






Effectiveness of genomic selection for improving provitamin A carotenoid content and associated traits in cassava

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Abstract

Global efforts are underway to develop cassava with enhanced levels of provitamin A carotenoids to sustainably meet increasing demands for food and nutrition where the crop is a major staple. Herein, we tested the effectiveness of genomic selection (GS) for rapid improvement of cassava for total carotenoids content and associated traits. We evaluated 632 clones from Uganda's provitamin A cassava breeding pipeline and 648 West African introductions. At harvest, each clone was assessed for level of total carotenoids, dry matter content, and resistance to cassava brown streak disease (CBSD). All clones were genotyped with diversity array technology and imputed to a set of 23,431 single nucleotide polymorphic markers. We assessed predictive ability of four genomic prediction methods in scenarios of cross-validation, across population prediction, and inclusion of quantitative trait loci markers. Cross-validations produced the highest mean prediction ability for total carotenoids content (0.52) and the lowest for CBSD resistance (0.20), with G-BLUP outperforming other models tested. Across population, predictions showed low ability of Ugandan population to predict the performance of West African clones, with the highest predictive ability recorded for total carotenoids content (0.34) and the lowest for CBSD resistance (0.12) using G-BLUP. By incorporating chromosome 1 markers associated with carotenoids content as independent kernel in the G-BLUP model of a cross-validation scenario, prediction ability slightly improved from 0.52 to 0.58. These results reinforce ongoing efforts aimed at integrating GS into cassava breeding and demonstrate the utility of this tool for rapid genetic improvement.

Keywords: breeding; carotenoids; genetic gain; genomic prediction; *Manihot esculenta*; vitamin A deficiency

Introduction

Cassava (*Manihot esculenta* Crantz), grown on approximately 18 million hectares in Africa, offers great potential to end extreme hunger, achieve food security and improve nutrition, if proven varieties are unconditionally accessed by producers and consumers (Kolawole et al. 2010). However, nutritionally cassava is deficient in essential micronutrients such as provitamin A carotenoids (Montagnac et al. 2009), which renders diets that heavily depend on the crop vulnerable to vitamin A deficiency (VAD). Indeed, VAD is a widespread nutritional challenge in sub-Saharan Africa, with women and children being the most affected (Gegios et al. 2010; Stephenson et al. 2010). For this reason, cassava varieties with elevated levels of provitamin A carotenoids are being developed and promoted (Pfeiffer and McClafferty 2007) as a cost-effective and sustainable approach to help communities burdened by VAD (Bouis et al. 2011; Talsma et al. 2013). The significant variation for total carotenoid content (TCC) (ranging from 1.02 to 10.4 $\mu\text{g g}^{-1}$), of which >90% is constituted by β -carotene, a provitamin A

carotenoid, reported in large collection (>2400 clones) of cassava accessions (Chávez et al. 2005; Nassar et al. 2007) could facilitate the effective breeding of nutrition-sensitive varieties.

A major drawback in our endeavors to breed cassava varieties with elevated provitamin A carotenoids has been the undesirable tendency of low dry matter content (DMC) in most provitamin A cassava clones (Njoku et al. 2015; Esuma et al. 2016a). Another worrisome and unique challenge to cassava breeding in East Africa is the menace of cassava brown streak disease (CBSD) caused by two *Ipomoviruses*: *Ugandan cassava brown streak virus* and *Cassava brown streak virus* (Alicai et al. 2016; Kawuki et al. 2016). Collectively, these conundrums of low DMC and CBSD that directly affect root quality hamper speedy development and dissemination of provitamin A cassava clones in eastern Africa. It is for such situations that tools for implementing efficient crop improvement programs to address intricate breeding obstacles are being optimized (Ahmar et al. 2020).

For example, the rapid advances in next generation sequencing (NGS) technologies have enabled the use of genome-wide

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markers for implementing genomic selection (GS), a tool that can significantly enhance the efficiency of crop breeding (Bhat *et al.* 2016). Essentially, GS aims to increase the speed and accuracy of selection in breeding programs by predicting the genetic value of individuals or lines at an early selection stage, or for individuals that cannot be directly phenotyped (Crossa *et al.* 2017). One of the salient features of GS is the use of high-density markers for prediction, which relies on genotyping at a high marker density to ensure most causal loci are in linkage disequilibrium (LD) with at least one marker (Jannink *et al.* 2010). By using dense markers to quantify Mendelian sampling, GS avoids the need for extensive progeny phenotyping, which saves cost and time by reducing the length of a breeding cycle, while enhancing genetic gain per selection cycle (Desta and Ortiz 2014). Consequently, GS can facilitate rapid crop improvement for relevant agronomic and end-user traits, as witnessed in different crops (Xu *et al.* 2018, 2020).

A broad review of GS in plant breeding, detailing requirements for training population and features of prediction models, has been elegantly provided by Desta and Ortiz (2014). In fact, response of GS in crop improvement may be affected by factors such as model performance, sample size and relatedness, marker density, heritability and genetic architecture of traits, and the extent and distribution of LD between markers and quantitative trait loci (QTL) on the accuracy of genomic estimated breeding values (GEBVs) (Zhong *et al.* 2009). Thus, predictive accuracy varies among GS models depending on their assumptions and treatments of marker effects (Ornella *et al.* 2012).

Indeed, results from both simulation and empirical studies have illustrated the efficacy and limitations of GS for several crops, including maize (Bernardo and Yu 2007), rice (Xu *et al.* 2018), barley (Nielsen *et al.* 2016), and wheat (Kristensen *et al.* 2018). Wolfe *et al.* (2017) reported some promising results depicting prospects for GS in cassava, with higher prediction accuracies for DMC (0.36–0.48) and cassava mosaic disease (CMD) (0.26–0.40) than those for fresh root yield attributes (<0.1). Relatedly, Kayondo *et al.* (2018) evaluated the accuracy of seven genomic prediction models using empirical data from 1301 cassava clones and reported predictive ability of 0.31–0.42 for CBSD severity in roots, indicating GS as a useful tool for CBSD resistance breeding. Furthermore, Ozimati *et al.* (2018) reported increased genomic prediction accuracies for CBSD resistance arising from optimized training populations and the prospect of East African training sets to predict CBSD in West African cassava germplasm.

Motivated by these promising results from the pioneering efforts on the application and use of GS in cassava breeding, our study was aimed at exploring genomic prediction as a strategy for the rapid development of carotene-rich cassava product profiles suited for the growing conditions in East Africa. Broadly, this study was part of an ongoing effort to optimize and integrate GS into the provitamin A cassava breeding scheme in Uganda for rapid genetic improvement. The provitamin A cassava breeding pipeline currently utilizes locally adapted germplasm (Esuma *et al.* 2016a), with occasional introductions and introgression from the West African germplasm, where remarkable success has been achieved in cassava biofortification. Thus, we evaluated genomic prediction ability within the base population for provitamin A carotenoids and assessed the effectiveness of this population to predict performance of subsequent breeding cycles and that of West African germplasm. Specifically, we tested (i) the predictive ability for TCC, DMC, and CBSD across different statistical models and (ii) the utility of QTL markers in genomic prediction for TCC.

Materials and methods

Plant materials

Three sets of germplasm were used in this study. First, a panel of 280 clones segregating for root quality and agronomic attributes were selected from a cassava breeding population at National Crops Resources Research Institute (NaCRRI), Uganda. This panel constituted the training population, hereafter referred to as Cycle 0. Second, a subset of 50 Cycle 0 clones with the highest breeding values for carotenoid content was identified (through cross-validation described hereafter) as progenitors and planted in a crossing nursery at Namulonge to generate cycle one (Cycle 1) population, during the period 2016/2017. Following seed germination and preliminary screening at nursery and seedling stages, 352 Cycle 1 genotypes were cloned and evaluated in 2018. Third was a set of 648 clones derived from 80 progenitors introduced from West Africa (WA) through International Institute for Tropical Agriculture (IITA); this population segregated for carotenoids and DMC.

Field trials and phenotypic data collection

The Cycle 0 population was evaluated at two locations, Namulonge (0.52166458°N, 32.608997564°E) and at Serere (1.4994°N, 33.5490°E) during the 2017/2018 cropping season in Uganda. Both locations are characterized by high prevalence of CBSD causing viruses and high whitefly vector populations (Alicai *et al.* 2019) known to transmit such viruses. Cycle 1 and WA populations were evaluated at one location (Namulonge), due to the limitation of planting material. Trials were laid out in augmented design with ~25–30 plots per block and four checks. Each plot was represented by a single row of 10 plants spaced at 1 × 1 m. In all trials, CBSD susceptible cultivar TME 204 was planted as spreader rows to provide source of virus inoculum.

At harvest (12 months after planting), all plants in a plot were uprooted. Total carotenoid content was assessed by visually scoring the intensity of pigmentation of the root parenchyma on a qualitative scale of 1–8 (Sánchez *et al.* 2006). We used the visual color scale for carotenoids content as it was the available high throughput phenotyping method the time of data collection and previous reports have indicated a strong positive correlation between carotenoid content assessed visually and quantitatively (Esuma *et al.* 2016a). Furthermore, all roots harvested per plot were pooled and screened for CBSD necrosis severity using a scale of 1–5 described by Hillocks and Thresh (2000), where: 1 = no necrosis, 2 = ≤5% of the root is necrotic, 3 = 6–10% of the root is necrotic, 4 = 11–25% of the root is necrotic and mild root constriction and 5 = >25% of the root necrotic and severe root constriction. DMC was estimated from approximately 200 g of fresh root samples that were oven-dried into a constant weight at 105°C for 24 hours. Subsequently, DMC was computed using the formula:

$$\text{DMC(\%)} = \frac{\text{DSW}}{\text{FSW}} \times 100$$

where DSW = dry sample weight and FSW = fresh sample weight.

Genotyping of samples using DArTseq technology

Leaf tissues were collected from all test clones evaluated and sent to Intertek and Diversity Array Technology Pty Ltd. (<http://www.diversityarrays.com/>) for DNA extraction and genotyping,

respectively. Briefly, DArTseq technology relies on a complexity reduction method to enrich genomic representations with single copy sequences and subsequently perform next-generation sequencing using HiSeq 2500 (Illumina, USA). Further detail of the DArTseq genotyping process has been described by Kilian et al. (2012).

In our case, sequences of the genomic representations were aligned to cassava reference genome v6.1. Eventually, 13,675 high-quality single nucleotide polymorphism (SNP) markers were selected using the following quality control parameters (Kilian et al. 2012): (i) the reproducibility of 100%, (ii) the overall call rate over 95%, and (iii) the polymorphic information content between 0.3 and 0.5. Genotypes were coded as 0 (homozygous for reference allele), 1 (heterozygous), and 2 (homozygous SNP). Genotype data were imputed to a panel of 23,431 SNPs using the Beagle 5.0 algorithm (Browning et al. 2018) and a reference panel of 20,733 mostly East African cassava haplotypes, derived from a combination of genotyping-by-sequencing and DArTseq-LD. The data are available here: ftp://ftp.cassavabase.org/marmin_datasets/nextgenImputation2019/.

Statistical analyses

Estimates of variance components and heritability:

Phenotypic dataset for each trial was considered independent and analyzed separately using a two-step genomic prediction approach. In the first step, we fitted linear mixed models accounting for each trial's design and extracted the best linear unbiased predictions (BLUPs) of the clone effects for TCC, DMC, and CBSDR root necrosis using the *lme4* package for R statistical software (Bates et al. 2015). For Cycle 0 that was evaluated in two locations, we fitted a model $y = X\beta + Z_g c + Z_{block(l)} b + Z_{g,l} l + \epsilon$, where vector β was the fixed effect for grand mean and location, with the corresponding incidence matrix X ; vector c and corresponding incidence matrix Z_g was the random effect for clones (g) such that $c \sim N(0, I\sigma_c^2)$; vector b with its corresponding incidence matrix $Z_{block(l)}$ represented random effect for blocks nested in locations (l) such that $b \sim N(0, I\sigma_b^2)$; vector l and incidence matrix $Z_{g,l}$ represented random effect of genotype-environment interaction; and ϵ was the residual such that $\epsilon \sim N(0, I\sigma_e^2)$. For Cycle 1 clones and the West African introductions, each evaluated in one location, we fitted a linear mixed model $y = X\beta + Z_g c + Z_{block^k} b + \epsilon$ where y was the vector of raw phenotypes, β was a fixed effect of grand mean, and $Z_{block^k} b$ represented the random effect for blocks.

Variance components were extracted from the models for estimation plot-based heritability (H) as:

$$H = \frac{\sigma_c^2}{(\sigma_c^2 + \sigma_{cl}^2 + \sigma_e^2)}$$

where σ_c^2 was the clone variance, σ_{cl}^2 was the variance attributed to genotype by location interaction (excluded for trials conducted in one location) and σ_e^2 was the model residual variance. Furthermore, we used the *mixed.solve* function in R to fit a single-stage genomic best linear unbiased prediction (G-BLUP) model, with the grand mean and location as fixed effects and clone effects treated randomly. Eventually, SNP-based heritability (h^2) was computed as:

$$h^2 = \frac{\sigma_a^2}{(\sigma_a^2 + \sigma_e^2)}$$

where σ_a^2 clone was the additive genetic variance and σ_e^2 was the residual variance.

The total genetic value of each individual was estimated as BLUP extracted from the mixed linear models following the procedure described by Garrick et al. (2009). To avoid applying shrinkage to the same data twice (at the first step and subsequent genomic prediction step), the BLUPs were de-regressed as:

$$\text{de-regressed BLUP} = \frac{\text{BLUP}}{1 - \frac{\text{PEV}}{\sigma_c^2}}$$

where PEV represented the prediction error variance for the BLUPs and σ_c^2 was the clone variance. The de-regressed BLUPs were used in subsequent genomic prediction analyses.

Population structure:

To assess population structure, we used 23,431 polymorphic DArTseq markers filtered to keep only SNPs with minor allele frequency (MAF) ≥ 0.01 . Using the *A.mat* function built in the *rrBLUP* R package (Endelman 2011), we constructed a realized genomic relationship matrix (K) from SNP data. Finally, principal component analysis (PCA) was done on the genomic relationship matrix, using the *prcomp* function in R. The first two principal components (PC1 and PC2) were used to visualize population structure.

Estimating the ability of genomic prediction models through cross-validation:

We used the BLUPs in a fivefold cross-validation scheme, with 10 replications, to evaluate prediction ability for TCC, DMC, and CBSDRs across four parametric GS models: (i) genomic BLUP (G-BLUP), (ii) BayesA, (iii) BayesB, and (iv) Bayesian Lasso. The main features of these genome-wide prediction models have been reviewed by Desta and Ortiz (2014). To perform cross-validation with G-BLUP, the *A.mat* function in the R package *rrBLUP* was initially used to construct a genomic realized relationship matrix from SNP marker dosages and GEBVs obtained after fitting a linear mixed model using the *mixed.solve* function in the same package. Cross-validations for Bayesian models were computed with the BGLR package (Perez and de los Campos 2014), with model parameters *niter* and *burnIn* fixed at 10,000 and 1000, respectively.

To achieve cross-validation for each fold in a replication, the total number of genotypes in a population was divided into five equal proportions such that four groups at a time formed the training set to build the prediction model, while the fifth group was the test set. For example, when cross-validation was performed for Cycle 0 ($n = 280$), 224 genotypes were used as training set while the remaining 56 individuals were used as validation set. This process was repeated for each of the five folds across 10 replications. Prediction abilities were computed as Pearson's correlation coefficients (r) between GEBVs predicted for the test set and the corresponding BLUPs obtained from the first step of the analysis.

GWAS-based cross-validation:

The purpose of GWAS analysis was to identify chromosome markers associated with each phenotype and use such information to design kernel-based cross-validation schemes. Thus, we surveyed for QTL for all the three traits using genome-wide association study (GWAS), using 632 individuals (constituted by Cycle 0 and Cycle 1) and 23,431 polymorphic markers filtered at MAF > 0.05 . To avoid any potential upward bias of prediction abilities, we performed GWAS in the training set of each fold across 10 replications of the cross-validation scheme, such that kernel-based

predictions were done in the test set of the respective fold. For example, for each fold, ~506 genotypes (training set) representing 80% of the 632 individuals were used for GWAS and the remaining ~126 (test set) used for kernel-based predictions.

Specifically, GWAS was implemented using a linear mixed model in the R package *rrBLUP* following the modified methodology described by [Isidro-Sánchez et al. \(2017\)](#). The linear mixed model fitted included a kinship matrix and the first three principal components to account for population structure. We generated quantile-quantile and Manhattan plots generated with the R package *qqman* ([Turner 2014](#)) and used them to evaluate the association mapping model and chromosome-wise association signals such that SNPs with *P*-values less than the 5% Bonferroni threshold were considered to be significantly associated with phenotypes.

Based on DArTseq markers used in this study, GWAS results for DMC and CBSDr did not show significant association signals. However, we identified one QTL for TCC on chromosome 1, which was consistently the same for each of the five folds across the 10 replications, with ~49 markers meeting the Bonferroni threshold of association significance (Supplementary Figure S1). Subsequently, we designed four GWAS-guided cross-validation schemes for TCC: (a) single-kernel with significant QTL markers on chromosome 1, (b) single-kernel with all chromosome 1 markers, (c) single-kernel excluding all markers on chromosome 1, and (d) multi-kernel model that included the significant QTL markers and the rest of the markers fitted as independent kernels in G-BLUP model ([Morota and Gianola 2014](#)). Finally, we performed GWAS with all the 632 individuals and used the output to predicted TCC in the West African population along the four prediction scenarios.

Data availability

All the raw phenotypic and genotypic data are available at the link provided for references. Supplementary material is available at figshare: <https://doi.org/10.6084/m9.figshare.12752405>.

Results

Heritability estimates and population structure

Total carotenoids content had the highest broad-sense, varying between 0.68 and 0.73, while CBSDr had the lowest estimates (0.36–0.50) ([Table 1](#)). We did not record DMC in Cycle 1 clones due to insufficient quantity of roots arising from small plot sizes. Similar trends were observed for narrow-sense (SNP-based) heritability estimates, with the highest values (0.61–0.71) recorded for TCC and lowest (0.29–0.40) for CBSDr ([Table 1](#)).

PCA was used to describe population structure in the genetic materials analyzed. Grouping clones by the first two PCs (PC1 and PC2) showed no clear-cut differentiation among the three populations ([Figure 1](#)), with the PCs explaining 28.2 and 15% of the total genetic variation, respectively. Nonetheless, some WA clones tended to drift from other populations along PC2 ([Figure 1](#)).

Cross-validation prediction accuracies for TCC, DMC, and CBSDr

We performed cross-validation under three scenarios: within (a) Cycle 0 ($n=280$ genotypes), (b) Cycle 0 combined with Cycle 1 ($n=632$ genotypes), and (c) the West African population ($n=648$ genotypes). Distribution of predictive abilities across the five folds and ten replications is presented in [Figure 2](#), with an overall trend showing the highest predictive ability for TCC and the lowest for CBSDr. For the cross-validation performed within Cycle 0, we

Table 1 Heritability estimates for traits measured at the clonal evaluation stage

Population	Number ^a	H ^b			h ^{2c}		
		TCC	DMC	CBSDr	TCC	DMC	CBSDr
C0_pVAC	280	0.68	0.58	0.46	0.64	0.55	0.40
C1_pVAC	352	0.73	—	0.50	0.61	—	0.29
WA	648	0.73	0.56	0.36	0.69	0.54	0.34

^a Number of clones evaluated.

^b Broad sense heritability.

^c Narrow sense heritability; TCC, total carotenoid content; DMC, dry matter content; CBSDr, cassava brown streak disease severity in roots. We did not analyze DMC in C1 due to insufficient quantity of roots arising from small plot sizes and high CBSDr root necrosis.

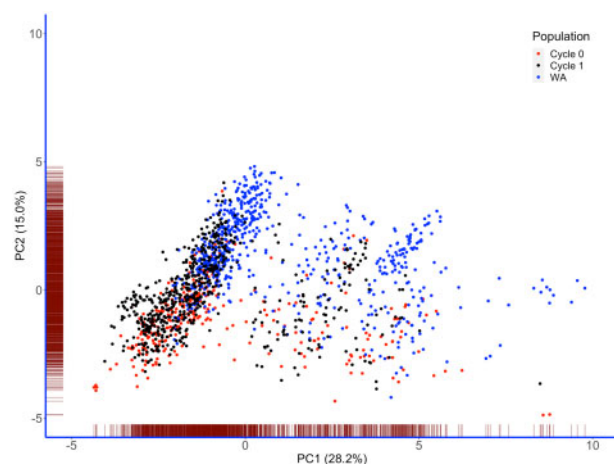


Figure 1 Plot of PC1 against PC2 for eigenvalue decomposition of DArTSeq marker genotypes for three sets of clones evaluated in this study.

recorded the highest mean prediction ability for TCC across all models, varying from 0.35 for BayesB and Bayesian Lasso to 0.44 for G-BLUP (Supplementary Table S1). Across all traits, G-BLUP had the highest mean predictive ability compared to the rest of the models tested across all three traits.

In the second cross-validation scenario that combined Cycle 0 combined with Cycle 1 clones, mean increase of 12.1% in predictive ability was recorded for TCC, with the highest increase (20%) recorded for Bayesian Lasso (Supplementary Table S1). In this scenario, the highest mean prediction ability of 0.52 was recorded for TCC with G-BLUP while the lowest value of 0.39 was noted for the trait with BayesB. Similarly, increase in training population size resulted into an increase of prediction ability for CBSDr, except for BayesB that had no appreciable (0%) change.

Cross validation within the West African population showed similar trend, with the highest predictive ability 0.47 recorded for TCC with G-BLUP. For this population, predictive abilities for CBSDr were remarkably low compared to values recorded in Cycle 0 and with Cycle 1.

Across population prediction accuracies for TCC, DMC, and CBSDr

We predicted each of the three traits across the four models under the following scenarios: Cycle 0 to predict performance of Cycle 1 and West African (WA) clones and a combination of Cycle 0 and with Cycle 1 to predict WA clones. First, we noted generally higher cross-population predictive ability for TCC than for other traits across all models, with the highest values recorded with G-

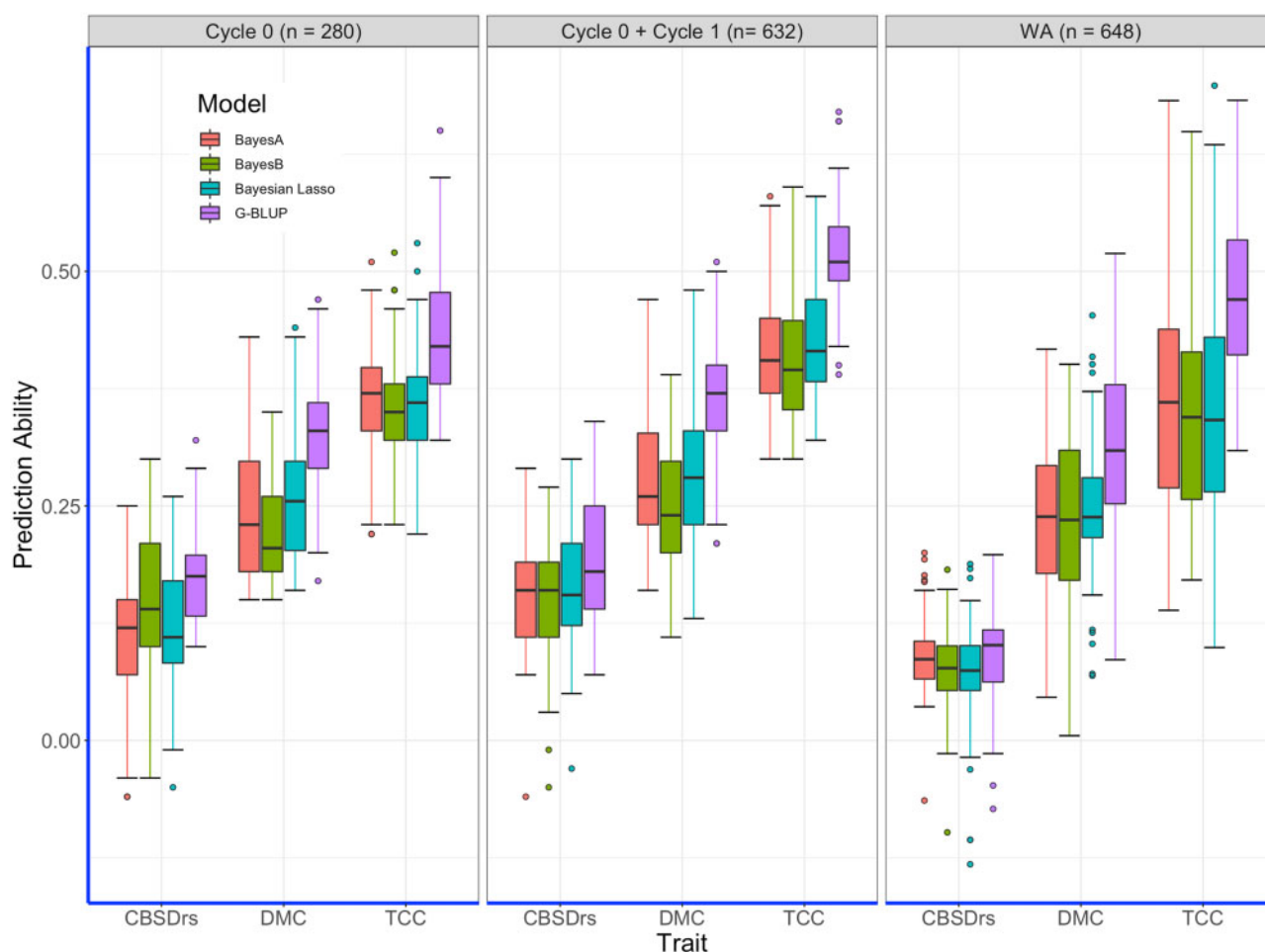


Figure 2 Prediction accuracies of five folds across 10 replications for all models evaluated in cross-validation schemes.

BLUP. For example, the highest predictive ability of 0.43 was recorded for TCC when using the Cycle 0 to predict the performance of Cycle 1 (Table 2). However, when the Cycle 0 and Cycle 1 were combined to predict TCC in WA population, the predictive ability was relatively low (0.27) compared to the value of 0.34 obtained when the prediction was performed with Cycle 0 alone. A similar trend of low predictive ability in the WA population was noted for TCC across the other three models, with the lowest value (0.16) recorded with Bayesian Lasso in the scenario.

Predictive abilities for CBSDRs were particularly low in all scenarios. For instance, the highest predictive ability (0.12) was only recorded when Cycle 0 was used to predict CBSDRs in Cycle 1 with G-BLUP; other models had negligible predictions for CBSDRs (Table 2). In fact, when Cycle 0 was used to predict CBSDRs in WA,

all results were close to zero, with the highest predictive ability recorded was 0.05 for G-BLUP (Table 2). Lastly, we only tested the scenario of Cycle 0 predicting DMC in WA, as this trait was not assessed in Cycle 1. In this case, the highest predictive ability was 0.34 with G-BLUP and the lowest was 0.25 from Bayesian Lasso (Table 2).

GWAS-guided genomic prediction

All marker-trait associations for DMC and CBSDRs were nonsignificant (data not shown). GWAS for each training set per fold consistently revealed one QTL for TCC on chromosome 1, with ~49 markers meeting the Bonferroni threshold for genome-wide association signal (Figure 3 and Supplementary Figure S1). Subsequently, GWAS-based prediction for TCC was performed in the respective test sets for each fold using cross-validation scheme with G-BLUP, which presented the highest prediction abilities compared to other models tested. The mean predictive abilities for the four scenarios of GWAS-guided cross-validation were 0.56 for single-kernel containing all chromosome 1 markers, 0.54 for single-kernel with significant chromosome 1 markers, 0.48 for single-kernel excluding all markers on chromosome 1, and 0.63 for the multi-kernel model that included the significant QTL markers and the rest of the markers fitted as independent kernels (Figure 4 and Supplementary Table S2). These scenarios led to better predictions than the case with naive model i.e., without segmentation of chromosome markers, with the exception where single kernel excluding all chromosome 1 markers was

Table 2 Mean prediction abilities for across population predictions for TCC, DMC, and CBSDRs

hba	Testing set	Trait	G-BLUP	BayesA	BayesB	BL
Cycle 0	Cycle 1	TCC	0.431	0.392	0.411	0.401
Cycle 0	Cycle 1	CBSDRs	0.117	0.052	0.031	0.052
Cycle 0	WA	TCC	0.401	0.216	0.223	0.247
Cycle 0	WA	DMC	0.342	0.282	0.274	0.251
Cycle 0	WA	CBSDRs	0.045	0.019	0.014	0.009
Cycle 0 + Cycle 1	WA	TCC	0.274	0.162	0.161	0.159
Cycle 0 + Cycle 1	WA	CBSDRs	0.001	-0.123	-0.107	-0.103

DMC = of provitamin A cassava breeding population; dry matter content; CBSDRs, cassava brown streak disease severity in roots; BL, Bayesian Lasso.

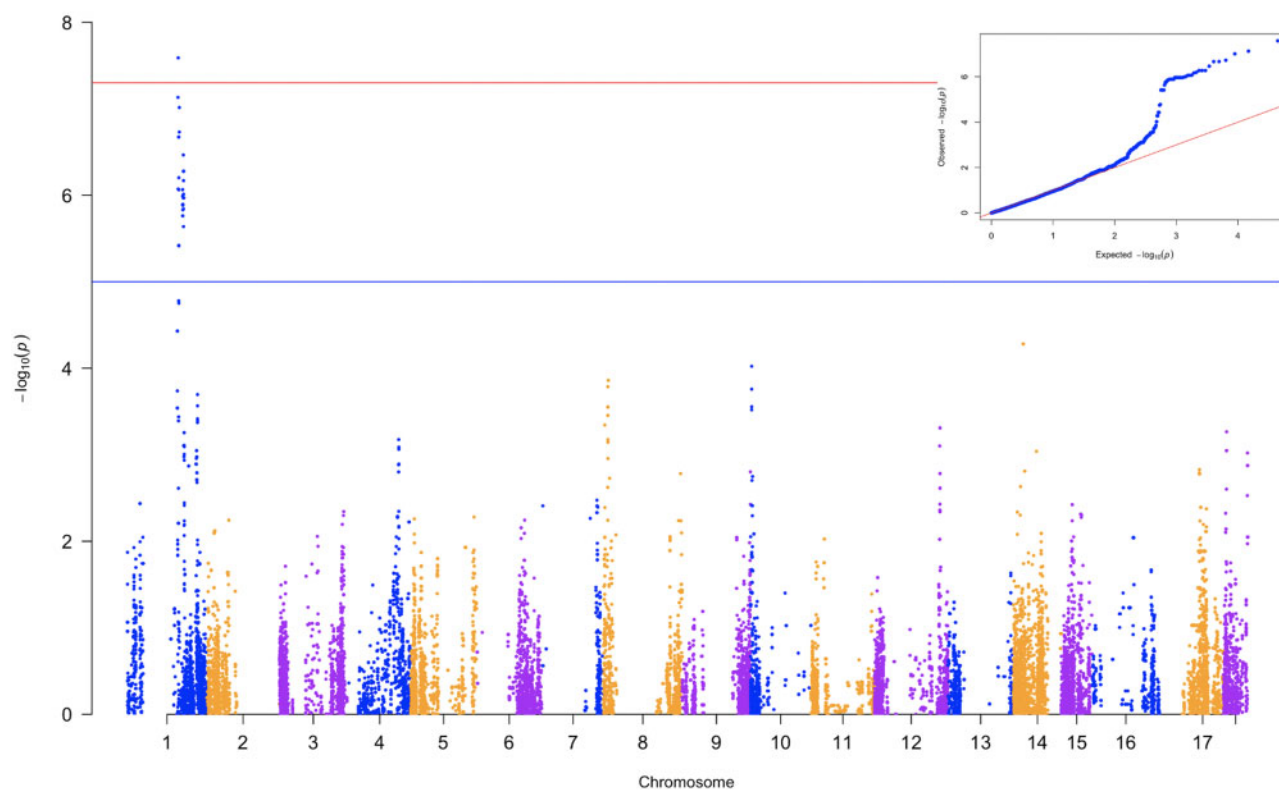


Figure 3 Manhattan plot for GWAS for total carotenoids content performed with 632 clones (constituted by Cycle 0 and Cycle 1). The horizontal blue line is the genome-wide significance (Bonferroni threshold); inset is the Q-Q plot for total carotenoid content.

used. Narrow-sense heritability estimates were proportionately higher for scenarios of predicting with markers on chromosome 1 and the multi-kernel approach than for the case of excluding chromosome 1 (Figure 4). When the GWAS-guided approach was used to predict TCC in WA, we noted similar pattern of the highest predictive ability (0.64) with multi-kernel scenario and the least (0.25) for single-kernel excluding chromosome 1 markers (Supplementary Table S2).

Discussions

The rapid increase in the global human population, predicted to reach 10 billion by 2050 (Bongaarts 2009; Henderson and Loreau 2019), highlights the urgent need for deployment of high yielding, resilient and nutritious crop varieties to vulnerable societies (Ray et al. 2013; Hickey et al. 2019). The nutritional impacts of biofortification evidenced through staples like orange-fleshed sweet potato (Jenkins et al. 2015) provide motivation for development of cassava enriched with provitamin A carotenoids as nutritious staple in sub-Saharan Africa. Collectively, TCC, DMC, and CBSD resistance are must-have traits for product profiles targeting deployment of provitamin A cassava varieties in eastern Africa.

Breeding efforts, including our current results, have indicated moderate to high broad-sense heritability (0.56–0.73) for TCC and DMC, implying the possibility of making meaningful genetic gains for both traits through phenotypic recurrent selection (Ceballos et al. 2013; Esuma et al. 2016b). Indeed, the high heritability for TCC has already facilitated the identification of cassava cultivars with high levels of β -carotene, which constitutes the bulk of carotenoids in cassava, with some varieties released for on-farm

production in West Africa (Ayinde et al. 2017; Eyinla et al. 2019). However, the high costs and drudgery associated with phenotyping cassava roots for TCC and DMC (Ceballos et al. 2013), the apparent negative correlation between TCC and DMC (Njoku et al. 2015; Esuma et al. 2016b), and the unique challenge of CBSD in eastern and southern Africa (Alicai et al. 2016, 2019) were a major motivation for testing the prospects for GS as a tool for improving these traits in our provitamin A cassava breeding pipeline.

We found relatively low broad-sense heritability (0.36–0.50) for severity of CBSD root necrosis, which relates to the trait's quantitative nature (Kulembeka et al. 2012; Masumba et al. 2017) strongly influenced by environmental factors, which complicates selection (Pariyo et al. 2015). However, heritability estimates above 0.5 have been reported by Kayondo et al. (2018) and Ozimati et al. (2019) for CBSD necrosis in populations evaluated for GWAS (1300 clones) and GS (922 clones), respectively. As expected, we noted lower estimates of SNP-based heritability for the three traits compared to the respective broad-sense heritability (Table 1). This could be attributable to under-tagging of causal loci by markers in our analysis (de los Campos and Toro 2017). Ozimati et al. (2019) observed similar trends for SNP-based heritability for several agronomic traits in a panel of >1000 clones evaluated for GS.

Based on the cross-validation results, predictive abilities were largely consistent across traits and prediction models. First, we noted a downward pattern for predictive ability TCC > DMC > CBSDRs across all models, and for both scenarios of small and increased size of training set. TCC is largely a qualitative trait (Welsch et al. 2010) for which genomic prediction is expected to be an effective tool (Zhang et al. 2019). Mean predictive abilities

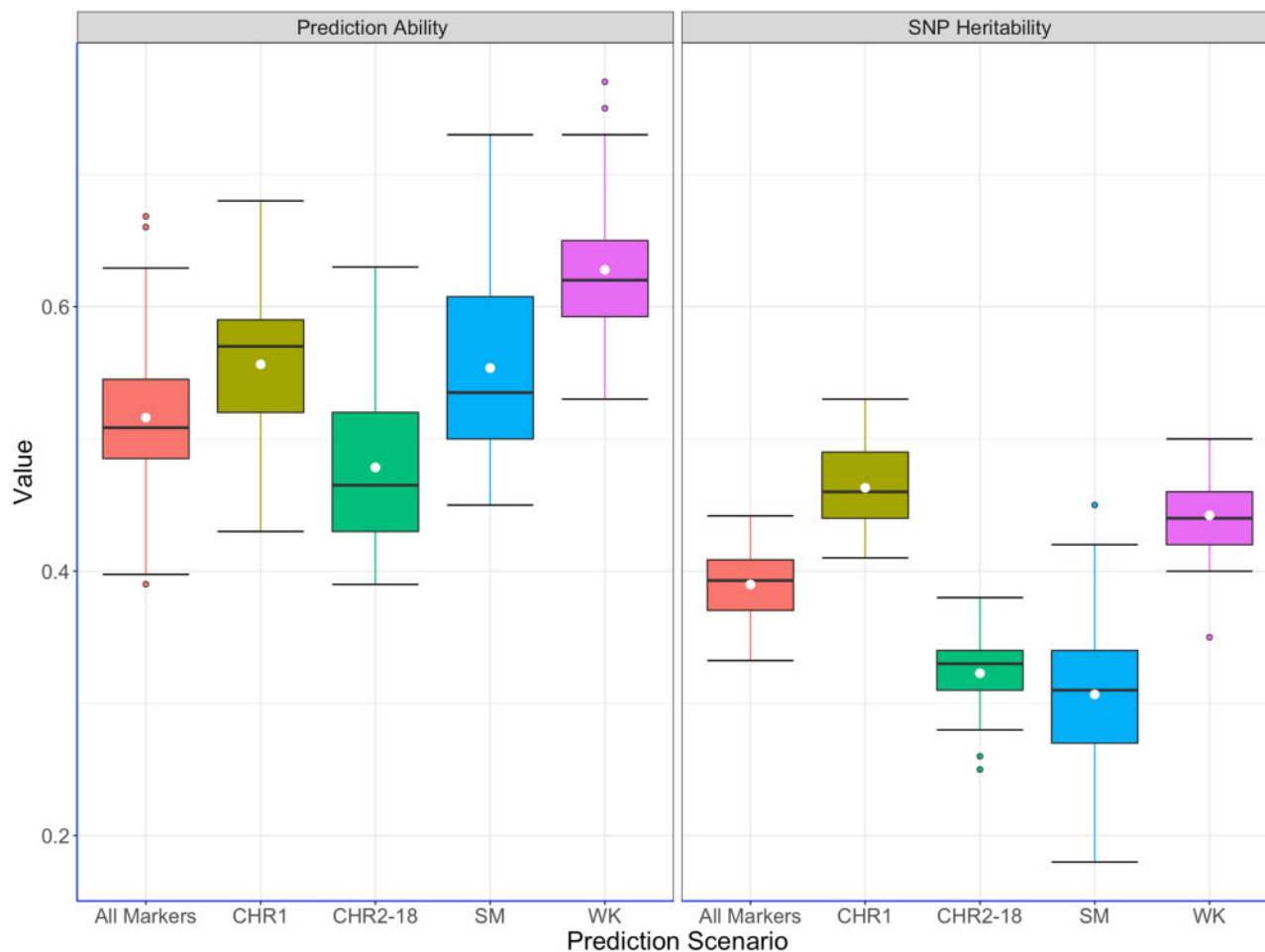


Figure 4 Prediction abilities for GWAS-guided scenarios for total carotenoids content for within-sub population cross-validation scheme. SM = scenario of predicting with significant QTL markers on chromosome 1 as single-kernel; CHR1 = scenario of predicting with all chromosome 1 markers as single-kernel; CHR2-18 = scenario of predicting with markers on chromosomes 2–18 only; WK = scenario of predicting with significant QTL markers on chromosome 1 and the rest of the markers as independent kernels.

for DMC were generally low, ranging between 0.21 and 0.33, similar to the range of 0.29–0.34 reported by Wolfe et al. (2017) and Ozimati et al. (2018). Interestingly, in the same report, Wolfe et al. (2017) showed high predictive ability for DMC (0.63–0.67) in a cassava breeding population of IITA. The discrepancies in predictive abilities for DMC across breeding populations may relate to variation in heritability arising from differences in phenotyping methods of using specific gravity and oven-drying method and/or genetic differences of clones evaluated. Subsequently, efforts are being made to optimize high throughput methods like near infrared spectroscopy for efficient phenotyping of root quality traits (Belalcazar et al. 2016; Ikeogu et al. 2017), which could improve reliability of GS for improving such traits.

Predictive abilities for CBSD resistance were generally low for all models tested in our analyses (mean of 0.20 for G-BLUP). Kayondo et al. (2018) highlighted GS as a promising tool to increase genetic gains for CBSD resistance in cassava, especially for nonparametric models like Random Forest and reproducing kernel Hilbert spaces regression, which capture both additive and nonadditive effects. It should be noted that Kayondo et al. (2018) used 1301 clones from NaCRRI's white-fleshed cassava breeding population phenotyped in at least three environments, which probably presented robust data for genomic prediction. Nonetheless, introgressing CBSD resistance into the provitamin A

cassava breeding population could increase the genetic merit of these clones for CBSD resistance and their use in developing varieties relevant for production in farmer's fields.

When we explored prospects of cross-population predictions, we noted higher predictive ability across Uganda's populations than in situations of predicting performance of WA clones. In evaluating the potential of East African training populations for genomic prediction of CBSD resistance in West African cassava germplasm, Ozimati et al. (2019) reported low predictive ability for the trait. Previous studies have indicated higher reliability of genomic predictions in populations that share ancestry with training sets than in situations of diverged genetic backgrounds where marker effects are likely to be different (De Roos et al. 2009; Lee et al. 2017). Indeed, a diagnosis of the second axis of PCA plot presented in Figure 1 shows an apparent drift of WA clones from the Ugandan clones, which is a manifestation of genetic difference. Nonetheless, this study revealed some possibility of predicting TCC and DMC in WA population using our cassava breeding population, with mean of 0.4 and 0.34, respectively, with G-BLUP. In this case, it would be possible to identify WA clones with superior GEBVs for TCC and DMC for further recombination with provitamin A cassava clones, which is a clever strategy to update a training set with useful alleles from external gene pools without disrupting genetic gain (Berro et al. 2019).

However, the low-predictive ability for CBSDRs in the WA population could indicate low genetic merit of using WA cassava germplasm for CBSD resistance breeding, which reinforces the need for CBSD pre-emptive breeding as a precautionary measure for the accidental introduction and spread of CBSD in WA.

Motivated by success attained by selection programs that exploit GWAS-aided prediction (Li *et al.* 2019), we assessed the suitability of this method for our GS scheme for provitamin A cassava. In this case, we noted increased predictive ability for TCC by incorporating its QTL as an independent kernel in the genomic prediction model. The QTL for TCC identified in this study is within the genomic vicinity of *Manes.01G124200.1*, a gene which encodes enzyme phytoene synthase known to catalyze accumulation of β -carotene in plant tissues (Welsch *et al.* 2010; Kandianis *et al.* 2013). In our study, the QTL represented biological information in the form of large-effect SNPs, which can enhance genomic prediction when fitted as an independent kernel. In this case, partitioning genomic markers into two relationship matrices, one kernel comprising the QTL markers associated with TCC on chromosomes 1 and the rest of the markers as the second kernel, led to better estimation of GEBVs (Morota and Gianola 2014). Li *et al.* (2019) reported increase in prediction accuracies of GS models resulting from increase in the number of most significant SNPs fitted as fixed effects in a maize breeding population. The apparent increase in predictive ability in our scenario of fitting effects of significant QTL markers on chromosome 1 and the rest of markers as independent kernels signifies the advantage of GS over traditional marker-assisted selection in utilizing whole-genome markers to account for phenotypic variance unexplainable by markers linked to QTL.

Taken together, the moderate to high predictive ability achieved for DMC and TCC in this study underpin the transformative ability of GS when adapted and integrated as a cassava breeding tool. We remark that GS offers a promise for rapid improvement of cassava for provitamin A carotenoids and DMC, especially when prediction models are properly chosen and tuned. In parallel, concerted efforts are required for further enrichment of provitamin A cassava breeding population with CBSD resistance alleles to make genomic prediction an effective tool for increasing gains for the trait. Results presented in this study complement ongoing efforts aimed at integrating the use of GS in cassava breeding programs.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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