

Development of diagnostic SNP markers for quality assurance and control in sweetpotato [*Ipomoea batatas* (L.) Lam.] breeding programs

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Author Contribution

DCG, GCY, SH and HC designed and managed the study, MNK, MD, DN managed tissue
sampling and genotyping, RTS, JS, GM, WG, EC, ROM, MIA developed the parents and
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approved the manuscript

Key Words

Sweetpotato, quality assurance, quality control, population structure, breeding programs

Key Message

A 36-SNP diagnostic marker set has been developed for quality assurance and control to support global sweetpotato breeding optimization efforts. Breeding population structure is shaped by sweetpotato virus disease prevalence.

Abstract

Quality assurance and control (QA/QC) is an essential element of a breeding program's optimization efforts towards increased genetic gains. Due to auto-hexaploid genome complexity, a low-cost marker platform for routine QA/QC in sweetpotato breeding programs is still unavailable. We used 662 parents of the International Potato Center (CIP)'s global breeding program spanning Peru, Uganda, Mozambique and Ghana, to develop a low-density highly informative single nucleotide polymorphism (SNP) marker set to be deployed for routine QA/QC. Segregation of the selected 30 SNPs (two SNPs per base chromosome) in a recombined breeding population was evaluated using 282 progeny from some of the parents above. The progeny were replicated from *in-vitro*, screenhouse and field, and the selected SNP-set was confirmed to identify relatively similar mislabeling error rates as a high density SNP-set of 10,159 markers. Six additional trait-specific markers were added to the selected SNP set from previous quantitative trait loci mapping. The 36-SNP set will be deployed for QA/QC in breeding pipelines and in fingerprinting of advanced clones or released varieties to monitor genetic gains in farmers fields. The study also enabled evaluation of CIP's global breeding population structure and the effect of some of the most devastating biotic stresses like sweetpotato virus disease on genetic variation management. These results will inform future deployment of genomic selection in sweetpotato.

Conflict of Interest

On behalf of all co-authors, the corresponding author declares no conflict of interest

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Introduction

Development of user-friendly, low-cost, high-throughput markers for quality assurance and control (QA/QC) in a genomic-assisted breeding era is a critically important aspect in crop improvement and germplasm conservation (Semagn et al. 2012; Ndjiondjop et al. 2018). This is because genetic fidelity and trueness-to-type are often not phenotypically obvious. The use of molecular markers for QC/QA has been implemented in several plants and animals and single nucleotide polymorphism (SNP) markers have become the markers of choice in germplasm characterization and QA/QC (Ertiro et al. 2015). For example, Cullingham et al. (2013) developed transcriptome-derived SNP markers for cost-efficient forest seed stock identification, Frey et al. (2013) developed ‘near-minimal’ sets of SNPs to differentiate operational taxonomic

units in fruit flies, while **Curk et al. (2015)** developed species-diagnostic SNP markers to analyze admixture structure of varieties and rootstocks in citrus. Here we define QA and QC according to **Gowda et al. (2017)** who defined QA as the process or set of processes used to measure the quality of a product and QC as the process of ensuring products and services meet consumer expectations. In plant breeding, QA would refer to all measures put in place to prevent errors and create a high-quality variety, while QC is the process of identifying the defects or errors in the quality of the breeding line, germplasm accession, variety, or any other product from the breeding pipeline. Solid QA/QC procedures are critical in plant breeding, as errors in the process of developing new varieties can lead to wasted time and resources, and also to reduce and/or cancel out genetic gains achieved because of genotype mix-ups along the breeding pipeline.

For most crops, the same SNP set can be used in a QA program to characterize germplasm, and study genetic diversity, genetic relationships and population structure, and in a QC program to evaluate genetic identity, genetic purity, parent-offspring identity, validation of crosses in nurseries and trait-specific testing (**Ertiro et al. 2015; Gowda et al. 2017**). Genotype misclassification is a common problem in most crops as has been reported in *Oryza spp* (**Orjuela et al. 2014**), *Brassica spp* (**Mason et al. 2015**) and sweetpotato (*Ipomoea batatas*; **Gemenet et al. 2019a**), and misclassification has consequences in breeding and variety development. QA/QC have become even more important with the advent of molecular markers for decision support in breeding programs. Whereas the importance of QC filtration methods for SNP markers are well established (**Forneris et al. 2015; Jarquin et al. 2019**) and methods put in place, QA/QC of the phenotypes that are combined with genotypes to predict performance are generally not very well established. The general lack of phenotype/genotype concordance has led to molecular decision

support tools for increasing genetic gains in plant breeding such as genomic selection not achieving their full potential. Although well acknowledged in human and animal genetic fields (Buyske et al. 2009; Smith et al. 2013), reports on the effects of poor QA/QC and genotype misclassification in plant breeding are generally lacking. With next- and third-generation sequencing methods enhancing rapid and cost-efficient development of large amounts of genomic data (van Dijk et al. 2018; Jarquin et al. 2019), one of the biggest challenges of plant breeding programs is putting in place highly precise mechanisms for QA/QC of the phenotyping/genotyping processes.

Sweetpotato is a crop of increasing importance in sub-Saharan Africa (SSA) contributing to both food and nutritional security, from both adapted, starchy, white-fleshed varieties and new improved high β -carotene, orange-fleshed varieties (Mwanga et al. 2011; Low et al. 2009, 2017). The International Potato Center (CIP), one of the centers of the Consultative Group on International Agricultural Research (CGIAR), runs a global sweetpotato improvement program. CIP is headquartered in Lima, Peru and has established three additional breeding support platforms in SSA. The support platform at Lima offers global technical support, while the east and central Africa platform focusses on end-user preferred varieties within this region including resistance to sweetpotato virus disease (SPVD), a major production constraint within the region. The southern Africa breeding support platform focusses on end-user preferred varieties in addition to drought tolerance which is the major production constraint in this region, while the west African support platform focusses on culinary aspects, especially the ‘less sweet’ sweetpotato which is preferred in this region (Low et al. 2017). Being mainly an auto-hexaploid, genomic-assisted breeding (GAB) tools are just starting to be mainstreamed into the breeding program due to genome complexity. Several genomic tools have been developed, in partnership

with several development partners and a molecular breeding team is currently stationed at CIP's regional office for SSA in Nairobi, Kenya to facilitate this mainstreaming. Recently, the first attempt of QA/QC identified misclassification errors of about 30% in one breeding trial while germplasm moved from *in-vitro* to greenhouse to field (**Gemenet et al. 2019a**). That study, using high density SNP markers, recommended putting in place QA/QC measures to enhance the likelihood of success applying GAB in sweetpotato breeding. The main objectives of the current study were: i) to characterize breeding population parents from global support platforms for population structure, ii) to estimate allele diversity and linkage disequilibrium among the breeding population parents from the four global support platforms iii) to develop a low-cost diagnostic SNP set for rapid QA/QC of sweetpotato breeding populations.

Materials and Methods

Genetic materials

We collected parents from all four global breeding support platforms of CIP: Peru, being the global support platform; Uganda, being the support platform for east and central Africa; Mozambique, being the support platform for southern Africa; and Ghana, being the support platform for west Africa. We had 331 parents from Peru, 126 parents from Uganda, 144 parents from Mozambique and 61 parents from Ghana, totaling 662 parents. The list of the breeding population parents is provided in **Online Resource 1**. Since our objective was to mainstream QA/QC in breeding trials, we used progeny from a breeding population to validate that the finally selected SNP set segregates in recombined individuals from parents. These validation materials were derived from a breeding population progeny developed from the east and central African support platform, named the Mwanga Diversity Panel (MDP) and described in **Gemenet et al. (2019a)**.

Genotyping and SNP calling

DNA from the breeding population parents was extracted at the Biosciences east and central Africa (BecA) laboratories based at the International Livestock Research Institute (ILRI), Nairobi. The extraction was done following a modified cetyl trimethylammonium bromide (CTAB) method optimized for sweetpotato. The DNA was treated for contaminating RNA using RNase A, quantified and normalized using standard protocols. The DNA was then submitted for sequencing using the Diversity Array Technology's DArTSeq method implemented by BecA's Integrated Genotyping Service and Support platform (IGSS) as described by **Kosmowski et al. (2018)**. IGSS is a subsidized genotyping platform supported by the Bill and Melinda Gates Foundation to enhance use of genomics in breeding for SSA. Sequencing was done at 96-plex, high density and SNP calling done using DArT's proprietary software DArTSoft (**Kosmowski et al. 2018**), with aligning to the diploid reference genome of *Ipomoea trifida*, a relative of sweetpotato (**Wu et al. 2018**). Given that most commercial genotyping platforms have allele depth coverage ~25x to 30x, previous studies (**Gemenet et al. under preparation**) have shown that this depth of coverage is not adequate to call allele dosage with confidence in genotype quality for hexaploid sweetpotato. The study also showed that in such cases, 'diploidized' biallelic loci which are informative enough performed almost as well as data with high confidence dosage information. Therefore, biallelic markers used in this study were called in a diploidized version. A total of 9,670 SNP markers were obtained. Since the aim of the study was to develop a low-density SNP set for QA/QC, we stringently filtered the genotype data to $\leq 25\%$ missingness ($\geq 75\%$ call rate), ≥ 0.25 polymorphic information content (PIC) and $\geq 10\%$ minimum allele frequency (MAF), for further data analysis. The data is provided as **Online Resource 2**.

Data analysis and validation

Population structure of International Potato Center's breeding parents

Since allele frequencies through genotype calling are biased when allele depth of coverage is relatively low (**Maruki and Lynch 2017**) and given that we used diploidized markers for a hexaploid, we used non-parametric methods as described by **Gao and Starmer (2007)**, to estimate allele sharing distance (ASD). These methods do not assume Hardy-Weinberg equilibrium or linkage equilibrium and were implemented using the program AWClust 3.1. The phylogenetic tree was constructed using MEGA X program (**Kumar et al. 2018**). For allele diversity, Nei's coefficients of inbreeding (F_{IS}) (**Nei 1977**) and Wright's inbreeding coefficients (F_{ST} or θ) according to **Weir and Cockerham (1984)**, were estimated in R. Additionally, linkage disequilibrium (LD) between pairs of markers used for parental population structure was done using the LDheatmap package in R, with the option of estimating r^2 .

Selection of a diagnostic SNP set

Given that our objective was to develop a QA/QC SNP set that would be diagnostic for the global breeding population, we selected SNPs identified from parents as described above but also validated the selected SNPs using progeny from the recombined MDP breeding population. The MDP population was developed by crossing 16 parents from the east and central African support platform. The parents were crossed in 8*8 without reciprocals following a B*A pseudo-heterotic grouping based on genetic distance established by simple sequence repeat (SSR) markers (**David et al. 2018**). With about 30 genotypes per family on average leading to ~2000 genotypes, about 5% of this population was selected for QA/QC, tracking the population from *in vitro*, through screen house and field (**Gemenet et al. 2019a**). To develop a rapid QA/QC intermediate marker set, we selected only high-quality SNP markers that were present in both the parents and the MDP breeding population progeny. The selected markers were confirmed if they kept the same

population structure of the parents and still identified the same error rate in the MDP population. Several studies have selected rapid QA/QC sets with as low as 10 SNP markers e.g. in Maize (Chen et al. 2016). However, the base chromosome number of sweetpotato is 15 and given that we were diploidizing hexaploid loci, our aim was to have a minimum of two markers per base chromosome. We performed principal component analysis of the intermediate marker set according to Chen et al. (2016), but no apparent grouping of the markers was determined. We therefore selected the final 30 SNP markers based on chromosome number and genetic distance, from an intermediate marker set of 85 SNP markers. To establish the utility of the 30 selected SNPs for rapid QA/QC, we compared the ASD of both parents and MDP populations based on the 30 selected SNPs and the ASD based on their respective original filtered marker sets (205 SNPs for parents and 10,159 SNPs for MDP), using DARwin 6.0.21 tree comparison function (Perrier and Jacquemoud-Collet 2006). The data, including the parental Full-SNP set (9,670 SNP), 205-SNP set, 85-SNP set, and 85-SNP set for MDP are provided in **Online Resource 2**. The full-SNP set for the MDP is published open-access together with Gemenet et al. (2019a).

Results

SNP profile from the parental population

The high-density genotyping resulted in 9,670 SNP markers (**Online Resource 2**) from 662 parents of CIP's breeding population. With filtration of $\leq 25\%$ missingness, ≥ 0.25 polymorphic information content (PIC) and $\geq 10\%$ minimum allele frequency (MAF) and an average of 30x allele depth of coverage we recovered 205 SNP markers that were deemed appropriate for analysis of the breeding population structure. **Fig. 1** shows quality attributes of the unfiltered and filtered SNP data. The number of filtered SNPs ranged from six to 18 per base chromosome.

Population structure of CIP's Breeding Population

We examined population structure of the parents using 205 SNP markers. As expected from a global breeding program, population structuring indicated evidence of germplasm transfer among the breeding support platforms, although there was also evident local adaptation to each support platform (**Fig. 2**). The global support platform in Peru had the highest number of parents in the current study. Clustering showed that there is a group of parents from Peru that are closely related to African breeding parents especially those from Ghana and Mozambique. However, an additional group was only unique to Peru (**Fig. 2**). This group can also be seen between the first and second dimensions of a 2D multidimensional scale (**Online Resource 3**). The east and central Africa support platform in Uganda had a distinct group of parents but also a small admixed group with Mozambique (**Fig. 2**). The Uganda platform did not have a lot of admixtures from Peru. Given that the west African support platform was recently established (ca. 2010), and is the smallest in terms of size, the clustering indicates intake of breeding materials from other breeding support platforms especially from Mozambique and Peru, with minimal transfer to Ghana from Uganda. However, on a higher level, the structure can be generalized into two, with one cluster made up of materials from Peru and Ghana, and the other made up of materials from Uganda, Mozambique and Peru.

Allele diversity and linkage disequilibrium

Nei's coefficients of inbreeding indicated an average of $F_{IS} = 0.14$ across all populations and that parents from Uganda had the highest inbreeding coefficient $F_{IS} = 0.33$, followed by Mozambique with $F_{IS} = 0.24$. Ghana, followed by Peru had the lowest coefficients of inbreeding at $F_{IS} = 0.008$ and $F_{IS} = 0.07$, respectively. The estimated variance components and fixation indices showed that the correlation of genes within individuals or inbreeding was $F = 0.18$, the correlation of

genes in different individuals within the same population was $\theta = 0.07$, and the correlation of genes within individuals within populations $f = 0.12$. Comparing θ values (F_{ST}) between pairs of populations (support platforms in this case) showed that Uganda was the most distinct group with $\theta = 0.08$, $\theta = 0.09$, and $\theta = 0.1$ with Ghana, Mozambique and Peru, respectively. The paired θ values among Ghana, Mozambique, and Peru were fairly consistent ranging from $\theta = 0.041$ to $\theta = 0.049$. Data is summarized in **Table 1**. Analysis of LD indicated minimal LD among the SNP markers used with the genome-wide LD having an average $r^2 \leq 0.1$. LD per chromosome is presented in **Fig. 3**. The results show that very few loci were in LD at $r^2 \geq 0.1$, as majority of loci within a chromosome also had $r^2 \leq 0.1$. This data indicated that the data set was adequate for analyzing population structure.

Identifying diagnostic markers for routine quality assurance and control of breeding populations

To develop QA/QC diagnostic markers from parents that can be used in routine QA/QC of breeding populations, we added an additional filtering step to the QA/QC parent SNP markers so as to include only those markers that were also present in genotypic data developed from a breeding population progeny (MDP). A random 5% (94 genotypes) of the MDP population had previously been genotyped for QA/QC and genetic fidelity as the population passed through *in vitro*, screenhouse and to the field experiments, using the same genotyping platform. The genotyping had been done at high density with approximately 41k SNPs filtered down to 10,159 SNPs. Genotype misclassification in the population was then previously estimated based on 10,159 SNPs (**Gemenet et al. 2019a**), which is ‘rich’ for routine QA/QC within most breeding programs. The desired low-cost, low-density QA/QC SNP set was therefore selected based on the following criteria: i) $\sim 30\times$ allele depth of coverage; ii) $\geq 75\%$ call rate; iii) ≥ 0.25 PIC; iv)

254 $\geq 10\%$ MAF; v) chromosome position known; vi) be present in a randomly selected population of
 255 progeny. This resulted in further filtration of the 205 SNP markers used for population structure
 256 of the parents above, down to 85 SNP markers (**Online Resource 2**), which could be used for
 257 ‘general QC’ in the sweetpotato breeding programs as proposed by **Chen et al. (2016)**. However,
 258 for routine QC, this marker number is still probably too high for most breeding programs.
 259 Principal component analysis of the 85 markers did not show any specific grouping of markers,
 260 with PC1 and PC2 only explaining 9.1% of the variation (**Online Resource 4**). Therefore, the
 261 final 30 SNP markers were selected based on genetic distance per chromosome. The set of 85
 262 markers were not evenly distributed for all chromosomes and chromosome 15 had only one
 263 marker. To achieve the target of two markers per base chromosome, we selected one marker
 264 from the original set of 205, based on genetic distance relative to the one marker present in the
 265 set of 85. Comparing the population structure of the parents using 205, 85 and 30 SNP markers
 266 indicated that the 30 markers kept the general structure of the populations, though the clustering
 267 was considerably different compared with the use of 205 SNPs (**Fig. 4**). Comparing trees
 268 indicated that the tree developed with 205 SNPs was 17.1% different from the tree with 30 SNPs
 269 when strict conditions were used. For validation of the selected marker set, we also compared the
 270 level of error identified in the breeding population progeny (MDP) using 10,159 SNPs, 85 SNPs
 271 and 30 SNPs (**Fig. 5**). Results show that 10,159 SNPs identified 27.7% misclassification, 85
 272 SNPs identified 29.8% misclassification and 30 SNPs identified 31.9% misclassification. Tree
 273 comparison between 10,159 SNPs and 30 SNPs indicated that they were 24.6% dissimilar when
 274 strict conditions were applied. Combined, these results suggest that the selected 30 SNPs could
 275 be used as a cost-effective rapid QA/QC set for sweetpotato in CIP’s breeding populations. The
 276 selected SNPs are listed in **Table 2**.

Addition of trait specific markers to the selected QC set

Previous studies had mapped quantitative trait loci (QTL) for yield and component traits (Pereira et al. 2019; Gemenet et al. 2019a) as well as quality-related traits (Gemenet et al. 2019b). From these QTL mapping results, we selected six SNP markers that were associated with dry matter, starch, β -carotene, flesh color and total root yield. The markers labeled ‘trait specific’ are shown in **Table 2**. The first four traits were selected because they are correlated and important contributors to culinary traits that affect adoption of new varieties in sweetpotato. Dry matter and starch are positively correlated but are negatively correlated to both β -carotene and flesh color, and this negative correlation affects ‘culinary quality’. Additionally, these traits are oligogenic hence results are repeatable within the QTL. Total storage root yield was selected as a primary trait and the selected marker of a QTL was found to be a constitutive marker for this trait across several environments based on multi-environment testing of a full-sib population (Gemenet et al. 2019a).

Discussion

Our genotyping efforts resulted in less than 10,000 bi-allelic SNP markers. Stringent filtering resulted in an even smaller data set of 205 SNP markers. The considerable reduction in highly informative markers can be associated with the difficulty in genotyping polyploids. With a mostly auto-hexaploid genome (Wu et al. 2018), sweetpotato presents allele dosage uncertainty due to ambiguous copy numbers of each allele. Additionally, the assumption of random inheritance of alleles may not hold true in this case due to uncharacterized consequences of whole genome duplication (Blischak et al. 2016, 2018). DArTSeq implements new protocols of sequencing complexity reduced representations (Altshuler et al. 2000) in combination with the next-generation sequencing methods (Baird et al. 2008; Elshire et al. 2011). Implementing

genotyping-by-sequencing-like procedures, DArTSeq involves a two-restriction enzyme system composed of a ‘rare-cutter’ and a ‘common-cutter’, mainly *Pst1-Mse1*, to enhance uniform complexity reduction within the genome (**Poland et al. 2012; Brouard et al. 2017**). In such next-generation sequencing methods, depth of sequencing determines genotyping quality as low depth of coverage results in genotyping errors, misalignments and a lot of missing data which eventually cause biases in downstream population-genetic analyses (**Fumagalli 2013; Maruki and Lynch 2017; Crawford and Lazzaro 2012**). For instance, **Ashraf et al. (2016)** showed that low sequencing depth resulted in SNPs that underestimated genomic heritability due to overestimation of inbreeding and underestimation of heterozygosity in rye-grass. We chose to use ‘diploidized’ data in the current analysis because the depth of coverage from most genotyping platforms is not adequate to reliably characterize heterozygous loci, such as those likely to be found in polyploids, for which deep sequencing is required (**Fresnedo-Ramirez et al. 2019**). Furthermore, analyses comparing genotypic data from DArT-Seq and those from a deep sequencing optimization platform for sweetpotato called GBSpoly (**Wadl et al. 2018**) have confirmed that highly informative ‘diploidized’ DArTseq data performed just as well as high confidence data with dosage in genomic predictions of sweetpotato depending on trait architecture (**Gemenet et al. under preparation**).

Population structure as well as allele diversity analyses in the current study indicated that parental genotypes from Uganda were the more distinct and inbred. This observation can be associated with the high sweetpotato virus disease (SPVD) pressure around the lake region of eastern Africa and a general lack of germplasm with high levels of resistance to SPVD necessitating the use of the same lines frequently as parents in the Ugandan breeding program (**Gibson et al. 1998a, 1998b; Ndunguru et al. 2009**). SPVD is the most important virus

complex in SSA and its effects are most pronounced in east Africa, causing yield losses of about 56-98% in farmers fields (**Mukasa et al. 2003; Ndunguru and Kapinga 2007**). **Gruneberg et al. (2015)** noted that SPVD prevalence in east Africa resulted in the failure of nearly all orange-fleshed varieties introduced into this region. SPVD is caused by a synergistic and complex infection by sweetpotato feathery mottle virus and sweetpotato chlorotic stunt virus, transmitted by aphids and white-flies, respectively (**Mwanga et al. 2002**). **Clarke et al. (2012)** indicated that different regions have different strains of the individual virus, and that east Africa has distinct strains. Studies have also showed that sweetpotato chlorotic stunt virus strains are more related in east and southern Africa and are distinct from those in the other regions of the world (**Hoyer et al. 1996**). These results are supported by our current population structuring which shows that Uganda has some ad-mixing with Mozambique, but very little admixing with either Peru or Ghana. These results have implications extending to other breeding decisions such as determining the effective population sizes especially for the Uganda breeding platform where migration of germplasm into the platform is restricted due to SPVD.

Different alleles are represented in different genetic backgrounds and our results show allele diversity between other support platforms with especially the Uganda population. Therefore, understanding population diversity especially of a global breeding program is important for breeding decisioning. Breeding programs are currently moving towards genomics-assisted breeding (GAB). Repeatability of quantitative trait loci in different genetic backgrounds is one prerequisite for the success of GAB methods such as QTL mapping, genome-wide association mapping, and genomic selection (**Azevedo et al. 2017; Wientjes et al. 2018**). In genome-wide association mapping, accounting for population structure avoids false positives and allows selection of causative variants, while accurate prediction of untested future genotypes in genomic

selection is only possible when familial relatedness is accounted for, allowing for a reliable association between markers and QTL (**Daetwyler et al. 2012**). In the case of our global breeding population, the current information will be important when designing a genomic selection scheme to facilitate decisions such as prediction within or across sub-populations. Similarities and differences in genetic architecture of complex traits between populations can also be understood by studying the genetic correlation between the populations (**Wientjes et al. 2018**). Our results indicate that the Ugandan sub-population was also the most distinct from the three others when θ values (F_{ST}) between pairs of populations was examined. This would imply that predictions may be carried out separately for the Ugandan populations in future GAB activities, while the predictions may be tested across the platforms in Peru, Mozambique and Ghana, given similar environmental conditions. Since GAB requires that markers be in LD with QTL, our results indicating very minimal LD among markers confirm that this marker density is not enough for making selection decisions (**Flint-Garcia et al. 2003; Vos et al. 2017**). Although the number of markers used in the current study are adequate for the purposes of the current objectives of population structuring, more dense markers along the genome will be required to reliably study the LD decay in sweetpotato. However, ‘high density’ has cost implications and hence the optimum number of markers required for routine GAB use will need to be reliably estimated through reducing within-haplotype density by selecting the minimum number of markers that can define common haplotypes (**Meng et al. 2003**).

In the current study, we used filtration and validation methods of DArTSeq developed markers to select a marker set of 30 SNPs that can be used for QA/QC purposes in sweetpotato. Our selection of informative markers included considerations for depth of coverage, missingness, chromosome position, genetic distances, validation for repeatability in progeny and inclusion of

trait specific markers to result in a total of 36 SNPs. Development of SNP sets for QA/QC has been done in several crops. Extensive tests were carried out to develop a SNP set for ‘broad’ and ‘rapid’ QC in maize (**Chen et al. 2016**). In their study, they showed that marker coverage between 2 and 15, markers with less than 20% missing values, including markers with chromosome positions, markers with less than 6% heterogeneity, inclusion of trait specific markers, and selection of markers from groups based on average group distance gave the best marker set towards developing a ‘rapid’ QC set, using DArTSeq markers. Prior to this, **Semagn et al. (2012)**, used about 1,597 SNP markers from the KASPar and GoldenGate platforms to select highly informative markers for low-cost QC genotyping in maize. They recommended a set of 50-100 SNPs for routine QC after finding about 29% heterogeneity in inbred lines. In rice, **Ndjiondjop et al. 2018** recommended a subset of 24-36 SNP markers filtered from DArTSeq developed markers for genetic purity analyses. In sweetpotato, QA/QC problems have recently been acknowledged (**Gemenet et al. 2019a**) by monitoring the rate of misclassification as materials moved through different stages of breeding trialing. That study indicated about 30% misclassification issues in one breeding population. QA/QC in sweetpotato breeding trials will improve precision and breeding efficiency through use of new methods like forward breeding and genomic selection currently being adopted by CGIAR programs. These new breeding strategies are aimed towards increasing the rate of genetic gains from breeding to address issues related with population increase and climate change. Therefore, QA/QC of breeding processes will improve the likelihood of success.

Since the real impact from breeding can only be measured by the improvements observed in farmers’ fields, controlling and assuring the quality of finished varieties is important to breeding programs. Issues with QA/QC of released varieties have been reported in sweetpotato and this is

exacerbated because the extent of adoption of new varieties cannot be determined accurately especially with informal seed systems where genetic integrity is seldom considered (Namanda et al. 2011). In Ethiopia, Kosmowski et al. (2018) used 17,220 DArTSeq developed markers to establish that about 20% of farmers confused local varieties for improved varieties and vice versa, and that farmers assigned different local names to the same variety or vice versa. Their study confirmed that data from survey studies (Labeyrie et al. 2014; Wossen et al. 2017) were mostly unreliable. Despite this important revelation, high density genotyping at 17,220 markers is not amenable for widespread routine use, therefore leaving household surveys as the predominant way of carrying out adoption studies. The currently developed marker set will therefore be useful in addressing also adoption-related needs in sweetpotato.

Towards increasing genetic gains in the sweetpotato breeding programs, QA/QC will need to be combined with other approaches of optimizing breeding schemes.

Data Availability

All data associated with this manuscript are provided together with the manuscript as supplementary (Online Resource 2).

Figure Captions

Fig. 1 Quality attributes of unfiltered (9,670; Top) and filtered (205; Bottom) SNPs from DArTSeq indicating call rate (A), frequency of homozygotes for the reference allele (B), frequency of homozygotes for the alternative allele (SNP; C), frequency of heterozygotes in the data (D), polymorphic information content of the SNP (E), and average polymorphic information content between reference and SNP alleles (F)

Fig. 2 Phylogenetic tree (Neighbor-Joining) showing the population structure of the International Potato Center (CIP)’s global breeding parents. Genotypes in Black represent parents from the global support platform in Peru, genotypes in Blue represent parents from the southern Africa support platform in Mozambique, genotypes in Green represent parents from the east and central Africa support platform in Uganda, while genotypes in Red represent parents from the west African support platform in Ghana. The tree was developed using MEGA X.

Fig. 3 Linkage disequilibrium among 205 markers used in population structure analysis, analyzed per chromosomes for the 15 base chromosomes of hexaploid sweetpotato

Fig. 4 Phylogenetic trees (Neighbor-Joining) comparing the clustering of the International Potato Center (CIP)’s breeding parents using 205 highly informative SNP markers (left), 85-SNP intermediate marker set (center) and the 30-SNP selected QA/QC set markers (right). Genotypes in Violet represent parents from the global support platform in Peru, genotypes in Green represent parents from the southern Africa support platform in Mozambique, genotypes in Orange represent parents from the east and central Africa support platform in Uganda, while genotypes in Blue represent parents from the west African support platform in Ghana. Trees were developed using DARwin 6.0.21

Fig. 5 Sankey diagrams showing mislabeling error as Mwanga diversity Panel (MDP) population moved from *in-vitro* to screen house to field, based on 10,159 SNPs, 85-SNP intermediate marker set and 30-SNP selected quality control (QC)-set. The Pink color indicates those that did not cluster (with mislabeling errors) while the grey color indicates those that clustered as expected, implying no mislabeling errors

Online Resource Captions

Online Resource 1 List of parental genotypes from the International Potato Center (CIP)'s global breeding program indicating breeding support platform of origin.

Online Resource 2 DArTSeq data used in the current study in separate excel sheets showing the original data set of 9,670 SNPs (Parents-Full), 205 stringently filtered and highly polymorphic SNPs (Parents-205), 85-SNP intermediate with highlighted 30-SNP selected QA/QC set based on parents (Parents-85&Selected QC Set), and 85-SNP intermediate marker set with highlighted selected QA/QC set based on the progeny of the Mwanga Diversity Panel (MDP-85&Selected QC Set)

Online Resource 3 Two-dimensional figure from multidimensional scaling of the International Potato center (CIP)'s global sweetpotato breeding parents as observed using 205 highly informative SNP markers

Online Resource 4 Principle component analysis (PCA) carried out on 85-SNP intermediate marker set to check for possible groupings to aid the selection of a 30-SNP quality control marker set

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Table 1. Allelic diversity parameters among parents of the International Potato Center (CIP)'s breeding parents from Ghana, Mozambique, Peru and Uganda.

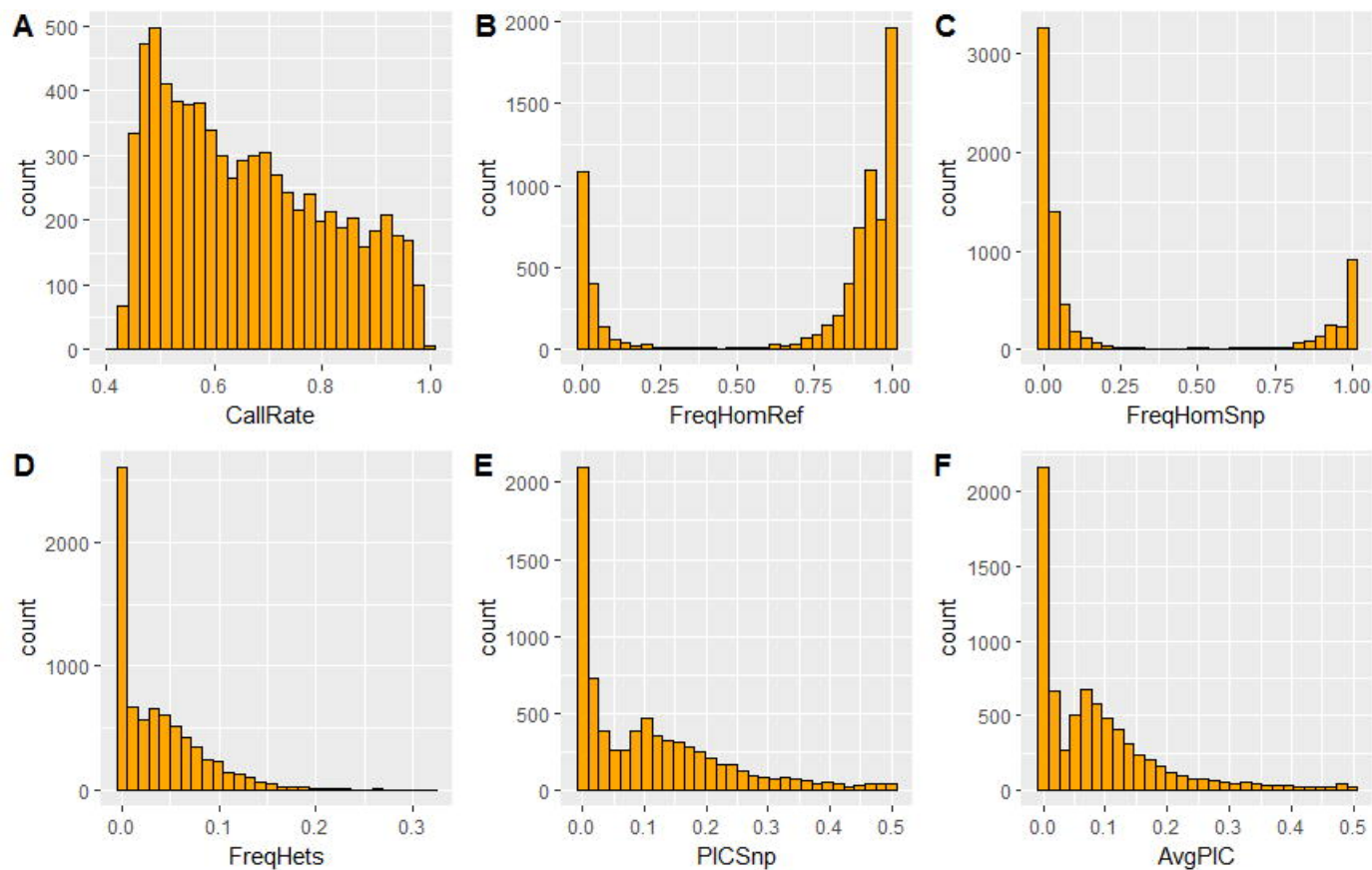
Nei's F _{IS}				
Ghana	Mozambique	Peru	Uganda	Average
0.008	0.24	0.07	0.33	0.14
Variance and fixation indices				
F=0.18	θ=0.07			f=0.12
F _{ST} (θ) among pairs of populations				
	Ghana	Mozambique	Peru	Uganda
Ghana	0			
Mozambique	0.049	0		
Peru	0.046	0.041	0	
Uganda	0.08	0.09	0.1	0

623 **Table 2** Details of the 36 SNPs selected for ‘rapid QC’ in sweetpotato. AlleleID refers to the identity of the specific allele on the
 624 DArT platform, AlleleSeq refers to the flanking sequence of the SNP, Chr indicates the chromosome number, Pos indicates the
 625 position of a SNP on the specific chromosome, SNP is single nucleotide polymorphism

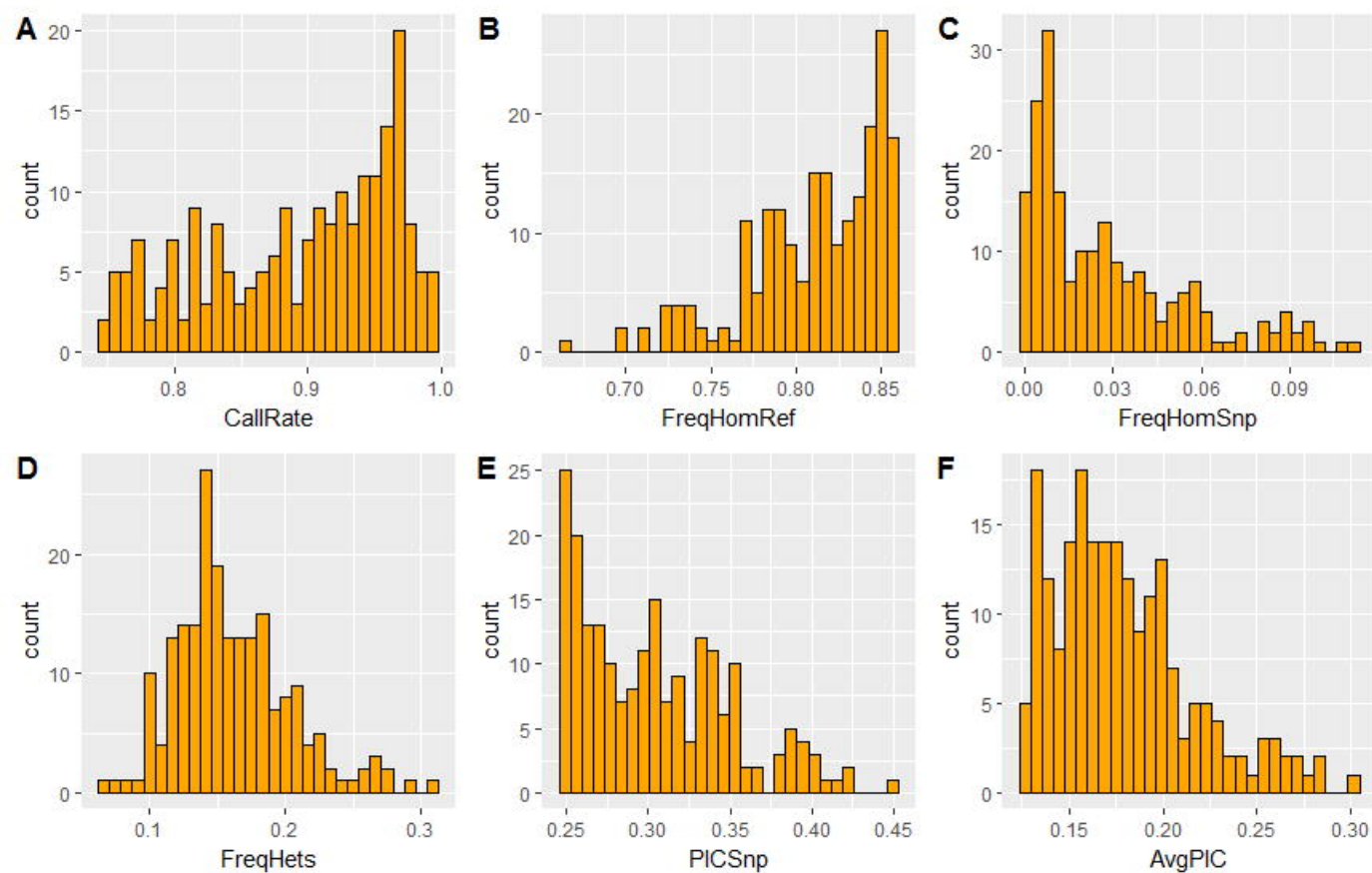
No	AlleleID	AlleleSeq	Chr	Pos	SNP
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2	100736260 F 0-18:C>T-18:C>T	TGCAGTCAGCGACTCTCTCCAATGATATTCTTCTTGGAGCTGAGTGGAACCTCTTTCTTTGATTCTA	Chr01	881461	18:C>T
3	7629110 F 0-28:G>T-28:G>T	TGCAGTCTTTGCTCTCAAAAGTTTCTTTGGAGTTCTCATATGAATTCTGAACATCACTAATTTGGATTG	Chr02	13184152	28:G>T
4	7609930 F 0-10:T>G-10:T>G	TGCAGTCATCTGTTGTCTGAAGCAATTAGCCTATGATCTTGTGAGCTGCTGTTGTCACTGCAATTC	Chr02	6247633	10:T>G
5	7629039 F 0-39:A>T-39:A>T	TGCAGTACAGAAAACCAACCAGCAGAGAAGATAATTTTATAATGAACAGCTCAGGAACCCAGTTGGCTAG	Chr03	24217089	39:A>T
6	7561292 F 0-28:A>G-28:A>G	TGCAGTTGACTCATCCCAACCGCCTACACATTATCAAAACAAATACAGATCGGAAGAGCGGTTACAGCA	Chr03	3304180	28:A>G
7	11826044 F 0-66:G>A-66:G>A	TGCAGTCCATATCAGAATGACAATTCTGTAGAGATTGCACAATCCTTTGGGTTTTCTTCTGCGTACGAT	Chr04	31341133	66:G>A
8	7569592 F 0-50:G>A-50:G>A	TGCAGAAGATGGTGGTTGCGACAGAATGAAAGAATGGAGTAAGCAGAGAAGGCCATTACCCCTTCTGAT	Chr04	5305718	50:G>A
9	7552489 F 0-18:T>G-18:T>G	TGCAGATAAAAGGTAAATCAAAACCACAAATCTAACTGTCTCTACATTCTTCTATCAAAATTTGG	Chr05	24475925	18:T>G
10	7562059 F 0-41:A>G-41:A>G	TGCAGATGAAATGAAATGAAAACCTTTAGTGCATATCATGTAAGCAATGTAATTGAAATCCACTAAGAG	Chr05	892499	41:A>G
11	9847708 F 0-17:G>C-17:G>C	TGCAGAAAAACATACGCGGTGGATTGATGTTCTCAAAACAAATGGAAGATGCAGAAAGTAAACCTGACT	Chr06	19672316	17:G>C
12	7558428 F 0-52:C>T-52:C>T	TGCAGCTACAACCTTTGACAAGCTGGCATCTATTAGTTACGTTTTGTTCCCTTCATGTGGCACTCTTGAT	Chr06	4639839	52:C>T
13	9845663 F 0-25:T>G-25:T>G	TGCAGTTTATCTAAGTAAGATGATATTCAGCGAGATGAAAACCTAGGATGAGTGTGAAGGAATACAAG	Chr07	23485155	25:T>G
14	7618077 F 0-38:G>A-38:G>A	TGCAGATCTTGAGCAGGTTGTAATAAAGTGAGAGTGGAATTAGTTACCACAATCTCTGTAAATTTAG	Chr07	5042144	38:G>A
15	100588703 F 0-44:T>C-44:T>C	TGCAGGCAACTTTATTGAAATGTTGACTAAAATCTGTTTTCTGTCAAGCTTCAACATAGACCTCATTG	Chr08	15171824	44:T>C
16	100512185 F 0-24:A>G-24:A>G	TGCAGTATCCGAAATCCCTTTCCAAATGTTTGCTTATAAGCTGGTTGAGAAGGAGAAAAGTTAGGGAA	Chr08	6218106	24:A>G
17	7568783 F 0-21:T>A-21:T>A	TGCAGTGCAATGCATGAGCCTCTGGCAACGTTGAGAAGTCAACCGCTTGCAAGTTTCTCGGTCACGTCGGT	Chr09	22534529	21:T>A
18	14313832 F 0-18:G>A-18:G>A	TGCAGATATAATGAAAAAGCACATAAAAAGTGACAAGAAATATCAAATTAGGTACACTTGCTGCATCT	Chr09	520352	18:G>A
19	7554048 F 0-9:G>A-9:G>A	TGCAGTATCGAAAGCAATGTCTTTGGTCTTCTGTTAGGTTCTCTTCTTCCATTTCTATTTTACA	Chr10	4446069	9:G>A
20	7574585 F 0-20:A>G-20:A>G	TGCAGAAACTCCAAAGGAGATAGGAAATTTGCATCATCTAAGGTACATTGATTACAGATCGGAAGAG	Chr10	6952705	20:A>G
21	7619107 F 0-63:G>T-63:G>T	TGCAGTGACGATTCTTCCAATTAGCTCTTCTGCCCTTGAACAACAATCAACATACTAGCTTGCTGTT	Chr11	18928235	63:G>T
22	7611165 F 0-24:G>T-24:G>T	TGCAGTCAATCAGATAGAACAATCGTTTAGTCTTATGTTATGGTGATTGATAGGGGGAGTATACGATTA	Chr11	2783237	24:G>T
23	7558251 F 0-66:C>A-66:C>A	TGCAGCCACGTGACACCAACAAACCCCTATTTTCCGCCAGTTTGTCTCACTTGGCGGGAAACCCC	Chr12	1719732	66:C>A
24	7619930 F 0-17:C>A-17:C>A	TGCAGAGGATAAAAGTTCTGTACCCAAACAGGGGCTTTTACAGATCGGAAGAGCGGTTACGAGGAAT	Chr12	24038510	17:C>A
25	7562142 F 0-54:C>T-54:C>T	TGCAGATTGTGAATCCCTTTAGAGTCAGCAACAGAGGCCTCTCGGTGATTCTCTTCTCATTATTATC	Chr13	22402544	54:C>T
26	100589662 F 0-45:C>T-45:C>T	TGCAGTAATGATTTGGATATAGCACATACACATATAAATTATATACAAATATAGTATTATTTTCAGCAA	Chr13	7074575	45:C>T
27	100619651 F 0-17:C>T-17:C>T	TGCAGTTGCTTAGCTTCCGCTACTTTGTTGGGTGGCCTTCTCTTGAGGTAATTTGAAGTACTAATCA	Chr14	17915206	17:C>T

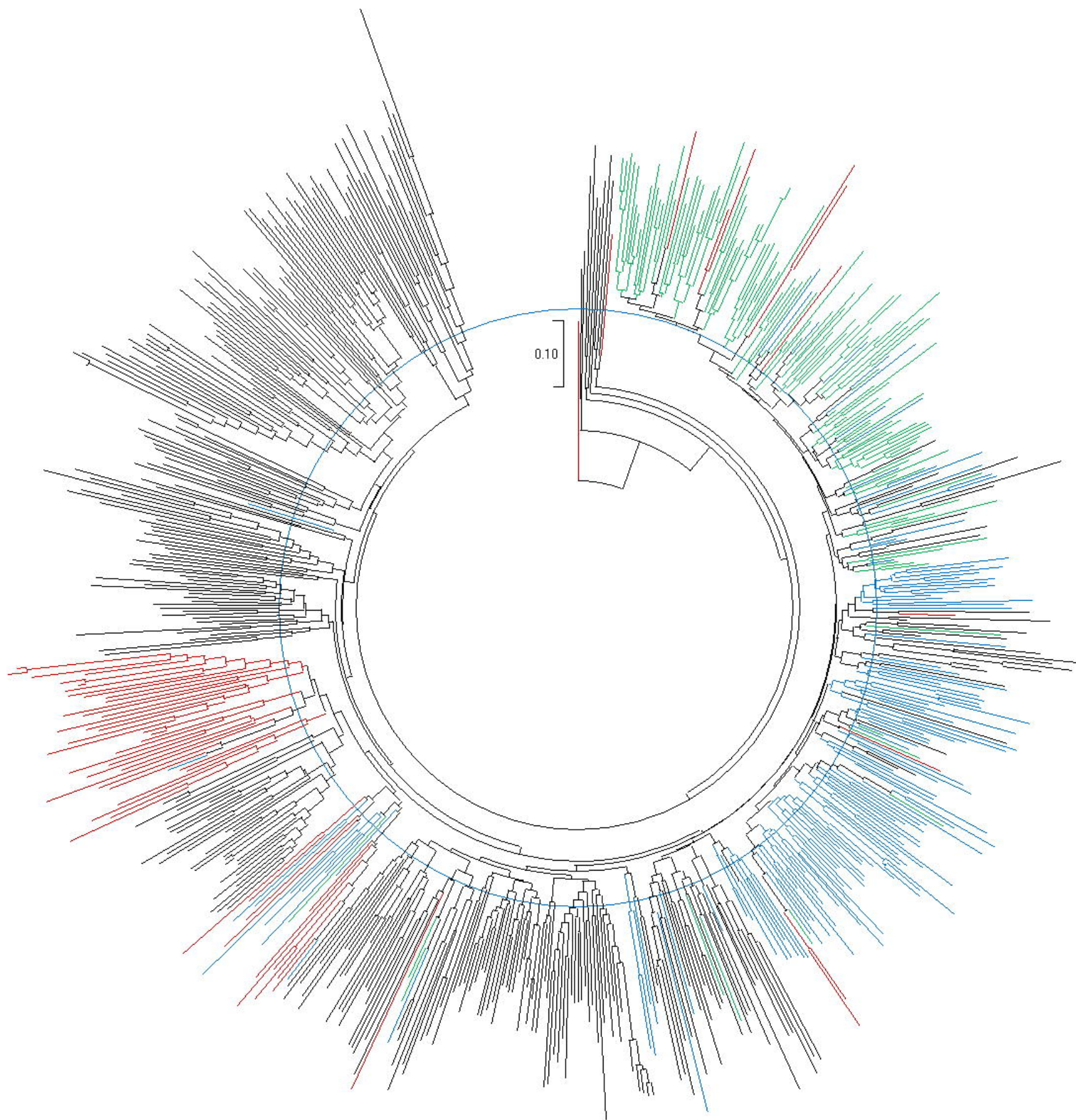
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30	9845617 F 0-25:C>A-25:C>A	TGCAGTTCCTGCACTTCCAGTGAACCCCGATATATGCTCTCCGCATATAACACTCAGCAATGAATTC	Chr15	8808402	25:C>A
Trait-Specific Markers					
	Trait	Genetic Position	Chr	Pos	SNP
31	Dry matter & Starch	37.44	Chr03	3185578	C>T
32	β-Carotene & Flesh				
32	color	36.14	Chr03	2994719	C>G
33	β-Carotene & Flesh				
33	color	146.02	Chr12	22131994	G>A
34	Starch	147.31	Chr12	22197168	T>A
35	Dry matter	150.05	Chr12	22369268	A>T
36	Storage root yield	4.19	Chr15	452966	A>C

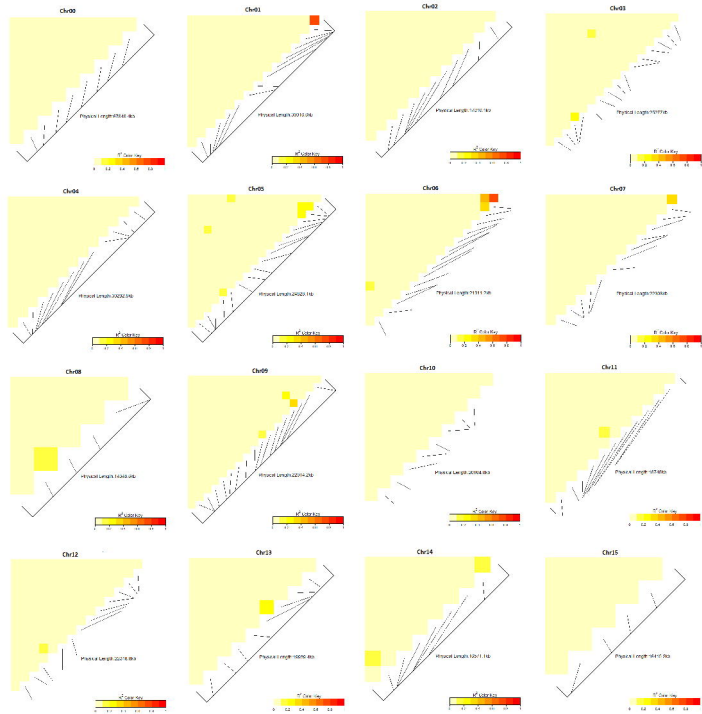
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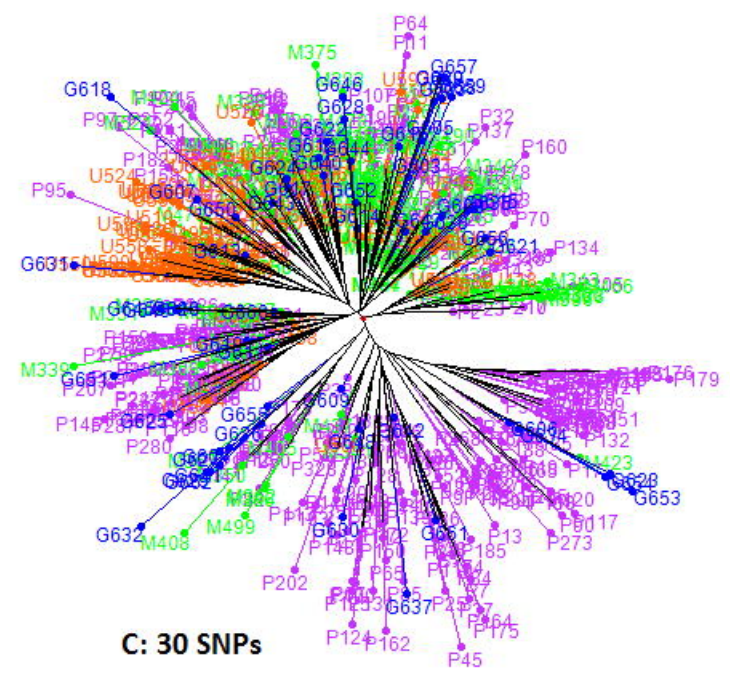
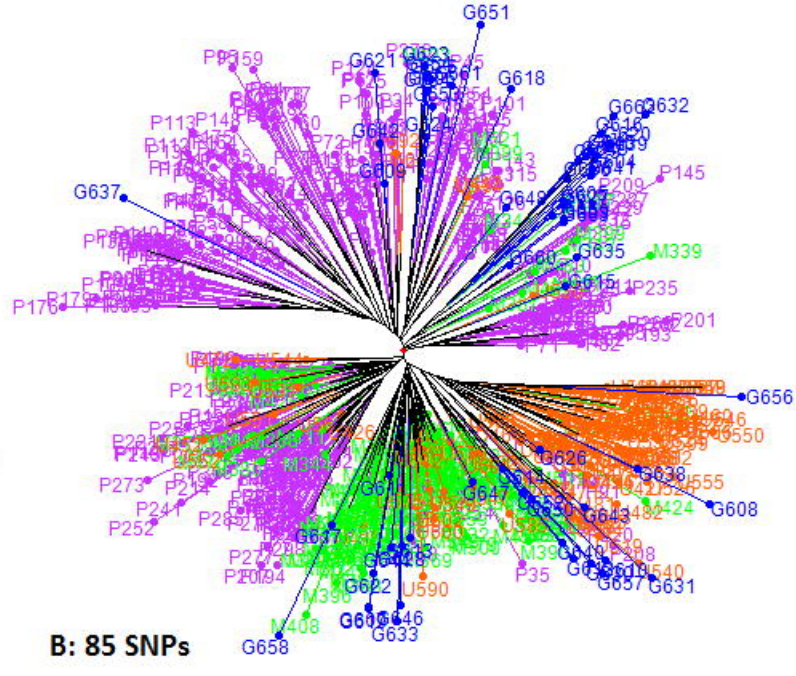
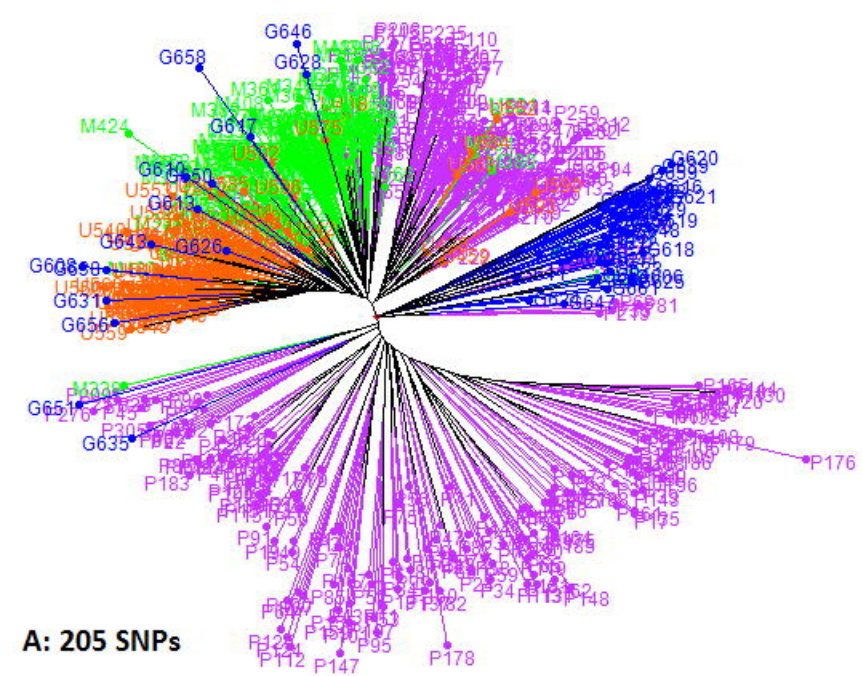


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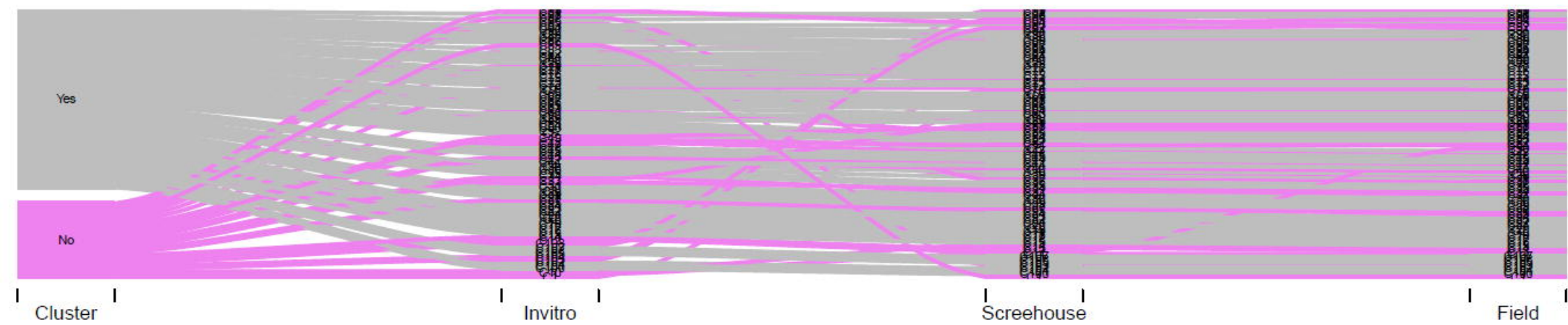




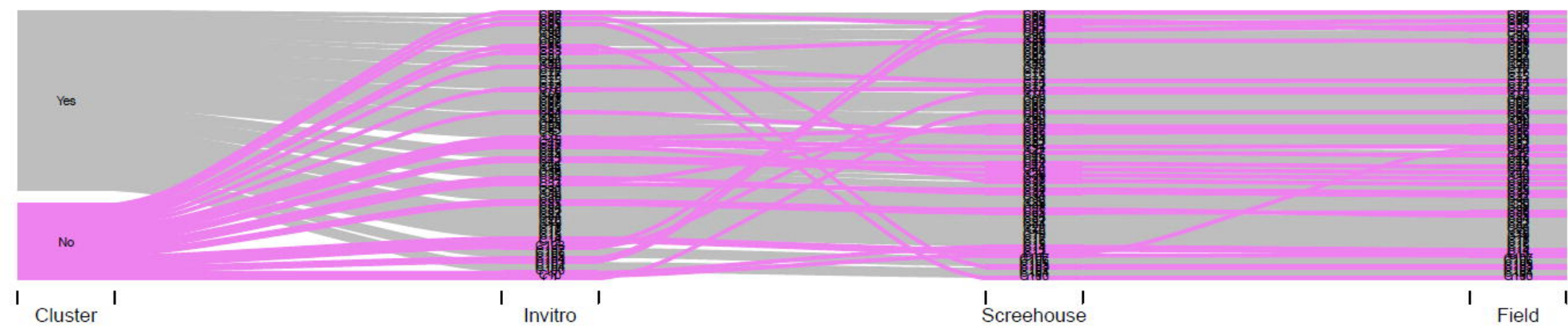




10,159 SNPs
Error = 27.7%



85 SNPs
Error = 29.8%



30 SNPs
Error = 31.9%