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Implementation of rapid cycle recurrent genomic selection for forage yield in perennial ryegrass

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Abstract

Opportunities exist to accelerate genetic gain in forage breeding using genome-wide selection approaches. In this study, we evaluated rapid cycle recurrent genomic selection (GS) as a means of improving genetic gain for value of annual forage yield. A small population of tetraploid half-sib families was evaluated for seasonal forage yield over two years, and the maternal parent plants were genotyped and genomic prediction models developed. The GS model for value of annual forage yield had a predictive ability of 0.23. An initial round of among-family selection based on field evaluations and within-family selection using genomic estimated breeding values was performed. This was followed by two further GS cycles. New synthetics were produced after each round of selection and were established in a field trial alongside the starting population. A positive response to selection was observed in new synthetics after two successive rounds of rapid cycle recurrent genomic selection before declining in the third round. The genetic gain for the value of annual forage yield was 2.4% from C_0 SYN-1 to C_1 SYN-1 and 6.4% from C_1 SYN-1 to C_2 SYN-1. In the case of C_0 to C_1, genetic gain was compounded by among-family selection based on field evaluations. The implementation of rapid cycle recurrent genomic selection offers an opportunity to increase genetic gain; however, the predictive ability is likely to decay rapidly as selection candidates become more distant from the training population.

Keywords: forage yield, genomic selection, *Lolium perenne*, perennial ryegrass.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most important forage grass species in Ireland and temperate regions of the world. Yield is an important trait for perennial ryegrass to provide natural low-cost feed for ruminants (Wilkins and Humphreys 2003, Humphreys 2005). High-yielding cultivars produce more herbage, making them a relatively cheap and high-quality feed for animals. However, in a typical grazing system, there is excess grass growth during the summer months and a growth deficit in spring and autumn relative to animal demand. Surplus yields from summer are typically harvested and stored as silage to compensate for feed deficits in spring and autumn. Silage production is an expensive process that decreases the overall profitability of farms (O’Kiely and Flynn 1987). Developing cultivars with increased yield in spring and autumn could potentially increase the overall grazing season and decrease supplemental feed costs (Wilkins and Humphreys 2003).

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Abbreviations: GS - genomic selection; GBS - genotyping by sequencing; HSF - half-sib family.

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McEvoy *et al.* (2011) developed a pasture profit index (PPI) to identify and rank cultivars for profitability at the farm level. PPI assigns economic values for key traits such as dry matter yield (spring, summer, and autumn yields), first and second cut silage yield, dry matter digestibility, and persistency. PPI was based on simulating a spring-calving dairy farm model over a period of 12 months. Currently, perennial ryegrass cultivars produce on average 12.3 ± 5.5 t(DM) ha^{-1} yr^{-1} (Gilliland *et al.* 2021) under simulated grazing and there is a potential to increase forage yield further. However, the genetic gain for annual dry matter yield is approximately 0.3 to 0.5% per year (Wilkins and Humphreys 2003, McDonagh *et al.* 2016) and these rates of genetic improvement are significantly lower than in cereals, which can be 1.0 to 1.5% per year (Öfversten *et al.* 2004).

Genomic selection offers an approach for accelerating genetic gain during breeding (Meuwissen *et al.* 2001) where molecular marker data is utilised to estimate breeding values. Genomic prediction has revolutionized animal breeding and has been successfully applied (Meuwissen *et al.* 2016); however, it is still in the early phase of plant breeding. Previous studies have demonstrated the potential of genomic prediction for diploid perennial ryegrass populations (Fè *et al.* 2015a,b; Lin *et al.* 2016, Byrne *et al.* 2017, Faville *et al.* 2018, Arojju *et al.* 2018, 2020; Esfandyari *et al.* 2020). However, there are limited empirical studies on genomic selection (Faville *et al.* 2022) and none have evaluated rapid cycle recurrent genomic selection in perennial ryegrass.

In this study, we empirically evaluated rapid cycle recurrent genomic selection by implementing three recurrent cycles of genomic selection (GS) to improve the value of annual forage yield. New synthetics produced after each round of GS were evaluated in a field trial along with the starting population to determine the response to genomic selection.

Materials and methods

Training population development and trial design: The tetraploid population used in this study was developed from a commercial cultivar, which has been on the Irish recommended list since 2012. The cultivar Kintyre was initially developed by intercrossing 75 plants from each of four full-sib families. A set of 120 plants from the cultivar was clonally propagated and each clone planted out in one of seven blocks in a polycross nursery and allowed to cross pollinate. Seeds from matching maternal parents were harvested and bulked, producing half-sib families. Out of the original 120 half-sib families (HSFs), 109 families produced enough seed for a replicated field trial with two managements. In each management regime, HSFs were planted out in one of seven experimental blocks, where each block contained two replicates of 16 test families and two control cultivars. The two control cultivars (Kintyre and Abergain) were consistent across experimental blocks. Plots were established in 2014 and data was collected in 2015 and 2016 at Oak Park, Carlow, Ireland. Yield was measured as fresh mass under two management schemes, *1*) simulated grazing management with seven harvest cuts per year (each cut every four weeks from March to October) and *2*) conservation management with silage cuts. Each plot size was 6 × 1.5 m and forage was harvested to approximately 4 cm above ground using a *Haldrup* forage plot harvester.

Phenotyping and data analysis: In the simulated grazing management, yield data were collected from at least seven harvest cuts in each of two years. Total yield in each year was the sum of all seven harvests. We estimated the value of the annual forage yield using weightings from the PPI (McEvoy *et al.* 2011). Cuts in the simulated grazing management were divided into spring yield (cuts 1 and 2), summer yield (cuts 3, 4, and 5), and autumn yield (cuts 6 and 7). Yields in spring, summer, and autumn were multiplied by the weightings 0.16, 0.04, and 0.11, respectively (McEvoy *et al.* 2014) to ensure yield in spring was valued higher than forage yield in autumn, and forage yield in autumn was valued higher than forage yield in summer. The value of annual forage yield was then calculated by summing spring, summer, and autumn yield. Under conservation management, we focussed on yield data of the first cut silage. In total five traits from the simulated grazing management (total annual yield, value of annual

Harvest dates for forage yield measurements under conservation and simulated grazing managements.When calculating the value of annual forage yield, a weighting was applied to each harvest depending on when the harvest took place. The weighting assigned to each cut is shown in the table.

	2015	2016	
Simulated grazing management			Weighting
Harvest 1	09/04 to 10/04	14/03 to 15/03	0.16
Harvest 2	29/04 to 01/05	18/04 to 19/04	0.16
Harvest 3	21/05 to 22/05	12/05 to 13/05	0.04
Harvest 4	10/06 to 19/06	07/06 to 08/06	0.04
Harvest 5	15/07 to 17/07	04/07 to 05/07	0.04
Harvest 6	10/08 to 12/08	04/08 to 05/08	0.11
Harvest 7	07/09 to 08/09	14/09 to 21/09	0.11
Conservation management			
First cut silage	21/05 to 22/05	12/05 to 13/05	na

forage yield, spring, summer and autumn yields) and one trait from the conservation management (first silage cut) were used for further analysis. Phenotypic analysis was carried out in two stages. In stage one, the mean of controls in each experimental block and mean of controls across all seven experimental blocks were calculated. An adjustment value was calculated for each experimental block by subtracting the mean of controls across all blocks from the mean of controls within a block. This adjustment value for each experimental block was then used to adjust values for each test family within that experimental block. In stage two, adjusted values were used to fit a mixed model with family as a random effect, and year as fixed effect, to obtain best linear unbiased predictors (BLUPs) for all six traits (calculated in *lme4* according to Bates *et al.* 2015). The heritabilities on a half-sib family mean basis were calculated as follows:

$$h^2 = \frac{\sigma_f^2}{\sigma_f^2 + \frac{\sigma_{fy}^2}{y} + \frac{\sigma_{res}^2}{r \cdot y}}$$

where σ_f^2 is half-sib family variance, σ_{fy}^2 is the half-sib family by year variance, σ_{res}^2 is the residual variance, y is the number of years, and r is number of replicates.

Genotyping and variant calling: DNA was extracted from leaf samples of maternal plants using a standard cetyl trimethyl ammonium bromide (CTAB) method (Doyle 1991). A genotyping by sequencing (GBS) approach based on genome complexity reduction with restriction enzymes (Elshire *et al.* 2011) was used for library preparation and was carried out as a service by *LGC Genomics* (Berlin, Germany). Sequence reads were quality filtered, demultiplexed and aligned to the reference perennial ryegrass genome (Byrne *et al.* 2015) available at the time. All GBS data has been submitted to the *NCBI SRA* under BioProject PRJNA1013016. After demultiplexing, genotype calling was done according to Li *et al.* (2014) where no distinction was made among the three heterozygous states. Briefly, for each SNP a minimum of 11 reads were required to call a homozygote (*i.e.*, AAAA). If fewer than 11 reads were present, the genotype was considered as missing to avoid misclassifying a triplex heterozygote (*i.e.*, AAAT) as homozygous. To call a SNP heterozygous, two reads per allele were required and the proportion of reads supporting the less frequent allele needed to be greater than 0.10, otherwise it was considered as a missing genotype. The data set was filtered to remove SNPs with a minor allele frequency less than 5% and more than 50% of the data missing. The final SNP set consisted of 45 569 genome wide markers.

Genomic prediction models and cross validations: Genomic prediction was carried out using the SNP data set, and the A.mat function of the *rrBLUP* package (Endelman 2011) was used to impute missing data. We used ridge regression BLUP (rrBLUP) with the mixed solve function of *rrBLUP* package to develop genomic prediction models for each trait described above. Monte Carlo cross-validation (1 000 iterations) was carried out with an 80:20 split between training and test set. Predictive ability was measured as the Pearson correlation coefficient of genomic estimated values and trait BLUPs determined from field evaluations averaged over 1 000 iterations. We assessed predictive ability for the following traits; spring yield, summer yield, autumn yield, annual forage yield, first cut silage, and value of annual forage yield.

Implementation of genomic selection for value of annual forage yield: In an initial round of within-family selection with genomics, seed from the top five HSFs for value of annual forage yield (one family with insufficient seed was replaced with next in line) were germinated and 240 seedlings from each family were selected for genotyping (Fig. 1). Genotyping and data analysis was carried out as described for the training population. Genomic estimated breeding values (GEBVs) were calculated for each plant using models developed for the value of annual forage yield on the complete training population. The GEBVs were ranked within each HSF and the top thirty seedlings from each family were selected and established in a field isolation in January and allowed to cross-pollinate (oats without application of a plant growth regulator was used as a pollen barrier). Seed (C1 SYN-1) was harvested from the isolation in August and seed germinated to establish 1 008 seedlings for genotyping. Again, GEBVs for the value of annual forage yield were determined for each seedling, ranked, and the top 100 candidates established in a field isolation, allowed to cross-pollinate, and C2 SYN-1 seed harvested. A third round of genomic selection was performed with 1 000 seedlings from the previous C2 SYN-1 and again the top 100 candidates were established in a field isolation, allowed to cross-pollinate, and C3 SYN-1 seed harvested.

Evaluation of new synthetic populations: In addition to the three SYN-1s produced (C_1 SYN-1, C_2 SYN-1, and C_3 SYN-1), SYN-2s were also developed from C_1 SYN-1 and C_2 SYN-1 seed. C_3 SYN-2 seed was not available when starting this evaluation trial. SYN-2 seed was produced through random mating of SYN-1 seed in field isolations. The synthetic populations were established in an experimental field plot trial (plot size 6 × 1.5 m) alongside the starting population of Kintyre (C_0). The trial was established as a randomized complete block design with four blocks. The plots were evaluated for forage yield (measured as fresh mass) in a simulated grazing management over two years following an establishment year. Each trait was analysed using a linear regression model in *R* (R Core Team 2013) with population ID, year, and block as model parameters. Estimates of the marginal means were calculated with the *emmeans* package (Lenth *et al.* 2023).

Results

Phenotyping a training population: The population used for training the genomic selection models was evaluated in two management regimes (conservation, simulated

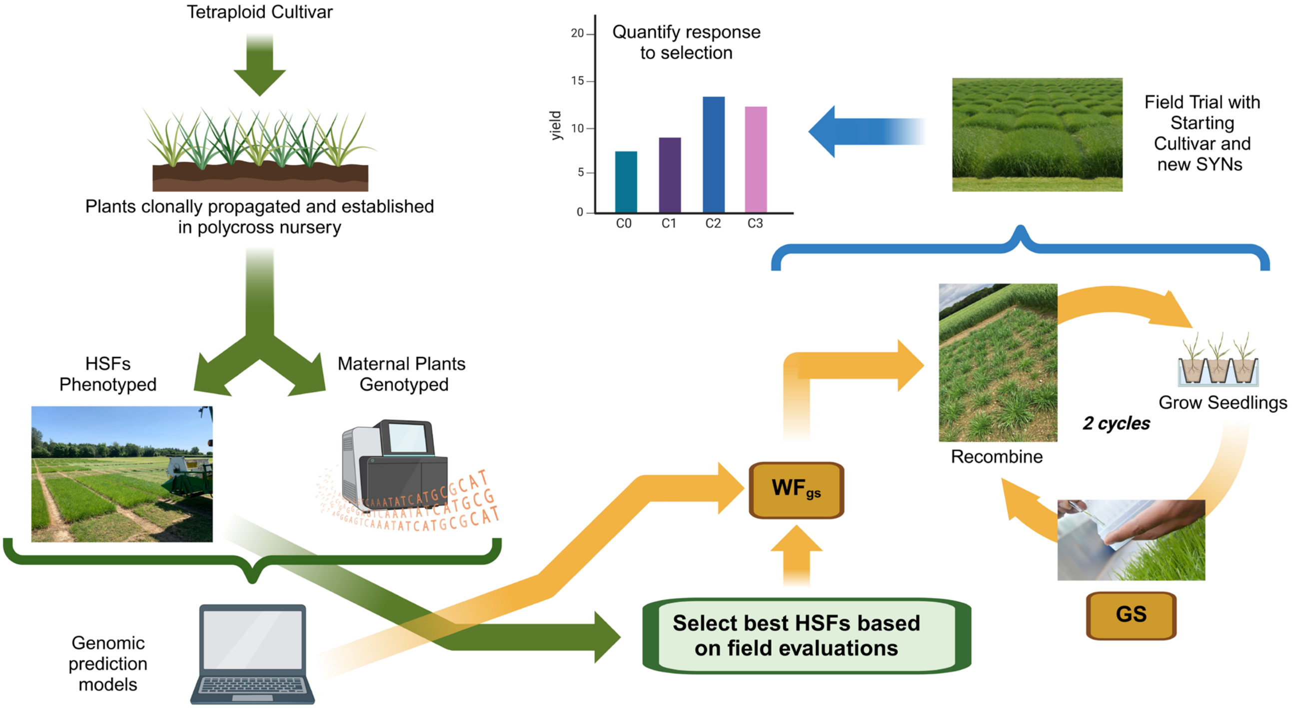

Fig. 1. Overview of reference population development and rapid cycle recurrent genomic selection implemented in this study. Genotyping was carried out using genotyping-by-sequencing based on complexity reduction with restriction enzymes. GS - genomic selection, WF$_{gs}$ - within-family genomic selection. Created with *BioRender.com*.

grazing), and the descriptive statistics are presented in Table 1. The heritability ranged between 0.22 for the first cut silage and 0.54 for value of annual forage yield (Table 1).

The correlation between annual yield and value of annual forage yield was high (Fig. 2), and heritability was similar for both traits (Table 1). The correlation between the first cut silage measured under conservation management and forage yield traits measured under simulated grazing management was low (Fig. 2). The highest correlation (0.19) was with spring forage yield and the lowest (0.09) with autumn forage yield, likely reflecting a greater difference in timing between first cut silage and autumn yields in comparison to spring yield. Furthermore, Kendall rank correlations between first cut silage and simulated grazing yields were all below 0.12.

Accuracy of genomic selection models for forage yield: A final SNP set, consisting of 45 569 SNPs, was identified in the training population. There was no clear clustering of maternal plants, as observed using principal component analysis (PCA) of the marker data (Fig. 3). The lack of structure in the population is not unexpected considering that it is composed of plants taken from a cultivar that was originally developed by intercrossing 75 plants from each of four full-sib families.

In this study, we first evaluated the accuracy of genomic prediction of forage yield traits measured under two management regimes. Under conservation management, first cut silage is the trait of greatest importance, and we obtained a mean predictive ability of 0.22. Under simulated grazing management, we determined the predictive abilities for spring yield, summer yield, autumn yield, annual yield, and value of annual forage yields. The mean predictive abilities ranged from 0.03 for summer yield to 0.28 for spring yield (Fig. 4).

Table 1. Summary of training population phenotypic data. Silage was measured under separate conservation management and all other traits under a simulated grazing management.

Trait	σ^2	Mean [kg plot^{-1}]	Min. [kg plot^{-1}]	Max. [kg plot^{-1}]	h^2
Spring yield	1.81	8.21	4.04	13.56	0.33
Summer yield	3.69	31.71	26.11	40.68	0.26
Autumn yield	2.37	13.91	4.12	17.56	0.44
Annual yield	11.98	53.84	43.65	70.15	0.53
Value of annual forage yield	0.11	4.12	3.23	5.51	0.54
Silage yield	9.70	31.67	11.87	45.20	0.22

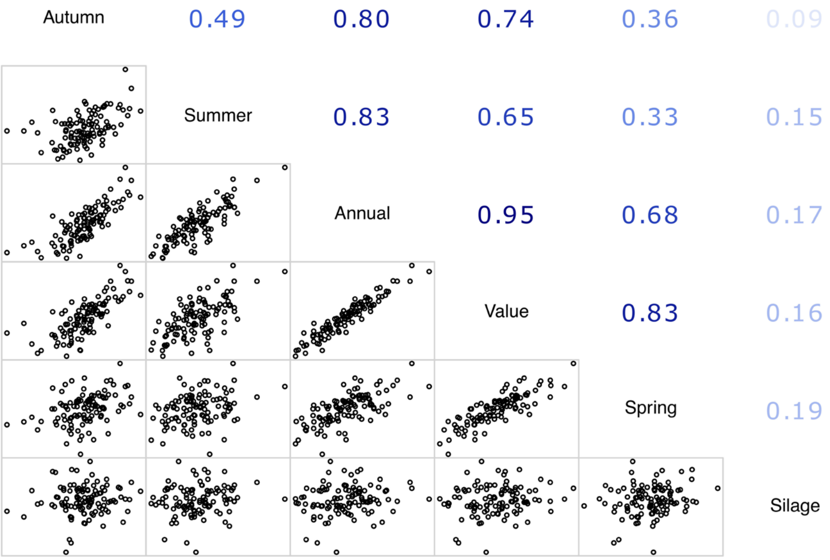

Fig. 2. Correlation of forage yield traits for half-sib families measured in a simulated grazing regime (spring, summer, autumn, and annual forage yield, and value of annual forage yield) and a conservation regime (first cut silage). Scatter plots and the Pearson correlation coefficients.

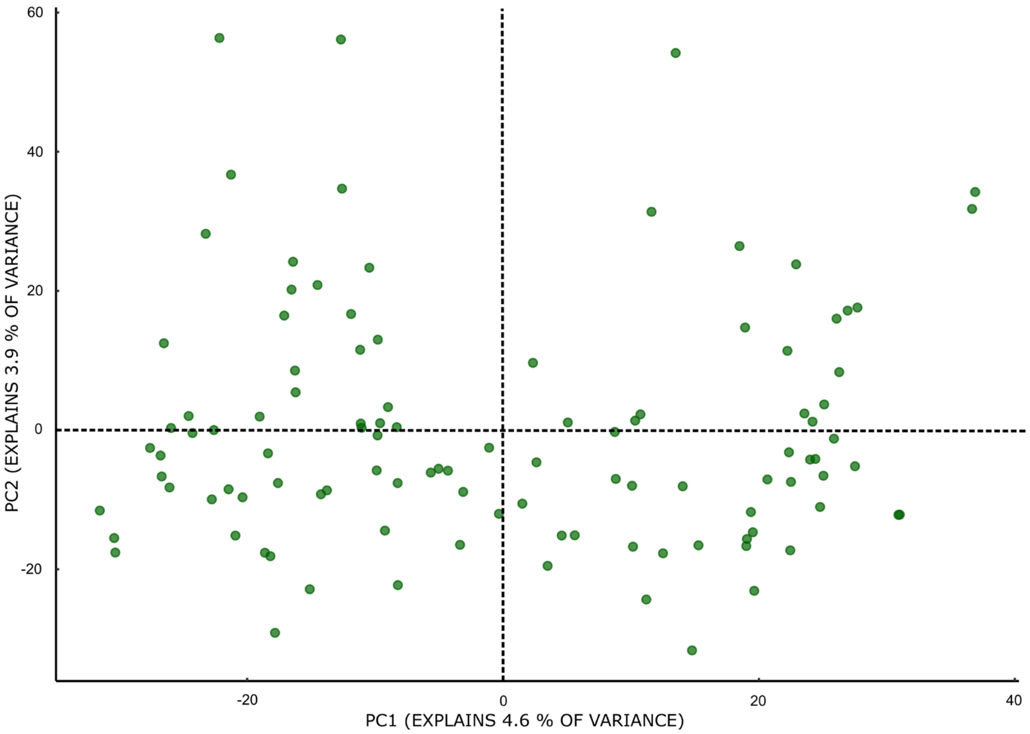

Fig. 3. Principle component analysis of genotype data on the maternal plants of the 109 half-sib families that made up the training population.

Empirical assessment of genomic selection for forage yield: A cycle of among-family selection based on field evaluations and within-family selection using genomics was carried out. This was followed by two further rounds of genomic selection. New synthetic populations were produced in field isolations using parents selected based on GEBVs. Three rounds of genomic selection were carried out over three years. These new synthetic populations (C_1 SYN-1, C_2 SYN-1, C_3 SYN-1, C_1 SYN-2, and C_2 SYN-2) were established in a field trial alongside starting population (C_0) and evaluated for seasonal forage yield over two years (Table 2).

Estimated marginal means for all traits and SYN-1s and SYN-2s are shown in Fig. 5. In the case of value of annual forage yield, which was the trait selected for, there was a statistically significant difference between

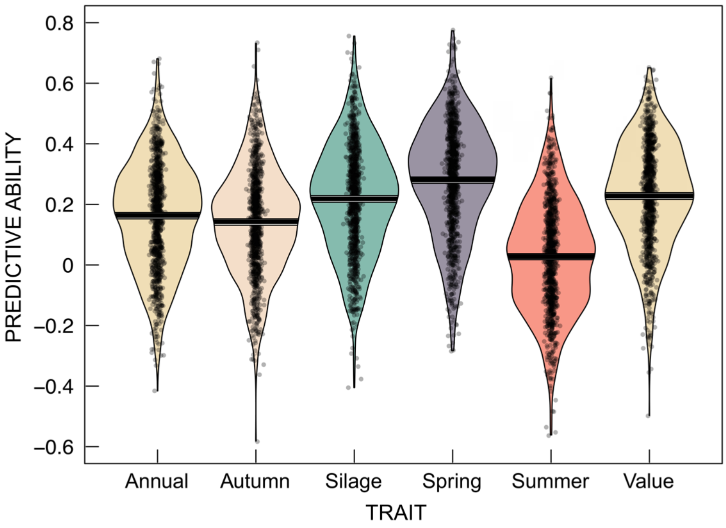

Fig. 4. Predictive ability (PA) measured as Pearson correlation between predicted and field evaluations. The mean, inference around the mean, smoothed density curve, and data points are shown. Genomic selection models developed using ridge regression BLUP and evaluated using Monte-Carlo cross validation (1 000 iterations) with 80:20 split between training and test set. The traits evaluated are spring, summer, autumn, and annual yield in a simulated grazing management regime, first cut silage yield, and the value of annual forage yield.

Table 2. Seasonal forage yield [t ha^{-1}] data across years. Means of four plots shown along with standard errors.

	C_0	C_1		C_2		C_3
	Kintyre	SYN-1	SYN-2	SYN-1	SYN-2	SYN-1
2021						
Spring yield	10.34 ± 0.32	11.33 ± 0.50	11.89 ± 0.47	12.00 ± 0.28	12.22 ± 0.51	12.00 ± 0.68
Summer yield	36.89 ± 0.84	36.78 ± 0.58	37.11 ± 0.30	38.00 ± 0.63	35.78 ± 0.62	36.33 ± 0.59
Autumn yield	12.22 ± 0.43	12.22 ± 0.08	12.44 ± 0.37	13.56 ± 0.21	12.78 ± 0.47	13.00 ± 0.26
Annual yield	59.44 ± 1.47	60.22 ± 1.08	61.33 ± 0.71	63.67 ± 0.86	60.67 ± 1.36	61.33 ± 1.46
Annual yield [€/plot]	4.03 ± 0.11	4.16 ± 0.10	4.27 ± 0.10	4.45 ± 0.07	4.31 ± 0.13	4.32 ± 0.14
First cut silage	51.33 ± 0.73	54.56 ± 1.78	52.78 ± 1.14	54.44 ± 0.53	55.00 ± 1.02	54.67 ± 1.02
2022						
Spring yield	10.27 ± 0.37	10.43 ± 0.56	10.99 ± 0.29	11.22 ± 0.26	10.84 ± 0.36	10.73 ± 0.37
Summer yield	31.78 ± 1.12	31.78 ± 0.69	32.00 ± 0.90	33.00 ± 0.09	31.11 ± 0.58	31.89 ± 1.03
Autumn yield	6.51 ± 0.36	6.69 ± 0.27	6.82 ± 0.21	7.01 ± 0.21	6.67 ± 0.21	6.53 ± 0.41
Annual yield	48.56 ± 1.62	48.89 ± 0.77	49.78 ± 1.11	51.22 ± 0.98	48.67 ± 1.03	49.11 ± 1.38
Annual yield [€/plot]	3.27 ± 0.10	3.31 ± 0.08	3.41 ± 0.07	3.49 ± 0.05	3.34 ± 0.08	3.34 ± 0.08
First cut silage	39.44 ± 1.21	39.44 ± 0.44	38.22 ± 2.76	39.56 ± 1.22	42.22 ± 1.20	36.89 ± 1.60

SYN-1s of C_0 and C_2 (Fig. 5). The gain from C_1 SYN-1 to C_2 SYN-1 was greater than the gain from C_0 SYN-1 to C_1 SYN-1. SYN-2s were also developed from C_1 and C_2 SYN-1s by random mating of SYN-1 plants. In the case of C_1 the SYN-2 was higher than the SYN-1, and in the case of C_2 the SYN-2 was lower than the SYN-1 for value of annual forage yield.

There was distinction between selection candidates in C_1 due to genotyping within five half-sib families (Fig. 1 Suppl.). Levels of relatedness between selection candidates (C_1, C_2, C_3) and training population (C_0) dropped as we moved through selection cycles (Fig. 2 Suppl.).

Discussion

Given the importance of perennial ryegrass in pastoral agricultural systems (Wilkins 1991), it is essential to accelerate genetic progress in the species for key traits such as forage yield. Studies to quantify the genetic gain in perennial ryegrass have highlighted modest gains in dry-matter yield of *ca.* 0.3% per year (Sampoux *et al.* 2011, McDonagh *et al.* 2016). A lack of progress in improving spring forage yield has also been highlighted (Sampoux *et al.* 2011), a key focus in grazing systems where forage supply in spring typically falls short of demand.

Genomic selection is a breeding tool that has the

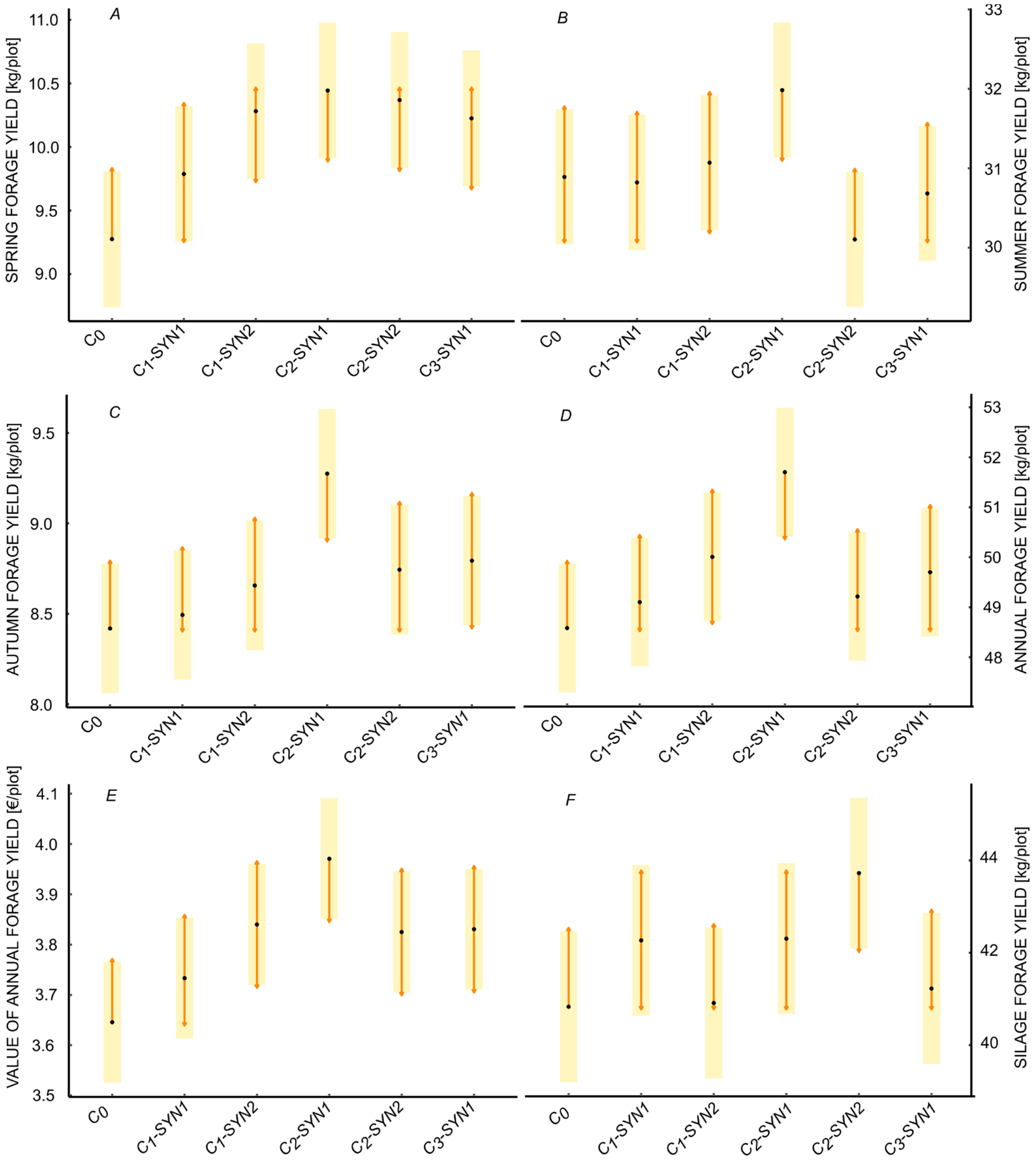

Fig. 5. Estimated marginal means (EMMs) for forage yield of starting population Kintyre (C_0) and SYN-1 and SYN-2 after recurrent rounds of genomic selection. *A* - spring forage yield [kg/plot]; *B* - summer forage yield [kg/plot]; *C* - autumn forage yield [kg/plot]; *D* - annual forage yield [kg/plot]; *E* - value of annual forage yield [€/plot]; *F* - first cut silage forage yield [kg/plot]. *Gold bars* are confidence intervals for EMMs and *orange arrows* are for pairwise contrasts (when arrow from mean of one group overlaps with another, the pairwise comparison is not significant at α of 0.05).

potential to accelerate genetic gain in forage species. The greatest benefit comes from rapid cycles of recurrent selection with genomics, where even modest predictive accuracies can potentially lead to a tripling of genetic gain (Annicchiarico *et al.* 2015). While such an approach takes full advantage of genomics to shorten the generation interval (and increase selection intensity), there is a risk that predictive accuracy will degrade over cycles as selection candidates become increasingly removed from the training population. In fact, there have been many studies demonstrating the importance of maintaining a strong relationship between training population and selection candidates (Goddard 2009, Speed and Balding 2015, Konkolewska *et al.* 2021). Empirical studies using genomics for within-family selection in perennial ryegrass have been reported (Faville *et al.* 2022), although there have not been any empirical reports on using genomics for rapid cycle recurrent genomic selection. However, there are limited examples of empirical studies of rapid cycle recurrent genomic selection in other species, and encouraging results have been reported (Zhang *et al.* 2017, Veenstra *et al.* 2020, Dreisigacker *et al.* 2023).

In this study, we implemented rapid cycle recurrent genomic selection in a perennial ryegrass half-sib family population to determine the response to genomic selection and evaluate the genetic gain.

The data for model training were collected across separate simulated grazing and conservation management regime, as it is known that perennial ryegrass cultivars perform differently under different management conditions (Gilliland and Mann 2000, McEvoy *et al.* 2010, McDonagh *et al.* 2016). This was seen here with little overlap in top-performing families identified in each management and low correlations between first cut silage and seasonal yield traits measured in simulated grazing management. This is likely due to a high degree of genetic independence between the yield during reproductive growth, which primarily contributes to silage yield, and the yield during vegetative growth, which primarily contributes to grazing yield (Wilkins and Humphreys 2003). The heritability based on a half-sib family mean basis ranged between 0.22 and 0.54, and these are in line with location specific heritabilities for forage yield traits (Fè *et al.* 2015b).

Genomic prediction models were initially developed for all forage yield traits using genotypes of maternal plants and phenotypes collected from the half-sib progeny. This focused on the additive genetic variation, of relevance in predicting parental breeding values during synthetic cultivar development. The predictive ability, as assessed *via* cross-validation within the training population ranged from 0.03 to 0.28, which is in line with predictions for forage yield traits from other studies of perennial ryegrass (Lin *et al.* 2016, Faville *et al.* 2018, Guo *et al.* 2018, Pembleton *et al.* 2018, Arojju *et al.* 2020), alfalfa (Annicchiarico *et al.* 2015, 2022), and switchgrass (Ramstein *et al.* 2016).

The focus of this study was on rapid cycle recurrent genomic selection for value of annual forage yield, where the mean predictive ability was estimated at 0.23, and results showed that we achieved genetic gain for this trait up to C2. Focussing on SYN-1s, the greatest difference was between C_0 and C_2, and this difference was statistically significant. The genetic gains from C_0 to C_2, as evaluated on their SYN-1s, were 12.6% for spring yield, 3.5% for summer yield, 10.2% for autumn yield, and 8.9% for value of annual forage yield. The gains for spring, summer, and autumn yield reflect the emphasis placed on each of these traits when calculating the value of annual forage yield in the development of GS models. It is important to highlight that the genetic gain from C_0 to C_1 is compounded by among-family selection based on field data, and at least part of the gain from C_0 to C_1 resulted from selecting plants from the best half-sib families. However, other studies have demonstrated the advantages of within-family genomic selection over random selection from remnant seeds (Faville *et al.* 2022). The genetic gain from C_1 to C_2 was not compounded by among-family selection, and gains were 6.7% for spring yield, 3.8% for summer yield, 9.2% for autumn yield, and 6.4% for value of annual forage yield. These gains from C_1 to C_2 were greater than the gains from C_0 to C_1, where gains were 5.5%, -0.2%, 0.9%, and 2.4% for spring yield, summer yield, autumn yield, and the value of annual forage yield, respectively.

A positive response to selection was not observed in the SYN-1s when moving from C_2 to C_3. While rapid cycle recurrent genomic selection maximizes the advantages of genomic selection, it requires that the predictive ability of models be maintained over multiple generations. It can be seen here that after two rounds of GS, and as we move away from the population upon which the model was developed, we lost predictive ability. This can be seen in the Genomic Relationship Matrix (GRM), where the relationships between C_3 and C_0 are lower than those between C_2 and C_0. However, further cycles of GS would need to be completed to confirm this trend. The SYN-2s were developed from random mating within each SYN-1 and an increase in value of annual forage yield was observed from C_0 to C_1 SYN-2, but no increase from C_1 SYN-2 to C_2 SYN-2. On average, SYN-1s are expected to outperform SYN-2s in both diploids and tetraploids (Reheul *et al.* 2003) but this was not the case here where the C_1 SYN-2 was higher yielding than C_2 SYN-1, albeit the difference was not significant.

In the first round of genomic selection, we did not exclusively select on GEBVs but rather ranked GEBVs within each of the five HSFs selected based on field evaluations. This was done to avoid restricting genetic diversity during the initial round of selection. After this point, selection was based purely on a plants GEBV ranking. It is unclear whether gains equivalent to the C_2 SYN-1 would have been achieved in C_1 if we had selected parents based purely on GEBV ranking in the initial round of genomic selection and without selecting an equal number of parents from each of the top five HSFs. A clear advantage of the above is that it saves a cycle of genomic selection and associated time and costs. A scenario could be envisioned where selecting the best plants within the best families is used to identify parents for the next round of selection, but where selection is based solely on GEBVs when producing new synthetics during product development. It is also possible that an additional round of GS (C_1 to C_2) offers the potential to further increase genetic gain during product development and warrants further research to validate in both HSF and full-sib family breeding scenarios.

The relative value of yield at different times of the year was captured using the pasture profit index (PPI) (McEvoy *et al.* 2010, 2011, 2014), which was developed in Ireland to provide economic value to cultivars. PPI is primarily aimed at helping farmers select new cultivars when reseeding pastures, but can also aid breeders in developing selection indexes to support the development of new cultivars for Irish production systems. Future implementations may benefit from treating seasonal yield as separate traits and incorporating a multi-trait selection index such as the Smith-Hazel index (Smith 1936, Hazel 1943) or base index (Williams 1962), ideally expanding traits beyond forage yield to other key traits such as forage quality. At least in the case of an Irish forage grazing system, the relative weightings required for such an index already exist (McEvoy *et al.* 2011).

Opportunities exist in perennial ryegrass to accelerate genetic gain with GS for complex traits such as forage yield. There are different approaches to implementing GS in forage breeding that enable breeders to take advantage of its benefits. One approach is rapid cycle recurrent genomic selection, and although it has the greatest potential to fully exploit GS, this approach comes with the greatest risk. Our study showed that rapid cycle recurrent genomic selection yielded a positive response to selection for only two successive rounds of GS, before declining in the third round as selection candidates became more distant from the training population. Encouragingly, the greatest gains were from C_1 to C_2, where genetic gain was not compounded by among-family selection based on field evaluations. This demonstrates that GS can be an effective tool in perennial ryegrass breeding, and further empirical studies are required to optimize GS strategies for population improvement and product development in both HSF and FSF breeding schemes.

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