Optimizing self-pollinated crop breeding employing genomic selection: from schemes to updating training sets

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Optimizing self-pollinated crop breeding employing genomic selection: from schemes to updating training sets

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19   **ABSTRACT**

20

21   Long-term breeding schemes employing genomic selection (GS) can boost the response to selection per year. Although
22   several studies show that GS delivers a higher response to selection, only a few analyze the best strategy to employ it,
23   specifically how often and in what manner the training set (TS) should be updated. Therefore, we used stochastic simulation
24   to compare in a long-term breeding program of a hypothetical self-pollinated crop five different strategies to implement GS
25   in the line fixation stage and four methods and sizes to update the TS. Moreover, among breeding schemes, we proposed a
26   new approach for using GS to select the best individuals in each F2 progeny based on genomic estimated breeding and
27   divergence and crossed them to generate a new recombination event. Finally, we compared these schemes to the traditional
28   phenotypic selection and drift. Our results showed that using GS in F2 followed by the phenotypic selection of new parentals
29   in F4 was the best scenario. Furthermore, adding a new set of training data every cycle (over 800) to update the TS maintains
30   the accuracy at satisfactory levels for many more generations, showing that more data is better than optimizing the genetic
31   relationship between TS and the targeted population in a closed system. Hence, we believe that these results may help breeders
32   optimize GS in their programs and improve genetic gain in long-term schemes.

33

34   **Keywords:** breeding scheme; Accuracy; sample size; response to selection;

35   **DECLARATIONS**

36

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39

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41

42   **Availability of data:** all the data is available as supplementary information.

43

44   **Code availability:** all the scripts are available as supplementary information.

45

46   **Ethical standards:** Not applicable

47

48   **AUTHOR CONTRIBUTION STATEMENT**

49   FS wrote the manuscript, discussion, contributing to ideas and graphs. JCD and JP contributed to the writing, mainly
50   discussion. RFN elaborated on the hypothesis, conducted the analyses, interpreted the results, and contributed to the writing.
51   All authors read and approved the final manuscript.

52

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56

57   **KEY MESSAGE**

58   Update the training set every cycle is a key factor in GS accuracy.

59

60 INTRODUCTION

61

62 Genomic selection (GS) has successfully been employed in plant and animal breeding (Heffner et al. 2011a; Spindel
63 et al. 2015; Wolc et al. 2015; Garner et al. 2016). In this technique, breeders build a training set (TS) containing genotyped
64 and phenotyped individuals to estimate the marker effects and use them to calculate genomic estimated breeding values
65 (GEBV) of only genotyped individuals (Meuwissen et al. 2001). Since the selection of individuals does not need their
66 phenotypic information, these genotypes can be selected early, which permits faster breeding schemes and increases the
67 genetic gain (Beyene et al. 2015; Voss-Fels et al. 2019). However, several parameters influence the accuracy of GEBV
68 prediction, including TS size (Heffner et al. 2011b; Lorenz 2013), population structure (Guo et al. 2014), the genetic
69 relationship between the TS and selection candidate genotypes (Lorenz and Smith 2015), and trait heritability (Combs and
70 Bernardo 2013).

71 Several studies have examined optimal employment of GS on self-pollinated crop breeding program (Lorenz et al.
72 2012; He et al. 2016; Michel et al. 2016). The self-pollinated crop breeding programs are composed of three phases: create
73 genetic variation, identify desirable recombinant lines within progenies, and stabilize and advance the desired genotype
74 (Brown et al. 2014). There are several breeding methods to increase the homozygosity during the line fixation step, and single
75 seed descent (SSD) is the method most suited to accelerating a breeding scheme. In rice, especially in the IRRI breeding
76 program, the rapid generation advanced (RGA) method, which consists of an SSD method conducted in greenhouses or
77 screenhouses, is employed to accelerate and shorten the growth cycle (Collard et al. 2017). The combination between GS and
78 the RGA can boost genetic gain in rice long-term breeding schemes principally due to shortening each cycle, which increases
79 the response to selection (Falconer and Mackay 1996).

80 Long-term breeding schemes should be analyzed concerning different genetic parameters, as genetic gain, response
81 to selection, genetic variance, and prediction accuracy to determine the best long-term strategy for maximizing results. Even
82 though there are several studies involving GS in long-term recurrent selection schemes (Goddard 2009; Jannink 2010; Muleta
83 et al. 2019), only a few test the best stage to apply it (Bassi et al. 2016; Gaynor et al. 2017). Mendonça et al. (2020) showed
84 that GS increases the efficiency in advanced phases in a soybean breeding program using a low-intensity selection for
85 quantitative traits in the early stages. Marulanda et al. (2016) verified different strategies to apply GS in hybrid breeding
86 programs in several self-pollinated crops. They concluded that using GS in stage 1 followed by a final phenotypic selection
87 stage produced the highest genetic progress. Hence, there is room to optimize the strategy and stage for using GS to boost
88 genetic gain and improve efficiency in a breeding program.

89   Another point to highlight in long-term breeding schemes employing GS is that prediction accuracy declines over
90 cycles (Jannink 2010; Muleta et al. 2019). This phenomenon might be due to the decrease in genetic relationship between
91 TS and selection candidate genotypes (Lorenz and Smith 2015), the breakdown of LD between marker and QTL due to
92 recombination (Jannink 2010; Müller et al. 2017), and the lack of genetic variability of the population. In this context, a few
93 studies claim the requirement of updating the TS (Jannink 2010; Muleta et al. 2019; DoVale et al. 2021) to reduce the accuracy
94 decline across breeding cycles. For instance, Neyhart et al. (2017) used different methods to select individuals to add into the
95 TS during recurrent selection cycles. They concluded that a better strategy to decrease accuracy decline is selecting
96 individuals genetically most related to selection candidate genotypes. Therefore, our study aimed to optimize the use of GS
97 and identify a method to update the training set to achieve that brings greater efficiency in self-pollinated crop breeding
98 programs. For that, we used stochastic simulation to compare five different strategies to implement GS in the line fixation
99 stage and four methods and sizes to update TS in a long-term breeding program of a hypothetical self-pollinated crop.
100 Moreover, among breeding schemes, we proposed a new method for using GS to select the best individuals in each F2 progeny
101 based on genomic estimated breeding and divergence and crossed them to generate a new recombination event. Finally, we
102 compared these schemes to the traditional phenotypic selection and drift.

103

104 MATERIAL AND METHODS

105

106 In our study, we compared different GS strategies applied in a long-term breeding program. For that, we used rice
107 (*Oryza sativa* L.) as a model self-pollinated crop and stochastic simulations performed by the *AlphaSimR* package (Gaynor
108 et al. 2021). Furthermore, we evaluated different strategies for updating the TS and how it influences genetic parameters over
109 breeding cycles.

110

111 Long-term breeding schemes employing genomic selection

112

113 *Historical population and genetic parameters*

114

115 The historical rice founder population was simulated as 1,000 unique diploid inbred individuals, with 12
116 chromosome pairs each using a Markovian Coalescent Simulator (MaCS) (Chen et al. 2009). 1,644 biallelic segregating sites
117 were considered, uniformly distributed across chromosomes and 360 segregating loci randomly sampled as quantitative trait
118 nucleotides (QTN) and 994 segregating loci as single-nucleotide polymorphism (SNP).

119 The target of simulation was a quantitative trait, such as yield. The genetic parameters obtained by Li et al. (2008)
120 were used. Each QTN received randomly-allocated additive and dominance effects. Genetic values for each genotype were
121 obtained by summing all additive and dominance effects for all QTN. Additive effects (a) were sampled of a gamma
122 distribution with scale and shape parameters equal to 1 and randomly assigned for each QTN. Similarly, dominance effects
123 (d) for each QTN were computed by multiplying the absolute value of its additive effect (a_i) by locus-specific dominance
124 degree (δ_i). Dominance degrees were sampled of a Gaussian distribution with $\delta_i \sim N(\mu_\delta, \sigma_\delta^2)$, where μ_δ is the average
125 dominance degree equal to 0.22 and σ_δ^2 is the dominance variance equal to 0.50. Dominance effects were assigned for each
126 QTN according to the equation below:

$$127 \quad d_i = \begin{cases} 0, & \text{if QTN is homozygous} \\ \delta_i \times |a_i|, & \text{if QTN is heterozygous} \end{cases}$$

128

129 Phenotypic values were obtained by adding a random error sampled of a Gaussian distribution with mean equal to
130 0 and variance (σ_e^2) equal to 1, which was defined by broad-sense ($H^2 = 0.53$) and narrow ($h^2 = 0.50$) heritabilities.

131

132

Base population and burn-in phase

Two parental group sizes (24 and 48 parental lines) were considered to in designing the crossing block, and further steps were conducted independently. Parental lines (24 or 48) were randomly sampled based on their phenotypic value to build the base population. As a starting point to consider a program representative of current 4-year rice breeding programs, five traditional recurrent selection cycles were simulated totaling 20 years of the program. First, the parental lines were randomly crossed to generate 30 F₁ plants, which were selfed to produce 230 F₂ plants from each cross (Cobb et al. 2019). SSD conducted the line fixation stage until the F₆, where the best individuals based on their phenotypic values were selected to found the next breeding cycle. After three recurrent breeding cycles, we obtained the initial population to evaluate the genomic recurrent selection breeding schemes.

The base TS was composed of 1,530 inbreed lines originated by crossing between individuals from the historical population. Markers effects were predicted using the ridge-regression best linear unbiased prediction (RRBLUP) (Endelman 2011) according to the equation below:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Z}_u\mathbf{u} + \boldsymbol{\varepsilon}$$

where $\mathbf{y}$ is the vector of individual phenotypic values from the TS; μ is the mean (intercept); $\mathbf{u}$ is the vector of marker effects, where $\mathbf{u} \sim N(\mathbf{0}, \mathbf{I}\sigma_u^2)$; and $\boldsymbol{\varepsilon}$ is the vector of random residuals. $\mathbf{1}$ is the vector of ones and $\mathbf{Z}_u$ is the incidence matrix of TS genotypes for m markers. $\mathbf{Z}_u$ is coded as 1 for homozygous A₁A₁, -1 for homozygous A₂A₂, and 0 for heterozygous A₁A₂.

To perform the GS, the genomic estimated breeding value (GEBV) were estimated using the following equation: $\mathbf{GEBV} = \mathbf{Mu}$, where $\mathbf{M}$ is the incidence matrix of selection candidate genotypes, and $\mathbf{u}$ is the vector of predicted marker effects.

Breeding schemes simulations

Simulations were based on the rice breeding program structure from the International Rice Research Institute (IRRI) (Collard et al., 2019) as a representative self-pollinated crop. For all scenarios, the line fixation phase was conducted by the single-seed descent (SSD) method, which consists of collecting one seed from each segregating plant to advance for the next stage until it reaches a high homozygosity level. Seven breeding schemes were compared, with different timelines and GS

162 procedures (Fig.1). Five schemes used GS in distinct stages during the line fixation phase, and two phenotypic breeding
163 schemes (traditional and drift) were used as baselines. Furthermore, two group sizes of parental lines (24 and 48) were
164 analyzed to populate the crossing block, totaling 14 different scenarios. In this case, we make a naïve assumption, which the
165 number of parents is a proxy to the effective population size (N_e).

166 In the first cycle, from crossing to F_2 generation were the same for all breeding schemes. Parental lines were crossed
167 to generate 30 random crosses between them. Then, F_1 plants were selfed to produce a F_2 segregating generation with 230 F_2
168 plants per cross (Cobb et al. 2019). From the F_2 phase, the different long-term breeding schemes were applied until the new
169 parental group selection for the next cycle. After the second cycle, each scheme followed independently and simulated over
170 20 breeding cycles and 40 replicates, totaling 800 estimates (Fig.1).

171

172

173 **Fig. 1** Scheme of the first cycle of the long-term breeding schemes. In the final of each scheme, a parental group was selected
174 for recycling and compound the next cycle that works independently

175

176 Traditional (Trad) and drift (Drift) schemes were conducted by the SSD method from F_2 to F_6 generation. Each
177 generation lasted about 180 days, totaling four years for each cycle. These two schemes differed on new parental group
178 selection, where they were selected based on the best phenotypic values (Trad) and a random sample (Drift). For breeding
179 schemes with GS, the line fixation phase was conducted by the rapid generation advanced (RGA) method. The RGA method
180 is an SSD method carried out in greenhouses or screenhouses to accelerate and shorten the growth cycle (~ 90-100 days).
181 Collard et al. (2017) present a good review of the RGA method. Below are details about each breeding scheme, along with
182 how GS was applied:

- 183
- GS_{F_2} : GS was performed in the F_2 stage, where all plants were genotyped. Individuals with the best GEBV
184 in F_2 were selected (24 or 48), regardless of progenies, to compound the next cycle (one year per cycle).
  - $GS_{F_2S_{F_4}}$: GS was performed in the F_2 stage, and genotypes with the best GEBV within each progeny were
186 selected for the next generation (F_3). So, 230 F_3 plants from the selected F_2 plant were conducted until F_4 .
187 In F_4 , individuals with the best phenotypic value were selected, regardless of progenies, to compound the
188 next cycle (2 years per cycle).
  - GS_{F_4} : GS was performed in the F_4 stage, where all plants were genotyped. Individuals with the best GEBV
189 in F_4 were selected (24 or 48), regardless of progenies, to compound the next cycle (2 years per cycle).
- 190

- GS_FSB: GS was performed in the F₂ stage, and the two individuals with the best GEBV within each progeny were selected and crossed to generate 230 F_{1seg} segregating plants. These plants were conducted until F₄, and individuals with the best phenotypic value were selected, regardless of progenies, to compound the next cycle (2.33 years per cycle).
- GS_FSD: GS was performed in the F₂ stage, and the best individuals (10%) based on GEBV were selected to calculate the Euclidean genetic distance between them. Within each progeny, the most divergent sibs were crossed to generate 230 F_{1seg} segregating plants. These plants were conducted until F₄, and individuals with the best phenotypic value were selected, regardless of progenies, to compound the next cycle (2.33 years per cycle).

The average genetic value, genetic value of the best genotype, additive genetic variance, and prediction accuracy were calculated for each breeding cycle. The prediction accuracy was calculated as a Pearson correlation between true genetic values and GEBV. For Trad and Drift schemes, the prediction accuracy was computed as the square root of heritability (h^2). Furthermore, the response to selection was calculated following the equation:

$$RS = \frac{X_i - X_0}{t}$$

where RS is the relative response to selection to the previous cycle; X_i is the genetic value mean of the parental group in the i cycle; X_0 is the genetic value mean of the previous parental group; and t is the breeding cycle time.

Training sets update scenarios

The influence of updating the TS on long-term GS breeding schemes was examined by tested different strategies to update this. The crossing block was fixed at 48 parental lines for each cycle and the GS_{F2}S_{F4} scheme used as the breeding strategy. As benchmark strategies, we used Trad and Drift schemes. Below are details about the updating scenarios:

- TSC₀: the TS was composed of 1,530 individuals. It was built just one time and used for all cycles, without updates. Markers effects were used to predicted selection candidate genotypes in the future cycles.
- TSC_N: TS was initially composed of 390 individuals. Each new cycle, a new TS was built with 13 random individuals within each F₄ progeny, totaling 390 individuals. Marker effects were estimated in each cycle and used to predicted selection candidate genotypes in the next cycle.

- 220   •   TS_{ALL}: TS was initially composed of 390 individuals. In every cycle, a new group was added consisting of
221   390 individuals (13 random individuals within each F₄ progeny). The TS thus increased by 390 individuals
222   each cycle (390, 780, 1170, ..., 3,900). Marker effects were estimated in each cycle and used to predicted
223   selection candidate genotypes in the next cycle.
- 224   •   TS_{GPO}: TS was composed of three sets of 390 individuals, one set per generation (grandparents, parents,
225   and offspring). Every cycle, the newest set, selecting 13 randomly individuals within each F₄ progeny
226   totaling 390 individuals, was included and the oldest one removed. Markers effects were estimated in each
227   cycle and used to predicted selection candidate genotypes in the next cycle.

228

229   Finally, only for the TS_{ALL} scenario, different TS sample sizes (0, 390, 780, and 1170) were simulated to add into
230   the TS every breeding cycle (individuals phenotyped and genotyped), aiming to recalibrate the marker effects and keep the
231   GS accuracy at satisfactory levels for more generations.

232   Each strategy to update the TS was simulated over 20 breeding cycles and replicated 40 times. Similarly to the
233   breeding schemes, the population mean, the genetic value of the best genotype, additive genetic variance, prediction accuracy,
234   and response to selection were calculated for each cycle.

235 Results

236

237 Influence of the breeding schemes and number of parental lines

238

239 The start point (intercept) of 24 and 48 parental lines was equal as the same burn-in was used for both (48). Then,
240 each parental group size was conducted independently in the further scenarios (Fig.2). In this context, GS-based methods
241 showed similar performances regardless of the number of parents, concerning population mean (genetic mean), the best
242 genotype performance, accuracy, and additive genetic variability (Fig.2a and 2b). Conversely, using 24 parents, the
243 population plateau was reached earlier for the traditional SSD than using 48 parents. GS_FSb and GS_{F2}S_{F4} were the best Gs-
244 based methods for performance indicators for both parental size scenarios, reaching the higher population mean after eight
245 breeding cycles (Fig.2).

246 Our results revealed that additive variance reduced over the breeding cycles for all breeding schemes regardless of
247 the number of parental lines (Fig.2c). Also, using 48 parental lines showed a slightly higher value of additive variance over
248 the cycles than 24 parental lines. Furthermore, the drift effect on the genetic variability was much more pronounced in 24
249 parents, which can vanish the genetic variability and genetic gains over the cycles (Fig.2c). On the other hand, all GS breeding
250 schemes revealed a reduction of accuracy across the breeding cycles (Fig.2d). Indeed, accuracy values in GS schemes
251 displayed negative values from the eighth cycle for 24 parentals, though for 48 parentals, only GS_FSd and GS_FSb showed
252 negative values from the eighth and tenth cycle, respectively.

253

254

255 **Fig. 2** Population mean (a), the best genotype (b), additive variance (c), and accuracy of selection (d) over 20 long-term
256 breeding schemes using two sizes of parental lines (N=24 and N=48). Each colored line represents a breeding scheme.

257

258 Concerning the response to selection after a defined period (ten years) of breeding, which is much more realistic
259 because each method has a different length, the GS_{F2}S_{F4} method outperformed the others, and specifically, the traditional
260 SSD the advantage of using that method was higher than 93% (Fig.3). GS_{F2}S_{F4} outperformed all the others among the GS
261 methods, being 44% better than fast recycling methods, such as GS_{F2}, highlighting the trade-off between the number of cycles
262 and the importance of updating the training sets. Moreover, the use of 24 parents was slightly better than 48, which, from our

perspective, perhaps reflects stochastic variation or does not compensate for the risks due to the drift. Therefore, for further comparisons, we considered 48 parents and the $GS_{F_2S_{F_4}}$ breeding scheme.

Fig. 3 Genetic gains after ten years of breeding using different selection schemes and two sizes of parental lines (N=24 and N=48.

Effect of training set update

After the breeding scheme evaluation, different ways to update the TS were compared, re-estimating the marker effects, to keep GS performances satisfactory in long-term breeding schemes. Overall, the updating methods (TSC_N , TS_{ALL} , and TS_{GPO}) produced similar responses in terms of accuracy over cycles and outperformed the TS_0 (no updates), which presented a steep decline after just two to four breeding cycles (Fig.4). Differences negligible among the updated scenarios, so TS_{ALL} was considered the best due to ease of implementation and a more stable trend over cycles. Also, if for any reason experiments were missed in a certain year and it was not possible to update the TS, TS_{ALL} might buffer and keep the accuracies at higher levels due to its size. Therefore, for the final comparisons, the latter scenario for updating TS was considered.

Fig. 4 Accuracy of selection over 20 breeding cycles via $GS_{F_2S_{F_4}}$ scheme considering 48 parental lines using different methods to update the training set. Each colored line represents an updating training set method.

Finally, we compared different sample sizes to update the TS every breeding cycle. Overall, by adding sample sizes of 800 (~10% of the breeding program the total breeding program population size after RGA) or more every cycle it is possible to keep the GS accuracy at satisfactory levels for more cycles (up 8 or 10), outperforming the TS_0 (no updates) or small sample sizes, such as 390 (Fig.5). After 8 or 10 cycles, there is a steady decay even for the best methods, which might be due to the lack of additive genetic variability in the population (Fig.2c).

Fig. 5 Accuracy of selection over 20 breeding cycles via $GS_{F_2S_{F_4}}$ scheme considering 48 parental lines, the TS_{ALL} method, and different sample sizes to update every cycle's training set. Each colored line represents an updating training sample size.

293 Discussion

294

295 Breeding schemes and number of parental lines

296

297 GS can speed up breeding schemes and increase genetic gain (Jannink et al. 2010; Varshney et al. 2017). However,
298 to maximize the breeding program efficiency, it is essential to decide on how and at which stage to apply GS. In this study,
299 different long-term breeding strategies for implemented GS were tested based on a simulated rice breeding program.
300 Furthermore, a new approach is proposed for using GS during the line fixation stage. In this approach, two individuals (best
301 ones - GS_FSb, and most divergent - GS_FSD) within each F_2 progeny are identified and crossed to generate a new
302 recombination event. These progenies are then fixed using the RGA method until F_4 to select new parents for the next
303 recurrent cycle.

304 Firstly, these results showed that the number of parental lines to compose the crossing block influences all genetic
305 parameters over breeding cycles. This influence was expected due to the relationship between the number of parental lines
306 with the effective population size (N_e) and the selection intensity. In the situations examined here, the number of crosses
307 (crosses = 30) was the same in both scenarios (24 and 48 parentals). However, a lower number of parents increases the number
308 of crosses with the same parental, which reduces the final N_e , and consequently, the genetic variance. Also, a smaller N_e
309 increases the effect caused by the drift (Hartl and Clark 2006), which brings a reduction in genetic variance in long-term
310 breeding cycles. The Drift scheme acted as one of the benchmarks for this purpose. In the Drift scheme, using 24 parentals
311 showed an additive genetic variance reduction over breeding cycles, whereas using 48 parental lines showed higher resilience
312 to this effect. Furthermore, the N_e is a crucial factor for breeders as programs with a smaller N_e will become inbred faster
313 and show no further response to selection (Cobb et al. 2019).

314 Several studies report the increase of response to selection in breeding schemes that use GS (Gorjanc et al. 2018;
315 Muleta et al. 2019). In this study, long-term breeding schemes including GS showed values between 200% (GS_{F_2}) and 608%
316 (GS_{FSD}) higher than phenotypic selection (Trad) in the first breeding cycle for response to selection per year (Fig.2c). This
317 increase was principally due to the shortening of the breeding cycle with the implementation of GS. However, as the breeding
318 cycles progress, they showed a rapid decrease in response to selection. Long-term breeding schemes with GS have been
319 observed to exhaust the genetic variance faster than phenotypic selection because of the tendency of GS to select closely-
320 related individuals, thereby accelerating inbreeding (Jannink 2010). These results also show a greater decline in genetic
321 variance under GS breeding schemes compared to a traditional breeding scheme, and consequently, their response to selection

322 decays faster (Fig.2). However, this effect can be mitigated and offset by preserving a larger N_e (48 parent scenario), reducing
323 the genetic variance consuming by GS over breeding cycles and permitting long-term genetic gain.

324 Genomic selection also increases genetic gain through shortening the breeding cycle, since phenotypic information
325 is not required to select the best genotypes (Jannink et al. 2010; Lorenz et al. 2011; Crossa et al. 2017). Thus, the breeder can
326 use methods to shrink the growth cycle and increase generations per year. Nevertheless, to shorten the growth cycle, the mode
327 of reproduction of the species associated with the breeding method must be considered to reach the desirable effect (Brown
328 et al. 2014). Rice does not currently have scalable and feasible procedures to generate doubled-haploid populations, so in this
329 study the RGA method was used to advance generations and increase endogamy in our GS breeding schemes. This method
330 reduces the rice growth cycle to 90-100 days, depending on the genotype (Collard et al. 2017). Differences in the number of
331 generations in the breeding schemes examined led to differences in the total cycle time, with the Trad scheme taking four
332 years to complete each cycle, whereas the GS_{F_2} lasted only six months. The $GS_{F_2}S_4$ was observed to yield 93% higher genetics
333 gain than the Trad scheme and 44% better than GS_{F_2} in terms of genetic gains in ten years of breeding (Fig.3). Another
334 interesting point is the best scenarios are composed of 2-stages selection, whereas all others are 1-stage or combined. This
335 result shows that besides longer, 2-stages, combining GS and phenotypic selection, compensate rather than apply rapid
336 cycling, using only GS at the stage-1 for recycling parents. Therefore, our results reinforce that to obtain sustainable long-
337 term genetic gain in long-term breeding schemes is crucial to consider a balance between genetic gain, accuracy, time, and
338 genetic variance over the breeding cycles (Jannink et al. 2010). In this context, some questions arise for further studies: do
339 we get progressive increases in effectiveness with 3, 4, 5 stages of selection? E.g., GS at F_2 + GS at F_4 + Phenotypic at F_6 ?
340 Do we need different types of selection? GS and phenotypic are used here (2 different types). Would the same response be
341 shown with, e.g., two rounds of GS?

342 Few studies explore the best phase or stage to apply GS to maximize the genetic gain in long-term breeding schemes
343 (Gorjanc et al.; Bassi et al. 2016). A new method was examined here employing GS in the line fixation stage in a rice breeding
344 program. In this scheme, a cross was simulated between two individuals selected by GS in the F_2 generation (GS_{FSb} and
345 GS_{FSd}) considering the best GEBVs (GS_{FSb}) and most divergent between best GEBVs (GS_{FSd}). Among the GS-based
346 schemes, the GS_{FSb} reached the highest population mean over the breeding cycles (Fig.2a). The strategy of crossing the
347 best individuals based on GEBV probably enriched the parents in which major-effect alleles were fixed since these individuals
348 may have the same genotype for these alleles (Zhong et al. 2009; Jannink 2010). Also, loci with minor effects, but important
349 for the trait, could be divergent between these individuals, and recombination between these loci may increase the probability
350 of a future line with this favorable allele in homozygosity. On the other hand, when we crossed the most divergent individuals,

major loci may have different alleles in each genotype. The probability of achieving a future line with the favorable allele in this locus is therefore lower, and indeed GS_FSd had one of the worst results considering genetic value over breeding cycles.

Even though GS_FSB has shown the highest population mean over breeding cycles, its values were close to the GS_{F₂S_{F₄}} scheme. However, further considering genetic gain, it is crucial to regard the practicality to realize each breeding scheme in real conditions. Making a new cross between two individuals from an F₂ progeny brings additional labor and costs. Hence, since the difference between GS_FSB and GS_{F₂S_{F₄}} is negligible, we consider the GS_{F₂S_{F₄}} the best scenario to employ GS.

Effect of training set update strategies

The second objective of this study was to identify a better strategy to update the TS to guarantee a higher genetic gain and avoid the decline of accuracy across long-term breeding cycles. As mentioned, all genomic breeding schemes showed a reduction of accuracy over breeding cycles (Fig.2d). After the first breeding cycle, the prediction accuracy showed a substantial decline, and by the last breeding cycles GS was practically random, with the accuracy was close to zero. This situation can hinder the selection of the best genotypes, and consequently, the genetic mean of the population does not increase and may sometimes decrease (Jannink 2010; Neyhart et al. 2017). This observation is consistent with several other studies showing the reduction of accuracy of genomic prediction in long-term breeding schemes (Jannink 2010; Müller et al. 2017; Muleta et al. 2019). Working with GS optimization in a sorghum breeding program, Muleta et al. (2019) verified that the prediction accuracy declined over breeding cycles, especially for oligogenic traits. The latter study also showed that updating the TS slowed the decline in accuracy over breeding cycles but did not prevent this entirely. In these results, all scenarios involving GS with TS update reached the best performance for population mean compared to phenotypic selection, at least until the sixth breeding cycle. In initial cycles, TSC₀ had the highest population mean values, which shows that a larger TS delivers a higher accuracy (Cericola et al. 2017; Norman et al. 2018) because a larger number of allelic observations is required to accurately predict a small effect QTL (Gilmour 2007). However, the TSC₀ displayed a greater decrease in accuracy across breeding cycles since that this scenario did not update the TS (Fig.3). This accuracy reduction may promote a lower population mean than other updating scenarios after the fourth cycle. Amongst scenarios that did include updating of the TS, the TSC_N scenario was unstable and slightly worse than TS_{GPO} or TS_{ALL}. These results show that it is crucial to update the TS keeping high the genetic relationship between the TS and selection candidate genotypes.

379 Another question about updating the TS is how and how many to select the individuals to add them to this. Neyhart
380 et al. (2017) studied different updating TS methods in a barley long-term breeding program. Updating the TS decreases the
381 decline in accuracy due to updating of LD between markers and QTL, but nonetheless accuracy continues to decline across
382 breeding cycles. Furthermore, it was found that the prediction accuracy was slightly higher when the TS contained only the
383 most recent data, whereas adding the best individuals from each cycle in long-term breeding schemes resulted in the highest
384 genetic gain (Neyhart et al. 2017). Our results revealed that updating methods had a similar genetic variance decreasing across
385 breeding cycles, with the lowest value for TS_{GPO} in the tenth breeding cycle.

386 All updating methods avoided an abrupt decline in prediction accuracy across breeding cycles, which corroborates
387 previous studies (Neyhart et al. 2017; Muleta et al. 2019). In the first cycle, TSC_0 accuracy presented the highest accuracy
388 (0.69), which can be explained from the TS size effect (Müller et al. 2017; Zhang et al. 2017; Norman et al. 2018). However,
389 over breeding cycles, TSC_0 accuracy presented the highest decline displaying negative values from the eighth cycle. TSC_0
390 scenario did not update the training set, and selection candidate genotypes were selected only using the initial marker effects.
391 Hence, the genetic relationship between the TS and selection candidate genotypes decreased across breeding cycles, resulting
392 in accuracy decline (Lorenz and Smith 2015).

393 When considering the maintenance of accuracy and genetic gain across breeding cycles, a balance needs to be
394 maintained between the genetic relationship between the TS and selection candidate genotypes and accurate estimates of LD
395 between markers and QTL. However, in long-term recurrent schemes, new recombination events occur at each breeding
396 cycle, which causes a breakdown of LD between markers and QTL, consequently decreasing accuracy (Jannink 2010; Müller
397 et al. 2017). Investigating the marker density effect on maize long-term breeding schemes, DoVale et al. (2021) pointed that
398 high-density marker panels reduce an abrupt decline of accuracy across breeding cycles and, consequently, they can deliver
399 higher genetic gain in long-term genomic recurrent schemes. The lower accuracy of low-densities marker panels in our long-
400 term breeding schemes employing GS may be explained by a lower probability of LD between the marker and the QTL when
401 there are fewer markers, which results in a smaller fraction of explained genetic variation (Solberg et al. 2008). Hence, high-
402 density marker panels could help to increase the probability of finding markers in LD with the same QTL across different
403 cycles (Daetwyler et al. 2010).

404 This study used a SNP chip containing 1,000 markers to simulate the SNP-panel optimized for the IRRI irrigated
405 breeding program (Arbelaez et al. 2019). Low-density SNP panels are attractive for GS due to the lower price per sample and
406 have been explored by several groups (Vallejo et al., 2018; Arbelaez et al., 2019; Al-Tobasei et al., 2021). In this context,
407 our results showed that updating the TS with an affordable size reduced the accuracy decline over breeding cycles, presenting

408 a lower decay than other studies (Neyhart et al. 2017; Muleta et al. 2019), even using a low-density marker set. Updating the
409 TS promoted more accurate estimates of LD between markers and QTL, since, in long-term breeding schemes, recombination
410 between marker and QTL causes an LD decrease, whereas selection and drift act to generate new LD or tighten the LD
411 between closely linked loci (Hill and Robertson 1968; Habier et al. 2007; Lorenz et al. 2011). This outcome is crucial as SNP
412 chips with few markers may permit breeding programs with limited resources to use GS in their pipeline. However, it seems
413 to be not so essential consider previous cycles to estimate the LD patterns between markers and QTL since TS_{ALL} and TS_{GPO}
414 obtained as similar to those obtained via TSC_N , which counts only the last breeding cycle. However, as described earlier, we
415 considered TS_{ALL} a straightforward and stable method. Furthermore, it might buffer and keep the accuracies at good levels in
416 cases of missing a year of trials.

417

418 **Conclusion**

419

420 In long-term breeding schemes employing genomic selection may deliver higher genetic gain over breeding cycles
421 and over a given period. However, it is crucial to determine where and when to implement the genomic selection since GS
422 can produce lower genetic gain than phenotypic selection, depending on the strategy. Applying genomic selection in an F_2
423 progeny followed by a phenotypic selection of new parentals in F_4 produced the highest genetic gain across breeding cycles.
424 Moreover, updating the training set allowed better maintenance of accuracy selection over recurrent breeding cycles. Adding
425 a new and proper amount of information every cycle into the training set allows re-estimation of the marker's effects. In other
426 words, updating the LD between markers and underlying QTL guarantees the highest genetic gain over recurrent selection
427 cycles.

428

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431

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538

Figures

Figure 1

Scheme of the first cycle of the long-term breeding schemes. In the final of each scheme, a parental group was selected for recycling and compound the next cycle that works independently

Figure 2

Population mean (a), the best genotype (b), additive variance (c), and accuracy of selection (d) over 20 long-term breeding schemes using two sizes of parental lines (N=24 and N=48). Each colored line represents a breeding scheme.

Figure 3

Genetic gains after ten years of breeding using different selection schemes and two sizes of parental lines (N=24 and N=48).

Figure 4

Accuracy of selection over 20 breeding cycles via GSF2SF4 scheme considering 48 parental lines using different methods to update the training set. Each colored line represents an updating training set method.

Figure 5

Accuracy of selection over 20 breeding cycles via GSF2SF4 scheme considering 48 parental lines, the TSALL method, and different sample sizes to update every cycle's training set. Each colored line represents an updating training sample size.

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