Empirical assessment of a genomic breeding strategy in perennial ryegrass

Marty J. FAVILLE1*, Jana SCHMIDTI, Michael TROLOVE2, Peter MORAN1, Won HONG1, Mingshu CAO1, Siva GANESH1, Richard GEORGE3 and Brent BARRETT1
1 AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand
2 AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
3 PGG Wrightson Seeds Ltd., Ruakura Research Centre, Private Bag 3214, Hamilton, New Zealand
*Corresponding author: marty.faville@agresearch.co.nz

Abstract
In genomic selection (GS) DNA markers and trait data are integrated in a model that then predicts genomic-estimated breeding values (GEBV’s) for individuals from marker information alone, improving breeding efficiency. This study assessed a genomic breeding strategy (AWF GS) for improving dry matter yield (DMY) in perennial ryegrass. In AWF GS, the best-performing half-sibling families (HS) are identified using phenotypic data and GS is used to select the best individuals within HS. Four selections were made from three breeding populations: Base (random sample of plants from all HS), HS p (random sample from the six phenotypically best HS), AWF GS and AWF GS-L (top or bottom 5% of plants, respectively, selected by GEBV from the six HS). Plants from within the selections were polycrossed, creating 12 experimental synthetics that were evaluated for DMY (n=7 harvests) at two locations over 18 months. In each population, mean DMY across locations and harvests showed a trend of AWF GS > HS p > Base, with AWF GS-L closest to Base performance. When averaged across populations, AWF GS increased DMY by 43% (P<0.05) compared to Base, more than twice the level of improvement achieved with HS p. These results showed that AWF GS can substantially improve selection response for a genetically complex trait from a single breeding cycle.

Keywords: among-and-within-family selection, breeding, dry matter yield, Lolium, genomic selection, genotyping-by-sequencing

Introduction
Perennial ryegrass (Lolium perenne L.) is the most important source of nutrition for ruminant livestock grown on New Zealand farms and contributes nearly $14.6B to annual GDP (NZIER 2016). Major goals for genetic improvement in perennial ryegrass are annual and seasonal dry matter yield (DMY) (Williams et al., 2007; Lee et al., 2012), with persistence, nutritive quality, disease and pest resistance and symbiont compatibility also targeted. Significant improvements in DMY have been achieved through plant breeding, but the rate of genetic gain (∆G) for this genetically complex trait has been moderate, estimated at only 0.3 – 0.7% per annum (Van Wijk and Reheul 1990; Easton et al., 2002; Sampoux et al., 2010). Genomic selection (GS), which is already implemented in livestock species (Meuwissen et al., 2016) and is under adoption in major economic plant species (Crossa et al., 2014; Lin et al., 2014), is a promising approach for improving ∆G for complex traits, such as DMY, in forages (Hayes et al., 2013).

To undertake GS a set of individuals or families, referred to as a training set, is genotyped using 10’s to 100’s of thousands of genome-wide DNA markers (single nucleotide polymorphisms, SNP) and phenotyped for the trait of interest. The genotypic and phenotypic data are combined to derive a statistical model, referred to as a genomic prediction model. The genomic prediction model is subsequently used to predict the trait or genomic-estimated breeding value (GEBV) for new individuals, by acquiring and entering their SNP genotype information into the model. Superior plants can therefore be selected based on GEBVs without recourse to expensive or long-term phenotyping, and then recombined by crossing to create the next breeding generation or a new cultivar.

The establishment in the last decade of cost-effective, universally available SNP genotyping methods, such as genotyping-by-sequencing (GBS) (Elshire et al., 2011), has provided a platform for GS to be developed in perennial ryegrass and other ‘orphan’ species that lack significant genomic resources. In perennial ryegrass, genomic prediction models have been developed for DMY (Fé et al., 2016; Grinberg et al., 2016; Faville et al., 2018; Pembleton et al., 2018), heading date (Fé et al., 2015; Byrne et al., 2017; Faville et al., 2018), various nutritive quality traits (Grinberg et al., 2016; Aroju et al., 2020), disease resistance (Aroju et al., 2018) and host plant compatibility with Epichloë endophyte (Gagic et al., 2018).

A challenge, once genomic prediction models are developed, is their practical implementation in GS breeding schemes. Faville et al. (2020) demonstrated that application of genomic prediction models for DMY...
or heading date in perennial ryegrass could improve these traits. However, the efficacy of GS generally diminished when applied into selection populations that were less related to the original training set. This suggested that the use of GS to reduce generation interval (speeding up breeding and improving ∆G by completing more selection cycles per unit time) is likely to have diminishing returns as the level of genetic relatedness between the training set and the selection population is expected to decline over multiple selection cycles. Based on the relatively small training sets currently available in forages (n = 200–1000), computer simulation estimated a 60–72% reduction in predictive ability for a perennial ryegrass DMY genomic prediction model between a first and second cycles of selection (Jahufer et al., 2021).

Within-cycle applications of GS, which aim to improve ∆G by increasing selection accuracy from a single breeding cycle are, therefore, appealing. Modelling predicts a substantial increase in ∆G for DMY by applying a within-cycle strategy called among (by phenotype) and-within (by genomic selection) half-sibling family selection (A<sub>PWF<sub>GS</sub></sub>) (Faville et al., 2018; Barrett et al., 2021). Here, the breeder uses DMY phenotypic data from sown plot trials to identify the best-performing half-sibling families (HS) (among-family selection) and then GS is applied to select the best individuals from within those top HS (within-family selection; Figure 1). The latter component is achieved by growing seedlings from saved seed of the HS, then genotyping and generating GEBV’s for the seedlings. In Figure 1 following the first breeding cycle there are options to implement a second cycle of GS, or to advance selected individuals to cultivar development, or to return to commence a new cycle. A second GS cycle would be based on application of the same GS model, without further training from field data (Jahufer et al., 2021), enabling selection of another generation of elite parental seedlings based on GEBV’s in the space of one year. Integration of additional traits for GS is also possible, by phenotyping the traits either on HS family plots or directly on the parental generation, as single plants.

The objective of the following work was to assess the potential of the A<sub>PWF<sub>GS</sub></sub> strategy for improving DMY in perennial ryegrass, by applying existing DMY genomic prediction models (Faville et al., 2018) in commercial selection populations and comparing progeny against those from a conventional breeding approach, phenotypic half-sibling family selection (H<sub>S</sub>)<sub>P</sub>). The H<sub>S</sub><sub>P</sub> system differed from A<sub>PWF<sub>GS</sub></sub> only in that, instead of selecting individuals from within the

![Figure 1](image-url)
best-performing HS by GEBV, a random sample of individuals was taken.

Materials and Methods
Plant material
Selection was undertaken in three Grasslands Innovation Ltd perennial ryegrass selection populations (SelPop I, SelPop III and SelPop V), which consisted of 96, 115 and 106 HS, respectively, that had been evaluated previously for DMY in multi-year, multi-environment plot trials (Faville et al., 2018). These HS were the progeny from the parental generation that had been used to train the DMY GS models in that study. The six highest ranking HS were identified in each population based on their DMY phenotypic values. DMY phenotypic values were an index of mean DMY calculated across seasons, years and locations, as detailed in Faville et al. (2020). Forty seeds (selection candidates) were randomly sampled from stored seed of each of the six selected HS, then germinated and grown under standard greenhouse conditions for 4 weeks, until two tillers had emerged.

Genotyping-by-sequencing and genomic selection
Approximately 100 mg of leaf tissue per seedling was sampled and DNA extraction completed using the method of Anderson et al. (2018). DNA samples were used to develop GBS libraries for the selection candidates. For methodological details of GBS library development, sequencing of GBS libraries, bioinformatic data processing, SNP genotype calling and genomic relationship matrix (GRM) development, see Faville et al. (2018). GBS data generated for selection candidates were merged with data from the original GS training set (Faville et al., 2018), SNP genotypes were determined, and these were used to estimate the genomic relationship matrix (GRM), consisting of selection candidates + training set individuals (Dodds et al., 2015). DMY GEBV’s for each of the 720 selection candidates were then derived by Genomic Best Linear Unbiased Prediction (GBLUP), as described in Faville et al. (2020). Briefly, five environment-specific DMY GEBV’s for each selection population individual were estimated using the genomic prediction models developed previously by Faville et al. (2018): Waikato standard management; Waikato severe summer grazing management; Manawatu standard management; Manawatu severe summer grazing management and Canterbury standard management (Table 1). Each environment-specific prediction model was trained using a multi-population training set composed of small numbers of HS from five breeding populations, including SelPop I, SelPop III and SelPop V (Faville et al., 2018).

For each selection candidate genomic estimated breeding values (GEBV’s) were generated for each of the five models. The GEBV values for an individual were then entered into a weighted index to generate a single GEBV value (Faville et al., 2020), on which selections were based.

Combination of the environment-specific GEBV’s into a weighted selection index (Faville et al., 2020) generated a single DMY GEBV for each individual, providing a measure of across-environment performance. For each selection population, an additional 30 seeds were randomly sampled from across all HS and propagated as described above – these plants were not genotyped. Four selections were

---

**Table 1** Predictive ability ($r^A$, the Pearson correlation coefficient between predicted and observed trait values) and narrow sense heritability ($h^2_n$), for five environment-specific dry matter yield genomic prediction models (Faville et al., 2018) in each of the three selection populations (SelPop I, III and V).

| Genomic prediction model | Wai STD | Wai SEV | Man STD | Man SEV | Can STD | Mean |
|--------------------------|--------|--------|---------|---------|---------|------|
| Selection index weighting | 0.30   | 0.30   | 0.10    | 0.20    | 0.10    | -    |

| Predictive ability ($r^A$) | SelPop I | SelPop III | SelPop V |
|-----------------------------|-----------|------------|----------|
|                             | 0.16      | 0.17       | 0.21     |
|                             | 0.25      | 0.21       | 0.31     |
|                             | 0.11      | 0.13       | 0.21     |
|                             | 0.24      | 0.19       | 0.23     |
|                             | 0.08      | 0.06       | 0.05     |
|                             | 0.14      | 0.13       | 0.16     |

| $h^2_n$ | SelPop I | SelPop III | SelPop V |
|---------|----------|------------|----------|
|        | 0.65     | 0.60       | 0.73     |
|        | 0.84     | 0.80       | 0.76     |
|        | 0.55     | 0.42       | 0.34     |
|        | 0.85     | 0.80       | 0.76     |
|        | 0.52     | 0.59       | 0.60     |
|        | 0.68     | 0.64       | 0.64     |

Wai = Waikato, Man = Manawatu, Can = Canterbury; STD = standard grazing; SEV = severe summer grazing.
made for each of SelPop I, III and V, resulting in 12 selection groups (SG):

1) Base: random sample of 30 plants across all HS in the population
2) HS<sub>p</sub>: random selection of two plants from within each of the phenotypically top-ranked six HS (n = 12 plants total)
3) A<sub>WF</sub><sup>GS</sup>: selection of two plants with highest GEBV from within each of the phenotypically top-ranked six HS (n = 12 plants total)
4) A<sub>WF</sub><sup>GS</sup>-L: selection of two plants with lowest GEBV from within each of the phenotypically top-ranked six HS (n = 12 plants total)

Population development
Individuals selected within each of the 12 SG’s were grown to maturity and polycrossed under isolation (no mixing between SGs) during spring 2017 at AgResearch Grasslands, Palmerston North. Syn-1 generation seed was harvested from individual plants within a SG polycross and a balanced bulk for the SG was created by combining equal seed quantities from each plant. This resulted in 12 Syn-1 experimental synthetic populations, one for each of the SG’s.

Field evaluation of SG synthetic populations
Field trials were conducted at AgResearch Ruakura in Waikato (37.78°S, 175.32°E) and AgResearch Grasslands in Manawatu (40.21°S, 175.37°E) from May 2018. Each of the SG synthetics, along with two control cultivars, were direct-drilled as 2 m rows (0.6 g seed per row) with 30 cm spacing between rows and 40 cm gaps at the ends of the rows, in a row-column design with three replicates. Soil fertility levels were adjusted to ensure nutrients did not limit plant growth. Nitrogen was applied (15-30 kg N/ha) at each defoliation. Superphosphate fertiliser (8.8 kg P/ha) was applied in late autumn each year. Trials were defoliated by sheep grazing whenever they reached the two to three leaf stage of development, except when DMY harvests were taken, in which case the plants were defoliated manually. Between November 2018 and May 2020, seven seasonal DMY harvests were completed at each site by manual cutting, drying and weighing herbage to determine grams of DMY per 2 m row, as described by (Faville et al., 2018).

Statistical analysis
Mean DMY for each SG across all harvests and locations was determined. Data were analysed by a linear mixed model, using the variance component analysis procedure residual maximum likelihood (REML) option in DeltaGen software v0.03 (Jahufer and Luo 2018). Repeated checks, location, harvest date, and SG were treated as fixed effects; replicates, rows, and columns were treated as random effects; and the model included SG-by-year, SG-by-location and SG-by-season interaction effects. The final DMY values for each SG synthetic were generated as best linear unbiased estimators (BLUEs).

Results and Discussion
This study is the first empirical assessment of a genomics-driven breeding strategy in perennial ryegrass, namely an among-and-within-HS selection method (A<sub>WF</sub><sup>GS</sup>), that leveraged phenotypic data for the among-family selection component and GS for and the within-family selection component. In forage breeding, among- and within-family selection methods enable utilisation of 100% of the additive (heritable) genetic variation within a population (Casler and Brummer 2008) but historically this has been difficult to implement, particularly for traits such as DMY. Commonly applied HS breeding approaches, such as HS<sub>p</sub>, use only the 25% of additive genetic variation that occurs amongst HS (Falconer 1989), usually based on phenotypic assessment of HS as sown plots in multiple locations over several years. The remaining 75% of the total additive genetic variation in a population occurs within the HS, but this is generally inaccessible to breeders for sward traits such as DMY. This is because within-family selection typically relies on measuring traits in single plants randomly sampled from within the HS. However, there is negligible correlation between single plant and sward DMY (Lazenby and Rogers 1964; Hayward and Vivero 1984) and so single plant DMY cannot be used to reliably select for sward DMY within families. Genomic selection makes the application of meaningful within-HS selection pressure for sward DMY possible on single plants, by using genomic prediction models trained using sown row or plot DMY data. Esfandyari et al. (2020) showed that the ability to accurately select single plants for sward traits, by using sward trait GEBV’s, substantially increased ∆G.

Selection
With reference to Figure 1, the first steps of the breeding scheme (HS family generation, phenotypic evaluation of HS, genotyping parents and GS model training) were achieved previously as described in Faville et al. (2018), enabling selection of the six top-ranked HS from SelPop I, III and V. Random selection of two individuals per HS followed and these were used to generate HS<sub>p</sub> GS synthetics for each selection population at among-family selection pressures of 6%, 5% and 5% for SelPop, III and V, respectively.

Genotypes were successfully called at 777k SNP loci for 701 individuals sampled from the selected HS (235 individuals from each of SelPop III and V and
231 from SelPop I) plus 566 training set individuals. This allowed generation of a DMY GEBV for mean performance across environments for the selection population individuals. Ranking of the plants by DMY GEBV enabled selection of the two top-ranked and two bottom-ranked individuals within HS samples from SelPop I, III and V. This provided within-family selection pressure of 5% in each selection population. The selected individuals were used to generate A_PWF_GS and A_PWF_GS-L SG synthetics, respectively, for each selection population. Overall, for these SGs the among and within-family selection pressures were 5-6% and 5%, respectively.

Field evaluation of SG synthetics
Evaluation of the 12 SG synthetics at two locations over 18 months generated DMY data from seven seasonal harvests at Manawatu and Waikato. The expectation from simulation studies (Barrett et al., 2021; Jahufer et al., 2021) was that the GS breeding system, A_PWF_GS, should deliver improved DMY outcomes compared with the conventional phenotypic selection approach, HS_p. That expectation was endorsed by the selection responses observed in the current study.

The magnitude of selection response differed by population, but in each of SelPopI, SelPop III and SelPop V DMY across locations and harvests showed a consistent trend for A_PWF_GS > HS_p > Base (Table 2), where the latter represented the source population. Greater selection responses in SelPopI and SelPopV may have been influenced by higher predictive abilities (r^2) for some of the DMY prediction models used (Table 1), particularly Wai SEV and Man SEV, but because the models were used as part of an index, it was not possible to assess that definitely. When averaged across all selection populations (All SelPop), applying HS_p improved DMY by 18% compared to the Base control (Table 2, Figure 2), although this difference was not significant. In contrast, A_PWF_GS increased DMY by 43% (P<0.05) from the Base population, more than double the level of improvement achieved using the conventional method.

These relative differences corresponded closely to those from a simulation study reported by Barrett et al. (2021), which showed that application of 5% among- and 5% within-HS selection pressure in A_PWF_GS doubled ∆G relative to HS_p in a perennial ryegrass selection population. Similarly, modelling by Esfandyari et al. (2020) showed that ∆G increased in GS schemes because it enabled more accurate selection of single plants for sward traits by using GEBV’s.

Table 2  Mean dry matter yield (DMY) for selection group (SG) Syn-1 synthetics from three individual selection populations (SelPopI, II, V) and averaged across all three populations (All SelPop).

| Measure   | SG         | SelPop I   | SelPop III  | SelPop V    | All SelPop |
|-----------|------------|------------|-------------|-------------|------------|
| Mean DMY  | Base       | 41.6 (7.45)^a  | 63.2 (7.42)^ab | 43.4 (6.67)^a | 49.8 (5.37)^a |
| g row⁻¹ (± SE) | HS_p      | 54.2 (7.42)^ab | 66.8 (7.42)^ab | 51.6 (7.39)^ab | 58.7 (5.37)^a  |
|           | A_PWF_GS   | 69.8 (7.45)^b  | 75.8 (7.45)^ab | 65.1 (7.45)^b  | 71.0 (5.38)^b  |
|           | A_PWF_GS-L | 52.8 (7.45)^ab | 55.6 (7.42)^b  | 41.2 (7.46)^a  | 51.0 (5.38)^a  |
| LSD_0.05  |            | 19.8        | 19.8         | 19.8         | 12.3        |
| Response relative | HS_p     | +30         | +6          | +19         | +18         |
| to Base (%) | A_PWF_GS  | +68         | +13         | +50         | +43         |

Data are BLUES (±SE) for DMY across multiple seasonal harvests (n=7) and two evaluation environments. DMY with different letters within a column (SelPop) are significantly different (P<0.05), supported by least significant difference (LSD_0.05). A_PWF_GS = among_p and-within half-sibling family_{gs} selection, (P = phenotypic, GS = genomic selection) selecting for high DMY; A_PWF_GS-L = among_p and-within half-sibling family_{gs} selection, selecting for low DMY; HS_p = half-sibling family selection; Base = source population.
The impact of $A_pWF_{GS}$ was exemplified further by comparison with a divergent selection, $A_pWF_{GS-L}$, which was based on selection of the two lowest-ranking individuals per HS as opposed to the two best plants. In all selection populations, except SelPopI, DMY performance of the $A_pWF_{GS-L}$ SG was significantly ($P<0.05$) lower than $A_pWF_{GS}$ (Table 2). When averaged across all selection populations (All SelPop), there was a 39% DMY differential between these SG (Table 2, Figure 2) and, in most cases, the DMY of $j_PWF_{GS-L}$ was close to that of the unimproved Base population. This result indicated that a high level of additive genetic variation for DMY existed within HS and illustrated the positive impact of being able to identify and eliminate poor candidates, and, hence, undesirable alleles, from the breeding programme.

Due to external factors, the field evaluation period was shorter than the three years typically employed for DMY assessment of perennial ryegrass (Easton et al., 2001). However, previous studies based on plots showed that ranking of trial entries was reliably consistent when comparing first year performance and DMY in later years (Easton et al., 2001; Chapman et al., 2015). Stability of diploid synthetics, according to the Hardy-Weinberg rule, is achieved from the Syn-2 generation and generations thereafter, provided mating is completely at random and there is no selection pressure (Allard 1960). The SG synthetics evaluated in the current study were a Syn-1 generation, therefore it was possible that the results were influenced by non-additive genetic effects, notably heterosis. This was mitigated by ensuring that selection sizes within HS were balanced, using the same number of Syn-0 individuals ($n = 12$) from each HS for each of the $A_pWF_{GS}$, $HS_p$ and $A_pWF_{GS-L}$ SG’s. However, the Base selections used 30 randomly-sampled Syn-0 parents – which was to enable capture of the overall within-population genetic diversity of the population (Kubik et al., 2001). In perennial ryegrass, a slight decrease in yield from Syn-1 to Syn-2 is often observed and the extent of decline depends on the number of parents in the Syn-0 and the inbreeding co-efficient of these parents (Wright 1922; Allard 1960; Becker 1988). Due to the discrepancies in Syn-0 parent numbers, a smaller decline in performance can be expected in the Syn-1 Base population relative to the nine other Syn-1 SG’s, when moving to Syn-2. However, given i), the number of parents in the nine selected Syn-0 SG populations were still quite high, ii), the Syn-0 parents were unlikely to be inbred, and iii), the proportion of non-additive genetic variance to additive genetic in ryegrass is thought to be minor (Breese and Hayward 1972), any upwards bias in performance of the Syn-1 SG populations was, most likely, negligible. Future development and assessment of Syn-2 generations from the 12 SGs, over a longer evaluation period, is required to validate the current results and enable fair comparison against industry cultivars.

The five genomic prediction models used in this study had low to moderate predictive ability, $r_A$ (Faville et al., 2018; 2020). Additional improvement in selection response from the $A_pWF_{GS}$ breeding strategy may be expected by increasing the $r_A$ of the genomic prediction models used. For example, modelling by Faville et al. (2018) estimated a 50% increase in $\Delta G$ for DMY by improving $r_A$ from 0.27 to 0.50 at a fixed selection pressure. Utilising larger training sets to develop the prediction models is one way to achieve higher $r_A$ and Esfandyari et al. (2020) demonstrated that, in a long term breeding programme, $r_A$ could be increased by enlarging the training set through recruitment of data from multiple breeding cycles started in consecutive years. That approach is compatible with the breeding system described here. Greater gains would also be expected through the application of higher selection pressures than those tested in the current study (Jahufer et al., 2021).

**Conclusions**

The results provided empirical evidence that a GS breeding approach ($A_pWF_{GS}$), when applied as an adjunct to a conventional breeding strategy ($HS_p$), considerably improved selection response for a genetically complex trait from a single breeding cycle. This was achieved not by reducing generation interval, but rather by improving selection accuracy and more accurately exploiting within-family additive genetic variation. It can be recommended that the seed industry adopt genomic breeding, supported by continued research to improve efficiency and accuracy, as well as on-farm evaluation to monitor real-world impact.

**ACKNOWLEDGEMENTS**

The research was funded by Pastoral Genomics Plus (grant number PSTG1501), a joint venture co-funded by Dairy New Zealand, Beef + Lamb New Zealand, Dairy Australia, AgResearch Ltd., Barenbrug Agriseeds Ltd. Grasslands Innovation Ltd. and the Ministry of Business, Innovation and Employment, New Zealand. The authors are grateful to Alan Stewart and Derek Woodfield (Grasslands Innovation Ltd.) for providing access to the ryegrass populations used in this study. The authors would like to thank Prue Taylor, Anna Larking, Jessica O’Connor, Bridget Wise, Derrick Wilson, Deborah Hackell, Martin Kear, Stuart Lindsey, Jan Sprosen, Trevor Watson and others for their assistance in field trial harvests and sample processing.
REFERENCES

Allard RW. 1960. Principles of Plant Breeding: John Wiley & Sons. https://doi.org/10.2134/aronj1962.0021962005400040037x

Anderson CB, Franzmayr BK, Hong SW, Larking AC, van Stijn TC, Tan R, Moraga R, Faville MJ, Griffiths AG. 2018. Protocol: a versatile, inexpensive, high-throughput plant genomic DNA extraction method suitable for genotyping-by-sequencing. Plant Methods 14: 75. https://doi.org/10.1186/s13007-018-0336-1

Arojju SK, Conaghan P, Barth S, Milbourne D, Casler MD, Hodkinson TR, Michel T, Byrne SL. 2018. Genetic prediction of crown rust resistance in Lolium perenne. BMC Genetics 19: 35. https://doi.org/10.1186/s12863-018-0613-z

Arojju SK, Cao M, Zulfi Jahufer MZ, Barrett BA, Faville MJ. 2020. Genomic predictive ability for foliar nutritive traits in perennial ryegrass. G3 10: 695-708. https://doi.org/10.1534/g3.119.400880

Barrett BA, Jahufer MZZ, Sise J, Faville MJ. 2021. The potential of genomic breeding for improved pastures. Journal of New Zealand Grasslands 83. https://www.uni-goettingen.de/de/document/download/68ce420eaf1dfbf598df74bd9b8159101.pdf/1988 Becker PBBReview Synthetics.pdf

Becker HC. 1988. Breeding synthetic varieties of crop plants. Plant Genetics and Breeding Review 1: 31-54. https://www.uni-goettingen.de/de/document/download/68ce420eaf1dfbf598df74bd9b8159101.pdf/1988 Becker PBBReview Synthetics.pdf

Breese EL, Hayward MD. 1972. The genetic basis of present breeding methods in forage crops. Euphytica 21: 326-336. https://doi.org/10.1007/BF00036773

Byrne SL, Conaghan P, Barth S, Arojju SK, Casler M, Michel T, Velmurgan J, Milbourne D. 2017. Using variable importance measures to identify a small set of SNPs to predict heading date in perennial ryegrass. Scientific Reports 7: 3566. https://doi.org/10.1038/s41598-017-03232-8

Casler MD, Brummer EC. 2008. Theoretical expected genetic gains for among-and-within-family selection models in perennial forage crops. Crop Science 48: 890-902. https://doi.org/10.2135/cropsci2007.09.0499

Chapman DF, Muir PD, Faville MJ. 2015. Persistence of dry matter yield among New Zealand perennial ryegrass (Lolium perenne L.) cultivars: insights from a long-term data set. Journal of New Zealand Grasslands 77: 177-184. https://doi.org/10.33584/jnzg.2015.77.463

Crossa J, Pérez P, Hickey J, Burgueño J, Ornella L, Cerón-Rojas J, Zhang X, Dreisigacker S, Babu R, Li Y, Bonnett D, Mathews K. 2014. Genomic prediction in CIMMYT maize and wheat breeding programs. Heredity 112: 48-60. https://doi.org/10.1038/hdy.2013.16

Dodds KG, McEwan JC, Brauning R, Anderson RM, van Stijn TC, Kristjánsson T, Clarke SM. 2015. Construction of relatedness matrices using genotyping-by-sequencing data. BMC Genomics 16: 1047. https://doi.org/10.1186/s12864-015-2252-3

Easton HS, Baird DB, Cameron NE, Kerr GA, Norriss M, Stewart AV. 2001. Perennial ryegrass cultivars: herbage yield in multi-site plot trials. Proceedings of the New Zealand Grassland Association 63: 183-188. https://doi.org/10.33584/jnzg.2001.63.2408

Easton HS, Amyes JM, Cameron NE, Green RB, Kerr GA, Norriss MG, Stewart AV. 2002. Pasture plant breeding in New Zealand: where to from here? Proceedings of the New Zealand Grassland Association 64: 173-179. https://doi.org/10.33584/jnzg.2002.64.2455

Elshrie RJ, Glauбитz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. 2011. A robust, simple Genotyping-by-Sequencing (GBS) approach for high diversity species. PLoS ONE 6: e19379. https://doi.org/10.1371/journal.pone.0019379

Falconer DS. 1989. Introduction to quantitative genetics. New York: Longman Scientific and Technical. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1471025/pdf/15342495.pdf

Faville MJ, Ganesh S, Cao M, Jahufer MZZ, Bilton TP, Easton HS, Ryan DL, Trehewey JAK, Rolston MP, Griffiths AG, Moraga R, Flay C, Schmidt J, Tan R, Barrett BA. 2018. Predictive ability of genomic selection models in a multi-population perennial ryegrass training set using genotyping-by-sequencing. Theoretical and Applied Genetics 131: 703-720. https://doi.org/10.1007/s00122-017-3030-1

Faville MJ, Cao M, Schmidt J, Ryan DL, Ganesh S, Jahufer MZZ, Hong SW, George R, Barrett BA. 2020. Divergent genomic selection for herbage accumulation and days-to-heading in perennial ryegrass. Agronomy 10: 340. https://doi.org/10.3390/agronomy10030340

Fè D, Cericola F, Byrne S, Lenk I, Ashraf BH, Pedersen MG, Roulund N, Asp T, Janss L, Jensen J. 2015. Genomic dissection and prediction of heading date in perennial ryegrass. BMC Genomics 16: 1-15. https://doi.org/10.1186/s12864-015-2163-3

Fè D, Ashraf BH, Pedersen MG, Janss L, Byrne S, Roulund N, Lenk I, Didion T, Asp T, Jensen CS, Jensen J. 2016. Accuracy of genomic prediction in a commercial perennial ryegrass breeding
program. *Plant Genome* 9. https://doi.org/10.3835/plantgenome2015.11.0110

Gagic M, Faville MJ, Zhang W, Forrester NT, Rolston MP, Johnson RD, Ganesh S, Koolaaer JP, Easton HS, Hudson D, Johnson LJ, Moon CD, Voisey CR. 2018. Seed transmission of *Epichloë* endophytes in *Lolium perenne* is heavily influenced by host genetics. *Frontiers in Plant Science* 9. https://doi.org/10.3389/fpls.2018.01580

Grinberg NF, Lovatt A, Hegarty M, Lovatt A, Skot KP, Kelly R, Blackmore T, Thorogood D, King RD, Armstead I, Powell W, Skot L. 2016. Implementation of genomic prediction in *Lolium perenne* (L.) breeding populations. *Frontiers in Plant Science* 7: 133. https://doi.org/10.3389/fpls.2016.00133

Hayes BJ, Cogan NOI, Pemberton LW, Goddard ME, Wang J, Spangenberg GC, Forster JW. 2013. Prospects for genomic selection in forage plant species. *Plant Breeding* 132: 133-143. https://doi.org/10.1111/pbr.12037

Hayward MD, Vivero JL. 1984. Selection for yield in *Lolium perenne*. II. Performance of spaced plant selections under competitive conditions. *Euphytica* 33: 787-800. https://doi.org/10.1007/BF00021905

Hayward MD, Abdullah IB. 1985. Selection and stability of synthetic varieties of *Lolium perenne*. *Theoretical and Applied Genetics* 70: 48-51. https://doi.org/10.1007/BF00264481

Jahufer MZZ, Luo D. 2018. DeltaGen: A comprehensive decision support tool for plant breeders. *Crop Science* 58: 1118-1131. https://doi.org/10.10135/cropsci2017.07.0456

Jahufer MZZ, Arojju SK, Faville MJ, Ghamkhar K, Luo D, Arief V, Yang W-H, Sun M, DeLacy IH, Griffiths AG, Eady C, Clayton W, Stewart AV, George RM, Hoyos-Villegas V, Barrett BA. 2021. Modelling the impact of genomic and phenomic selection in forage breeding to improve the rate of genetic gain for dry matter yield. *Scientific Reports* 11: 13265. https://doi.org/10.1038/s41598-021-92537-w

Kubik C, Sawkins M, Meyer WA, Gaut BS. 2001. Genetic diversity in seven perennial ryegrass (*Lolium perenne* L.) cultivars based on SSR markers. *Crop Science* 41: 1565-1572. https://doi.org/10.2135/cropsci2001.4151565x

Lazenby A, Rogers HH. 1964. Selection criteria in grass breeding: II. Effect, on *Lolium perenne*, of differences in population density, variety, and available moisture. *Journal of Agricultural Science* 62: 285–298. https://doi.org/10.2135/S0021859600060937

Lee JM, Matthew C, Thom ER, Chapman DF. 2012. Perennial ryegrass breeding in New Zealand: a dairy industry perspective. *Crop and Pasture Science* 63: 107-127. https://doi.org/10.1071/CP11282

Lin Z, Hayes BJ, Daetwyler HD. 2014. Genomic selection in crops, trees and forages: a review. *Crop and Pasture Science* 65: 1177-1191. https://doi.org/10.1071/CP13363

Meuwissen T, Hayes B, Goddard M. 2016. Genomic selection: A paradigm shift in animal breeding. *Animal Frontiers* 6: 6-14. https://doi.org/10.2527/af.2016-0002

NZIER. 2016. How valuable is that plant species? Application of a method for enumerating the contribution of selected plant species to New Zealand’s GDP., 212 p. https://www.mpi.govt.nz/dmsdocument/14527/direct

Pemberton LW, Inch C, Baillie RC, Drayton MC, Thakur P, Ogaji YO, Spangenberg GC, Forster JW, Daetwyler HD, Cogan NOI. 2018. Exploitation of data from breeding programs supports rapid implementation of genomic selection for key agronomic traits in perennial ryegrass. *Theoretical and Applied Genetics*. https://doi.org/10.1007/s00122-018-3121-7

Reheul D, Baert J, Ghesquiere A, Waters B, Humphreys M, Van Wijk AJP, Scheller H, Röbl L. 2003. Progress in breeding perennial fodder grasses 2. Differences between SYN1 and SYN2 varieties of *Lolium perenne* L.. *Czech Journal of Genetics and Plant Breeding* 39 57-63. https://www.cabdirect.org/cabdirect/abstract/20123027683

Sampoux J-P, Mètral R, Ghesquière M, Baudouin P, Bayle B, Béguier V, Bourdon P, Chosson J-F, Brujin K, Deneufbourg F, Galbrun C, Pietraszek W, Tharel B, Viguët A. 2010. Genetic improvement in ryegrass (*Lolium perenne*) from turf and forage breeding over the four past decades. In: Huyghe C Ed. *Sustainable use of Genetic Diversity in Forage and Turf Breeding*, pp. 325-330. Springer Netherlands. https://doi.org/10.1007/978-90-481-8706-5_46

Van Wijk AJP, Reheul D. Achievements in fodder crops breeding in maritime Europe. In: Nijs APM, Elgersma AW Ed. https://doi.org/10.1007/978-90-481-8706-5_46

Williams WM, Easton HS, Jones CS. 2007. Future options and targets for pasture plant breeding in New Zealand. *New Zealand Journal of Agricultural Research* 50: 223-248. https://doi.org/10.1080/00288230709510292

Wright S. 1922. The effects of inbreeding and crossbreeding on guinea pigs. I. Decline in vigor. II. Differentiation among inbred families. *US Department of Agriculture Bulletin*: 1-59. https://books.google.co.nz/books?hl=en&lr=&id=RVJCQAAMAAJ&oi=fnd&pg=PA1&dq=wright+1922+The+effects+of+inbreeding&ots=bFdxHegN1B&sig=-SGq0zeWrzb3j3w6P8_25eHT4&redir_esc=y - v=onepage&q=wright%201922%20The%20effects%20of%20inbreeding&f=false