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1 *Detection of dynamic QTLs for traits related to organoleptic quality during*  
2 *banana ripening*

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18

19 **Abstract**

20 Fruit quality traits are directly linked to consumer acceptability, and thus key targets for banana breeding programs.

21 We explored the genetic control of three major organoleptic and ripening-related traits, namely pulp acidity (pH),

22 firmness (PF) and dry matter content (DMC), over a 7-day ripening period and three production cycles in a banana

23 segregating population genotyped by sequencing. Significant broad-sense heritabilities were estimated with 0.77,

24 0.46 and 0.81 values for pH, PF and DMC, respectively. QTL detection was first performed on the whole dataset to

25 analyze their dynamics. In a second approach, per-cycle data were considered to evaluate the stability across

26 production cycles. Finally, a meta-analysis was performed. Various QTLs were detected, as well as many QTL

27 colocalizations, while 12 of these QTLs were more prominent as they were detected in several approaches and/or

28 explained over 15.0% of the phenotypic variation. Candidate genes were proposed for 10 of these QTLs. The QTL

29 with the largest contribution to pulp acidity ( $R^2 = 19.3\text{-}50.6\%$ ) was located on LG1\_7 on the genetic map of Pisang

30 Madu, i.e. a parent that is closely related to Cavendish, the world's most cultivated dessert banana cultivar group.  
31 This QTL is located on a chromosome derived from a reciprocal translocation that does not recombine in Pisang  
32 Madu, which is a favorable context for molecular marker monitoring. These first results will provide a relevant basis  
33 for marker-assisted selection in banana improvement programs.  
34  
35 **Keywords:** dry matter content, organoleptic banana quality, Musa, pulp acidity, pulp firmness, dynamic QTL

## 36 1. Introduction

37 Banana (*Musa* spp.) is a major economically important fruit that is grown throughout the world's tropical  
38 and subtropical regions. It is a cash crop that is sold on local and international markets, while also being staple food  
39 for several million people worldwide. Most world production is based on a few groups of cultivars derived from  
40 somaclonal variation, which is why bananas are particularly vulnerable to pests and diseases (Bakry et al. 2009).  
41 Banana cultivars are generally triploid, and sometimes diploid or tetraploid, with a basic chromosome number of  
42  $x=11$ . They are derived from hybridization between several *Musa* species and subspecies (Carreel, et al. 2002,  
43 Perrier et al. 2009), and their genome consists of complex inter(sub)specific mosaics (Baurens et al. 2019; Martin et  
44 al. 2020a; Cenci et al. 2021). The main target of banana breeding programs is to produce disease resistant cultivars  
45 while maintaining good fruit quality.

46 However, banana breeding is particularly complex since cultivars need to be sterile or have very low  
47 fertility to produce seedless edible fruits, and since triploid is the most agronomically efficient ploidy level.  
48 Improving cultivars by successive crossing steps among the best cultivars is therefore not an option. Banana breeders  
49 thus use existing diploid cultivars to exploit their residual fertility and/or for artificial tetraploidization to recover  
50 fertility before crossing with wild or cultivated diploids to produce triploid cultivars. The genome complexity of  
51 banana cultivars and the lack of knowledge on the genetic determinism of the targeted traits are further banana  
52 breeding constraints. The rare QTL and GWAs studies performed so far in banana have focused on the seedless  
53 phenotype (i.e. parthenocarpy combined with female sterility, Sardos et al. 2016), bunch weight component traits  
54 (Nyine et al. 2019) and *Fusarium* wilt resistance (Ahmad et al. 2020).

55 Several large reciprocal translocations have been reported in *Musa* species and subspecies (Martin et al.  
56 2017; Baurens et al. 2019, Dupouy et al. 2019, Martin et al. 2020b, Šimoníková et al. 2020). These translocations at  
57 an heterozygous state induce various extents of segregation distortion and recombination reduction that involve only  
58 the breakpoint region, a chromosome arm or an entire chromosome. They are frequently found at an heterozygous  
59 state in cultivars and breeding materials (Martin et al. 2020b), thereby at least partly explaining the major segregation  
60 distortions observed in the few genetic *Musa* maps developed so far (Fauré et al. 1993, Hippolyte et al. 2010; DHont  
61 et al. 2012; Mbanjo et al. 2012; Nombissié et al. 2016).

62 In recent decades, ecophysiological studies have generated insight into the morphological, physicochemical  
63 and sensory characteristics of edible *Musa* varieties, in order to understand banana consumers' preferences and

64 facilitating breeding (Gibert et al. 2009; Bugaud et al. 2011, 2013). As for most fleshy fruit, sweetness, sourness,  
65 firmness, mealiness and aroma are the five most relevant attributes for consumer acceptance (Bugaud et al. 2011,  
66 2016). Pulp acidity is an important aspect of banana organoleptic quality since it is related to the perception of  
67 sourness and sweetness based on the sugar-acid ratio, which has a high impact on consumer acceptability (Bugaud et  
68 al. 2016). pH is a practical measurement for high throughput phenotyping as this indicator is able to predict  
69 accurately the sensory characteristics sourness (Bugaud et al., 2013). During ripening, processes involved in the  
70 metabolism and accumulation of organic acids in pulp cells are under both genetic and environmental control  
71 (Etienne et al. 2013a, b, 2014, 2015). Pulp texture—mostly firmness and melting—is the second most important  
72 factor regarding the eating quality of bananas (Bugaud et al. 2016). Fruit texture and softening, associated with fruit  
73 cell wall disassembly during ripening, are also crucial fruit shelflife and postharvest quality factors (Brummell et al.  
74 1999; Wakabayashi 2000). In recent decades, there has been an increased focus on the pulp dry matter content,  
75 which has been found to be linked to various quality traits, namely textural traits (Vázquez-Ovando et al., 2012;  
76 Gibert et al. 2010), soluble solids (Bugaud et al. 2009) and sugars (Phillips et al., 2021); with sugars, organic acids  
77 and non-structural carbohydrates being the main components of pulp dry mass. The dry matter content (DMC) is also  
78 a useful trait to discriminate cooking and dessert bananas (Gibert et al. 2009). The sensory texture of boiled bananas  
79 noted by consumers can be predicted by this criterion (Kouassi et al., 2021).

80 Phenotyping of organoleptic fruit quality characteristics is difficult and time-consuming in cultivated  
81 material and very hard to assess in fruits from wild material since they have very low pulp contents. The aim of our  
82 study was to analyze the genetic determinism of organoleptic quality traits in banana and to identify the genomic  
83 regions involved so as to be able to develop marker assisted selection for these traits and to improve breeding  
84 schemes. To this end, we performed QTL detection of three banana quality criteria: pH, firmness and DMC. This  
85 analysis was based on a segregating population obtained from a cross between two diploid parents that serve as elite  
86 genitors in several of the very few banana breeding programs existing around the world. The parent Pisang Madu is a  
87 cultivar closely related to Cavendish, the most commercially important dessert cultivar group, and the parent Galeo  
88 is a cooking banana cultivar. Phenotypic variation and heritability of the studied traits were exploited in a dynamic  
89 QTL analysis encompassing all days of the fruit ripening process and the various production cycles. The stability of  
90 QTLs across production cycles was also investigated. A meta-analysis was then performed to identify QTL

91 clustering and colocation, and to refine the confidence intervals. Several candidate genes related to the studied  
92 quality traits were identified and the implications of these findings for banana breeding programs are discussed.

93

## 94 **2. Material and Methods**

### 95 *2.1. Plants materials*

96 The progeny consisted of 156 individuals generated from crosses between the diploid Pisang Madu cultivar,  
97 used as female, and the diploid Galeo cultivar. This population was developed at the CIRAD Neufchateau station in  
98 Guadeloupe (French West Indies). Due to the reduced fertility observed in the parents, this population was obtained  
99 by mixing seeds produced from 5 bunches of Pisang Madu bananas (5 inflorescences corresponding to around 500  
100 female flowers, with 100,000 ovules potentially hybridized with Galeo pollen). Embryo rescue was performed, as  
101 described by Bakry (2008), to maximize the recovery of all viable progeny. After greenhouse acclimation, the plants  
102 were transferred to the field from October 2013 to December 2014. The banana trees used for these experiments  
103 were grown in duplicated randomized blocks under the same field conditions, using plant suckers as planting  
104 material for block 2. These individuals were grown at the CIRAD Neufchateau station in Guadeloupe (elevation 250  
105 m; andosol; rainfall 3,500 mm/year; latitude 16° 4'46.63" N, longitude 60° 35'29.35" O) using conventional field  
106 practices.

107

### 108 *2.2. Fruit harvesting, ripening induction and sampling*

109 Fruits were harvested from the field when the 'first yellow finger' stage was visible on the banana bunch.  
110 Postharvest fruit treatment and ripening induction were performed as described in Hubert et al. (2014). Bananas were  
111 then placed in a climatic room at 20°C for 48 h minimum. Fruits were exposed to 1,000 ppm acetylene for 24 h at  
112 20°C to initiate ripening. After treatment, fruits were kept at 20°C and ambient humidity during the phenotyping  
113 phase. For each production cycle, at least 60 fruits were sampled per bunch.

114

### 115 *2.3. Physicochemical measurements*

116 The progeny was studied during three successive production cycles for the three distinct criteria.  
117 Measurements were performed over a 7-day ripening period (day 0 = the day of ripening initiation), each day for

118 pulp pH and firmness, and at days 1, 3 and 7 for pulp dry matter content (DMC). For each biological replicate  
 119 (corresponding to one harvested bunch), three analytical replicates (corresponding to three fruits) were performed for  
 120 pulp firmness, while two analytical replicates were performed for pulp pH and DMC. Rheological characteristics for  
 121 pulp firmness was assessed using a TA-XT2 penetrometer with a 20 mm cylindrical probe on fresh banana, as  
 122 described by Chillet et al. (2008). The pulp pH and DMC were measured on two of the three fruits used in the  
 123 rheological analysis. The pulp pH was measured by direct insertion of a pH sensor into transversely cut fruit pulp.  
 124 DMC was determined by measuring the weight loss of 2 g of fresh pulp after oven drying at 70°C for 16 h.

125

#### 126 2.4. Phenotypic data analysis, BLUPs and heritability

127 All statistical analyses were performed using R software v.2.9.2 (R development Core team, 2020,  
 128 <http://www.R-project.org>), except for the mixed linear model, which was built using PROC MIXED in SAS  
 129 software, Version 9.4 (SAS Institute Inc., Carey, NC, USA). The phenotypic data were checked visually for their  
 130 normal distribution and descriptive statistics were generated, including mean, range and standard deviation (SD).  
 131 Pearson correlation coefficients for the measured traits within and between cycles were also calculated using the  
 132 `cor.test()` function. The effect of genotype, days, cycle and their interactions on the organoleptic variables was  
 133 estimated using the following global mixed model, which was selected according to minimization of Akaike's  
 134 information criterion (AIC) (Akaike, 1974):

$$135 P_{ijkf} = \mu + G_i + D_j + B_k + C_f + (G \times D)_{ij} + (G \times C)_{if} + \varepsilon_{ijkf} \quad (1)$$

136 where  $P_{ijkf}$  is the phenotypic value of genotype  $i$  at the ripening day  $j$ , for the production cycle  $k$  and the  
 137 block  $f$ .  $\mu$  is the overall mean of the progeny,  $G_i$  is the random effect of genotype  $i$ ,  $D_j$  is the fixed effect of the  
 138 ripening day  $j$ ,  $B_k$  is the random effect of the block  $k$ ,  $C_f$  is the random effect of the production cycle  $k$ ,  $(G \times D)_{ij}$  and  
 139  $(G \times C)_{if}$  are their random interactions and  $\varepsilon_{ijkf}$  is the random residual error effect.

140 The residual distribution normality was checked afterwards. Broad-sense heritability ( $H^2$ ) was then  
 141 calculated using the equation proposed by Cullis et al. (2006) for unbalanced data:

$$142 \underline{H}_C^2 = 1 - \frac{\bar{v}BLUP}{2\sigma_g^2} \quad (2)$$

143 where  $\sigma_g^2$  is the genotypic variance,  $\bar{v}BLUP$  is the mean variance of a difference of two best linear unbiased  
 144 predictions (BLUPs; Henderson, 1950).

145

146       2.5. *Ploidy assessment*

147             The nuclear DNA content of the progenies (i.e, 156 offspring) and the two parents was assessed by flow  
148 cytometry. A leaf sample of each plant was chopped with leaf tissue of *Oryza sativa* L. ssp. *japonica* cv. Nipponbare  
149 as internal reference in modified LB01 buffer (Doležel et al. 1989) in which mercaptoethanol was substituted by 40  
150 mM sodium sulfite (Na<sub>2</sub>SO<sub>3</sub>). Analyses were performed with a PAS II flow cytometer (Partec GmbH, Münster,  
151 Germany), as described by Doležel and Bartoš (2005).

152

153       2.6. *Parentage verification*

154             Young leaves of the 156 F1 individuals and parents were sampled, and DNAs were extracted from 3 g of  
155 leaf material ground in liquid nitrogen according to the modified CTAB/MATAB method (Risterucci et al. 2000).  
156 Parentage verification was performed using a total of 15 SSR loci spread over the 11 *M. acuminata* chromosomes  
157 based on the DH-Pahang reference genome sequence (Lagoda et al. 1998; Hippolyte et al. 2010; D'Hont et al. 2012).  
158 Genotyping was carried out using the protocol described by Christelová et al. (2011) and the Applied  
159 Biosystems\_3500xL Genetic Analyzer and GeneMapper v4.1 software (Applied Biosystems;  
160 www.appliedbiosystems.com). Automated data scores were corrected by manual checks.

161

162       2.7. *High-throughput genotyping and genetic mapping*

163             Genotyping by sequencing (GBS) libraries were constructed using the PstI and MseI restriction enzymes  
164 and sequencing was performed at the GeT-PlaGe platform (<https://get.genotoul.fr/>) using the Illumina Hiseq3000  
165 sequencer. Raw GBS sequencing data from the F1 progeny were demultiplexed using GBSX version 1.2 (Herten et  
166 al. 2015) and adaptors were removed with cutadapt (Martin 2011). Variant calling was performed using the  
167 vcfhunter toolbox (available at <https://github.com/SouthGreenPlatform/VcfHunter/>) (Garsmeur et al., 2018) as  
168 described in Dupouy et al. (2019). Mapping was performed on the *M. acuminata* reference sequence assembly,  
169 version 2 (Martin et al. 2016). For each accession, sites with read depths of <10 and >3,000 were excluded. The  
170 procedure performed to select SNPs useful for the construction of both parental maps is schematically presented in



171 Supplemental Fig. 1. SNP markers with >10.0% missing data and individuals with >30.0% missing data were  
172 removed from the mapping dataset to avoid loss of accuracy during the genetic mapping process.

173 The remaining SNPs were separated into 3 sets of markers to create parental maps for cv Pisang Madu and  
174 cv Galeo using JoinMap 5.0 (Van Ooijen, 2006):  $lm \times ll$  (markers from the female parent),  $nn \times np$  (markers from the  
175 male parent), and  $hk \times hk$  markers (markers heterozygous in both parents). Parental markers were phased and  
176 extracted for use as a cross pollinated population type. SNPs included in these three classes were then merged in one  
177 file and used to construct the two parental maps. All identical loci were removed and then linkage groups were  
178 determined with a LOD threshold of 15, a recombination fraction threshold of 0.35, a ripple value of 1.0 and a jump  
179 threshold of 1.0. Markers were ordered using the regression-based parameters; and Kosambi's mapping function was  
180 used to calculate genetic distances among loci (Kosambi, 2016).

181

## 182 2.8. QTL detection and meta-analysis

183 In the present study, two QTL mapping approaches were applied on the best linear unbiased predictors  
184 (BLUPs) extracted from the selected mixed linear model (Equation 1). When significant, BLUPs for the GxD and  
185 GxC interactions, according to the studied step, were computed for each day and cycle. Quantitative trait loci  
186 analysis was performed using the multi-QTL model (MQM) in R/qtl (Broman and Sen 2009; Arends et al. 2010) and  
187 interval mapping based on the marker distance. The stepwise QTL function was used to conduct a forward-backward  
188 search and account for epistasis with a maximum of 5 QTLs. For each trait, the LOD threshold at a significance level  
189 of  $p = 0.05$  was calculated based on 1,000 permutations. QTLs with a LOD above the threshold of 3 were declared  
190 significant. The percentage of phenotypic variance explained ( $R^2$ ) of a single QTL was estimated based on a  
191 maximum likelihood estimation, and those with a  $R^2 \geq 15.0\%$  were considered as major QTLs.

192 All QTL analyses were performed for each day of the ripening process. The two QTL mapping approaches  
193 were applied independently for each trait, while the meta-analysis was performed with the three traits pooled. In the  
194 first approach, QTL detection was performed on the whole dataset, including all of the production cycles, to study  
195 QTL stability during fruit ripening. In the second approach, QTL analysis was performed on the three cycle datasets  
196 (C1, C2 and C3) separately to assess the stability of QTLs across these production cycles. For a given trait, when a  
197 QTL was detected in the same region at different days of the ripening process, they were pooled into a single QTL  
198 (consensus QTL), and the consensus QTL was then refined using BioMercator software V4

199 (<http://moulon.inra.fr/index.php/en>) and then named 'consensus QTL', with the following nomenclature:  
200 Trait<sup>datasetID</sup>QTL-MapName-LinkageGroup ID'. For example, DMC<sup>W</sup>QTL-PM5 corresponds to a QTL related to  
201 DMC detected from Pisang Madu polymorphism on linkage group 5 in the whole dataset. The meta-analysis  
202 performed using BioMercator software V4 was based on QTLs obtained from the two first approaches. Five different  
203 meta-analysis models (1-, 2-, 3-, 4-, or N-QTL) are proposed on BioMercator® and the best model choice was based  
204 on Akaike's information criterion (AIC) values (the model with the lowest AIC-value was considered optimal).  
205 Finally, the consensus QTL presented by the optimum model was considered and named MQTL (Arcade et al.  
206 2004).

207

## 208 *2.9. Identification of potential candidate genes*

209 Research on potential candidate genes was only performed on QTLs and MQTLs showing a confidence  
210 interval of 10 Mb or less. Genes coding for enzymes or transporters directly linked to acids, sugars, cell wall and  
211 hormone metabolism were assessed according to Gene Ontology terms related to these functions available at  
212 <http://geneontology.org/>. From the functional annotation of the Banana Genome Hub (BGH, [https://banana-genome-](https://banana-genome-hub.southgreen.fr/)  
213 [hub.southgreen.fr/](https://banana-genome-hub.southgreen.fr/)) a systematic search of the location on the physical map of the list of identified GO terms was  
214 conducted. Colocation between a QTL and a candidate gene was identified when the latter was located in the  
215 confidence interval of the consensus QTL or MQTL using the BEDTools intersect function (Quinlan and Hall 2010).

216

## 217 *2.10. Identification of chromosomal structures associated with pH variation*

218 Both Pisang Madu haplotypes for chromosome 1 were searched in the progenies using the methodology  
219 described in Martin et al. (2020b). Two classes of individuals were obtained for each of these structures: individuals  
220 with haplotype 1 of Pisang Madu (with the 1/7 translocation) and individuals with haplotype 2 of Pisang Madu (with  
221 the reference sequence structure). For each of these classes, the mean pH at day 7 (corresponding to ripe fruit) and  
222 standard deviation were calculated. These values were then plotted using a box plot representation. pH averages for  
223 the two haplotypes were compared using the Tukey test.

224

## 225 **3. Results**

226           3.1. *Genotyping and genetic map*

227           The progeny consisting of 156 individuals was genotyped by sequencing, resulting in 2.4 million  
228 polymorphic SNPs after variant calling (Supplemental Fig. 1). Seven individuals were removed from the dataset due  
229 to the extent of missing data (> 20.0%). After the filtering steps, 4,663 bi-allelic SNPs were kept, with 1,250 (6.4%)  
230 corresponding to SNPs heterozygous only in Galeo, 2,287 (11.6%) to SNPs heterozygous only in 'Pisang Madu', and  
231 1,126 (5.7%) to SNPs heterozygous in both parents. Loci were further separated into two sets of uniparental  
232 configurations to create parental maps. Linkage groups (LGs) were named according to the reference *M. acuminata*  
233 genome assembly (D'Hont et al. 2012).

234           The Galeo genetic map (Supplemental Table 1), consisted of 1,148 SNPs assembled in 11 linkage groups,  
235 as expected. They were distributed among 937 independent loci due to cosegregation. The map had a total length of  
236 1,345 cM, corresponding to an average interval of 1.5 cM between markers. However, several gaps larger than 10.0  
237 cM or 20.0 cM were observed (Supplemental Table 1). Comparison of the genetic positions of the markers to the  
238 physical ones on the *M. acuminata* reference genome assembly v2.0 (data not shown), revealed no large genome  
239 rearrangements in Galeo compared to the reference genome assembly. This also showed that the genetic map  
240 covered 379 Mb overall, corresponding to 95.4% of the *M. acuminata* genome assembly v2.0.

241           The Pisang Madu genetic map (Supplemental Fig. 1) consisted of 1,319 SNPs distributed among 990  
242 independent loci assembled in 10 linkage groups. The Pisang Madu map had a total length of 1,154 cM,  
243 corresponding to an average interval of 1.5 cM between markers. However, several gaps larger than 10.0 cM or 20.0  
244 cM were observed (Supplemental Table 1). Comparison of the genetic positions of the markers to the physical ones  
245 on the *M. acuminata* reference genome assembly v2.0 revealed that all markers corresponding to reference  
246 chromosome 1 were genetically mapped in the middle of a linkage group that corresponded to reference  
247 chromosome 7. This linkage group was named LG1\_7 (Supplemental Table 1, Fig. 1a). In addition, no  
248 recombination was detected between markers from reference chromosome 1. The genetic map covered 359 Mb,  
249 corresponding to 97.4% of *M. acuminata* genome assembly v2.0 (Supplemental Table 1).

250

251           3.2. *Analysis of phenotyping data*

252 The 156 individuals of the studied progeny were phenotyped during fruit ripening for three quality traits  
253 measured in the pulp, i.e. pH, firmness and DMC, during three cropping cycles (Supplemental Table 2). 147, 125,  
254 and 46 progeny individuals were phenotyped at cycles 1, 2 and 3, respectively. Phenotypic data of progeny  
255 individuals showed a wide distribution for all traits during fruit ripening (Fig. 2). Marked variations in pH and DMC  
256 were measured at each fruit ripening day, while for PF there was very little variation within the progenies after day 3.  
257 For each trait at each ripening day, the phenotypic data tended towards a normal distribution, except for PF which  
258 showed a right-skewed distribution from the third ripening day (Fig. 2e-h). The range of values measured for these  
259 traits and the slopes observed during fruit ripening were similar between cycles, as confirmed by the non-significant  
260 cycle effect (Table 1 and Fig. 3).

261 Variance analysis and decomposition (Table 1) revealed highly significant genotypic effects ( $p < 0.0001$ ),  
262 as well as high interaction effects between genotype and the ripening day and genotype and the plant cycle ( $p <$   
263  $0.0001$ ). The proportions of variance explained (PVE) by the different factors were, however, extremely variable  
264 between traits. Genotypic effects were the strongest ones for DMC and pH (61.3% and 39.0%, respectively), but  
265 explained the lowest part of variance for PF (12.2%). Similarly, the interaction between the genotype and ripening  
266 day ranged from 7.5% for DMC to 23.4% for PF. The PVE of the interaction between the genotype and crop cycle  
267 was more stable, varying from 13.7% for DMC to 18.5% for PF. DMC and pH finally displayed high heritability  
268 values, of 0.81 and 0.77, respectively, whereas PF had the lowest one, with a value of 0.46 (Table 1).

269 The curve of pulp acidity determined from the pH measurements first decreased until the fourth ripening  
270 day, and then stabilized over a period corresponding to the beginning of the second part of fruit ripening process,  
271 followed by a rise until the end of ripening, i.e. generally considered as the ready-to-eat stage (Fig. 2a-d,  
272 Supplemental Fig. 2a). The pH increase was especially marked in Pisang Madu pulp, contrary to Galeo in which it  
273 remained low. PF showed a quick decrease until day 4 of ripening and then stayed almost unchanged at low values  
274 during the second part of fruit ripening until the end of this process (Fig. 2e-h, Supplemental Fig. 2b). The DMC  
275 tended to decrease during fruit ripening, with quite low variations compared to the other traits (Fig. 2i-l,  
276 Supplemental Fig. 2c). All quality traits were found to be positively correlated, regardless of the fruit ripening day,  
277 except for DMC and pH at day 7 (Table 2). Furthermore, pH had the highest correlation with PF at days 1 and 3 ( $r =$   
278  $0.48$ ), while the correlation between PF and DMC was intermediate and relatively similar for the three measurement  
279 days ( $r = 0.3$  to  $0.39$ ).

280

281 *3.3. QTLs detected for each of the three quality traits over the ripening period*

282 The QTL detection results obtained with the whole dataset (first approach) are summarized in Supplemental  
283 Table 3 and Fig. 3a. A total of 36, 15 and 10 significant associations were detected for pH, PF and DMC,  
284 respectively, which corresponded to 5, 4 and 2 consensus QTLs (QTLs detected in the same region at different  
285 ripening days), respectively (Supplemental Table 3). They were located on all linkage groups (LG), except LG2.  
286 More QTLs were detected in Pisang Madu than in Galeo, except for PF. Variations in  $R^2$  values were observed for  
287 several QTLs detected during fruit ripening (Supplemental Table 3). Most of the QTLs were not detected every day  
288 of the ripening period, many (43.0% for Pisang Madu polymorphism and 30.0% for Galeo polymorphism) were  
289 detected only for one or two days. The number of QTLs detected each day ranged from 1 to 10, with the highest  
290 number detected at the end of the ripening period (Pisang Madu map). The  $R^2$  values determined for a single QTL,  
291 depending on the ripening day, ranged from 2.2 to 50.6% for pH, 7.9 to 30.0% for PF, and 6.8 to 26.1% for DMC  
292 (Supplemental Table 3).

293 For pulp pH, QTLs were detected on LG1\_7, 3, 4, 6, 9 and 10 (Fig. 1b and Fig. 4). On LG1\_7 of Pisang  
294 Madu, QTLs were detected in two distinct regions (Fig. 1b-c). QTLs detected in the central region of LG1\_7 were  
295 detected for all fruit ripening days ( $\text{pH}^{\text{W}}\text{QTL-PM1-7.1}$ :  $R^2 = 19.3\text{-}50.6\%$ ,  $\text{CI} = 27.4\text{ Mb}$ ). This central region of  
296 LG1\_7 corresponded to the part of the reference chromosome 7 and the entire reference chromosome 1 that does not  
297 recombine in Pisang Madu (Fig. 2a-c). The second region at the bottom of the LG1\_7, corresponding to reference  
298 chromosome 7, was detected for all fruit ripening days, except for days 0 and 6 ( $\text{pH}^{\text{W}}\text{QTL-PM1T7.2}$ :  $R^2 = 3.2\text{-}6.9\%$ ,  
299  $\text{CI} = 2.2\text{ Mb}$ ). These QTLs combined with those detected for pH reached a total  $R^2$  value of 67.8% for day 6 and  
300 58.5% for day 7.

301 For pulp firmness, QTLs were detected on LG1, 3, 4, 5, 6, and 11 (Fig. 1b and Fig. 4). The total  $R^2$  values  
302 of QTLs for PF ranged from 17.7% at day 3 (2 QTLs) to 53.8% at day 6 (4 QTLs). Similar values of QTLs for PF  
303 were found at days 5 ( $R^2 = 47.2\%$ ; 2 QTLs) and 7 ( $R^2 = 41.5\%$ ; 3 QTLs). QTLs with high  $R^2$  values (Table 3 and  
304 Supplemental Table 3) were observed for PF close to the end of fruit ripening, i.e. on LG6 at day 5 ( $\text{PF}^{\text{W}}\text{QTL-G6}$ :  $R^2$   
305  $= 30.0\%$ ,  $\text{CI} = 9.4\text{ Mb}$ ) and on LG11 at days 6 and 7 ( $\text{PF}^{\text{W}}\text{QTL-PM11}$ :  $R^2 = 14.2\text{-}17.8\%$ ,  $\text{CI} = 1.0\text{ Mb}$ ).

306 For pulp dry matter content (DMC), QTLs were detected on LG5, 6, 8, 9, 10, and 11 (Fig. 1b and Fig. 4).  
307 Total  $R^2$  values were 60.4%, 31.4% and 59.2% at days 1, 3 and 7, respectively, corresponding to 5, 2 and 5 distinct

308 QTLs, respectively (Supplemental Table 3). Two QTLs with major effects (Table 3) were detected at the top of LG9  
309 on the Pisang Madu map, i.e. the first one at days 1 and 3 (DMC<sup>W</sup>QTL-PM9.1: R<sup>2</sup> = 18.0-22.1%, CI = 0.9 Mb), and  
310 the second at day 7 (DMC<sup>W</sup>QTL-PM9.2: R<sup>2</sup> = 26.1%, CI = 1.2 Mb).

311 Several QTLs for the different quality traits were found in the same regions. Two colocalizations were  
312 identified on the Pisang Madu map, i.e. at the top of LG3 with QTLs for pH and PF, and at the top of LG9 with  
313 QTLs for pH and DMC. One colocalization was identified from Galeo polymorphism at the bottom of LG6 with  
314 QTLs for pH and PF (Supplemental Table 3).

315

#### 316 *3.4. Consistency of QTLs throughout production cycles over the ripening period*

317 QTL stability over the successive production cycles was investigated by comparing consensus QTLs  
318 detected in the same region at different ripening days throughout all cycles to those detected at each cycle (second  
319 approach). 110 cycle-specific QTLs were detected overall, including 21, 35 and 54 for cycles 1, 2 and 3, respectively  
320 (Fig. 2b-c and Supplemental Table 4). The maximum number of QTLs (51) was detected for PF, followed by pH (39  
321 QTLs) and DMC (20 QTLs) (Fig. 2b-c and Supplemental Table 4). The detected QTLs were usually specific to one  
322 cycle (Supplemental Table 4). Six QTLs detected at one cycle were also detected at a second cycle, i.e. one for pulp  
323 pH and the others for the PF trait. Two consensus QTLs (PF<sup>W</sup>QTL-G1 and pH<sup>W</sup>QTL-PM1\_7.1) were identified for  
324 all cycles (Fig. 1b, Fig. 4, and Table 3). Ten of the 23 QTLs found in the whole dataset (Supplemental Table 3) were  
325 detected using the cycle-specific approach (Table 3). In addition, QTL detection at each cycle allowed the  
326 identification of eight additional consensus QTLs that were not detected in the first approach, i.e. two for pH, five for  
327 PF and one for DMC. Unlike the first approach, QTLs on LG2 were detected for each trait via this cycle-specific  
328 analysis (Supplemental Table 4).

329

#### 330 *3.5. QTL meta-analysis*

331 Interestingly, many QTLs detected in this study colocalized within or between traits, and several were  
332 overlapped, thereby increasing the size of the confidence interval. Thus, to mine MQTLs and refine QTL intervals,  
333 all QTLs from the two approaches were pooled and projected on each map to run a meta-analysis. MQTLs were  
334 projected on a consensus map in Biomecator.V4 except for LG1\_7. The meta-analysis results are shown in

335 Supplemental Table 5. The meta-analysis successfully reduced the number of QTLs to investigate in this study as  
336 well as their confidence intervals. A total of 33 MQTLs were distributed unevenly across the genome, 9 of which  
337 were related to QTLs detected using both the first approach on the whole dataset and the cycle-specific approach  
338 (Fig. 4 and Table 3). Among the MQTLs (Supplemental Table 5), three were related to pH only (MQTL-G5.2,  
339 MQTL-PM1\_7.3, MQTL-PM3.2), and five were related to PF only (MQTL-G4.1, MQTL-G8.1, MQTL-G9.2,  
340 MQTL-PM10.1 and MQTL-PM11.2). Remarkably, the confidence intervals obtained for MQTL-PM8.1 and MQTL-  
341 PM11.2—both related to DMC—were at least 85.0% lower than that detected for the whole dataset (DMC<sup>W</sup>QTL-  
342 PM8\_1:  $R^2 = 9.9\%$ , CI = 28.8 Mb; and DMC<sup>W</sup>QTL-PM11:  $R^2 = 13.5\%$ , CI = 19.2 Mb).

343 The number of MQTLs per LG ranged from one MQTL on LG2 to six on LG10. MQTL-G1 as well as  
344 MQTL-PM8.2 covered eight QTLs, while MQTL-PM9.1 and MQTL-PM7.2 covered 15 and 18 QTLs, respectively.  
345 Many of the MQTLs identified in this study were associated with two or three traits. 13 of these MQTLs were related  
346 to pH and PF, 3 to pH and DMC, and 3 to PF and DMC. Moreover, 4 MQTLs were found to be related to all traits  
347 (Supplemental Table 5).

348

### 349 *3.6. Colocalization between QTLs and candidate genes (with CI lower than 10 Mb)*

350 Colocalization between QTLs linked to pH and a selection of candidate genes culled from the literature  
351 allowed the identification of genes potentially involved in pulp organic acid metabolism (Table 3, Supplemental  
352 Tables 3 and 5). On the Pisang Madu map, genes related to PEPC and PEPCCK were identified in the pH<sup>W</sup>QTL-PM9  
353 and pH<sup>W</sup>QTL-PM1\_7.2 confidence intervals. In addition, the latter detected by MQTL analysis, i.e.  
354 MQTL-PM1\_7.3, was associated with genes involved in Aco, GAD and Mal activities (Table 3 and Supplemental  
355 Table 5). The gene related to V-ATPase was identified in a QTL detected by the two approaches and in the MQTL-  
356 PM3.2 confidence interval (Table 3). On the Galeo map, most of these genes were identified in pH<sup>W</sup>QTL-G10 and  
357 MQTL-G5.2 confidence intervals (Supplemental Tables 3 and 5). Surprisingly, no candidate gene linked to citrate  
358 synthase was found for all QTLs linked to pH.

359 Candidate genes involved in cell wall metabolism (division and expansion) and hormones regulating fruit  
360 ripening were detected in seven QTLs linked to PF. Most of these genes were related to auxin regulation and  
361 peroxidase activity, as for PF<sup>W</sup>QTL-PM11 and PF<sup>W</sup>QTL-G6 and for MQTL-G4.1 (1 Mb) (Tables 3 and  
362 Supplemental Table 3). In addition, candidate genes involved in the ethylene response (ETR) were identified for

363 PF<sup>W</sup>QTL-G11 (1.5 Mb) (Table 3). Genes involved in cell wall metabolism were found in QTL intervals on both  
364 parental maps, PF<sup>W</sup>QTL-PM3 and PF<sup>W</sup>QTL-G6, and on several MQTLs related to several quality traits, including  
365 PF.

366           Based on their functions, enzymes involved in glycolysis, sugar metabolism, transport, and specific  
367 hormonal activity constituted obvious candidate genes for DMC. Candidate genes involved in cell walls—  
368 corresponding to structural dry matter—were detected on two QTLs from the Pisang Madu map, i.e. PF<sup>W</sup>QTL-  
369 PM9.1 and -PM9.2 (Table 3). Five MQTLs linked to DMC collocated with genes coding for enzymes catalysing  
370 carbohydrate synthesis or degradation (Supplemental Table 5). The same MQTLs except for MQTL-M11.2  
371 collocated with aconitate hydratase activity (Aco). Sucrose synthase activity (SuSy) collocated with MQTL-PM4.1  
372 and MQTL-PM9.3, while UDP collocated with MQTL-PM4.1 and MQTL-PM11.2. In addition, genes linked to auxin  
373 metabolism collocated with the two MQTLs linked to DMC only (MQTL-G7 and MQTL-PM11.2).

374

### 375           3.7. *Identification of chromosomal structures associated with pH variation*

376           The QTL detected for pH with the greatest effect (pH<sup>W</sup>QTL-PM1\_7.1) was identified on LG1\_7 of Pisang  
377 Madu (Fig. 2b-c). Pisang Madu is structurally heterozygous for translocation between reference chromosomes 1 and  
378 7. We analyzed the correlation between the presence of each of these chromosome haplotypes (chromosome 1 vs  
379 chromosome 1T7) with the pH at day 7—corresponding to the ready-to-eat stage—in the population. In the progeny,  
380 the mean pH calculated on individuals with chromosome 1T7 was significantly higher than that for individuals with  
381 chromosome 1 (Fig. 2d). The higher pH of pH<sup>W</sup>QTL-PM1\_7.1 was thus associated with chromosome 1T7.

382

## 383           4. **Discussion**

### 384           4.1. *Dynamic QTL mapping approaches and meta-analysis for banana quality traits*

385           The phenotypic data analysis revealed high heritability values for two of the measured traits ( $\approx 0.8$  for pulp  
386 pH and DMC~), and a slightly less one for pulp firmness (0.46), which were suitable for further QTL detection. We  
387 detected significant genotypic effects, but also strong genotype–production cycle and genotype–ripening day  
388 interactions. This led us to implement two QTL mapping approaches. The first one exploited the whole dataset to  
389 analyse QTLs over the fruit ripening period, as reported in dynamic QTLs analyses for other fruits (peach (Desnoves



390 et al., 2016), apple (Costa et al., 2010) and tomato (Sun et al., 2012)). The second one exploited data by cycle to  
391 evaluate the stability of the detected consensus QTLs according to the production cycle. These approaches resulted  
392 in detection of numerous QTLs, but the confidence intervals were broad, and lot of them were overlapping. So an  
393 additional meta-analysis of QTLs identified in these approaches was performed that enabled us to refine the QTL  
394 intervals and study potential QTLs colocalization. Meta-analysis approaches were initially developed to identify fine  
395 consensus QTLs across multiple studies for a single trait (Wu and Hu 2012), but they were also recently used to  
396 study colocalization between QTLs for distinct traits (Shen, Xiang et al. 2018)

397  
398 The construction of the two parental genetic maps, used to perform QTL detection, led to 11 linkage groups  
399 for Galeo as expected, but to only 10 linkage groups for Pisang Madu, with LG1\_7 associating markers from  
400 reference chromosome 1 as well as reference chromosome 7 (Fig. 2). This particularity was linked to the presence of  
401 a large reciprocal translocation involving reference chromosomes 1 and 7 that has been reported in heterozygous  
402 state in Pisang Madu and that prevents recombination on chromosome 1 (Martin et al. 2020b). The translocation  
403 breakpoint was localized in the pericentromeric region for chromosome 7, which explains the central position of  
404 chromosome 1 markers in LG1\_7.

405 Among the various detected QTLs, 12 seemed highly promising (Table 3), nine of which were confirmed in  
406 the meta-analysis, with a marked reduction in CI enabling us to research candidate genes. Among these nine QTLs,  
407 three corresponded to major consensus QTLs with  $R^2$  values over 25.0% ( $pH^W$ QTL-PM1\_7.1,  $DMC^W$ QTL-PM9.2,  
408 and  $PF^W$ QTL-G6). Moreover, these three QTLs were involved in three MQTLs linked to several quality traits, thus  
409 highlighting colocalization between QTLs for these distinct traits. Relationship between such traits were previously  
410 reported in other fruits such as peach (Quilot et al. 2005; Desnoues et al. 2016), apple (Liebhard et al. 2003; Kenis et  
411 al. 2008) or tomato (Chaïb et al. 2006; Saliba-Colombani et al. 2001). Finally, two additional notable QTLs with  $R^2$   
412 values higher than 15.0%, were detected but only from the first approach, they concerned  $DMC^W$ QTL-PM9.1 and  
413  $PF^W$ QTL-PM11. The latter was detected in particular at the end of fruit ripening. The tenth QTL with an  $R^2$  close to  
414 15.0%, which was not identified in the meta-analysis, was detected throughout the fruit ripening period ( $pH^W$ QTL-  
415 G6). We presume that this difference in QTL detection between the two approaches was related to the number of  
416 phenotyping data, which was higher in the first approach, thereby facilitating QTL detection. The variation in QTLs  
417 identified between production cycles in the second approach could be related to the different environmental

418 conditions. The impacts of climatic conditions—which could vary between crop cycles—are well-documented for  
419 organoleptic fruit quality in banana (Peacock 1980; Daniells et al. 1992; Ambuko et al. 2006; Abd el Moniem et al.  
420 2008; Hailu et al. 2013), as well as for other fruit such as tomato (Guichard et al. 2001; Mulholland et al. 2003;  
421 Panthee et al. 2012) and melon (Bernillon et al. 2013). In addition to environmental effects, this difference could at  
422 least partly be explained by the fact that in each cycle not all the same progeny individuals were phenotyped and the  
423 number of progeny individuals phenotyped per cycle varied. Accordingly, QTLs detected in cycle 3, which involved  
424 the lowest number of individuals, should be regarded with caution since the number of QTLs detected was reported  
425 to increase as the population size decreased (Vales et al., 2005).

426

#### 427 *4.2. Genetic control of banana quality traits*

428 Flavor, including taste and aroma, is the first quality perception criterion for consumer acceptance. Flavor  
429 mainly depends on sugar and acid contents, and particularly on the sugar/acid ratio, which plays a role in both  
430 sourness and sweetness perception (Bugaud et al. 2011, 2016). The majority of pH QTLs were detected on the  
431 Pisang Madu genetic map, using both the whole dataset and per-cycle data. The increase in pH at the end of fruit  
432 ripening, as observed in Pisang Madu, has been related to the decrease in acid contents, and described in banana  
433 cultivars with low citrate levels and sometimes with high malate levels (Etienne et al. 2013a, 2014). The pH profile  
434 observed for cv Galeo was typical of these acidic bananas and could be associated with high citrate contents (Etienne  
435 et al., 2014).  $\text{pH}^{\text{W}}\text{QTL-PM1}_{7.1}$  had the highest estimated effect for QTLs detected at the optimal maturity stage,  
436 with  $R^2$  values of 50.6 and 44.0% at days 6 and 7, respectively. The effects of additional pH QTLs were relatively  
437 lower with, however, some pH QTLs having marked effects, especially during the first part of ripening on LG3  
438 ( $\text{pH}^{\text{W}}\text{QTL-PM3}$ : 9.0%) and LG6 ( $\text{pH}^{\text{W}}\text{QTL-G6}$ : 5.6 to 14.4%).

439 Genes coding for enzymes involved in acid metabolism were found in the confidence interval of QTLs for  
440 pH. They corresponded especially to the tricarboxylic acid cycle, namely with Aco, GAD, Mal, POX, PEPC and  
441 PEPCCK. A previous modelling study on banana acidity highlighted the potential role of NAD-malic enzyme and  
442 mitochondrial malate carriers in the genotypic variability of citrate concentration differences (Etienne et al. 2014,  
443 2015). These genes were linked to QTLs related to organic acid variations in other fruits like tomato (Causse et al.  
444 2004), apple (Ban and Xu, 2020) and grapeberries (Chen et al., 2015). For malic acid variations among cultivars, a  
445 similar approach highlighted the importance of proton pump activity and especially the free energy of ATP

446 hydrolysis (Etienne et al. 2014). This activity could be studied in relation to the pH MQTL-PM3.2, where gene  
447 coding to V-ATPase was detected. QTL regions with these genes were reported to influence malate accumulation in  
448 other fruits such as apple (Jia et al. 2018) and tomato (Causse et al. 2004).

449  
450 After flavor, fruit texture—which can be assessed by pulp firmness—is the first consumer quality  
451 perception criterion for bananas (Bugaud et al. 2013). In addition, texture is essential for consumers with regard to  
452 shelflife and transportability (Bugaud et al. 2013; Jaiswal et al. 2014). The detected genetic effect of pulp firmness in  
453 the studied progeny was particularly low compared to other traits (PVE 12.2% and  $H^2$  0,46), while presenting the  
454 highest interaction effects and residuals (PVE ~ 40.0%), which could be attributed to high variability between  
455 analytical replicates and a greater environmental influence on this trait compared to others. According to several  
456 studies on banana, fruit firmness depends on several factors, such as genotype (Saliba-Colombani et al. 2001),  
457 growth conditions (Ambuko et al. 2006; Bugaud et al. 2009), maturity stage at harvest and postharvest conditions  
458 (Ambuko et al. 2006; Salvador et al. 2007; Bugaud et al. 2007). Some cycle-specific QTLs were detected from the  
459 first two cycles, during the second part of ripening, close to the optimum stage of consumption.  $PF^{C1}QTL.G1$  and  
460  $PF^{C1}QTL.PM10$  were both found at days 6 and 7, and  $PF^{C2}QTL.PM8$  and  $PF^{C2}QTL.PM1_7.2$  were detected on 4  
461 different days from days 5 to 7. Several genes involved in cell wall metabolism were found in QTL intervals related  
462 to PF on the two maps, i.e.  $PF^WQTL-PM3$  and  $PF^WQTL-G6$ . Genes involved in the auxin response and auxin  
463 signaling pathway that could regulate cell wall metabolism and influence fruit softening (Kumar et al. 2014) have  
464 been identified with  $PF^WQTL-PM4$  and  $PF^WQTL-G4$  within 0.9 Mb and 4.7 Mb, respectively. In tomato, through the  
465 *SlARF4* gene, auxin has been found to control fruit firmness by regulating the pectin structure and tissue architecture  
466 (Jones et al. 2002; Sagar et al. 2013). Moreover, genes involved in the ethylene response (ETR) were found within  
467 the 1.5 Mb interval of  $PF^WQTL-G11$ . Ethylene and its biosynthetic genes have been implicated in fruit softening and  
468 shelflife maintenance regulation in several fleshy fruits (Xiong et al. 2005; Nishiyama et al. 2007; López-Gómez et  
469 al. 2009), e.g. via the expression of cell wall-related genes in tomato (Bu et al. 2013) apple (Wei et al. 2010), and  
470 peach (Hayama et al. 2006).

471  
472 DMC is an important determinant with regard to several aspects of fruit quality such as fruit texture,  
473 including fruit firmness, storage and taste (Ferris et al. 1999; Bugaud et al. 2007). Bananas with higher pulp DMC

474 have thus been related to firm bananas and consumers have shown a preference for fruit with higher DMC, including  
475 plantain and cooking bananas at the green stage (Ferris et al. 1999; Gibert et al. 2009; Bugaud et al. 2013).

476 DMC presented high genetic determinism in the studied progeny (61.3% PVE), thus confirming the  
477 potential of this trait for dessert and cooking banana breeding schemes. In addition, DMC measured at harvest was  
478 correlated with DMC at the optimal stage ( $r = 0.72$ ; data not shown). The high stability of DMC at the end of fruit  
479 growth and ripening had been pointed out in previous studies on banana (Ferris et al. 1999; Yomeni et al. 2004;  
480 Bugaud et al. 2007, 2012; Gibert et al. 2010) and mango (Lechaudel et al. 2005). This relationship prevails especially  
481 in climacteric fruit, like bananas, that accumulate starch during growth, which is converted into soluble sugars during  
482 ripening (Xiao et al. 2018). Consequently, although DMC was measured at three days of fruit ripening (days 1, 3 and  
483 7), a significant number of QTLs were detected in the whole dataset. Among the QTLs associated with very low  
484 confidence intervals, most were detected for DMC, including those on LG9 (DMC<sup>W</sup>QTL-PM9.1 and -PM9.2) and  
485 LG10 (DMC<sup>W</sup>QTL-PM10 and -G10). Other QTLs detected presented high confidence intervals of over 19.0 Mb,  
486 which would hamper molecular breeding tool development. About 30.0% of DMC is attributed to structural dry  
487 mass, and cell walls (Etienne et al., 2014), which could explain why genes involved in cell wall dynamics  
488 colocalized with four DMC QTLs, including two MQTL (MQTL-PM8.1 and -PM11.2). Interestingly, genes related  
489 to several hormone metabolisms, such as auxin transport, ethylene response and gibberellin, colocalized with  
490 MQTL-PM11.2 within a 2.9 Mb region. This clustering of genes involved in the fruit development and growth phase  
491 may have a similar effect on the structural and non-structural dry matter content.

492  
493 Several QTL colocalizations were identified for different quality traits on the two parent maps, suggesting  
494 physiological relationships between them. The closest correlations between pH and firmness values were observed  
495 during the first part of fruit ripening when these traits showed a concomitant decrease. The high number of QTL  
496 colocalizations observed for pH and PF could hence be explained by the links between ripening-related changes in  
497 pH and fruit texture. Several studies have suggested that changes in pH and mineral composition during fruit  
498 ripening may regulate cell wall hydrolase activity (Almeida and Huber 1999; Prasanna et al., 2007). The pH  
499 acidification trend during ripening has been found to be involved in fruit softening of several species (Johnston et al.,  
500 2002).

501 The colocalization between pH and DMC was expected as acids are major dry matter components. Frequent  
502 colocalizations have been previously observed between QTLs for pulp dry matter and acidity in tomato (Paterson et  
503 al. 1991; Saliba-Colombani et al. 2001) and peach (Dirlewanger et al. 1999). In such cases, it is difficult to  
504 distinguish the pleiotropic effect of a single gene from the effects of the nearest genes.

505 The colocalization between PF and DMC could be explained by the hydrolysis of starch, i.e. the main DMC  
506 compound, into soluble sugars during banana ripening (Bhuiyan et al., 2020). This causes an increase in osmotic  
507 pressure and involves a decrease in turgor pressure associated with softening, as observed during berry (Thomas et  
508 al., 2008) and tomato ripening (Saladié et al., 2007). Moreover, the pulp dry mass can be assessed by the sum of the  
509 dry weights of the main non-structural compounds, such as soluble sugars, starch and acids, and the structural  
510 compounds (Etienne et al, 2014), i.e. the constitutive part of pulp cells. This relationship between the structural dry  
511 matter component and cell walls could explain the indirect links between DMC and PF.

512 Finally, we also noted significant QTL clustering, including a large cluster containing QTLs for all  
513 measured traits localized on LG10. This clustering may be due to tight linkage between QTLs (clustering of different  
514 genes) and/or may indicate the presence of a single QTL with pleiotropic effects. Similar fruit quality QTL clusters  
515 have been reported in other fruit species, including peach (Quilot et al. 2005; Desnoues et al. 2016), apple (Liebhard  
516 et al. 2003; Kenis et al. 2008) and tomato (Chaïb et al. 2006).

517

## 518 **5. Conclusion**

519 High heritability was found in the progeny for the three studied traits, although pulp firmness seemed to be  
520 more affected by environmental factors. Breeding and prebreeding approaches should thus permit to improve these  
521 characters.

522 Several promising QTLs ( $R^2 > 15.0\%$ ) were detected for the three characters, including three major QTLs  
523 ( $R^2 > 25.0\%$ ) –one per trait- ( $pH^WQTL-PM1\_7.1$ ,  $DMC^WQTL-PM9.2$ , and  $PF^WQTL-G6$ ). They should now be  
524 validated in other genetic contexts and their confidence intervals would need to be refined before envisioning MAS  
525 selection.

526 One particularly interesting QTL is QTL  $pH^WQTL-PM1\_7.1$  that had the highest estimated effect at the  
527 optimal maturity stage, with an  $R^2$  of 50.6 and 44.0% at days 6 and 7, respectively. We showed that the  
528 corresponding haplotype in chromosome 1T7 of Pisang Madu was associated with higher pH at fruit maturity, which

529 is a sweet dessert banana characteristic. This haplotype in Pisang Madu is located, in a region of Chr 1T7 that does  
530 not recombine in this heterozygous context and corresponds to reference chromosome 1. This could thus be an  
531 advantage for MAS, since selection for this QTL could be performed using any specific markers from chromosome 1  
532 in this progeny and potentially in other progeny of parents structurally heterozygous for the 1T7 chromosome  
533 structure. The effects of this QTL in other genomic contexts have yet to be tested, but could potentially explain the  
534 success of the 1T7 chromosomal structure to which it is associated and which has been found in many dessert  
535 cultivars including Cavendish, that accounts for 50.0% of world banana production (Martin et al., 2020b).

536

537

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543

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545 **Stella Biabiany**: Investigation, Formal analysis, Writing - Original Draft ; **Emilie Araou**: Resources, Methodology,  
546 Investigation ; **Fabien Cormier**: Methodology, Validation; **Guillaume Martin**: Software, Data curation, Formal  
547 analysis, Writing – review and editing; **Françoise Carreel**: Methodology, Validation; **Catherine Hervouet**:  
548 Resources; **Frédéric Salmon**: Methodology, Resources; **Jean-Claude Efile**: Methodology, Resources; **Felicie**  
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### 559 **Declaration of Competing Interest**

560 The authors declare that they have no conflict of interest.

561

### 562 **Availability of data and material**

563 The data that support the findings of this study are available from the authors upon request.

564

565

### 566 **References**

567

568 Abd el Moniem EA, A. S. E. Abd-Allah, Ahmed MA (2008) The combined effect of some organic manures, mineral N fertilizers and  
569 algal cells extract on yield and fruit quality of Williams banana plants. *Agric & Environ Sci* 4:417-426

570 Ahmad F, Martawi NM, Poerba YS, de Jong H, Schouten H, Kema GH (2020) Genetic mapping of Fusarium wilt resistance in a  
571 wild banana *Musa acuminata* ssp. *malaccensis* accession. *Theoretical and Applied Genetics* 133:3409-3418

572 Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19:716-723

573 Almeida DPF, Huber DJ (1999) Apoplastic pH and inorganic ion levels in tomato fruit: A potential means for regulation of cell wall  
574 metabolism during ripening. *Physiologia Plantarum* 105:506-512

575 Ambuko JL, Sekozawa Y, Sugaya S, Itoh F, Nakamura K, Gemma H (2006) EFFECT OF SEASONAL VARIATION, CULTIVAR AND  
576 PRODUCTION SYSTEM ON SOME POSTHARVEST CHARACTERISTICS OF THE BANANA. 712 edn. International Society for  
577 Horticultural Science (ISHS), Leuven, Belgium, pp 505-510

578 Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) BioMercator: integrating genetic maps  
579 and QTL towards discovery of candidate genes. *Bioinformatics* 20:2324-2326

580 Arends D, Prins P, Jansen RC, Broman KW (2010) R/qtl: high-throughput multiple QTL mapping. *Bioinformatics* 26:2990-2992

581 Bakry F (2008) Zygotic embryo rescue in bananas. *Fruits* 63:111-115

582 Bakry F, Carreel F, Jenny C, Horry J-P (2009) Genetic improvement of banana. *Breeding plantation tree crops: tropical species*.  
583 Springer, pp 3-50

584 Baurens F-C, Martin G, Hervouet C, Salmon F, Yohomé D, Ricci S, Rouard M, Habas R, Lemainque A, Yahiaoui N (2019)  
585 Recombination and large structural variations shape interspecific edible bananas genomes. *Molecular biology and evolution*  
586 36:97-111

587 Bernillon S, Biais B, Deborde C, Maucourt M, Cabasson C, Gibon Y, Hansen TH, Husted S, de Vos RCH, Mumm R, Jonker H, Ward  
588 JL, Miller SJ, Baker JM, Burger J, Tadmor Ya, Beale MH, Schjoerring JK, Schaffer AA, Rolin D, Hall RD, Moing A (2013)  
589 Metabolomic and elemental profiling of melon fruit quality as affected by genotype and environment. *Metabolomics* 9:57-77

590 Bhuiyan F, Campos NA, Swennen R, Carpentier S (2020) Characterizing fruit ripening in plantain and Cavendish bananas: A  
591 proteomics approach. *Journal of Proteomics* 214:103632

592 Broman KW, Sen S (2009) *A Guide to QTL Mapping with R/qtl*. New York: Springer

- 593 Bu J, Yu Y, Aisikaer G, Ying T (2013) Postharvest UV-C irradiation inhibits the production of ethylene and the activity of cell wall-  
594 degrading enzymes during softening of tomato (*Lycopersicon esculentum* L.) fruit. *Postharvest Biology and Technology* 86:337-  
595 345
- 596 Bugaud C, Belleil T, Daribo M-O, Génard M (2012) Does bunch trimming affect dry matter content in banana? *Scientia*  
597 *Horticulturae* 144:125-129
- 598 Bugaud C, Cazevielle P, Daribo M-O, Telle N, Julianus P, Fils-Lycaon B, Mbéguié-A-Mbéguié D (2013) Rheological and chemical  
599 predictors of texture and taste in dessert banana (*Musa* spp.). *Postharvest Biology and Technology* 84:1-8
- 600 Bugaud C, Daribo MO, Dubois C (2007) Climatic conditions affect the texture and colour of Cavendish bananas (*Grande Naine*  
601 cultivar). *Scientia Horticulturae* 113:238-243
- 602 Bugaud C, Daribo M-O, Beauté M-P, Telle N, Dubois C (2009) Relative importance of location and period of banana bunch  
603 growth in carbohydrate content and mineral composition of fruit. *Fruits* 64:63-74
- 604 Bugaud C, Deverge E, Daribo MO, Ribeyre F, Fils-Lycaon B, Mbéguié-A-Mbéguié D (2011) Sensory characterisation enabled the  
605 first classification of dessert bananas. *Journal of the Science of Food and Agriculture* 91:992-1000
- 606 Bugaud C, Maraval I, Daribo M-O, Leclerc N, Salmon F (2016) Optimal and acceptable levels of sweetness, sourness, firmness,  
607 mealiness and banana aroma in dessert banana (*Musa* sp.). *Scientia Horticulturae* 211:399-409
- 608 Carreel F, De Leon DG, Lagoda P, Lanaud C, Jenny C, Horry J-P, Du Montcel HT (2002) Ascertaining maternal and paternal lineage  
609 within *Musa* by chloroplast and mitochondrial DNA RFLP analyses. *Genome* 45:679-692
- 610 Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A  
611 genetic map of candidate genes and QTLs involved in tomato fruit size and composition. *Journal of Experimental Botany*  
612 55:1671-1685
- 613 Causse M, Saliba-Colombani V, Lecomte L, Duffé P, Rousselle P, Buret M (2002) QTL analysis of fruit quality in fresh market  
614 tomato: a few chromosome regions control the variation of sensory and instrumental traits. *Journal of Experimental Botany*  
615 53:2089-2098
- 616 Causse M, Saliba-Colombani V, Lesschaeve I, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 2.  
617 Mapping QTLs for sensory attributes. *Theoretical and Applied Genetics* 102:273-283
- 618 Cenci A, Sardos J, Hueber Y, Martin G, Breton C, Roux N, Swennen R, Carpentier SC, Rouard M (2020) Unravelling the complex  
619 story of intergenomic recombination in ABB allotriploid bananas. *Annals of Botany* 127:7-20
- 620 Chaïb J (2006) Stability over genetic backgrounds, generations and years of quantitative trait locus (QTLs) for organoleptic  
621 quality in tomato. *Theoretical and applied genetics* v. 112:pp. 934-944-2006 v.2112 no.2005
- 622 Chillet M, de Lapeyre de Bellaire L, Hubert O, Mbéguié-A-Mbéguié D (2008) Mechanical characterisation of banana fruits. *Fruits*  
623 63:51-52
- 624 Christelová P, Valárik M, Hřibová E, Van den houwe I, Channelière S, Roux N, Doležel J (2011) A platform for efficient genotyping  
625 in *Musa* using microsatellite markers. *AoB PLANTS* 2011
- 626 Costa F, Peace CP, Stella S, Serra S, Musacchi S, Bazzani M, Sansavini S, Van de Weg WE (2010) QTL dynamics for fruit firmness  
627 and softening around an ethylene-dependent polygalacturonase gene in apple (*Malus domestica* Borkh.). *Journal of*  
628 *Experimental Botany* 61:3029-3039
- 629 Cullis BR, Smith AB, Coombes NE (2006) On the design of early generation variety trials with correlated data. *Journal of*  
630 *Agricultural, Biological, and Environmental Statistics* 11:381
- 631 D'hont A, Denoeud F, Aury J-M, Baurens F-C, Carreel F, Garsmeur O, Noel B, Bocs S, Droc G, Rouard M (2012) The banana (*Musa*  
632 *acuminata*) genome and the evolution of monocotyledonous plants. *Nature* 488:213-217
- 633 Daniells J, Lisle A, O'Farrell P (1992) Effect of bunch-covering methods on maturity bronzing, yield, and fruit quality of bananas in  
634 North Queensland. *Australian Journal of Experimental Agriculture* 32:121-125



- 635 Desnoues E, Baldazzi V, Génard M, Mauroux J-B, Lambert P, Confolent C, Quilot-Turion B (2016) Dynamic QTLs for sugars and  
636 enzyme activities provide an overview of genetic control of sugar metabolism during peach fruit development. *Journal of*  
637 *Experimental Botany* 67:3419-3431
- 638 Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999) Mapping QTLs controlling fruit  
639 quality in peach (*Prunus persica* (L.) Batsch). *Theoretical and Applied Genetics* 98:18-31
- 640 DOLEŽEL J, BARTOŠ J (2005) Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size. *Annals of Botany* 95:99-110
- 641 Dolezel J, Binarovk P, Lucretti S (1989) Analysis of nuclear DNA content in plant cells by flow  
642 cytometry. *Biol Plant* 31:113-120
- 643 Dupouy M, Baurens F-C, Derouault P, Hervouet C, Cardi C, Cruaud C, Istace B, Labadie K, Guiougou C, Toubi L (2019) Two large  
644 reciprocal translocations characterized in the disease resistance-rich burmannica genetic group of *Musa acuminata*. *Annals of*  
645 *botany* 124:319-329
- 646 Etienne A, Génard M, Bancel D, Benoit S, Bugaud C (2013a) A model approach revealed the relationship between banana pulp  
647 acidity and composition during growth and post harvest ripening. *Scientia Horticulturae* 162:125-134
- 648 Etienne A, Génard M, Bancel D, Benoit S, Lemire G, Bugaud C (2014) Citrate and malate accumulation in banana fruit (*Musa* sp.  
649 AA) is highly affected by genotype and fruit age, but not by cultural practices. *Scientia Horticulturae* 169:99-110
- 650 Etienne A, Génard M, Bugaud C (2015) A process-based model of TCA cycle functioning to analyze citrate accumulation in pre-  
651 and post-harvest fruits. *PLoS one* 10:e0126777
- 652 Etienne A, Génard M, Lobit P, Mbéguié-A-Mbéguié D, Bugaud C (2013b) What controls fleshy fruit acidity? A review of malate  
653 and citrate accumulation in fruit cells. *Journal of experimental botany* 64:1451-1469
- 654 Fauré S, Noyer J-L, Horry J-P, Bakry F, Lanaud C, de León DG (1993) A molecular marker-based linkage map of diploid bananas  
655 (*Musa acuminata*). *Theoretical and Applied Genetics* 87:517-526
- 656 Garsmeur O, Droc G, Antonise R, Grimwood J, Potier B, Aitken K, Jenkins J, Martin G, Charron C, Hervouet C, Costet L, Yahiaoui  
657 N, Healey A, Sims D, Cherukuri Y, Sreedasyam A, Kilian A, Chan A, Van Sluys M-A, Swaminathan K, Town C, Bergès H, Simmons B,  
658 Glaszmann JC, van der Vossen E, Henry R, Schmutz J, D'Hont A (2018) A mosaic monoploid reference sequence for the highly  
659 complex genome of sugarcane. *Nature Communications* 9:2638
- 660 Gibert C, Génard M, Vercambre G, Lescouret F (2010) Quantification and modelling of the stomatal, cuticular and crack  
661 components of peach fruit surface conductance. *Functional Plant Biology* 37:264-274
- 662 Gibert O, Dufour D, Giraldo A, Sanchez T, Reynes M, Pain J-P, Gonzalez A, Fernandez A, Diaz A (2009) Differentiation between  
663 cooking bananas and dessert bananas. 1. Morphological and compositional characterization of cultivated Colombian Musaceae  
664 (*Musa* sp.) in relation to consumer preferences. *Journal of Agricultural and Food Chemistry* 57:7857-7869
- 665 Guichard S, Bertin N, Leonardi C, Gary C (2001) Tomato fruit quality in relation to water and carbon fluxes. *Agronomie* 21:385-  
666 392
- 667 Hailu M, Tilahun Seyoum Workneh, Belew D (2013) Review on postharvest technology of banana fruit. *African Journal of*  
668 *Biotechnology* 12
- 669 Hayama H, Shimada T, Fujii H, Ito A, Kashimura Y (2006) Ethylene-regulation of fruit softening and softening-related genes in  
670 peach. *Journal of Experimental Botany* 57:4071-4077
- 671 Henderson CR (1950) Estimation of genetic parameters. *Ann Math Stat* 21:309
- 672 Herten K, Hestand MS, Vermeesch JR, Van Houdt JJK (2015) GBSX: a toolkit for experimental design and demultiplexing  
673 genotyping by sequencing experiments. *BMC Bioinformatics* 16:73
- 674 Hippolyte I, Bakry F, Seguin M, Gardes L, Rivallan R, Risterucci A-M, Jenny C, Perrier X, Carreel F, Argout X (2010) A saturated  
675 SSR/DaRT linkage map of *Musa acuminata* addressing genome rearrangements among bananas. *BMC plant biology* 10:1-18

- 676 Hubert O, Piral G, Galas C, Baurens F-C, Mbéguié-A-Mbéguié D (2014) Changes in ethylene signaling and MADS box gene  
677 expression are associated with banana finger drop. *Plant Science* 223:99-108
- 678 Jaiswal P, Jha SN, Kaur PP, Bhardwaj R, Singh AK, Wadhawan V (2014) Prediction of textural attributes using color values of  
679 banana (*Musa sapientum*) during ripening. *Journal of Food Science and Technology* 51:1179-1184
- 680 Jia D, Shen F, Wang Y, Wu T, Xu X, Zhang X, Han Z (2018) Apple fruit acidity is genetically diversified by natural variations in three  
681 hierarchical epistatic genes: MdSAUR37, MdPP2CH and MdALMTII. *The Plant Journal* 95:427-443
- 682 Johnston JW, Hewett EW, Hertog MLATM (2002) Postharvest softening of apple (*Malus domestica*) fruit: A review. *New Zealand  
683 Journal of Crop and Horticultural Science* 30:145-160
- 684 Jones B, Frasse P, Olmos E, Zegzouti H, Li ZG, Latché A, Pech JC, Bouzayen M (2002) Down-regulation of DR12, an auxin-  
685 response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *The  
686 Plant Journal* 32:603-613
- 687 Kenis K, Keulemans J, Davey MW (2008) Identification and stability of QTLs for fruit quality traits in apple. *Tree Genetics &  
688 Genomes* 4:647-661
- 689 Kosambi DD (2016) The Estimation of Map Distances from Recombination Values. In: Ramaswamy R (ed) DD Kosambi: Selected  
690 Works in Mathematics and Statistics. Springer India, New Delhi, pp 125-130
- 691 Kouassi HA, Assemand EF, Gibert O, Maraval I, Ricci J, Thiemele DEF, Bugaud C (2021) Textural and physicochemical predictors of  
692 sensory texture and sweetness of boiled plantain. *International Journal of Food Science & Technology* 56:1160-1170
- 693 Kumar R, Khurana A, Sharma AK (2014) Role of plant hormones and their interplay in development and ripening of fleshy fruits.  
694 *Journal of Experimental Botany* 65:4561-4575
- 695 Lagoda PJL, Dambier D, Grapin A, Baurens F-C, Lanaud C, Noyer J-L (1998) Nonradioactive sequence-tagged microsatellite site  
696 analyses: A method transferable to the tropics. *ELECTROPHORESIS* 19:152-157
- 697 Léchaudel M, Génard M, Lescourret F, Urban L, Jannoyer M (2005) Modeling effects of weather and source–sink relationships  
698 on mango fruit growth. *Tree Physiology* 25:583-597
- 699 Lescot T (2020) Banana genetic diversity. *Fruitrop* 269:98-102
- 700 Liebhard R, Kellerhals M, Pfammatter W, Jertmini M, Gessler C (2003) Mapping quantitative physiological traits in apple (*Malus ×  
701 domestica* Borkh.). *Plant Molecular Biology* 52:511-526
- 702 López-Gómez R, Cabrera-Ponce JL, Saucedo-Arias LJ, Carreto-Montoya L, Villanueva-Arce R, Díaz-Perez JC, Gómez-Lim MA,  
703 Herrera-Estrella L (2009) Ripening in papaya fruit is altered by ACC oxidase cosuppression. *Transgenic research* 18:89-97
- 704 Martin G, Baurens Fc, Hervouet C, Salmon F, Delos Jm, Labadie K, Perdereau A, Mournet P, Blois L, Dupouy M (2020b)  
705 Chromosome reciprocal translocations have accompanied subspecies evolution in bananas. *The Plant Journal* 104:1698-1711
- 706 Martin G, Baurens F-C, Droc G, Rouard M, Cenci A, Kilian A, Hastie A, Doležel J, Aury J-M, Alberti A, Carreel F, D'Hont A (2016)  
707 Improvement of the banana “*Musa acuminata*” reference sequence using NGS data and semi-automated bioinformatics  
708 methods. *BMC Genomics* 17:243
- 709 Martin G, Cardic C, Sarah G, Ricci S, Jenny C, Fondi E, Perrier X, Glaszmann JC, d'Hont A, Yahiaoui N (2020a) Genome ancestry  
710 mosaics reveal multiple and cryptic contributors to cultivated banana. *The Plant Journal* 102:1008-1025
- 711 Martin G, Carreel F, Coriton O, Hervouet C, Cardic C, Derouault P, Roques D, Salmon F, Rouard M, Sardos J (2017) Evolution of the  
712 banana genome (*Musa acuminata*) is impacted by large chromosomal translocations. *Molecular biology and evolution* 34:2140-  
713 2152
- 714 Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *2011* 17:3
- 715 Mbanjo EGN, Tchoumboungang F, Mouelle AS, Oben JE, Nyine M, Dochez C, Ferguson ME, Lorenzen J (2012) Molecular marker-  
716 based genetic linkage map of a diploid banana population (*Musa acuminata* Colla). *Euphytica* 188:369-386

- 717 Mulholland BJ, Edmondson RN, Fussell M, Basham J, Ho LC (2003) Effects of high temperature on tomato summer fruit quality.  
718 *The Journal of Horticultural Science and Biotechnology* 78:365-374
- 719 Nishiyama K, Guis M, Rose JKC, Kubo Y, Bennett KA, Wangjin L, Kato K, Ushijima K, Nakano R, Inaba A, Bouzayen M, Latche A,  
720 Pech J-C, Bennett AB (2007) Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. *Journal of*  
721 *Experimental Botany* 58:1281-1290
- 722 Noubissié G, Chabannes M, Bakry F, Ricci S, Cardi C, Njembele J-C, Yohoume D, Tomekpe K, Iskra-Caruana M-L, d'Hont A  
723 (2016) Chromosome segregation in an allotetraploid banana hybrid (AAAB) suggests a translocation between the A and B  
724 genomes and results in eBSV-free offsprings. *Molecular Breeding* 36:38
- 725 Nyine M, Uwimana B, Akech V, Brown A, Ortiz R, Doležel J, Lorenzen J, Swennen R (2019) Association genetics of bunch weight  
726 and its component traits in East African highland banana (*Musa* spp. AAA group). *Theoretical and Applied Genetics* 132:3295-  
727 3308
- 728 Panthee DR, Cao C, Debenport SJ, Rodríguez GR, Labate JA, Robertson LD, Breksa AP, van der Knaap E, McSpadden Gardener BB  
729 (2012) Magnitude of Genotype × Environment Interactions Affecting Tomato Fruit Quality. *HortScience horts* 47:721-726
- 730 Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors  
731 underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics* 127:181-197
- 732 Peacock BC (1980) Banana ripening: effect of temperature on fruit quality. *Queensland Journal of Agricultural and Animal*  
733 *Sciences (Australia)* 37:39-45
- 734 Perrier X, Bakry F, Carreel F, Jenny C, Horry J-P, Lebot V, Hippolyte I (2009) Combining biological approaches to shed light on the  
735 evolution of edible bananas
- 736 Phillips KM, McGinty RC, Couture G, Pehrsson PR, McKillop K, Fukagawa NK (2021) Dietary fiber, starch, and sugars in bananas at  
737 different stages of ripeness in the retail market. *PLoS ONE* 16(7): e0253366
- 738 Prasanna V, Prabha TN, Tharanathan RN (2007) Fruit Ripening Phenomena—An Overview. *Critical Reviews in Food Science and*  
739 *Nutrition* 47:1-19
- 740 Quilot B, Kervella J, Génard M, Lescourret F (2005) Analysing the genetic control of peach fruit quality through an  
741 ecophysiological model combined with a QTL approach. *Journal of Experimental Botany* 56:3083-3092
- 742 Quinlan AR, Hall IM (2010) BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841-842
- 743 Risterucci A-M, Grivet L, N'Goran JA, Pieretti I, Flament M-H, Lanaud C (2000) A high-density linkage map of *Theobroma cacao* L.  
744 *Theoretical and Applied Genetics* 101:948-955
- 745 Sagar M, Chervin C, Mila I, Hao Y, Roustan J-P, Benichou M, Gibon Y, Biais B, Maury P, Latché A, Pech J-C, Bouzayen M, Zouine M  
746 (2013) SIARF4, an Auxin Response Factor Involved in the Control of Sugar Metabolism during Tomato Fruit Development *Plant*  
747 *Physiology* 161:1362-1374
- 748 Saladié M, Matas AJ, Isaacson T, Jenks MA, Goodwin SM, Niklas KJ, Xiaolin R, Labavitch JM, Shackel KA, Fernie AR, Lytovchenko  
749 A, O'Neill MA, Watkins CB, Rose JKC (2007) A Reevaluation of the Key Factors That Influence Tomato Fruit Softening and  
750 Integrity. *Plant Physiology* 144:1012-1028
- 751 Saliba-Colombani V, Causse M, M., Langlois D, Philouze J, BURET M (2001) Genetic analysis of organoleptic quality in fresh  
752 market tomato. 1. Mapping QTLs for physical and chemical traits. *TAG Theoretical and Applied Genetics* 102:259-272
- 753 Salvador A, Sanz T, Fiszman SM (2007) Changes in colour and texture and their relationship with eating quality during storage of  
754 two different dessert bananas. *Postharvest Biology and Technology* 43:319-325
- 755 Sardos J, Rouard M, Hueber Y, Cenci A, Hyma KE, Van Den Houwe I, Hribova E, Courtois B, Roux N (2016) A genome-wide  
756 association study on the seedless phenotype in banana (*Musa* spp.) reveals the potential of a selected panel to detect candidate  
757 genes in a vegetatively propagated crop. *PLoS One* 11:e0154448

- 758 Shen Y, Xiang Y, Xu E, Ge X, Li Z (2018) Major Co-localized QTL for Plant Height, Branch Initiation Height, Stem Diameter, and  
759 Flowering Time in an Alien Introgression Derived Brassica napus DH Population. *Frontiers in Plant Science* 9
- 760 Šimoníková D, Němečková A, Čížková J, Brown A, Swennen R, Doležel J, Hřibová E (2020) Chromosome Painting in Cultivated  
761 Bananas and Their Wild Relatives (*Musa* spp.) Reveals Differences in Chromosome Structure. *International journal of molecular*  
762 *sciences* 21:7915
- 763 Sun YD, Liang Y, Wu JM, Li YZ, Cui X, Qin L (2012) Dynamic QTL analysis for fruit lycopene content and total soluble solid content  
764 in a *Solanum lycopersicum* x *S. pimpinellifolium* cross. *Genetics and molecular research : GMR* 11:3696-3710
- 765 Team RC (2020) R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna,  
766 Austria.
- 767 Thomas TR, Shackel KA, Matthews MA (2008) Mesocarp cell turgor in *Vitis vinifera* L. berries throughout development and its  
768 relation to firmness, growth, and the onset of ripening. *Planta* 228:1067
- 769 Vales MI, Schön CC, Capettini F, Chen XM, Corey AE, Mather DE, Mundt CC, Richardson KL, Sandoval-Islas JS, Utz HF, Hayes PM  
770 (2005) Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. *Theor Appl Genet*  
771 111:1260-1270
- 772 Van Ooijen J (2006) JoinMap(R) 4, software for the calculation of genetic linkage maps in experimental population
- 773 Vázquez-Ovando A, Andrino-López D, Adriano-Anaya L, Miguel S, Ovando-Medina I (2012) Sensory and physico-chemical quality  
774 of banana fruits “Grand Naine” grown with biofertilizer. - *African J Agricultural Research* 7:4620-4626
- 775 Wakabayashi K (2000) Changes in Cell Wall Polysaccharides During Fruit Ripening. *Journal of Plant Research* 113:231-237
- 776 Wei J, Ma F, Shi S, Qi X, Zhu X, Yuan J (2010) Changes and postharvest regulation of activity and gene expression of enzymes  
777 related to cell wall degradation in ripening apple fruit. *Postharvest Biology and Technology* 56:147-154
- 778 Wu X-L, Hu Z-L (2012) Meta-analysis of QTL Mapping Experiments. In: Rifkin SA (ed) *Quantitative Trait Loci (QTL): Methods and*  
779 *Protocols*. Humana Press, Totowa, NJ, pp 145-171
- 780 Xiao Y-Y, Kuang J-F, Qi X-N, Ye Y-J, Wu Z-X, Chen J-Y, Lu W-J (2018) A comprehensive investigation of starch degradation process  
781 and identification of a transcriptional activator MabHLH6 during banana fruit ripening. *Plant Biotechnol J* 16:151-164
- 782 Xiong A-S, Yao Q-H, Peng R-H, Li X, Han P-L, Fan H-Q (2005) Different effects on ACC oxidase gene silencing triggered by RNA  
783 interference in transgenic tomato. *Plant Cell Reports* 23:639-646
- 784 Yomeni M, Njoukam J, Tchango Tchango J (2004) Influence of the stage of ripeness of plantains and some cooking bananas on  
785 the sensory and physicochemical characteristics of processed products. *Journal of the Science of Food and Agriculture* 84:1069-  
786 1077

Figure captions:

**Fig. 1** Genetic map and QTL detected on LG 1\_7. **a:** comparison of the genetic linkage map of LG1\_7 with the physical map of reference chromosomes 1 and 7, **b:** location of the QTL related to the quality traits studied detected in the two approaches (W: whole dataset, C: production cycle datasets, with C1, C2 and C3 for cycles 1, 2 and 3, respectively) and the meta-analysis (MQTL), **c:** detail of QTLs for each day of the fruit ripening process that contributed to the two consensus pH QTLs identified in the first two approaches and confirmed by the meta-analysis, **d:** box plot representation of mean pH values at day 7 (corresponding to ripe fruit) of individuals with haplotype 1 (1/7 translocated chromosome structure) of Pisang Madu and individuals with haplotype 2 (reference chromosome structure) of Pisang Madu. Different letters indicate that haplotype averages are significantly different at  $P < 0.05$  (according to Tukey's multiple comparison test)

**Fig. 2** Boxplots of pulp pH, firmness (PF, in N) and dry matter content (DMC, in %) measured at the various studied fruit ripening days for all progeny individuals, either at all production cycles (**a, e, i**), or at cycles 1 (**b, f, j**), 2 (**c, g, k**) and 3 (**d, h, l**)

**Fig. 3** Number of QTLs detected for the three quality traits, i.e. pulp pH, firmness (PF, in N) and dry matter content (DMC, in %) on Pisang Madu (black) and Galeo (white) maps in the first QTL mapping approach on the whole dataset (**a**) and in the second approach on the three production cycle datasets (**b** and **c**) from Pisang Madu polymorphism (**b**) and Galeo polymorphism (**c**) for each cycle

**Fig. 4** Physical map based on reference chromosomes (except 1 and 7) and QTLs detected in both QTL mapping approaches and the meta-analysis, and QTLs from the first approach (whole dataset) with an  $R^2$  higher than 15%. QTLs are identified according to the quality trait, i.e. pulp pH (red), firmness (green) and dry matter content (blue, the dataset (W: whole dataset, C: production cycle datasets, with C1, C2 and C3 for cycles 1, 2 and 3, respectively) and the origin of linkage groups with Galeo (G) and Pisang Madu (PM). The location of SNP markers, QTLs, and MQTLs (grey) are indicated

**Fig. 1**

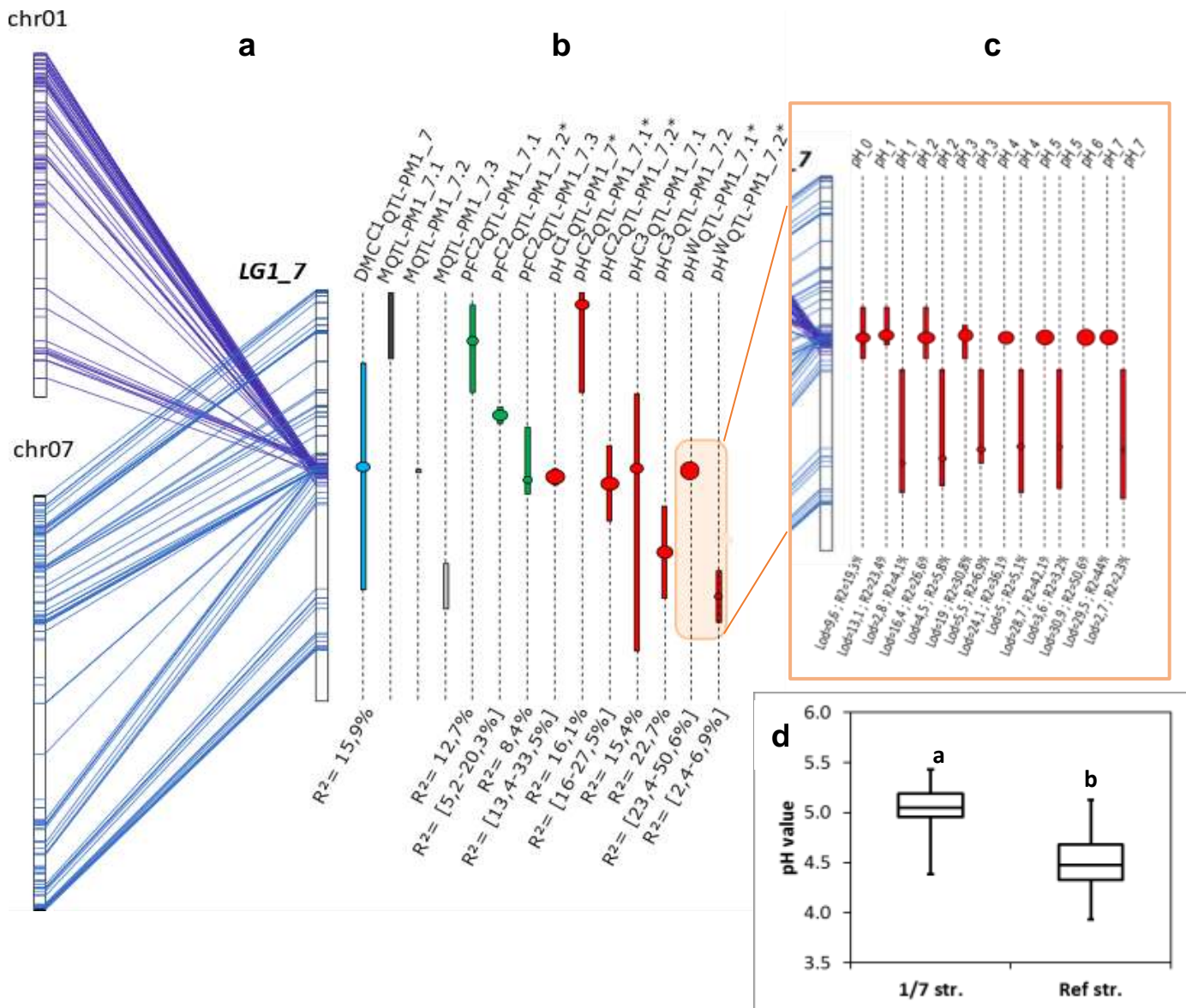


Fig. 2

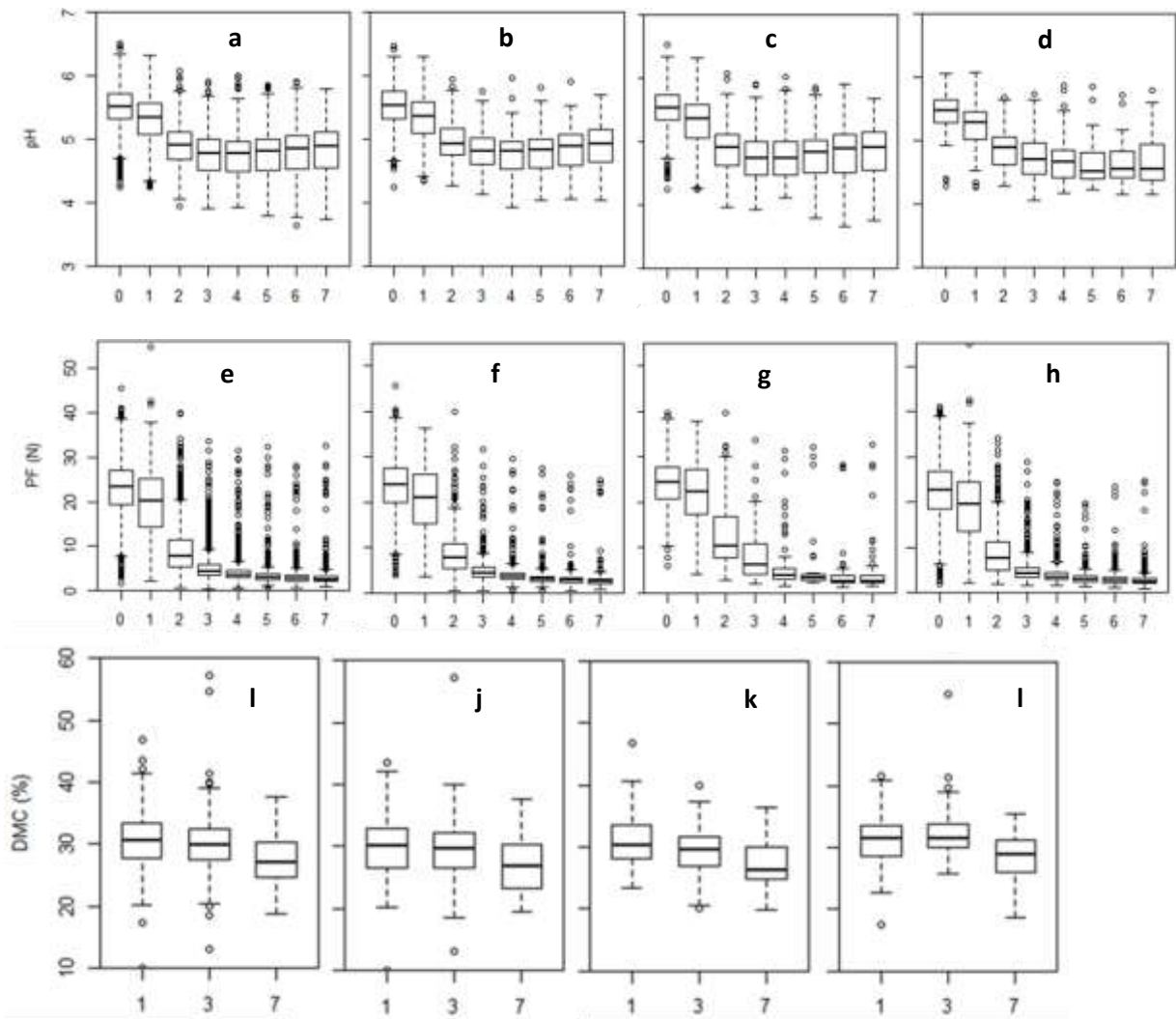


Fig. 3

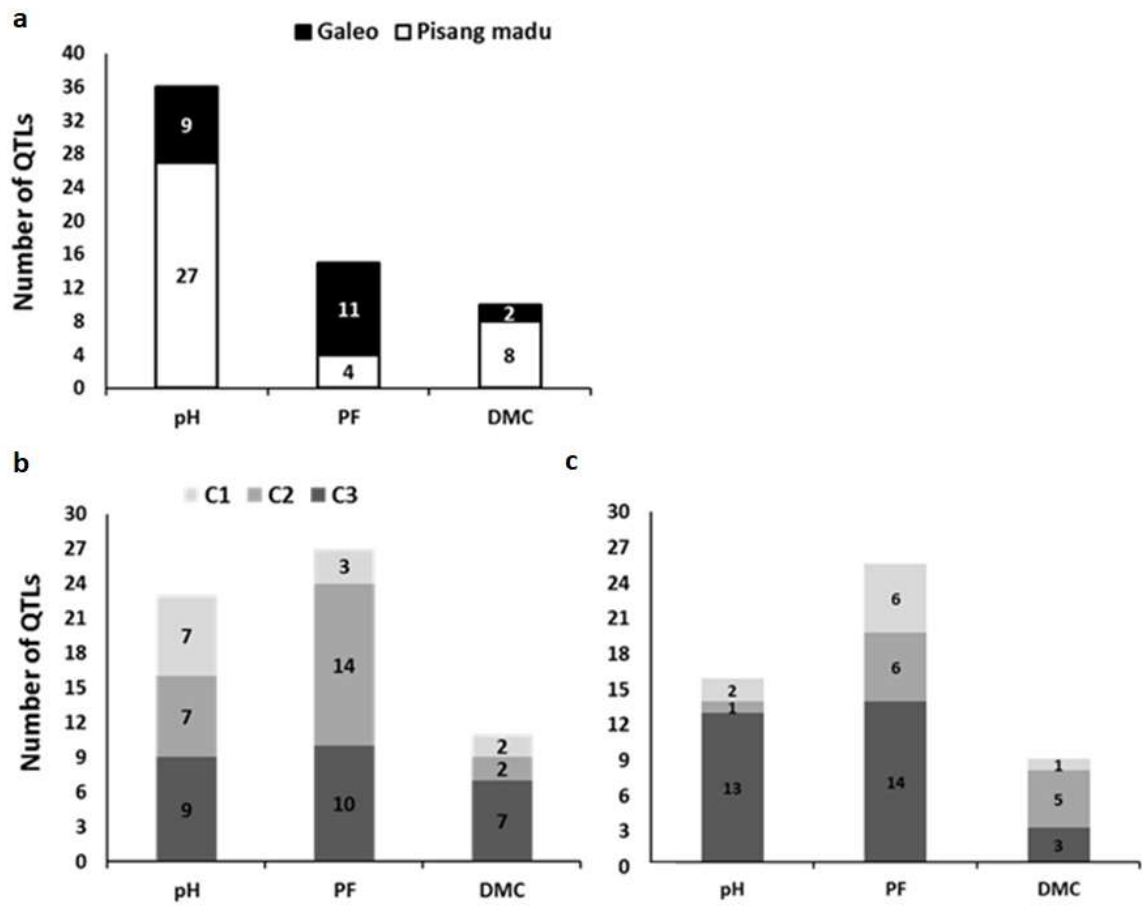
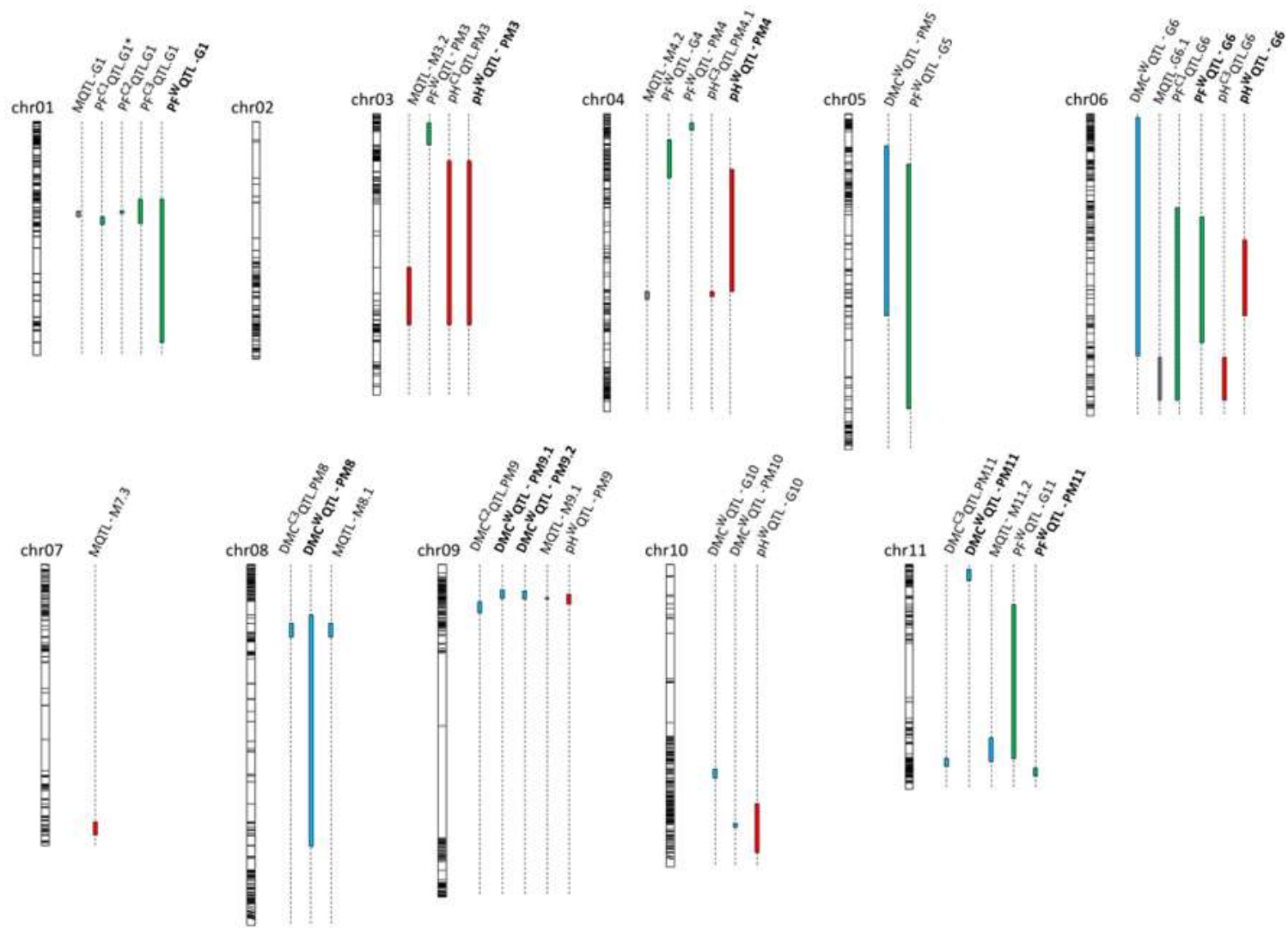




Fig. 4



**Table 1:** Variance decomposition for pH, pulp firmness (PF) and pulp dry matter content (DMC) in the studied progeny, and estimated generalized heritability (Cullis and al., 2006). The proportion of variance explained (PVE, in %) by each factor, i.e. progeny individuals, block, production cycle, interactions between two factors, and residuals were calculated as the percentage of the corresponding sum of squares of each variable to the total sum of squares.

	pH	PF	DMC
Individuals	39.0***	12.2***	61.3***
Block	6.3	0.4	1.5
Cycle	3.2	3.9	0.0
Hybrid*Day	14.5***	23.4***	7.5***
Hybrid*Cycle	15.4***	18.5***	13.7***
Residual	21.6***	41.5***	16.0***
Generalized Heritability	0.77	0.46	0.81

\*P < 0.005; \*\*P < 0.01; \*\*\*P < 0.001

**Table 2:** Correlation between quality traits, i.e. pulp pH, firmness (PF) and dry matter content (DMC) at the various studied fruit ripening days

	<b>day 0</b>	<b>day 1</b>		<b>day 2</b>	<b>day 3</b>		<b>day 4</b>	<b>day 5</b>	<b>day 6</b>	<b>day 7</b>	
	<b>PF</b>	<b>PF</b>	<b>pH</b>	<b>PF</b>	<b>PF</b>	<b>pH</b>	<b>PF</b>	<b>PF</b>	<b>PF</b>	<b>PF</b>	<b>pH</b>
<b>pH</b>	0.32***	0.48***	na	0.45***	0.48***	na	0.35***	0.3***	0.31***	0.23***	na
<b>DMC</b>	na	0.39***	0.33***	na	0.3***	0.24***	na	na	na	0.36***	0.09

\*\*\*P < 0.001

**Table 3:** Description of QTLs detected in both QTL mapping approaches and the meta-analysis, and QTLs from the first approach (whole dataset) with an R<sup>2</sup> higher than 15%. The day of fruit ripening, trait variability explained by the QTL (R<sup>2</sup>), position (Pos), identification (ID), including the quality trait, i.e. pulp pH, firmness (PF) and the dry matter content (DMC), dataset, origin of linkage groups with Galeo (G) and Pisang Madu (PM), and candidate genes collocated with the QTLs are indicated.

Trait	QTL Mapping Approach 1				QTL Mapping Approach 2				Meta-analysis		Candidate genes	
	QTL ID	Day	R <sup>2</sup>	Pos	QTL ID	Day	R <sup>2</sup>	Pos	MQTLs	Pos		Quality traits
pH					pH <sup>C1</sup> QTL-PM1_7	0567	[13.39-33.47]	56.85				
	pH <sup>W</sup> QTL-PM1_7.1	01234567	[19.27-50.64]	54.94	pH <sup>C2</sup> QTL-PM1_7.2	567	[16 - 27.5]	58.85	MQTL-PM1_7.2	55.05	pH, PF, DMC	
					pH <sup>C3</sup> QTL-PM1_7.1	7	15.4	54.2				
	pH <sup>W</sup> QTL-PM1_7.2	0123457	[3.17-6.92]	93.85	pH <sup>C3</sup> QTL-PM1_7.2	0	22.74	80	MQTL-PM1_7.3	90.53	pH	Aco; GAD; Mal; POX; PEPCK; PEPC
	pH <sup>W</sup> QTL-PM3	0	8.96	64.00	pH <sup>C1</sup> QTL-PM3	0	13.47	65	MQTL-PM3.2	64.66	pH	Aco; POX; AG; V-ATPase
	pH <sup>W</sup> QTL-PM4	4567	[2.25-9.13]	68.24	pH <sup>C3</sup> QTL-PM4.1	5	18.21	78	MQTL-PM4.2	78.01	pH, PF	Aco; PEPC; AuxREs
	pH <sup>W</sup> QTL-G6	01234567	[5.56-14.41]	111.9	pH <sup>C3</sup> QTL-G6	3	24.52	152				
PF					PF <sup>C1</sup> QTL-G1*	67	[5.48 - 11.79]	82.42				
	PF <sup>W</sup> QTL-G1	36	[8.92-9.25]	82.68	PF <sup>C2</sup> QTL-G1	6	[9.28-15.46]	74.38	MQTL-G1	78.06	pH, PF	GAD; POX
					PF <sup>C3</sup> QTL-G1	3	9.97	72.06				
	PF <sup>W</sup> QTL-G6	155	[8.24-30.00]	113.84	PF <sup>C1</sup> QTL-G6	7	9.62	136	MQTL-G6.1	149.20		Aco; CW; CW organization; POX; Mal; PEPCK; PEPC
				PF <sup>W</sup> QTL-PM11	67	[14.19-17.8]	87.09					POX
DMC					DMC <sup>C3</sup> QTL-PM8	7	22.37	63.23	MQTL-PM8.1	63.94	DMC	Aco; CW
					DMC <sup>W</sup> QTL-PM9.1	13	[17.95-22.10]	11.18				
	DMC <sup>W</sup> QTL-PM9.2	7	26.12	16.00	DMC <sup>C2</sup> QTL-PM9	3	26.12	16	MQTL-PM9.1	14.92	pH, PF, DMC	CW
	DMC <sup>W</sup> QTL-PM11	3	13.49	50.00	DMC <sup>C3</sup> QTL-PM11	1	27.38	73	MQTL-PM11.2	69.19	DMC	CW; AT; ETR; Gibberellin; UDP; CW biogenesis

Abbreviations for candidate genes: Aco, aconitate hydratase activity; AuxREs, response to auxin; CW, cell wall; ETR, response to ethylene; GAD, Glu decarboxylase; Mal, malate metabolic process; PEPC, phosphoenolpyruvate carboxylase activity; PEPCK, phosphoenolpyruvate carboxykinase (ATP) activity; POX, peroxidase activity; V-ATPase, vacuolar proton-transporting V-type ATPase complex