MEAT & LIVESTOCK AUSTRALIA

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Phalaris Pre-Breeding

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Abstract

Phalaris (*Phalaris aquatica*) is an important perennial grass sown for meat production in temperate Australia. The species has a reputation for being persistent and able to support high levels of animal production. In contrast to species such as perennial ryegrass where major efforts have been undertaken to develop genetic and genomic technologies to increase the rate of genetic gain in the species, genetic research in Phalaris has focussed in recent decades on the development of new cultivars to address requirements of farming systems such as winter-activity, aluminium tolerance and grazing tolerance.

The main purpose of this project was to describe new pre-breeding traits and develop breeding technologies for the phalaris species that will enable the rate of genetic gain in phalaris breeding programs to be increased.

Three work packages (sets of discrete activities for delivery of a program sub-component) of co-ordinated activities will contribute to the development of tools and technologies to increase the rate of genetic gain in phalaris breeding programs and will achieve the following outcomes:

- Genetic gain in phalaris described and quantified in economic terms
- Phalaris breeding programs prepared for genomics assisted breeding strategies
- The use of phalaris as a model for the deployment of improved breeding

The following objectives were delivered:

1. Described the phenotypic and genetic diversity in phalaris (bred both within Australia and internationally) germplasm to guide the design of future breeding programs, with a focus on the winter-active germplasm pools including the development of molecular marker panels suitable for cultivar identification and genetic diversity studies. These pools had been identified as priority areas for further breeding based on consultations with seed companies.
2. Developed a methodology for genomic selection for complex traits such as yield and persistence (based on the regression of allelic variation in genetic markers with phenotypic variability) in phalaris breeding programs, in collaboration with private seed companies, which will allow for more efficient selection of quantitative traits relevant to the economic value of phalaris. The most economically important traits of total yield and seasonal yield distribution were used in the model.
3. Developed markers for seed retention as a mechanism to increase the rate of genetic gain in phalaris breeding programs.
4. Used 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 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 traits such as seasonal yield and persistence.
    c. Calculate the economic benefit of new sowings to producers.

5. Private seed companies using these technologies (genomic selection methodologies, gene markers for seed retention, genetic diversity analyses) in their own breeding programs.

As a result of this project

- More than 140,000,000 bp of reference data have been sequenced from the phalaris genome and more than 500,000 putative SNPs identified at a frequency of 1 SNP per 262 bp. This is by far the largest contribution of phalaris sequence information and will act as a resource for further research and practical outcomes in phalaris breeding.
- An economic model to assess differences in the value of phalaris has been developed based on the relative importance of seasonal yield and could be used as the basis of a genomic selection program. This information could also be combined with cultivar evaluation data from the Pasture Trial Network (PTN) to develop a Forage Value Index for phalaris cultivars similar to that which has been successfully released for perennial ryegrass cultivars for the dairy industry.
- Proof of concept of the use of genomic information to select for yield and seed characteristics has been obtained.
- Lessons/ key messages
    - Population structure in phalaris germplasm means that the initial application of genomic selection methods will require that reference populations are developed based on the diversity analysis within this project. This situation is analogous to 'within breed' vs 'across breed' selection models in cattle.
    - Depending on company relationships and breeding priorities there is the opportunity to pool resources during the development and evaluation of reference populations.
    - Genomic sequence information can be used to accurately identify phalaris cultivars.
- The use of economic weightings to measure the economic value of genetic gain and the relative importance of seasonal production in meat production systems could be used to develop a "Breeding Objective" for use in selection that would be aligned with a "Forage Value Index" used in cultivar evaluation that would integrate selection and measurement throughout the genetics supply chain.

Executive summary

Phalaris (*Phalaris aquatica*) is an important perennial grass sown for meat production in temperate Australia. The species has a reputation for being persistent and able to support high levels of animal production. In contrast to species such as perennial ryegrass where major efforts have been undertaken to develop genetic and genomic technologies to increase the rate of genetic gain in the species, genetic research in phalaris has focussed on the development of new cultivars to address requirements of farming systems such as winter-activity, aluminium tolerance and grazing tolerance. Further gains in various traits in phalaris will be possible by continued selection based on quantitative genetics principles but the new field of genomic selection enabled by advances in rapid genome sequencing offer clear prospects of increased rate of genetic gain and is attracting interest worldwide not only in crop species and major pasture species like perennial ryegrass (*Lolium perenne* L.), but also in species such as switchgrass (*Panicum virgatum* L.) which commercially on a world scale can be considered a minor species like phalaris.

This project was initiated with the following objectives that would develop the tools to enable genomics assisted breeding in phalaris through the provision of publicly available genomic sequence data, the assessment of the practicality of genomic selection in phalaris and the development of a model to value genetic gain in phalaris.

Specifically, the objectives of this projects were to have:

1. Described the phenotypic and genetic diversity in phalaris (bred both within Australia and internationally) germplasm to guide the design of future breeding programs, with a focus on the winter-active germplasm pools including the development of molecular marker panels suitable for cultivar identification and genetic diversity studies.
    a. The genetic and phenotypic diversity in phalaris was assessed and shown to be similar to that seen in other forage species such as perennial ryegrass.
    b. There was evidence that although like-cultivars clustered together that there was sufficient diversity to allow selection.
    c. SNP based molecular maker panels suitable for cultivar identification and genetic diversity studies for phalaris were developed and exemplified.
2. Developed a methodology for genomic selection for complex traits such as yield and persistence (based on the regression of allelic variation in genetic markers with phenotypic variability) in phalaris breeding programs, in collaboration with private seed companies, which will allow for more efficient selection of quantitative traits relevant to the economic value of phalaris.
    a. A genomic selection method for seasonal forage yield was developed using winter-active phalaris as the example reference population.
    b. The collaboration with seed companies in development of this was via individual consultation prior to the commencement of the project and at regular intervals throughout the project.

3. Developed markers for seed retention as a mechanism to increase the rate of genetic gain in phalaris breeding programs.
    a. Eight potential markers for seed retention were identified but require further validation. These markers were identified in the commercially important cultivar Landmaster and given the interrelatedness of cultivars from the CSIRO phalaris program they are likely to be able to be used within all populations from this program and in crosses using this germplasm.
    b. Markers have been published an are available for use and validation in any breeding program.
4. Used 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 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 traits such as seasonal yield and persistence.
    c. Calculate the economic benefit of new sowings to producers.
5. Private seed companies using these technologies (genomic selection methodologies, gene markers for seed retention, genetic diversity analyses) in their own breeding programs.

These objectives cover the basic tools for the development of a genomics assisted breeding program for phalaris.

Objective 1.

In this objective, the largest database of genomic sequence information from phalaris was assembled and published on a publicly available database that is freely available to all breeding companies and genomics services providers for customisation to their specific needs. This sequence information can also be used to identify all phalaris cultivars or breeding lines, even if they are closely related.

Specifically, a filtered set of 89,738 SNPs was identified from 290 genotyped samples in the training population, along with the associated custom bioinformatics pipeline capable of handling missing data and the segmental allotetraploid nature of phalaris. A total of 14 phalaris cultivars and populations were used to develop an initial understanding of genomic relationships in current cultivars and breeding populations. These revealed close affinities reflective of known aspects of the cultivars' breeding history. From the diversity of populations that are present in the training/reference population, Advanced AT is the most distinct cultivar, while the other cultivars share a higher level of similarity (Landmaster, Holdfast, Holdfast GT). Consequently, two or more prediction equations may be required to increase accuracy for selection purposes depending on the specific germplasm used in any breeding program. Similar results have been observed in other pasture and animal species.

Objective 2

With the construction of a large scale genomics database it is possible to undertake genomics assisted breeding for complex traits such as yield and persistence. The use of genomic selction has revolutionised animal and plant breeding programs. In this project we have demonstrated the

applicability of genomic selection in commercially relevant populations of phalaris with accuracies similar to those achieved in perennial ryegrass.

Specifically, a genotyping-by-sequencing approach using skim sequencing of the transcriptome was developed and implemented in this project. A filtered set of 89,738 SNPs was identified from 290 genotyped samples in the training population, along with the associated custom bioinformatics pipeline capable of handling missing data and the segmental allotetraploid nature of phalaris. A total of 14 phalaris cultivars and populations were used to develop an initial understanding of genomic relationships in current cultivars and breeding populations. These revealed close affinities reflective of known aspects of the cultivars' breeding history. From the diversity of populations that are present in the training/reference population, Advanced AT is the most distinct cultivar, while the other cultivars share a higher level of similarity (Landmaster, Holdfast, Holdfast GT). Consequently, two or more prediction equations may be required to increase accuracy for selection purposes.

The training population for this project was based on several modern winter-active cultivars: 50% on crosses among and within Holdfast GT, Advanced AT and Landmaster, 32% on crosses among progeny of Advanced AT and a related population, and 18% on a commercial population derived from crosses between Landmaster and Advanced AT with a Holdfast-related population. The resulting training population comprising 290 half-sib families should be applicable to future commercial breeding programs in the seed-retaining, winter-active phalaris pool. A partial sequence based on RNA (transcriptome) was completed for phalaris and ~63,000 single nucleotide polymorphism (SNP) markers identified to genotype training population individuals. The training population was phenotyped for seasonal herbage biomass at sites in southern-eastern Australia. Biomass data fom the Canberra site are summarised for this report. Accuracies (correlation between GEBV and observed phenotype) of 0.545, 0.302, 0.401 and 0.174-0.6 where achieved for heading date, summer activity, average biomass and seasonal biomass, respectively. Based on these results genomic selection was considered practical with accuracies similar to those achieved with similar population sizes in perennial ryegrass and switchgrass.

Objective 3.

One of the constraints to breeding phalaris and the broader commercialisation of phalaris is the fact that many populations are not fixed for the seed retention trait and seed shattering plants are still produced that have much lower commercial seed yield.

We used a genome-wide study on the intact rachilla seed retention trait based on 288 Landmaster plants and identified 8 putative markers related to aspects of seed retention. Further work is required to validate these markers but they provide the basis for marker assicted selection for seed retention in phalaris.

Objective 4.

A model to value genetic gain in phalaris was developed based on the relative value of seasonal changes in dry matter production with respect to lamb and beef production systems. This model could be used as a basis for a "Forage Value Index" similar to those used for perennial ryegrass in the dairy industry or for the basis of a 'Breeding Objective' in a phalaris breeding program.

Each genetic gain of 1kg of DM was shown to be worth between $0.16 (summer) and $0.31 (winter) on a prime lamb production system demonstrating the importance of genetic gain in the appropriate seasons for meat production systems.

Calculation of the economic values were generally lower using the "change in stocking rate" method than for alternative "change in livestock production" method. Both methods suggested that genetic gain in seasonal DM production is highest in value during the winter season in south-eastern Australia, supporting the adoption of a training population based on the "winter-active" pool of phalaris cultivars. The change in livestock method better accounts for the variation in seasonal value of DM compared to the replacement cost method whilst not having such onerous and specific data requirements of a whole farm model. The change in livestock production method was considered more suitable for estimating economic values in a forage selection index at the industry scale. DM Yield and its seasonal distribution were chosen as the initial traits to value as all other traits such as disease resistance contribute to this outcome trait. However, the model is robust and general and could be expanded to include other traits.

Objective 5.

The populations and methods used in this project were developed in conjunction with seed companies and they were regularly briefed during the course of the project to ensure that they were aware of the research and its outcomes. The project has developed the tools to allow commercial application in relevant backgrounds but the lack of co-ordinated breeding activities in the private sector has limited the uptake of the technologies developed in the program. For companies to adopt these technologies it is likely that they will partner with genomics service providers, rather than bring these 'in-house' this model has been used by independent ryegrass breeding programs in Australia, New Zealand and Denmark.

In order to ensure the maximum outcomes from this research it is recommended that:

- MLA consider the development of a 'Forage Value Index' similar to those used by the dairy industry in Australia, New Zealand and Ireland. These indices are being used by farmers to choose new cultivars and also by breeding companies as the basis for trait weightings in their breeding programs. These use of this data does not require companies to adopt genomic selection and can be used in traditional selection approaches.
- That companies are made aware of the location of the genomic sequence data and that it is freely available.
- That a further information session be organised with interested seed companies through the Australian Seeds Federation to discuss the data described in this report and its application to breeding.

Table of contents

1 Background

Phalaris (Phalaris aquatica L.) is the most important perennial pasture plant species in medium (500-750mm per annum) rainfall regions of southern Australia. Progress has been made in improving seasonal growth, grazing tolerance and seed production through traditional plant breeding methods (Smith 2013). A large number of genes control the actions of most or all of these traits. Conventional selection based on quantitative genetics principles can make gradual but slow improvement in these traits (Smith 2013).

Genomic selection has shown promise as a method of improving rates of gain if used in combination with traditional selective breeding of animals (Hayes, et al. 2009) and plants (Resende, et al. 2014). Novelty of the method means there are currently few published studies that have examined genomic selection in forage plants (Smith 2013). Genomic selection was concluded to be of greatest value when there are: difficulties evaluating the phenotype of plants under sward conditions, when it is difficult or impossible to apply selection pressure within families, or when traits require long cycle times for phenotypic evaluations (Resende, et al. 2014). Genomic selection could therefore be used to improve the rates of gain in key phalaris traits of interest to sheep and beef farmers. This is because the three aforementioned situations which make genomic selection valuable occur in the breeding of phalaris for dry matter yield, and persistence (Smith 2013).

Whereas marker assisted selection may be used for simple traits controlled by relatively few loci (seed retention) experience in major crop species has shown that breeding programs are limited in the number of marker trait associations that can be managed. Marker assisted selection is also not suited for complex traits such as yield where genomic selection is the practical method of choice. In genomic selection allelic affects across the whole genome are estimated in a reference population that is chosen to represent the future breeding population and evaluated in a target environment/s. These affects are then used to estimate the genomic breeding value of new individuals.

The economic value of changes in forage plant traits through genomic selection is an important consideration when deciding the most appropriate resources to direct toward traits of most benefit to farmers. Forage plants are an indirect product in a farm system (Abadi Ghadim and Morrison 1992) as they are converted into other products such as meat, wool and milk using ruminants. This makes pasture plants difficult to calculate economic values for. Recently, efforts have been directed toward developing tools to assess pasture traits (Lewis, et al. 2013; Ludemann, et al. 2013). The main methods of assessing economic value of plant traits have included the replacement cost method (Johnson and Hardin 1955; Ludemann, et al. 2013) and the change in production method (Moore, et al. 2009).

2 Project objectives

1. Described the phenotypic and genetic diversity in phalaris (bred both within Australia and internationally) germplasm to guide the design of future breeding programs, with a focus on the winter-active germplasm pools including the development of molecular marker panels suitable for cultivar identification and genetic diversity studies.

2. Developed a methodology for genomic selection for complex traits such as yield and persistence (based on the regression of allelic variation in genetic markers with phenotypic variability) in phalaris breeding programs, in collaboration with private seed companies, which will allow for more efficient selection of quantitative traits relevant to the economic value of phalaris.
3. Developed markers for seed retention as a mechanism to increase the rate of genetic gain in phalaris breeding programs.
4. Used 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
5. Assess the genetic merit of individual plants and cultivars.
6. Assess the value of individual sub-traits (such as pest and disease tolerance) that contribute to pasture traits such as seasonal yield and persistence.
7. Calculate the economic benefit of new sowings to producers.
8. Private seed companies using these technologies (genomic selection methodologies, gene markers for seed retention, genetic diversity analyses) in their own breeding

3 Methodology

3.1 Genetic and phenotypic diversity in phalaris described to guide the design of future breeding programs with a focus on winter-active germplasm

3.1.1 Field trials and the selection of germplasm for evaluation

Key to employing genomics in plant breeding is the matching of genotype with accurate phenotyping. In the project proposal we stated our intention to make seasonal production the trait of highest priority in this project following more than two decades of breeding work in CSIRO introducing specific traits related mainly to persistence factors (e.g. Al tolerance, grazing tolerance). Persistence factors nevertheless remain a high priority and phenotyping to develop genomic relationships for Al tolerance and grazing tolerance is also being undertaken. The training population for this project was based on several modern winter-active cultivars following discussions with seed companies that indicated either a current interest in Phalaris breeding or a likely future interest in breeding. 50% on crosses among and within Holdfast GT, Advanced AT and Landmaster, 32% on crosses among progeny of Advanced AT and a related population, and 18% on a commercial population derived from crosses between Landmaster and Advanced AT with a Holdfast-related population. The resulting training population comprising 290 half-sib families should be applicable to future commercial breeding programs in the seed-retaining, winter-active phalaris pool. Advanced AT has 10-15% of its genetic background from Phalaris arundinacea which may explain its distinctness in the genotypic data.

Activities related to phenotyping yield and persistence traits were undertaken during the project. Seed retention remains an important commercial trait enabling easier production of high quality seed, a view confirmed in a recent discussion between Heritage Seeds and CSIRO. Markers for the seed retention trait were targeted in a genome-wide association study. The project deliberately did not target potential chemical causes of phalaris toxicity due to uncertainty of all the compounds

involved but we have begun an attempt at genomic selection for known alkaloids by phenotyping the training population parents.

Seasonal Yield

Trials to evaluate seasonal yield were conducted at 3 sites, Canberra, Hamilton and Maryborough. Canberra and Hamilton represent major phalaris production areas in southern NSW and Western Victoria. Maryborough is a slightly drier, less productive environment but is in an important phalaris production area. This site was in almost continuous drought through 2014 and 2015 so that observations were limited in those years. High rainfall in 2016 enabled the collection of yield data for the final year but spatial variation was high due to the earlier drought.

The training population families plus 15 controls (total entries=305) were sown in rows spaced 40 cm apart in May (Canberra, Maryborough) or October (Hamilton) 2014 with 3 replicates at all sites at a sowing rate within the row equivalent to 5 kg/ha of viable seed. Row length was 4 m at Canberra and 2 m at Hamilton and Maryborough. A row-column design amenable to spatial analysis was used at all sites. Yield was assessed by calibrated visual estimates because of the large number of rows.

Dates on which measurements were taken are shown in Table 3.1.1. There was a complete set of within growing season measurements for Canberra and Hamilton supplemented with summer measurements at Canberra. Only one yield assessment was possible at Maryborough in 2015 due to drought but complete within growing season measurements were obtained in 2016. At Canberra, rows were scored for DM yield by 2 observers on a 10-point scale and scores were calibrated by cutting 15-20 row segments 0.5 m in length from across the range. The row trial was grazed off by 100-300 Merino sheep over 2-3 days after each assessment. Heading date was assessed in 2014 as the date in October when 50% of stems had emerged heads.

Fertiliser applications at Canberra were: 40 kg N/ha as urea in Novermber 2014, 25 kg N/ha in March 2016, 55 kg N/ha as urea in August 2015 and 2016, and 150 kg/ha single superphosphate in March 2016.

Table 3.1.1. Phenotypic measurements obtained on training populations rows at 3 sites.

Measurement	Canberra	Hamilton	Maryborough
Herbage DM (estimated)	Seedling	Seedling	Seedling
	Summer 2014/15	*	*
	Autumn 2015	Autumn 2015	*
	Winter 2015	Winter 2015	*
	Spring 2015 (1)	Spring 2015 (1)	Winter/Spring 2015*
	Spring 2015 (2)	Spring 2015 (2)	*
	Summer 2015/16	*	*

	Autumn 2016	Autumn 2016	Autumn 2016
	Winter 2016	Winter 2016	Winter 2016
	Spring 2016 (1)	Spring 2016 (1)	Spring 2016 (1)
	Spring 2016 (2)	Spring 2016 (2)	*
Row density	2-Oct-14	*	12-Nov-14
	9-Sep-15	*	23-Oct-15
	13-Sep-16	*	17-Jun-16
Heading Date	Oct-14 (rows) Oct-16 (parents)	*	*
Summer tillering activity	18-Feb-16	*	*

*Only one measurement in 2015 due to severe drought

To support our calibrated visual estimates for DM in rows, we ran a comparison of the row estimates with estimates made in plots. This comparison of swards vs. rows, conducted at Canberra only, included 33 lines, 8 cultivars, 2 summer-dormant breeding populations and 23 training population families for which there was sufficient seed. Sowing rate was 4 kg/ha of viable seed and there were 4 replicates. Plots were scored visually by 2 observers whenever the row trial was assessed. At least 15 quadrats of 0.5 m x 0.5 m dimensions were cut to calibrate the scores.

Canberra May 2015

December 2015

Hamilton late October 2015

Maryborough March 2016 June 2016

Fig. 3.1.1. Views of the three sites.

3.1.1.1 Aluminium tolerance

Al toxicity is a key factor which affects establishment and survival of phalaris in acid soils due to stunting of root growth. Two cultivars in the training population, Advanced AT and Landmaster, have significantly increased tolerance of soluble Al and are the recommended cultivars for acid soils (pHCa<4.4). Parents of the training population were screened for Al tolerance in a hydroponics system.

Al tolerance screening was conducted by breaking off 10 tillers from each parent plant during winter, cutting the root system at approx. 1 cm from the plant base, and planting these in tanks containing a low ionic strength nutrient solution similar to that used by Requis and Culvenor (2004) either without Al (control) or with 100 µM Al at pH 4.2. There were 5 tanks of each treatment with one tiller per parent in each tank. Each tank held 96 tillers in 36 L of solution changed twice per week for 2 weeks. At the end of each run each plant was given a root condition score on a 1-9 scale where 1 indicated no root growth and 9 indicated a healthy, fine, vigorous root system, root system length was measured, and roots separated from shoots for DM measurement. Screening of the training population required 3 runs to do 282 of the parents with a sensitive and tolerance control in each tank. Two screening runs were performed in June and July 2015 and the third run in July 2016.

3.1.1.2 Persistence under grazing

Holdfast GT was the first winter-active phalaris cultivar bred specifically for improved persistence under heavy grazing pressure in response to criticisms of earlier winter-active cultivars not persisting as well as the Australian cultivar. In theory, genomic selection conducted in annual cycles is ideal for speeding selection for persistence due to the length of each conventional cycle, which was 4 years (3 full grazing seasons) in each of 3 cycles during the development of Holdfast GT.

There was sufficient seed of 181 of the 290 training population families to sow a persistence experiment. This was sown at 4 kg/ha of viable seed in 5 m x 0.9 plots replicated 3 times during August 2014 at Canberra and was continuously stocked mostly at 18 hoggets/ha from May to mid-December 2015 when it was destocked due to very low herbage mass under Animal Ethics criteria. Sheep were returned in mid-February for 3 weeks but removed again due to low herbage mass. The trial was stocked at 16.5 wethers/ha from Jun-Oct 2016 and then stocked at 20 wethers/ha until late January 2017 The trial was stocked with 103 hoggets for 4 days in April to control weeds growth after good rains and then continuously stocked at 16.5 ewes/ha from 21 April until 19 May 2017, and 18 ewes/ha from 21 June to 1 December 2017 and from 3 January to 22 February 2018. All sheep were Merinos. Granulock 15 (15%N, 12%P) was applied at 200 kg/ha each except for 2016 when 150 kg/ha was applied.

Basal frequency in 2 fixed quadrats of dimension 1 m x 0.75 m per plot was measured in October 2014, June 2015, July 2016 and May 2017 by counting the number of 10 cm x 15 cm cells in which phalaris base was present and expressing as a percentage. The 15 cm dimension better matched the row spacing than the 10 cm we normally use. Seedling vigour was scored in December 2014 and green herbage mass in May 2015 and February 2016. Phalaris density was also scored visually in June 2017.

Family means for basal frequency in 2017 are shown in Fig. 3.1.3. The differences largely reflect changes in frequency over the period 2014-17. The lower persistence of the Advanced AT pool of families is clear (AT crossed to AT). Families sown from seed of Advanced AT-related plants mixed with Holdfast GT and Landmaster-related plants were intermediate between Advanced AT and Holdfast GT suggesting that the progeny of these plants were crosses.

The grazing persistence experiment has continued into 2018 outside of the duration of this project when final measurements of basal frequency and a visual density score will be made during winter.

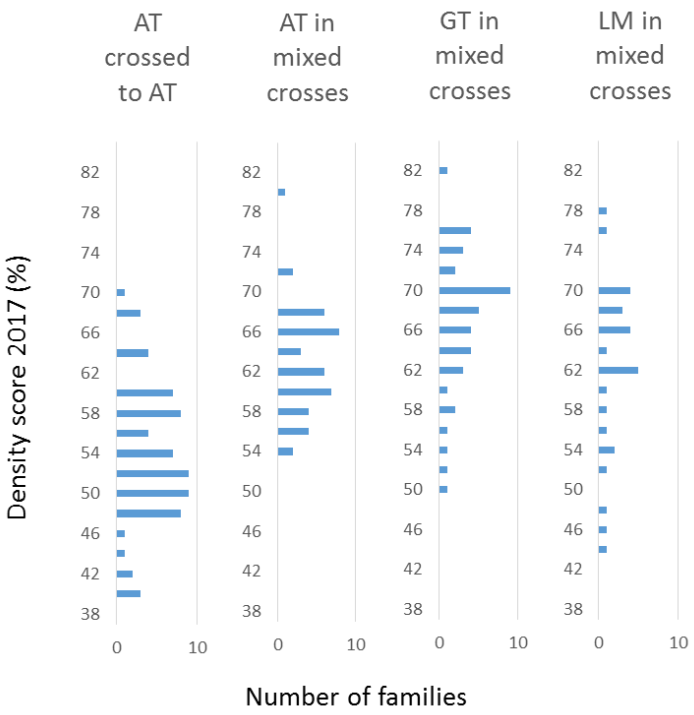


Fig. 3.1.3. Density score (0-100 scale) in June 2017 for heavily grazed plots of training population families sown in 2014 at Canberra

Plots under grazing, late October 2015

Plots just before destocking, December 2015

Fig. 3.1.4. Grazing tolerance phenotyping trial, Canberra

3.1.1.3 Seed retention

Identification of markers for the intact-rachilla type of seed retention currently in more recent CSIRO-bred cultivars has the potential to increase the rate of genetic gain in phalaris by avoiding the loss of a year to observe the trait which can only be assessed after seed set. A spaced plant trial of 800 plants was established in May 2015 to act as a resource in which good seed retainers or non-retainers could be identified. Table 3.1.2 shows numbers of phenotypes identified during the 2015/16 summer. Selected plants were genotyped in 2015.

Table 3.1.2. Plant numbers in seed retention categories in Canberra spaced plants.

Category	Advanced AT	Holdfast	Holdfast GT	Landmaster	Cell 14 polyx	Cell 16 polyx
Good retainers	42	20	44	25	19	16
Good non-retainers	9	38	18	39	5	11
Other retainers	23	9	15	9	8	7
R-R minus, R minus	20	13	22	15	13	12
Partial retainers	8	7	8	11	5	9
PR-N	10	11	5	7	3	
Other non-retainers	1	16	7	9	4	4

A further 350 Landmaster individuals were planted in late August 2016 to identify more retainer and non-retainers in the 2016/17 summer to help develop a genomic relationship for seed retention. 288 of these plants were rated in the field during January after which 4 heads were cut from each plant for more intensive examination. Sampled heads were examined under low power magnification and the proportion of visible florets containing seed after several sharp flicks to remove seed was visually estimated. Heads of each plant were then threshed and the number of seeds remaining in the heads counted with an electronic seed counter. Retained seed was expressed per unit of head length.

3.1.1.4 Alkaloids

Causes of the various forms of phalaris toxicity are not known with certainty, in particular the cause of the important 'sudden death" form of toxicity. However, it is likely that some presently known alkaloids are causes of toxicity. For example, tryptamine-related alkaloids have been clearly implicated in phalaris staggers (Bourke et al. 1990, AVJ 67, 356) and CSIRO has endeavored to select against the tryptamine-related alkaloids in its winter-active cultivars. We therefore decided to screen the training population parents for the level of at least 7 known alkaloids: N,N-dimethyltryptamine, 5-methoxy- N,N-dimethyltryptamine, bufotenine, gramine, tyramine, N-methyltyramine, hordenine. A sampling methodology which involved combining reps (compositing) of most lines and analyzing individual reps only of some lines to obtain an error estimate was employed (Smith et al. 2011 Appl. Statist. 60, 437-455). Clones of 285 training population parents plus 2 Holdfast and one Australian plants as controls in 2 replicates were established in the field in September 2015. Plants were sampled in May 2016 by plucking the youngest fully expanded leaf blade from 8-16 tillers. This sample was chopped into ca. 1 cm lengths and 4 g FW subsample immersed in 20 ml of 0.1 M HCl and placed in a cool room. The extracts were shaken once daily for 5 days, filtered and the supernatant frozen awaiting analysis. Samples were analysed by Southern Scientific Services at Hamilton using HPLC for chemical separation and UV spectra for identification of alkaloids against standards. Results indicate that the plants were unexpectedly low in alkaloids at the time of sampling and the data for some alkaloids is dominated by zero values which has presented difficulties in statistical analysis which is on-going.

October 2015 December 2015

Fig. 3.1.5. Views of the alkaloid nursery at Canberra.

3.1.2 Development of transcriptome sequence resource for phalaris

3.1.2.1 Plant materials

A single plant from the cultivar Landmaster (identified as #D19-17) was selected as the reference genotype for transcriptome assembly. Clonal copies of the plant were produced by vegetative propagation and grown in standard potting mix in 200 mm plastic pots at 22 ± 2°C with a photoperiod of 16/8-h (light/dark) in a glasshouse.

A total of 11 cDNA libraries were developed from vegetative tissues, tips and mid-sections of individual leaves 1, 2 and 3 from a single tiller; whole pseudostem (designated pseudostem 1); lower pseudostem (pseudostem 2); upper pseudostem (pseudostem 3); root tip and mid-root. In addition, 4 cDNA libraries were constructed from tissues associated with reproductive development: elongated stems prior to flowering; early emerging flowering head; whole head prior to anthesis; and whole head at seed set (Fig. 4)

3.1.2.2 De novo transcriptome sequence assembly

RNA extraction was performed using the RNeasy (Qiagen, Hilden, Germany) protocol following manufacturer's instructions. RNA-Seq libraries with an insert size of c. 350 bp were generated using the SureSelect Strand-Specific RNA Library Prep Kit and evaluated using the TapeStation 2200 platform with D1000 ScreenTape System (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Each RNA-Seq library was generated with a unique barcode, and an equal mass of each sequencing library was combined to create a single pooled sample for sequencing. The pooled sample was quantified using the KAPA library quantification kit (KAPA Biosystems, Boston, USA). Libraries were pair-end sequenced using the HiSeq 2000 or 3000 or NextSeq 500 systems (Illumina Inc., San Diego, USA).

Following fastq data generation, the raw sequence reads were filtered using a custom perl script as well as the Cutadapt v1.4.1 software [48]. The filtered reads were then *de novo* assembled using SOAPdenovo-Trans v. 1.03 [49] using a k-mer of 71. Fork, bubble and complex loci from the SOAPdenovo-Trans assembly were further combined using CAP3 assembler v 12/21/07 with 95% identity to produce longer, more complete consensus sequences.

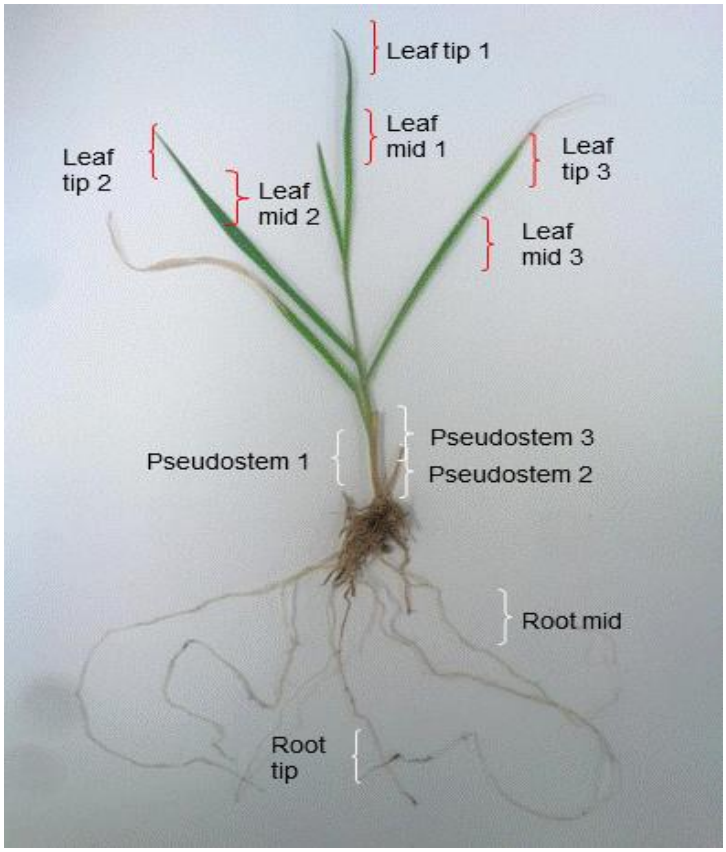


Figure 3.1.6. Pictorial representation and identification of the tissues of origin for vegetative sequencing libraries. See Baillie et al. (2017) for more details.

3.1.2.3 De novo transcriptome sequence annotation and tissue-specific expression

The assembled transcripts were compared by use of BLASTX to the UniRef90 database (Altschul *et al.* 1997) and the highest match was recorded. All transcripts that showed a primary significant match to a non-plant species were removed. For further annotation, assembled transcripts were also BLASTN compared to the coding sequences (CDSs) of the Poaceae model species, using the *B. distachyon* (v1) and rice (*Oryza sativa* ssp. *japonica* group) (GCA_000005425.2 Build 4) databases with an E-value threshold of 10^{-10}. Degree of completeness of the assembly was assessed by comparison to the early access Plantae reference set of orthologs using BUSCOv1.1b1. In addition, the assembled transcripts were assessed using gene ontology (GO) terms, via the BLAST2GO PRO software program (Conesa *et al.* 2005) with an E-value threshold of 10^{-10}, using Nr annotations.

Once the reference assembly was generated, trimmed, unassembled sequence reads from each of the individual tissue samples were aligned against the reference using the BWA software package and the mem algorithm (Li 2013). The data was normalised on the 75th percentile, as described previously (Sudheesh *et al.* 2015).

3.1.2.4 Identification of long transcripts and molecular phylogenetic analysis

All transcripts were processed through the emboss getorf software package (Rice et al. 2000), generating sets of protein sequences with a minimum of 300 amino acids. The protein sequences that were generated were then compared to the genome assemblies of B. distachyon and rice using BLASTP with an E value threshold of 10-20. The output of the BLASTP analysis was recorded as a table with the single highest match recorded in each instance. A sub-set of 500 proteins was

identified in which the BLASTP alignment started at the first amino acid in the phalaris reference set as well as both of the model species, and which were therefore were amenable to comparative sequence alignment. All of the protein sequences from the three species were then aligned using Clustalw2 (Larkin et al. 2007) and neighbour joining trees based on percentage identity were generated for a selected sub-set of aligned protein sequences, selected on the basis of sequence annotation.

3.2 Development of a methodology for genomic selection in phalaris

3.2.1 Principles

Traits of economic importance in forage species such as yield, persistence and quality are generally controlled by many genes each of small effect (polygenic traits). Genomic selection (GS) is a form of marker-assisted selection ideal for polygenic traits because it employs large numbers of markers across the whole genome such that most if not all genes affecting the trait are linked to at least one marker in each chromosome segment. The effects of alleles linked to these markers can then be summed over all markers to give a Genomic Estimated Breeding Value (GEBV) which is correlated with phenotype (level of a trait). Once a robust prediction equation is developed, one to a few rapid cycles of selection can be undertaken before further recalibration, reducing the need for long and expensive phenotypic evaluation in each cycle. GS also allows the selection of individual plants for traits that can normally be evaluated only in swards.

The implementation of GS depends on the development of an accurate relationship between phenotype and genotype in a 'training' or 'reference' population. This calibration can be tested for accuracy in a 'validation' population and then applied to the breeding population of interest. Resende *et al.* (2014) emphasize the importance of high accuracy in the training population for long term gains from GS. Accuracy depends on the proportion of variation explained by the markers, the relatedness of the training population with the candidate breeding population, the size of the training population and the heritability of the trait (Jannink *et al.* 2010; Resende *et al.* 2014; Hayes *et al.* 2014; Lin *et al.* 2014).

Hayes *et al.* (2013) discuss the deployment of GS for temperate forage grasses such as perennial ryegrass. The obligate outcrossing nature of perennial ryegrass results in high levels of genetic diversity, large effective population sizes and relatively high levels of recombination among chromosome segments resulting in more rapid decay of associations (linkage disequilibrium) between genetic markers and target genes than occurs in inbreeding species such as wheat and rice. Low levels of linkage disequilibirum point to a need for large training populations (in the 1000's) and large numbers of markers for GS to be successful in populations of diverse, unrelated individuals. However, structure and relatedness in the training and breeding populations as well as population-wide linkage disequilibrium contribute to accuracy (Jannink *et al.* 2010). Attention to population structure e.g. use of full- or half-sib families, can markedly reduce effective population size and hence training population size required to achieve useful levels of accuracy although the resulting prediction equations will be applicable to a narrow set of families (Hayes *et al.* 2013). Importantly, Hayes *et al.* (2013) and Resende *et al.* (2014) conclude that GS remains significantly advantageous in theory over conventional breeding in many situations even if the training population size and number of markers are smaller than desirable.

3.2.2 Training population for phalaris

Like perennial ryegrass, phalaris is an obligate outcrossing species but with potentially higher genetic diversity due its being a tetraploid species (perennial ryegrass is a diploid). Effective population size is therefore likely to be high and population-wide linkage disequilibrium likely to be low in phalaris, pointing to a need for large training populations and high marker density if selecting among a population of unrelated individuals. However, Lin *et al.* (2014) note that although livestock species tend to have high *past* effective population size, domestication and selection have resulted in much smaller effective population sizes making them amenable to GS. Forage grasses have a much shorter history of selection than livestock but forage breeding programs tend to be based on elite cultivars and other germplasm with considerable selection history, potentially facilitating GS.

Factors considered when designing the training population for the phalaris project were the relation between the training and subsequent breeding populations, the size of the population, heritability of the target traits and design of the evaluation trials. A study of genetic diversity among a relevant group of phalaris cultivars and breeding populations was also conducted to shed light on how genomic relationships within this group were likely to affect implementation of GS.

3.2.3 Relation between training and breeding populations

The accuracy of GS increases and therefore the required size of the training population decreases for a given level of accuracy the more closely related the training population is to the target breeding population (Heffner *et al.* 2009; Jannink *et al.* 2010; Nakaya and Isobe 2012; Lin *et al.* 2014). This raises the question of whether the training population should have narrow focus on a particular candidate breeding population, with loss of broader applicability, or whether it should be more broadly applicable to a range of likely future breeding populations (Lin *et al.* 2014). For the phalaris project, this required judgement of breeding directions in the short-medium term by commercial breeders.

We decided in the Phalaris Pre-breeding Plan that we would pursue as our top priority gain in seasonal yield of winter-active phalaris, which offers the potential for highest gains in production particularly during the winter feed gap when extra feed is of most value (Smith 2013). We also decided that in the first instance we would put less emphasis on the North African-based pool for the drier margins, concentrating on the most productive main phalaris zone (>550 mm LTA rainfall). The general purpose germplasm pool represented by Holdfast and related cultivars such as Landmaster, Holdfast GT and Advanced AT appeared most suitable for this purpose. Since maintaining improved pasture at its most productive level for as long as possible is important for economic return (Malcolm *et al.* 2014), we did not want to ignore recent selection for persistence factors such as shallow soils (Landmaster), strongly acid soils (Advanced AT) and heavy grazing pressure (Holdfast GT). Further, we knew of one commercial breeding program (PGGWrightson Seeds) based on germplasm derived from Holdfast-Landmaster-Advanced AT crosses with aims including improved production for acid soils. Against this background, the training population for the project is based 50% on crosses among Holdfast GT, Advanced AT and Landmaster (which is closely related to Holdfast), 32% on crosses among Advanced AT progeny and progeny of a later generation (AT04) selected at an acid soil site, 10% from a commercial population derived from crosses between selected Landmaster plants and a Holdfast-related population, and selected Advanced AT plants and

the same Holdfast-related population. The last two groups were produced by PGGWrightson Seeds. The resulting training population should be applicable to the contributing commercial breeding program and anticipates future breeding in the most advanced seed-retaining, winter-active phalaris pool.

3.2.4 Size of the training population

Studies of GS consistently conclude that the accuracy of genomic prediction is related to the size of the training population and numbers in the 1000-5000 range can be found for outcrossing species in the literature. Resende *et al.* (2014) recommend that the training population be as large as possible without compromising the integrity of the selection criterion. This qualification is important since traits such as yield and persistence cannot usually be evaluated on spaced plants due to low correlations with swards. Thus Resende *et al.* (2014) considered a smaller training population (200) more appropriate for evaluation in sward plots than a larger (1000) population evaluated as spaced plants if the correlation between spaced plant yield and sward yield is low, as is often the case. Their analyses of a range of GS strategies concluded that GS can give benefits in selection efficiency with "modestly sized" training populations. Breeders with limited resources could start at his level and gradually increase the size of the training population over time. A prominent researcher in GS for forage species and consultant to the phalaris project, Prof. Michael Casler, has adopted this strategy with promising results in swtichgrass, starting with a population of 144 half-sib families, then 150 families then 109 then 110 families from unrelated populations added over time.

The phalaris project is constrained by the need to prove the efficacy of GS in phalaris in a limited time frame (3 years) for traits that require evaluation preferably in sward plots or a realistic equivalent, for which seed of half- or full-sib families is preferable. Clonal stocks of cultivar parents and related progeny held by CSIRO were intercrossed 6 open-pollinated groups of plants in the summer for 2013/14, which resulted in seed of 237 half-sib families to evaluate. This was augmented by 53 families from a commercial breeding program run by PGG Wrightson Seeds, Ballarat, giving a total of 290 families for evaluation of seasonal yield and slightly lower numbers for evaluation and under heavy grazing pressure due to limitations on seed quantity.

3.2.5 Heritability of target traits

Accuracy of prediction in GS increases with the heritability of the trait (Iwata and Jannink 2011; Lin *et al.* 2014). Larger training populations are therefore required for traits of low heritability. Although traits of economic importance in forage species tend to be in the low (<0.3) to moderately high range (0.4-0.6), a number of reviews have concluded that this range is unlikely to limit use of GS so long as enough markers and large enough training populations are used (Hayes *et al.* 2013; Lin *et al.* 2014). Importantly, simulations studies have shown that genotype-based methods such as genomic selection have their highest advantages over conventional phenotypic methods at low heritabilities (Iwata and Jannink 2011; Resende *et al.* 2014). Resende *et al.* (2014) note that at heritabilities <0.3, which often apply to traits in forage species, genotypic methods contribute valuable information where the phenotypic information is less reliable. Oram and Culvenor (1994) list narrow-sense heritabilities, mostly predicted from half- or full-sib analyses, of various traits in phalaris. These ranged from 0.1 for rhizomatous spread to 0.8 for sward seed yield. Values for seedling size were around 0.2, bud dormancy 0.4, maturity time 0.5, winter herbage yield 0.14 in spaced plants and

0.56 in sward plots. Heritability for sward yield among 50 half-sib families across 3 acid soil sites was around 0.5 and for basal frequency after 3 years was 0.8 (Culvenor *et al.* 2004). Persistence over 4 years of grazing was shown to be a heritable trait (Culvenor *et al.* 2009) with heritability of final basal frequency estimated from variances presented by Culvenor *et al.* (2007) and other unpublished data to be around 0.3-0.4 across sites where phalaris was adapted (Southern Tablelands-Western Victoria). Note that in these cases, GS could replace the need for 3-4 years of field evaluation for at least one cycle of selection.

3.2.6 Design of evaluation trials

In contrast to conventional phenotypic selection where replication within and across environments is important for evaluation of whole plant performance, GS evaluates marker or allele effects in the training population (Lin *et al.* 2014). In this situation, replication of alleles can be achieved by increasing population size without necessarily replicating plants or growing them at all sites (Lorenz 2013). Jannink *et al.* (2011) quote several studies which concluded that accuracy is maximized by evaluating as many unreplicated individuals as possible rather than replicating fewer individuals. Lorenz (2013) achieved similar results also using simulation but noted that, in contrast to conventional marker-assisted selection, resource allocation using GS was in fact more flexible and allocating more resources to replication could improve gains when heritability was low.

A trade-off between number of lines and replication has not strongly influenced the design of the trials to evaluate seasonal yield in the phalaris training population. It was not possible to easily increase the number of parent plants in the crosses we undertook. Conversely, plot numbers were not considered a strongly limiting factor with this number of families were evaluated in rows rather than swards. We also note that all studies recommending increasing training population size at the expense of replication are simulation studies rather than actual field-based studies where real-life field variation operates. Given that we did not feel to be strongly limited by numbers of plots, and given our experience of field variation under Australian conditions, it was decided to evaluate progeny of the 290 parents employing 3 replicates at each of 3 sites, Canberra, Maryborough and Hamilton.

One concession to resources was the use of row plots rather than sward plots. Row plots are easier to evaluate than swards and their use allowed us to maximise the number of families included since some families which had insufficient seed for replicated sward plots at all sites. Smith *et al.* (2001) explored correlations between single-row plots and swards in perennial ryegrass. Correlation between herbage yields for the different plot arrangements were significant for all but 1 of 13 harvests whether row yield was directly measured or visually assessed. Although the range of values differed for the two methods, the relative ranking of lines was similar. Row plots have been used in the phalaris breeding program before and much of the development of the successful cultivar, Landmaster, was done using rows (Oram 1996).

3.3 Development of markers for seed retention

A large cohort of plants (288) from a specific genetic background (Landmaster) was established at CSIRO Canberra. Landmaster was chosen because of its historic role in the CSIRO breeding program and the fact that this population was segregating for a range of seed retention traits such as seed

yield and spikelet filling. The genotypes were also scored on a qualitative scale for seed retention from non-retainers, partial retainers through to genotypes with complete seed retention. The GBS processing of the samples was completed and bioinformatic analysis was performed to define the resulting SNP set, following by use of the relevant algorithms for genomic selection prediction analysis.

3.4 Use economic analysis to develop robust estimates of the economic importance of genetic gain in phalaris

3.4.1 Approach

The replacement cost (Johnson and Hardin, 1955) and change in livestock production (Ludemann *et al.*, In Press) methods were used to calculate economic values for seasonal DM yield of phalaris on Australian sheep and beef farms. The results were used to help describe the similarities, advantages and disadvantages of each method.

3.4.2 Replacement cost method

Metabolisable energy (ME) expressed in megajoules (MJ) is a common unit of measure for assessing changes in DM yield. This is because it accounts for both the quantity and quality of feed sources (Moore *et al.*, 2009). In this project the replacement cost method of assessing the value of seasonal phalaris DM yield was described using MJ as its common unit.

The replacement cost method uses the market value of alternative feed in order to estimate the marginal value of the change in DM yield (Johnson and Hardin, 1955). There is limited data on the cost and quality of pasture (expressed as energy concentration of pasture DM) sold either as hay or through agistment (payment for grazing). A sheep or beef farmer in need of energy for their farm system may also be able to purchase supplementary feeds such as barley, oats or sheep pellets. These types of feed are more rigorously tested for quality in Australia than hay or agisted pasture. More data is therefore available for the cost of barley, oats and sheep pellets per MJ of energy, so data from these non-pasture sources were used to estimate the 'market value' of feed energy. Monthly barley prices and annual prices for oats and sheep pellets were taken from Waterman and Creese (2014). Economic values for seasonal DM yield of phalaris were calculated based on the three contrasting sources of energy for comparison.

The economic value (in Australian dollars AUD) for a one-kg increase in phalaris DM yield (EV_DM_t) for any period of time (t) using the replacement cost method, followed:

$$EV\_DM_t = \Delta DM_t \times E_t \times Feed\$_t \quad [1]$$

where: 't' represents the period of time the value was calculated for, from either autumn, winter, spring or summer, ΔDM_t is the change in DM yield in 't' period (arbitrarily set to one-kg of DM across all seasons), and E_t is the energy concentration of the additional phalaris DM yield in each period of time expressed in MJ of ME per kilogram of DM (MJME/kg DM) (with values for each period of time shown in Table 1). The $Feed\$_t$ is the cost of utilisable energy (in Australian dollars-AUD per utilised MJ) from purchased feed for a certain period of time (t) following an equation from Ludemann *et al.* (2013):

$$\text{Feed\$}_t = \frac{\text{MPF}_t + \text{AFE}_t}{1000\text{kg\_tonne} \times \text{Prop}_{DM} \times \text{Prop}_U \times \text{EC}_t} \quad [2]$$

where, MPF_t is the market price of the feed at 't' point in time, AFE_t is any additional feed expenses (such as transport, repairs and maintenance on feeding equipment, or any additional labour for feeding out) in dollars per tonne fresh weight for the purchased feed in 't' period of time, 1000kg_tonne represents the kilogram to tonne conversion factor, $Prop_{DM}$ is the feed DM as a proportion of fresh weight, $Prop_U$ is the proportion of purchased feed DM that is generally utilised by livestock, and EC is the energy concentration of the feed in MJ/kg DM in 't' period. Values for variables used in Equation 1 and Equation 2 are shown in Table 1. The costs associated with depreciation of plant and machinery for feeding the supplements to livestock were not included in Equation 2 so that the EV_DM_t was a conservative estimate.

Table 3.4.1. Summary of assumptions used in assessing the value of seasonal dry matter (DM) yield of phalaris using the replacement cost method using barley, oats and sheep pellets for the replacement cost where $ indicate Australian dollars

Para-meter	Description	Value used in analysis when using the various types of feed			Reference
		Barley	Oats	Sheep pellets	
ΔDM_t	Change in DM yield in any (t) period- kg	1	1	1	One unit change
E_{aut}	Energy concentration of phalaris in autumn-MJ/kg DM	9.1	9.1	9.1	Moore *et al.* (2006)
E_{win}	Energy concentration of phalaris in winter-MJ/kg DM	11.8	11.8	11.8	" "
E_{spr}	Energy concentration of phalaris in spring-MJ/kg DM	10.9	10.9	10.9	" "
E_{es}	Energy concentration of phalaris in summer-MJ/kg DM	8.7	8.7	8.7	" "
MPF_{aut}	Market price of feed in autumn-$/t fresh weight[1]	243	210	280	Waterman and Creese (2014);DEPI VIC (2014)
MPF_{win}	Market price of feed in winter-$/t fresh weight	239	210	280	" "

MPF_{spr}	Market price of feed in spring-$/t fresh weight	251	210	280	" "
MPF_{es}	Market price of feed in summer-$/t fresh weight	252	210	280	" "
AFE_t	Additional feed expenses in any (t) period-$/t fresh weight	25	25	25	Ludemann *et al.* (2013)
$Prop_{DM}$	Feed DM as a proportion of fresh weight	0.88	0.88	0.90	Ludemann, *et al.* (2013); DEPI VIC (2014)
$Prop_U$	Proportion of purchased feed DM utilised by livestock	0.90	0.90	0.90	Ludemann, *et al.* (2013)
EC	Energy concentration of purchased feed (MJ/kg DM)	13	11	13	Rayner (2007)

3.4.3 Change in livestock production method

The effect of changes in stocking rate or changes in sheep and beef liveweight gains on the economic value of a one-kg increase in phalaris DM yield were assessed for the 'change in livestock production' method.

3.4.3.1 Changing stocking rate

Annual stocking rates in a livestock system are generally determined by the period with the most severe feed deficit (Moore *et al.*, 2009). The timing of the most severe feed deficit can vary by production system and climatic conditions. Management in the production system will influence the number of livestock and the fertility of the soils, which can affect the demand and supply of feed. Climatic conditions affect the supply of feed, through availability of soil moisture and temperatures. The interaction between the supply and demand of feed will determine which times of year have a feed deficit. In areas of Australia where phalaris is grazed, few sheep and beef farmers feed their livestock supplements (such as grain) except under drought conditions (Waterman and Creese, 2014). This means the effect of climatic conditions on the supply of pasture for feed is crucial for the timing of severe feed deficits.

If an increase in DM yield occurs at a time of year when there is not a severe feed deficit, then management practices other than an increase in stocking rate could be used to increase production. This could include utilising the additional DM yield through greater livestock production such as through greater liveweight gains in non-capital livestock.

In each of the four seasons (autumn, winter, spring and summer) a one-kg increase in phalaris DM yield was assumed to be managed by increasing stocking rate, or increasing the liveweight gains of 'trading' livestock (livestock kept for growing meat and wool rather than for their offspring).

The economic value for a one-kg increase in phalaris DM yield in t period of time using the change in stocking rate method (SR_EV_DMt) was calculated using:

$$SR\_EV\_DM_t = \frac{\Delta DM_t \times Prop_{UPasture} \times E_t}{ME\_DSE} \times GM\_DSE \quad [3]$$

Where ΔDM_t was the arbitrary one-kg of DM change in phalaris yield, $Prop_{UPasture}$ was the proportion of pasture DM yield increase that was utilised by livestock throughout the year (0.6)(Gout, 2008), E_t is the mean energy concentration of phalaris (in MJ of ME per kg DM) for 't' season (shown in Table 1), ME_DSE is the ME requirements of one dry sheep equivalent (DSE) for 't' season, and GM_DSE is the gross margin per DSE (in AUD) for 't' season. Complex interactions which affect the capacity for pasture intake such as stocking rates, pasture growth, class of livestock grazing the pasture and time of year were assumed to be taken into account in the 0.6 value for $Prop_{UPasture}$. It is acknowledged using this value for $Prop_{UPasture}$ is a simplification, so sensitivity analysis of the $Prop_{UPasture}$ assumption was included in the results. A DSE is defined as equivalent to the energy requirements of a 2 year old 50kg wether to maintain its liveweight, which is 2737.5 MJ per year (DPI NSW, 2010) or 684.4 MJ per season (for ME_DSE). Energy requirements for 1 DSE for the particular season was used rather than the annual energy requirements, as it was assumed only the season under analysis had a severe feed deficit. A surplus of feed in the other seasons would cover energy requirements of the additional DSE throughout the rest of the year. While this is a simplistic assumption, it allows the economic value of additional DM yield in each season to be estimated independently.

The GM_DSE is the gross margin per DSE per season which is the total gross margin for the year equally divided by the four seasons. Mean gross margins per DSE from the Waterman and Creese (2014) survey were used. For a primarily wool producing system an annual gross margin of AUD23/DSE was used, for dual purpose sheep production AUD20/DSE was used and for beef production a AUD13/DSE gross margin was used. The annual gross margins per year were divided by the four seasons to estimate the GM_DSE on a dollar per season basis.

3.4.3.2 Changing liveweight gain of livestock

There will be variation in the seasons in which capital stock such as ewes or cows are mated, in order to best fit the demand of energy with the supply of pasture energy (which is in turn dependent on climatic conditions). Therefore, there will be variation in the times at which offspring are born and are at a suitable liveweight to be sold for further growing (finishing), or for slaughter if they obtain a suitable carcase weight. Given this variation, it is necessary to assess the marginal value of one extra kilogram of phalaris DM when it has been utilised by growing animals in a range of seasons. Four contrasting classes of growing animals were used as examples for how the economic value of additional DM yield from phalaris may be utilised. These were the change in liveweight of lambs sold at the store market (store lambs) or to the meat processors (prime lambs), weaned calves sold at a fixed sale date, and heavy (520kg liveweight) steers sold to slaughter, as shown in Table 2. Assumptions for production of the lambs sold as either store or prime lambs followed target market specifications from AWI and MLA (2011). This included the sale of store lambs at 6 months of age at 36kg liveweight, and prime lambs sold to a heavy weight market at 9 months of age when they reached a liveweight of 54 kg (Table 2). Ewe lambs were chosen for the lamb growth rate scenarios in order to conservatively estimate changes in production. If ram lambs were chosen, higher growth rates would need to be assumed (CSIRO, 2007). Heifers were chosen for simulating 'weaner calves' and steers were chosen for simulating heavy trading cattle (Table 2). Assumptions for the percentages of liveweight at slaughter as carcase weight (dressing out percentage –DO%) and pasture utilisation are shown in Table 2.

Table 3.4.2. Key parameters for the classes of livestock used to assess the economic value of phalaris dry matter (DM) yield using the change in livestock production method

Class of livestock	Age (days)		Liveweight (kg)		Mean liveweight gain (kg/day)	DO%[1]	Pasture DM utilised (%)
	Start	End	Start	End			
Female store lambs	90	180	22.5	36.0	0.15	43%	60%
Female prime (heavy) lambs	180	270	36.0	54.0	0.20	45%	60%
Female weaner calves	90	180	100.0	170.0	0.78	53%	60%
Male heavy steers	570	660	430.0	520.0	1.00	53%	60%

[1]Dressing out percentage expressed as the mass of saleable carcass weight as a percentage of the equivalent liveweight at slaughter (this is a theoretical value for store lambs and weaner calves)

Estimates of production for growing livestock (Table 2) were assumed to be the same in each of the four seasons they were calculated for. This is because if management was changed in order to have the classes of livestock shown in Table 2 grown in another season, it would be because the farm manager believed the seasonal pasture production was adequate for that livestock growing enterprise. Given the variation in seasonal pasture production and management between regions, using trading livestock as a method of estimating the economic value of seasonal DM yield may not be applicable to every region or season. Calculating separate economic values for each season allows farmers to choose the most appropriate economic values for their farm system. For instance, a sheep and beef farmer in one region of Australia may sell prime lambs in spring, whereas other farmers may only be able to sell store (restocker) lambs in that season.

It was assumed there were no additional costs for growing animals when they converted additional phalaris DM into liveweight because it was assumed management did not have to change as the change in pasture DM was marginal (livestock were sold at the same time), the change in liveweight did not require any additional transportation or processing costs, and the incremental change in liveweight did not result in a change in the average price per kilogram sold.

The economic value of the change in DM yield in 't' season using changes in livestock liveweight gain (LWG_EV_DMt) was calculated as:

$$\mathrm{LWG\_EV\_DM_t} = \Delta\mathrm{DM_t} \times \Delta\mathrm{Wt_{Sold}} \times \$\mathrm{Wt_{Sold}} \quad [4]$$

where, ΔWt_{Sold} was the change in weight of animal (lamb or head of cattle) for the one-kg increase in phalaris DM yield, and $\$Wt_{Sold}$ was the net monetary value (in AUD per kg) of the additional weight

of animal sold. It was assumed for store lambs and weaner calves, 'prime' lambs and finishing beef cattle the ΔWt_{Sold} was in kg of saleable carcase weight, because this was the unit of value used in the product price data (MLA, 2014). Livestock were sold at the same age, regardless of whether there was a change in the DM yield of phalaris in that season or not. It was also assumed liveweight gains did not result in the animal going into another pricing category (i.e. into a different carcase weight grade in the processor slaughter price schedule for prime stock) when sold. For store lambs the price per kilogram of carcase weight of the 'restocker lamb' was used as the $\$Wt_{Sold}$, the 'heavy lamb' (>22kg carcase weight) prices per kg of carcase weight were used as the $\$Wt_{Sold}$ for prime lambs (MLA, 2014). For weaner cattle the 'Eastern Young Cattle Indicator' (EYCI) was used as the price per kg of carcase weight for $\$Wt_{Sold}$ (as prices specifically for weaned calves were not available), whereas for finishing beef cattle, the price of a 'grown steer' in AUD per kg of carcase weight (weighing in excess of 500 kg liveweight, or 320-400kg carcase weight) was used (MLA, 2014). The mean real prices per kg of carcase weight (in each month) over the years were used for $\$Wt_{Sold}$ values.

The ΔWt_{Sold} was calculated using:

$$\Delta \mathrm{Wt}_{\mathrm{Sold}} = 1 \div \Delta \mathrm{ME\_LWT} \div \mathrm{E_t} \div \mathrm{Prop}_{\mathrm{UPasture}} \div \mathrm{DO\%} \div 100 \quad [5]$$

where ΔME_LWT is the change in ME requirements for a one-kg increase in liveweight gain of the respective class of livestock. Similarly to in Equation 3, the $Prop_{UPasture}$ values were 0.6 for both lambs and cattle in Equation 5, and the respective values for DO% for each class of livestock shown in Table 2 were used. The ΔME_LWT was calculated through the energy requirements for the livestock with one extra kilogram of liveweight gain less the energy requirements for the livestock with the 'Base' (no change in) liveweight gains (depicted in Table 2) using CSIRO (2007) equations.

4 Results

4.1 Genetic and phenotypic diversity in phalaris described to guide the design of future breeding programs with a focus on winter-active germplasm

4.1.1 Development of Genomic Resources in Phalaris

The development of these genomic resources for phalaris has been published and made available for broader use (Baillie et al. 2017). The results are summarised below.

4.1.1.1 De novo sequence assembly of the phalaris transcriptome

The reference unigene set for the selected cv. Landmaster genotype reference was obtained from a total of 553,566,274 sequence reads. For the initial SOAPdenovo assembly, a range of k-mers were empirically tested and a value of 71 was identified as delivering the optimal assembly, based on size of assembly compared to mean and median contig and scaffold size. The initial 71k-mer assembly was 233,054,090 bp in length (not including Ns) from 437,776 contigs and scaffolds, with a mean length of 577 bp and N50 values of 1304 bp from 52,492 contigs. Following this initial assembly, contigs that were <149bp were removed, as the single sequence read length was 150 bp and so these contigs are likely to be spurious features within the complete data set. Contigs that were <250

bp were required to have >10 sequence reads associated with the assembly, otherwise they were also removed from the assembly. This filtering step removed a large number of contigs, and left 217,707 contigs and scaffolds (49.7% of total).

The remaining contigs and scaffolds were then filtered by performing a BLASTx analysis in comparison to the uniref90 database. A total of 107,463 scaffolds and contigs were identified as being of plant origin (78,713 scaffolds relating to 26,467 loci and 28,750 contigs), while 18,470 contigs were of non-plant origin and 91,774 failed to return any match. Any contig or scaffold that failed to return a match to a known plant protein was removed. These included 9,154 contigs identified as deriving from the *Puccinia* genus of foliar rust-causing fungal pathogens (which includes the stem rust pathogen *P. graminis*, known to infect phalaris), as well a substantial number of insect and bacterial origin. The assembled scaffolds identified as forked bubble or complex in nature were individually assembled with CAP3, after manual sequence assembly and evaluation was performed on a limited set to assess the degree of sequence variation between locus-related contigs. Manual evaluation was performed to evaluate the potential for co-assembly of homeologous variants of a gene locus, given the predicted allopolyploid constitution of phalaris. However, minimal sequence divergence was identified from the range of examined loci. If the observed loci were generated as a result of coalescence between homoeologous variants this limited sequence divergence would make sub-genome sequence-specific assembly technically impossible. Processing of these scaffolds through the CAP3 assembler generated 14,324 scaffolds relating to 12,668 loci, as well as 18,020 scaffolds that were unable to be assembled. Representatives of a range of complex loci that were unassembled were therefore manually analysed and aligned. In all instances a single contig was able to be generated for the locus from the scaffolds. A common issue was identified as the presence of large predicted gaps in the scaffolds filled with Ns. As a result, the longest scaffold derived from the unassembled complex locus was entered into the reference.

Following extensive sequence analysis and filtering, a final reference of 56,873 sequences, corresponding to 58,174,765 bp, was created. The assembled reference has N50 values of 11,945 sequences $\geq$ 1,558 bp in length, with a GC content of 49.11%. The final assembled reference represents only 24.9% of the sequence length of total unfiltered assemblies, and only 13% of the initial contig and scaffold number (Table 1). Examination of the Uniref90 results relating to the final reference identified a total of 37,687 different proteins. A further examination of the taxonomic distribution of uniref90 proteins revealed that >69% of all retained sequences displayed a highest BLASTX match to a protein from Poaceae species (either *B. distachyon* or the cereal species *T. aestivum* L. (bread wheat), *Hordeum vulgare* L. (barley) or *Aegilops tauschii* L., all of which belong to the sub-family Pooideae of the Poaceae family, along with phalaris (Fig. S1, Fig. S2). Comparison of the final 56,873 reference transcripts to a core set of 956 single copy plantae orthologs revealed 64% as complete, 24% fragmented, and only 11% missing (BUSCO notation: C:64%[D:17%], F:24%, M:11%, n:956).

Table 4.1.1. Overview of sequencing outputs and assembly.

Primary assembly - SOAPdenovo-Trans	
Total number of filtered reads	553,566,274
Total number of contigs	437,776
N50 length	1,304
Total basepairs	233,054,090
Secondary assembly - CAP3 and filtering	
Total number of scaffolds and contigs	56,873
N50 length	1,558
Total basepairs	58,174,765

4.1.1.2 Sequence annotation of the phalaris transcriptome

Following the initial assembly, a targeted comparison was made to both the *B. distachyon* and rice gene complements. A total of 31,771 of the phalaris reference sequences generated a significant match to 17,573 *B. distachyon* CDSs, while comparison to CDSs of rice (which is taxonomically more distant from phalaris) only identified 23,513 sequences matching 14,200 rice genes. A total of 20,860 of phalaris sequences identified significant matches to both Poaceae model genomes.

The phalaris unigene set was assigned GO terms based on sequence similarity to the Nr databases. BLAST searches showed highest similarity to rice, followed by maize (*Zea mays* L.)*, Aegilops tauschii and Brachypodium distachyon* (Fig. 1), with 72.5% of transcripts (41,286) being allocated at least one GO term. Within this group, assignments to the biological process category was highest (42%), followed by cellular function (40%) and molecular function (18%; Fig. 2). Among the biological process sub-categories, metabolic process (27%) and cellular process (23%) were prominently represented (Fig. 2, Table S2) indicating that tissues used in this study were undergoing extensive metabolic activity. A moderate number of transcripts were also involved in the single-organism process (17%), biological regulation (8%), response to stimulus (7%), regulation of biological process (8%), biogenesis and localisation (5%) categories. Under the molecular function category, catalytic activity (51%) and binding (49%) were the most common (Fig. 2, Table S1). For the cellular component category, the majority of the transcripts were assigned to the cell (26%), cell part (26%), organelle (23%) and membrane (10%) categories, while much smaller proportions (<5%) were assigned to membrane part, organelle part and macromolecular complex (Fig. 2, Table S1).

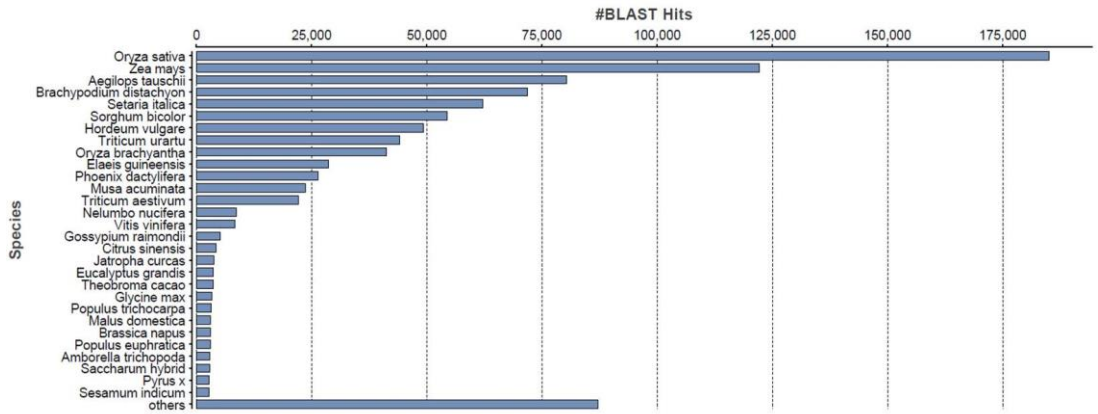


Figure 4.1.1. Species-specific distribution of highest matches for GO ontology.

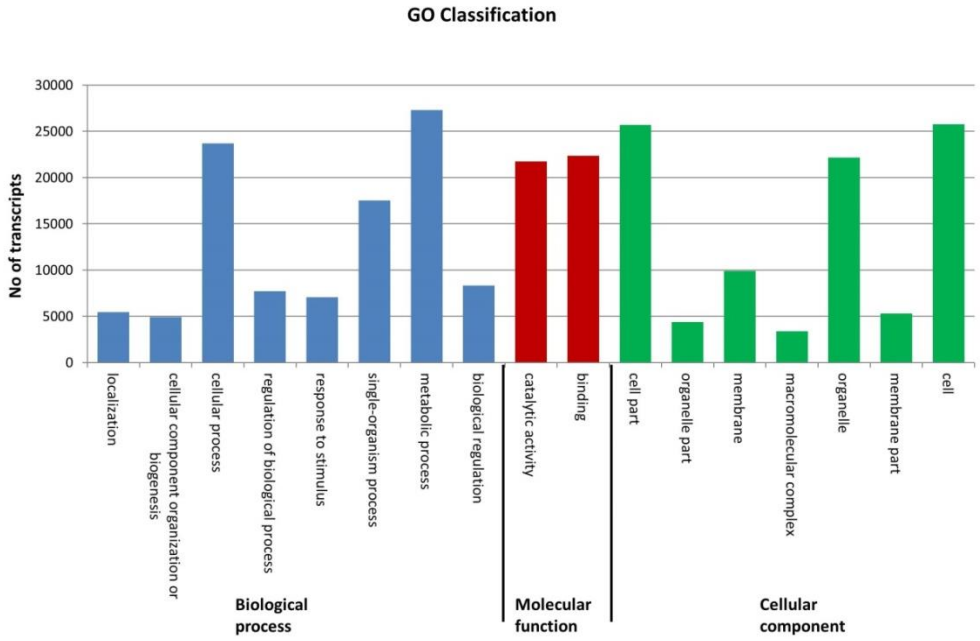


Figure 4.1.2. GO classification categories for phalaris unigenes.

4.1.1.3 Tissue-specific gene expression analysis in phalaris

The reference unigene set was used to analyse global gene expression. Individual samples were reference aligned against the assembled sequences, and a normalised count was generated. A total of 20,593 unigenes (36.2%) were identified as being expressed in all tissue samples, and a further 18,874 (33.2%) were identified in all samples with the exception of one. From the 15 tissue-specific samples that contributed to transcriptome assembly, the lowest level of expression, or the most tissue-specific pattern, was displayed by a cohort of three unigenes that were only detected in two of the tissues. A tissue-type analysis was subsequently performed, in which data was combined into three groups: vegetative tissues, root tissues and reproductive tissues. A total of 53,978 (94.9%) unigenes were detected in all three groups, while a further 2,804 were detected in both the vegetative and reproductive groups and 64 were detected in both the vegetative and root groups. A

single unigene was detected only in reproductive tissues, and no gene was detected only in root tissues.

4.1.1.4 Identification of candidate's genes for agronomic traits in the phalaris transcriptome

In order to exemplify the value of the dataset in terms of candidate gene identification, a text-based search of the reference unigene set was made for specific genes that related to agronomic traits of interest, on the basis of the highest recorded BLASTX match to the Uniref protein database, and associated description term. Specific candidates were identified with high confidence that relate to processes of flowering (including *CONSTANS*-like genes), tolerance to aluminium toxicity (including organic acid transporter and MATE efflux protein family genes), herbage quality (including genes for lignin biosynthesis) and toxin production. The relative gene expression levels for each candidate within a given class were determined, revealing tissue-specific expression patterns capable of correlation with anticipated biological activity.

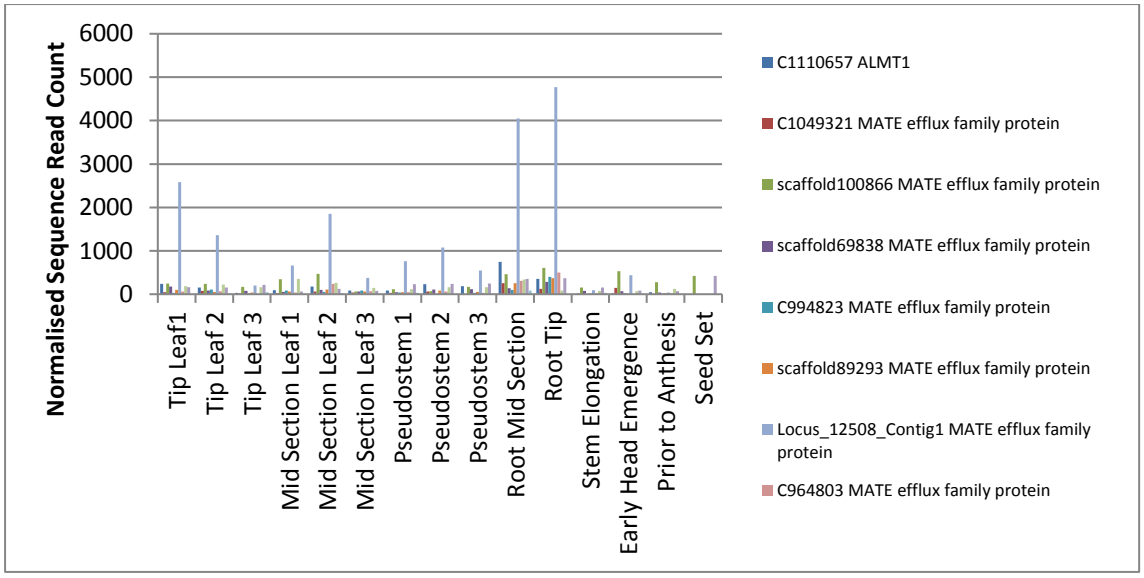
4.1.1.5 Identification of long transcripts and molecular phylogenetic analysis

Although the candidate genes for specific agronomic traits were identified on the basis of highly significant BLASTX E-values, none of the gene classes was represented in their entirety by full-length transcripts, and as a consequence, detailed phylogenetic analysis through comprehensive sequence comparison to the predicted orthologues in other Poaceae genomes was not possible. However, proof-of-concept for such analysis was obtained by filtering the unigene set for transcripts of significant length, leading to identification of 9,584 protein sequences (Table S2). Of this sub-set, 9,782 generated matches to *B. distachyon* and 9,527 generated matches to rice. Only 35 (0.35%) of the predicted proteins failed to generate a match to either of the model species, while 9,490 (96.3%) generated matches to both. The sequence set was further refined by identification of proteins containing predicted conserved start codon locations for all three species. Of these 500 proteins, phylogenetic affinity between phalaris, rice and *B. distachyon* was determined for a number of representative genes with plausible functional annotations: a cellulose synthase, involved in cell wall biosynthesis; a chitinase-like protein, involved in defence against fungal pathogens; and a cytokinin-N-glucosyltransferase, involved in plant growth regulation. In each instance, the relative affinities of the corresponding genes were consistent with taxonomic distance, being lower between phalaris and rice than between phalaris and *B. distachyon* (Fig. S3). This finding demonstrates the capacity to identify and exploit comparative genomics for trait improvement in phalaris, given the ability to isolate full-length genes, either directly from transcriptome assemblies, by PCR or from whole genome sequences.

Table 4.1.2. Summary information on identification of candidate genes for key agronomic traits

Trait Category/ Common Gene Name	Uniref90 Description	*Brachypodium* BLAST match	Rice BLAST match
Flowering			
Q gene	Floral homeotic protein	Bradi1g03880.1	Os07g0235800
	CONSTANS-like protein CO6		
	CONSTANS-like protein CO6	Bradi3g05800.1	Os06g0654900

	CONSTANS-like protein CO6		
	CONSTANS-like protein CO6	Bradi1g31280.1	Os04g0497700
	CONSTANS-like protein	Bradi5g14600.1	Os04g0497700
	CONSTANS-like protein	Bradi5g14600.1	Os04g0497700
	CONSTANS-like protein	Bradi1g43670.1	
	CONSTANS-like protein		
	CONSTANS	Bradi1g43670.1	
Aluminium Tolerance			
*ALMT*1	*ALMT*1	Bradi5g09690.1	Os04g0417000
	MATE efflux family protein	Bradi2g17260.1	
	MATE efflux family protein	Bradi1g69120.1	Os03g0227966
	MATE efflux family protein		
	MATE efflux family protein		
	MATE efflux family protein	Bradi1g69120.1	Os03g0227966
	MATE efflux family protein	Bradi2g17260.1	Os05g0554000
	MATE efflux family protein		Os02g0676400
	Aluminum-activated malate transporter 12	Bradi3g33980.1	Os10g0572100
	Aluminum resistance transcription factor 1		
	Aluminum resistance transcription factor 1		
Toxin Biosynthesis			
	Putative Cyanogenic beta-glucosidase		
	(R)-mandelonitrile lyase 2	Bradi1g31250.1	
Herbage Digestibility			
	Cinnamyl alcohol dehydrogenase 7		
	Cinnamyl alcohol dehydrogenase 3	Bradi4g29770.1	Os09g0400400
	Cinnamyl alcohol dehydrogenase	Bradi4g29770.1	Os09g0399800
	Cinnamoyl-CoA reductase	Bradi3g36890.1	
	Cinnamoyl-CoA reductase	Bradi3g36890.1	Os08g0441500
	Cinnamoyl-CoA reductase	Bradi3g36890.1	Os08g0441500
	Cinnamoyl coenzyme A reductase (Fragment)		


Normalised Sequence Read Count
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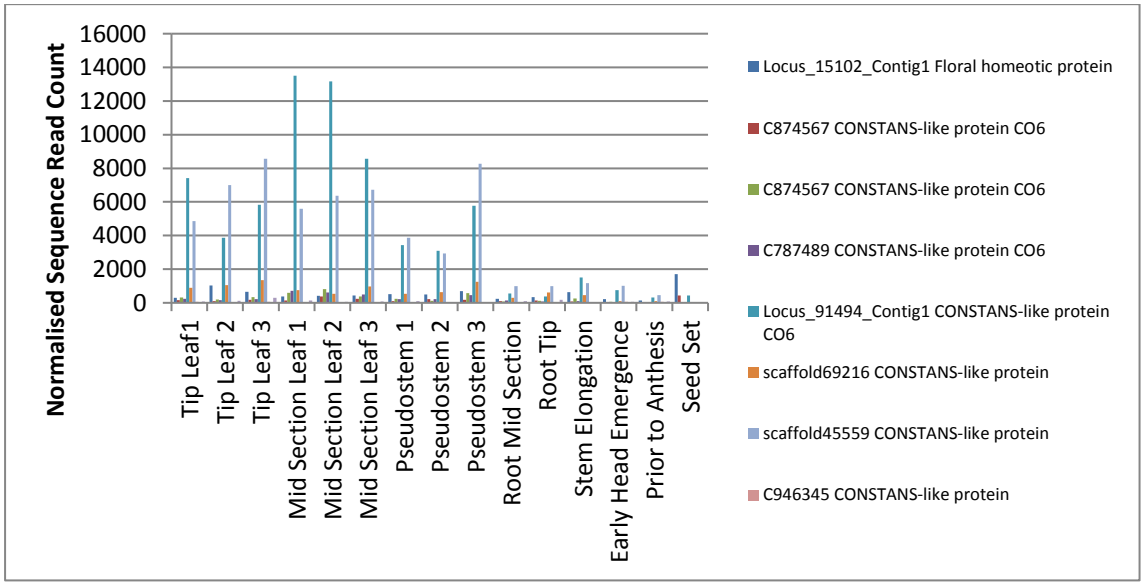
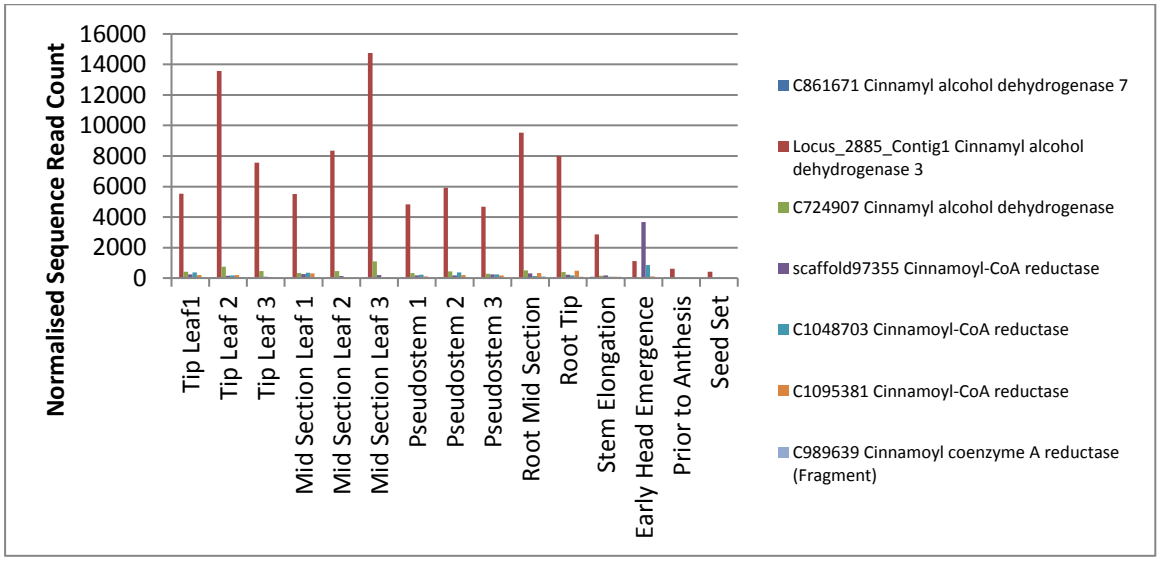
5000
4000
3000
2000
1000
0
Tip Leaf1
Tip Leaf 2
Tip Leaf 3
Mid Section Leaf 1
Mid Section Leaf 2
Mid Section Leaf 3
Pseudostem 1
Pseudostem 2
Pseudostem 3
Root Mid Section
Root Tip
Stem Elongation
Early Head Emergence
Prior to Anthesis
Seed Set
C1110657 ALMT1
C1049321 MATE efflux family protein
scaffold100866 MATE efflux family protein
scaffold69838 MATE efflux family protein
C994823 MATE efflux family protein
scaffold89293 MATE efflux family protein
Locus_12508_Contig1 MATE efflux family protein
C964803 MATE efflux family protein

A


Normalised Sequence Read Count
16000
14000
12000
10000
8000
6000
4000
2000
0
Tip Leaf1
Tip Leaf 2
Tip Leaf 3
Mid Section Leaf 1
Mid Section Leaf 2
Mid Section Leaf 3
Pseudostem 1
Pseudostem 2
Pseudostem 3
Root Mid Section
Root Tip
Stem Elongation
Early Head Emergence
Prior to Anthesis
Seed Set
Locus_15102_Contig1 Floral homeotic protein
C874567 CONSTANS-like protein CO6
C874567 CONSTANS-like protein CO6
C787489 CONSTANS-like protein CO6
Locus_91494_Contig1 CONSTANS-like protein CO6
scaffold69216 CONSTANS-like protein
scaffold45559 CONSTANS-like protein
C946345 CONSTANS-like protein

B


Normalised Sequence Read Count
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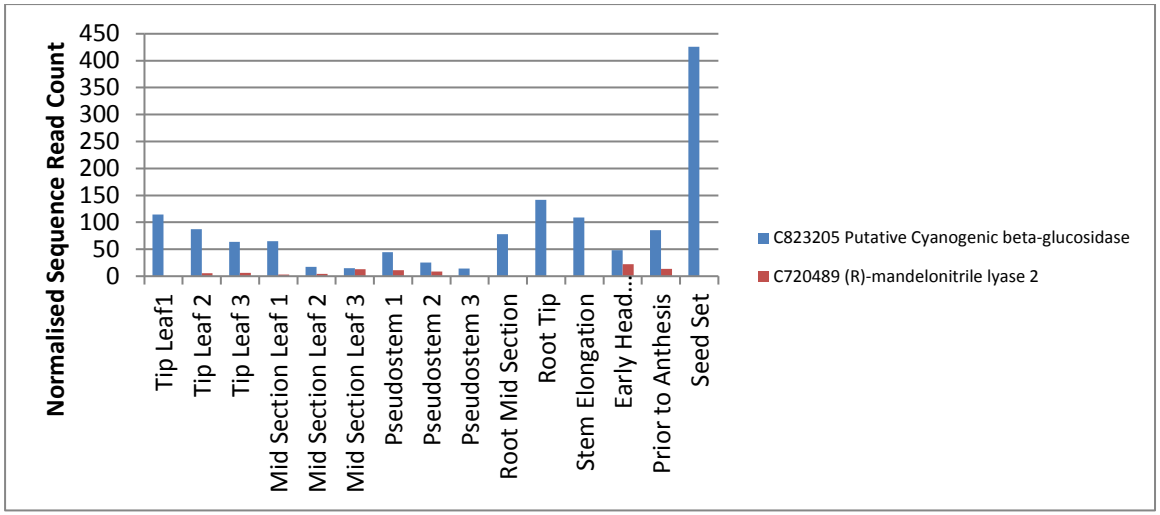
14000
12000
10000
8000
6000
4000
2000
0
Tip Leaf1
Tip Leaf 2
Tip Leaf 3
Mid Section Leaf 1
Mid Section Leaf 2
Mid Section Leaf 3
Pseudostem 1
Pseudostem 2
Pseudostem 3
Root Mid Section
Root Tip
Stem Elongation
Early Head Emergence
Prior to Anthesis
Seed Set
C861671 Cinnamyl alcohol dehydrogenase 7
Locus_2885_Contig1 Cinnamyl alcohol dehydrogenase 3
C724907 Cinnamyl alcohol dehydrogenase
scaffold97355 Cinnamoyl-CoA reductase
C1048703 Cinnamoyl-CoA reductase
C1095381 Cinnamoyl-CoA reductase
C989639 Cinnamoyl coenzyme A reductase (Fragment)

C



D

Figure 4.1.3 Expression profiles of selected candidate genes for various trait-specific categories: aluminium tolerance; (B) reproductive development; (C) lignin biosynthesis; (D) toxin production.

The identities of sampled tissue types are shown on the y = 0 axis, while the x = 0 axis represents transcript abundance in terms of normalized sequence read count.

4.1.2 Use of genomic diversity to identify phalaris cultivars and its consequences for genomics assisted breeding

A total of 14 phalaris cultivars and populations were used to develop an initial understanding. The transcript-based genotyping-by-sequencing approached was used on multiple plants per population. The computational package StAMPP (freely available as an R package) was used for the efficient statistical analysis of the genotype data. Nei's genetic distance was calculated using StAMPP and a neighbour joining tree was constructed in DARWin (Fig. 8.1.1). Many of the close affinities observed on the NJ-tree are reflective of known aspects of the cultivars' breeding history.

From the diversity of populations that are present in the training/reference population, Advanced AT is the most distinct cultivar, while the other cultivars share a higher level of similarity (Landmaster, Holdfast GT, and a population closely related to Holdfast GT, PWA; Fig. 8.1.2). Consequently, two or more prediction equations are likely to be required to increase the accuracy across the genetic backgrounds.

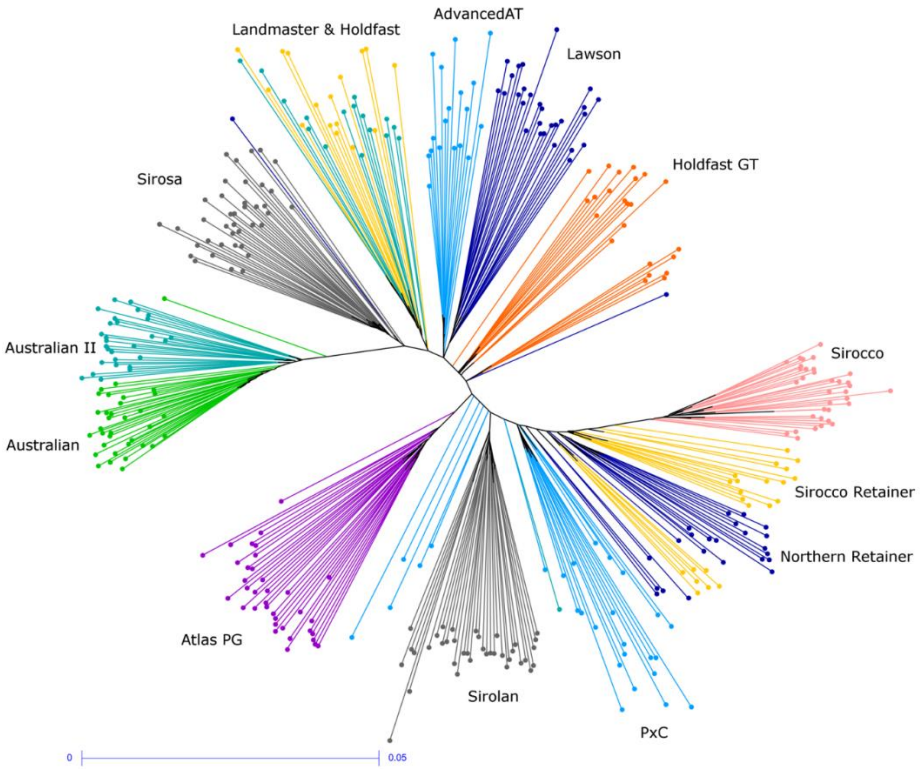


Fig. 4.1.4. Neighbour-joining tree of 14 varieties and populations of phalaris calculated using the StAMPP package.

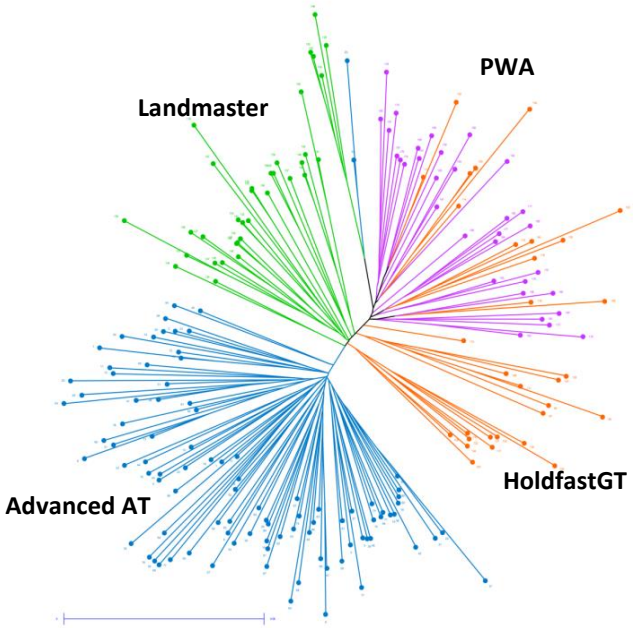


Fig. 4.1.5. Neighbour-joining tree of the germplasm present in the training/reference population of phalaris calculated using the StAMPP package. The PWA population was selected from the same generation as Holdfast GT but with more emphasis on survival at Tamworth.

4.2 Development of a methodology for genomic selection in phalaris

4.2.1 Initial validation of the genomic selection model

Field trials of the training population established in multiple locations in south-eastern Australia were evaluated for agronomically relevant traits such as flowering time, establishment, vigour and herbage biomass yield. As an example of the data collected, a summary of herbage biomass yield at the Canberra site is presented in Table 4.2.1 reproduced from Culvenor et al. (2017). The genotype effect was highly significant (P<0.001) in analyses of variance at all times of observation for both rows and the limited sward comparisons. The range among training population families usually exceeded the range for the winter-active cultivars (excluding Australian and Australian II; Table 1). Sirolan was usually the highest yielding cultivar in the main growing season (autumn, winter, spring) and the semi-winter-dormant cultivars, Australian and Australian II, were the lowest (Table 1). Australian and Australian II were the most productive cultivars at the late spring assessment in both years.

Table 4.2.1. Seasonal herbage DM for the highest (TP max) and lowest (TP min) training population (TP) families and 8 cultivars in single rows (g/m) and sward plots (kg/ha) at Canberra.

Entry	Summer		Autumn		Winter		Spring (main)		Late spring	
	Row	Sward	Row	Sward	Row	Sward	Row	Sward	Row	Sward
Year 1 - 2015										
TP max	77	-	117	-	93	-	168	-	84	-
TP min	45	-	38	-	39	-	104	-	20	-
Holdfast	62	1458	101	1869	87	1782	166	3214	65	536
Landmaster	61	1507	85	1749	71	1560	146	3214	59	715
Holdfast GT	67	1443	113	1630	98	1520	163	2818	52	511
Advanced AT	64	1219	94	1717	78	1550	151	2667	50	528
Sirosa	61	1662	80	2032	89	1819	152	3105	61	731
Sirolan	61	1630	110	2331	100	2191	158	3184	67	803
Australian	51	880	29	842	34	674	83	1985	58	1115
Australian II	44	1108	19	887	33	864	103	2074	59	1255
lsd	*11*	*332*	*28*	*313*	*15*	*251*	*19*	*389*	*16*	*290*
Year 2 - 2016										
TP max	38	-	55	-	108	-	165	-	171	-
TP min	14	-	30	-	54	-	91	-	45	-
Holdfast	27	546	46	784	91	1985	152	2734	97	1356
Landmaster	27	451	42	755	75	1758	136	2477	93	1465
Holdfast GT	23	412	46	810	90	1990	145	2709	106	1556
Advanced AT	23	390	43	707	75	1726	130	2398	73	1070
Sirosa	24	470	43	747	93	2034	149	2658	97	1219
Sirolan	26	562	53	1107	99	2288	144	2837	113	1517
Australian	18	304	36	511	61	1220	104	1800	114	2090
Australian II	19	415	34	685	57	1357	96	1951	76	1663
lsd	*7*	*104*	*7*	*173*	*15*	*246*	*20*	*306*	*32*	*480*

Correlations between rows and swards for 33 cultivars and families were highest in autumn and winter (~0.8), slightly lower in spring (~0.7), and lower again in late spring and summer (Table 4.2.2). Among the 8 cultivars shown in Table 4.2.1, correlations over the two years were higher in autumn, winter and spring (0.86-0.97) than in summer (r=0.65-0.81) and late spring (r=0.22-0.54). Scoring

was found to be more difficult in late spring and summer when variable amounts of stem were present. Correlations between rows and swards were similar to those reported by Smith et al. (2001) for perennial ryegrass families physically harvested or visually estimated. While harvested swards are the ideal for grass evaluation, the much larger number of rows that can be evaluated for a given level of resources makes them a necessary technique in small programs.

Table 4.2.2. Correlation coefficients between visually estimated DM yield in rows and swards for 33 entries. Values in italics, P<0.01; values in bold, P<0.001.

Observation period	Year 1	Year 2
Summer	**0.62**	**0.67**
Autumn	**0.77**	**0.80**
Winter	**0.79**	**0.82**
Spring (main)	**0.72**	**0.69**
Late spring	**0.78**	*0.53*

After filtering c. 63,000 high quality SNP loci remained and missing data was imputed using a linkage disequilibrium k-nearest neighbor imputation algorithm. To test and validate genomic selection a 5-fold cross validation approached was implemented for the traits heading date, summer activity, average biomass and seasonal biomass fitting a BayesA model, using the R package BGLR to estimate marker effects. Accuracies (correlation between GEBV and observed phenotype) of 0.545, 0.302, 0.401 and 0.174-0.6 where achieved for heading date, summer activity, average biomass and seasonal biomass, respectively (Fig. 8.1.3 & Table 8.1.1). Although the population size used in this study was small, it highlights the ability to rapidly develop genomic prediction equations with moderate accuracies for orphan species with complex genomic structures such as phalaris. Further work is required to expand the reference population and explore prediction accuracies across difference environments.

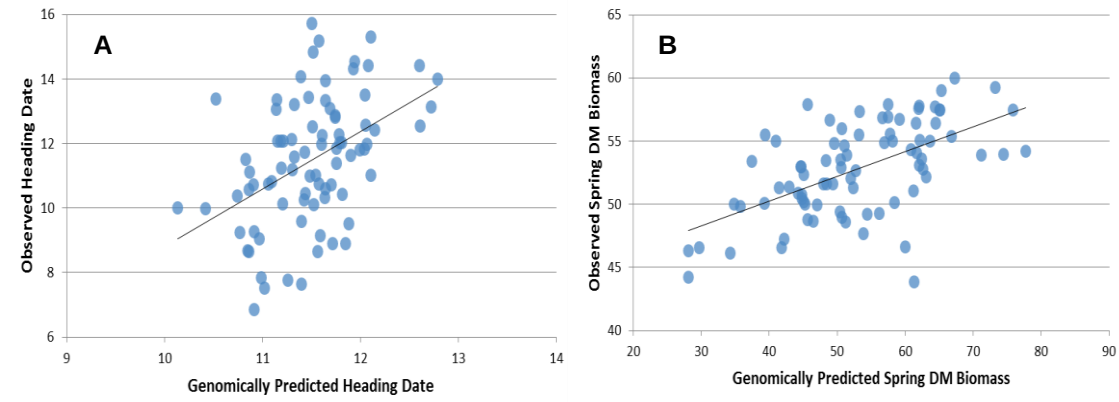


Fig. 4.2.3. Correlation between genomically predicted and phenotypically observed heading date (A) and spring DM biomass (B) of phalaris.

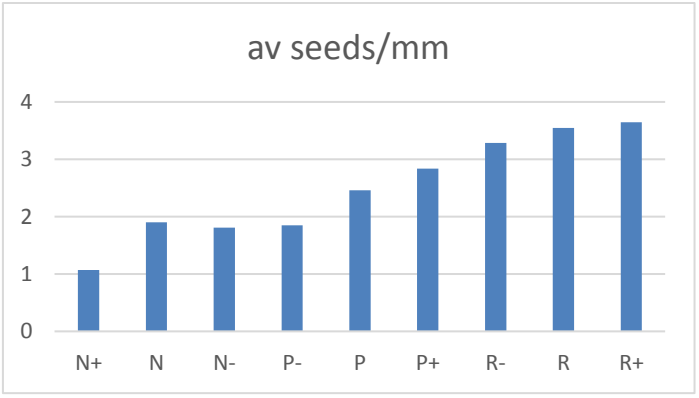
Table 4.2.4. Genomic prediction accuracies for agronomic traits in phalaris

	HD	Average Biomass	Autumn Biomass	Winter Biomass	Spring Biomass	Summer Biomass	Summer Activity
Trait H^{2*}	0.696	0.272	0.135	0.307	0.280	0.145	0.221
Prediction Accuracy	0.545	0.401	0.174	0.193	0.600	0.552	0.302
S.E.	0.067	0.071	0.066	0.094	0.042	0.070	0.123

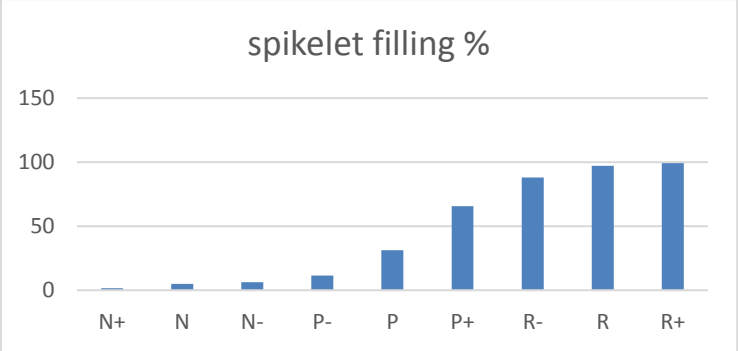
*Heritability values were calculated from a field trial containing the Advanced AT training population as well as additional genotypes not included in the presented genomic prediction calculations.

4.3 Development of markers for seed retention

An initial assessment based on a qualitative scoring scale (divided into retainers, non-retainers and various intermediate classes) was performed for a small set of genotypes across the cultivars listed in Table 3.1.2. The GBS-derived set of c. 82,000 SNPs was generated for this group of plants as a reference population for genomic selection. However, analysis of this dataset failed to obtain reliable prediction equations based on the relatively low number of indivduals that were classified into some of the phenotypic categories in the initial assessment. As a consequence, more accurate data (including quantitative measurements of average numbers of seeds per seed head and proportions of spikelet filling – Figure 4.3.1) was obtained for a larger cohort of plants (288) from a specific genetic background (Landmaster). The GBS processing of the samples was completed and bioinformatic analysis was performed to define the resulting SNP set, following by use of the relevant algorithms for genomic selection prediction analysis.



(A)



(B)

Figure 4.3.1. Assessments of average numbers of seeds per seed head (A) and percentage spikelet filling (B) in plants of the Landmaster population. The classes for seed retention refer to clear non-retainers (N+), clear retainers (R+) and the various intermediate classes of non-retainers, partial retainers and retainers between these extreme phenotypes.

From this data 8 putative markers related to aspects of seed retention were identified (Table 4.3.1). Further work is required to validate these loci as practical markers for intact-rachilla seed retention. These markers could then be used to complement either traditional or genomic selection programs in Phalaris

Table 4.3.1 Putative markers for seed retention traits in phalaris

Trait	Threshold	Marker
NS	5.94	C963307_POS_129
NS	5.85	Locus_12803_Contig1_POS_99
NS	5.73	Locus_6886_Contig3_POS_1159
SD	5.94	C963307_POS_129
SD	5.94	Locus_13491_Contig1_POS_23
SD	5.73	Locus_13491_Contig1_POS_23
SD	5.73	Locus_6886_Contig3_POS_1159
SR2	5.29	Locus_15070_Contig1_POS_960

4.4 Use economic analysis to develop a robust estimate of the economic importance of genetic gain in phalaris

The development of this model has been peer reviewed and published (Ludemann and Smith 2015) A comparison of methods to assess the likely on-farm value for meat production systems of pasture traits and genetic gain through plant breeding using phalaris (*Phalaris aquatica* L.) as an example. *Grass and Forage Science* http://onlinelibrary.wiley.com/doi/10.1111/gfs.12164/) . The following section is a summary of the key results presented in this paper.

4.4.1 Replacement cost method

The cost of feed (Feed$) and subsequent economic values for seasonal DM yield (EV_DM) are shown in Table 3. Monthly price data were available for barley only, so the cost of utilised energy based on oats or sheep pellets were assumed to be constant across seasons. The cost of barley energy varied by a small (AUD0.001/MJ utilised) amount between seasons with AUD0.026/MJ utilised in autumn and winter, and AUD0.027/MJ utilised in spring and summer. In contrast, the cost of oat energy was AUD0.027/MJ utilised, year round compared to AUD0.029/MJ utilised for sheep pellets.

Table 4.4.1. Economic values for seasonal dry matter (DM) yield of phalaris using the replacement cost method where $ indicate Australian dollars

			Value used in analysis when using the various types of feed		
Parameter	Description	Units	Barley	Oats	Sheep pellets
$Feed\$_{aut}$	Cost, utilised feed energy, autumn	$/utilised MJ	0.026	0.027	0.029
$Feed\$_{win}$	Cost, utilised feed energy, winter	$/utilised MJ	0.026	0.027	0.029
$Feed\$_{spr}$	Cost, utilised feed energy, spring	$/utilised MJ	0.027	0.027	0.029
$Feed\$_{es}$	Cost, utilised feed energy, summer	$/utilised MJ	0.027	0.027	0.029
EV_DM_{aut}	Economic value of a 1kg increase in autumn DM yield of phalaris	$/kg DM	0.238	0.246	0.265
EV_DM_{win}	Economic value of a 1kg increase in winter DM yield of phalaris	$/kg DM	0.303	0.319	0.342
EV_DM_{spr}	Economic value of a 1kg increase in spring DM yield of phalaris	$/kg DM	0.291	0.293	0.314
EV_DM_{sum}	Economic value of a 1kg increase in summer DM yield of phalaris	$/kg DM	0.234	0.235	0.252

Economic values for seasonal DM yield were greatest (AUD0.252/kg DM to AUD 0.342/kg DM) when using the sheep pellet replacement cost of utilised energy. This reflects the fact that sheep pellets had the greatest cost per unit of energy (AUD0.029/MJME utilised). Economic values for seasonal DM yield were lowest in summer using the barley replacement cost method (AUD0.234/kg DM). The economic value of seasonal DM yield using the replacement cost of oats ranged between AUD0.235/kg DM and AUD0.319/kg DM.

The relative magnitude of economic values for each season and type of feed used for the replacement cost would change if variables such as the energy concentration of the purchased feed or if the proportion of purchased feed that was utilised changed. For instance, a 0.5 MJ/kg DM increase in energy concentration of sheep pellets would reduce the economic values for seasonal DM yield by between AUD0.017/kg DM (in summer) and AUD0.024/kg DM (in winter) (Table 4). A 0.05 increase in the proportion of purchased sheep pellets utilised by livestock would decrease the economic value of seasonal DM yield by between AUD0.013/kg DM in summer and AUD0.018/kg DM in winter.

Table 1.4.2. Sensitivity analysis of the economic value of seasonal dry matter (DM) yield of phalaris when using sheep pellets as the replacement cost where $ indicate Australian dollars

Parameter	Description	Units	Economic value in each scenario		
			Sheep pellets (Base)	0.5 increase in pellet energy concentration	0.05 increase in utilisation of pellets
EV_DM_{aut}	Economic value of a 1kg increase in autumn DM yield of phalaris	$/kg DM	0.265	0.246	0.251
EV_DM_{win}	Economic value of a 1kg increase in winter DM yield of phalaris	$/kg DM	0.342	0.319	0.324
EV_DM_{spr}	Economic value of a 1kg increase in spring DM yield of phalaris	$/kg DM	0.314	0.293	0.298
EV_DM_{sum}	Economic value of a 1kg increase in summer DM yield of phalaris	$/kg DM	0.252	0.235	0.239

4.4.2 Change in livestock production method

The energy requirements for the various changes in livestock production are shown in Table 5 for a one-DSE increase in stocking rate, or for changes in the carcase weight of lambs or cattle that could be achieved with one-kg extra DM yield of phalaris. The greatest change in carcase weight for one-kg extra phalaris DM yield was for a weaner calf sold in winter with a 0.12 kg increase in carcase weight. The least change in carcase weight for one-kg extra phalaris DM yield was for a store or prime lamb sold in summer with a 0.03 kg increase in carcase weight. Pasture energy concentration was greatest (11.8 MJ/kg DM) in winter which meant the greatest liveweight gain response of calves at weaning occurred in this season. The low liveweight gain response to the one-kg increase in phalaris DM yield in summer reflects the low energy concentration of pasture DM (8.13MJ/kg DM) in that season.

Table 4.4.3. Key parameters calculated in the estimation of economic values for seasonal dry matter (DM) yield of phalaris using the change in production method where $ indicate Australian dollars

				Aspect of production changed:			
Parameter	Description	Units	Stocking rate	Store lamb LWG	Prime lamb LWG	Weaner calf LWG	Heavy steer LWG
ME_DSE_t	Metabolisable energy requirements of a dry stock equivalent in one season	MJME/DSE/autumn	684.4	N/A	N/A	N/A	N/A
$\Delta Wt_{Soldaut}$	Change in weight of animal for the one-kg increase in phalaris DM yield in autumn	kg carcase weight	N/A	0.04	0.04	0.09	0.05
$\Delta Wt_{Soldwin}$	Change in weight of animal for the one-kg increase in phalaris DM yield in winter	kg carcase weight	N/A	0.06	0.06	0.14	0.08
$\Delta Wt_{Soldspr}$	Change in weight of animal for the one-kg increase in phalaris DM yield in spring	kg carcase weight	N/A	0.06	0.05	0.12	0.07

$\Delta Wt_{Soldsum}$	Change in weight of animal for the one-kg increase in phalaris DM yield in summer	kg carcase weight	N/A	0.03	0.03	0.07	0.04
$\$Wt_{Soldaut}$	Net monetary value of the additional weight gain in autumn	$/kg	N/A	5.29	5.15	3.68	1.88
$\$Wt_{Soldwin}$	Net monetary value of the additional weight gain in winter	$/kg	N/A	4.78	4.92	3.76	1.91
$\$Wt_{Soldspr}$	Net monetary value of the additional weight gain in spring	$/kg	N/A	4.90	4.61	3.69	1.92
$\$Wt_{Soldsum}$	Net monetary value of the additional weight gain in summer	$/kg	N/A	4.93	4.87	3.61	1.84

Table 6 shows the range of economic values for one-kg increases in phalaris DM yield in the four seasons (EV_DM_t) based on changes in livestock production. The greatest economic value based on the change in livestock production was in winter for live weight gains of weaner calves (AUD 0.515/kg DM). This was AUD 0.479/kg DM greater than the economic value based on a change in stocking rate in summer (the lowest economic value based on the change in livestock production method) and AUD 0.173/kg DM greater than the highest economic value based on the replacement cost method (using sheep pellets in the winter period-AUD 0.342/kg DM).

Table 4.4.4. Economic values for seasonal dry matter (DM) yield of phalaris using the base pasture utilisation ($Prop_{UPasture}$)(0.6) value across various change in livestock production methods, where $ indicate Australian dollars

			Aspect of livestock production changed				
Parameter	Description	Units	Stocking rate	Store lamb LWG[1]	Prime lamb LWG[1]	Weaner calf LWG[1]	Heavy steer LWG[1]

EV_DM_{aut}	Economic value of a 1kg increase in autumn DM yield of phalaris	$/kg DM	0.040	0.215	0.202	0.319	0.090
EV_DM_{win}	Economic value of a 1kg increase in winter DM yield of phalaris	$/kg DM	0.052	0.310	0.310	0.515	0.146
EV_DM_{spr}	Economic value of a 1kg increase in spring DM yield of phalaris	$/kg DM	0.048	0.272	0.248	0.435	0.126
EV_DM_{sum}	Economic value of a 1kg increase in summer DM yield of phalaris	$/kg DM	0.036	0.162	0.155	0.256	0.071

[1]Liveweight gains

4.4.3 Relative economic values of seasonal variation in dry matter production

The economic values for DM yield in each season, when expressed as a percentage of the sum of the economic values across all four seasons (relative economic values) are shown in Table 7. The greatest range in relative economic values for seasonal DM yield traits using the replacement cost method within seasons was 0.7 percentage points. This was between the economic value for winter using the barley replacement cost method (28.4%) and the economic value for winter using the oat replacement cost method (29.2%). Between seasons there was a 7.7 percentage point range in relative economic values using the three replacement cost methods. The results in Table 7 indicate there was less variation between seasons for relative economic values calculated using the replacement cost method than between seasons using the change in livestock production methods. Moreover, on average, the economic values calculated using the various change in liveweight gain methods were consistently highest in winter and lowest in summer.

Table 4.4.5. The relative economic values of seasonal dry matter yield traits using a range of methods of calculation

	Method of calculating economic value of phalaris dry matter yield							
	Barley replacement	Oat replacement	Sheep pellet replacement	Stocking rate	Store lamb LWG[1]	Prime lamb LWG[1]	Weaner calf LWG[1]	Heavy steer LWG[1]
	Relative economic values (contribution of economic values in each season, as a percentage of the whole year)							
Autumn	22.3%	22.6%	22.6%	22.9%	22.4%	22.1%	20.9%	20.7%
Winter	28.4%	29.2%	29.2%	29.6%	32.3%	33.9%	33.8%	33.7%
Spring	27.3%	26.8%	26.8%	27.2%	28.4%	27.1%	28.5%	29.1%
Summer	21.9%	21.5%	21.5%	20.4%	16.9%	16.9%	16.8%	16.5%

[1]Liveweight gains

4.4.4 Sensitivity analysis

There was a 16.7% reduction in all economic values when a $Prop_{UPasture}$ of 0.5 was used, as opposed to the Base $Prop_{UPasture}$ value of 0.6. The relative economic values therefore did not change when the assumption for $Prop_{UPasture}$ was reduced by 0.1. When a $Prop_{UPasture}$ assumption of 0.4 was used there was a 33.3% reduction in economic values as compared to using the Base $Prop_{UPasture}$. There was no change to the relative economic values when the $Prop_{UPasture}$ was changed to 0.4.

5 Discussion

5.1 Development of a methodology for genomic selection in phalaris

The application of molecular markers and quantitative genetics to predict plant performance has the potential to radically change plant breeding particularly in species with long selection cycles. Whether this potential can be realised for relatively minor species such as phalaris will depend on the cost effectiveness of applying marker-assisted techonologies compared with traditional selection strategies which themselves have considerable costs, notably in field testing. Additionally, currently little is known about the genetic relationships present within and between varieties and breeding germplasm of less-studied species such as phalaris. For this reason, the approach used in this project is an appropriate one where a comprehensive study of the genomic variation within the pool of germplasm that is routinely used for breeding can be used to identify cultivars and importantly the likely allocation of resources to selection pools based on genomic similarity/dissimilarity. New

genomic tools such as genotyping-by-sequencing now enable the rapid and cost effective development of genomic resources for previously 'orphan' forage species such as phalaris. Through the application of these tools with genomic selection methodologies, the ability to replace lengthy phenotypic evaluation trials with prediction of performance, increased accuracy of selection, and an understanding of the genetic relationships between breeding germplasm will become possible. Ultimately this will lead to the development of more elite and better adapted cultivars faster and more efficiently that are aligned with industry breeding objectives that can be evaluated and validated through increased performance in national evaluation programs such as the Pasture Trials Network.

In order to successfully implement genomic selection within a phalaris breeding program a genotyping-by-sequencing approach that uses skim sequencing of the transcriptome was developed and implemented in this project. A filtered set of 89,738 SNPs was identified from 290 genotyped samples in the training population, along with the associated custom bioinformatics pipeline capable of handling missing data and the segmental allotetraploid nature of phalaris. Preliminary analysis of genotypic data has highlighted a level of population structure. To be able to successfully implement genomic selection, a comprehensive understanding of the genetic diversity and relationships in the phalaris germplasm is required. A total of 14 phalaris cultivars and populations were used to develop an initial understanding which revealed close affinities reflective of known aspects of the cultivars' breeding history. Thereby providing guidance as to how to proceed with selection within these groups of cultivars and also with respect to the genomic/screening strategies required should breeders wish to combine attributes from diverse cultivars within the overall population. From the diversity of populations that are present in the training/reference population, Advanced AT is the most distinct cultivar, while the other cultivars share a higher level of similarity (Landmaster, Holdfast GT). Consequently, two or more prediction equations may be required to increase the accuracy across the genetic backgrounds. There is ample diversity to select within each pool but combining the attributes of Advanced AT and Holdfast GT was a common goal of breeding companies surveyed throughout this project so there is a desire to bring these pools together. This could be achieved either through genomic selection within each pool and subsequent intercrossing or through investing the resources in a much larger combined reference population. It is most likely that the use of genomic information will be utilised within pools in the first instance.

Although the training population was relatively small compared to those used in major crop species, analysis of herbage biomass from the Canberra site indicated that genomic selection is practical for the selection of traits both with low heritability (yield) and high heritability (flowering time) with accuracies similar to those achieved with similar population sizes in perennial ryegrass (e.g. Faville et al. 2017) despite the more complex genome of phalaris. Accuracies were also of a similar magnitude to those found in switchgrass (Lipka et al. 2014; Casler and Ramstein 2017). Even with demonstrated accuracies in the order of 0.1-0.2, Casler and Ramstein (2017) predicted rates of genetic gain in various switchgrass breeding populations in the order of double or triple that possible with field-based half-sib family selection due to the ability to exploit more of the additive variance and shorter selection cycle time.

5.2 Development of markers for seed retention

Some difficulties were initially experienced when trying to relate genotype to phenotype across a range of cultivars due to issues with identifying large enough numbers of individuals in each phenotypic class and thus reducing the power to identify informative sequence variations but later work on a large number of plants confined to single cultivar, Landmaster, resulted in the discovery of 8 putative markers for intact rachilla seed retention. There was not time within this project to validate these putative markers but there is now a well-defined line of enquiry to pursue.

5.3 Use economic analysis to develop robust estimates of the economic importance of genetic gain in phalaris

Results of this study on economic values for seasonal DM yield of phalaris indicate the value from genetic gain in pastures if realised on-farm. Calculation of the economic values were generally lowest (AUD0.04 to AUD0.05 per kg increase in phalaris DM yield) using the change in stocking rate method. This is a reflection of the relatively low gross margins used (AUD20 per DSE) (Waterman and Creese, 2014) in the calculation of these economic values. A proportionate increase in these economic values would ensue if the gross margins per DSE were increased.

Economic values by the replacement cost method using three types of alternative feed (barley, oats and sheep pellets) were similar between seasons. The economic values using barley as the replacement cost for instance had the lowest economic value in summer (AUD0.234) and the greatest economic value in winter (AUD0.303). The price of barley did not change substantially (<6%) between seasons. Therefore, the variation in economic values for phalaris DM yield between seasons was a reflection of the variation in phalaris ME concentration of DM. For instance, the lowest ME concentration for phalaris (8.7 MJME/kg DM) was in summer and the greatest ME concentration (11.8 MJME/kg DM) was in winter.

Compared to economic values calculated based on the replacement cost method, there was greater variation in absolute economic values between seasons using the change in livestock production methods. Economic values calculated using the weaner calf change in livestock production method had a AUD0.259/kg DM range in economic value for a one-kg increase in phalaris DM (with AUD0.515/kg DM in winter and AUD0.256/kg DM in summer).

While the absolute economic values for pasture traits may vary by the method of calculation, it is the relative economic values (as well as the heritability of traits and their correlations between traits) that are important for assessing what genetic progress in seasonal DM yield could be made in a pasture plant breeding program. The relative economic values were calculated as the economic value for the change in phalaris DM yield in a specific season as a percentage of the sum of all four seasonal economic values. The timing and frequency of the four seasonal DM yield traits did not need to be scaled by the frequency or timing of each trait for inclusion into a breeding objective (McClintock and Cunningham, 1974). This is unlike some traits in animal breeding objectives because all four seasonal DM yield traits are expressed within the same year. If traits with different timing and/or frequency of expression were to be included in the phalaris breeding objective (ie. pasture persistence) then, the economic values would need to be converted to economic weights. Economic

values can be converted to economic weights through multiplication with discounted genetic expression coefficients (Amer, 1999).

Variation in economic values for seasonal DM yield is supported by other workers who have calculated economic values for pasture traits. In New Zealand, typical dairy farm systems were modelled to estimate the economic values for an additional one-kg of DM yield across 5 seasons including winter, early spring, late spring, summer and autumn (Chapman *et al.*, 2012). Four representative dairy farms (situated in four different regions of New Zealand) were modelled. For the 'Upper North Island' region the economic values for a one-kg increase in pasture DM was NZD0.30 in winter, NZD0.48 in early spring, NZD0.21 in late spring, NZD0.40 in summer and NZD0.41 in autumn. For comparative purposes if the mean of the two spring (early and late spring) seasonal economic values were used, the relative economic value for: winter would be 20.6%, spring would be 23.7%, summer would be 27.5%, and autumn would be 28.2%. The relative economic values from Chapman *et al.* (2012) were generally greater for autumn and summer and lower in winter and spring than results of this study. This can be explained by the fact that in New Zealand dairy systems greater pasture ME concentrations can be maintained in summer and autumn to improve animal production when DM increases by one-kg. Results from the present study indicates there is greater value in additional DM yield in winter for sheep and beef farms in south-eastern Australia compared to New Zealand dairy conditions (based on relative economic values). This is because a feed deficit in this period can limit the stocking rates of sheep and beef farms in south-eastern Australia where phalaris is grown. The difference in the relative economic value of seasonal DM yield between the dairy farms in New Zealand and sheep and beef farms in Australia mean plant breeders in Australia may need to place more emphasis on winter DM yield than what may be bred for dairy farms in New Zealand.

McEvoy *et al.* (2011) calculated the economic values of DM yield for Irish dairy systems for three time periods. These included spring (€0.152/kg DM), mid-season (summer)(€0.03/kg DM), and autumn (€0.103/kg DM). The relative economic value for seasonal DM yield was therefore weighted heavily toward spring, followed by autumn and mid-season. The differences in relative economic values between seasons, is a reflection of the differences in farm systems under comparison. In Irish dairy farm systems, dairy cattle are typically housed indoors over winter, and spring is an important time to harvest DM yield through grazing or as silage where grass silage is a principal winter feed (McEvoy *et al.*, 2011). Lack of grazing in winter explains why there was no economic value calculated for DM yield in this season.

Chapman *et al.* (2012) and McEvoy *et al.* (2011) calculated economic values for pasture DM traits in dairy systems. These results came from models with more defined production systems where the main focus was how the increase in pasture DM affected milk production. The present study in contrast took into account the role of pastures in creating economic value from rearing and growing lambs and beef cattle. It must be acknowledged that the change in livestock production method used in the present study may not be applicable to every farm or season. Nor will these scenarios take into account the inter-temporal trade-offs of the availability of feed for females and how this may affect their performance in the following season(s).

5.3.1 Advantages and disadvantages of 'replacement cost' method

The replacement cost method takes into account the least cost method of otherwise obtaining energy for livestock. Calculating economic values by this method is simple as there are prices for alternative feeds available. However, the replacement cost method does not take into account how critical the feed is for the farm system in that period. In most cases stocking rates on a pasture-based livestock system will be limited by the period of limited availability of pasture ME. This can vary based on the climatic conditions experienced on the farm (which affect pasture growth and quality) and how livestock are managed. For instance, Moore *et al.* (2009) compared the typical patterns of feed supply and demand for self-replacing beef cattle enterprises in Australia. In south west Victoria, the period of energy deficit occurred in January and February. In the drier mid north of South Australia, the energy deficit lasted for much longer (October to April), whereas on the south west slopes of New South Wales energy deficits occurred in November to February and again in April.

The economic value of DM yield based on the replacement cost method may also not take into account the opportunity cost of lost production when there is an energy deficit. In autumn a feed deficit may result in ewes not obtaining adequate body condition for mating. Energy deficits in the period preceding the mating of capital livestock can have flow-on effects to the fertility of the flock or herd which could limit the number of offspring available for sale as weaner or finished livestock. Energy limitations in the period when offspring are growing (such as spring and summer for growing steers or lambs) could result in these animals taking longer to get to slaughter (requiring more maintenance energy) or being slaughtered at a lower carcase weight when slaughtered at the same age. Differences in the replacement cost of feeds such as barley, oats or sheep pellets throughout the year may therefore not necessarily reflect how critical energy is to the efficiency of the production system.

This study supports the hypothesis that economic values of pasture traits using the replacement cost method do not take into account the seasonal availability (and hence value to farmer) of pasture supply, because it does not take into account the opportunity cost of the pasture DM which could be converted into products. In the example of phalaris the greatest economic value for winter DM yield was AUD0.342/kg DM using the sheep pellet replacement cost method. This same DM yield could have gone toward increasing weaner calf production with an economic value of AUD0.515/kg DM. As a consequence, there was less variation between seasons for relative economic values calculated using the replacement cost method than between seasons using the two change in livestock production methods (change in stocking rate and change in livestock growth). Yet, although the absolute economic values for the seasonal DM yield traits varied between methods of estimation, the relative economic values were consistent between methods. This suggests robust relative economic values for seasonal DM yield could be calculated over a range of methods. However, the use of the change in stocking rate method may be most applicable to sheep and beef farm operations because this method does not assume animals are being finished year round and the change in liveweight gain methods does not account for the whole farm system in the same way as assuming an increase in stocking rate.

5.3.2 Advantages and disadvantages of 'change in livestock production' method

The change in livestock production method is an intermediary between the replacement cost method and a detailed farm system experiment or modelling exercise. This is because the change in livestock production method requires less data than a detailed model of a farm system, but more data than the replacement cost method. The intermediary nature of the data requirements for the change in livestock production method therefore makes economic value results using this method not as generalised as the replacement cost method, and less specific than a farm experiment or detailed model of a farm system. The change in livestock method better accounts for the variation in seasonal value of DM compared to the replacement cost method whilst not having such onerous and specific data requirements of a whole farm model. The change in livestock production method is therefore less generalised than the replacement method, yet is applicable to a wider range of farm systems compared to detailed farm experiments or detailed models of farm system. This makes the change in livestock production method suitable for estimating economic values in a forage selection index at the industry scale.

6 Conclusions/Recommendations

6.1 Genomic Resources and Genomic Breeding Strategies for Phalaris

This project has developed a significant genomic resource that is available for use in molecular breeding of phalaris. This kind of genomic resource will allow the development of genomic selection strategies to greatly increase the rate of genetic gain in phalaris as has been seen in other animal and crop species and is being implemented in perennial ryegrass. A successful genomic selection program could utilise the genomic resources identified in this program to characterise a target reference population/populations of relevance to a company's specific breeding priorities. The reference population sizes used in this research were on the low side of those required for practical implementation of genomic selection but have demonstrated that the model proposed is practical for the selection of traits both with lowly heritability (yield) and highly heritability (flowering time) with accuracies similar to those achieved with similar population sizes in perennial ryegrass (e.g. Faville et al. 2017) despite the more complex genome of phalaris. The structure that is present in phalaris germplasm pools and first identified in this project means that greater accuracy will be achieved by having more defined reference populations than with for instance, one broadly-based winter active pool. For instance, targeting Holdfast GT and derivatives or Advanced AT and derivatives

Whether or not these reference populations could be shared across companies in a model such as the Beef Information Nucleus is a point for further discussion across the industry. Although companies generally share breeding goals but there is not a defined breeding objective that is commonly shared and selected for. It is more likely that reference populations will be specific to individual companies or consortia of companies.

Work is continuing to relate data on aluminium tolerance, grazing tolerance and alkaloid composition to genotype. It has been found necessary to extend the grazing experiment in which

grazing tolerance of training population families is being phenotyped beyond the life of the project to obtain meaningful changes in persistence.

6.2 Use of Sequence Variation to Identify Phalaris Cultivars

We have demonstrated the utility of SNP based variation to discriminate between even very closely related culivars. This process could be used for a number of outcomes as seen in other species; including but not limited to

- Quality assurance during breeding and commercialisation
- Support for Plant Breeders Rights and other IP protection policies
- Identification of plants sown in paddocks
- Agronomic experimentation

6.3 Development of Markers for Seed Retention

A set of putative molecular markers have been identified that require further validation. Different loci were associated with contrasting aspects of the seed retention trait therefore it is recommended that future phenotypic data collection focus on all aspects of seed retention. The use of these validated markers would allow breeders to use these as a screening tool to identify seed retaining genotypes. These seed retaining genotypes could then be used in either traditional or genomic selections programs allowing them to proceed more efficiently as resources would not be used in screening and evaluating germplasm that would ultimately be discarded due to seed shattering.

6.4 Development of a Model to Value Genetic Gain in Phalaris

A model to value genetic gain in phalaris has been developed based on the relative value of seasonal changes in dry matter production with respect to lamb and beef production systems. This model could be used as a basis for a “Forage Value Index” similar to those used for perennial ryegrass in the dairy industry or for the basis of a ‘Breeding Objective’ in a phalaris breeding program. The model suggested that genetic gain in seasonal DM production is highest in value during the winter season in south-eastern Australia, supporting the use of a training population based on the “winter-active” pool of phalaris cultivars.

7 Key Messages

This project was initiated to

- Increase the amount of genomic sequence information available for Phalaris with an emphasis on understanding the diversity of agronomically adapted Phalaris to assist with future breeding efforts
- To validate the applicability of geneomic selection within Phalaris populations of interest to commercial breeding companies
- To develop and publish an economically based breeding objective for phalaris

- To provide 'proof of concept' for the application of genomic selection technologies in Phalaris

As a result of this project

- A world's first resource of more than 140,000,000 base pairs of reference data have been sequenced from the phalaris genome and more than 500,000 putative SNPs identified at a frequency of 1 SNP per 262 bp
- An economic model to assess differences in the value of Phalaris has been developed and could be used as the basis of a genomic selection program and to evaluate the relative on-farm value of existing cultivars
- Proof of concept of the use of genomic information to select for yield and seed characteristics has been obtained.
- Putative markers for seed-retention traits have been identified for further validation.

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