

1 **Improving root characterisation for genomic prediction in cassava**

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13

14 **Abstract**

15 Cassava is widely cultivated due to its high drought tolerance and high carbohydrate-containing
16 storage roots. The lack of uniformity and irregular shape of storage roots within and between
17 genotypes poses significant constraints on harvesting and post-harvest processing. Routine
18 assessment of storage root size and shape in breeding plots relies on visual scores.

19 Here, we phenotyped the Genetic gain and offspring (C1) populations from the International
20 Institute of Tropical Agriculture (IITA) breeding program for root shape and size-related traits
21 using image analysis of storage root photographs taken in the field.

22 In our study, using univariate genome-wide association analysis, we detected for most shape
23 and size related traits, significant QTL regions located on chromosomes 1 and 12. The QTL
24 region on chromosome 12 has been previously associated, using IITA breeding populations, to
25 cassava mosaic disease (CMD) resistance.

26 Because the uniformity in size and shape of cassava roots is an important breeding goal, we
27 calculated the standard deviation of individual root measurements per clone. The use of
28 standard deviation measurements allowed the identification of new significant QTL for
29 Perimeter, Feret and Aspect Ratio on chromosomes 6, 9 and 16. Using genomic prediction
30 cross validation, the accuracies of root size and shape-related traits were lower than those
31 previously reported for dry matter content (DM) and cassava mosaic virus resistance (CMD).
32 Predictive accuracies of the mean values of root size and shape image-extracted traits were
33 mostly higher than yield trait prediction accuracies in the C1 population. This study aimed to
34 evaluate the feasibility of the image phenotyping protocol and to assess the use of genome-

35 wide analyses for size and shape image-extracted traits. The methodology described here and
36 the results obtained in this study are promising and open up the opportunity to apply high-
37 throughput methods in cassava.

38

39

40 **Introduction**

41 Cassava (*Manihot esculenta* Crantz), a tropical root crop with origins in Latin America, ranks
42 as the 3rd most important crop in the tropics after rice and maize (Guira *et al.*, 2017). In Africa,
43 more than 800 million people rely on cassava as a primary source of calories (Howeler *et al.*,
44 2013). Cassava is widely cultivated due to its high drought tolerance and high carbohydrate-
45 containing storage roots, and although most of the production is for human consumption, its
46 use extends to animal feed and industrially processed products (Hahn, Reynolds and Egbunike,
47 1992; Howeler *et al.*, 2013; Lukuyu *et al.*, 2014). In addition to the edible, high-starch storage
48 roots, cassava plants produce thin fibrous roots, which function to absorb water and nutrients
49 from the soil (Alves, 2002). The development and differentiation of fibrous roots, as well as
50 the mechanism that triggers root storage formation in cassava, are poorly understood.

51 Cassava storage roots are morphologically diverse, the lack of uniformity and irregular shape
52 between and within genotypes poses significant constraints on harvesting and post-harvest
53 processing. The irregularity of root shape results in considerable losses of valuable root yield
54 (Hahn, Reynolds and Egbunike, 1992). The waste of tuber flesh and the inefficiency of hand
55 peeling could be avoided by peeling mechanization. However, breeding for root characteristics
56 that facilitate this process requires a thorough understanding of the genetic basis of cassava
57 root morphology. Several studies have attempted to characterize cassava root shape to support
58 the development of peeling mechanization (Onwueme, 1978; Ejovo N. Ohwovoriolè *et al.*,
59 1988). The root characteristics that were evaluated in those studies include root diameter,
60 weight, length and peel thickness.

61

62 Routine assessment of storage root size and shape in breeding plots relies on visual scores
63 (www.cassavabase.org/search/traits). The categorical scores for root size are 3, 5 and 7 for
64 small, medium and large roots, respectively. A single categorical score is given to a harvested
65 plot based on the most frequent size in that plot. The visual rating of shape is 1 (conical), 2
66 (conical-cylindrical), 3 (cylindrical), 4 (fusiform), 5 (Irregular), and 6 (Combination of shapes).
67 Similar to root size, the shape scoring is based on the most common observation in a plot. These
68 categorical scores suffer from person to person subjectivity and inability to describe the

69 variation in size and shape within a plot. Thus, image analysis of roots offers a more objective
70 means of obtaining unbiased quantitative data on important root traits.

71

72 Image analysis software tools for high-throughput phenotyping have gained increased
73 relevance due to the need in crop improvement to keep up with the advances in genotyping
74 technologies (Furbank and Tester, 2011; Hartmann *et al.*, 2011; Fahlgren, Gehan and Baxter,
75 2015). In Maize, imaging under controlled illumination followed by automatic image-analysis
76 has been successfully used to study root system architecture traits (Colombi *et al.*, 2015). In
77 cereals, grain shape is an important target for genetic improvement, because it is usually related
78 to quality, consumer appeal or the intended end usage (Lestrel, 2011). For rice grain shape
79 description, SHAPE, a program based on Elliptical Fourier Descriptor (EFDs) has been used
80 to derive shape-related phenotypes for genome-wide association and genomic prediction (Iwata
81 *et al.*, 2015b, 2015a).

82

83 Genomic selection (GS) is a method first introduced in animal breeding to select candidates for
84 crossing in the breeding program using only genomic information. GS is particularly relevant
85 for the improvement of polygenic traits (Heffner, Sorrells and Jannink, 2009) because its
86 implementation can lead to a reduction in cost and time compared to traditional plant breeding
87 programs (Jannink, Lorenz and Iwata, 2010). Because cassava is an outcrossing species mostly
88 propagated by stem cuttings, conventional breeding methods can take more than five years to
89 produce superior performing clones (www.nextgencassava.org). Genome-wide association
90 studies (GWAS) are complementary to GS as they have proven effective for the identification
91 of QTL regions associated with several traits that are critical for cassava breeding, including
92 cassava mosaic disease resistance (CMD) (Wolfe *et al.*, 2016), cassava brown streak disease
93 resistance (CBSD) (Kayondo *et al.*, 2018), and beta-carotene content and dry matter content
94 (Rabbi *et al.*, 2017).

95

96 In this study, size and shape related traits describing cassava roots were obtained through
97 automated image analysis. We first estimated their heritability and conducted a genome-wide
98 association study to explore the genetic architecture of cassava roots shape characteristics; then
99 we compared the genomic prediction accuracy of image size and shape traits to those of root
100 yield. Our research contributes to a better understanding of cassava root shape and explores the
101 possibility of high-throughput phenotyping that would allow breeders to use GS to select
102 varieties for quantitative root characteristics.

103 **Materials and methods**

104 **Germplasm**

105 We processed and analyzed cassava roots images taken from several field trials conducted by
106 the International Institute for tropical agriculture (IITA) as part of their genomic selection
107 breeding program. The cassava germplasm collections that we analyzed are known as Genetic
108 Gain (GG) and the progeny of the first genomic selection event (C1), which are thus progeny
109 of a subset of the GG population. The GG constitutes a large collection of important landraces,
110 breeding lines and released improved varieties of cassava developed by IITA over the last four
111 decades. More detail about the origins and constituency of these populations is available in
112 several published studies (Wolfe *et al.*, 2016; Wolfe *et al.*, 2017).

113

114 A summary of the trials used in the present study is presented in Table 1. The first set of trial
115 was the GG trial which comprised 805 plots planted in the summer of 2014 in Ubiaja, Nigeria
116 using an augmented design with two checks planted in each incomplete block. The trial
117 comprised of 758 unique clones. Each plot consisted of 10 stands in a single row with spacing
118 of 1 m between rows and 0.8 m within rows. The second set of trials consisted of 88 clones
119 selected from the GG population and planted as preliminary yield trial (PYT) across four
120 locations (Ibadan, Ikenne, Ubiaja and Mokwa) using a randomized complete block design with
121 two replicates. Plot size was similar to that of the GG trial. It is important to note here that
122 these clones were used as parents for the GS cycle 1 population. The third set of trials involved
123 GS cycle 1 clones that were split into three sets and planted separately in three locations:
124 Ibadan, Ikenne and Mokwa. Each set was planted as a clonal evaluation trial (CET) using an
125 incomplete block design with common checks in each block. All trials had at least 10 clones in
126 common. Plants were harvested after 12 months in all trials.

127

128 **Table 1.** Summary of trials used in the present study including the trial names, design,
129 locations, number of plots and number of unique clones in each trial.

<i>Trial</i>	<i>Design*</i>	<i>Location</i>	<i>Plots</i>	<i>Unique entries</i>	<i>Plot size</i>
<i>GG.C0.UBJ</i>	CET, augmented	Ubiaja	805	738	10 plants, single row
<i>GS.C1.EC.IBA</i>	CET, augmented	Ibadan	293	265	20 plants (4 x 5)
<i>GS.C1.EC.IKN</i>	CET, augmented	Ikenne	331	307	20 plants (4x5)

<i>GS.CI.EC.MOK</i>	CET, augmented	Mokwa	329	278	20 plants (4x5)
<i>Crossing block.C0 CI.UBJ</i>	CET augmented	Ubiaja	243	218	

130 * CET = clonal evaluation trial

131

132 **Image acquisition**

133 The roots from four plants per plot were spread across a green board (160 cm by 120 cm). It
134 was important that the roots were not touching each other and also not touching the board edges
135 to get an individual root value (Supplementary Figure 1). Five circles, each 7.5 cm in diameter
136 were painted on the left and right sides of the board. Those circles were used as a reference to
137 transform the final result from the pixel unit to cm. Labels were placed on the board for each
138 image allowing images to be identified and renamed for further processing.

139

140 **Image processing and phenotype acquisition**

141 First, the images were coded to assign each photo to the plot from which the roots were taken.
142 In some cases, several images were required per plot, to capture all roots from all the plants.
143 For the GG collection, after quality control we obtained 805 images of cassava roots for 738
144 clones of which 665 had genotypic information. For the C1 population, we had images
145 originating from four locations and a total of 1091 root images for 997 clones. All the image
146 processing was performed with ImageJ Java version 1.8.0_11 (64-bit). The images were copied
147 in two folders, one for processing and measuring the roots and the second for scaling the
148 measurements. Thus, each image was processed and analysed twice.

149

150 **Image processing**

151 The first step of the image processing was to convert our RGB colour images into HSB stacks
152 (hue, saturation and brightness images). We obtained three slices, but we only kept the first
153 slice (the hue image). We then set a threshold from 0 to 255 for the roots and from 125 to 255
154 for the reference scaling circles before proceeding to run the “threshold” followed by the “make
155 binary” commands. This threshold was determined by doing individual tests on some images.
156 At the end of the processing, each image was binary, with our objects of interest (roots and
157 scales) represented as white pixels and everything else as black. Most steps in the procedure
158 were automated using customized ImageJ macros.

159

160 ***Phenotypes acquisition and description***

161 The “analyze particles” command in ImageJ counts each contiguous area of white pixels within
162 a binary image and gives some additional basic measurements. With the aim to get shape
163 related traits, we used the “extended particle analyzer” function in the BioVoxel Toolbox
164 plugin (http://imagej.net/BioVoxel_Toolbox#Extended_Particle_Analyzer). This function
165 computes useful parameters of which we chose to keep seven for downstream analysis: Area,
166 Perimeter, Feret, Circularity, Solidity, Roundness, and the Aspect Ratio (AR). The area and
167 the perimeter describe the size of a root. The Feret, is the longest distance between any two
168 points along the selection boundary, also known as maximum caliper. Circularity, Solidity,
169 Roundness and aspect ratio (AR) describe shape.

170 The shape descriptors are ratio values that ranged from 0 to 1 except AR, which is not bounded.
171 In addition, the shape descriptors do not have a unit, while area, perimeter, and feret are
172 parameters expressed in pixels. The mean area value of the circles was used as a reference to
173 convert pixels to centimetres (scaling coefficient). Since the exact diameter in centimetres of
174 each circle was known, we used this value to calculate the mean number of pixels per cm² for
175 each image.

176

$$177 \text{Scaling coefficient} = \sqrt{(\text{Area (pixel}^2)/\text{Area (cm}^2))}$$

178

179 **Genomic analyses**

180 We performed a two-step approach for the genomic analysis. In the first step, we used a linear
181 mixed model to account for the variability in the field design and calculate the broad-sense
182 heritability. The input data was: 1) the mean phenotype value for each plot (average phenotype
183 of all imaged roots), 2) the same as (1) but adjusted to account for the potential effects of
184 variation in cassava mosaic disease (CMD) severity among plots and 3) the standard deviation
185 of the root shape and size measurements (across all imaged roots) per plot, also adjusted to
186 remove the effect of CMD. We fit two different models, with CMD correction and without
187 CMD correction, for each of the two focal populations (GG or C1).

188

189 For GG, the following models were fitted:

$$190 (1) \mathbf{y} = \mathbf{Xm} + \mathbf{Z}_{\text{clone}}\mathbf{c} + \mathbf{Z}_{\text{range}}\mathbf{r} + \varepsilon$$

191

$$192 (2) \mathbf{y} = \mathbf{Xn} + \mathbf{Z}_{\text{clone}}\mathbf{c} + \mathbf{Z}_{\text{range}}\mathbf{r} + \varepsilon$$

193

194 In both models \mathbf{y} is a vector of phenotypes, $\mathbf{Z}_{\text{clone}}$ and $\mathbf{Z}_{\text{range}}$ are respectively the incidence
195 matrices of the clones and range both fit as random with their effects vector $c \sim N(0, \mathbf{I}\sigma_c^2)$ for
196 clones and $r \sim N(0, \mathbf{I}\sigma_r^2)$ for range. \mathbf{X} is the incidence matrix for the fixed effects. In model 1,
197 the number of harvested plants per plot (NOHAV) and CMD were accounted for as fixed and
198 the vector m contains the effect estimates. In model 2, we did not correct for CMD, \mathbf{X} and n
199 therefore only reference NOHAV.

200

201 For C1, model (3) and (4) were fitted:

202 (3) $\mathbf{y} = \mathbf{X}m + \mathbf{Z}_{\text{clone}}c + \mathbf{Z}_{\text{loc:range}}r + \varepsilon$

203 (4) $\mathbf{y} = \mathbf{X}n + \mathbf{Z}_{\text{clone}}c + \mathbf{Z}_{\text{loc:range}}r + \varepsilon$

204 In model (3) and (4), we replace the range variable with the combination of the location and
205 the range (Loc:range, i.e. range is nested in location) as the C1 population was planted in
206 several locations unlike the GG. In all models, for all traits, \mathbf{y} corresponded to the log
207 transformation of the original phenotypic values. Additional explanation of the models fitted
208 for these populations can be found in Wolfe et al. (2017).

209

210 From these models, we extracted the clone-effect BLUP, which estimates the total genetic
211 value (EGV) of each line and de-regressed the EGV by dividing them by their reliability to
212 obtain the de-regressed BLUP. Broad-sense heritability values were calculated using the
213 variance components estimated using the mixed-models described above. EGV and de-
214 regressed EGV are used in downstream analyses as described below.

215

216 **Genotyping data**

217 Both populations (GG and C1) were genotyped using the genotyping-by-sequencing (GBS)
218 method (Elshire *et al.*, 2011). TASSEL 5.0 GBS pipeline v2 (Glaubitz *et al.*, 2014) was used
219 for SNP calling. Alignment of GBS reads was to the cassava reference genome v6.1
220 (<http://phytozome.jgi.doe.gov>; ICGMC, 2015). The condition for the genotype calls was the
221 presence of a minimum of four reads. Extracted SNPs were filtered to remove clones with
222 >80% missing and markers with >60% missing genotype calls. Markers were also removed
223 when they had an extreme deviation from Hardy-Weinberg equilibrium ($\chi^2 > 20$).

224 A combination of custom scripts and common variant call file (VCF); manipulation tools were
225 used to accomplish the above pipeline. The missing data were imputed using Beagle v4.0

226 (Browning and Browning, 2016). For the GG and C1 populations, we had 112,082 and 179,041
227 markers, respectively, with $MAF > 0.01$.

228

229 **Genomic prediction**

230 We estimated genomic prediction accuracy using 5-fold cross-validation repeated 25 times
231 similar to what is described in Wolfe et al. (2017). Briefly, the for each replicate of the process,
232 the population was split into five approximately equal chunks (folds). Five genomic predictions
233 were then made in which each fold (fifth of the population) in turn served as the test set (no
234 phenotypes) and were predicted by the remaining four-fifths (training set, with phenotypes).
235 Prediction accuracy for each fold was defined as the correlation of the genome-estimated
236 breeding values (GEBVs, which are BLUPs from the test-sets of each fold), with the de-
237 regressed EGVs from the pre-adjustment stage of the analysis.

238

239 For genomic prediction, we used a mixed-model with a genotype (clone) random effect with
240 covariance proportional to the genomic relationship matrix, also called GBLUP. The genomic
241 relationship matrix was constructed using the function *A.mat* in the R package rrBLUP
242 (Endelman, 2011; Endelman and Jannink, 2012) De-regressed BLUPs were used as the
243 response variable and the GBLUP models were fit with the function *emmreml* in the R package
244 EMMREML (Akdemir and Okeke, 2015).

245

246 **GWAS analyses**

247 Genome-wide association mapping (GWAS) analyses were performed using a linear mixed-
248 model analysis (MLMA) implemented in GCTA (Version 1.90.0beta) (Yang *et al.*, 2011).
249 Specifically, we followed a leave-one-chromosome-out approach and tested all markers with
250 $MAF > 0.05$. The leave-one-chromosome-out approach involves excluding all markers on the
251 chromosome of the current candidate SNP from the genomic relationship matrix (GRM) used
252 to control population structure when estimating their marker effects. Manhattan plots were
253 generated using the R package *qqman* (Turner, 2014) with a Bonferroni threshold of 6.28.

254

255 Candidate gene identification was performed using the significant GWAS results of the
256 standard deviation + CMD correction GWAS results. Using the phytozome 12 portal link to
257 biomart (<https://phytozome.jgi.doe.gov/biomart/>) we searched for genes located 10kb around
258 the top SNP hits.

259

260 **Multivariate GWAS analysis**

261 We used a multivariate linear mixed model as implemented in GEMMA (mvLMM) (Zhou and
262 Stephens, 2014). We tested marker associations with multiple phenotypes that are fitted jointly
263 in the mvLMM while controlling for population stratification. Different combinations of
264 phenotypes were fitted in six models, the phenotypes that were fitted together were selected
265 based on their phenotypic correlation. Model 1: Circularity, Round, Solidity; Model 2: Area,
266 Feret, Circularity, Solidity, AR; Model 3: Area, Perimeter, Round, Solidity, AR; Model 4:
267 Area, Perimeter, Feret, Circularity, Round, Solidity, AR; Model 5: Area, Perimeter, Feret;
268 Model 6: Circularity, Round, Solidity, AR.

269

270 **Results**

271 **Phenotypes distribution**

272 Using the plugin BioVoxel in ImageJ, we extracted quantitative measurements of the Area,
273 Perimeter, Feret, Circularity, Solidity, Roundness, and the aspect ratio (AR) from root images
274 collected in the field. The raw value datasets show similar ranges for root shape and size
275 descriptors in GG and C1 populations (Supplementary Table 1). The individual root
276 measurements with the maximum and minimum value of each trait in both populations are
277 presented in Figure 1.

278 The frequency distribution of the mean value per plot of the GG and C1 populations is
279 presented in Supplementary Figure 2 and the mean values per trait within population are
280 presented in Supplementary Table 1. Some genotypes exhibited large differences in their mean
281 values for Area, Perimeter and Feret. For example, the maximum mean root Area in GG
282 population was 339 cm² while the mean Area of the GG population was 121.5 cm². Similarly,
283 those genotypes exhibited a maximum mean root Perimeter of 135 cm and a maximum mean
284 Feret of 50 cm while the mean Perimeter and Feret value in the GG population were 66 cm and
285 26 cm, respectively.

286 In the C1 dataset, the maximum mean value for the root area in the C1 dataset was 372 cm²,
287 while the mean area of that population was 128 cm². The maximum values for Perimeter and
288 Feret were 132 and 49 cm while the C1 population mean value for the two traits was 68 cm
289 and 28 cm respectively.

290

291

292

293 **Correlation plots**

294 Phenotypic correlations were calculated pairwise using de-regressed BLUPs of the mean values
295 for each population separately (Figure 2). In the GG dataset, the highest correlation within yield
296 traits corresponded to root number and root weight ($r^2=0.79$). Similarly, root number and root
297 weight were highly correlated in C1 population ($r^2=0.88$). In both datasets, correlations
298 between yield traits were significant and high ($r^2 > 0.5$) and these traits were also positively
299 correlated with Area, Perimeter and Feret. However, a low correlation ($r^2 < 0.1$) was observed
300 between yield traits and root shape descriptors such as Circularity, Roundness, Solidity and
301 AR in both populations.

302

303 Size-related traits derived from root images (Area, Perimeter and Feret) showed the highest
304 positive correlation ($r > 0.7$) with each other. In both datasets, the highest correlation between
305 size-related traits corresponded to Perimeter and Feret ($r=0.97$). Additionally, Feret and
306 Perimeter were negatively correlated with shape-related traits (Circularity, Roundness and
307 Solidity) and positively correlated with AR. In the GG dataset, Area showed a negative
308 correlation with Circularity ($r= -0.26$), Roundness ($r= -0.21$), Solidity ($r= -0.19$), and a positive
309 correlation with AR ($r= 0.19$). While in the C1 population, a low correlation was observed
310 between Area and shape descriptors.

311 Within the shape related traits, the highest correlation was found between Circularity and
312 Roundness (GG $r = 0.89$, C1 $r = 0.86$) and Solidity (GG $r = 0.87$, C1 $r = 0.84$). AR showed a
313 negative correlation with Circularity, Solidity and Roundness in both datasets.

314

315 **Broad-sense heritability**

316 Broad-sense heritability values (H^2) for root shape and yield-related traits were calculated for
317 each population (Table 2). In the GG population, without adjusting the phenotypes for their
318 CMD score, H^2 of root shape related traits ranged from 0.17 (Perimeter and Circularity) to 0.46
319 (aspect ratio) and for yield traits, H^2 ranged from 0.29 root weight (RTWT) to 0.44 shoot weight
320 (SHTWT). In the GG dataset, Perimeter, Circularity and Solidity exhibited the lowest
321 heritability values at 0.17, 0.17 and 0.12, respectively.

322

323 In the C1 population, the heritability of shape-related traits ranged from 0.36 (Perimeter) to
324 0.54 (Circularity) while for yield traits H^2 ranged from 0.36 (SHTWT) to 0.61 (RTWT). The
325 heritability of most traits was higher in the C1 population than GG except for Area (0.39 to

326 0.38) and SHTWT (0.44 to 0.36). The inclusion of the CMD in the calculation of the variance
327 components always reduced the heritability of all the traits in both populations by around 10%.

328

329 **Genome-wide association study of root traits**

330 Using a univariate genome-wide association approach for root image traits (root size and shape)
331 and root yield traits we identified significant loci for all traits except for area (Figure 3). We
332 detected a total of 91 SNP markers exceeding the significance threshold ($-\log_{10} P \geq 6.28$).
333 The Manhattan plots of the univariate GWAS results for yield traits are shown in
334 Supplementary Figure 3 and detailed information on the significant markers is summarized in
335 Supplementary Table 2.

336 We detected markers associated with Perimeter and Feret on chromosome 12, and with Solidity
337 on chromosome 1, whereas for AR we identified significant loci on chromosome 1 and
338 chromosome 12. Similarly, for Circularity and Roundness, we detected significant loci on
339 chromosome 1 and chromosome 12.

340 For most shape-related traits several other regions on chromosomes 3, 4, 8, 9, 14, 15 and 18
341 did not reach the significance threshold but showed a $-\log_{10} P \geq 5$ (Figure 3). For root yield
342 traits we detected a QTL on chromosome 12 associated to root number (RTNO) and RTWT
343 (Supplementary Figure 3, Supplementary Table 2). Notably, using the CMD adjusted
344 phenotype removed the significance of the QTL on chromosome 12 but did not identify new
345 QTL for the image traits shape phenotypes (Supplementary Figure 4) it detected new loci
346 associated with root number and shoot weight (Supplementary Table 3).

347 Significant SNP markers ($-\log_{10} P \geq 6.28$) were detected for the standard deviation-derived
348 traits of Perimeter (per-sd), Feret (feret-sd) and Aspect Ratio (AR-sd) (Figure 4). For per-sd, a
349 significant QTL was detected on chromosome 16, though it was not observed in the GWAS
350 model with the mean values nor in the GWAS model with mean values with CMD adjusted
351 phenotypes. For feret-sd, two significant QTL were identified, one on chromosome 9 and one
352 on chromosome 6 and for AR-sd one significant QTL was found on chromosome 8
353 (Supplementary Table 4).

354 Different markers were significant in the multivariate GWAS model dependent on which
355 phenotypes were included in the multivariate linear mixed model (mvLMM). Although the
356 multivariate model can increase the power for detecting pleiotropic variants when using
357 correlated traits, we identified few significant markers above the Bonferroni threshold
358 (Supplementary Fig 5-10). Nonetheless, when P-values were corrected for multiple testing by
359 computing Benjamini-Hochberg q-values, four SNPs were identified as significant in the

360 multivariate analysis. In the multivariate analysis using Area, Perimeter, Feret, Circularity,
361 Round, Solidity and AR in the mvLMM (Model 4) we identified a significant marker at the
362 same location on chromosome 4 (Supplementary figure 8). Similarly, using model 6
363 (Circularity, Round, Solidity, Aspect ratio) we identified one significant marker located on
364 chromosome 4 (Supplementary figure 10). When Area, Perimeter and Feret were included in
365 the mvLMM (model 5) we identified significant markers on chromosomes 6 and 9 using a q-
366 value threshold of < 0.1 (Supplementary figure 9).

367

368 **Genomic prediction**

369 Using the parental (GG) and offspring generation (C1) datasets independently, we calculated
370 the prediction accuracies of size and shape image traits and compared those to root yield traits
371 accuracies using de-regressed BLUPs of 1) the mean phenotype value (average phenotype of
372 4 plants) (Figure 5, Supplementary table 5), 2) the mean root size and shape phenotypes
373 adjusted to account for the potential effect of cassava mosaic disease (CMD) on these traits
374 (Figure 6, Supplementary Table 5) and 3) the standard deviation of the root shape and size
375 measurements adjusted to remove the effect of CMD (Figure 6, Supplementary Table 5).

376 Prediction accuracy, calculated as the correlation between the genome estimated breeding
377 values (GEBVs) and the de-regressed BLUPs of the mean phenotype value, ranged from 0.32
378 (SHTWT) to 0.43 (RTNO) in the GG population and from 0.12 (RTNO) to 0.46 (AR) in the
379 C1. For yield traits, accuracies in GG were higher than in C1 but were not different between
380 populations for the shape and size related traits. In the GG population, the shape descriptors
381 Circularity (mean = 0.40), Roundness (mean=0.39), Solidity (mean =0.37) and AR (mean
382 =0.38) showed slightly higher accuracies than the size descriptors Area (mean =0.33),
383 Perimeter (mean = 0.34) and Feret (mean = 0.33). In the C1 population, size and shape image
384 traits exhibited a higher prediction accuracy than root yield traits. Among the size descriptors,
385 Feret showed the highest accuracy (mean=0.34) and Area the lowest (mean=0.29). Among
386 shape descriptors, AR showed the highest predictive value (mean=0.46) and Solidity the lowest
387 (mean=0.33) (Supplementary Table 5). When the mean root size and shape phenotypes were
388 adjusted to account for the effect of CMD, we observed a minimal decrease in predictive
389 accuracy (Supplementary Table 5). A lower predictive accuracy was obtained for standard
390 deviation of size and shape traits adjusted for CMD, in both populations. In the GG population,
391 the decrease was pronounced with a maximum reduction of up to 55% for root perimeter (0.27
392 mean to 0.12 CMD adjusted) while in the C1 population the largest reduction was of 73% for
393 circularity (0.41 mean to 0.11 CMD adjusted) (Supplementary Table 5).

394 **Discussion**

395 Root number and root weight are among the most important targets for improvement in cassava
396 breeding programs. Although cassava root characterisation has been the subject of several
397 studies (Adetan, Adekoya and Aluko, 2003; Padonou, Mestres and Nago, 2005; Anggraini *et*
398 *al.*, 2009), the genetic architecture underlying cassava root shape remains unexplored. This
399 study aimed to evaluate the feasibility of the image phenotyping protocol and to assess the use
400 of genome-wide analyses for size and shape image-extracted traits.

401 Here, we phenotyped the GG and C1 populations from the International Institute of Tropical
402 Agriculture (IITA) breeding program for root shape and size-related traits using image analysis
403 of storage root photographs taken in the field. In both populations, the storage roots exhibited
404 a wide range of shape variation. Root-size related traits (Area, Perimeter and Feret) obtained
405 through image analysis showed significant but low correlation ($r \leq 0.5$) with cassava root yield
406 components (RTNO, RTWT). Roots with a large area were generally heavier and the circularity
407 of storage roots was mostly inversely correlated to its area. These results, suggest that rounded-
408 shaped roots in cassava are generally smaller and hence lighter in weight. More importantly,
409 the lack of correlation between size-related traits and shape related traits increases the interest
410 in shape related traits as a target for selection.

411

412 In radish, rice and wheat, imaging-based studies of root shape and size traits have demonstrated
413 first, that these have different genetic architectures (Iwata *et al.*, 2000) and second, that shape
414 phenotyping can aid the identification of pleiotropic QTL. In our study, using univariate
415 genome-wide association analysis, we detected for most shape and size related traits,
416 significant QTL regions located on chromosomes 1 and 12. The QTL region on chromosome
417 1 has been previously shown to be segregating for an introgressed segment from *M. glaziovii*
418 (Bredeson *et al.*, 2016). Furthermore, the QTL region on chromosome 1 has been associated,
419 in the IITA genetic gain population, with other root traits such as dry matter and total carotenoid
420 content (Rabbi *et al.*, 2017).

421 For root weight and root number, we identified a significant QTL associated with those traits
422 on chromosome 12. The QTL region on chromosome 12 has been previously associated, using
423 IITA breeding populations, to cassava mosaic disease (CMD) resistance (Wolfe *et al.*, 2016).
424 The effect of cassava mosaic disease (CMD) on root yield has been previously investigated in
425 fully and partly infected stands of cassava (Seif, 1982; Otim-Nape, Thresh and Shaw, 1997;
426 Owor *et al.*, 2004). In those studies, fresh stem, leaf and root yields and the number of tuberous

427 roots were influenced by the health status of the plants harvested and that of their nearest
428 neighbours. In our study, when we adjusted the size and shape phenotypes according to their
429 CMD score we did not identify new QTL but a reduction in marker significance, which suggest
430 that the CMD2 locus in chromosome 12 does not participate in the regulation of size and shape
431 phenotypes. Nonetheless, the identification of new QTL for root number and shoot weight,
432 when these traits were adjusted according to the CMD score, support the notion that CMD can
433 have an effect on root yield traits.

434 Because the uniformity in size and shape of cassava roots is an important breeding goal we
435 calculated the standard deviation of individual root measurements per clone. The use of
436 standard deviation measurements allowed the identification of new significant QTL for
437 Perimeter, Feret and Aspect Ratio on chromosomes 6, 9 and 16. For the new QTL regions
438 located on chromosomes 9 and 16 we identified candidate genes related to the tocopherol and
439 carotenoids pathways which are known regulators of plant development (Nisar *et al.*, 2015)
440 (Supplementary table 6). On chromosome 6, the most promising candidate is
441 Manes.06G078700 a root meristem growth factor 1 related gene.

442 Together our GWAS results suggest that 1) root-related traits have in common the genetic
443 control under few large effect loci and many small effect loci, 2) a possible correlation between
444 disease severity and yield loss and, 3) that introgressed regions contain gene clusters which
445 control root yield and root size/shape traits.

446 To increase the power of our study and to detect pleiotropic loci for size and shape traits (Korol
447 *et al.*, 2001; Korte *et al.*, 2012), we used a multivariate linear mixed model approach which
448 included groups of correlated root size and root size/shape traits. Considering multiple
449 phenotypes in the mvLMM enabled us to identify new candidate loci on chromosomes 4, 6 and
450 9 that were not identified in the univariate analyses.

451

452 The potential of GS as a breeding tool to increase the rates of genetic gain was recently tested
453 in three Next Generation Cassava Breeding programs (Marnin D. Wolfe *et al.*, 2017). The study
454 showed promising results particularly for traits with consistent heritability values across
455 programs and stable large-effect quantitative trait loci. Prediction accuracies for RTNO, RTWT
456 and SHTWT were similar with those reported in previous cassava cross-validation analyses (
457 Wolfe *et al.*, 2017). Root size and shape-related trait accuracies were lower than those reported
458 for dry matter content (DM) and cassava mosaic virus resistance (CMD) (Wolfe *et al.*, 2017).

459

460 Although the heritability of yield traits was higher in the offspring (Cycle 1, C1) than the
461 parental generation (Genetic Gain, GG), the predictive accuracy of traits extracted from root
462 images showed intermediate to high values in both populations. However, the C1 yield traits
463 accuracies being lower than the GG, suggests that because the C1 had been selected strongly
464 for these yield traits, its variance was diminished.

465

466 Nonetheless, predictive accuracies of the mean values of root size and shape image-extracted
467 traits were mostly higher than yield trait prediction accuracies in the C1 population. Adjusting
468 the mean and standard deviation phenotypes for the effect of CMD reduced the predictive
469 accuracy. However, that correction is necessary to unlink the effect of CMD from the causal
470 loci that are responsible for the regulation size and shape root traits.

471

472 Although these measurements were laborious in the field and not high-throughput, the analyses
473 of the images are automated and quantitative, they avoid subjectivity in scoring and other
474 human-errors and most importantly, they improve cassava root characterisation. The
475 methodology described here and the results obtained in this study are promising and open up
476 the opportunity to apply high-throughput methods in cassava. The image capture and analysis
477 can now be performed using the OneKK (one thousand kernels) app
478 (<https://github.com/PhenoApps/OneKK>), an inexpensive and user-friendly tool for automated
479 measurement of seed size, shape, and weight using smart phones. The app is developed under
480 the BREAD PhenoApps project and supported by the National Science Foundation. Still, there
481 is a need to explore the use of image-based phenotyping in multiple environments to estimate
482 the effect of the environment on root shape related traits and to automate the collection of root
483 images in the field further.

484

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488 Breeding Project”, and CGIAR-Research Program on Roots, Tubers and Bananas. The
489 authors acknowledge the support of Andrew Smith Ikpan, Ogunpaimo Kayode and Cynthia
490 Idhigu in acquiring the root images. We also acknowledge the PhenoApp project led by Jesse
491 Poland, Kansas State University, for technical support in image-based phenotyping using
492 their OneKK (one thousand kernel) application.

493

494 **Competing interests**

495 **The author(s) declare no competing interests.**

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511 **Figures and tables**

	GG min	GG max	C1 min	C1 max
AREA	 10.25 cm ²	 717.47 cm ²	 7.540 cm ²	 738.559 cm ²
PERIMETER	 12.86 cm	 334.97 cm	 12.272 cm	 228.268 cm
FERET	 4.54 cm	 86.51 cm	 4.492 cm	 100.718 cm
CIRCULARITY	 0.028	 0.864	 0.039	 0.988
ROUNDNESS	 0.071	 0.907	 0.050	 0.965
SOLIDITY	 0.363	 0.989	 0.223	 0.988
ASPECT RATIO	 1.104	 14.13	 1.026	 20.073

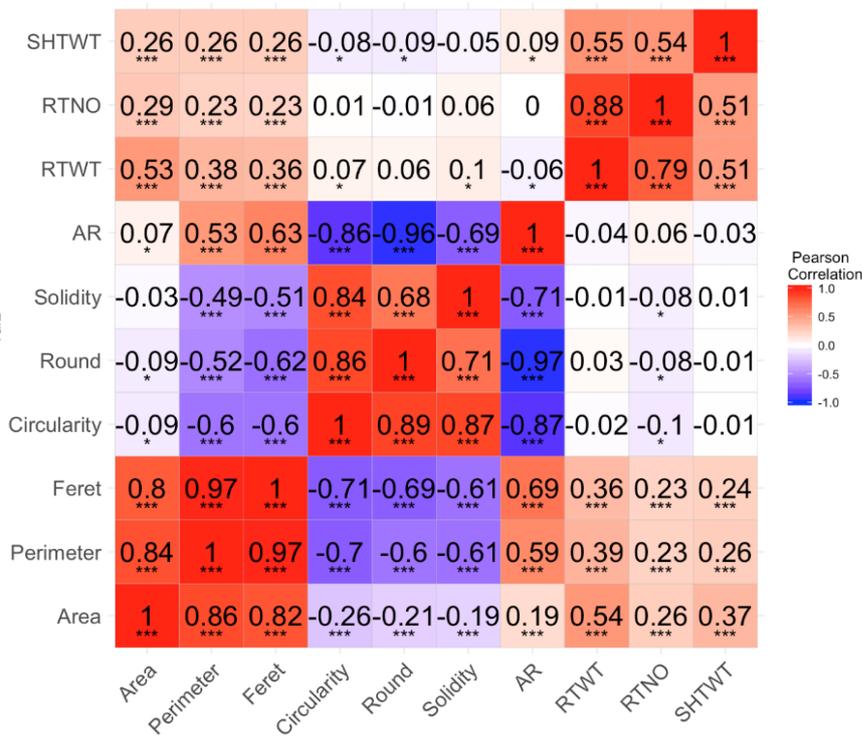
512

513

514 **Figure 1** : Phenotype description obtained using the extended particle analyzer plugin in
 515 ImageJ: Individual root measurements with the maximum and the minimum value of each
 516 trait in Genetic gain (GG) population and Cycle1 (C1) population, represented to highlight
 517 the range of values for each trait.

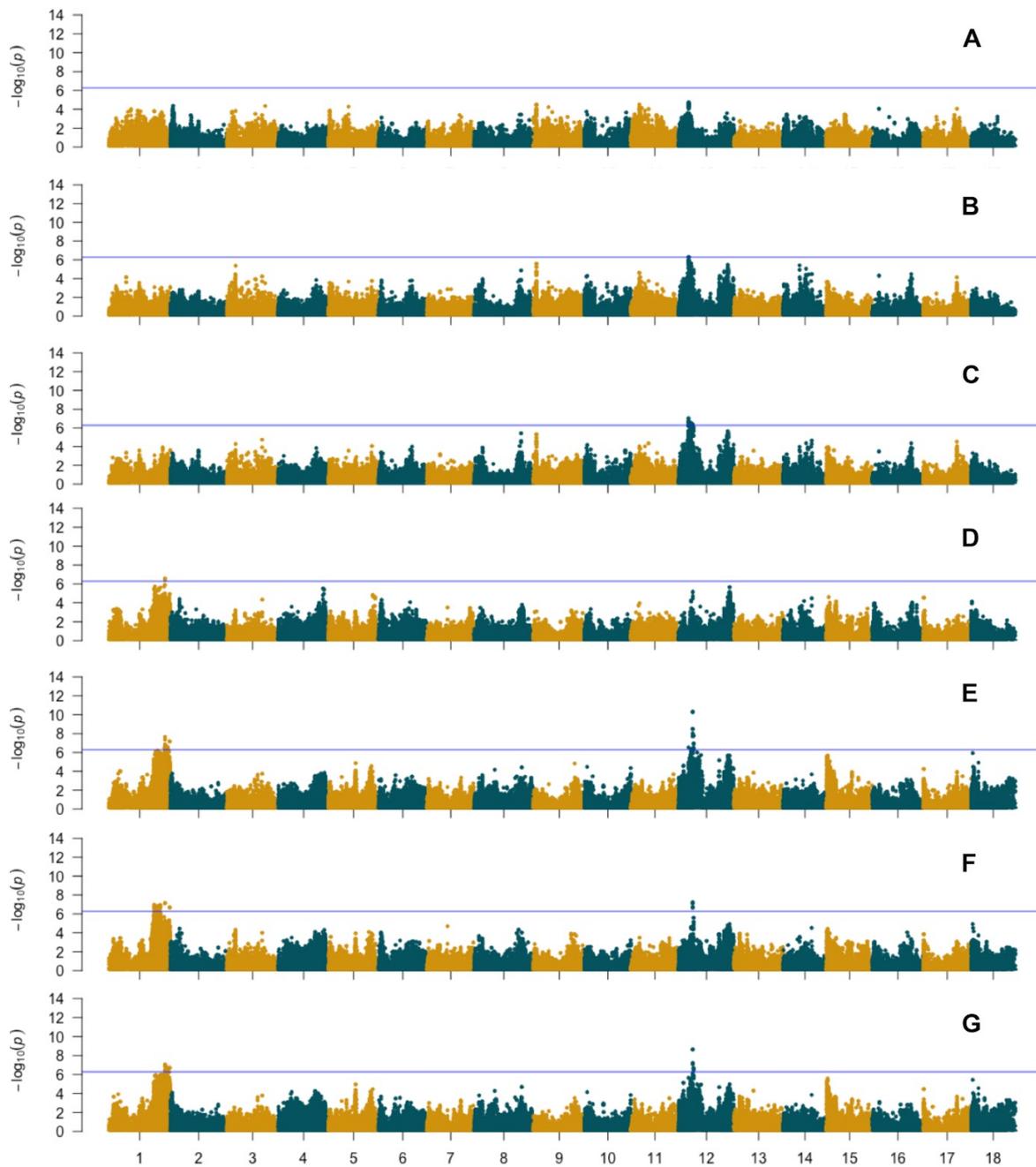
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520

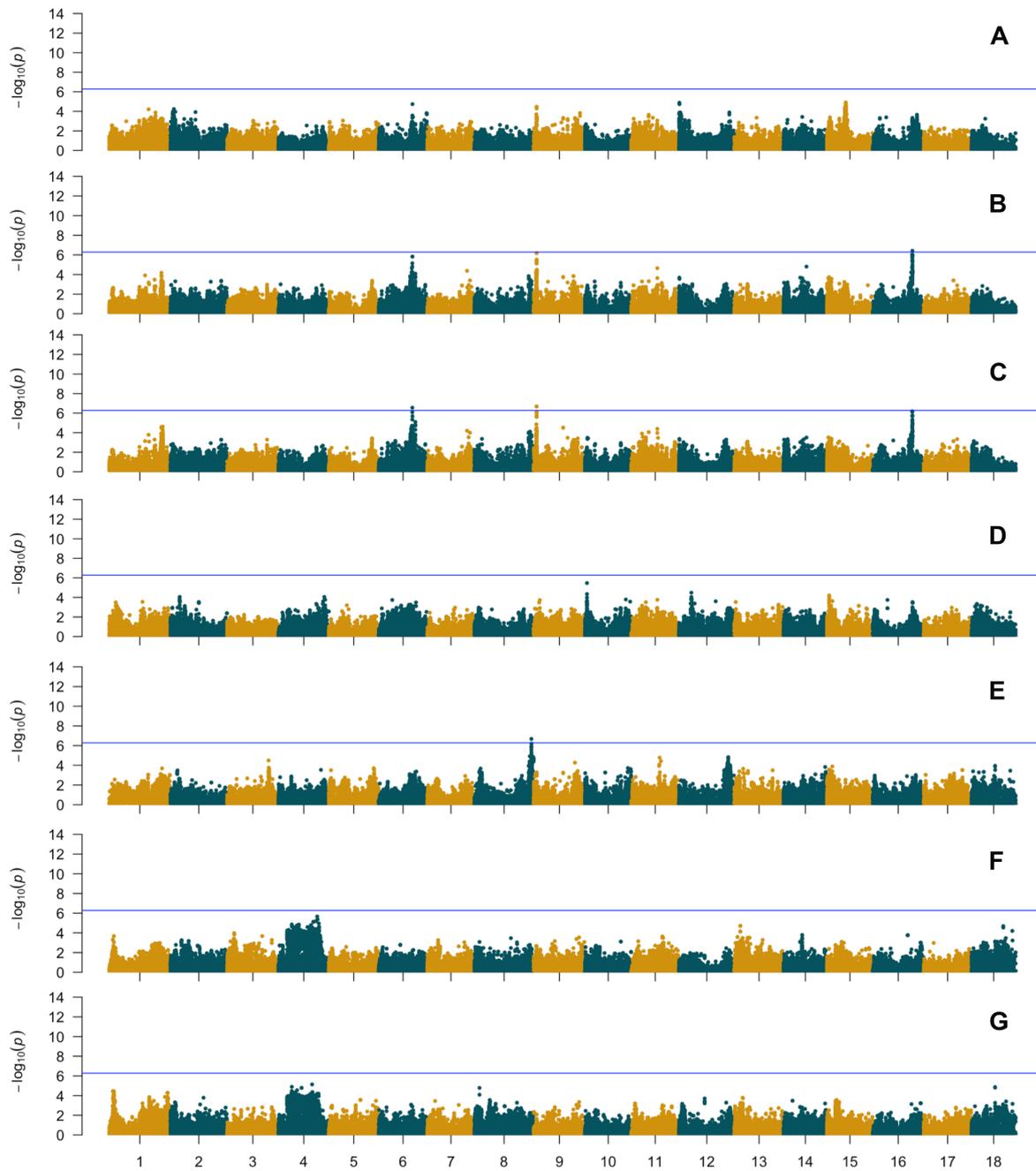
521 **Figure 2:** Heatmap with Pearson correlation coefficient: Trait correlation using the de-
 522 regressed BLUP value of GG dataset (lower triangle) and C1 dataset (upper triangle). The
 523 stars depict the significance according the p-value (** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$)



524

525 **Figure 3.** Genome-wide association results of size and shape-related traits using de-regressed
526 BLUPs of mean values (not corrected for CMD). A. Area; B. Perimeter; C. Feret; D. Solidity;
527 E. Aspect ratio; F. Circularity; G. Roundness. Blue horizontal line indicates the Bonferroni
528 statistical threshold.

529



530

531

532 **Figure 4.** Genome-wide association results of standard deviation-derived size and shape-

533 related traits using de-regressed BLUPs of mean CMD-corrected values. A. Area; B.

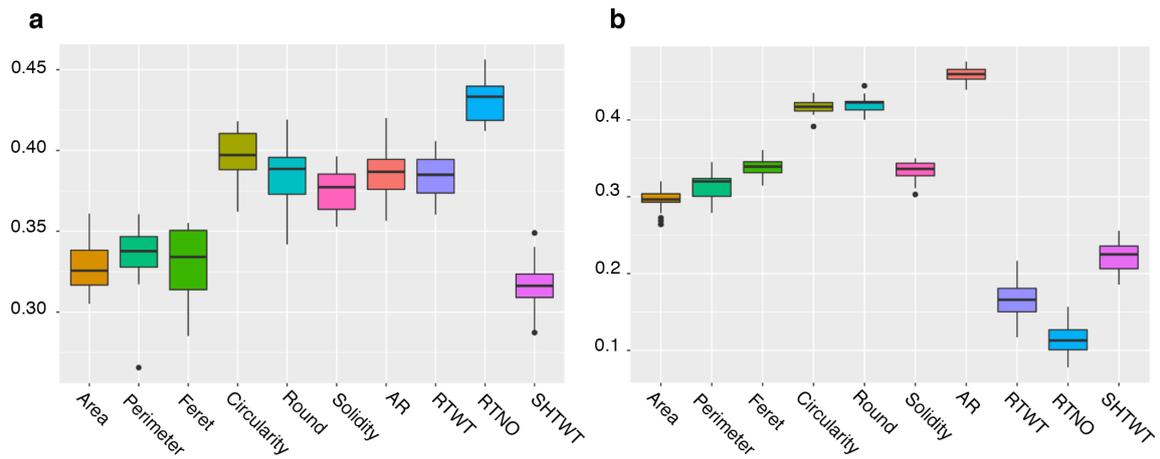
534 Perimeter; C. Feret; D. Solidity; E. Aspect ratio; F. Circularity; G. Roundness. Blue

535 horizontal line indicates the Bonferroni statistical threshold.

536

537

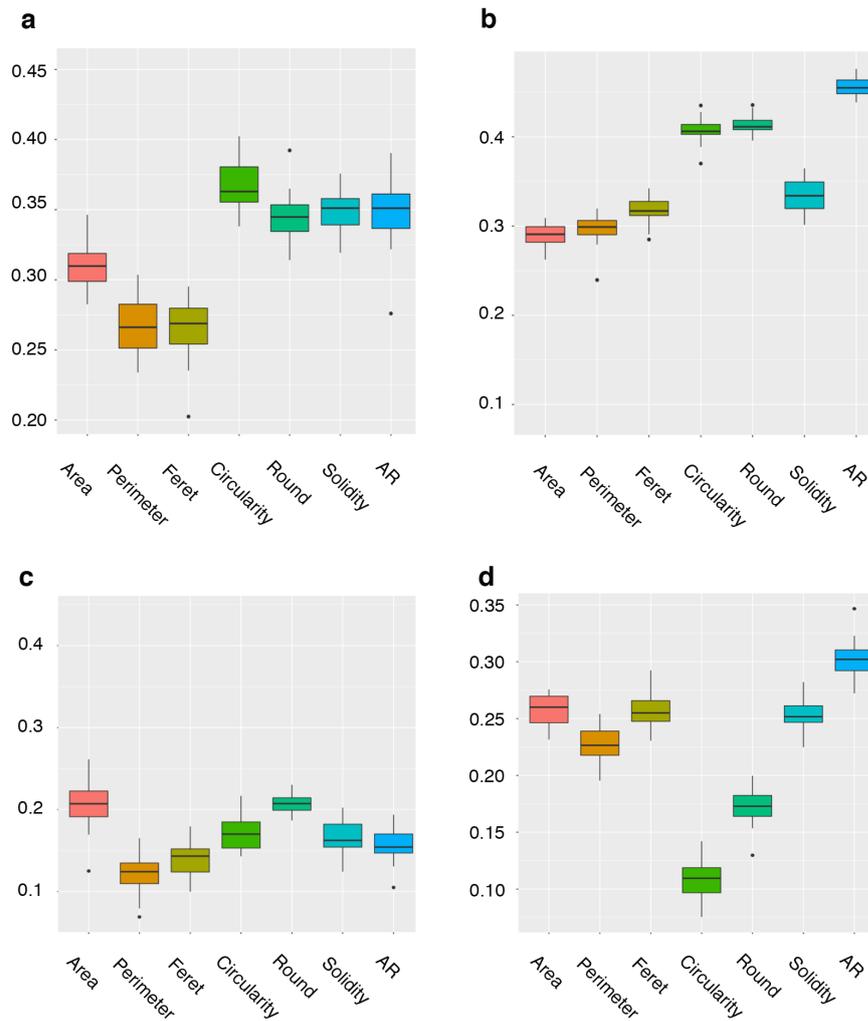
538



539

540 **Figure 5:** Prediction accuracy of root size and shape and yield traits. Predictive accuracies
541 were obtained with 5 fold-cross-validation analysis using a GBLUP model in the (a) GG
542 dataset and in the (b) C1 dataset.

543



544

545 **Figure 6.** GBLUP model predictive accuracy of root size and shape traits. a) GG population

546 CMD adjusted phenotypes, b) C1 population CMD adjusted phenotypes, c) GG population

547 standard deviation + CMD correction, d) C1 population standard deviation + CMD correction

548

Trait	GG		C1	
	no correction	+MCMDS	no correction	+MCMDS
Area	0.39	0.33	0.38	0.36
Perimeter	0.17	0.12	0.36	0.33
Feret	0.33	0.17	0.40	0.35
Circularity	0.17	0.15	0.54	0.53
Round	0.39	0.26	0.52	0.49
Solidity	0.12	0.12	0.48	0.48
Aspect Ratio	0.46	0.31	0.56	0.54
RTWT	0.29	0.23	0.60	0.50
RTNO	0.39	0.37	0.61	0.54
SHTWT	0.44	0.39	0.36	0.33

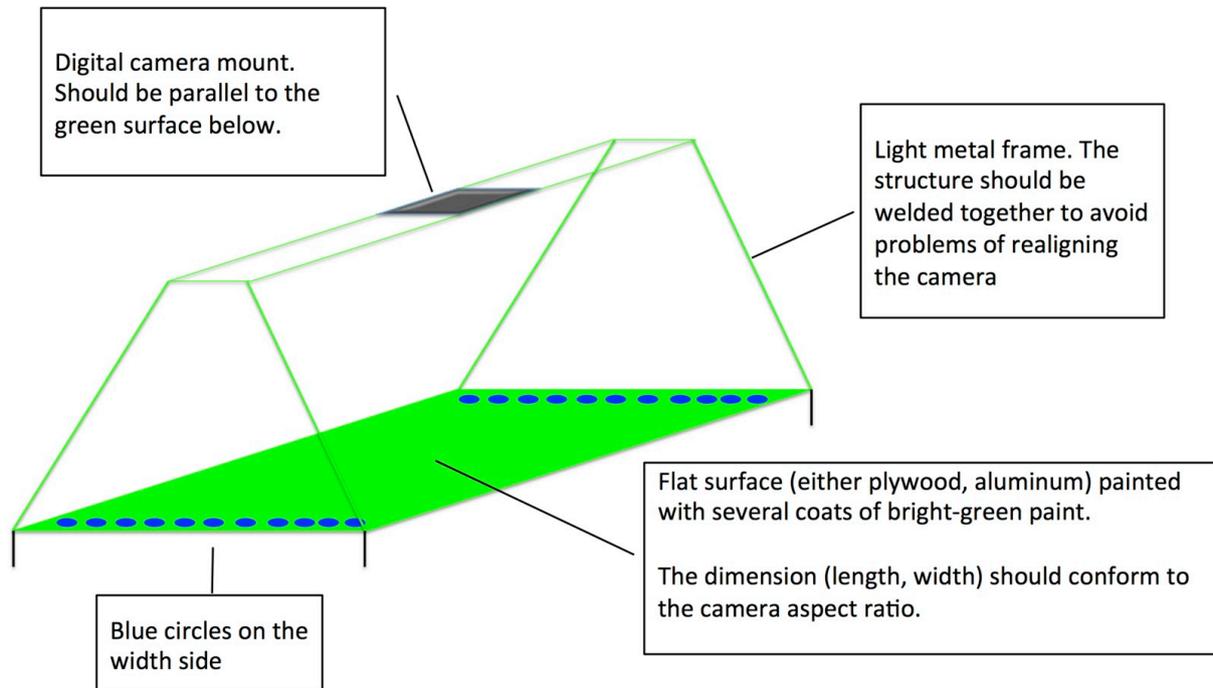
549

550 **Table 2:** Broad-sense heritability values of root shape and root yield traits for the Genetic
551 gain and cycle 1 breeding populations. (RTWT: root weight; RTNO: root number; SHTWT:
552 Shoot weight).

553

554 **Supplementary figures**

555

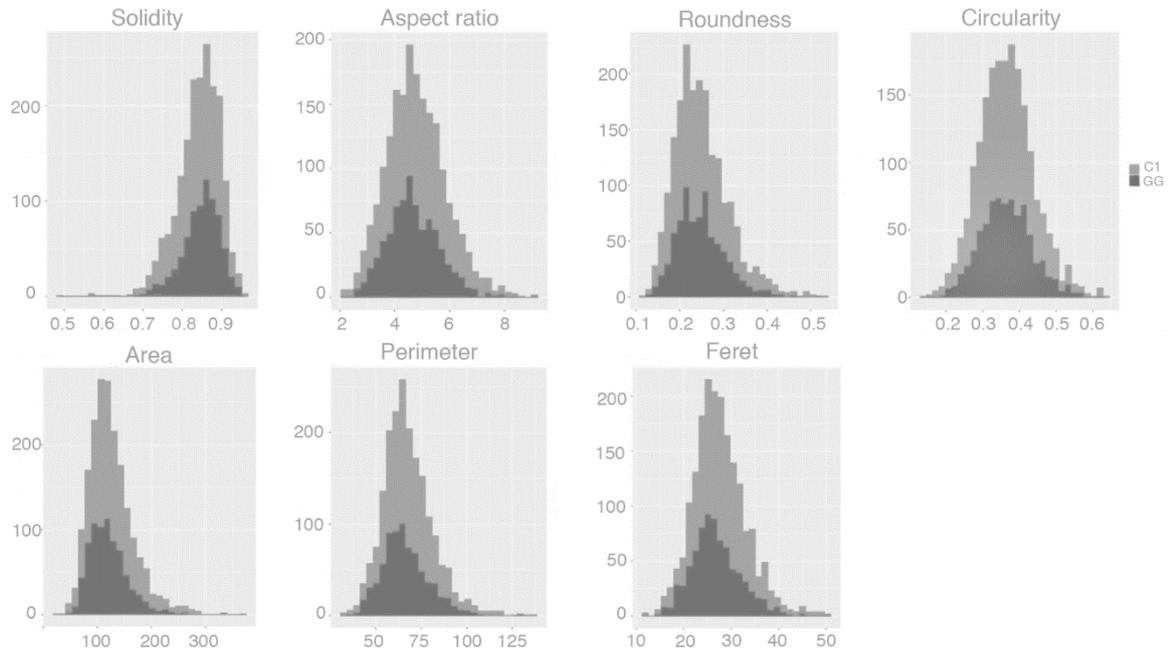


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557

558 **Supplementary figure 1:** Schematic of the green board used as a background to take
559 photographs in the field.

560



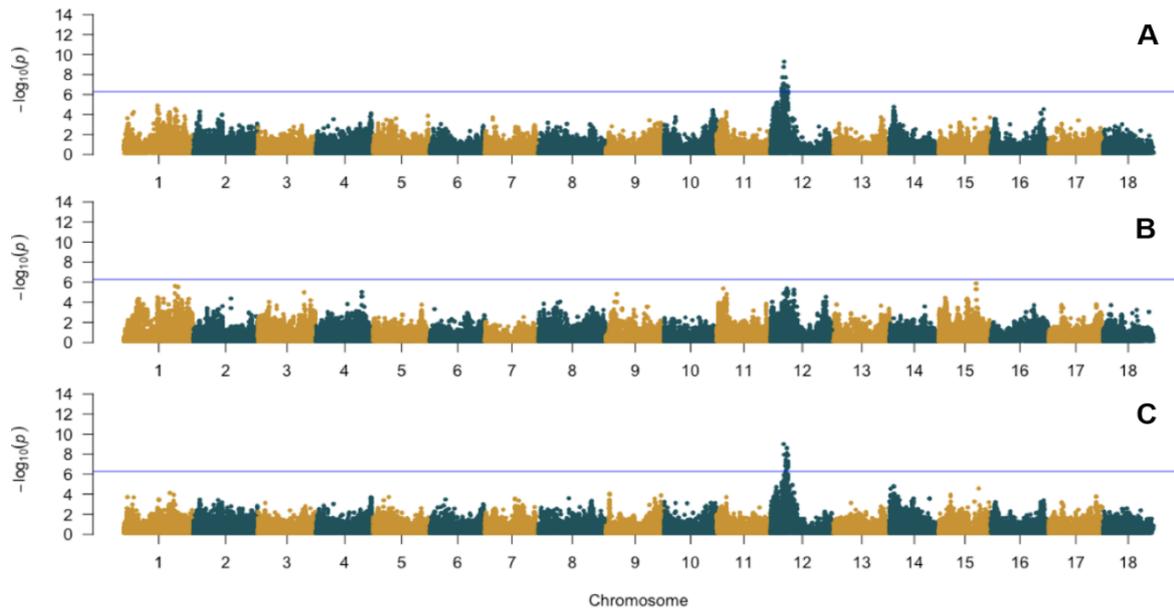
561

562 **Supplementary Figure 2.** Trait distribution of mean root values. Root values of each

563 phenotype extracted from photographs from each genotype were averaged and the

564 distribution plotted. Dark gray: GG, light gray : C1.

565



566

567

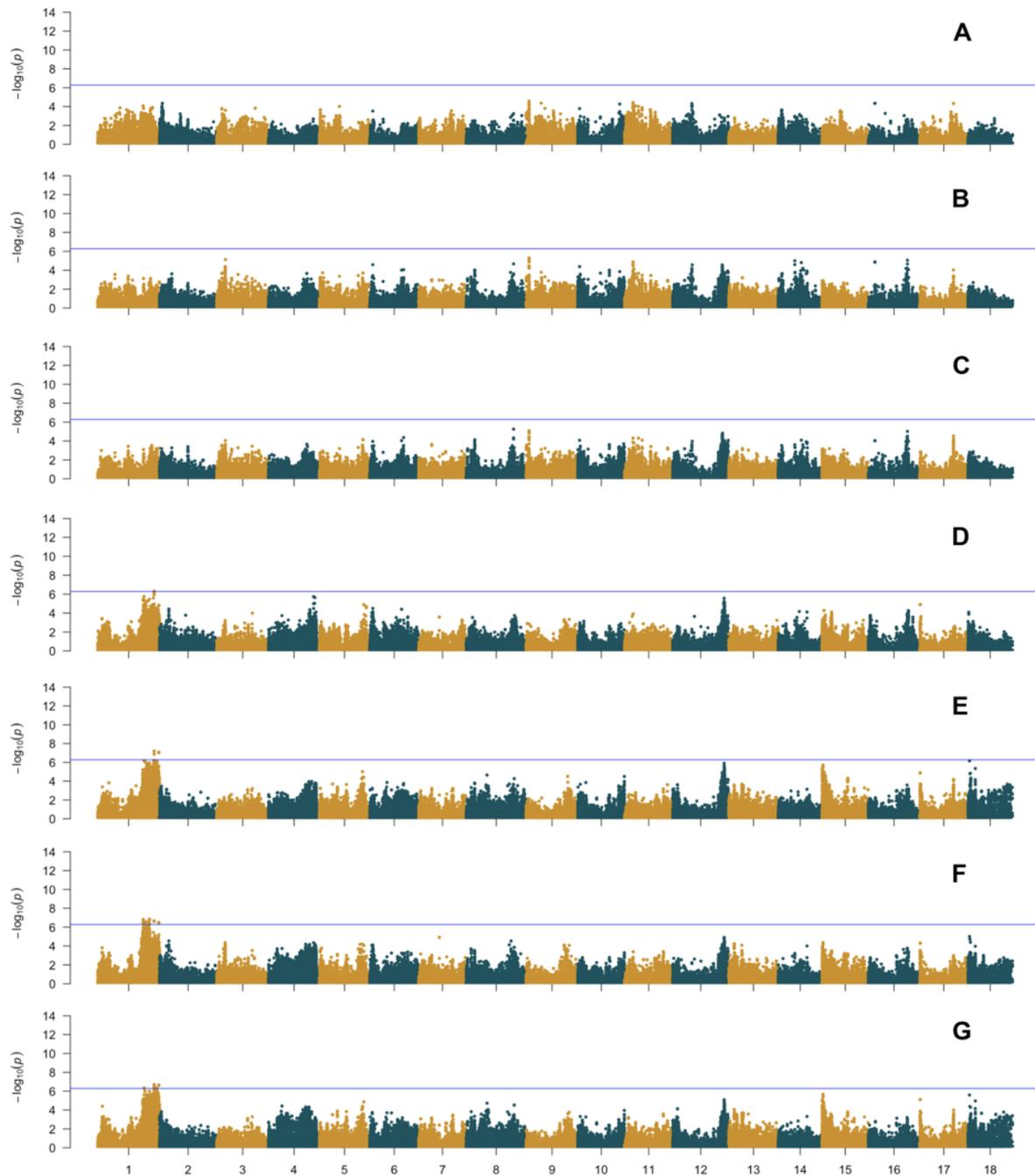
568 **Supplementary figure 3.** Yield traits GWAS results using the IITA Genetic Gain and Cycle

569 1 breeding populations. Manhattan plots of $-\log_{10}(P\text{-value})$ of A. Root weight, B. Shoot

570 weight, C. Root number. Blue horizontal line indicate the Bonferroni statistical threshold ($-\log_{10}(P\text{-value}) > 6.28$)

571 $\log_{10}(P\text{-value}) > 6.28$)

572



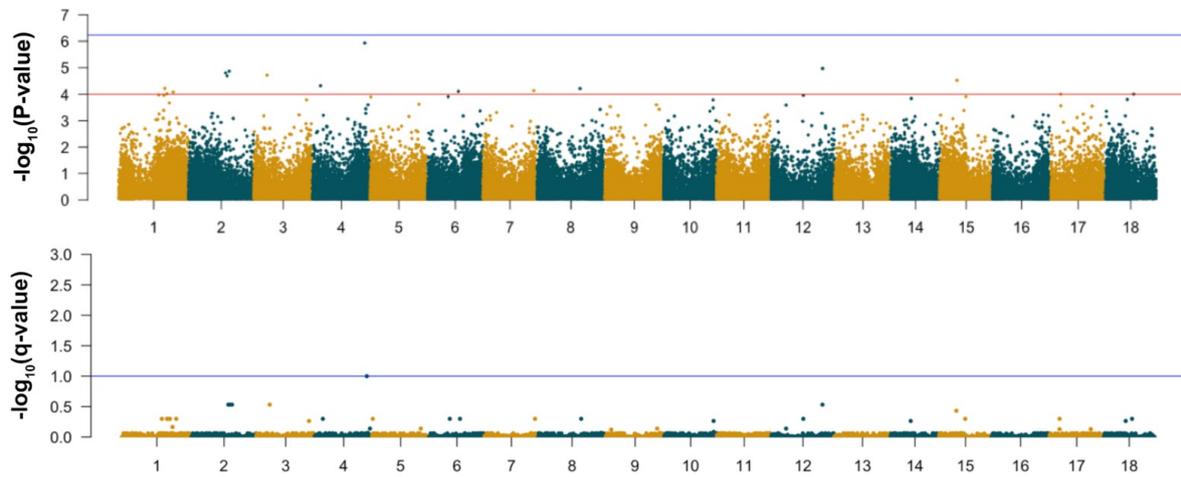
573

574

575 **Supplementary figure 4.** Size and shape GWAS results using the IITA Genetic Gain and
576 Cycle 1 breeding populations using CMD score as a covariate. Manhattan plots of $-\log_{10}(P$ -
577 value) of A. Area; B. Perimeter; C. Feret; D.Solidity; E. Aspect ratio; F. Circularity; G.
578 Roundness. Blue horizontal line indicates the Bonferroni statistical threshold.

579

580



581

582 **Supplementary Figure 5.** Multivariate GWAS of Circularity, Round and Solidity (model 1).

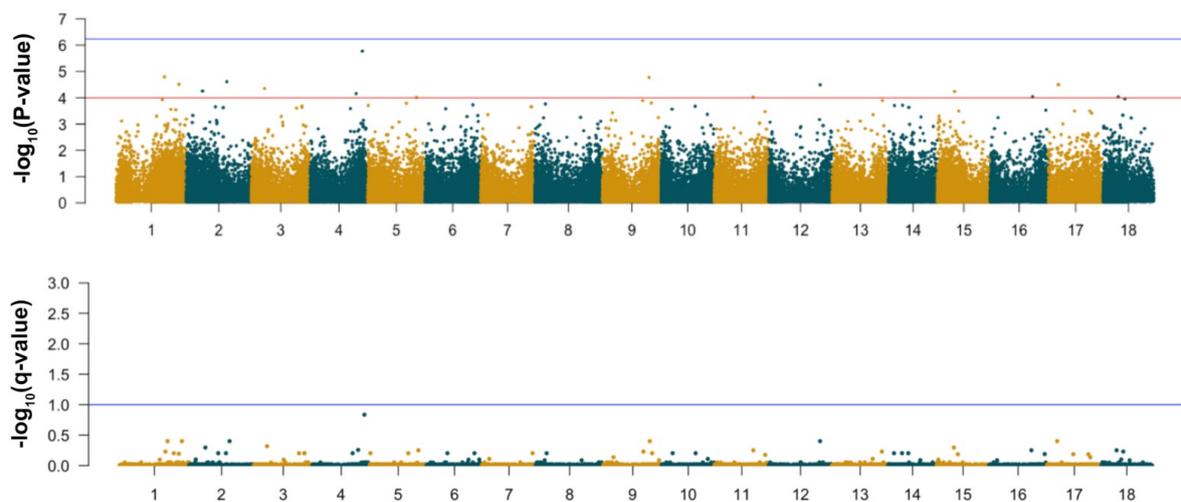
583 Manhattan plots of $-\log_{10}(\text{P-value})$ (top panel) and $-\log_{10}(\text{q-value})$ (bottom panel). In the top

584 panel the blue horizontal line indicates the Bonferroni statistical threshold and the red line

585 indicate a $-\log_{10}(\text{p-value}) = 4$. In the bottom panel de the blue line indicates the significant

586 threshold of the q-value.

587



588

589 **Supplementary Figure 6.** Multivariate GWAS of Area, Feret and Circularity (model 2).

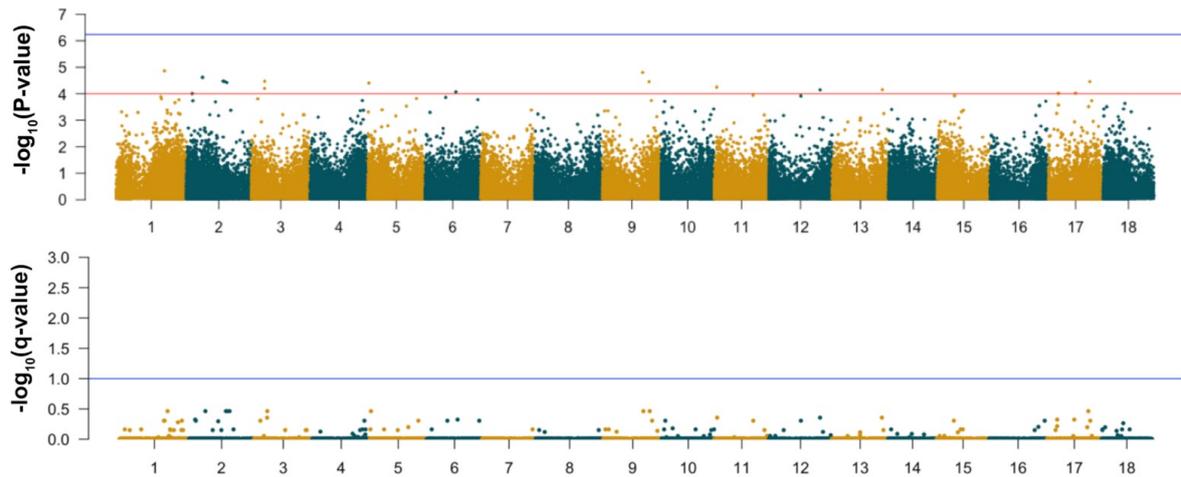
590 Manhattan plots of $-\log_{10}(\text{P-value})$ (top panel) and $-\log_{10}(\text{q-value})$ (bottom panel). In the top

591 panel the blue horizontal line indicates the Bonferroni statistical threshold and the red line

592 indicate a $-\log_{10}(\text{p-value}) = 4$. In the bottom panel de the blue line indicates the significant

593 threshold of the q-value.

594



595

596 **Supplementary Figure 7.** Multivariate GWAS of Area, Perimeter, Round, Solidity and AR

597 (model 3). Manhattan plots of $-\log_{10}(\text{P-value})$ (top panel) and $-\log_{10}(\text{q-value})$ (bottom panel).

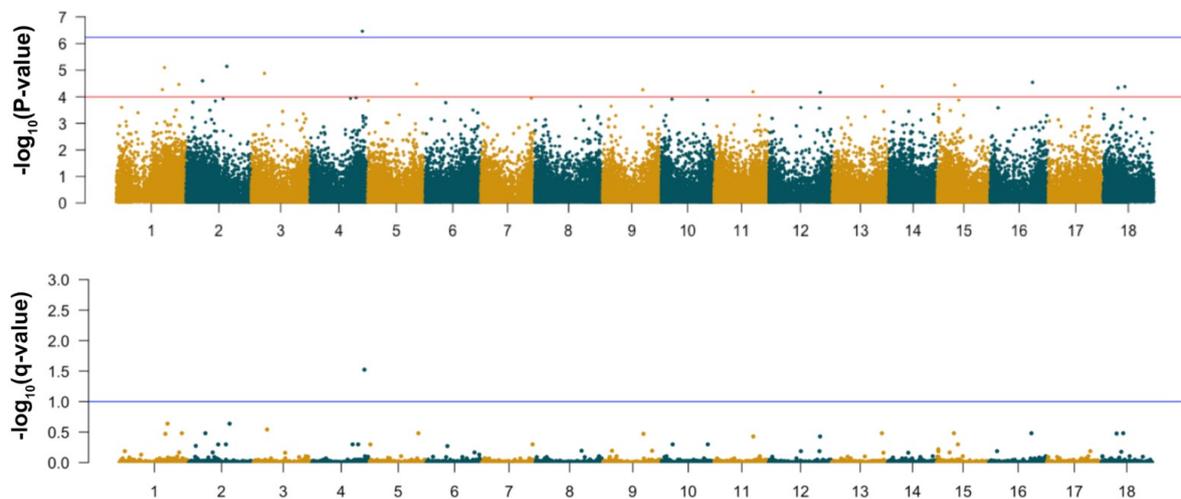
598 In the top panel the blue horizontal line indicates the Bonferroni statistical threshold and the

599 red line indicate a $-\log_{10}(\text{p-value}) = 4$. In the bottom panel de the blue line indicates the

600 significant threshold of the q-value.

601

602



603

604 **Supplementary Figure 8.** Multivariate GWAS of Area, Perimeter, Feret, Circularity, Round,

605 Solidity and AR (Model 4). Manhattan plots of $-\log_{10}(\text{P-value})$ (top panel) and $-\log_{10}(\text{q-value})$

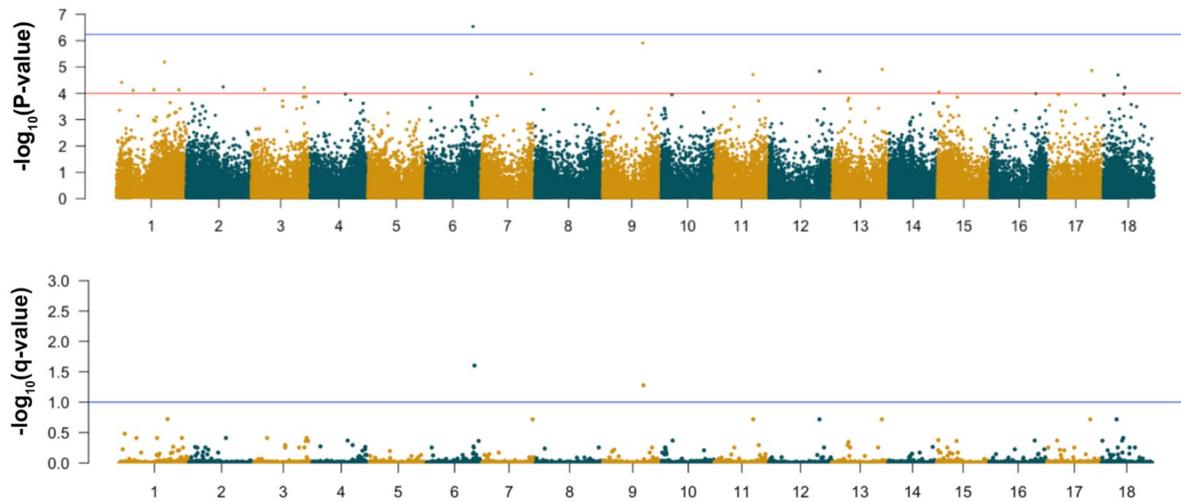
606 (bottom panel). In the top panel the blue horizontal line indicates the Bonferroni statistical

607 threshold and the red line indicate a $-\log_{10}(\text{p-value}) = 4$. In the bottom panel de the blue line

608 indicates the significant threshold of the q-value.

609

610



611

612 **Supplementary Figure 9.** Multivariate GWAS of Area, Perimeter and Feret (model 5).

613 Manhattan plots of $-\log_{10}(\text{P-value})$ (top panel) and $-\log_{10}(\text{q-value})$ (bottom panel). In the top

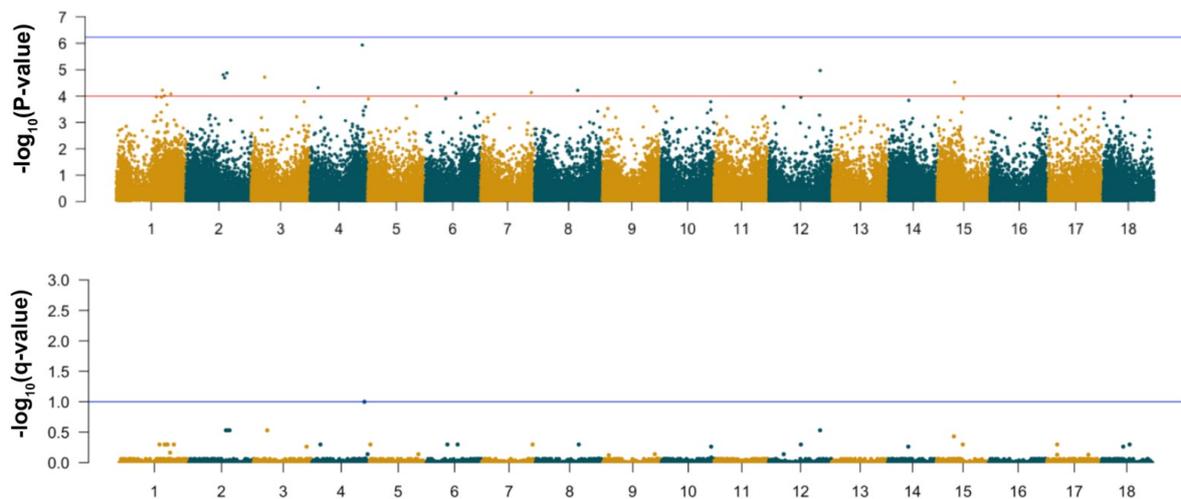
614 panel the blue horizontal line indicates the Bonferroni statistical threshold and the red line

615 indicate a $-\log_{10}(\text{p-value}) = 4$. In the bottom panel the blue line indicates the significant

616 threshold of the q-value.

617

618



619

620 **Supplementary Figure 10.** Multivariate GWAS of Circularity, Round, Solidity and AR

621 (model 6). Manhattan plots of $-\log_{10}(\text{P-value})$ (top panel) and $-\log_{10}(\text{q-value})$ (bottom panel).

622 In the top panel the blue horizontal line indicates the Bonferroni statistical threshold and the

623 red line indicate a $-\log_{10}(\text{p-value}) = 4$. In the bottom panel de the blue line indicates the
624 significant threshold of the q-value.

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631 **Supplementary tables**

632 **Supplementary table 1.** Mean values of raw and plot shape and size root measurements. The
633 plot value was calculated as the average of five plants per plot, the raw value was the
634 individual root value per genotype

635

636 **Supplementary table 2.** GWAS results of image-extracted and yield-related traits. Results in
637 bold are SNP p-values that surpassed the Bonferroni threshold.

638

639 **Supplementary table 3.** GWAS results of image-extracted and yield-related traits using
640 CMD adjusted phenotypes. Results in bold are SNP p-values that surpassed the Bonferroni
641 threshold.

642

643 **Supplementary table 4.** GWAS results of the standard deviation of image-extracted traits
644 using CMD adjusted phenotypes. Results in bold are SNP p-values that surpassed the
645 Bonferroni threshold.

646

647 **Supplementary table 5.** Summary results genomic prediction. Input phenotypes: mean
648 values per genotype/plot, mean adjusted phenotypes (mean + CMD correction), standard
649 deviation per genotype/plot of adjusted phenotypes (sd + CMD correction).

650

651 **Supplementary table 6.** Candidate gene annotation of significant QTL regions in
652 chromosomes 6,9 and 16 using the GWAS results of the standard deviation + CMD
653 correction of root size and shape traits.

654

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