persistence traits resulting in distinct improvements in the cultivars it has released. Some outputs from extensive germplasm evaluation and intensive breeding effort by CSIRO have been:

- Release in 1967 of the first cultivar, Sirocco, with enhanced summer dormancy and high winter growth rates for the cropping zone
- The release of two highly successful cultivars, Sirosa in 1974 and Sirolan for cropping areas in 1978, resulting from a major breeding effort involving population construction and recurrent selection during the 1960’s and 1970s. This introduced higher seedling vigour for easier establishment, higher winter activity for improved productivity, and lower tryptamine alkaloid content. Both remain important public domain cultivars to the present day
- Discovery of ‘intact-rachilla’ seed retention, an important domestication step which facilitates production of high quality seed. The first seed-retaining cultivar was Uneta in 1983. All CSIRO cultivars since Uneta have been bred with intact-rachilla seed retention, which is a trait largely controlled by recessive alleles located at four distinct loci.
- Release of cv. Holdfast in 1991 combining the production attributes of Sirosa with greatly improved seed retention provided by intact-rachilla retention. Holdfast has been the top-selling certified cultivar in Australia since the early 1990’s.
- Release of Atlas PG in 1996, which combines higher summer dormancy for drier areas with intact rachilla seed retention
- A focus since 1990 on persistence under realistic grazing and soil conditions to make phalaris a more reliable and profitable perennial grass for graziers. Soil and climatic stresses can interact with heavy grazing pressure to reduce persistence. Breeding outputs have included:
    - Landmaster in 1996 for shallow, stony and moderately acidic soils in recharge areas
    - Advanced AT in 2008 with high aluminium tolerance for strongly acidic soils incorporating genes from a closely related species
    - Holdfast GT in 2008, a winter-active cultivar with improved tolerance of heavy grazing pressure
- Development of early-flowering, higher summer dormancy populations with high seeding vigour and winter growth rates currently under test in low rainfall areas. Use in the cropping zone and under climate change scenarios is envisaged.

Private companies in Australia or New Zealand began showing interest in phalaris breeding in the late 1990s, but with relatively small-scale activity to date. Several re-selections, such as those emanating from old stands, based on cv. Australian are now marketed (e.g. Grazier, Fosterville, Australian Gold, Australis Australian) which, with seed-retaining forms of cv. Australian bred by CSIRO (Uneta and Australian II) has led to a proliferation of cultivars essentially similar to cv. Australian in terms of production characteristics. While trying to exploit the reputation of cv. Australian for long-term persistence under grazing, all these cultivars also carry the disadvantages of Australian relative to the winter-active cultivars, that is, they have slower winter growth, lower seedling vigour, high tryptamine alkaloid levels and low seed retention. Alkaloid content in phalaris are believed to be the reason why the grass is relatively free of insect problems (Carlson *et al.* 1996). However, there are no observations suggesting that newer cultivars which are low in tryptamine alkaloids are more susceptible to insect attack. Seasonal high levels of alkaloid can sometimes cause grazing stock to avoid some phalaris cultivars such as Australian, particularly when small plots of different cultivars are grazed in common (e.g. Culvenor and Boschma 2005a). The high persistence of cv. Australian under grazing is believed to be mainly due to its growth habit (Culvenor 1993). Nevertheless, Culvenor *et al.* (2009) found that tryptamine alkaloid level increased slightly during selection for grazing tolerance, suggesting that the very low levels found in cultivars such as Holdfast may affect persistence.

Upper Murray Seeds in NSW developed cv. Stockman by crossing an Australian type with a winter-active cultivar apparently without directional selection except to eliminate “off-types” up to the F_3 generation (information supplied by IP Australia). More recently, PGGWrightson Seeds has commenced phalaris breeding at its Victorian base with plans to increase activity in the future (J. Sewell, pers. comm.). PGGWrightson previously bred cv. Persister in New Zealand from a cross between Uneta and Holdfast but finally withheld it from the Australian market in recognition of the value of the CSIRO program (A. Stewart, pers. comm.).

2.2 International breeding programs

Phalaris cultivar development has also occurred in the USA, Argentina, Uruguay, New Zealand, Tunisia and Greece. Oram *et al.* (2009) describe much of this history and list 26 commercial cultivars that were available worldwide by 2009, including 12 developed in Australia by CSIRO. The only programs outside Australia known to continue to the present day are in Argentina, in which cv. Mate was derived from the CSIRO cultivar Sirosa by Gentos, and cv. BarLaris was derived from the CSIRO cultivar Holdfast by Barenbrug, and is now marketed in Australia as ‘Lawson’ by Heritage Seeds (R. Prusa, pers. comm.). Phalaris is not used widely in South American countries at present, tall fescue and cocksfoot being more common. The Noble Foundation in Oklahoma, USA, became interested in phalaris for winter herbage in the drought-prone southern Great Plains in the late 1990’s. They conducted a selection program in the 2000s (Hopkins *et al.* 2006) but this ceased due to concerns over phalaris toxicity.

The relatively minor effort that currently exists in phalaris breeding in countries outside Australia means that Australian growers cannot expect overseas-based seed companies to import ready-made cultivars for Australian conditions, nor can Australian phalaris breeders necessarily expect to import elite germplasm pools with which to commence breeding. Oram *et al*. (2009) concluded that a cultivar selected in one country is often not optimal for other countries.

2.3 Phalaris toxicity

The potential for phalaris to cause occasional toxicity has been recognised for at least 70 years (McDonald 1942). However, as noted by McDonald, “the rarity of the disease contrasted with the common use of the plant”. Although toxicity is highly sporadic and total losses are small, significant losses to individual graziers sometimes occur (Gallagher *et al.* 1967; Bourke *et al.* 2003). The main effect of these outbreaks on an industry-wide basis is failure to capture benefits in productivity and sustainability of sowing phalaris through fear of toxicity. For example, fear of toxicity was the largest single factor deterring farmers from adopting phalaris in north-central Victoria and both users and non-adopters of phalaris were well aware of the problem (Baird 1993). In this sample, 54% of the 37% of non-adopters of phalaris, or 20% of all farmers surveyed, gave fear of toxicity as the main reason for non-adoption.

Phalaris toxicity is considered to present in two distinct forms:

- Chronic toxicity (“staggers”) – This is a nervous condition thought to be caused by tryptamine alkaloids and their derivatives which can occur at high levels in herbage typically soon after the break of season. It mainly affects sheep but can also affect cattle, and is the more common form of toxicity. This form is preventable by dosing with cobalt bullets or spraying cobalt solution on the pasture. The CSIRO breeding program has expended much effort over many years breeding for low levels of tryptamine alkaloids and modern cultivars display much lower levels than cv. Australian and cultivars selected from it (Culvenor *et al.* 2005b).
- Very acute toxicity (peracute toxicity or “sudden death”) – This is a condition with potentially high mortality that is responsible for the largest losses on phalaris stands and the form with the highest potential to deter adoption. Sudden death characteristically occurs in hungry sheep (more usually) or cattle within 12-48 hours of grazing short, freshly-shooting phalaris affected by dry conditions and frost (Bourke 1998). Stock die from high ammonia levels in the blood and brain caused by the combination of a sudden increase in nitrogen load and reduced capacity to metabolise nitrogen due to an as yet unidentified inhibitor of nitrogen metabolism (Bourke *et al.* 2005). Surviving stock become accustomed to the nitrogen load and toxin after about 48 hours. Management includes avoidance of the likely circumstances and test grazing with less valuable stock. Sudden death can occur on all cultivars including those selected for low levels of tryptamine alkaloids and the causal factors of the syndrome have not been identified.

Finding the chemical cause of sudden death would be a major breakthrough which would facilitate breeding and subsequently adoption by growers. It would allow (i) measurement of toxicity risk in pastures, and (ii) breeding of safer cultivars. CSIRO and collaborators such The Mackinnon Project have sought funding from either AWI or MLA to find the cause of sudden death on at least 3 occasions from 2000 to the present. We continue to believe that research in this area is important, and flag the intention of seeking funding as further opportunities arise. Regardless of when the genetic basis of the syndrome become clear, this trait can be added to the targets using the technologies developed in this project.

2.4 Recent cultivars

The most recent cultivars released by the CSIRO phalaris program address two important issues affecting persistence: (i) tolerance (or growth on) acid soils; (ii) grazing tolerance of the winter-active cultivars. Both were released in 2008 and have been available commercially for about 3 years.

2.4.1 Advanced AT for strongly acid soils:

Soil acidity is widespread in south-eastern Australia and is a major cause of reduced persistence in sown pasture species. Phalaris is more sensitive than cocksfoot and ryegrass to ionic aluminium (Al^{3+}) that becomes available at soil pH_{Ca} in the range 4-4.5. Cv. Landmaster was the first cultivar with substantially improved acid soil and Al tolerance. An important result from early screening work was that reed canarygrass (*Phalaris arundinancea* L.) is considerably more Al tolerant than *P. aquatica*. However, reed canarygrass is too summer active for good survival in many Australian locations, and becomes unpalatable in summer. Consequently *P. aquatica* was hybridized with *P. arundinacea* in an attempt to transfer Al tolerance into a *P. aquatica* genetic background, backcrossed to *P. aquatica* and subjected to cycles of selection in nutrient solution and at acid field sites to give cv. Advanced AT. Advanced AT is morphologically similar to winter-active phalaris cultivars such as cv. Holdfast and shares a degree of sensitivity to continuous grazing with these cultivars.

Cv. Advanced AT is the most Al-tolerant phalaris cultivar to be developed with a *P. aquatica* morphology and growth pattern. The benefit of this improved tolerance in strongly acid soils has been found to depend on soil moisture conditions during the first spring, when the plants are establishing their root system, with the largest benefit being observed in dry years (Culvenor *et al.* 2011). In higher rainfall years, Advanced AT and Landmaster generally established with similar basal frequency by the second year and with higher basal frequency than less tolerant cultivars, but Advanced AT was more productive than cv. Landmaster in winter of the second year. The key benefits of the higher Al tolerance of cv. Advanced AT therefore are more assured establishment on acid soils under variable moisture and improved establishment under late winter-early spring sowing.

Possible breeding to improve Advanced AT could focus on (i) improvement of grazing tolerance by selection under grazing similar to Holdfast GT (see below), and (ii) combining the good performance of Landmaster on lower fertility, skeletal soils with the high Al tolerance of Advanced AT.

2.4.2 Holdfast GT – a winter-active cultivar with improved grazing tolerance:

The winter-active cultivars can produce up to double the autumn-winter herbage of the “semi winter-dormant” cv. Australian. However, it gradually became apparent from the 1980’s onwards that these cultivars were not persisting as well as cv. Australian, partly due to increasing levels of soil acidity but also to lower tolerance of continuous grazing or frequent low defoliation. A program to breed a winter-active cultivar with improved grazing tolerance was commenced in 1989 and resulted in the release of Holdfast GT.

Cv. Holdfast GT was bred by subjecting three unrelated populations of winter-active phalaris, cv. Holdfast, a broadly-based, seed-retaining population and a progenitor of the more summer-dormant cv. Atlas PG, to two cycles of between and within half-sib family selection for high persistence under heavy, largely continuous grazing pressure at sites varying in soil fertility and acidity. Holdfast GT therefore has a broad genetic base. The 19 parents of Holdfast GT were selected by testing Cycle 2 half-sib progeny of each population in western Victoria and the Southern Tablelands and North-West Slopes of NSW. Selection was based on persistence after four years mainly in western Victoria and southern NSW, reasonable second yield winter growth potential, and on seed retention. Cv. Holdfast GT differs morphologically from other winter-active phalaris cultivars in having narrower and slightly shorter leaves, a denser tillering habit and slightly shorter stems. It is best adapted to areas with at least 600 mm average annual rainfall.

Average persistence in the fourth year of Cycle 2 families under continous grazing at sites in Western Victorian (mean annual stocking rate 17 sheep/ha) and Southern Tablelands (mean annual stocking rate 12 sheep/ha) was 30-40% higher than that of earlier winter-active cultivars (Culvenor *et al.* 2007). However, survival at a North-West Slopes site was low due to the hot and dry conditions that occurred during the evaluation. Current work is confirming the higher persistence of Holdfast GT compared with earlier winter-active cultivars under a high rate of continuous grazing. Winter yield after a spell in the second year (sheep excluded for about 2 months) was lower than cvv. Sirosa and Sirolan, slightly less than cv. Holdfast, similar to cv. Landmaster but clearly higher than cvv. Australian and Australian II.
Future breeding could aim to increase yield potential while maintaining grazing tolerance.

2.5 Phalaris germplasm pools

A number of germplasm pools are available for breeding, the most important for Australian conditions being those developed over the last 50 years by CSIRO. The pools are:

i. Winter-active - general purpose for main phalaris regions
ii. Winter-active – specialised for acid and skeletal soils
iii. Winter-active - higher summer dormancy for drier margins
iv. Semi-winter dormant (Australian type) for continuous grazing in higher rainfall areas

v. Other – accessions and breeding populations with potential utility
vi. South American origin

2.5.1 General-purpose winter-active pool

This is a genetically diverse pool developed by CSIRO over many years to provide growers with phalaris cultivars which have vigorous seedlings for easier establishment, higher winter activity for improved productivity, lower tryptamine alkaloid content and seed retention. All cultivars in this group were subjected to several-to-many cycles of recurrent selection. The main ones are:

- **Sirosa** – based on a population created by crossing 30 accessions from the Mediterrean region to cv. Australian and a Turkish accession similar in type to cv. Australian, followed by intense selection for seedling vigour and autumn-winter production. Still being one of the most productive cultivars in fertile soil, Sirosa suffers from being one of the most sensitive cultivars to acid soils. Semi-erect and variable in morphology, more sensitive to heavy continuous grazing that cv. Australian like most cultivars in this group
- **Sirolan** – based on progenitor generation for cv. Sirosa crossed with summer-dormant cultivar, Sirocco, and selected for production and survival in cropping zone. Very high production in autumn-winter with unusually low summer dormancy given its Sirocco background. Heavily selected for low tryptamine alkaloid content.
- **Holdfast** – first winter-active cultivar with intact-rachilla seed retention, a trait largely controlled by recessive alleles at 4 loci. Based on three cycles of outcrossing of the original seed-retaining plant of cv. Australian to various winter-active populations: cycle 1, Sirolan progenitor; cycle 2, Sirosa and Sirolan; cycle 3, 15 diverse Mediterranean accessions. In each cycle, the progeny were backcrossed to the retaining parent to enrich alleles for seed retention. Selected for fast winter growth and low alkaloid content in each cycle of outcrossing. Final generations selected for high seedling vigour, production in higher and lower rainfall environments, and for Al tolerance in nutrient solution. Holdfast remains a resource of high importance for future breeding.
- **Holdfast GT** – diverse cultivar based 16% on Holdfast, 52% on Atlas PG progenitor (see below) , 32% on a diverse winter-active population, subjected to 3 cycles of selection for persistence under heavy grazing resulting in slightly lower productivity under infrequent grazing. Each individual population was selected separately before combining to create Holdfast GT and seed of these separate populations also selected for 3 cycles has been saved. As well, intermediate generations have been saved in their individual famailies and are potentially available for retrospective study depending on seed amounts and viability.

2.5.2 *Winter-active pool for acid and skeletal soils*

This germplasm pool contains some of most recent developments in the CSIRO program which has aimed to combat soil-related persistence problems:

- **Landmaster** – high contribution from cv. Holdfast, selected for Al tolerance and productivity on low fertility, skeletal soils with proven success. Could be used to improve performance of related cultivars such as Holdfast and Advanced AT on low fertility soils, e.g. northern foothill country in Victoria.
- **Advanced AT** – specialised cultivar for high Al soils containing an estimated 13% of its parentage from *P. arundinacea.* A very erect cultivar, could benefit from selection for improved grazing tolerance; currently recommended for rotational grazing only. Crosses to other cultivars would require selection for Al tolerance to maintain current tolerance level.

2.5.3 *Winter-active pool with higher summer dormancy for drier margins*

This pool from North-West Africa has traditionally been aimed at the cropping zone of southern Australia but represents an important resource to address possible higher temperatures and increased drought incidence under climate change scenarios. Cultivars and accessions in this pool are generally highly productive in autumn and winter but have lower growth response to summer storms. Nevertheless, they will respond to high summer rainfall if accompanied by a spell of cool weather.

- **Sirocco** – Moroccan accession selected for improved seed production. No longer commercially available but breeder's seed has been retained. A seed-retaining form of Sirocco has been bred in recent years and is being evaluated in lower rainfall environments in the Future Farm Industries CRC.
- **El Golea (CPI 19305)** – Based on very early flowering accession from low rainfall area of south-central Morocco, selected for improved seed production. This was never commercialised. Seed-retaining form bred in recent years and is being evaluated in Future Farm Industries CRC.
- **Atlas PG** – first seed-retaining summer-dormant cultivar, currently recommended for medium rainfall cropping zone. Later flowering than Sirocco which may explain lower performance than expected on north-west Slopes of NSW.
- **Northern Retainer population** – based on Moroccan and Tunisian accessions from DPI Victoria collection with the highest persistence at two sites on the north-west Slopes of NSW, crossed to an Atlas PG × Sirocco population to introduce seed retention. Under evaluation in Future Farm Industries CRC at low rainfall sites.
- Atlas PG × Sirocco population - Under evaluation in Future Farm Industries CRC at low rainfall sites.

Evaluation in the Future Farm Industries CRC project is yet to identify the best population for cultivar release. However, a CSIRO study at Yanco in the NSW Riverina has demonstrated higher survival and subsequent production compared with Atlas PG from the Northern Retainer, 19305 Retainer and Atlas PG × Sirocco populations (Culvenor *et al.* 2012). Discussions on commercialisation have commenced with Heritage Seeds/Seedmark on the basis of this information.

All cultivars and populations in this group are likely to be more susceptible to soil acidity than the general-purpose pool and could benefit from selection for improved acid soil tolerance for use in present acid soil areas under climate change.

2.5.4 Semi-winter dormant (Australian) pool

This pool is less productive in autumn-winter than the winter-active type and high in tryptamine alkaloids but has excellent persistence under heavy grazing pressure in areas above 550-600 mm rainfall. Based on the original introduction of phalaris; relatively narrow-based but nevertheless variable as expected of an outcrossing tetraploid species. It has been shown to be responsive to selection for attributes such as seedling size but lacks useful variation in tryptamine alkaloid content.

2.5.5 Other germplasm held in Australia (CSIRO and DPI Victoria)

CSIRO holds seed of various breeding populations most of which contain genes for seed retention. Examples include populations selected for low levels of all known toxins ("LoTox"[1]), low levels of red sap which may be related to toxicity ("Green Sap"), several populations containing *Phalaris arundinacea* background selected for high rhizomatous spread or on strongly acid soils, and a (Holdfast × Sirolan) × Holdfast population to further reduce the level of inbreedng in Holdfast. CSIRO also holds accessions from higher rainfall areas in Sardinia collected by DPI Victoria, and from low rainfall areas in southern Sardinia collected by NSW DPI. The latter have not been evaluated. The former contain accessions with high ground cover and grazing toleance but lower productivity than cv. Australian (Reed *et al.* 2008). DPI Victoria at Hamilton hold seed of accessions from Sardinia, Morocco and Tunisia (Cunningham collection).

2.5.6 South American pool

The most consistent breeding effort outside of Australia has occurred in Argentina and, to a lesser extent, Uruguay. Unless selected in Australia, South Amercan material is likely to be less well-adapted than locally-selected material (Oram *et al.* 2009). As an example, CSIRO released Seedmaster in 1965 based on an Australian-like cultivar from Argentina, which was said to be derived in turn from cv. Australian (Oram 1990). Despite being very similar morphologically to the highly persistent Australian (except for its seedhead characteristics), Seedmaster performed poorly in Australia probably because of poor adaptation to Australian soils and possibly climatic differences. The New Zealand-bred cultivar, Grasslands Maru, was based on lines from South America and performs well in Australia. A cultivar released recently

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[1] The cause of the most severe form of toxicity ("sudden death") is not known

by Heritage Seeds, Lawson, is said to be derived from cv. Holdfast by a South American program and is said to have been tested in Australia.

It is possible that material of use in Australia could be present in South American germplasm. However, given the high level of variation usually found within phalaris populations, it is likely than more progress can be made by selection within Australian germplasm pools using efficient selection procedures.

Cocksfoot

Cocksfoot is also a major species of temperate Australia in areas unsuitable for perennial ryegrass, second to phalaris in area overall and slightly higher in area than phalaris in Victoria (Hill and Donald 1997). In particular, cocksfoot has advantages in low fertility and acidic soils. Subsp. *glomerata* (e.g. Porto) in areas >600 mm average rainfall of southern Australia and *glomerata x hispanica* types (e.g. Currie) in slightly lower rainfall areas are the main agricultural forms of cocksfoot and *glomerata* certainly receives breeding attention overseas. Subsp. *hispanica* is more drought tolerant, depending on higher levels of summer dormancy than phalaris for its summer survival in lower rainfall areas. This type receives less breeding attention overseas although there is current interest in the Mediterranean and north Africa. It was the *hispanica* type that was said to be of potential interest for prebreeding effort at the MLA Prioritisation Workshop held in October 2011.

Although ideally both phalaris and cocksfoot are worthy of breeding effort for Australian conditions, we have decided that the introduction of genomics-assisted selection to increase rate of genetic gain can only be undertaken initially in one species, that is phalaris, for the following reasons:

1. We believe that the limit on expenditure of $1.5M is insufficient to undertake the research we plan in more than one species at realistic costing. Even in phalaris, this is insufficient to conduct needed research on toxicity in addition to research aimed at increasing rate of genetic gain.
2. Of the temperate perennial grasses used in southern Australia, phalaris was identified at the 2011 Prioritisation Workshop as the species most prone to market failure in terms of its breeding due to its unique importance in Australia and absence of breeding attention overseas. This gives it precedence if there is to be a choice between species.
3. While both phalaris and cocksfoot are considered as “minor species” in the world forage seed trade compared with perennial ryegrass, for example, Australian seed sales of phalaris are much higher than those of cocksfoot at the moment (e.g. 54 t of cocksfoot certified seed compared with 337 t of phalaris certified seed in 2011/12; Australian Seeds Authority 2012)
4. To undertake a genomics-based prebreeding program to accelerate genetic gain requires the existence of a scientifically-based recurrent selection breeding program. However, most Australian cultivars of cocksfoot such as Currie, Porto and Kasbah are the product of ecotype selection with a minimal amount of within ecotype selection for uniformity and seed production. The TasGlobal *hispanica* cocksfoot cultivars, Uplands and Sendace, were the

product of a recurrent selection program in a Spanish ecotype undertaken by Tasmanian Institute of Agricultural Research. This program will focus on winter-active germplasm pools under the leadership of Dr Rowan Smith with the support of TIA and TasGlobal Seeds. In contrast, phalaris has been the subject of a major recurrent program over many years and has material suitable for the genomic selection activities we have in mind.

5. *Hispanica* cocksfoot has received some attention in recent years in the CRC for Plant-based Management of Dryland Salinity and Future Farm Industries CRC. The *hispanica* cocksfoot cultivars selected by Mr Steve Clark, DPI Victoria at Hamilton (Clark *et al.* 2012),, will come on the market in the next few years. Note that these are also the product of ecotype selection in contrast to methodical recurrent selection required for genomic selection.

Against this background, we have decided to concentrate our limited resources in the area of genomics-assisted phalaris prebreeding.

Developing a Genomics-Assisted Selection Strategy for Phalaris

The cultivars and breeding populations created by the CSIRO program will be the focus of proposed activities since, to our knowledge, overseas-bred cultivars do not contain specific traits of interest not present in Australian material and their breeding has targeted non-Australian environments. Germplasm pools created by CSIRO have undergone selection for Australian conditions and capture the specific traits introduced by CSIRO over many years.

Although the pools created by CSIRO were based on diverse germplasm, nevertheless many of the populations and even distinct pools share common ancestories to varying degrees and have been subjected to varying intensities of recurrent selection and crossing bottlenecks which have the potential to reduce the level genetic variation essential to the selection activities we wish to undertake. We therefore believe it is appropriate to commence the project by examining the genetic diversity in CSIRO-bred material and comparing it with a broad range of germplasm (overseas cultivars, accessions) using modern molecular genetic techniques. This will give us information on whether genetic variation has become restricted in the CSIRO material, whether it would be desirable to introduce new germplasm at his stage and, very importantly, which populations and pools can be combined in the teaching nurseries that are established to introduce genomic selection methods.

Irrespective of the information derived from the diversity study, we propose that the project will concentrate on the winter-active germplasm pools since these offer the potential for highest gains in production particularly during the winter feed gap when extra feed is of most value. Certainly the Australian pool offers high grazing tolerance and persistence in cooler areas but is relatively restricted and appeals to growers who want the “original” phalaris with fewer changes the better.

We believe it is the right time to elevate production over seasons and years as the outcome trait of highest priority following more than two decades of breeding work in CSIRO introducing specific traits related mainly to persistence factors (e.g. Al tolerance, grazing tolerance) sometimes with acknowledged loss of some production potential as a trade-off. Persistence will remain a high priority and we hope to maintain the gains already made. Indeed, families with high production and persistence were observed during the breeding of Holdfast GT but could not be easily exploited at the time.

Reviews by MLA (eg. Banks *et al.* 2005) have highlighted that a major deficiency in most forage plant breeding programs has been the low use of quantitative genetics and other tools to develop and implement robust economically-based breeding objectives as have been used to greatly increase the rate of genetic gain in sheep and beef cattle breeding. This deficiency has also led to the problems that the rate of genetic gain is hard to define in economic terms and that it is relatively hard to prioritise between individual traits in a breeding or prebreeding program. Better definition of the value of genetic gain in pasture breeding and evaluation programs was identified as a priority in the MLA prioritisation process (Smith 2012).

Most breeding programs for perennial forage programs in public sector and commercial companies operate using a combination of half-sib family and post-synthesis selection to develop new cultivars. Whilst this breeding system is useful in that it attempts to select between elite half sib-families and allows for evaluation of pre-release synthetics as swards, it has several limitations. Half-sib evaluations only allow ¼ of the additive genetic variance to be assessed and the system is not usually based on recurrent selection so either resulting or not resulting in varieties. This means that new base populations are not based on elite parents but rather random selections within cultivars or breeding pools (Vogel and Pedersen 1993, Casler and Brummer 2008, Conaghan and Casler 2011, Vogel 2013).

There are a number of alternative selection models in forage breeding that are more efficient than the Half-Sib Progeny Test (HSPT), such as Restricted Recurrent Phenotypic Selection (RRPS) and Between and Within Family Selection (B&WFS), these increases in predicted genetic gain are based on the proportion of the additive genetic variance (the component of genetic control of traits that responds to selection) and hence greatly increase the rate of genetic gain. All of these strategies are able to be facilitated through genomics-assisted selection.

This project proposes to use economic analyses to develop robust estimates of the economic importance of genetic gain in phalaris in meat production systems that can be used for a range of outcomes including

- An assessment of the genetic merit of individual plants and cultivars
- The calculation of the economic benefit of new sowings
- An assessment of the value of individual sub-traits (such as pest and disease tolerance) that contribute to pasture traits such as seasonal yield and persistence that drive the economic value of pastures.

In the absence of these genetic and economic analyses the objectives of programs have been poorly defined and attempts to prioritise between component traits have been based on various levels of economic analysis of the potential value of individual traits and then the use of expert opinion to rank a long list of potential targets, but this approach is not well suited to the concurrent selection of traits as practised in breeding programs as it does not allow the appropriate weightings to be placed on the traits under selection.

The traits of most interest in phalaris (production, persistence, higher levels of Al tolerance, summer dormancy, etc) are all quantitative traits controlled by many gene, the majority of small effect. An exception is the intact-rachilla seed retention trait, which is largely controlled by 4 distinct genes. Seed retention is an important domestication and enabling trait in phalaris, which has the downside of slowing rate of progress in phalaris breeding because plants must be allowed to flower and set seed before the trait can be selected. One priority of this project will be to develop markers for this trait to improve rate of breeding progress.

Traditional molecular marker-assisted selection as deployed in self-pollinated crops is unsuitable for quantitative traits, but the new method of genomic selection (or genome-wide selection) is eminently suitable. It is this methodology that the project will introduce to grass breeding in Australia. Success in phalaris will be transferrable to cocksfoot and other grasses.

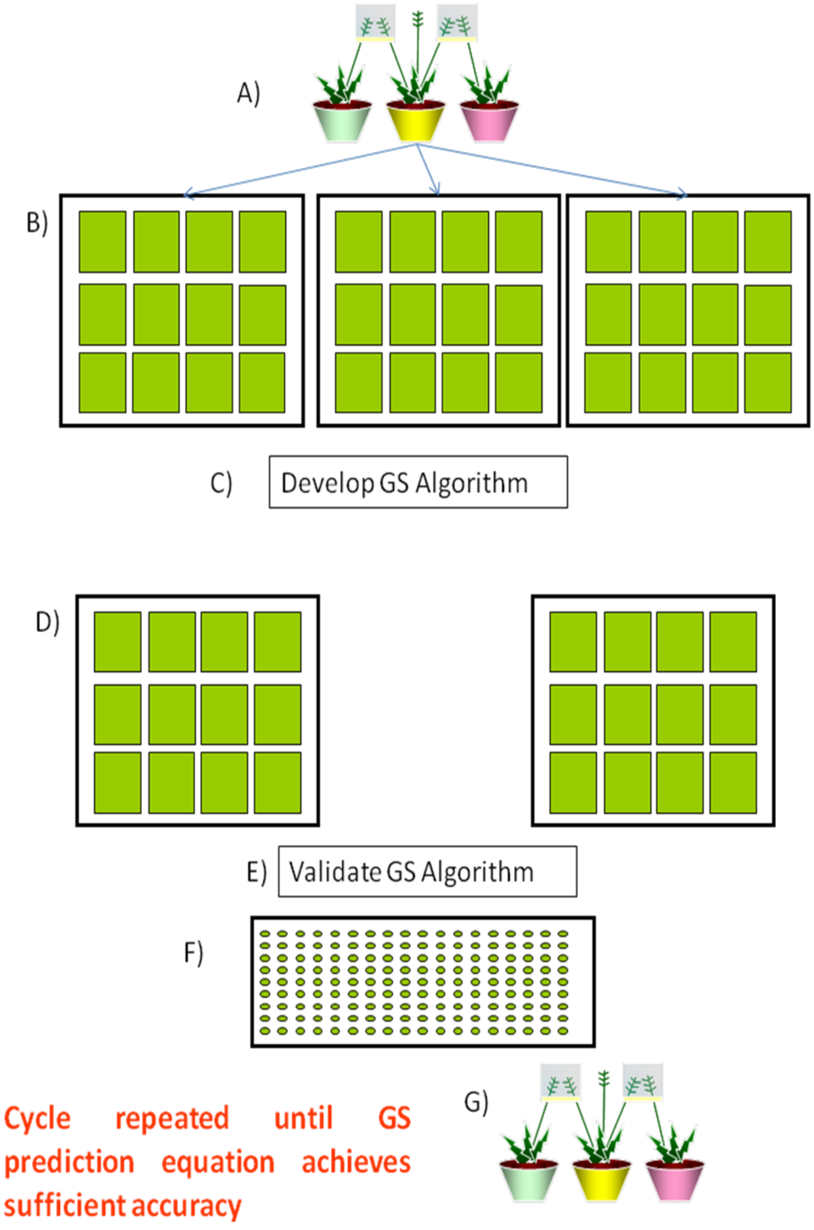
The project team have extensive experience in the development of practical genomics tools and genomics-assisted selection strategies in allopolyploid forage species such as white clover (Hand et al, 2008) and tall fescue (Hand et al, 2012), and this knowledge will assist in the development of these techniques in phalaris, which is an allotetraploid with diploid-type (disomic) inheritance patterns. Identification, characterisation and interpretation of single nucleotide sequence variation in allopolyploid crop plant species was reviewed by Kaur et al, 2012. These elements these include the development of sub-genome specific SNP markers and a greater understanding of progenitor genomes in terms of trait expression. Autotetraploid species such as lucerne require other strategies, to measure allele dosage and other specific features for comprehensive understanding.

We acknowledge that the methodology is new and relatively untried in forage plant breeding, but the project team has access to the skills of Prof. Mike Goddard and Dr. Ben Hayes who led the development of genomic slection strategies in dairy cattle and have recently worked with Prof John Forster to develop strategies for genomic selection in forage plant breeding (Hayes et al. 2013). We are encouraged by the participation of highly reputable overseas crop and forage breeders (e.g. Dr Mike Casler, USDA, Madison WI) in studies similar to the one we propose to undertake in species with relatively few genomic resources. The project team were in regular dialogue with the leaders in the development and application of genomics-assisted selection during the development of this project and this will continue throughout the course of the program. We acknowledge that transfer of this new methodology to private breeders will be essential to the long term success of the project and have been in discussion with the most likely companies to be formally appointed as partners to the project in the first year.

We have decided that the project will not address two important areas at this stage. The first is toxicity which we regarded as prohibitively expensive to combine with the proposed work, and which requires a more significant research effort. As already stated, we believe research in this area to be of high importance and will pursue opportunities at a later date. The second area is that of herbage quality which has received relatively little attention in phalaris. At least one company flagged this as an area of interest. Again, constraints of cost and limited prior research and breeding to build on in phalaris meant delaying attention to forage quality to a later date. However, both these areas are acknowledged as important and the technologies developed here will be suitable for application to these traits in the future.

An illustration of the proposed methodology is given in the following figure

Proposed Genomic Selection Strategy for Phalaris (modified from Hayes *et al.* 2013)



In this model the following steps/activities will be undertaken

A) Development of *"Teaching/Reference"* Population. The purpose of this population is the development of progeny for phenotypic evaluation and subsequent development of a genomic selection algorithm. For genomic selection to be effective this population must represent a large proportion of the likely populations in which selection will be implemented. We propose to develop this population based on a set of plants that includes the parents of modern CSIRO winter-active cultivars such as Holdfast GT and Advanced AT that are augmented with other relevant families from the previous CSIRO program. Consultations with industry have confirmed the desire to combine attributes of these cultivars in future breeding and also the need to focus on the winter-active pool.

B) The progeny of this cross will then be evaluated for dry matter yield and survival across years and seasons in multi-environment trials. The progeny will be sown as rows or plots under competitive conditions. Dry matter yield will be measured as calibrated cuts, survival and yield will be combined to give a measure of persistence and productivity.

C) In genomic selection the unit of selection is the combination of allelic affects at all marker loci across the genome. The teaching population will be genotyped using a set of SNP markers and these will be regressed against phenotypic data to give the genomic selection algorithm.

D) The genomic selection algorithm will be validated and augmented using one or both of the following methodologies:
- if company nurseries are available the algorithm will be tested against the data and selections from these nurseries, and updated based on these results
- if no company selection nurseries are available within the timeframe a between and within family selection model will be implemented on the project nurseries and a new reference population formed based on these selections.

These data will be used to update the genomic selection algorithm E) and develop new crosses F) and G). If the selected families come from company nurseries they can also form the basis of new synthetic cultivars that are then entered into standard cultivar testing programs.

In summary, the project will by 2017:

1. Describe genetic diversity in Australian-bred phalaris germplasm to guide the design of future breeding programs with an emphasis on winter-active germplasm pools
2. Develop a methodology of genomic selection in phalaris for Australian breeding programs, in collaboration with private seed companies, to more efficiently select for the quantitative traits which dominate the economic value of phalaris
3. Develop markers for seed retention as a tool to increase the rate of gnetic gain in phalaris breeding programs.
4. Use economic analyses to develop robust estimates of the economic importance of genetic gain in phalaris in red meat production systems that can be used to

a. assess of the genetic merit of individual plants and cultivars
b. assess of the value of individual sub-traits (such as pest and disease tolerance) that contribute to pasture traits such as seasonal yield and persistence
c. calculate the economic benefit of new sowings to producers

3. Detailed response to terms of reference

3.1 A description of the organisations to implement the R&D plans including their roles and responsibilities (i.e. lead organisation, participating, etc)

This team is led by 4 of Australia's leading researchers in grass genetics, genomics and breeding.

Prof Kevin Smith (University of Melbourne) has 25 years experience in forage grass genetics and breeding with a particular emphasis on the development and application of new breeding technologies in conjunction with private sector breeding programs. He has been actively involved in the development and application of the national research and development programs for red meat and dairy. He has trained more than 10 Ph.D. students in forage breeding, published more than 70 refereed papers and bred cultivars of perennial ryegrass, tall wheatgrass and white clover. Key achievements related to this project include the ability to link quantitative, molecular and economic data in breeding programs and the development of effective linkages with the private sector.

Prof German Spangenberg (Executive Director of Biosciences Research at DPIV and Professor of Biosciences at LaTrobe University). Is a world authority on the development of biotechnologies for application to plant industries – particularly forages and a Fellow of the Australian Academy of Technological Sciences and Engineering. He has been actively involved in the development and application of the national research and development programs for red meat and dairy. He has trained more than 25 Ph.D. students in forage breeding, published more than 130 refereed papers and is listed as inventor on 45 patents.

Prof John Forster (Research leader for molecular genetics at DPIV and Professor of Molecular Genetics at La Trobe University). John is Leader and Principal Research Scientist in Molecular Genetics for the Victorian Department of Primary Industries' Biosciences Research Division, Professor of Molecular Genetics at La Trobe University, and Research Leader for the Designer Forages sub-program of the Dairy Futures CRC. He leads a group of 30 staff and students applying DNA sequencing and genotyping technologies for improvement of a broad range of domestic animals, agricultural microbes and crop species, using state-of-the-art equipment platforms. John's personal research work has focused on developing and implementing genomics-assisted tools for breeding improvement of forage grasses and legumes. In this capacity, he has pioneered the construction of genetic maps, use of high-throughput genetic markers, studies of genetic diversity and tagging of key agronomic genes. He has widely collaborated and presented several keynote addresses at international research conferences He has trained more than 15 Ph.D. students in forage molecular genetics and breeding, and published more than 95 refereed papers.

Dr Richard Culvenor (Senior Scientist at CSIRO in Canberra). He has led the CSIRO phalaris breeding program with a particular emphasis on the development of winter-active phalaris cultivars with adaptation to hostile soils and improved grazing tolerance. Key achievements include (i) breeding of Advanced AT, the most acid-soil tolerant cultivar yet developed, (ii) Holdfast GT, a winter-active cultivar with improved grazing tolerance, (iii) a cultivar for the low rainfall margins to replace Atlas PG (yet to be named), and (iv) physiological and grazing research on persistence factors in phalaris. He was also involved in the breeding of Landmaster, Atlas PG and Australian II. He has published more than 40 refereed journal papers. Dr Culvenor will be mainly responsible for phenotyping phalaris populations for association with molecular markers developed at La Trobe University. This will include running of the main “teaching nursery” for genomic selection and involvement in other nurseries run at other locations, e.g. to account for GxE interactions, company nurseries.

3.2 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.

The proposed team (CSIRO, DPI and UofM) are leaders in this area of research and development with a recognised reputation for delivery of outcomes. Our experience is that cost-effective and flexible projects are best managed through dialogue between all investors and project partners. It is not proposed that any other research agencies will be involved in the project in this phase but linkages with the private sector are crucial.

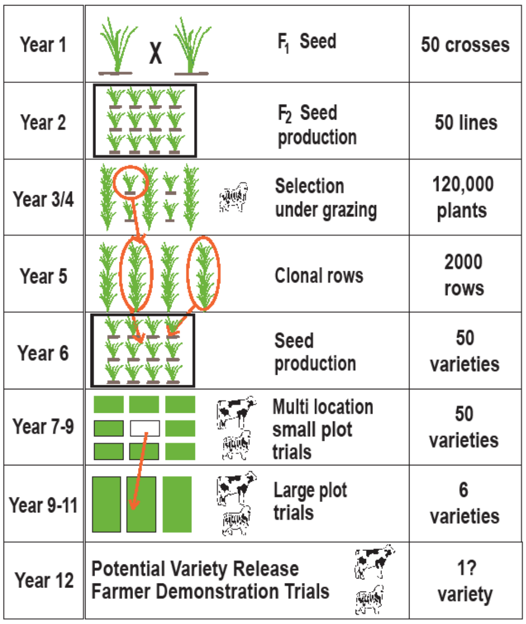
Whilst there are other groups with experience in molecular genetics and/or forage breeding there is no combination of scientists in Australia with more experience in the development and application of quantitative and molecular genetic techniques to outcrossing perennial grass species and in direct experience with phalaris breeding and phenotyping.

3.3 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.

Australia has historically been the main country where phalaris breeding has occurred, largely through the ongoing breeding program at CSIRO Plant Industry (see section A).

Most breeding programs for perennial forage programs in public sector and commercial companies operate using a combination of half-sib family and post-synthesis selection similar to this example based on the perennial ryegrass breeding program at Agriseeds NZ.

Breeding Programme Outline
Perennial Ryegrass



Year 1	F_1 Seed	50 crosses
Year 2	F_2 Seed production	50 lines
Year 3/4	Selection under grazing	120,000 plants
Year 5	Clonal rows	2000 rows
Year 6	Seed production	50 varieties
Year 7-9	Multi location small plot trials	50 varieties
Year 9-11	Large plot trials	6 varieties
Year 12	Potential Variety Release Farmer Demonstration Trials	1? variety

Whilst this breeding system is useful in that it attempts to select between elite half sib-families and allows for evaluation of pre-release synthetics as swards, it has several limitations. Half-sib evaluations only allow ¼ of the additive genetic variance to be assessed and the system is not usually based on recurrent selection so that it either results in varieties or it doesn't. This means that new base populations are not based on elite parents but rather random selections within cultivars or breeding pools (Vogel and Pedersen 1993, Casler and Brummer 2008, Conaghan and Casler 2011).

There are a number of alternative selection models in forage breeding that are more efficient than the Half-Sib Progeny Test (HSPT) such as Restricted Recurrent Phenotypic Selection (RRPS) and Between and Within Family Selection (B&WFS), these increases in predicted genetic gain are based on both the proportion of the additive genetic variance that can be utilised (Table 1).

Scheme	**Genetic gain per cycle**	**Gain/year (% of σ^2_A)**
RRPS	$k\,\sigma^2_A(\sigma_{PS})^{-1}$	**33.3**
HSPT	$k\,\frac{1}{2}\sigma^2_A(\sigma_{PFM})^{-1}$	**7**
B&WFS	$k_1\,\frac{1}{4}\sigma^2_A(\sigma_{PFM})^{-1} + k_2\,\frac{3}{4}\sigma^2_A(\sigma_{PW})^{-1}$	**25**

The adoption of these technologies has been limited by the complexity of maintaining pedigrees and a lack of confidence in making selections on the performance of individual plants within families.

The adoption of genomics-assisted selection strategies will allow the development of much more efficient breeding strategies in forage plants through the following attributes

- The assessment of relationships between individuals (pseudo-pedigree)
- The facilitation of B&WFS strategies
- The identification of allelic affects across genetic backgrounds
- The facilitation of recurrent selection procedures

The use of estimated breeding values and profit indices has not been practiced in forage breeding. However, the Forage Value Index developed by Dairy NZ for perennial ryegrass cultivars is an example of the industry moving in this direction. Many of the components of an EBV based system are in place or currently under development in Australia, such as:

- BLUP and BLUE estimates of genetic effects exemplified in perennial ryegrass (Smith et al 1998, Smith and Casler 2004)
- Prioritisation of individual component traits in a range of pasture species (Smith and Fennessy 2011)
- Economic assessment of genotypic effects on pasture performance in white clover, lucerne and perennial ryegrass (Lewis et al. 2013, Ludemann *et al.* 2013)
- Assessment of marker effects on key traits and attributes exemplified in perennial ryegrass (Pearson et al 2011, Wang et al 2011, Shinozuka et al 2012)

These developments have largely been achieved in major perennial species such as perennial ryegrass and white clover, but the knowledge applies broadly to all cross-pollinated perennial species.

A particular focus of the project will be the introduction of genomic selection (or genome-wide selection) to phalaris breeding. Unlike the QTL-based form of marker-assisted selection which utilises only statistically significant QTLs, genomic selection relates variation in all available markers across the whole genome to phenotype to yield a “genomic estimated breeding value”(Heffner et al. 2009). It is considered to be a much superior technique to traditional marker-assisted selection for quantitative traits determined by variation at many loci, as is normal for traits of interest in grass breeding. Genomic selection has become possible only in recent years with the continuing reduction in the costs of developing and implementing genetic markers and the development of appropriate statistical techniques. For traits such as yield and persistence which take years to measure, a major attraction of genomic selection is the capacity to undertake rapid cycles of selection once relationships between traits and markers have been developed in “teaching nurseries” set up for this purpose. These relationships are then applied to nurseries composed of appropriate commercial breeding populations. A number of grass breeding groups overseas are now developing projects around genomic selection including perennial ryegrass (VDEPI) and switchgrass for biofuel (USDA).

What is required now is to integrate these components into a pre-breeding program for phalaris in Australian environments.

3.4 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

The program will be managed as three integrated work packages:

3.4.1 Work Package 1 - Genomics Analysis (DPIV Lead - Forster)

This work package will develop the essential knowledge of the genetic diversity within phalaris to define effective populations for further breeding, develop markers for genomic regions controlling seed retention, yield and persistence in phalaris and the development of genomic selection algorithms for phalaris.

Tools & Techniques

- Utilising sequencing technology to identify and validate genetic markers
- Genotyping-by sequencing (GBS) as a cost-effective means for generic marker implementation

Outputs and outcomes:

Development of pseudo-pedigree and relationship matrices

- Knowledge of the population structure and genetic diversity in phalaris

- Better use of genetic resources, including knowledge of relationships between species within the *Phalaris* genus
- Discovery of markers for specific traits that contribute to important outcomes for meat producers e.g. persistence, seasonal production and quality
- Tools to greatly increase rate of genetic gain in phalaris breeding programs

Workplan and Budget

Assessment of genetic diversity within and between phalaris cultivars and breeding lines

This activity will see phalaris cultivars and breeding lines screened for genetic diversity and cross-relatedness. The activity to include all winter-active cultivars, Australian and the key CSIRO breeding populations. (2013 – 2014)

Development of molecular markers for seed retention in phalaris

Phenotypic data from Work Package 2 and genome sequence data will be combined and analysed to determine associations between markers and genes for seed retention in phalaris. Seed retention is an important “enabling trait” in phalaris which CSIRO has attempted to breed into all of its more recent populations. Seed retention facilitates ease of production of seed with high average quality because all seed in the head can be allowed to ripen before harvest. Markers for seed retention will overcome the loss of one year each time a breeder selects for seed retention (trait can only be observed after flowering). It is proposed that genome-wide association studies (GWAS) be used to identify these marker trait associations based on the fact that the trait is controlled by relatively few loci, as opposed to traits such as yield and persistence. (2014 – 2015).

Development of genomics-assisted selection algorithms for phenotypic traits (seasonal yield, persistence measured on the information nurseries)

Following phenotypic analysis associations between genotypic diversity will be assessed and the correlation between phenotypic and genomic data will be quantified using methods as described in Hayes *et al.* 2013 (2014-2017).

Development of genomics-assisted selection algorithms for phenotypic traits (seasonal yield, persistence) validated in commercial nurseries

Genomic selection algorithms will be validated in commercial nurseries (2014-2017).

3.4.2 Work Package 2 – Trait validation and development (CSIRO lead – Culvenor)

This work package is the phenotypic assessment component of the project. This will involve the measurement of a range of traits within a nursery of phalaris plants that will be used as a 'teaching' population for genome assisted selection in phalaris. This teaching will lead to the development of selection equations that can then be used by commercial breeders in their own programs.

Tools & Techniques:

- Field and greenhouse screening of phalaris reference populations
- Application of tools from work package 1
- Traits prioritized through consultation with industry (Smith 2012) will be refreshed through consultation with the breeding companies but may include aspects of persistence, stress tolerance, and associated field performance

Outputs and outcomes

- Improved germplasm for use in cultivar development by commercial partners
- Genetic and phenotypic assessment of traits and correlations between traits
- Capacity to breed safer phalaris cultivars and to monitor at risk phalaris pastures to secure phalaris uptake

Workplan and Budget

Assembly of winter-active populations of phalaris for the information nursery

(2013 – 2014) The nurseries will include existing breeding populations and families and/or de novo crosses based on the results of activity 1.1. The size and composition of the nurseries will likely be in the order of 200-300 families depending on the results of the diversity analysis and will cover all major winter active cultivars to ensure linkage with breeding companies.

Establishment of phalaris information nurseries

(2014 – 2017) Sites will be established at Canberra, Maryborough and/or Hamilton depending on seed availability following crosses in 2013/2014. These proposed sites represent both target environments for phalaris and proximity to researchers to facilitate the collection of high quality phenotypic data for yield, persistence and disease resistance across seasons. Phenotypic data relating to yield and persistence will be analysed to predict the breeding value of individual genotypes and used to develop genomic selection algorithms (Work Package 1) and economic analysis of traits and cultivars (Work Package 3)

3.4.3 Work Package 3 – Economic and Genetic Modelling (UofM lead – Smith)

In this work package, economic analyses will be used to determine the value of genetic gain in phalaris for meat production systems. These findings will be relevant for other perennial grass species sown in these systems, such as cocksfoot. The detailed economic analyses will include risk and seasonal variation to not only determine average values but also look at their sensitivity and variability.

Tools & Techniques:

- Combined economic and genetic modelling to determine the value of genetic gain in phalaris.
- Application of tools from work packages 1 & 2
- Traits incorporated into value indices to allow the selection of plans or cultivars to raise the productivity/profitability of red meat production systems
- Phalaris reference populations established for phenotypic assessment and the development of genomics assisted breeding strategies in phalaris

Outputs and outcomes

- Genetic gain described and quantified in economic terms
- Phalaris breeding programs prepared for genomics assisted breeding strategies
- Phalaris will become a model for the deployment of improved breeding methodologies in other perennial grass species eg cocksfoot

Workplan and Budget

Assessment of genetic diversity within and between phalaris cultivars and breeding lines in economic terms

(2013 – 2016) Models will be developed to equate variation in seasonal yield, persistence and lifetime productivity of phalaris to economic value in two key meat production systems (Prime Lamb and Beef Growth). The models will include seasonal variation and risk and will be based on those being developed by the project team to value genetic gain and specific traits in the dairy and grain industries where bioeconomic models are developed based on known and predicted performance across a broad range of years within a given environment (Lewis *et al.* 2013, Ludemann *et al.* 2013, Smith *et al*. 2014).

Development of a profit based breeding index for phalaris

(2014 – 2017) Phenotypic, genomic and economic data will be combined to develop a profit index for phalaris in the two production systems described above. These indices will be made available to breeding companies for use during selection and used to describe the value of genetic gain in phalaris breeding. The purpose of this index is to allow a breeding company to make informed decisions about the relative value of individual traits within the potential parents of new cultivars, it is related to but not the same as a "Forage Value Index" for phalaris cultivars as this index requires the analysis of performance data from completed cultivars in the first instance.

Budget Summary

	2013/14	**2014/15**	**2015/16**	**2016/17**	**TOTAL**
MLA	**434,217**	**410,136**	**347,510**	**306,843**	**1,498,706**
UofM	27,300	27,300	27,300	27,300	**109,200**
DPIV	137,833	137,833	137,833	137,833	**551,332**
CSIRO (In kind)	213,886	199,631	204,622	168,550	**786,690**
Total Research Organisation contributions	**379,019**	**364,764**	**369,755**	**333,683**	**1,447,221**

Interest (IP proportions)

MLA	51%
Research Organisations	49%

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

Heritage Seeds, Seed Force and PGWrightson Seeds have indicated that they will provide a letter of support for this investment plan. During discussions between the seed companies and Drs Smith and/or Culvenor the companies made the following indications of their requirements of a phalaris prebreeding program.

- The focus should be on winter-active germplasm with Holdfast GT portrayed as the industry benchmark. Limited (if any) commercial breeding interest would be placed on the Australian-type germplasm pool.
- Key traits requested by the companies were persistence, adaptation and absence of anti-quality factors (alkaloids)
- Markers for seed retention were seen as valuable tools for nursery management and genetic gain.
- Future work should focus on the causes and mechanisms of ‘sudden death’ syndrome and developing genetic solutions for this problem.
- The phalaris market is small and on the edge of commercial sustainability therefore it is essential that the value of new cultivars be known, demonstrated and marketed and that the market is not flooded with a suite of ‘me-too’ cultivars.

Rather than a Communication/Extension Plan *per se* it is proposed that the project team work in conjunction with the commercial partners (see Section 8) and that the results of this integration be presented in project management and milestone reports.

Skills development and training of commercial breeders will be essential if this project is to be successful. The Principal Investigators have individually or in project teams acted in this role for most of the major seed companies operating in the pasture market in Australia (including Heritage Seeds, PGW, Seed Force and Vicseeds) so this model will be viable regardless of both the number of and which companies are involved in phalaris breeding in the future.

3.5 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

Unlike animal breeding, in which EBV-based indices based on BLUP predictions of individual merit to improve the accuracy of the estimation of the genetic merit of an individual have been developed for commercial breeding programs (eg Breedplan, Lambplan) the development and implementation of EBV based indices in forage plant breeding is in its infancy but there is no reason to believe that the gains achieved in animal breeding will not be achieved, in fact this program will combine the advances that would arise from the use of EBVs with those achieved/predicted with the use of Genomic Selection in animal breeding through factors such as decreased generation intervals and more accurate pedigree.

Forest tree breeders have been early adopters of EBVs and whole genome selection (eg Resende *et al.* 2012a, 2012b) and models have been proposed for outcrossing perennial forage grasses (Hayes *et al.* 2013).

Whilst there are no published papers on the application of genomic selection in forages due to the novelty of the methods, a recent simulation study (Resende and Casler 2014) made the following conclusions:

"Application of genomic selection methods to forage breeding is expected to be of greatest value under the following circumstances: (1) when phenotypic evaluation of individual plants is incapable of predicting performance under sward conditions, (2) when it is difficult or impossible to apply meaningful selection pressure within families, or (3) time-intensive phenotypic evaluations necessitate long cycle times, e.g. 4 to 5 years."

Each of these situations applies in phalaris breeding for forage yield and persistence, where the traits need to be measured under sward conditions over a number of years.

The project team will use experiences from forages, dairy, beef and sheep to develop these tools and technologies for phalaris breeding and to quantify the value of these technologies during the project.

Specific areas where the project will enable enhanced genetic gain in phalaris include:

- Enabling between and within family selection to increase the proportion of additive genetic variation that is captured during the selection cycle.
- Increasing selection intensity by removing non-retainers from the breeding nurseries so that more seed retaining plants can be evaluated for the same cost.
- Increased accuracy of phenotyping and appropriate weightings for traits in breeding programs.

3.6 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

One of the key limitations to assessing the economic impact of this project is the lack of tools to assess the marginal impact of new pasture genetics on farm. Many studies compare a run-down or obsolete pasture with a new pasture to assess the effects of pasture renovation *per se* and often these studies also incorporate changes in the farming system (stocking rates, turn off times etc) to take advantage of the new pasture (Lewis *et al.* 2012). An alternative approach is to assume a base case and then test sensitivity to varying a number of parameters such as stocking rate (Lewis *et al.* 2012), using this model to assess the results of the triple pasture systems and the improved ryegrass pastures of the Evergraze project at Hamilton the effect of improved pasture systems on mean net cash flow was predicted to be $100/ha with no increase in stocking rate from 16.2 DSE/ha over the base case. Whereas the increase was in the order of $200/ha if stocking rate was increased to 21 DSE/ha with an associated increase in variability (risk) (Lewis *et al.* 2012). Another approach is the use of bioeconomic modelling to assess the partial values of incremental change in components of the pasture system to give marginal values for improvements in factors such as pasture growth and utilisation within a grazing system, this approach has been used to model pasture growth and utilisation within a perennial ryegrass based prime lamb system in SW Victoria (Young *et al.* 2010) where partial values for an extra kg of DM/day production were shown to vary between $0.90 and $10.30 per hectare depending on growing season and whether the pastures were grazed by first cross or composite ewes.

In the absence of these detailed studies for phalaris it is not possible to provide accurate estimates of the potential value of increased rates of genetic gain in phalaris and the project includes a significant suite of activities in this area of impact analysis. However, based on a hypothetical analysis whereby 10% of the value of an improved system (Lewis *et al.* 2012) could be achieved through the development of better cultivars based on the tools developed in this project a conservative projection could be made that the project will contribute to these MLA objectives in two main areas

1. *Increased Rate of Genetic Gain*

Whilst the benefits of this genetic gain will not be realised on-farm by 2020, the benefits of increased rate of genetic gain will be captured through increased productivity per hectare. For instance if 150,000 hectares of phalaris continue to be sown per annum and the benefit of increased genetic gain were only $10 per hectare this would equate to $1.5M pa. The project will develop robust methodologies to value genetic gain in phalaris in on-farm production systems.

2. Description of the Value of Phalaris Cultivars in Economic Terms

The activities in Work Package 3 will allow the value of novel cultivars to be objectively described and this data could be used to promote the benefits of pasture cultivars to red meat producers and may then lead to an increase in the currently very low rates of pasture renovation. This technology could be applied to existing cultivars and could deliver benefits by 2020. For instance, if phalaris use increased by 10% to replace 1,500 ha of run down pasture and the average benefit of improved phalaris over run-down pastures was $150/ha the benefit would be $225,000 pa. So even at these conservative estimates would equate to approximately 10% of MLA's target. Again more accurate economic analyses are proposed for the project to add rigour to these estimates.

3.7 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

This project will develop potentially valuable intellectual property (IP) including, but not limited to:

- Genome sequence information from phalaris
- SNP and SSR markers for varietal identification and marker assisted selection
- A relationship matrix between individuals and cultivars
- Molecular diagnostics for key traits under relatively simple genetic control such as seed retention
- Haplotype and phenotype associations for genomics assisted selection
- Phenotyping protocols for novel traits
- Germplasm containing/enriched for favourable alleles

It is the aim of the project team to see these outputs deployed widely for the benefit of Australian red meat producers. However, because of the broad range of IP developed in the project, the project team does not believe that the best model to achieve this is to make all of the IP available for free and without constraint to any company that intends to commercialise phalaris. From the beginning of the discussions of this project with MLA it was clear that the project should also include a significant element of training for the private sector as well, the project will move the sector from phenotypic selection to an era of genomics assisted quantitative genetics. This transition took decades in animal breeding.

The project will develop generic tools that can be made broadly available to all interested parties including markers for varietal ID, sequence data and analysis services, phenotyping protocols for desired traits, tools for validating genetic gain - all of these tools would be published in the scientific literature given that the data will be published they will be freely available for all who wish to utilise them in some way, regardless of any analysis service that may be provided.

For genomics-assisted selection it is proposed that MLA and the project partners use the first year of the project to undertake an expression of interest process to identify the primary commercial partner/s (it is not the aim of the project to select any predetermined number of partners) for this project for the following reasons:

- The commercial breeding market is best served when this pre-breeding project focuses on tools and technologies rather than germplasm enhancement. If this project were to develop elite germplasm it would actually encourage the rapid release of this germplasm by a multiplicity of partners rather than the partners further developing the germplasm and losing the early release advantage.
- No commercial company today has an established phalaris breeding program therefore it is critical that companies are encouraged to develop commercial nurseries concurrently with this germplasm enhancement project.
- It is our experience that projects with companies who are active partners are more successful than those where the commercial sector is represented by a consortium with little active investment in the project area.
- Individual companies may elect to prioritise different traits and germplasm pools in their programs. This is better facilitated through bilateral agreements as opposed to proposing that one size fits all.

It is proposed that the “expression of interest process” will be undertaken in partnership with MLA and the assessment criteria for seed company partners will include.

- Scale of proposed phalaris breeding program
- Demonstrated willingness to establish selection nurseries that are well linked to the information nurseries in 2014
- Technical and commercial capability of the proposed breeding programs to incorporate the pre-breeding technologies in consultation with the pre-breeding team.

The proposed relationship model then becomes similar to the “information nucleus”/”stud breeder” model that has evolved in the beef and sheep industries. In time the companies may take on the cost of the information nucleus allowing the research partners to focus on new traits and technologies.

The proposed process is very similar to that has been used by MLA for its co-investments in base animal breeding technologies such as Breedplan and Lambplan. The seed industry may wish to see the model evolve to one where selection algorithms and processes become ‘in-house’ processes as opposed to a centralised testing service like the one provided by Sheep Genetics Australia. The project team believes that the likely outcome is somewhere in the middle of these extremes but some form or central data analysis and interpretation is desirable to allow an industry wide analysis of the relative value of pasture genetics. This business model will be developed with MLA and industry over the life of this project.

3.8 Identify synergies regarding technology development with the team that is currently developing the R&D plan for pre-breeding for annual legumes

Differences in genetic architecture due to taxonomic distance mating behaviour (cross-pollinated vs. self-pollinated) between phalaris and the annual legumes (medics and sub-clover) mean that there are few direct technical synergies between the two projects. It is proposed that the project leader will work with the pillar leader to ensure that experiences are shared between the project teams.

The two teams would be able then to share their extensive experience in pasture plant breeding, but particularly the perennial grasses team will be able to share both its past experience with private:public partnerships and the development of technology (grass and endophyte) in partnership with seed companies with the legume team along with the lessons of developing and implementing genomics assisted breeding in perennial forage plants that will be developed in this and related projects.

The development of consistent IP protection and commercialisation strategies between the two projects would also be desirable.

3.9 Draft plans to be submitted to MLA who will distribute for comment as part of a consultation process. MLA will collate responses for inclusion into final version of the R&D plan. Revised plans (including responses to received comments) to be submitted to MLA.

This draft investment plan was made available to MLA for circulation to the Feedbase Steering Committee. Circulation to any other parties requires the consent of the submitting agencies.

3.10 Milestones and schedule

1. Report detailing progress in establishing training nursery and preliminary economic models (30 June 2014)

- Knowledge of genomic diversity in phalaris
- Confirmation of SNP incidence in phalaris
- Knowledge of the degree to which CSIRO program has sampled overall diversity
- Markers for varietal and species identification
- Guidelines for the design and extent of training population/s

- Preliminary estimates of economic value including analysis of the role of seasonal productivity versus persistence.
- Model for engagement with seed companies described and developed

2. Interim report describing progress towards deliverables due June 2015 and beyond . (30 Dec 2014)

3. Project progress report describing the following deliverables (30 June 2015)
    - Approximately 15,000 SNPs available for genome wide association studies
    - Commencement of genotyping of Training Nursery and Commercial Selection Nurseries
    - Estimates of economic value including analysis of the role of seasonal productivity versus persistence adjusted for risk.
    - Experimental design and breadth of germplasm reported and shared with MLA and seed companies
    - Protocols for measuring seasonal yield, persistence, seed retention and incidence of pests and diseases documented.

4 Interim report describing progress towards deliverables due June 2015 and beyond. (30 Dec 2015)

5. Project progress report describing the following deliverables. (30 Jun 2016)
    - Initial genome wide association studies for seed retention, seasonal yield and persistence
    - Definition of a "Breeding Objective" for target environments and production systems, this breeding objective to be outcome based and hence applicable to phalaris, cocksfoot and any other perennial grass species sown in these environments.
    - Measurement seasonal yield, persistence, seed retention and incidence of pests and diseases documented.
    - Collection of data from seed company nurseries for curation and analysis

6. Project progress report describing the following deliverables. (30 Dec 2016)
    - Genome wide association studies for seed retention, seasonal yield and persistence
    - Definition of a genomics assisted breeding strategy for phalaris including estimated breeding values
    - Definition of the value of genetic gain in phalaris in terms of specific traits and an overall index.
    - Measurement seasonal yield, persistence, seed retention and incidence of pests and diseases documented.
    - Collection of data from seed company nurseries for curation and analysis

Final Report: Detailing progress towards developing and implementing genomic selection strategies in phalaris including phenotypic and genomic analyses and the description of a robust breeding system for the target environments and production systems. (30 Sep 2017 - 3 months after project completion).

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5. Brief CVs for Principal Investigators on MLA Co-Funded Phalaris Pre-Breeding Program

Phalaris 2020 Vision – Better Cultivars Faster

Developing the Tools and Strategies for Genomics Assisted Selection in
Phalaris aquatica

Professor Kevin Smith
The University of Melbourne
Project Leader and Principal Investigator

Relevant Experience and Role in Project

Prof Kevin Smith (Professor of Pasture Agronomy (Plant Breeding - University of Melbourne) has 25 years experience in forage grass genetics and breeding with a particular emphasis on the development and application of new breeding technologies in conjunction with private sector breeding programs. He has been actively involved in the development and application of the national research and development programs for red meat and dairy. He has trained more than 10 PhD students in forage breeding, published more than 70 refereed papers and bred cultivars of perennial ryegrass, tall wheatgrass and white clover. Key achievements related to this project include the ability to link quantitative, molecular and economic data in breeding programs and the development of effective linkages with the private sector. Prof Smith will lead the project and bring relevant expertise in quantitative genetics and molecular plant breeding.

Significant publications last 5 years

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Dr Richard Culvenor
CSIRO
Principal Investigator

Relevant Experience and Role in Project

Dr Richard Culvenor (Senior Scientist at CSIRO in Canberra). He has led the CSIRO phalaris breeding program with a particular emphasis on the development of winter-active phalaris cultivars with adaptation to hostile soils and improved grazing tolerance. Key achievements include (i) breeding of Advanced AT, the most acid-soil tolerant cultivar yet developed, (ii) Holdfast GT, a winter-active cultivar with improved grazing tolerance, (iii) a cultivar for the low rainfall margins to replace Atlas PG (yet to be named), and (iv) physiological and grazing research on persistence factors in phalaris. He was also involved in the breeding of Landmaster, Atlas PG and Australian II. He has published more than 40 refereed journal papers. Dr Culvenor will be mainly responsible for phenotyping phalaris populations for association with molecular markers developed at La Trobe University. This will include running of the main “teaching nursery” for genomic selection and involvement in other nurseries run at other locations, e.g. to account for GxE interactions, company nurseries.

Significant publications last 5 years

Haling, R.E., Simpson R.J., Culvenor R.A., Lambers H., Richardson A.E. Field application of a DNA-based assay to the measurement of roots of perennial grasses. Plant and Soil. 358: 183-199. 2012.

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Professor German Spangenberg
Department of Environment and Primary Industries
Principal Investigator

Relevant Experience and Role in Project

Prof German Spangenberg (Executive Director of Biosciences Research at DEPI and Professor of Biosciences at LaTrobe University). Is a world authority on the development of biotechnologies for application to plant industries – particularly forages and a Fellow of the Australian Academy of Technological Sciences and Engineering. He has been actively involved in the development and application of the national research and development programs for red meat and dairy. He has trained more than 25 PhD students in forage breeding, published more than 130 refereed papers and is listed as inventor on 45 patents. Prof Spangenberg will contribute significant expertise in the development and application of genomics technologies in plant breeding to the project.

Significant publications last 5 years

Boden SA, Langridge P, Spangenberg G, Able JA (2009) *TaASY1* promotes homologous interactions and is affected by deletion of Ph1. *The Plant Journal* 57:487-497.

John UP, Polotnianka RP, Sivakumaran KA, Chew O, Mckin L, Kuiper MJ, Talbot JP, Nugent GD, Mautord J, Schrauf GE, Spangenberg GC (2009) Ice recrystallisation inhibition proteins (IRIPs) and freeze tolerance in the cryophilic Antarctic hair grass *Deschampsia antarctica* E. Desv. *Plant, Cell and Environment* 32:336-348.

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Professor John Forster
Department of Environment and Primary Industries
Principal Investigator

Relevant Experience and Role in Project

Prof John Forster (Research leader for molecular genetics at DPIV and Professor of Molecular Genetics at La Trobe University). John is Leader and Principal Research Scientist in Molecular Genetics for the Victorian Department of Primary Industries' Biosciences Research Division, Professor of Molecular Genetics at La Trobe University, and Research Leader for the Designer Forages sub-program of the Dairy Futures CRC. He leads a group of 30 staff and students applying DNA sequencing and genotyping technologies for improvement of a broad range of domestic animals, agricultural microbes and crop species, using state-of-the-art equipment platforms. John's personal research work has focused on developing and implementing genomics-assisted tools for breeding improvement of forage grasses and legumes. In this capacity, he has pioneered the construction of genetic maps, use of high-throughput genetic markers, studies of genetic diversity and tagging of key agronomic genes. He has widely collaborated and presented several keynote addresses at international research conferences He has trained more than 15 PhD students in forage molecular genetics and breeding, and published more than 95 refereed papers. Prof Forster will lead the genome sequencing and analysis sections of this project.

Significant publications last 5 years

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Pembleton, L.W., Wang, J., Cogan, N.O.I., Pryce, J.E., Ye, G., Bandaranayake, C., Hand, M.L., Baillie, R.C., Drayton, M.C., Lawless, K., Erb, S., Dobrowolski, M.P., Sawbridge, T.I., Spangenberg, G.C., Smith, K.F., Forster, J.W. (2013) Candidate gene-based association genetics analysis of herbage quality traits in perennial ryegrass (*Lolium perenne* L.). *Crop and Pasture Science* 64: 244-253.

Pembleton, L.W., Cogan, N.O.I, Forster, J.W. (2013) StAMPP: an R package for calculation of genetic differentiation and structure of mixed ploidy level populations. *Molecular Ecology Resources* 13: 946-952.

“Career 10 relevant best”

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