Developing new tools for pasture plant breeding

B.A. BARRETT, M.J. FAVILLE, K. GHAMKHAR and M.J. CARENA
AgResearch Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand
brent.barrett@agresearch.co.nz

Abstract

The rate of genetic gain represented in the Forage Value Index of perennial ryegrass (*Lolium perenne*) is a major factor underpinning sustained profitability in pastoral farming. Effective new technologies for trait data acquisition and parent plant selection are used in many animal and crop improvement programmes to lift the rate of gain, but have yet to be developed and integrated in forage breeding. For forage improvement, hypotheses tested were: a) genomic selection (GS) offers a viable breeding strategy, and b) key enabling technologies for non-destructive, high-throughput phenotyping (HTP) in the field will improve trait data acquisition. To evaluate GS, extensive molecular marker and phenotypic datasets in structured populations of perennial ryegrass were developed. Phenotypic data for seasonal dry matter yield (DMY), the core trait in the Dairy NZ Forage Value Index, were obtained replicated field trials. Data on heading date (HD) as a useful trait to assess the efficacy of GS for simply inherited traits, were also collected. Genomic prediction models were developed for seasonal DMY and HD. Application of GS for HD was effective in selecting for both early and late heading, with movement of up to 7 days in a single generation of selection. The HTP research used iterative development of computational methods supporting a repeatable, non-invasive means of accurately and rapidly measuring DMY of perennial ryegrass in single row plots. These findings demonstrate effective genetic prediction and phenotyping approaches which may enable breeders to lift the rate of genetic gain in perennial ryegrass.

Keywords: forage, genomic selection, phenomics, LIDAR

Introduction

The genetic potential of pasture is a major determinant of farm system productivity and profitability. In New Zealand dairy systems, the financial value of the highest ranked perennial ryegrass (*Lolium perenne*) genetics compared to the lowest ranked is currently estimated to be in the hundreds of dollars/ha/year (Chapman *et al.* 2017).

These economic models, and realised benefits on farm, justify continued industry investment in genetic improvement. Of particular interest is the long-term rate of genetic improvement, commonly referred to as rate of gain, for seasonal dry matter yield (DMY) of perennial ryegrass. This is arguably the single most important plant trait for New Zealand farmers.

There have been historic studies of the rate of gain for DMY in cool season pasture species, showing a range of results varying from nil for lucerne (*Medicago sativa*) to recent studies indicating up to 0.76% per annum for dry matter yield in perennial ryegrass monocultures (Brummer & Casler 2014; Harmer *et al.* 2016).

The rate of gain in pasture species is constrained by a number of factors. Compared to most crops, forage genetics is relatively complex. Breeders face heterogeneous populations of heterozygous individuals, and strong environmental and management effects on expression of traits controlled by many genes in a perennial species over time. In ryegrass, managing the genetic interaction between host plant and its *Epichloë* fungal endophytic symbiont (Faville *et al.* 2015) creates an additional level of complication for plant breeding (Lee *et al.* 2012). Finally, being a perennial species used in a mixed sward under a range of environmental conditions creates further complexity in breeding for cultivars with predictable, robust performance on-farm. Pasture species such as perennial ryegrass are minor species in terms of global seed sales, limiting seed industry investment which constrains implementation of improved breeding techniques and further limits the rate of gain.

The cost of collecting accurate trait data on large numbers of selection candidates under realistic sward conditions is high, meaning that direct selection for DMY is rarely undertaken. Breeders usually rely on indirect measures such as visual preference among individual spaced plants, which show no or weak correlation with sward performance (Hayward & Vivero 1984; Waldron *et al.* 2008). Breeders less commonly use sown single row plots, which show moderate correlation ($R^2 = 0.59$) with plot performance (M.Z.Z. Jahufer pers. comm.).

Genomic selection (GS), a breeding strategy that uses genomic marker data to predict trait performance (Meuwissen *et al.* 2001), is a compelling option for improving the rate of genetic gain, by enabling selection much earlier than in conventional breeding as well as allowing breeders to access the full spectrum of genetic variation present in a population (Casler & Brummer 2008).

In recognition of these opportunities, and the desire

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to increase the rate of gain, a research programme has been developed to address the use of calibrated pasture sensors for efficient collection of DMY trait data, and genomic prediction of breeding values among selection candidates. Recognising the complexity of DMY, and that it is expressed over multiple years, an initial GS approach is being validated for Heading Date (HD), a trait that is simpler both in terms of its genetic control and measurement. This paper reports preliminary findings on development and evaluation of GS for HD, and a light distance and range (LIDAR)-based approach to DMY trait data collection, for use in pasture plant breeding.

Methods

Genomic selection model development and evaluation for heading date

A model for genomic prediction of heading date (HD) in ryegrass (Faville *et al.* 2018) was used to make selections in five breeding populations from the Grasslands Innovation Ltd perennial ryegrass breeding programme. These populations (Pop Ia, Ib, Ic, IIIa, and IIIc) were related to two breeding pools (Pop I and III) that were part of the training set used to develop the HD genomic prediction model. In each case 'a' is the next generation after the training set generation. Generations 'b' or 'c' represent advanced generations of Pop I or Pop III that were produced after two cycles of recurrent selection from the same population.

DNA was isolated from between 137 and 259 seedlings/population using a semi-automated method (Anderson *et al.* 2018). Genotyping-by-sequencing (GBS) was used to generate single nucleotide polymorphism (SNP) markers for a total of 1069 seedlings, using methods described in Faville *et al.* (2018). The SNP data were used to estimate a genomic relationship matrix for individuals of the five populations and those in the full training set, using KGD methodology (Dodds *et al.* 2015). The genomic prediction model for HD (Faville *et al.* 2018) was then applied using this matrix to calculate genomic estimated breeding values (GEBV's) for HD using Genomic Best Linear Unbiased Prediction (GBLUP).

Following estimation of GEBV's for individuals in the five populations, 12 individuals were randomly removed from each population to create a control (non-selection group). From amongst the remaining individuals in each population, the top 10% (high GEBV group) and bottom 10% (low GEBV group) were identified based on HD GEBV. Twelve individuals in each of the low and high GEBV groups were randomly selected for polycross isolation to produce progeny for evaluation. A total of 15 polycrosses (five populations x low GEBV, high GEBV and non-selection groups) were completed at AgResearch Grasslands in Palmerston North during 2016-2017. Seed was harvested and 50 seeds/selection group randomly chosen, germinated in a greenhouse and grown on for evaluation.

Field trials were established at AgResearch Ruakura, in Waikato and AgResearch Grasslands, in Manawatū. At each location a spaced-plant trial, with 40 cm spacing between plants, was established by transplanting in May 2017. Each trial consisted of 420 plants in a row-column design, including 25 individuals from each of the 15 selection groups and 45 repeated clonal checks (three check plants x 15 clones). The three check plants were chosen to represent a range of HD, and in this experiment were measured as +4.7, +10.5 and +28.5 days after 22nd October, respectively, on average, across both locations. Plants were grazed by sheep, except during late-September to late-December to allow flowering to proceed (trials were closed to grazing at both sites in 8th September 2017). At both sites, HD was recorded as the number of days after 22nd October 2017 until five fully-emerged flower heads were observed. The HD data were analysed as a linear mixed model, using the variance component analysis procedure residual maximum likelihood (REML) option in GenStat (GenStat 2006). Repeated clonal checks and location were treated as fixed effects while selection group, rows and columns were treated as random effects. Final means for each selection group were calculated as best linear unbiased predictors (BLUPs) (White & Hodge 1989).

LIDAR dry matter yield platform development and field evaluation

As described (Ghamkhar *et al.* 2018), a system was developed targeting DMY in single row plots of perennial ryegrass, a standard format used by forage plant breeders to evaluate selection candidates. In brief, spatial features and soil surface variation via sensor-based field screening were examined on a mobile platform, a high throughput method to estimate DMY developed; linked with automated data processing and analysis to continuously estimate DMY in real-time.

An indoor LIDAR scanning unit (LMS400 Pro, SICK Vertriebs-GmbH, Germany) was adapted for use in the field (Figure 1). To minimise variation due to variable ambient light, the sensor was placed under a mobile screen. The unit was powered using a battery and connected to a computer, where custom-made software captured raw data from the LIDAR unit. These data were processed in real-time and displayed during scanning, allowing users to monitor data acquisition in the field.

Algorithms were developed to automatically segment the LIDAR data from a sequence of raw distance measures into volumetric estimates for each single row plot in a particular scanning phase. The

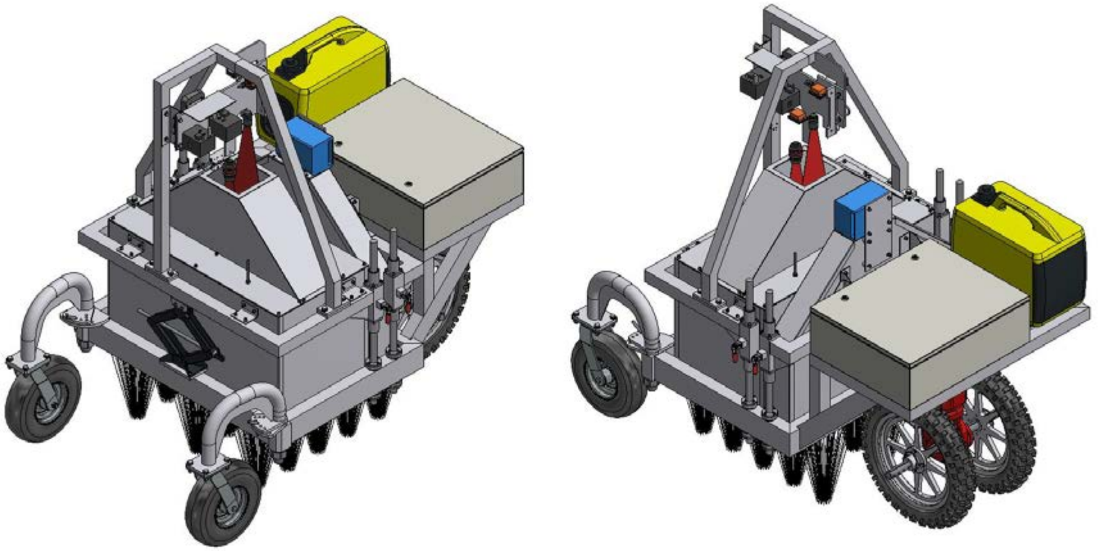

Figure 1 Technical drawing showing two views of the integrated mobile LIDAR system developed for non-destructive, real-time measurement of dry matter yield in single row plots of perennial ryegrass.

ground height was calculated independently for each single row plot, as it varies among plots. The ryegrass component of the data are then segmented from the surrounding soil background and any sparse weeds. The LIDAR data in each ryegrass region is then further processed to calculate the biomass estimate for each plot by converting the two dimensional height and location measurement data to a three dimensional volumetric model. This package allows the user to capture, monitor, and process LIDAR data in the field and acquire real-time, non-destructive estimates of DMY, termed LIDAR Volumetric (LV) estimates.

The LIDAR platform was used to acquire LVs in single row plots of perennial ryegrass during a regrowth phase following defoliation. The trial consisted of 86 single row plots of perennial ryegrass. Each plot was 2 m long and planted by seed on 50 cm centres in March 2015 at Kimihia Research Centre (-43.62, 172.46) near Lincoln, New Zealand. The trial had been grazed by sheep eight times before this experiment. The most recent defoliation before the experiment was 23rd March 2016. The LIDAR was used to scan each plot 83 days later, immediately before mechanical harvesting of each plot to a residual height of 3 cm above the soil. Fresh weight (FW) yield data were collected from harvested samples by weighing at harvest time, with samples subsequently dried and DMY data calculated. The LVs were analysed and compared to the FW and DMY data.

Initial analysis of FW, DMY and LV data was carried out using Microsoft Excel 2013 to align and clean data, and calculate correlations of FW and DMY data with LVs, with subsequent plotting of data using SigmaPlot.

Results

Genomic selection for heading date

Significant differences ($P<0.05$) in mean HD were observed between divergent selection groups derived via GS from five populations (Table 1). The impact of GS varied among populations.

Populations Ia and IIIa of the generation immediately subsequent to that used to train the genomic prediction model showed large (24-37%), significant ($P<0.05$) differences in mean heading date between low and high GEBV selections. Divergence from the non-selected group was significant in only one direction and this differed for Pop Ia (only high GEBV > non-selected) and Pop IIIa (only low GEBV < non-selected).

When GS was applied in advanced generations of the same populations the effect was generally consistent, but smaller. For Pop IIIb, there was a significant ($P<0.05$) 11% difference in heading date between low and high GEBV groups, but neither of those groups was significantly different to the non-selected group. For one advanced generation of Pop I (Pop Ib) there was a 10%, but non-significant difference ($P>0.05$), in heading date between low and high GEBV groups, and neither was significantly different to the non-selected group. In contrast, for a second Pop I advanced generation population (Pop Ic) there was large (41%) difference in heading date between low and high GEBV selection groups, with the non-selected group intermediate to these, and all differences were significant ($P<0.05$).

LIDAR estimation of dry matter yield

Among the 86 harvested rows, measured FW ranged from 57 to 849 g/row; and DMY ranged from 19 to

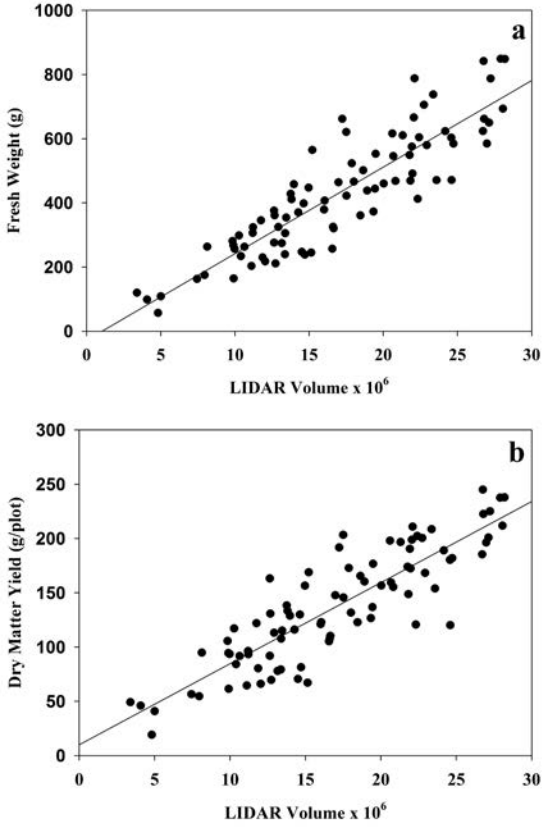

Figure 2 Correlation between LIDAR Volume and (a) fresh weight and (b) dry matter yield across 86 plots in a single-row perennial ryegrass evaluation trial. Correlations are significant (P<0.05), R2 values are 0.89 and 0.87, respectively.

245 g/row. Measured LVs ranged from 8.39 x 10^6 to 28.19 x 10^6. The R^2 between LV and FW was 0.89; which decreased slightly to 0.87 between LV and DMY (Figure 2). FW and DMY were correlated at $R^2 = 0.97$ (data not shown).

Discussion

Based on initial evaluation of GS and LIDAR technologies, there may be potential to address two major bottlenecks in forage breeding: acquisition of DMY trait data for selection candidates in breeding populations, and prediction of the breeding value of selection candidates. Individually, or in tandem, these may enable breeding strategies that substantially increase the rate and predictability of genetic gain for DMY in perennial ryegrass.

Heading Date is not regarded as a high priority trait for GS in itself, as it has high heritability and improvement by conventional phenotypic selection is effective. However, the use of HD to evaluate GS has allowed an early assessment of potential of GEBVs to predict breeding value in complex populations. A second year of data from this experiment will be acquired to validate this; and assessment of GS for a more complex trait, DMY, is currently in progress. The initial finding supporting the ability to predict HD within the same generation, and after multiple cycles of selection, is reassuring. The predictive ability of the model used in the complex population in this study (0.52) compares favourably with those reported in the literature (Byrne *et al.* 2017; Fè *et al.* 2015) of 0.73 to 0.90, when taking into account that those studies were based principally on prediction within genetically simpler F_2 families and synthetic cultivars, with large training population sizes (445 - 1757 individuals). There are no published cases where efficacy of GS for HD or any trait in ryegrass is evaluated empirically to compare the realised response to selection; the current study represents the first public presentation of such data.

The accuracy demonstrated with the LIDAR system, when fully validated, will offer breeders and agronomists a major advance in using sensors to non-

Table 1 Heading date BLUP-adjusted means (n = 25; SE in brackets) for plants selected using genomic selection (high GEBV, low GEBV) and non-selected controls in five perennial ryegrass breeding populations. BLUP's in the same row with different superscript letters are significantly different (P<0.05). NS = not significant (P>0.05). $LSD_{5\%}$ = least significant difference.

	Selection group mean heading date (days)			
Population	High GEBV	Non-selected	Low GEBV	Difference (High-Low GEBV)
Pop Ia	26.5^a (2.36)	20.3^b (2.34)	19.3^b (2.34)	+ 7.2 days (37%)
Pop Ib	26.7^{ab} (2.36)	28.6^a (2.34)	24.2^b (2.35)	+ 2.5 days (10%) NS
Pop Ic	24.0^a (2.35)	20.2^b (2.34)	17.0^c (2.35)	+ 7.0 days (41%)
Pop IIIa	29.5^a (2.34)	29.2^a (2.34)	23.7^b (2.33)	+ 5.8 days (24%)
Pop IIIb	35.7^a (2.37)	34.5^{ab} (2.38)	32.2^b (2.38)	+ 3.5 days (11%)
$LSD_{0.05}$	3.10			

destructively measure yield in the field, in real time. Findings of $R^2 = 0.87$ for DMY compare favourably with other reports in cool season grasses (Andersson *et al*. 2017; Ghamkhar *et al.* 2018; Schaefer & Lamb 2016) where accuracies range from $R^2 = 0.56$ to 0.78, and approach the $R^2 = 0.93$ recently reported in wheat (*Triticum aestivum*) (Jimenez-Berni *et al.* 2018).

These findings will help address three fundamental limitations in current forage breeding practice: a) breeding value of selection candidates is rarely evaluated in a systematic way, b) when it is evaluated, the number of candidates is limited, and c) the quantity and consistency of DMY data are limited.

However, these emerging technologies are in the early stages of development and validation. For LIDAR, there is extensive testing to be completed across a range of environmental and genetic variation, and scaling the technology to enable measurement in larger plots. There is also the need to address the challenge of estimating DMY in a way that is highly correlated with on farm experience including grazing and mixed swards, while being relatable back to individual selection candidates.

There are also other phenotypic traits of value that are difficult or expensive to measure, which precludes their systematic evaluation in breeding programmes: forage quality, stress response, nutrient use efficiency, water use efficiency and methanogenic potential are all examples of valuable traits for which sensors may be developed and deployed.

For GS, this is the first validation in New Zealand forage breeding of an approach that is well established for animal and plant breeding in a number of species. The critical questions for further development of GS relate to scalability, optimisation and efficiency. The effectiveness of GS in complex traits, in contrast to the genetically simpler HD trait tested here, is unknown. To address this, GS for DMY, analogous to the HD experiments described, is currently in progress and will begin to generate data from divergent selections during 2018.

The effectiveness of genomic prediction models across multiple cycles of selection in cool season grasses is also largely unknown. Data produced in the current study indicate that efficacy of genomic prediction models, developed at the modest scale utilised to date (Annicchiarico *et al.* 2015; Faville *et al.* 2018; Grinberg *et al.* 2016) diminishes in populations that are 2-3 generations removed from the generation used to train the model. Establishing limits and optima for training population size, re-training frequency and data quality are therefore essential. There is also a need to investigate the potential of GS to address multiple traits in index selection.

The development of simulation tools such as DeltaGen and QuLinePlus (Hoyos-Villegas *et al*. 2018; Jahufer & Lou 2018) allow exploration of many of these questions and will act as a guide toward optimal approaches for use of GS and LIDAR. However, a substantial need for empirical evaluation and implementation will remain. Regardless of the scope and efficiency of technology developed for breeding; they are no substitute for access to genetic diversity, the other major driver of efficiency in breeding. To that end, the use of germplasm collections and pre-breeding in national programmes to introgress new traits is valuable, as is access to novel traits via wide hybridisation (Hussain *et al.* 2016) or biotechnology (Winichayakul *et al.* 2013).

Conclusion

Effective technological solutions to substantial constraints to breeding for dry matter yield in perennial ryegrass may soon be available and validated. Based on initial findings, these technologies need and warrant continued evaluation and development. Extracting value from these developments will rely on a coherent strategy to deliver benefits on farm, germplasm offering genetic diversity for key traits, and use of pre-breeding strategies to bring relevant germplasm into elite breeding programmes.

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