Genomic selection strategies to increase genetic gain in tea breeding programs

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Abstract

Genomic selection (GS) can improve the efficiency of tea breeding compared to phenotypic selection (PS) by shortening the generation interval, increasing selection accuracy, and shortening the duration of the entire breeding program, especially at early stages. Tea (Camellia sinensis (L.) O. Kuntze) is mainly grown in low- to middle-income countries (LMIC) and is a global commodity. Breeding programs in these countries face the challenge of increasing genetic gain because the accuracy of selecting superior genotypes is low and resources are limited. Recurrent phenotypic selection has traditionally been the primary method for developing improved tea varieties and can take over 16 years. Therefore, the main objective of this study was to investigate the potential of implementing GS in tea breeding programs to speed up genetic progress despite the low labour costs in LMIC. We used stochastic simulations to compare three GS breeding programs with a commercial PS program over a 40-year breeding period. All GS breeding programs achieved higher genetic gains compared to PS. Seed-GSconst, in particular, proved to be the most cost-effective strategy for introducing GS into tea breeding programs. It introduces GS at the nursery stage, thereby increasing the predictive accuracy at the early stage of the breeding program. It also shortens the duration of the entire breeding program by three years and reduces the generation interval to two years. Our results indicate that GS is a promising strategy to improve genetic gain per unit time and cost in tea breeding programs.

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Core ideas

- Genomic selection could result in 1.6 times greater genetic gain than phenotypic selection in tea breeding programs.
- All genomic selection strategies achieved higher genetic gains per unit time and cost than phenotypic selection method.
- Seed-GSconst is the most cost-effective strategy for introducing GS into tea breeding programs.

Abstract

Genomic selection (GS) can improve the efficiency of tea breeding compared to phenotypic selection (PS) by shortening the generation interval, increasing selection accuracy, and shortening the duration of the entire breeding program, especially at early stages. Tea (*Camellia sinensis* (L.) O. Kuntze) is mainly grown in low- to middle-income countries (LMIC) and is a global commodity. Breeding programs in these countries face the challenge of increasing genetic gain because the accuracy of selecting superior genotypes is low and resources are limited. Recurrent phenotypic selection has traditionally been the primary method for developing improved tea varieties and can take over 16 years. Therefore, the main objective of this study was to investigate the potential of implementing GS in tea breeding programs to speed up genetic progress despite the low labour costs in LMIC. We used stochastic simulations to compare three GS breeding programs with a commercial PS program over a 40-year breeding period. All GS breeding programs achieved higher genetic gains compared to PS. Seed-GSconst, in particular, proved to be the most cost-effective strategy for introducing GS into tea breeding programs. It introduces GS at the nursery stage, thereby increasing the predictive accuracy at the early stage of the breeding program. It also shortens the duration of the entire breeding program by three years and reduces the generation interval to two years. Our results indicate that GS is a promising strategy to improve genetic gain per unit time and cost in tea breeding programs.

Abbreviations

ACT, Advanced Clonal Trial stage; ECT-GS, Elite Clonal Trials Genomic Selection Breeding Program; ECT, Elite Clonal Trial stage; GS, Genomic selection; LMIC, low- to middle-income countries; PS, Phenotypic selection; Seed-GSconst, Constrained Seedlings Genomic Selection Breeding Program; Seed-GSunconst, Unconstrained Seedlings Genomic Selection Breeding Program

1 Introduction

Tea (*Camellia sinensis* (L) O. Kuntze) is mainly grown in tropical and subtropical regions in low- to middle-income countries (LMIC) (Han, Li, and Ahammed 2018). It is an important crop for the economies of these countries as it provides a source of income for many smallholder farmers and those employed in tea processing companies (Mukhtar and Ahmad 2000). Additionally, the tea growing areas (mainly rural) have benefitted from improved social infrastructure such as good road networks, schools and hospitals. All tea varieties currently grown in the world originated in India and China and were either directly or indirectly imported from these two countries to other countries (Meegahakumbura et al. 2016). The world population is steadily increasing and is expected to reach 9 billion people by 2050 (Perroy 2015) leading to an increase in demand for food and beverages (Valin et al. 2014). Conventional tea breeding is well established in the major tea growing countries such as India, China and Kenya and has led to the development of many superior varieties (Meegahakumbura et al. 2016). Tea varieties developed through breeding have superior yield, quality and are resistant to drought compared to seedling genotypes (Corley and Tuwei 2018). However, to sustain long-term tea production and the increasing demand for tea, tea breeders need to continuously bring new improved varieties to the market. The objectives of tea breeding vary among major tea-growing countries, depending on local needs. However, the most important breeding objective is to develop varieties with high yield and improved quality (colour, aroma, taste and mouthfeel) (Kamunya et al. 2012, Mondal 2014). Currently, tea productivity is seriously threatened by climate change, which is already causing yield losses (Gunathilaka, Smart, and Fleming 2017, Sitienei, Juma, and Opere 2017), and decreased quality (Han et al. 2017). Climate change has led to extreme and unpredictable weather patterns, resulting in longer dry spells, heavy rainfall, more hail, and higher temperatures (Marx, Haunschild, and Bornmann 2017, Batley and Edwards 2016). Additionally, the changing climate has led to increased attacks of pests and diseases. Therefore, effective tea breeding strategies are needed to

develop high-yielding and high-quality tea varieties that are also tolerant to biotic and abiotic stresses (Mondal 2011, Muoki et al. 2020).

Tea breeding programs use recurrent phenotypic selection (PS) to select the best individuals based on phenotypic values estimated from the *per se* performance of clones in clonal evaluation trials. This involves the creation of genetic variation through crossing, followed by many years of recurrent selection aimed at determining the genetic value of promising genotypes, leading to the identification of new parents for crossing and the release of commercial varieties to farmers. In the initial phase of the breeding program, new genotypes are first tested as seedlings in single bush (preliminary) trials. Then, genetically identical teas (clones) are generated from selected seedlings through clonal propagation (cuttings), allowing genotypes to be tested in clonal plots in multiple replications, at multiple locations, and in multiple years (Carr 2018). PS has been quite successful in delivering improved tea varieties over many years (Mondal 2014). However, this is a time-consuming process as it takes 16 years to develop a tea variety from cross to release (Figure 1).

In modern times, plant breeding has started to move from complete reliance on PS to genomic-assisted selection due to improved molecular biology and high-throughput genotyping technologies (Leng, Lübberstedt, and Xu 2017). Quantitative trait loci (QTL) mapping (Kamunya et al. 2010, Malebe et al. 2021) and association mapping (Jin et al. 2016, Fang et al. 2021) have been tested in the genetic improvement of tea, and several QTLs associated with yield and quality have been identified. However, these QTLs have not been successfully applied in the genetic improvement of tea (Xia et al. 2020), since marker-assisted selection (MAS) methods do not account for the effects of minor QTLs influencing quantitative traits. Only a few major QTLs have been identified in tea (Fang et al. 2021, Yamashita et al. 2020), while the minor QTLs which influence important quantitative traits are ignored (Heffner, Sorrells, and Jannink 2009). Many complex traits, including yield, quality and drought tolerance are controlled by many genes with small effects, and therefore MAS is of limited use due to low statistical power to detect individual QTLs (Bernardo and Yu 2007).

Genomic selection (GS) uses all available (genome-wide) markers to predict breeding values (Meuwissen, Hayes, and Goddard 2001) and offers a great potential for identifying the best parents for crossing and superior clones for variety development in tea breeding programs. Genomic estimated breeding values (GEBVs) are calculated by summing marker effects that may or may not be in linkage disequilibrium with one or more QTLs across the entire genome (Bernardo and Yu 2007). GS uses a prediction model that is first trained using a population of genotyped and phenotyped individuals. The trained model is then used to predict GEBVs of selection candidates with genotyping information but no phenotypes. By then correlating estimated phenotypic values based on GEBVs with the actual phenotypic data, it is possible assess the accuracy of the genomic selection model (Heffner, Sorrells, and Jannink 2009, Meuwissen, Hayes, and Goddard 2001). For example, Lubanga, Massawe, and Mayes (2021) investigated the potential use of GS to improve tea quality. They reported higher prediction accuracies for all genomic prediction models compared to the pedigree model. Similar findings were also reported by Yamashita et al. (2020), who also investigated the potential of GS for improving tea quality. They found moderate prediction accuracies for the 6 GS models tested. In a tea breeding program, GS can be used in four ways:

1. to reduce the generation interval as new parents can be selected at the Seedlings stage. Genotypes in the nursery can be genotyped. Superior genotypes can then be selected based on GEBVs and planted in the germplasm garden for population improvement (Figure 2 and Figure 3),
2. to increase the accuracy of selecting superior tea genotypes at the Seedlings stage,
3. to increase the selection intensity. More seedlings can be genotyped at the nursery stage and promising ones predicted accurately compared to PS,
4. to shorten the entire breeding program by eliminating some of the stages in a breeding program to enable faster release of varieties (Figure 2 and Figure 3).

However, the implementation of GS in LMIC faces limitations. In most of these countries, the cost of phenotypic selection is much lower compared to Europe and North America because the local population provides cheap labour. In addition, most breeding programs have limited investment budgets for conducting research. In addition, there is a lack of qualified personnel who are trained and understand the technique of GS and its practical implementation in breeding programs. The implementation of GS in tea breeding should therefore take into account the particular challenges of these programs.

Plant breeders have traditionally relied on field trial experiments to inform their decisions (Rutkoski et al. 2015). However, evaluating these field trials takes a long time and is also expensive (Wang and Wolfgang H 2007). Simulations are useful in determining the best breeding strategies and can also be used to study the genetic gain, predictive accuracy, and cost-effectiveness of GS under different scenarios (Gaynor, Gorjanc, and Hickey 2021). Stochastic simulations have been conducted for many crops, including wheat (Gaynor et al. 2017), clonally propagated crops (Werner et al. 2020), maize (Powell et al. 2020), sorghum (Muleta, Pressoir, and Morris 2019), and trees (Iwata, Hayashi, and Tsumura 2011). However, to our knowledge, no simulation studies have been published integrating GS into tea breeding programs to investigate their feasibility and long-term outcomes.

This study aims to test the feasibility of implementing GS into a tea breeding program using stochastic simulations. To this end, we used a PS breeding program as a baseline in which the number of crosses, seedlings, replicates, and locations mimicked an actual commercial tea breeding program (Unilever Tea Kenya). We estimated variance parameters from real field data. We developed three new breeding programs based on the PS breeding program that integrated GS. Using simulations, we then compared the baseline PS breeding program with three GS breeding programs. All simulations were performed using AlphaSimR (Gaynor, Gorjanc, and Hickey 2021). Our objectives in this study were (i) to investigate the potential of implementing GS in tea breeding programs despite the limited breeding program resources and low labour costs in LMIC, (ii) to compare different strategies for

implementing GS in tea breeding programs at the same cost as PS, (iii) to investigate whether shortening the tea breeding generation interval and the entire breeding program duration by incorporating GS in breeding programs leads to higher genetic gains. In addition, we also evaluated two different strategies of parent selection.

2 MATERIALS AND METHODS

We used stochastic simulations to evaluate the possibility of implementing GS in tea breeding programs. We compared a PS breeding program and three breeding strategies incorporating GS. We subdivided the materials and methods section into simulation of the founder genotype population and simulation of the breeding programs.

We simulated the founder genotype population as follows:

i. Genome simulation: a genome sequence was simulated for a hypothetical diploid tea species (*Camellia sinensis* (L) O. Kuntze).
ii. Simulation of founder genotypes: the simulated genome sequences were used to generate a base population of 20 diploid founder genotypes.
iii. Simulation of genetic values: a single trait representing yield was simulated for all founder genotypes by summing the additive effects with 2400 quantitative trait nucleotides (QTN).
iv. Simulation of phenotypes: the phenotypes of all founder genotypes were simulated by adding random error to the total genetic value of the tea genotypes.

We simulated the breeding programs as follows:

i. Recent (burn-in) breeding phase: a PS breeding program for tea was simulated for a period of 40 years (burn-in) to provide a shared starting point for the future breeding phase.
ii. Future breeding phase: three different GS breeding programs were simulated and compared to the PS breeding program for an additional 40 years of breeding.

2.1 Simulation of the founder genotype population

2.1.1 Genome simulation

We simulated a genome sequence with 15 pairs of chromosomes for a hypothetical diploid tea species (*Camellia sinensis* (L) O. Kuntze). We then assigned a physical length of 10^8 base pairs and a genetic length of 1 Morgans to these chromosomes. We generated the chromosome sequences using the Markovian coalescent simulator (MaCS) (Chen, Marjoram, and Wall 2009) implemented in AlphaSimR (Gaynor, Gorjanc, and Hickey 2021). We estimated recombination rate as the ratio between genetic length and physical genome length (i.e., 1 Morgans / 10^8 base pairs = 10^{-8}). We set the per-site mutation rate to 2.5 x 10^{-8} mutations per base pair. We set the effective population size (Ne) to 100, as described by Werner et al. (2020).

2.1.2 Simulation of founder genotypes

We used the simulated genome sequences to generate a base population of 20 diploid founder genotypes in Hardy-Weinberg equilibrium. These genotypes were formed by randomly sampling 15 chromosome pairs per genotype. A set of 160 biallelic quantitative trait nucleotides (QTNs) and 600 single nucleotide polymorphisms (SNPs) were randomly selected along each chromosome, to simulate a quantitative trait that was controlled by 2400 QTN and an SNP marker array with 9000 genome-wide SNP markers.

2.1.3 Simulation of genetic values

We simulated genetic values for a single trait representing yield by summing the additive genetic effects at 2,400 randomly sampled QTN. We sampled additive genetic effects (a) from the standard normal distribution and scaled them to obtain an additive genetic variance of $\sigma_a^2 = 1$ in the founder population, as described in detail by Gaynor, Gorjanc, and Hickey (2021). The environmental effect represented the environmental component of the genotype by year (G x Y) interaction and was sampled for each year of the simulation from the standard normal distribution as described by Gaynor et al. (2017).

2.1.4 Simulation of phenotypes

We calculated the phenotypic values for yield by adding G x Y and random error to the additive genetic values. Therefore, the phenotypic value of genotype *i* grown in stage *k* of a breeding program in year *j* was calculated as;

$$y_{ij} = g_i + (gy)_{ij} + e_{ijk},$$

where g_i is the additive genetic value of genotype *i*; $(gy)_j$ is G x Y interaction effect associated with genotype *i* and year *j*; and e_{ij} is error associated with genotype *i*, year *j*, and stage *k*. The random error was sampled from the standard normal distribution with mean zero and an error variance σ_e^2 defined by the target level of heritability at each testing stage of the tea breeding program. In the founder population, we calculated the entry-mean values based on real data from Unilever Tea Kenya breeding program for narrow-sense heritability (h^2) at each of the breeding stages. The h^2 at the Seedling and PT stages was 0.05, 0.45 in the advanced clonal testing stage (ACT) and 0.65 in the elite clonal testing (ECT) stage. Narrow-sense heritabilities in later testing stages were higher because of the increased number of replicates per genotype. We calculated narrow-sense heritability as

$$\frac{\sigma_a^2}{(\sigma_a^2 + \sigma_{gy}^2/e + \sigma_e^2/er)},$$

where σ_a^2 is the additive genetic variance, σ_{gy}^2 is the G x Y interaction variance, σ_e^2 is the residual variance and e and r are the number of environments and replicates within each environment, respectively.

2.2 Recent (burn-in) breeding phase

We simulated a PS breeding program over a 40-year period (burn-in) to establish a common baseline for the future tea breeding phase. The structure of the PS breeding program was based on the Unilever Tea Kenya breeding program (Figure 1). A description of the Unilever Tea Kenya breeding program can also be found in Corley and Tuwei (2018). To fill the breeding pipeline and provide a

starting point for the burn-in phase, we performed 16 crossing and selection cycles prior to the burn-in phase. Each of these 16 cycles started with the same twenty founder genotypes in the crossing block to perform 100 bi-parental crosses and 100 pollinations per cross (10,000 crosses in total). Based on our experience, we assumed that 2,000 (approximately 20%) seedlings germinated and were grown in the nursery for one year. In the third year, seedlings were planted in the field as preliminary trials (PT), followed by a three-year evaluation period. Five hundred superior clones were selected and planted as advanced clonal trials (ACT) and yield data were recorded for 5 years. Forty high yielding clones were selected, advanced to the elite clonal trial (ECT) and yield data recorded for 6 years (Figure 1). Selection of new parents and best clones in each testing stage were based on phenotypic records. In the burn-in phase, the selection of new parents was done at the ECT stage in the year 16. Each year, we replaced the 5 genotypes in the crossing block with the oldest *per se* performance with new high yielding varieties from the ECT stage. The total duration of the PS breeding program was 16 years (Figure 1).

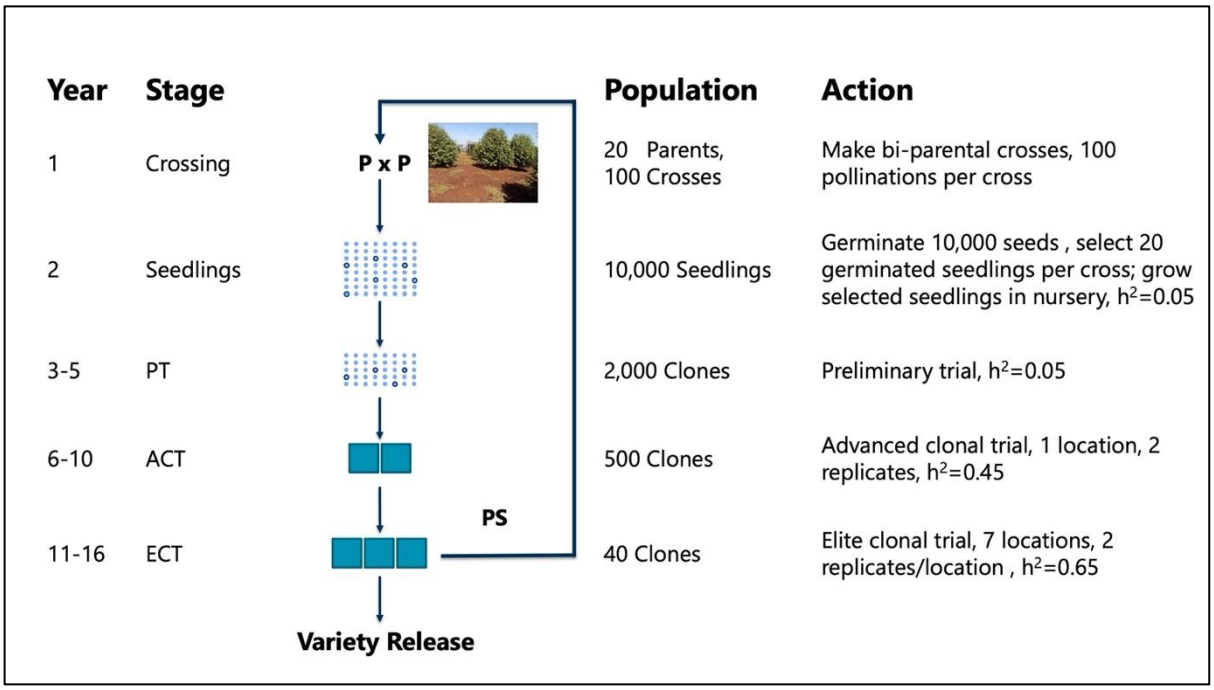

Figure 1. Schematic overview of the phenotypic selection breeding program (PS). This program is based on the commercial breeding program (Unilever Tea Kenya). The solid line represents the stage at which the 5 or 20 new parents are selected based on phenotypic information. PT, Preliminary Trial stage; ACT, Advanced Clonal Trial stage and ECT, Elite Clonal Trial stage.

2.3 Future Breeding Phase

We used the future breeding phase to evaluate the PS breeding program and the three GS breeding programs. We simulated each breeding program for an additional 40 years after the burn-in breeding phase to evaluate each program with an equivalent starting point. The three GS strategies were Seed-GSconst, Seed-GSunconst, and ECT-GS (see their description below). The GS programs replaced PS with GS at different stages of the PS breeding program. The costs of the three GS strategies were equalized to the estimated cost of the PS breeding program ($71,880). Table 1 shows the sizes and costs of the breeding programs. Equalization of operating costs in GS programs was done using the estimated costs of genotyping and reducing program sizes at different breeding stages. Briefly, the PT stage was eliminated for the Seed-GSconst and Seed-GSunconst programs, while the PT and ACT

stages were eliminated for the ECT-GS program. A summary of the key differences between the breeding programs can be found in Table 2. A complete schematic description of the programs is shown in Figures. 1, 2, 3. We assumed that the cost of genotyping per individual was $15 (http://techservicespro.com/test-locations/). Phenotyping costs were estimated based on the Unilever Tea Kenya breeding program.

Table 1. Summary of the tea breeding program sizes and annual costs of simulated breeding programs.

Breeding program	Number of parents	Seedlings	PT	ACT	ECT	Cost ($)
PS	20	2,000	2000	500	40	71,880
Seed-Gsconst	20	800	0	300	40	69,980
Seed-GSunconst	20	2,000	0	500	40	100,980
ECT-GS	20	800	0	0	90	72,970

PT, Preliminary Trial stage; ACT, Advanced Clonal Trial stage; and ECT, Elite Clonal Trial stage; PS, phenotypic selection breeding program; GS, genomic selection; Seed-GSconst, seedlings GS breeding program; Seed-GSunconst, seedlings GS breeding program with unconstrained budget; and ECT-GS, elite clonal GS breeding program.

We also compared two parent replacement methods for each strategy, namely:

1. replacing 25% of the parents after each breeding cycle,
2. replacing all the parents after each breeding cycle.

Table 2. Summary of the key differences between the four breeding programs.

Breeding Program	Parent selection stage	Number of parents	Generation interval / program duration (years)	Parent selection	Key features
PS	ECT	15 old, 5 best parents	16/16	Phenotype	Conventional breeding
Seed-GSconst	Seedlings	15 old, 5 new parents	2/13	GS	PT stage removed; 800 seedlings genotyped
Seed-GSunconst	Seedlings	15 old, 5 new parents	2/13	GS	PT stage removed; 2,000 seedlings genotyped
ECT-GS	Seedlings	15 old, 5 new parents	2/8	GS	PT and ACT stages removed; increased

					number of clones tested in ECT stage

PT, Preliminary Trial stage; ACT, Advanced Clonal Trial stage; and ECT, Elite Clonal Trial stage; PS, phenotypic selection breeding program; GS, genomic selection; Seed-GSconst, seedlings GS breeding program; Seed-GSunconst, seedlings GS breeding program with unconstrained budget; and ECT-GS, elite clonal GS breeding program.

2.3.1 Constrained Seedlings Genomic Selection Breeding Program (Seed-GSconst)

The Seed-GSconst program introduced genotyping and GS at the earliest Seedlings stage and eliminated the PT stage (Figure 2). Eight hundred (8 per family) seedlings from the 2,000 germinated seeds in the nursery were randomly selected for genotyping. Genomic selection was used to advance genotypes to the next stage and to select the best 5 or 20 genotypes to replace the oldest parents in the crossing block. The PT stage was eliminated to avoid three years of field testing, and hence, GS was used to advance the best 300 genotypes from the Seedlings stage to the ACT stage. Yield trials were recorded at the ACT stage for 5 years. Genomic selection was used to advance 40 promising clones to the ECT stage. The yield trials at the ECT stage were recorded for another 6 years. The Seed-GSconst program has a 2-year generation interval and lasts a total of 13 years, which is three years shorter compared to the PS breeding program.

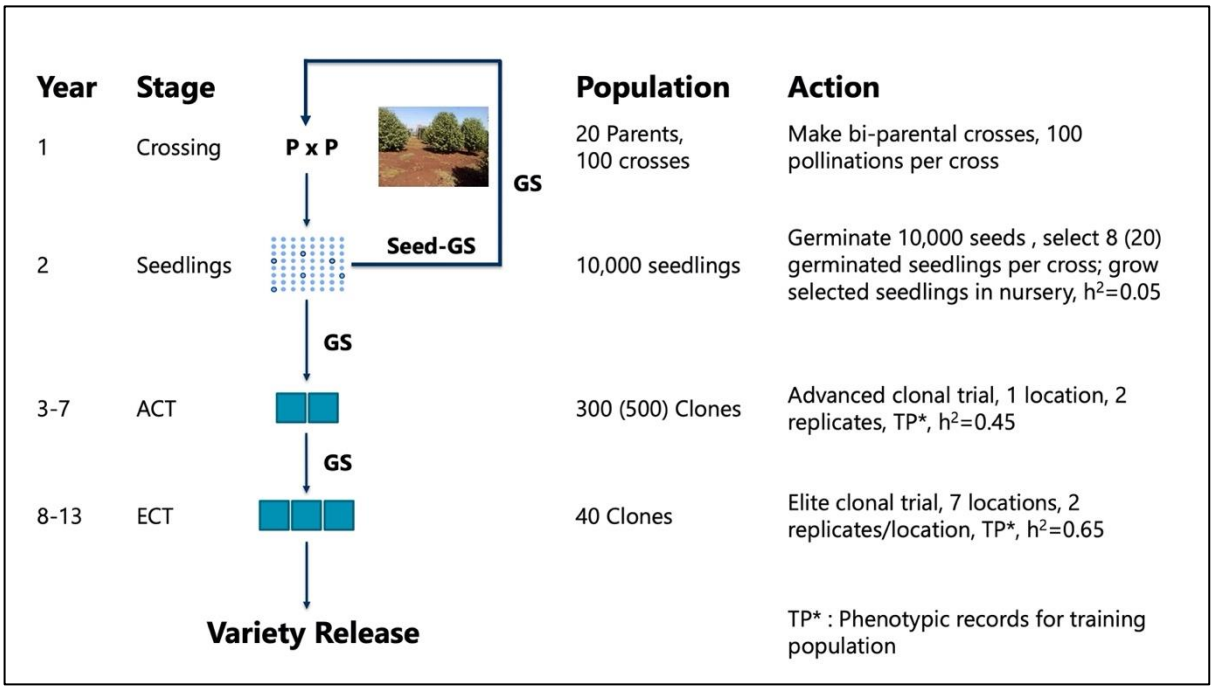

Figure 2. Schematic overview of the seedlings GS breeding program with constrained costs (Seed-GSconst) and unconstrained costs (Seed-GSunconst). The solid line represents the stage at which the 5 or 20 new parents are selected based on genomic prediction. The values outside parenthesis relate to Seed-GSconst and the values inside parenthesis relate to Seed-GSunconst. ACT, Advanced Clonal Trial stage; and ECT, Elite Clonal Trial stage.

2.3.2 Unconstrained Seedlings Genomic Selection Breeding Program (Seed-GSunconst)

The Seed-GSunconst program used a similar strategy to the Seed-GSconst program, only that it used an increased operating budget (Figure 2). There are two main differences between the two programs: (i) all 2,000 seedlings inside the Seedlings stage were genotyped and predicted with GS, and (ii) GS is used to advance a total of 500 genotypes (instead of 300) to the ACT stage. The Seed-GSunconst program also has a 2-year generation interval and lasts a total of 13 years, which is three years shorter compared to the PS breeding program.

2.3.3 Elite Clonal Trials Genomic Selection Breeding Program (ECT-GS)

The ECT-GS program introduced genotyping and GS at the earliest Seedlings stage and eliminated the PT and ACT stages (Figure 3). The two stages were eliminated to avoid eight years of testing and to reallocate the resources into genotyping of all 2,000 seedlings at the Seedlings stage as in the PS program. Compared to the previous two GS programs, the seedlings were planted in the nursery for an additional year to produce enough cuttings for direct planting at the ECT stage. Genomic selection was then used to advance genotypes to the next stage and to select the best 5 or 20 genotypes to replace the oldest parents in the crossing block. Ninety promising clones were advanced from the Seedlings stage to the ECT stage, where they were evaluated for 6 years. The ECT-GS breeding has a 2-year generation interval and lasts a total of eight years, which is eight years shorter compared to the PS breeding program.

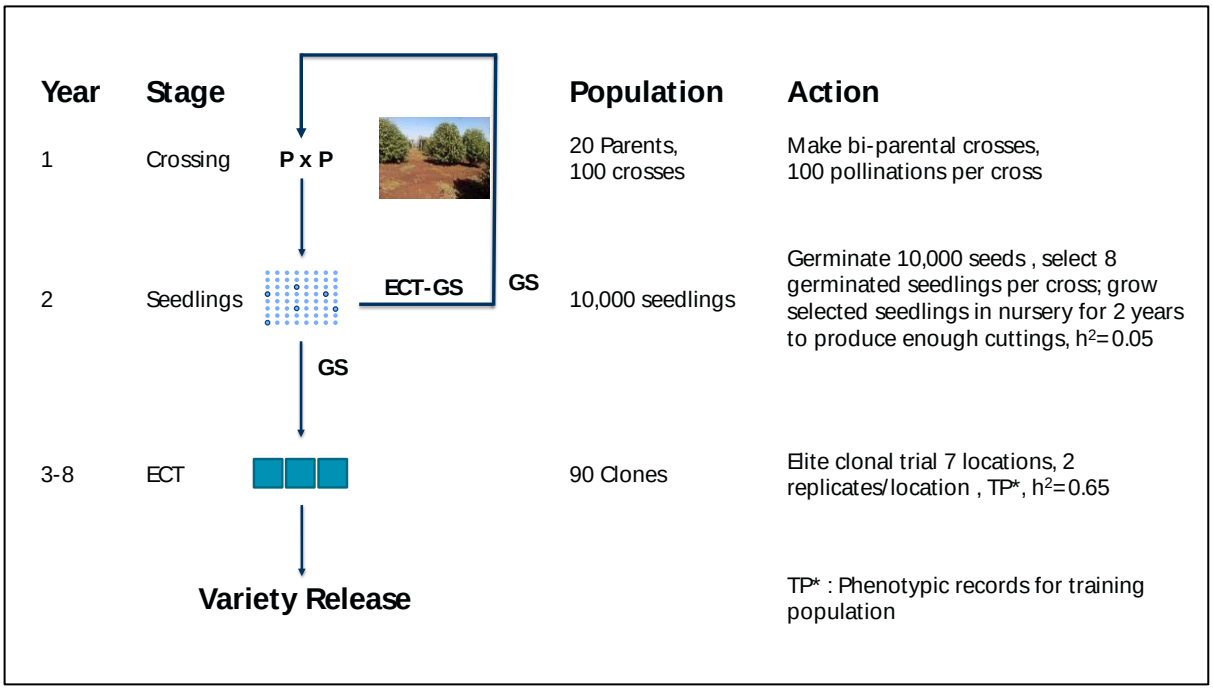

Figure 3. Schematic overview of the elite clonal breeding program with GS (ECT-GS). The solid line represents the stage at which the 5 or 20 new parents are selected based on genomic prediction. ACT, Advanced Clonal Trial stage; and ECT, Elite Clonal Trial stage.

2.4 Training population & Genomic Selection Model

To initialize the training population, the last 6 years of the burn-in phase were used to collect phenotypic data at the ACT stage for training the GS model (in year 41 and onwards). The initial training population consisted of 6,000 phenotypic records. In subsequent years, new phenotypic data were added to the training population as new yield trials were recorded. For the Seed-GSunconst and Seed-GSconst programs, ACT and ECT data were used to update the training population, while only ECT data were used to update the ECT-GS program. After 40 years of future breeding, the training population records grew to 24,600 (Seed-GSunconst), 16,600 (Seed-GSconst), and 6,600 (ECT-GS) records. We estimated genomic predictions using a ridge regression best linear unbiased prediction model (RR-BLUP) (Meuwissen, Hayes, and Goddard 2001). In the model, we fitted year as a fixed effect and allowed for heterogeneous error variance for each breeding stage. The predicted additive

316 SNP effects at each marker locus were used to estimate the average effect of allele substitution for each
317 SNP. The allele substitution effects were then summed to estimate GEBVs. GEBVs of each genotype
318 were calculated by summing the predicted additive SNP effects at each marker locus.

319 2.5 Evaluation and comparison of the tea breeding programs

320 We compared the efficacy of the three GS breeding programs with the PS program by measuring
321 the mean genetic values of the newly developed genotypes at the Seedlings stage over time. All
322 simulations for each strategy were replicated 10 times. We examined the genetic values of seedlings at
323 the Seedlings stage as this is the earliest stage at which all programs evaluate new crosses (F1
324 seedlings). We also evaluated genetic variance and selection accuracy in all breeding programs. We
325 evaluated genetic gain and genetic variance by plotting the mean and variance of seedling population
326 genetic values over time. We calculated the prediction accuracy for the GS breeding programs as the
327 correlation between the true genetic values and their GEBVs at the Seedlings stage. Conversion
328 efficiency was plotted as the change in genetic gain over genic variance.

329 3 RESULTS

330 We showed that all GS programs outperformed the PS breeding program. The Seed-Gsunconst
331 program had the highest overall genetic gain. All GS breeding programs had higher selection
332 accuracies compared to the PS breeding program. Although genetic variance decreased over time for
333 all breeding programs, the GS programs had a large decrease in genetic variance compared to the PS
334 breeding program. Replacement of all parents resulted in a slightly higher genetic gain, with a small
335 decrease in genetic variance compared to replacement of 25% of parents. The PS breeding program
336 had the highest conversion efficiency, but the lowest genetic gain compared to the GS programs.

337 3.1 Genetic gain

The GS breeding programs (Seed-GSunconst, Seed-GSconst, and ECT-GS) achieved greater genetic gain compared to the PS breeding program, regardless of the number of parents replaced. This is shown in Figure 4, where the population mean genetic value is plotted against the number of years of breeding at the Seedlings stage. The first plot shows the trends for the mean genetic values of all replicates for each of the tea breeding programs evaluated in the future breeding component when 25% of the parents are replaced. The second plot shows the same trend when all parents are replaced after each cycle for all breeding programs. Seed-GSunconst showed the greatest genetic gain compared to all other programs. Both plots show that the overall ranking of the breeding programs in terms of total genetic gain was consistent across the two proportions of parents replaced. The ranking of the breeding programs from highest to lowest mean genetic gain was as follows: Seed-GSunconst, Seed-GSconst, ECT-GS and PS.

The breeding programs where all parents were replaced showed slightly higher genetic gain than when 25% of the parents were replaced. When all parents were replaced, the best program, Seed-GSunconst, generated 1.6 times the genetic gain of the PS breeding program. Seed-GSconst and ECT-GS generated 1.53 and 1.50 times the genetic gain of the PS breeding program, respectively. When 25% of the parents were replaced, Seed-GSUnconst generated 1.43 times the genetic gain of the PS breeding program. On the other hand, Seed-GSconst and ECT-GS generated 1.39 and 1.36 times the genetic gain of the PS breeding program, respectively. All GS breeding programs had a generation interval of 2 years compared to 16 years generation interval of the PS breeding program (Figure 4).

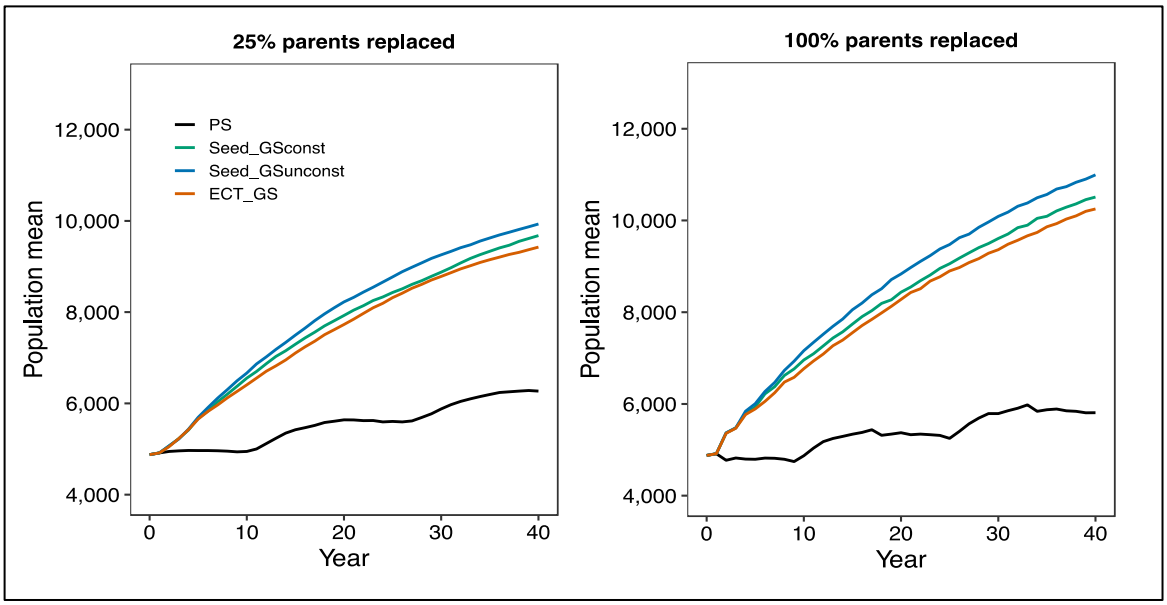

Figure 4. Genetic gain over time for the four simulated breeding programs. Results are shown for 25% (left) and 100% (right) parents replaced separately. The lines within each of the two panels represent the four breeding programs where each line represents population mean of genetic values for the 10 simulated replicates and the shaded area showing 95% confidence intervals of the mean. Genetic gain was measured at the seedlings stage. The black-coloured line is the phenotypic breeding program (PS), the green-coloured line represent the seedlings GS breeding program (Seed-GSconst), the blue-coloured line represent the GS breeding program with increased funding (Seed-GSunconst) and the red-coloured line represent the elite clonal GS breeding program (ECT-GS).

3.2 Selection accuracy

GS increased selection accuracy compared to PS, as shown in Figure 5, which plots the correlations between true and estimated genetic values for seedling entries over time. The first plot shows the selection accuracy for all breeding programs when 25% of all parents were replaced, and the second plot shows the selection accuracy when all parents were replaced. All GS breeding programs had higher selection accuracy compared to the PS breeding program, regardless of the number of parents replaced. Higher selection accuracy was observed when 25% of parents were replaced than

when all parents were replaced. Selection accuracy was the same across years and the two methods of parent replacement. In the early years of future breeding, selection accuracy for the ECT-GS program was lower compared to the other GS breeding programs, but then gradually increased until it reached a plateau in year 20. Selection accuracy for the Seed-GSunconst, Seed-GSconst, and PS breeding programs remained constant over time. Both plots show that the ranking from highest to lowest mean selection accuracy is as follows: Seed-GSunconst, Seed-GSconst, ECT-GS, and PS (Figure 5).

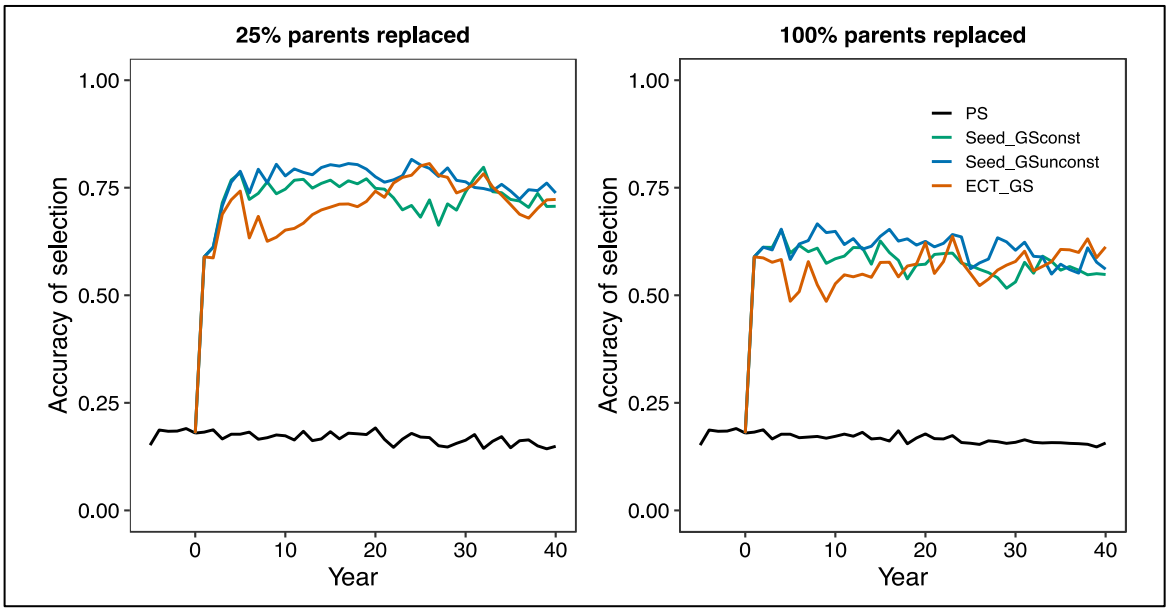

Figure 5. Accuracy of selection over time for the four simulated breeding programs. Results are shown for 25% (left) and 100% (right) parents replaced separately. The lines within each of the two panels represent the four breeding programs where each line represents the mean accuracy of selection for the 10 simulated replicates and the shaded area showing 95% confidence intervals of the mean. Accuracy of selection was measured at the seedlings stage. The black-coloured line is the phenotypic breeding program (PS), the green-coloured line represent the seedlings GS breeding program (Seed-GSconst), the blue-coloured line represent the GS breeding program with increased funding (Seed-GSunconst) and the red-coloured line represent the elite clonal GS breeding program (ECT-GS).

3.3 Genetic variance

The change in genetic variance over time is shown in Figure 6. The first plot shows the mean genetic variance over time when 25% of the parents are replaced. The second plot shows the same breeding programs when all parents are replaced. All breeding programs showed a decrease in genetic variance over time. However, the rate of loss of genetic variance varied from breeding program to breeding program. All GS breeding programs showed a tremendous decrease in genetic variance, while the phenotypic selection program showed a slow and gradual decrease in genetic variance over time. The difference in genetic variance when 25% and 100% of parents were replaced was small, except during the transition period when GS was introduced and 25% of parents were replaced. Both plots also show that the rank order from highest to lowest genetic variance was as follows: PS, Seed-GSconst, ECT-GS and Seed-GSunconst (Figure 6).

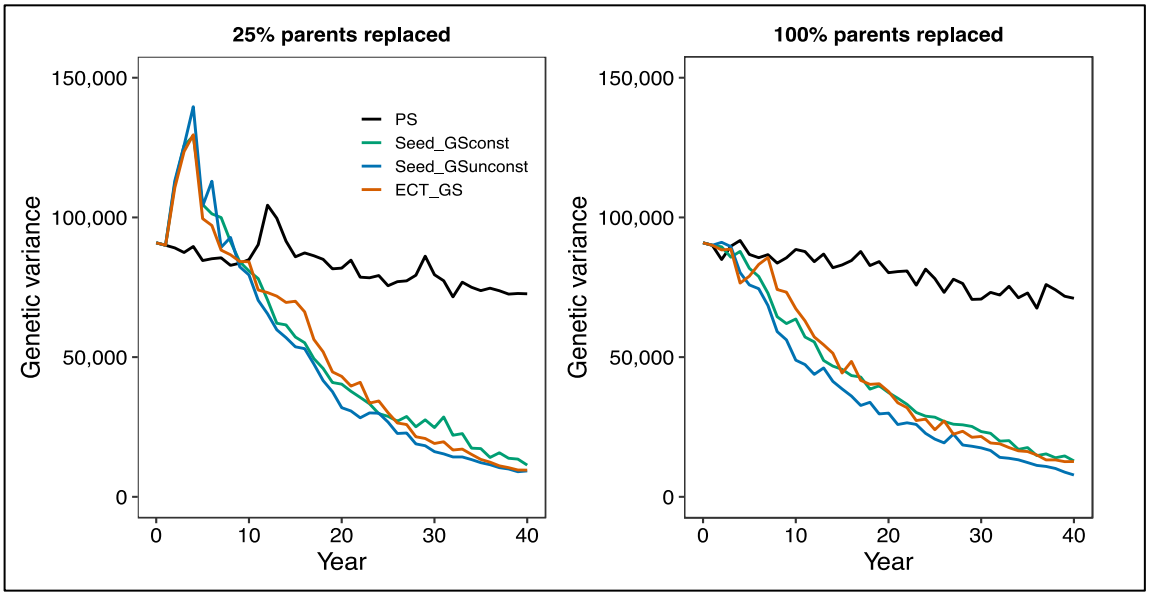

Figure 6. Genetic variance over time for the four simulated breeding programs. Results are shown for 25% (left) and 100% (right) parents replaced separately. The lines within each of the two panels represent the four breeding programs where each line represents mean genetic variance for the 10 simulated replicates and the shaded area showing 95% confidence intervals of the mean. Genetic variance was measured at the seedlings stage. The black-coloured line is the phenotypic breeding program (PS), the green-coloured line represent the seedlings GS breeding program (Seed-GSconst),

406 the blue-coloured line represent the GS breeding program with increased funding (Seed-GSunconst)
407 and the red-coloured line represent the elite clonal GS breeding program (ECT-GS).

408 **3.4 Conversion efficiency**

409 All breeding strategies had almost similar conversion efficiencies when all parents were replaced.
410 When 25% of the parents were replaced, the PS breeding program had more than twice the conversion
411 efficiency (56) but about 3.5 times less genetic gain than the GS breeding programs. This is illustrated
412 in Figure 7, which shows the long-term genetic gain in standard deviation units when all genic variance
413 is exhausted and is calculated by regressing realized genetic gain on lost genic variance over 40 years
414 of tea breeding. The first plot shows the change in genetic mean over genic standard deviation when
415 25% of the parents are replaced. The second plot shows the change in genetic mean over the genic
416 standard deviation when all parents are replaced. The slope of the change in genetic mean over the
417 change in the genic standard deviation quantifies the efficiency of converting genetic diversity into
418 genetic gain. The ranking of the breeding programs in terms of conversion efficiency when all parents
419 were replaced from highest to lowest was as follows: Seed-GSconst (34), PS (33), ECT-GS (32), and
420 Seed-GSunconst (31). The ranking of conversion efficiency of breeding programs when 25% of parents
421 were replaced from highest to lowest was as follows: PS (56), Seed-GSconst (28), Seed-GSunconst
422 (25), and ECT-GS (25).

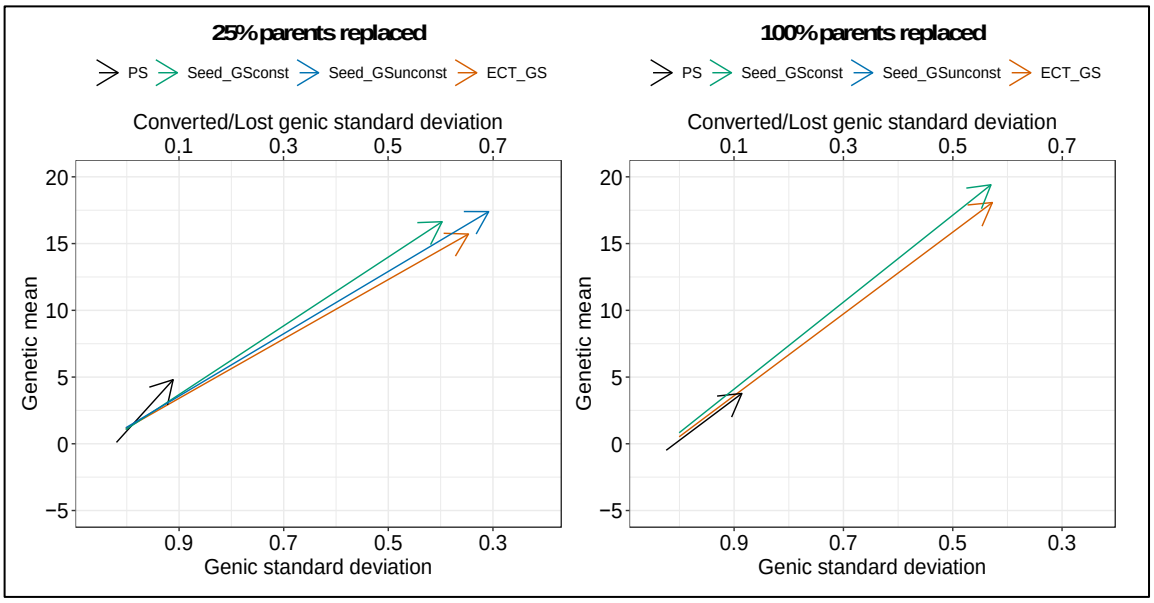

Figure 7. Efficiency plot showing change of genetic mean and genic standard deviation over time for the four simulated breeding programs. Results are shown for 25% (left) and 100% (right) parents replaced separately. The black-coloured line is the phenotypic breeding program (PS), the green-coloured line represent the seedlings GS breeding program (Seed-GSconst), the blue-coloured line represent the GS breeding program with increased funding (Seed-GSunconst) and the red-coloured line represent the elite clonal GS breeding program (ECT-GS).

4 DISCUSSION

Tea breeding programs require the integration of efficient genomic-assisted breeding approaches to increase the rate of genetic progress. However, currently, it is not clear how these could be integrated into existing programs and whether the additional costs these approaches incur are worth the effort. In our study, we used stochastic simulations for the first time to show that tea breeding programs in LMIC can benefit from genomic selection despite low labour costs and limited research budgets. We developed three different genomic selection breeding programs (Seed-GSconst, Seed-GSunconst and ECT-GS) and compared them with a phenotypic selection (PS) program based on the commercial breeding program from Unilever Tea Kenya. To discuss our results, we examine the effects of each breeding strategy on genetic gain, genetic variance, selection accuracy, and conversion

efficiency. We also compared the effects of replacing all parents and 25% of parents after each breeding cycle. Our results confirm that the use of GS increases genetic gain compared to traditional PS in tea breeding by shortening the generation interval and increasing the accuracy of selection of superior parents in the Seedlings stage. GS also allowed shortening the entire breeding program by eliminating some of the stages. For example, in Seed-GSconst and Seed-GSunconst, we eliminated the PT stage, hence saving 3 years, while in ECT-GS we removed the PT and ACT stages, resulting in an 8-year reduction in the duration of the breeding program.

4.1 Genetic gain

Phenotypic selection is a very slow process in tea breeding as it takes 16 years to develop an improved tea variety (Figure 1). This is because tea is a perennial crop with a long generation interval – it takes between 3-6 years for tea bush to grow from seed to flower (Kamunya 2010). The multi-year testing of clones at many locations is also a time-consuming process. The selection accuracy at the Seedlings and PT stage is very low because selection is based on *per se* performance of single bush unreplicated seedlings. This contributes to the slow genetic progress in tea breeding programs and hence, genomic-assisted breeding approaches need to be considered.

Our results showed that all breeding programs using GS achieved greater genetic gain compared to the PS breeding program, hence showing the potential of improving the rate of genetic gain in tea breeding programs. This may be attributed to the improved prediction accuracy of selecting superior parents by the GS model and the short generation interval (Cobb et al. 2019). All GS breeding programs had a generation interval of two years, compared to 16 years for the PS breeding program. This demonstrates the importance of reducing generation interval to increase genetic gain in tea breeding programs. Our results showed a perfect rank correlation between generation interval and genetic gain. Similar findings were reported by Bančič et al. (2021), who used stochastic simulations to investigate the potential of using GS to improve yield during intercropping. They found that all

programs using GS produced significantly more yield than the PS breeding program mainly because of reduced generation interval and increased prediction accuracy. Gaynor et al. (2017), using stochastic simulations, also reported that both conventional GS breeding methods and the two-part GS strategy significantly increased grain yield of inbred wheat compared to the PS breeding program. Reducing cycle time has the advantage of increasing the frequency with which haplotypes are recombined and exposed for selection, increasing the likelihood that a superior allele combination will emerge and be selected (Atlin, Cairns, and Das 2017). Although Seed-GSunconst produced the most genetic gain, the difference was not large when compared to the Seed-GSconst program, in which only a limited proportion of seedlings were genotyped due to cost constraints. This was less expected and suggests that genotyping more seedlings and testing more genotypes at ACT stage did not improve the genetic gain. Replacing all the parents in the crossing block with new improved parents produced a higher genetic gain, suggesting that substituting the poorest yielding and old parents with new higher yielding parents increases the probability of combining favourable alleles in tea breeding programs.

Selection of superior seedlings is the major challenge in tea breeding programs due to high selection intensity and extremely low selection accuracy at the seedlings stage. In the PS breeding program, seedlings are selected based on *per se* performance, so the selection accuracy is very low. In this study we estimated broad sense heritabilities at the PT, ACT and ECT stages from real data. The heritability at the PT stage was 0.05 compared to the ACT (0.45) and ECT (0.65) stages. The PT stage consists of a single bush (unreplicated) trial, and hence the low heritability. The ACT and ECT stages are clonal trials with larger plots consisting of more clones of each seedling. The ACT stage is also a multilocational trial. In clonal breeding programs, many seedlings are usually selected, thus the increased selection intensity (Werner et al. 2020). For instance, at Unilever Tea Kenya, 500 seedlings are selected each year in the PS breeding program to advance to the ACT stage (Corley and Tuwei 2018). The PS breeding program showed a cyclical pattern of genetic gain over time. This is because the parents in the PS breeding program are updated after 16 years, whereas in the GS breeding

programs, they were updated every two years, making genetic progress more continuous. In practice, this means that the GS breeding programs can deliver new varieties adapted to new biotic and abiotic stress factors and market requirements more quickly.

4.2 Selection accuracy

All GS breeding programs had higher selection accuracy compared to the PS program. The prediction accuracy is high in the GS breeding program because selection at the Seedlings stage is based on the predicted performance of the seedlings as clones, since the GS model is trained using data from the clonal testing stages (Werner et al. 2020). In our simulation, the 500 clones from the ACT stage were used as the initial training population using 6 years of historical phenotypic records (3,000 records). This improved the selection accuracy of parents because the phenotypic records at the ACT stage used to train the prediction model had a higher heritability than the individual bushes at the Seedlings stage in the PS breeding program. Additionally, there is increased accuracy of advancing the promising genotypes from the Seedlings to ACT and from the ACT to ECT stages using GEBVs. There is also a strong relationship between the training population and the selection candidates in the case of GS prediction model (Neyhart et al. 2017). We also updated the training population each year with new data from the previous cycle. As breeding cycles progress, the required linkage disequilibrium (LD) between quantitative trait loci and markers is expected to change as a result of recombination, selection and drift, leading to a decay in prediction accuracy (Lorenz et al. 2011). Therefore, the training population should be regularly updated during recurrent selection to maintain the prediction accuracy, as was also the case in our GS breeding programs. Previous research has identified the need to update the training population using new data that may capture new LD generated over breeding cycles. For instance, Neyhart et al. (2017) evaluated several methods for updating the training population in a long-term GS. They reported that using a smaller but more recent training population provided a slight advantage in prediction accuracy and genetic gain.

In the early years of the future breeding phase, selection accuracy was lower for the ECT-GS program compared to the other two GS breeding programs. This could be because fewer clones were used to update the training population each year. Each year, only 90 clones from the ECT stage in the ECT-GS breeding program were used to update the training population. In the Seed-GSunconst and Seed-GSconst breeding programs, clones from the ACT and ECT stages were used to update the training population model. The number of clones used to update the Seed-GSunconst and Seed-GSconst breeding programs was 540 and 340 clones, respectively. The Seed-GSunconst breeding program had the highest overall prediction accuracy, confirming that training population size is an important factor in the development of GS breeding programs. This is consistent with the results of previous studies that showed that a large training population is required to accurately estimate marker effects (Zhang et al. 2017, Combs and Bernardo 2013). We also observed higher selection accuracy when 25% of parents were replaced than when 100% parents were replaced. This could be because when all the parents are replaced, there is a large shift in LD pattern leading to a decay in prediction accuracy. Selection over time causes a change in LD between the quantitative trait loci (QTL) and the markers. Shifts in the pattern of QTL-marker LD, if not captured, can result in decreased prediction accuracy (Lorenz et al. 2011).

4.3 Genetic variance and conversion efficiency

In our simulation, all breeding programs showed a decrease in genetic variance over time. However, all the GS breeding programs had a huge decrease in genetic variance compared to the PS breeding program, which showed a slow and gradual decrease over time. Similar results were reported by Tessema et al. (2020), who used stochastic simulations based on real data to quantify the increase in genetic gain by implementing GS in a traditional wheat-breeding program. They reported a significant decrease in genetic variance over a 25-year period for the breeding strategies using GS. The loss of genetic variance is due to increased selection accuracy in the early stages of a breeding program

as well as shorter generation interval. Increased selection accuracy results in the Bulmer effect, which decreases genetic variance under directional selection due to the build-up of negative linkage-disequilibrium between causal loci (Bulmer 1971). Selection causes changes in in genetic variances, allele frequencies and LD relationships between markers and QTL (Muir 2007).

Our results showed that the GS breeding programs had lower conversion efficiency compared to the PS breeding program when 25% of the parents were replaced. The conversion efficiency of the PS breeding program decreased significantly when all parents were replaced, while it increased slightly for the GS breeding programs when all parents were replaced. This implies that the PS program converted the genetic gain over loss efficiently compared to the GS programs. A large reduction in genetic variance limits long-term genetic gain in plant breeding because genetic variance is important for continuous and sustained progress (Cobb et al. 2019). Genomic selection strategies that balance the rates of genetic gain and loss of diversity and could be implemented in tea breeding programs include optimal contribution selection (Sonesson, Woolliams, and Meuwissen 2012), optimal cross selection (Gorjanc, Gaynor, and Hickey 2018), optimal contribution selection with branching (Santantonio and Robbins 2020), optimal population value selection (Goiffon et al. 2017) and expected maximum haploid breeding value selection (Müller, Schopp, and Melchinger 2018).

Interestingly, little difference in genetic variance was observed when 25% and 100% of the parents were replaced, except for the transition period when GS was introduced and 25% of parents were replaced. This is mostly because tea is a highly outcrossing and has an extremely highly heterozygous nuclear genome (Xia et al. 2020). Wang et al. (2020) reported that hybridization increased the heterozygosity and wide-ranging gene flow among tea populations with the spread of tea cultivation.

4.4 Simulation constrains and practical implementation of GS in tea breeding programs

We used a real commercial tea breeding program of Unilever Tea Kenya and its parameters as a baseline to test the possible integration of GS. Our key constraints were the low operating cost of the breeding program due to cheap labour and limited resources for research. Kenya is one of the LMICs where labour costs are significantly lower compared to countries with advanced economies. For example, the average daily wage of a field worker at Unilever Tea Kenya is ~$5 (based on the ccollective bargaining aagreement (CBA) between the workers and tea companies in Kenya) while in the UK the hourly wage is ~$8-12 (based on salary reports from glassdoor.co.uk). Our simulations showed that despite these constraints, it is possible to use GS in tea breeding. Our results provide guidance for several important decisions regarding resource allocation to increase genetic gain in tea breeding programs:

1. Genotyping seedlings in nurseries and selecting the best parents based on GEBVs can increase genetic gain by reducing generation interval and increasing selection accuracy.
2. Elimination of preliminary evaluation (PT) stage reduced the cost of breeding and shortened the breeding cycle by 3 years. The saved costs could be reallocated to genotyping more seedlings in the nursery.
3. Eliminating the PT and ACT stages in the ECT-GS program reduced the duration of the breeding cycle by 8 years. However, genetic gains were lower compared to the other GS strategies.
4. If a breeding program has extra budget ($30,000), genotyping all the seedlings and increasing the number of genotypes evaluated at the ACT stage can increase genetic gain, however, only slightly.
5. GS is cost-effective for tea breeding programs with limited budgets when genotyping costs are $15. We expect the benefit of GS to increase in the future when costs of high-throughput genotyping decrease even more - this will increase the selection intensity in a breeding program.

6. GS can also be used to advance superior genotypes for variety development, e.g., from the PT to ACT and from ACT to ECT stages at a higher selection accuracy.

In addition, breeders will also need to consider whether all necessary facilities and equipment are available on site (e.g., freezers, sterile laboratories), train field technicians appropriately, determine whether genotyping can be done on site or else transportation costs should be considered, potentially collaborate with biometricians to optimize field trials, and develop GS pipelines for prediction using an appropriate modelling framework. Standardization and digitization of phenotyping protocols to ensure the best data quality will also be an important challenge, as this can greatly improve the predictive ability of GS.

5 Genomic selection for improvement of tea quality and practical implementation

Tea quality is an important attribute as it is the main determinant of price at the tea auction. It is measured based on colour, aroma, taste and mouthfeel of tea liquor and the appearance of dry tea (Zheng et al. 2016). These sensory attributes originate from biochemical compounds present in fresh tea shoots such as catechins, alkaloids, amino acids and volatile compounds (Borse and Jagan Mohan Rao 2012). Sensory evaluation using professional tasters is traditionally the main method used to evaluate, grade and determine the price of tea (Liang et al. 2003). Although sensory evaluation is quick and practical to use, it is limited since it requires identification and training to produce skilled and experienced professional tasters (Stone and Sidel 2004), who are not easily found (Corley and Chomboi 2005). It is also time-consuming, the tasters sometimes get exhausted and the approach is susceptible to many sources of variation because of individual tasters' preferences and moods (Sinija and Mishra 2011). Chemical and physical analytical methods have also been developed for identifying biochemical components associated with tea quality (Liang et al. 2008) and they include; liquid chromatography coupled with mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), near infrared (NIR) spectroscopy and chromatographic methods such as high-performance liquid chromatography (HPLC)

608 and gas chromatography (GC) (Yashin et al. 2015, Zheng et al. 2016). Most of these techniques are
609 objective, repeatable and reproducible (Chen et al. 2015). However, most of these analytical techniques
610 are expensive to acquire and maintain and require specialized expertise to operate.

611 GS could be used to improve the selection of superior quality tea varieties (Lubanga, Massawe,
612 and Mayes 2021). The best way to implement GS in tea breeding programs is to integrate it into an
613 existing PS program. GS could be incorporated into an existing PS program by genotyping seedlings
614 at the nursery stage and predicting their genetic values using a GS model. Professional testers could be
615 used to obtain sensory data for training the GS model. Biochemical traits could also be measured at the
616 nursery stage using highly reproducible equipment such as HPLC, NMR or LC-MS. Samples (between
617 100-200) from ACT and ECT stages can be used for training the GS model using both genotypic and
618 phenotypic data. Low-cost genotyping platforms such as genotyping by sequencing (GBS) could be
619 used to obtain SNPs. Seedlings at the nursery stage could then be genotyped and predicted using the
620 trained model using only the genotypic data. Parents with high quality attributes could be selected for
621 crossing at the nursery stage while poor quality seedlings can be discarded at the seedlings stage. GS
622 could be implemented as described in Seed-GSconst, Seed-GSunconst and ECT-GS programs.

623 The use of GS for breeding of high-quality tea varieties can reduce the disadvantages associated
624 with sensory evaluation methods and analytical techniques. For instance, high quality seedlings can be
625 predicted at the nursery stage without the need to be tested by professional testers or analysed by
626 analytical technical techniques. This increases the accuracy of predicting superior seedlings based on
627 GEBVs, hence reducing the subjectivity associated with the professional testers. Additionally, the
628 expensive costs associated with analytical equipment can be eliminated.

629 Our study used a practical breeding program at Unilever Tea Kenya and cost estimates to
630 measure the cost-effectiveness of implementing genomic selection in tea breeding programs. All the
631 programs in this study were constrained to equal operating costs, and therefore we can conclude that
632 implementing genomic selection in tea breeding programs can increase the rate of genetic gain despite

the challenges experienced in LMIC. Methods such as optimal cross selection (Gorjanc, Gaynor, and Hickey 2018) could be used to ensure that the newly introduced diversity is not quickly eliminated through genomic selection. It optimises the efficiency of converting genetic diversity into genetic gain through reducing the loss of genetic diversity and reducing the drop of genomic prediction accuracy with rapid cycling (Gorjanc, Gaynor, and Hickey 2018).

6 Conclusion

Our study provides excellent insights into the implementation of genomic selection in tea breeding programs for yield in LMIC. The genomic selection scenarios and results will help tea breeders with knowledge on how to design genomic selection strategies in breeding programs. We show that incorporating GS in tea breeding programs can increase genetic gain up to 1.6 times more than PS program, despite the low labour cost in LMIC. Moreover, the integration of GS does not significantly change the structure of the existing tea breeding program. Rather, it can significantly shorten its' duration. The increase in genetic gain in the GS breeding programs was due to higher prediction accuracy and reduced generation interval. After 40 years of future breeding, the GS breeding programs had lower genetic variance compared to PS, indicating the need to incorporate strategies that balance genetic gain and genetic variance, such as the optimal contribution algorithm. We also observed that replacing all parents resulted in higher genetic gain without significant loss of genetic diversity. Tea quality is a very important attribute, but expensive and difficult trait to phenotype and predict in breeding programs. We recommend further research to determine the most cost-effective pipeline for implementing GS to improve tea quality and yield simultaneously.

7 Author contributions

N.L., J.B, F.M. S.M. and G.G designed the research and wrote the manuscript. N.L. and J.B contributed to the writing of the scripts for the tea breeding simulations.

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