

1 **Identification of genomic regions of dry bean (*Phaseolus vulgaris* L.) associated with**  
2 **agronomic and physiological traits under drought stressed and well-watered conditions**  
3 **using genome-wide association study**

4

5

6 **Bruce Mutari<sup>1,2\*</sup>, Julia Sibiyi<sup>1</sup>, Admire Shayanowako<sup>1</sup>, Charity Chidzanga<sup>3</sup>, Prince M. Matova<sup>4</sup>, Edmore**  
7 **Gasura<sup>5</sup>**

8

9 **1** University of KwaZulu-Natal, School of Agricultural, Earth and Environmental Sciences, Scottsville,  
10 Pietermaritzburg, South Africa, **2** Crop Breeding Institute, Department of Research and Specialist Services,  
11 Harare, Zimbabwe, **3** School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, Australia,  
12 **4** Mukushi Seeds (Pvt) Ltd, Mt Humpden, Harare, Zimbabwe, **5** University of Zimbabwe, Mt Pleasant, Harare,  
13 Zimbabwe

14

15 \*brucemutari@gmail.com (BM)

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

## 31 **Abstract**

32 Understanding the genetic basis of traits of economic importance under drought stress (DS)  
33 and well-watered (NS) conditions is important in enhancing genetic gains in dry beans  
34 (*Phaseolus vulgaris* L.). This research aims to: (i) identify markers associated with agronomic  
35 and physiological traits for drought tolerance and (ii) identify drought-related putative  
36 candidate genes within the mapped genomic regions. An Andean and Mesoamerican diversity  
37 panel (AMDP) comprising of 185 genotypes was screened in the field under drought stress  
38 (DS) and well-watered (NS) conditions for two successive seasons. Agronomic and  
39 physiological traits, *viz.*, days to 50% flowering (DFW), plant height (PH), days to  
40 physiological maturity (DPM), grain yield (GYD), 100-seed weight (SW), leaf temperature  
41 (LT), leaf chlorophyll content (LCC) and stomatal conductance (SC) were phenotyped.  
42 Principal component and association analysis were conducted using filtered 9370 Diversity  
43 Arrays Technology sequencing (DArTseq) markers. The mean PH, GYD, SW, DPM, LCC and  
44 SC of the AMDP was reduced by 12.1, 29.6, 10.3, 12.6, 28.5 and 62.0%, respectively under  
45 DS. Population structure analysis revealed two sub-populations, which correspond to the  
46 Andean and Mesoamerican gene pools. Markers explained 0.08 – 0.10, 0.22 – 0.23, 0.29 –  
47 0.32, 0.43 – 0.44, 0.65 – 0.66 and 0.69 – 0.70 of the total phenotypic variability ( $R^2$ ) for SC,  
48 LT, PH, GYD, SW and DFW, respectively under DS conditions. For NS,  $R^2$  varied from 0.08  
49 (LT) to 0.70 (DPM). Overall, 68 significant ( $p < 10^{-03}$ ) marker-trait associations (MTAs) and  
50 22 putative candidate genes were identified across DS and NS conditions. Most of the identified  
51 genes had known biological functions related to regulating the response to moisture stress. The  
52 findings provide new insights into the genetic architecture of moisture stress tolerance in  
53 common bean. The findings also provide potential candidate SNPs and putative genes that can  
54 be utilized in gene discovery and marker-assisted breeding for drought tolerance after  
55 validation.

56

57

58

59

60

61

## 62 Introduction

63 Common bean (*Phaseolus vulgaris* L.,  $2n = 2x = 22$ ) is one of the major pulse crops consumed  
64 worldwide with a relatively small diploid genome size of approximately 473 Mb [1]. It is a  
65 cheap source of proteins and important micronutrients such as iron (Fe) and zinc (Zn) for  
66 millions in many African and Latin American countries [2, 3]. Beebe et al. [4] reported that  
67 Sub-Saharan Africa (SSA) and Latin America produce the largest volume of common beans,  
68 representing more than 60% of the world's bean production. Common bean was subjected to  
69 two parallel domestication events on the American continent, resulting in two different primary  
70 gene pools namely the Andean and the Mesoamerican [5, 6]. The Andean gene pool originated  
71 from the Andes mountains of South America and consists of medium (25 - 40 g per 100 seeds)  
72 or large ( $\geq 40$  g per 100 seeds) seeded genotypes [7]. On the other hand, the Mesoamerican  
73 gene pool is native to Central America and Mexico, and comprises of small seeded genotypes  
74 ( $\leq 25$  g per 100 seeds). According to Bitocchi et al. [8], there is more genetic variation within  
75 the Mesoamerican gene pool compared to the Andean gene pool.

76 Common beans are notably sensitive to climatic and environmental variations. This is  
77 aggravated by the fact that most bean growing regions in the world experience different  
78 production constraints including intermittent and terminal drought stress which adversely  
79 affect grain yield [9–12]. As reported by Katungi et al. [13], 73% of common bean production  
80 in SSA occurs in environments which experience moderate to severe drought stress. Beebe et  
81 al. [4], Hoyos-Villegas et al. [14] and Valdisser et al. [15] reiterated that drought stress is the  
82 most important grain yield-limiting abiotic factor of dry bean worldwide. It is predicted from  
83 various climate models that the duration and frequency of droughts are expected to increase in  
84 SSA [16]. Drought stress reduces stomatal conductance, total chlorophyll content, leaf  
85 expansion, number of days to physiological maturity, seed yield and biomass, number of pods  
86 and seeds per plant, seed size and harvest index [17–22]. According to Asfaw et al. [23], severe  
87 drought stress can result in grain yield losses of up to 80%. In Zimbabwe, grain yield reductions  
88 of more than 50% were reported by Mutari et al. [24] under terminal drought stress.

89 As reported by Mutari et al. [25], bean farmers in Zimbabwe have been using different  
90 mitigation strategies to minimize grain yield losses due to terminal drought stress. These  
91 strategies include soil mulching, ridging, cultivating the soil to retain more moisture and  
92 reducing the area under the bean crop. However, host plant resistance is a more sustainable,  
93 environmentally friendly and labour saving technology for managing drought stress in common  
94 beans compared to the multiple cultural practices. For this reason, most dry bean breeding

95 programmes aim to introduce drought tolerance into new cultivars to address the needs and  
96 preferences of smallholder farmers in the face of climate change [26].

97 Several researchers have successfully used different types of deoxyribonucleic acid  
98 (DNA)-based marker systems in association mapping of complex traits in common beans. The  
99 most widely used marker systems include simple sequence repeats (SSRs; [27–29]), amplified  
100 fragment length polymorphisms (AFLPs; [28, 30]), single nucleotide polymorphisms (SNPs;  
101 [3, 14, 31–34]) and microarray based Diversity Arrays Technology (DArT; [15, 35]) markers.  
102 However, SNP markers are widely preferred in marker assisted selection (MAS), genetic  
103 diversity analyses, genomic selection, haplotype mapping, genome wide association studies  
104 (GWAS), linkage map construction and population genetics [36]. They are widely preferred  
105 because they exhibit high level of polymorphism and occur in abundance (cover the whole  
106 genome) as differences of individual nucleotides between individuals.

107 Understanding the underlying genetic architecture of agronomic and physiological  
108 traits under drought stress (DS) and well-watered (NS) conditions is a fundamental prerequisite  
109 for the genetic improvement of these traits in common beans using MAS. Thus, dissecting the  
110 genetic basis of multiple polygenic traits of economic importance such as drought tolerance  
111 with respect to the genomic regions and/or genes involved and their effects is important to  
112 improve genetic gains in breeding for superior grain yield in dry beans under DS and NS  
113 environments. This can be accomplished through complementary approaches such as GWAS  
114 and genomic prediction models [6]. Genome wide association study is a powerful tool for  
115 characterizing the genetic basis of quantitative traits, and identifying multiple candidate genes  
116 (marker alleles) associated with variation in quantitative traits (marker-trait associations;  
117 MTA) of interest in crop species using high density DNA markers at high level of genetic  
118 resolution [34, 37–41].

119 Genome wide association study is also known as association mapping (AM) or linkage  
120 disequilibrium (LD) mapping [42]. It is based on linkage LD and historical recombination  
121 events of alleles of detected quantitative trait loci (QTL) at relatively high level of genetic  
122 resolution due to high genetic variability in the diverse population such as landraces, elite  
123 breeding lines and improved cultivars [43, 44]. The historical recombination events would have  
124 naturally occurred during the evolution and domestication of the crop, and crop improvement  
125 (several generations) [33]. With GWAS, the mapping resolution is increased as a result of the  
126 high number of recombination events in the genetically diverse genotypes within the natural  
127 population [45]. Therefore, it is inexpensive and reduces research time (no need to develop a  
128 mapping population) with greater allele numbers. The identification of genomic regions and

129 diagnostic genetic markers associated with grain yield and yield-attributing traits under DS and  
130 NS conditions will facilitate trait introgression and marker assisted selection (MAS).

131 Genome wide association study has been successfully used to detect MTAs and QTLs  
132 in common bean. Several QTLs associated with disease and insect pest tolerance have been  
133 identified in dry bean [32, 46–50]. Similarly, MTAs were identified for drought tolerance traits  
134 in dry bean [14, 15, 51–53]. Also, MTAs were identified for nutritional composition-related  
135 traits [6, 33], symbiotic nitrogen fixation [54], cooking time [55] and photosynthetic traits [34,  
136 56] in dry bean. Genomic regions governing agronomic traits in DS and yield potential  
137 environments were also identified in dry bean [1, 6, 14, 34, 57]. Even though several significant  
138 MTAs were identified in previous GWAS studies for agronomic traits in DS environments, the  
139 use of very low thresholds ( $-\log_{10} p\text{-value} \geq 3.0$ ) in most of the studies in determining  
140 significant MTAs might have resulted in many false positives. In addition, despite the fact that  
141 several QTLs/MTAs associated with agronomic traits have been identified in dry bean, further  
142 genetic studies are required using different genetic backgrounds to reach a saturation point.  
143 Moreover, most of the reported putative genes for agronomic and physiological traits were  
144 detected under yield potential environments.

145 Additionally, some of the previous mapping studies [14, 17, 51, 58–61] conducted on  
146 agronomic and physiological traits used a small population size and a limited number of  
147 molecular markers. This resulted in QTL with low resolution or poor estimation of marker  
148 effects, making it difficult to make inferences on putative candidate genes correlated with the  
149 identified QTL. Moreover, some of the previously identified QTLs explained low total genetic  
150 variance [23], and were sometimes not stable across environments due to genotype by  
151 environment interaction (GEI) [52]. Thus their potential for MAS in developing genotypes that  
152 are tolerant to drought stress was inconclusive. Therefore, additional studies are required to  
153 dissect the genetic basis of agronomic and physiological traits in dry bean under DS and  
154 optimal environments for increased genetic gains. The objectives of this study were: (i) to  
155 identify single nucleotide polymorphism (SNP) markers significantly associated with  
156 agronomic and physiological traits for drought tolerance and; (ii) to identify drought-related  
157 putative candidate genes associated with traits within the mapped genomic regions.

158

## 159 **Materials and Methods**

### 160 **Description of the study location**

161 The field experiments (drought stress; DS and well-watered; NS) were conducted at the  
162 screening site for moisture stress tolerance located at Save Valley Experiment Station (SVES),  
163 Zimbabwe. The experiments were carried out during the 2019 and 2020 dry winter seasons  
164 (April – July). Save Valley Experiment Station is characterised by clay soils and is located in  
165 the drier lowveld region of Zimbabwe where dry beans are commercially produced during the  
166 dry winter season (Table 1). The research station receives an average annual rainfall of 450  
167 mm that is usually distributed between the months of December and April. In both seasons, no  
168 precipitation was received during the trial evaluation period. Historically, SVES presents few  
169 rainfall occurrences during the dry winter season [24]. Daily temperatures (°C) and relative  
170 humidity (%) were recorded with a digital weather station (Table 1) during the growing  
171 seasons. More details on the agro-ecological characteristics of SVES are outlined in Table 1.  
172

### 173 **Germplasm**

174 A total of 185 dry bean genotypes constituted the Andean and Mesoamerican diversity panel  
175 (AMDP). The AMDP comprised of landrace collections (25), released cultivars (18) and elite  
176 breeding lines (142) of different market classes such as sugars, calimas, small whites, large  
177 whites and large red kidneys (S1 Table). The genotypes were sourced from public and private  
178 breeding institutions located in different geographic regions. These included the Alliance of  
179 Bioversity International and International Center for Tropical Agriculture (ABC) in Colombia  
180 (87), ABC in Malawi (67), ABC in Uganda (18), Ethiopian Institute of Agricultural Research  
181 (EIAR) in Ethiopia (3), Crop Breeding Institute in Zimbabwe (6) and Seed-Co, also in  
182 Zimbabwe (4) (S1 Table).  
183

184 **Table 1. Geographic information system, monthly weather conditions and soil characteristics during the growing seasons at Save**  
 185 **Valley Experiment Station, Zimbabwe (April to July, 2019 and 2020).**

Parameter	2019 dry season				2020 dry season				
		April	May	June	July	April	May	June	July
Temperature (°C)	Max	33.00	29.00	28.00	30.00	31.00	28.50	27.00	32.00
	Min	9.00	9.50	10.00	12.00	11.50	8.00	8.5.00	12.50
Relative Humidity (%)	Max	82.00	95.00	69.00	91.00	74.00	85.00	69.00	71.00
	Min	42.00	56.00	44.00	25.00	46.00	59.00	50.00	30.00
Total Rainfall (mm)		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soil type		Clay				Clay			
Latitude		20°32'S				20°43'S			
Longitude		33°09'E				33°03'E			
Altitude (m.a.s.l)		452				449			

186  
 187 masl = meters above sea level, mm = millimetres, ppm = parts per million, Max = maximum and Min = minimum.  
 188

189

190

191

192

## 193 **Field phenotyping of the diversity panel**

### 194 **Experimental design, irrigation scheduling and trial management**

195 The AMDP was evaluated side by side under DS and NS treatment conditions during the 2019  
196 and 2020 dry winter seasons. In both seasons, the genotypes in both DS and NS treatments  
197 were established in a 5 x 37 alpha lattice design with two replications. The seepage of water  
198 from the NS treatment to the DS treatment was minimized by maintaining a 30 m buffer zone  
199 between the two treatments. Each genotype was hand planted in four-row plots of 3 m in length,  
200 and an inter-row spacing of 0.45 m. Compound D (N = 7%, P = 14%, K = 7%) was applied at  
201 planting at a rate of 300 kg/ha. Ammonium nitrate (34.5% N) was applied in both DS and NS  
202 treatments as a top-dressing fertilizer thirty days after emergence at a rate of 100 kg/ha. An  
203 overhead sprinkler irrigation system was used to irrigate both DS and NS treatments during  
204 both seasons of evaluation. The irrigation cycles in both DS and NS treatments were as  
205 described by Mutari et al. [24]. In both seasons, recommended agronomic practices were  
206 followed for the management and control of pests such as diseases, insects and weeds.

207

### 208 **Collection of data on agronomic and physiological traits**

209 At the flowering stage of growth, the number of days from planting to 50% flowering (DFW)  
210 were recorded in both treatments. The DFW was recorded when 50% of the plants in a plot had  
211 at least one or more open flowers. At mid-pod filling, leaf temperature (LT; °C), stomatal  
212 conductance (SC; mmol m<sup>-2</sup> s<sup>-1</sup>) and leaf chlorophyll (LCC) content were collected on all  
213 genotypes in both DS and NS treatments. The LT and SC data were recorded from the surface  
214 of the uppermost fully expanded young leaf between 11:00 am to 14:00 pm using a FLUKE  
215 precision infrared thermometer (Everest Interscience, Tucson, AZ, USA) and a hand-held leaf  
216 porometer (Decagon Devices®, Pullman, WA, USA), respectively. Three readings were  
217 collected on three different randomly chosen plants from each plot per replicate in both the DS  
218 and NS treatments. The three measurements were averaged to obtain one final reading per plot.  
219 Phenotyping for LT and SC was done for an average of six days on clear, sunny days with  
220 minimal wind. Regarding the LCC, this was measured using a soil and plant analysis  
221 development (SPAD) chlorophyll meter (SPAD-502*Plus*, Konica-Minolta, Osaka, Japan) on  
222 two fully developed leaves of three plants in each plot. Then, the average value was calculated.  
223 At physiological maturity, the following traits were recorded from the two inner rows from  
224 every plot for every genotype in both treatments and seasons: plant height (PH; cm), days from  
225 planting to physiological maturity (DPM), grain yield (GYD; kg/ha) and 100-seed weight (SW;



226 g). Plant height which was measured from the base of the plant (soil surface) to the top node  
227 bearing at least one dry pod with seed was averaged from three plants per plot. The DPM were  
228 recorded as the average number of days from planting to when 95% of pods in a plot lose their  
229 green colour. Grain yield was recorded from the two middle rows in each plot using a weighing  
230 scale, and converted to kilograms per hectare (kg/ha) at 12.5% moisture basis. The SW was  
231 determined using a beam balance weighing scale by measuring the weight of 100 seeds  
232 randomly from each plot harvest.

233

## 234 **Statistical analysis of phenotypic data**

235 Before conducting analysis of variance, normality tests were conducted in Genstat® Discovery  
236 18th Edition [62] using residuals of the agronomic and physiological traits. The agronomic and  
237 physiological traits were analysed in Genstat® Discovery 18th Edition [62] using mixed  
238 models from which the best linear unbiased predictors (BLUPs) were obtained. The BLUPs  
239 were estimated for the studied traits to minimize the environmental and seasonal effects. The  
240 BLUPs for each entry were estimated through individual environment (DS or NS) analysis,  
241 and by combined analysis (across water regimes). In the first step of analysis (single-  
242 environment analysis), the phenotypic data of each individual environment were analysed  
243 using a mixed linear model (MLM). In this model, blocks and genotypes were treated as  
244 random effects, and replications were considered as fixed effects. Genotype effects were  
245 declared to be random to enable the calculation of BLUPs and broad-sense heritability ( $H^2$ ).  
246 The MLM presented below was fitted:

247

$$248 \quad Y_{ijl} = \mu + g_i + r_j + b_{lj} + e_{ijl} \quad (1)$$

249

250 where  $Y_{ijl}$  = is the phenotypic observation of the genotype  $i$  in replicate  $j$  in block  $l$  within  
251 replicate  $j$ ,  $\mu$  = grand mean effect,  $g_i$  = random effect associated with genotype  $i$ ,  $r_j$  = fixed  
252 effect associated with replicate  $j$ ,  $b_{lj}$  = random effect associated with block  $l$  nested within  
253 replicate  $j$ , and  $e_{ijl}$  = residual effect associated with observation  $ijl$ . For a combined or multi-  
254 environment analysis, a MLM was used. In this model, blocks nested within replications,  
255 replicates nested within environments, genotypes and their interactions with environments  
256 (GEI) were considered as random effects. Environments, defined as year x water regime  
257 combination were considered as fixed effects. The MLM presented below was fitted:

258

$$259 \quad Y_{ijkl} = \mu + G_i + E_j + R_{k[j]} + B_{l[jk]} + GE_{ij} + e_{ijkl} \quad (2)$$

260 where  $Y_{ijkl}$  = effect of genotype  $i$  in environment  $j$  and  $k$ th replication within environment  $j$  and  
261  $l$ th block nested within replicate  $k$  and environment  $j$ ,  $\mu$  = grand mean,  $G_i$  = random effect of  
262 the  $i$ th genotype,  $E_j$  = fixed effect of the  $j$ th environment,  $R_{k[j]}$  = random effect associated with  
263 the replicate  $k$  nested within environment  $j$ ,  $B_{l[jk]}$  = random effect of block  $l$  nested within  
264 environment  $j$  and replicate  $k$ ,  $GE_{ij}$  = random effect of the interaction between genotype  $i$  and  
265 environment  $j$ , and  $e_{ijkl}$  = random error associated with observation  $ijkl$ . The analysis was  
266 performed using the Restricted Maximum Likelihood (REML) method implemented in  
267 GenStat 18th edition [62]. Broad-sense heritability estimates for the agronomic and  
268 physiological traits were calculated following the formula proposed by Cullis et al. [63].  
269 Heritability was classified as low when less than 30 %, moderate when between 30-60 % and  
270 high when more 60 % [64]. Drought intensity index (DII) at the location, percentage GYD  
271 reduction (%GYR) due to DS, drought susceptibility index (DSI), geometric mean productivity  
272 (GMP) and drought tolerance index (DTI) of each entry were calculated as described by Mutari  
273 et al. [24]. A ranking method was used to select superior drought tolerant genotypes by  
274 calculating the mean rank of each genotype across all the studied indices.

275

## 276 **Genotyping of the diversity panel**

277 Genomic DNA of the 185 genotypes was extracted from young leaves of 2-week old bean  
278 plants following the plant deoxyribonucleic acid (DNA) extraction protocol for Diversity  
279 Arrays Technology (DArT; [65]). A NanoDrop Spectrophotometer (ND-8000, NanoDrop  
280 Technologies, Inc.) was used to determine the concentration of the DNA. The agarose gel (1%  
281 agarose gel) electrophoresis was used to evaluate the quality of the DNA. The DNA from the  
282 samples used in this study were genotyped using the Diversity Arrays Technology Sequencing  
283 (DArTseq) protocol using a set of 24,450 silico DArT markers. The DArT markers used were  
284 evenly distributed across all 11 chromosomes of common bean. Genotyping by sequencing  
285 (GBS) was done at the Biosciences Eastern and Central Africa (BecA) Hub of the International  
286 Livestock Research Institute (BecA-ILRI) in Kenya. The silico DArTs used had polymorphic  
287 information content (PIC) values ranging from 0.01 to 0.50, reproducibility values of 1.00, and  
288 the proportion of missing data per marker was 7% (mean call rate of 93%, ranging from 81 to  
289 100%). The entire data set of SNP markers was filtered in TASSEL v5.2 [66] to remove SNP  
290 loci with unknown physical positions on the common bean genome, monomorphic SNPs, and  
291 SNP markers with more than 20% missing data and minor allele frequency (MAF) of less than  
292 5% (<0.05) threshold [15, 49, 67]. A final total of 9370 (38%) DArTseq SNPs distributed

293 across the 11 chromosomes were retained after filtering for use in association analysis and  
294 population structure analysis via principal component analysis (PCA).

295

## 296 **Inference of population structure**

297 The genotypic data was imputed for missing alleles of SNPs on the KDCCompute online sever  
298 (<https://kdcompute.igs-africa.org/kdcompute/>) using the optimal imputation algorithm to  
299 increase the power of the study. KDCCompute was also used to graphically visualize the  
300 distribution of SNPs across the common bean genome. The population genetic structure was  
301 determined based on the Bayesian model-based clustering approach using the Bayesian  
302 inference program in STRUCTURE software version 2.3.4 [68]. A subset of additionally  
303 filtered SNP markers (4095) at or near Hardy-Weinberg equilibrium ( $r^2 < 0.8$ ) and that covered  
304 the entire genome were used in population structure analysis with STRUCTURE [14, 15, 31].  
305 This was done to reduce the background and admixture linkage disequilibrium (LD) owing to  
306 linked loci [68].

307 Settings for the STRUCTURE program were set as follows to derive the population  
308 structure: a burn-in period length of 10,000, and after burn-in, 10,000 Markov Chain-Monte  
309 Carlo (MCMC) repetitions. The number of sub-populations or clusters (K) was set from 1 to  
310 10, with ten independent runs for each K [3, 48, 55]. The best K-value explaining the population  
311 structure was inferred using the Delta K ( $\Delta K$ ) method in Evanno et al. [69] implemented in the  
312 on-line tool structure harvester software [70]. Genotypes with ancestry probability/coefficient  
313  $\geq 0.90$  ( $\geq 90\%$ ) (pure genotypes) for the Andean sub-population were allocated to the Andean  
314 gene pool [31, 71] (S1 Table). On the other hand, genotypes with ancestry probability  $\geq 0.90$   
315 for the Mesoamerican sub-population were allocated to the Mesoamerican gene pool. Those  
316 with ancestry probability  $< 0.90$  were considered as admixed [71]. The clustering of the AMDP  
317 was further assessed and visualized in a 3D scatter plot using PCA in prcomp R 3.0 function  
318 [72].

319

## 320 **Marker-trait association tests and linkage disequilibrium analyses**

321 The filtered 9370 SNPs and the adjusted trait means (BLUPs) for each of the environments  
322 (DS and NS) were used as input data in marker-trait association (MTA) analysis. The more  
323 conservative compressed mixed linear model (CMLM) procedure in the genome association  
324 and prediction integrated tool (GAPIT) (v3) program of R software was used to determine the  
325 MTAs following the  $Q + K$  model according to Lipka et al. [73]. *Phaseolus vulgaris* is

326 characterised by a strong genetic structure necessitating the need to use the Q + K model [74].  
327 The CMLM incorporated both the population structure ( $Q$ ; fixed effect) and kinship ( $K$ ; random  
328 effect) matrices as covariates to correct the population structure, increase statistical power of  
329 the analysis and minimize false positives (spurious MTAs) [67, 72, 75]. The K matrix was  
330 included in the association analysis to correct for cryptic relatedness within the AMDP [54,  
331 67]. The threshold for significant MTA was set at  $p < 0.001$  to reduce the risk of false MTAs.

332 The Manhattan plots drawn using the CMplot package in R 3.5.3 were used to visualise  
333 the significant MTAs for each environment. The p-values were plotted as  $-\log_{10}(p)$  to generate  
334 the Quantile-Quantile (Q-Q) and Manhattan plots using the CMplot package in R package [76].  
335 The Q-Q plots were produced from the observed and expected logarithm of the odds (LOD)  
336 scores for each trait. The LD Heatmap package in R 3.0 was used to generate the LD Heatmaps  
337 for the significant markers of each trait [77, 78]. Alleles with positive additive effects resulting  
338 in higher values of GYD, SZ and LCC were described as “superior alleles” under both DS and  
339 NS conditions, whereas alleles resulting in decreased GYD, SZ, and LCC were “inferior  
340 alleles”. On the other hand, alleles with negative effects resulting in lower values of DFW,  
341 DPM, LT and SC were considered to be “superior alleles” under DS conditions. The Jbrowse  
342 feature on Phytozome v13 was used to browse the *P. vulgaris* G19833 v2.1 reference genome  
343 sequence [1] to gain insight into potential putative candidate genes associated with significant  
344 SNPs for each trait. The functional annotation of the gene was checked on Phytozome v13  
345 website (<http://phytozome.net>) to postulate the role of the gene in the control of a target trait.

346

## 347 **Putative candidate gene prediction**

348 Plausible candidate genes were identified based on the window size of 200 kb (maximum  $\pm$   
349 100 kb) on either side (upstream and downstream) of the significant marker [74, 79]. The  
350 window size of 200 kb is the average LD [74, 79]. A gene was considered a potential candidate  
351 using the following criteria: (i) if the gene contained a significant SNP or the gene contained a  
352 SNP that was in LD with a significant SNP [3], and (ii) if the gene had a known role related to  
353 regulating moisture stress response and plant growth and development under water deficit  
354 based on gene ontology term descriptions in Phytozome v13. For the positional candidate genes  
355 that did not have adequate functional annotation information on Phytozome v13, the sequence  
356 data of the significant SNP was used against NCBI database using the basic local alignment  
357 search tool for nucleotide (BLASTn; <https://blast.ncbi.nlm.nih.gov/blast/blast.cgi>).

358

## 359 **Results**

360

### 361 **Variations of agronomic and physiological traits under two water** 362 **regimes**

363 The descriptive statistics and  $H^2$  estimates for the agronomic and physiological traits under DS  
364 and NS environments are shown in Table 2. Residual maximum likelihood analysis revealed  
365 highly significant ( $p < 0.001$ ) genotypic main effects on all the studied traits under both DS  
366 and NS environments supporting the use of the AMDP for GWAS purposes. Overall,  
367 phenotypic variability was observed among the genotypes for DFW, LCC, LT, SC, PH, DPM,  
368 GYD and SW under DS and NS conditions. High  $H^2$  estimates (0.83 - 0.97) were observed for  
369 all the studied traits under DS, except for SC ( $H^2 = 0.32$ ), LT ( $H^2 = 0.46$ ), and LCC ( $H^2 = 0.54$ ).  
370 Under NS conditions, high  $H^2$  estimates (0.88 – 0.98) were observed for all the traits except  
371 for LCC ( $H^2 = 0.14$ ), SC ( $H^2 = 0.33$ ), and LT ( $H^2 = 0.42$ ).

372 In general, the observed  $H^2$  estimates under both environments revealed that much of  
373 the observed phenotypic variation was due to the genetic component, supporting the suitability  
374 of the AMDP for GWAS studies. Grain yield was highest under NS (1016 kg/ha;  $H^2 = 0.88$ ),  
375 and lower under DS (715 kg/ha;  $H^2 = 0.92$ ). The SW also varied among the environments at  
376 34.98 g/100 seeds ( $H^2 = 0.97$ ), and 31.39 g/100 seeds ( $H^2 = 0.97$ ) under NS and DS,  
377 respectively. The AMDP had a shorter duration (lower values) under DS (DPM = 90.97 days),  
378 compared to NS (DPM = 104.10 days). The same trend was observed for PH, LCC, and SC.  
379 On the other hand, LT was lower (19.75 °C) under NS environments, compared to DS  
380 environments (25.22 °C). Under DS, GYD ranged from 39.4 kg/ha to 2134 kg/ha, and exhibited  
381 a narrower range than in NS where GYD ranged from 55.0 kg/ha to 2586.0 kg/ha. The  
382 coefficient of variation (CV) ranged from 5.32 to 5.58%, 2.55 to 3.71%, 33.68 to 36.57%, 12.45  
383 to 16.91%, 22.53 to 28.13%, 16.21 to 16.62%, 7.12 to 9.42%, and 18.90 to 35.93% for DF,  
384 DPM, GYD, SW, PH, LCC, LT, and SC, respectively. Low standard deviations (SD) were  
385 observed for LT and LCC under both environments.

386

387

388 **Table 1. Phenotypic summary statistics, coefficient of variation and broad-sense heritability of the measured traits for all the 185**  
 389 **dry bean genotypes based on the best liner unbiased prediction (BLUP) value grown under drought stressed and non-stressed**  
 390 **conditions.**

Traits	Treatment												AC
	Drought Stress						No stress						
	Average	SD	Range	Wald statistic (genotype)	CV (%)	$H^2$	Average	SD	Range	Wald statistic (genotype)	CV (%)	$H^2$	
DFW	43.32	7.11	32-60	139.64***	5.58	0.96	41.28	5.69	32.50-60.00	95.66***	5.32	0.98	0.94
DPM	90.97	8.40	71.50-106	187.89***	3.71	0.85	104.10	9.31	83.50-120.20	210.74***	2.55	0.94	0.93
GYP	715.40	457.80	39.4-2134	600772.00***	36.57	0.92	1016.00	555.00	55.00-2586.00	797047.00***	33.68	0.88	0.92
SW	31.39	11.61	14.25-60.00	420.66***	16.91	0.97	34.98	12.27	16.75-65.00	463.60***	12.45	0.97	0.98
PH	50.05	16.75	25.25-102.2	963.70***	28.13	0.83	56.97	18.46	28.5-125.00	1145.90***	22.53	0.92	0.88
LCC	31.12	3.80	18.17-44.15	53.51***	16.62	0.54	43.55	4.46	33.10-62.43	78.08***	16.21	0.14	0.35
LT	25.22	2.59	16.85-30.95	29.29***	9.42	0.46	19.76	1.15	17.23-24.90	4.78***	7.12	0.42	0.37
SC	96.66	13.97	59.38-141.4	760.10***	18.90	0.32	254.50	75.69	64.00-465.00	23883.00***	35.93	0.33	0.24

391 AC = across environments (drought stress and well-watered), SD = standard deviation of the trait means, CV = coefficient of variation,  $H^2$  = broad-sense heritability, DFW =  
 392 days to flowering, DPM = days to physiological maturity, GYP = grain yield (kg/ha), SW = 100 seed weight (g), PH = plant height (cm), LCC = leaf chlorophyll content, LT  
 393 = leaf temperature (°C), SC = stomatal conductance (mmol m<sup>-2</sup> s<sup>-1</sup>), \* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$  and \*\*\* =  $p \leq 0.001$ .

394 Combined GYD data over two seasons across environments revealed that the highest yielding  
 395 genotype was G184 (DAB91 - 2222,7 kg/ha) followed by G176 (DAB302 – 2097.5 kg/ha) and  
 396 G147 (CIM-SUG07-ALS-S1-3 - 2080,1 kg/ha) (Table 3).

397

398 **Table 2. Drought tolerance indices and predicted genotype values for grain yield**  
 399 **(across environments) of top 20 drought tolerant genotypes.**

Genotype	Gene pool	GYD (kg/ha)	DSI	GMP	DTI	%GYR	Mean rank
G184	Andean	2222.7	0.16	2205.3	4.74	4.69	25.5
G176	Andean	2097.5	0.35	2084.0	4.25	10.60	29.9
G147	Andean	2080.1	1.26	1995.6	4.03	37.83	66.3
G146	Andean	2067.4	1.18	1994.5	4.02	35.49	61.5
G158	Admixed	2017.1	-0.01	1979.5	3.82	-0.26	24.5
G135	Andean	1968.7	-0.37	1956.9	3.75	-11.17	19.8
G101	Andean	1964.8	1.32	1890.6	3.78	39.72	69.3
G138	Andean	1846.8	0.22	1826.1	3.25	6.50	31.0
G180	Andean	1838.9	0.09	1789.6	3.13	2.57	29.5
G162	Andean	1828.7	0.57	1814.3	3.20	16.97	40.5
G124	Andean	1815.0	-0.03	1805.5	3.19	-0.78	26.0
G173	Andean	1792.6	0.68	1780.4	3.07	20.40	46.0
G115	Andean	1788.9	0.40	1765.4	3.04	11.87	35.5
G150	Andean	1758.8	0.40	1750.8	2.98	12.03	36.5
G127	Andean	1750.0	-0.02	1733.0	2.94	-0.73	29.0
G159	Andean	1743.3	1.12	1694.7	2.90	33.67	65.3
G113	Andean	1683.8	1.76	1548.0	2.35	52.77	91.5
G125	Andean	1628.2	-0.25	1590.2	2.49	-7.39	26.8
G181	Andean	1614.1	1.03	1585.2	2.44	31.00	64.5
G104	Andean	1608.8	0.58	1601.1	2.49	17.37	45.3

400 GYD = grain yield, DSI = drought susceptibility index, GMP = geometric mean productivity, DTI = drought  
 401 tolerance index and %GYR = percent grain yield reduction. Note: Mean Rank is the mean rank of a genotype  
 402 across all the drought tolerance indices. Admixed includes genotypes that are 10 to 90% Andean or Mesoamerican  
 403 according to the structure analysis results.

404

405 The drought tolerance indices for the 185 genotypes based on mean GYD are summarised in  
 406 Table 3 (top 20 drought tolerant genotypes) and S2 Table (all study genotypes). The severity  
 407 of DS at SVES across the 2 seasons of evaluation was moderate (DII of 0.30). Among the  
 408 evaluated genotypes, G158 (SWEET WILLIAM/DAB287), G135 (DAB539), G124  
 409 (DAB487), G127 (CIM-SUG07-ALS-2), G125 (CIM-RM09-ALS-BSM-12), G138 (CZ104-

410 72) and G184 are some of the genotypes that were less sensitive to DS based on their low DSI,  
411 %GYR and overall mean ranks across the indices. These genotypes had DSI values ranging  
412 from -0.37 (G135) to 0.16 (G184) and %GYR ranging from -11.17 (G135) to 4.69 (G184). In  
413 summary, all the top 20 drought tolerant genotypes were members of the Andean gene pool,  
414 except for G158 which is an admixture (Table 3).

415

## 416 **Population structure analysis**

417 The STRUCTURE analysis results and Evanno test ( $\Delta K$ ) revealed the presence of two major  
418 sub-populations (highest  $\Delta K$  value occurred at  $K = 2$ ) within the AMDP of dry bean (Figs 1A,  
419 1B). The two sub-populations correspond to the Andean and Mesoamerican domesticated gene  
420 pools. The minimum ancestry or membership coefficient to a particular cluster was 0.63 (Fig  
421 1B and S1 Table). Most of the genotypes (90) clustered within the Mesoamerican gene pool  
422 (Fig 1B). Seventy-six genotypes clustered within the Andean gene pool (Fig 1B and S1 Table).  
423 On the other hand, 19 were Andean-Mesoamerican admixed genotypes of the two gene pools  
424 (10 to 90% Andean or Mesoamerican). The admixed genotypes included SMC16, SMC21,  
425 NUA674, NUA59-4, G75, DAB115, DAB63, DAB142, DAB477, CIM-RM02-36-1, CIM-  
426 RM09-ALS-BSM-11, CIM-RM02-134-1, Sweet William, ZABRA16575-60F22,  
427 GLP585/MLB49-89A-3, RWR2154, SAB792, NAVY LINE 22, and CIM-SUG07-ALS-S1-3  
428 (S1 Table).

429

430 **Fig 1. Population structure of 185 Andean and Mesoamerican Diversity Panel (AMDP) from different**  
431 **models.** Note: A = The  $\Delta K$  determined by the Evanno method showing the stratification of the 185 AMDP into  
432 two main sub-populations. The cluster with the largest  $\Delta K$  ( $K = 2$ ) was used to determine the number of sub-  
433 populations in the AMDP of dry bean and the existence of two-sub-populations was inferred; B = Population  
434 structure of 185 AMDP of dry bean genotypes based on 4095 SNP markers ( $K = 2$  gives the best separation) as  
435 determined from STRUCTURE analysis. Red and green represents Andean and Mesoamerican sub-populations,  
436 respectively; C = Three dimensional principal component analysis (PCA) scatter plot illustrating the population  
437 structure of 185 AMDP of dry bean genotypes based on 9370 SNP markers; D = Screen plot showing the  
438 percentage of variation explained by the different principal components.

439 The genetic structure result of the AMDP was verified with the PCA based on SNP marker  
440 data and is illustrated by a 3D scatter plot (Fig 1C). The first principal component (PC)  
441 accounted for more than 55% of the observed genotypic variability in the AMDP, while the  
442 second and third PCs separately accounted for less than 5% of the overall genetic variance in  
443 the AMDP (Fig 1D). The PCA also divided the genotypes into two distinct clusters (Andean



444 and Mesoamerican sub-populations) as were found with STRUCTURE output (Fig 1C).  
445 Further, the Andean-Mesoamerican admixed genotypes (positioned between the two groups)  
446 were isolated from the Andean and Mesoamerican sub-groups by PCA (Fig 1C).

447

## 448 **Analysis of marker-trait associations under drought stressed** 449 **conditions**

450 The significant MTAs and their respective statistical parameters for agronomic and  
451 physiological traits are summarised in Table 4. In this study, the threshold for significant MTA  
452 was set at  $p < 0.001$  to reduce the risk of false MTAs. Under DS conditions, 29 significant  
453 MTAs were identified for six traits (excluding DPM and LCC) with  $p < 10^{-03}$ . The associations  
454 are shown in Fig 2. The quantile-quantile (QQ) plots for the studied traits revealed that the  
455 expected and observed probability values were normally distributed (S3 Fig). The highest  
456 number of significant MTAs were observed on *P. vulgaris* (*Pv*) chromosome *Pv11* (28%),  
457 followed by *Pv8* (17%), with the least on chromosomes *Pv6* and *Pv4*, both with 3%. No  
458 significant associations for DPM and LCC were identified under DS conditions in this study.  
459 The highest number of significant MTAs were identified for PH (15), and the SNPs were  
460 distributed across six different chromosomes (*Pv1*, *Pv5*, *Pv7*, *Pv8*, *Pv10*, and *Pv11*).  
461 Additionally, the allele effect of these SNPs ranged from -16.03 cm (SNP 8198531) to 17.82  
462 cm (SNP 100101387).

463

464 **Fig 2. Manhattan plots indicating the significant marker-trait associations, their p-values and candidate**  
465 **genes for agronomic and physiological traits in 185 dry bean genotypes evaluated under drought stressed**  
466 **conditions.** Note: A = Grain yield, B = Seed size, C = Days to 50% flowering, D = Plant height, E = Leaf  
467 temperature, F = Stomatal conductance. \*Chr represents Chromosome, x-axis represents the physical map  
468 locations of the SNPs on each chromosome and the y-axis ( $-\log_{10}$  p-values) represents the degree to which a  
469 SNP is associated with a trait.

470

471 Four SNPs (SNPs 2362591, 2362591, 45231105, and 40802478) that have a significant  
472 association with GYD were also identified, and these were located on chromosomes *Pv4* and  
473 *Pv11*, with allele effect ranging from -174.56 kg/ha (SNP 3381526) to 202.90 kg/ha (SNP  
474 3382688). Notably, 75% of the SNPs that were significantly associated with GYD were located  
475 on chromosome *Pv11*. The sum of the SNPs with a significant positive effect on GYD was  
476 341,88 kg/ha and -351,23 kg/ha for all the SNPs with a significant negative effect on GYD  
477 (Table 4). For SW, two SNPs that were significantly associated with this trait were identified

478 on chromosomes *Pv03* and *Pv08*, with allelic effects ranging from -2.41 g per 100 seeds (SNP  
479 3383047) to 4.46 g per 100 seeds (SNP 16647170). Regarding physiological traits, SNPs were  
480 identified that have a significant association with LT distributed across two chromosomes (*Pv6*  
481 and *Pv8*), with allele effect ranging from -1.23°C (SNP 100065202) to 1.34°C (SNP  
482 100106140).

483

484 **Table 3. Single nucleotide polymorphism (SNP) markers associated with agronomic**  
485 **and physiological traits in dry bean genotypes under drought stress conditions.**

Phenotype	SNP name	CH	SNP position on genome (bp)	MAF	Allele	Effect of allele	-log <sub>10</sub> (P) value	R <sup>2</sup>	Candidate gene
LT	100106140	06	14389438	0.25	A/C	1.34	0.000	0.23	
	100065202	08	52504423	0.12	G/A	-1.43	0.000	0.22	
DFW	100132383	03	47240686	0.04	A/G	3.76	0.000	0.70	
	3381050	02	25978891	0.03	C/T	3.85	0.000	0.70	Phvul.002G122100
	8204238	10	42089084	0.06	A/G	2.78	0.000	0.70	
	8212194	10	42105474	0.04	A/T	3.54	0.000	0.69	
GYD	3384334	11	2362591	0.1	A/G	-176.67	0.000	0.44	
	3381526	11	2362591	0.09	A/G	-174.56	0.000	0.44	
	3382688	04	45231105	0.09	G/A	202.90	0.000	0.43	Phvul.004G150500
	100061855	11	40802478	0.42	T/G	138.98	0.000	0.43	
PH	100101387	05	34925013	0.03	G/A	17.82	0.000	0.32	
	8198531	07	9701750	0.04	G/A	-16.03	0.000	0.31	
	100060987	01	42938094	0.04	G/A	15.57	0.000	0.31	Phvul.001G172300
	100181735	08	22152034	0.23	G/A	7.57	0.000	0.30	Phvul.008G133100
	3379684	07	5239949	0.22	T/A	-5.62	0.000	0.30	
	16650827	07	51719432	0.09	T/C	-8.41	0.000	0.30	
	3380814	11	11934462	0.11	C/T	7.15	0.000	0.30	
	100119463	08	6003908	0.03	G/A	15.56	0.000	0.30	Phvul.008G065700
	8196298	11	12212674	0.12	T/G	-0.67	0.000	0.30	
	3379078	08	7823952	0.02	C/G	17.75	0.000	0.30	Phvul.008G080600
	3377272	11	9410740	0.16	T/C	-6.15	0.000	0.29	
	3379350	11	43494132	0.16	C/T	6.35	0.000	0.29	
	100063156	10	7307165	0.44	T/C	4.85	0.000	0.29	
	3379405	05	4782514	0.24	G/A	-7.80	0.000	0.29	
3377900	11	9691109	0.14	T/A	-6.02	0.000	0.29		
SW	16647170	08	36620996	0.11	T/C	4.46	0.000	0.66	
	3383047	03	50229319	0.33	G/A	-2.41	0.000	0.65	Phvul.003G263200
SC	3380850	01	50427390	0.08	T/C	-10.79	0.000	0.10	Phvul.001G254100
	3381030	02	33669423	0.04	G/A	-10.33	0.000	0.08	

486 CH = chromosome, DFW = days to flowering, GYD = grain yield (kg/ha), SW = 100 seed weight (g), PH = plant  
487 height (cm), LT = leaf temperature (°C), SC = stomatal conductance (mmol m<sup>-2</sup> s<sup>-1</sup>), SNP = single nucleotide  
488 polymorphism, MAF = minor allele frequency, R<sup>2</sup> = proportion of the total phenotypic variation explained by the  
489 significant SNP marker after fitting the other model effects and -log<sub>10</sub>(P) = p value of the association.

490 Notably, two SNPs on chromosomes *Pv1* and *Pv2* were significantly associated with SC, with  
491 allele effect ranging from -10.79 mmol m<sup>-2</sup> s<sup>-1</sup> (SNP 3380850) to -10.33 mmol m<sup>-2</sup> s<sup>-1</sup> (SNP  
492 3381030). Common regions associated with multiple traits on chromosomes were not  
493 identified under DS environments in this study. Markers explained 0.08 – 0.10, 0.22 – 0.23,  
494 0.29 – 0.32, 0.43 – 0.44, 0.65 – 0.66 and 0.69 – 0.70 of the total phenotypic variability ( $R^2$ ) for  
495 SC, LT, PH, GYD, SW and DFW, respectively. Overall, the  $R^2$  varied from 0.08 (SC: SNP  
496 3381030) to 0.70 (DFW: SNPs 100132383, 3381050 and 8204238).

497

## 498 **Analysis of marker-trait associations under non-stressed** 499 **environments**

500 The significant MTAs and their respective statistical parameters for agronomic and  
501 physiological traits are summarised in Table 5. Under NS conditions, 39 significant MTAs  
502 were detected for six traits (excluding SW and SC) with  $p < 10^{-03}$ . The associations are shown  
503 in Fig 3.

504

505 **Fig 3. Manhattan plots showing significant marker-trait associations, their p-values and candidate genes**  
506 **for agronomic and physiological traits under well-watered conditions.** Note: A = Days to 50% flowering, B  
507 = Grain Yield, C = Days to physiological maturity, D = Plant height, E = Leaf chlorophyll content, F = Leaf  
508 temperature. \*Chr represents Chromosome, x-axis represents the physical map locations of the SNPs and the y-  
509 axis ( $-\log_{10}$  p-values) represents the degree to which a SNP is associated with a trait.

510 The quantile-quantile (QQ) plots for the studied traits revealed that the expected and observed  
511 probability values were normally distributed (S4 Fig). The highest number of significant MTAs  
512 were observed on *Pv11* (15%), followed by chromosomes *Pv3* and *Pv4* (both with 18%), with  
513 the least on *Pv2* and *Pv10* (both with 3%). No significant markers for SW and SC were detected  
514 under NS conditions in this study. The highest number of significant MTAs were observed on  
515 PH (14), with markers accounting for 0.39 – 0.40 of the total trait variation. Additionally, the  
516 allele effect of these SNPs ranged from -10.46 cm (SNP 13121517) to 9.30 cm (SNP  
517 13121517). Interestingly, 38% of the markers that were significantly associated with PH were  
518 located on chromosome 11. For DFW, a total of 12 significant associations were identified,  
519 with markers explaining 0.45 – 0.46 of the observed trait variation. Additionally, the significant  
520 SNPs for DFW were located on chromosomes *Pv1*, *Pv3*, *Pv4*, *Pv5*, *Pv6*, *Pv7* and *Pv11*, with  
521 allele effect ranging from -2.27 days (SNP 100175933) to 2.23 days (SNP 100175934).

522

523 **Table 4. Single nucleotide polymorphism (SNP) markers associated with agronomic**  
 524 **and physiological traits in dry bean genotypes under non- stressed conditions.**

Phenotype	SNP name	CH	SNP position on genome (bp)	MAF	Allele	Effect of allele	-log <sub>10</sub> (P) value	R <sup>2</sup>	Candidate gene
DFW	3372129	04	43770691	0.20	C/T	1.85	0.000	0.46	
	3368616	01	48386869	0.29	C/G	2.19	0.000	0.46	
	8212932	04	43742237	0.35	C/A	-1.39	0.000	0.46	
	3379964	03	48424846	0.37	C/T	-1.61	0.000	0.46	
	100175933	06	31464277	0.27	A/G	-2.27	0.000	0.46	
	100175934	06	31464277	0.27	A/T	2.23	0.000	0.45	
	16647096	03	19481003	0.28	A/C	1.49	0.000	0.45	
	3378741	03	1178534	0.38	A/C	1.56	0.000	0.45	Phvul.003G011400
	100140152	04	43939513	0.32	A/G	1.67	0.000	0.45	Phvul.004G037700
	3374827	11	47036209	0.34	T/G	1.63	0.000	0.45	Phvul.011G166300
	3381380	05	1315962	0.27	T/C	-2.05	0.000	0.45	
100122216	07	23590138	0.39	A/T	1.79	0.000	0.45	Phvul.007G144000	
DPM	100117381	02	24161867	0.18	A/T	2.90	0.000	0.70	Phvul.002G112700
GYD	100124606	01	32783904	0.17	T/A	199.11	0.000	0.50	
LCC	8198945	06	30370228	0.16	T/C	2.18	0.000	0.12	Phvul.006G209700
	100167635	08	44516286	0.32	T/G	1.90	0.000	0.11	Phvul.008G163600
PH	3383709	11	23343020	0.28	A/G	7.30	0.000	0.41	
	100123206	03	41669536	0.27	G/T	9.02	0.000	0.40	Phvul.003G192800
	13121517	11	5699564	0.08	C/T	9.30	0.000	0.40	
	100164602	03	32040779	0.43	A/C	-4.72	0.000	0.40	
	100065600	11	38863980	0.27	C/G	-8.10	0.000	0.40	
	100181804	07	37529193	0.42	G/T	-4.77	0.000	0.40	Phvul.007G253400
	100124008	03	36956076	0.38	T/C	5.98	0.000	0.40	
	100101486	04	38236692	0.37	T/C	4.92	0.000	0.39	
	100073620	11	42969050	0.35	C/T	-7.00	0.000	0.39	Phvul.011G152000
	100068647	01	39765027	0.38	T/G	5.76	0.000	0.39	
	3382850	10	40091053	0.39	A/G	4.52	0.000	0.39	
	13121517	11	5699564	0.06	T/C	-10.46	0.000	0.39	
	3379157	06	30312046	0.44	T/C	-4.39	0.000	0.39	Phvul.006G208800
	13121469	01	44975217	0.49	C/A	5.29	0.000	0.39	Phvul.001G190800
LT	100101691	03	18922335	0.28	G/A	-0.56	0.000	0.08	
	100070187	04	12643816	0.21	A/G	0.80	0.000	0.15	
	100061661	01	19177470	0.16	T/A	0.69	0.000	0.09	
	100071816	04	33722284	0.45	A/G	0.39	0.000	0.08	
	100100644	08	26794110	0.21	A/C	0.54	0.000	0.08	
	100102687	04	7507744	0.14	G/A	-0.61	0.000	0.08	Phvul.004G055500
	100120897	08	20306142	0.06	C/A	-0.71	0.000	0.08	
	100167520	05	23720983	0.36	C/G	0.46	0.000	0.08	
100161682	05	18689401	0.17	G/A	-0.58	0.000	0.08	Phvul.005G077500	

525 CH = chromosome, DFW = days to flowering, DPM = days to physiological maturity, GYD = grain yield (kg/ha), PH = plant height (cm),  
 526 LCC = leaf chlorophyll content, LT = leaf temperature (°C), SNP = single nucleotide polymorphism, MAF = minor allele frequency, R<sup>2</sup> =  
 527 proportion of the total phenotypic variation explained by the significant SNP marker after fitting the other model effects, -log<sub>10</sub>(P) = p value  
 528 of the association.  
 529

530 Notably, one SNP (SNP 100124606) on chromosome *Pv01* was significantly associated with  
531 GYD, with a large positive allelic effect of 199.11 kg/ha. In addition, this SNP had a MAF of  
532 0.17 in the population. Regarding physiological traits, SNPs were identified that have a  
533 significant association with LCC distributed across two chromosomes (*Pv6* and *Pv8*), with  
534 positive allele effects ranging from 1.90 (SNP 100167635) to 2.18 (SNP 8198945). For LT,  
535 nine significant associations were detected, with markers accounting for 0.08 – 0.15 of the trait  
536 variation. The significant SNPs for LT were located on chromosomes *Pv1*, *Pv3*, *Pv4*, *Pv5* and  
537 *Pv8*, with allele effect ranging from -0.71°C (SNP 100102687) to 0.80°C (SNP 100070187).  
538 Additionally, the sum of the SNPs with a significant positive effect on LT was 2.88°C and -  
539 2.46°C for all the SNPs with a significant negative effect. A locus (SNP 100117381) on  
540 chromosome *Pv02* explained the highest proportion of the phenotypic variation (0.70) among  
541 the studied traits and was associated with DPM. In addition, SNP 100117381 had a MAF of  
542 0.18 in the population and a large positive effect (2.90 days) on DPM. On the other hand, nine  
543 significant SNPs for LT on chromosomes *Pv3*, *Pv4*, *Pv8* and *Pv5* explained the least proportion  
544 of the observed phenotypic variation (0.08) among the studied traits. Common regions  
545 associated with multiple traits on chromosomes were not identified under NS environments.  
546 Overall,  $R^2$  varied from 0.08 (LT – SNPs 100101691, 100071816, 100100644, 100102687,  
547 100102687, 100167520 and 100161682) to 0.70 (DPM - SNP 100117381) (Table 5).

548

## 549 **Identification of putative candidate genes associated with** 550 **significant single nucleotide polymorphism**

551

### 552 **Drought stressed environments**

553 A total of eight potential candidate genes (DFW - 1; GYD - 1; PH - 4; SW - 1; SC – 1) were  
554 identified under DS environments (Table 4 and Fig 2). The candidate genes for DFW  
555 (*Phvul.002G122100*), SC (*Phvul.001G254100*), SW (*Phvul.003G263200*) and GYD  
556 (*Phvul.004G150500*) were identified on chromosomes *Pv02*, *Pv01*, *Pv03* and *Pv04*,  
557 respectively (Table 4). These genes had diverse putative functions ranging from RNA  
558 recognition motif or RNP domain functions (DFW), NADPH dehydrogenase/NADPH  
559 diaphosare activity (SW), helicase activity and CCCH zinc finger protein domain functions  
560 (SC) to Phosphoethanolamine N-methyltransferase activity (GYD), respectively. On the other  
561 hand, the candidate genes for PH were identified on chromosomes *Pv01* (*Phvul.001G172300*)  
562 and *Pv08* (*Phvul.008G133100*; *Phvul.008G065700*; *Phvul.008G080600*) (Table 4). These

563 genes had diverse putative functions ranging from calcium transporting ATPase 1 activity,  
564 peptidyl prolyl cis trans isomerase activity, acyl-coenzyme A thioesterase activity to  
565 centrosomal protein nuf function, respectively.

566

### 567 **Non-stressed environments**

568 A total of fourteen potential candidate genes (DFW - 4; DPM - 1; LCC - 2; PH - 5; LT - 2)  
569 were identified under NS environments (Table 5 and Fig 3). The candidate genes for DFW  
570 were identified on chromosomes *Pv03* (*Phvul.003G011400*), *Pv04* (*Phvul.004G037700*), *Pv07*  
571 (*Phvul.007G144000*) and *Pv11* (*Phvul.0011G166300*), whereas the candidate gene for DPM  
572 was identified on chromosome *Pv02* (*Phvul.002G112700*) (Table 5). Candidate genes for DFW  
573 had diverse putative functions related to SORTING NEXIN-13, transcription factor TCP 13,  
574 U6 SNRNA-associated SM LIKE PROTEIN LSM4 and NHL domain containing protein. On  
575 the other hand, the candidate gene for DPM had a putative function related to the activity of  
576 thiol disulphide oxidoreductase. Chromosomes *Pv4* and *Pv5* harboured the two candidate genes  
577 for LT namely *Phvul.004G055500* and *Phvul.005G077500*, respectively (Table 5). These  
578 genes had diverse putative functions related to the mitochondrial transcription termination  
579 factor family protein and leucine rich repeat protein associated with apoptosis in muscle tissue,  
580 respectively.

581 The genes *Phvul.006G209700* and *Phvul.008G163600* for LCC were identified on  
582 chromosomes *Pv06* and *Pv08*, respectively. These genes had diverse putative functions, such  
583 as premnaspirodiene oxygenase or hyoscyamus muticus premnaspirodiene oxygenase activity  
584 and nucleoside triphosphate hydrolases activity, respectively. On the other hand, the candidate  
585 genes for PH were identified on chromosomes *Pv01* (*Phvul.001G190800*), *Pv03*  
586 (*Phvul.00G192800*), *Pv06* (*Phvul.006G208800*), *Pv07* (*Phvul.007G253400*), and *Pv11*  
587 (*Phvul.011G152000*) (Table 5). These genes also had diverse putative functions, such as f-box-  
588 like domain superfamily functions, protein NRT1 or PTR family related functions,  
589 phosphatidylserine decarboxylase activity, tyra-like translation elongation factor svrs-related  
590 functions, and inactive g-type lectin s-receptor like serine or threonine protein kinase activity,  
591 respectively.

592

### 593 **Linkage disequilibrium analysis using significant SNP markers**

594 The analysis of LD using SNP markers is shown in Fig 4. A high and extensive LD was  
595 observed for the common bean genome, which is expected in self-pollinated crops such as

596 common bean. The results show that the overall LD decay across the genome of 185 common  
597 bean genotypes was 30 bp, at a cut-off of  $r^2 = 0.4$ . Generally, there was a slow decay of LD  
598 throughout the common bean genome, and the LD extended to several mega-bases as shown  
599 in Fig 4. The population structure usually affects the extent of LD decay.

600

601 **Fig 4. Linkage disequilibrium (LD,  $r^2$ ) decay plot in genome of dry beans based on 9370 single nucleotide**  
602 **polymorphisms (SNPs) in 185 diverse genotypes.**

603

## 604 **Discussion**

### 605 **Variations in agronomic and physiological traits**

606 The low to moderate  $H^2$  estimates observed for SC, LT and LCC under DS and NS conditions  
607 imply that these physiological traits might be influenced by a number of genes (polygenic  
608 inheritance) and the production environment. Therefore, direct selection for SC, LT and LCC  
609 under DS and NS conditions could be a challenge to dry bean breeders. On the other hand, the  
610 high  $H^2$  estimates (97%) for seed size observed under DS and NS environments reflect the  
611 predominance of additive gene action (genetic control of this trait) across environments. The  
612 current findings are in agreement with Assefa et al. [80] and Hoyos-Villegas et al. [14] who  
613 reported  $H^2$  estimates of 77 and 93.4%, respectively under NS conditions. In this study, drought  
614 stress reduced PH, GYD, SW, DPM, LCC and SC by 12.1, 29.6, 10.3, 12.6, 28.5 and 62.0%,  
615 respectively, highlighting the detrimental effect of moisture stress under field conditions. These  
616 findings corroborate previous reports by Assefa et al. [80], Darkwa et al. [22], Assefa et al.  
617 [81], and Mathobo et al. [82] in common bean. Mathobo et al. [82] reported reductions of 48  
618 and 39% in SC and LCC, respectively under DS conditions. Darkwa et al. [22], using navy  
619 beans, reported reductions of 10.7, 14.8, 12.7 and 26.1% in SW, PH, DPM and LCC under DS  
620 conditions. Assefa et al. [80], using navy beans, also reported reductions of 12% and 17.6% in  
621 SW and DPM, respectively under DS conditions.

622 Crop plants close their stomata when exposed to drought stress to minimize excessive  
623 water loss and avoid dehydration. However, the closing of stomata reduces stomatal  
624 conductance, and also affects cooling mechanisms resulting in increased leaf or canopy  
625 temperature. Therefore, in this study, drought stress increased LT by 21.6%. Drought stress  
626 also reduced GYD by 30%, close to the GYD reductions reported by Schneider et al. [83]  
627 [26%], Darkwa et al. [22] [30%] and Mutari et al. [24] [28%] in dry bean drought tolerance  
628 screening trials. Breeding for enhanced GYD under both DS and NS environments is one of

629 the greatest challenges faced by dry bean breeders [15]. Therefore, one of the most important  
630 contribution of this study was to indicate drought tolerant genotypes (DAB91, DAB302,  
631 AFR703, CIM-SUG07-ALS-51-3, DAB487, DAB287, CIM-RM09-ALS-BSM-12 and  
632 DAB539) with consistent outstanding and stable GYD performance under both DS and NS  
633 environments. Terminal drought stress is an important factor limiting common bean  
634 productivity in the SSA region. Therefore, the identification and subsequent release of drought  
635 tolerant genotypes will positively impact on socio-economic, food and nutrition security in  
636 SSA. These genotypes could also serve as important genetic resources in drought tolerance  
637 breeding programmes to improve released cultivars. Both DAB287 and AFR703 were released  
638 in Zimbabwe as Sweet William and Gloxinia, respectively. Among the drought tolerant  
639 genotypes with superior GYD performance under water deficit conditions, most of the top 20  
640 genotypes were of the Andean gene pool, coded as drought Andean (DAB lines) (Table 3 and  
641 S2 Table). Notably, all the DAB lines evaluated in this study were developed for improved  
642 tolerance to drought by the Alliance of Bioversity International and International Centre for  
643 Tropical Agriculture in Colombia. The current observation suggests that progress in improving  
644 drought tolerance in the Mesoamerican gene pool has been limited compared to the Andean  
645 gene pool. The current findings are in agreement with Assefa et al. [81] who reported that  
646 progress in improving drought tolerance in navy beans (Mesoamerican gene pool) worldwide  
647 has been limited compared to the other commercial classes of small seeded Mesoamerican  
648 beans.

649

## 650 **Population structure and Linkage disequilibrium analysis**

651 The AMDP was delineated into two distinct major sub-populations based on the genotypes'  
652 genetic ancestry, and this corresponded to the Andean and Mesoamerican gene pools (Figs 1B,  
653 C). This is expected considering that the domestication of dry beans on the American continent  
654 in two main centres of origin (Andean and Mesoamerican regions of America) resulted in two  
655 major and diverse gene pools [59, 84]. Cichy et al. [31, 55], Raggi et al. [74], Tigist et al. [48],  
656 Nkhata et al. [49], Ojwang et al. [71], Keller et al. [6] and Liu et al. [85] also observed two sub-  
657 populations (Andean and Mesoamerican gene pools) in their GWAS studies.

658 A number of the identified Andean-Mesoamerican admixed genotypes carrying  
659 genomic regions from both gene pools are released cultivars in Rwanda (RWR2154), Malawi  
660 (NUA59-4), Zimbabwe (SMC16, NUA674, and Sweet William), Eswatini (NUA674) [86–89].  
661 Further, most of the admixed genotypes have commercial seed types, are biofortified



662 (RWR2154, SMC16, SMC21, NUA674 and NUA59-4) and drought tolerant (Sweet William,  
663 DAB115, DAB63, DAB142 and DAB477). Singh [90], Beebe et al. [4, 20] and Beebe [84]  
664 reported that interracial hybridizations between races or sister species (*Phaseolus coccineus*,  
665 *Phaseolus acutifolius* and *Phaseolus dumosus*) of *Phaseolus vulgaris* have been widely used  
666 in dry bean improvement programmes when breeding for enhanced grain yield, micronutrient  
667 density and drought tolerance. For example, the biofortified admixed genotype NUA674 is a  
668 product of an inter-gene pool cross between AND277 (Andean gene pool) and G21242  
669 (Andean-Mesoamerican inter-gene pool landrace) made at the Alliance of Bioversity  
670 International and International Centre of Tropical Agriculture (ABC) in Colombia [87]. Islam  
671 et al. [91] and Beebe [84], also reported that one of the parents to NUA674, G21242 (source  
672 of high seed iron in biofortification breeding programmes) is a product of Andean–  
673 Mesoamerican inter-gene pool hybridization, validating the current findings. Therefore, the  
674 current observation suggests that most of the admixed genotypes identified in this study  
675 resulted from deliberate breeding efforts (inter-gene pool hybridizations) to introgress genes  
676 for enhanced grain yield, drought tolerance and micronutrient density. Similar findings were  
677 reported by Hoyos-Villegas et al. [14] and Tigist et al. [48] in common bean.

678 The biofortified and drought tolerant admixed genotypes identified in this study may  
679 be used as a bridge to transfer favourable alleles for micronutrient density and drought  
680 tolerance into either the Andean or Mesoamerican seed types. The extent and structure of LD  
681 decay in the study germplasm usually determines the resolution of GWAS. The slow decay of  
682 LD observed in this study is expected in self-pollinating crop species, such as common bean  
683 because of the loss of recombination, which results in a homozygous genetic background.  
684 According to Vos et al. [92], recombination events in crops with a homozygous genetic  
685 background are ineffective to cause LD decay, resulting in extended (large) and slow decay of  
686 LD. The slow decay of LD, and the large extent of LD observed in this study corroborates  
687 previous reports in dry bean [32, 85].

688

## 689 **Marker-Trait Associations**

690 In dry bean, it is important to enhance moisture stress tolerance by identifying genotypes with  
691 high grain yield potential under water deficit conditions, and by introgressing desirable alleles  
692 conferring drought tolerance. The mean call rate (93%) and reproducibility (100%) of the silico  
693 DArTs used in this study were consistent with previous reports [15, 49], thus demonstrating  
694 the reliability and high quality of this set of silico DArTs. A higher number of significant MTAs

695 were detected under NS conditions, corroborating previous reports in bread wheat (*Triticum*  
696 *aestivum* L.) [93, 94] and dry bean [15]. The observed trend could be due to the fact that drought  
697 tolerance is a complex polygenic trait which is highly influenced by the production  
698 environment, resulting in unpredictable performance of genotypes (genotype-by-environment  
699 interaction [GEI]) under different environments (DS and NS). Even though a smaller number  
700 of significant MTA was observed under DS compared to the NS condition, novel genomic  
701 regions associated with key agronomic and physiological traits were detected under DS  
702 conditions. Notably, no significant SNPs for all the studied agronomic and physiological traits  
703 were consistent across DS and NS treatments. Similar findings were reported in wheat ([93] –  
704 plant height and spike length) and dry bean ([15] – grain yield) under DS and NS treatments.  
705 The observed trend suggests that some markers may influence the expression of phenotypic  
706 traits differently under DS and NS environments. Further, the GEI could have confounded the  
707 identification significant SNPs that are consistent across DS and NS treatments.

708 The highest number of significant SNPs were identified for PH. Similar findings were  
709 reported by Sukumaran et al. [95] who observed 30 significant MTAs for PH in durum wheat  
710 (*Triticum turgidum* L. ssp. *Durum*). Some of the SNPs identified in this study were located on  
711 genomic regions that had been previously reported to be harbouring genes and QTLs for the  
712 studied traits. For example, in this study, chromosomes *Pv01*, *Pv03*, *Pv04*, *Pv06* and *Pv07*  
713 harboured 1 SNP, 4 SNPs, 3 SNPs, 2 SNPs and 1 SNP, respectively that were significantly  
714 associated with DFW under optimal conditions. These results are consistent with Dramadri et  
715 al. [34], Nkhata et al. [49] and Keller et al. [6]. Dramadri et al. [34] identified 2 QTLs that were  
716 associated with DFW on *Pv03* under DS and NS conditions. Nkhata et al. [49] identified 2 and  
717 5 SNPs that were significantly associated with DFW on *Pv03* and *Pv06*, respectively under NS  
718 conditions. Further, Keller et al. [6] identified 6 SNPs, 1 SNP and 1 SNP that were significantly  
719 associated with DFW on *Pv01*, *Pv04* and *Pv07*, respectively under optimal conditions. These  
720 findings suggest that the aforementioned QTL regions are stable across different environments  
721 and genetic backgrounds. In addition, these findings also suggest that chromosomes *Pv01*,  
722 *Pv03*, *Pv04*, *Pv06* and *Pv07* harbour genes for controlling flowering.

723 In this study, only one marker (SN 1667170) was significantly associated with SW on  
724 chromosome *Pv08* under DS conditions. These results are in accordance with Moghaddam et  
725 al. [57], and Valdisser et al. [15] who identified significant MTAs for SW on chromosome *Pv8*  
726 under DS and NS environments, suggesting that this QTL is stable across different  
727 environments and genetic backgrounds. On the contrary, several significant MTAs for SW  
728 were previously identified under DS on chromosome *Pv01*, [52], chromosome *Pv03* [51],

729 chromosome *Pv09* [14], and chromosomes *Pv2* to *Pv4* and *Pv6* to *Pv11* [15]. Thus, the  
730 detection of significant MTAs for SW on different chromosomes and locations indicates high  
731 genetic diversity in common bean with respect to genomic regions associated with SW under  
732 drought stress. In this study, the identified SNPs that were significantly associated with GYD  
733 under DS were located on chromosomes *Pv04* (SNP 3382688) and *Pv11* (SNP 3384334 and  
734 SNP 3381526). Similarly, Dramadri et al. [34] identified significant QTL signals for GYD and  
735 yield components on chromosomes *Pv01*, *Pv02*, *Pv03*, *Pv04*, *Pv06*, and *Pv11* under DS  
736 conditions. Oladzad et al. [96] also identified SNPs that were significantly associated with  
737 GYD, placed on chromosomes *Pv03*, *Pv08*, and *Pv11* under heat stress. Further, Valdisser et  
738 al. [15] found 25 QTLs that were associated with GYD on chromosomes *Pv02*, *Pv03*, *Pv04*,  
739 *Pv08*, *Pv09* and *Pv11* under NS conditions, in agreement with the current findings. These  
740 findings suggest that chromosomes *Pv04* and *Pv11* harbour genes for controlling GYD.

741 The identification of SNPs associated with GYD, under moisture stress, would  
742 significantly contribute to the development of molecular tools for MAS and identification of  
743 genes of interest for edition. The proportion of the total phenotypic variation ( $R^2$ ) explained by  
744 the significant SNP markers for LCC and LT was generally low (0.11 – 0.12 for LCC under  
745 NS and 8 – 15% for LT under NS). Therefore, to account for the missing variation, it might be  
746 worthwhile to complement the SNP-based GWAS by haplotype-based GWAS [97].

747

## 748 **Candidate genes**

749

### 750 **Drought stressed**

751 The functional annotation revealed that the candidate gene for SC, *Phvul.001G254100* on  
752 chromosome *Pv01* encodes the CCCH zinc finger family protein which plays an important  
753 function in response of plants to biotic and abiotic stresses [98–101]. This functional gene also  
754 plays an important role in physiological and plant developmental processes [101]. Similar  
755 findings were reported in *Brassica rapa* [98], common bean [15] and Barley (*Hordeum vulgare*  
756 L.) [101]. Wang et al. [102], Seong et al. [103] and Selvaraj et al. [104] reported that several  
757 types of CCCH zinc family finger millet genes such as *OsC3H10*, *OsC3H47*, and *OsTZF5* are  
758 involved in the regulation of tolerance to moisture stress in rice (*Oryza Sativa* L.). According  
759 to Lin et al. [105], the CCCH zinc finger family gene confers drought tolerance in plants by  
760 regulating the opening and closing of stomata. They further reiterated that genotypes that are  
761 tolerant to drought stress have abnormal and lower stomatal conductance under moisture

762 stressed conditions. In this study, the marker SNP 3380850 for the gene *Phvul.001G254100*  
763 which confers tolerance to drought stress exhibited negative allelic effects ( $-10.79 \text{ mmol m}^{-2} \text{ s}^{-1}$ )  
764 on SC.

765 The functional annotation revealed that the candidate gene for DFW,  
766 *Phvul.002G122100* on chromosome *Pv02* encodes an RNA-recognition motif protein, which  
767 plays a comprehensive biological function (critical modulators) in abiotic stress (drought, heat  
768 flooding, cold and high salinity) responding processes in plants [106]. Zhou et al. [107]  
769 observed that the RNA-recognition motif gene “OsCBP20” from rice confers abiotic stress  
770 tolerance in *escherichia coli*. Therefore, the candidate gene *Phvul.002G122100* identified in  
771 this study may play a protective role under DS conditions. Candidate genes such as  
772 *Phvul.003G263200* (*Pv08*) for SW which encodes for NADPH dehydrogenase plays an  
773 important role in mechanisms which protect plants against nitro-oxidative stresses generated  
774 by biotic and abiotic stresses such as drought, low temperature, heat, and salinity [108]. Under  
775 DS, the seed is significantly affected by oxidative damages, and oxidative damages are  
776 minimized by the activity of NADPH dehydrogenase [109].

777 The candidate gene for GYD, *Phvul.004G150500* on chromosome *Pv04*, encodes the  
778 enzyme, phosphoethanolamine N-methyltransferase in plants. This catalytic enzyme plays an  
779 important role in the response of plants to abiotic stresses such as drought and salt tolerance by  
780 catalysing the methylation of phosphoethanolamine to phosphocholine [110]. Studies  
781 conducted by Wang et al. [110] in transgenic tobacco revealed that phosphoethanolamine N-  
782 methyltransferase improved the drought tolerance of transgenic tobacco. Notably, the marker  
783 (SNP 3382688) for this candidate gene *Phvul.004G150500* had large positive allelic effects  
784 ( $202.90 \text{ kg/ha}$ ) on GYD. The candidate gene for PH, *Phvul.001G172300* encodes the calcium  
785 transporting ATPase, which plays an important role in growth and development processes,  
786 opening and closing of stomata, hormonal signalling, and regulation of responses to biotic and  
787 abiotic stresses in plants [111]. In summary, these results further confirmed that the identified  
788 putative potential candidate genes were associated with moisture stress tolerance of dry bean.  
789 Therefore, the putative candidate genes identified in the current AMDP under DS conditions  
790 are important genetic resources. The candidate genes could be utilized in drought tolerance  
791 breeding programmes by creating and introgressing new genetic variability into commercial  
792 cultivars.

793

## 794 **Well-watered conditions**

795 The functional annotation revealed that the candidate gene for PH “*Phvul.011G152000*” on  
796 chromosome *Pv11* encodes the threonine protein kinase, which is associated with enhanced  
797 tolerance to biotic and abiotic stresses in plants [15]. Similar results were reported in dry beans  
798 by Valdisser et al. [15]. In rice, kinase causes dwarfism by reducing plant height [112].  
799 Similarly, in this study, the marker SNP 100073620 for the gene “*Phvul.001G152000*”  
800 exhibited negative allelic effects (-7.00 cm) on PH. According to Zhang et al. [112], kinases  
801 also has an impact on grain yield. The candidate gene *Phvul.004G037700* which was found on  
802 chromosome *Pv04* in association with DFW encodes transcription factor TCP<sub>13</sub>. The  
803 transcription factor families are strongly involved in abiotic and biotic stress responses,  
804 including zinc-finger, dehydration-responsive element-binding (DREB), and basic helix-loop-  
805 helix (bHLH) families which regulate plant growth in leaves and roots under water deficit  
806 conditions [113]. Studies conducted by Urano et al. [113] in *Arabidopsis thaliana* revealed that  
807 TCP<sub>13</sub> induces changes in leaf (leaf rolling and reduced leaf growth) and root morphology  
808 (enhanced root growth). This results in enhanced tolerance to dehydration stress under osmotic  
809 stress. The candidate gene *Phvul.004G055500* which was found in association with LT on  
810 chromosome *Pv04* encodes mitochondrial transcription termination factor family protein.  
811 According to Kim et al. [114], the mitochondrial transcription termination factor family protein  
812 enhances thermo-tolerance in *Arabidopsis*.

813

## 814 **Conclusions**

815 This study contributes many significant MTAs in common bean for agronomic and  
816 physiological traits under DS and NS environments. The present study identified a total of 68  
817 SNPs that were significantly ( $p < 10^{-03}$ ) associated with key agronomic and physiological traits  
818 under DS and NS conditions. The highest number of significant MTAs were observed on  
819 chromosome *Pv11* in both environments. For the two environments (DS and NS), no common  
820 SNPs for the studied traits was detected. Overall, twenty-two potential candidate genes were  
821 identified across environments. Most of the identified genes had known biological functions  
822 related to regulating drought stress response, and growth and development under drought  
823 stress. The information generated from this study provides insights into the genetic basis of  
824 agronomic and physiological traits under DS stress and NS conditions, and lays the foundation  
825 for future validation studies of drought tolerance genes in dry bean. Thus, the significant MTAs

826 identified in this study should be explored and validated further to estimate their effects using  
827 segregating populations and in different genetic backgrounds before utilization in gene  
828 discovery and marker-assisted breeding for drought tolerance. Further, functional  
829 characterization and the application of gene knockout to the identified putative candidate genes  
830 would further confirm their roles in regulating drought stress response, and growth and  
831 development under DS and NS conditions. More powerful statistical genetics tools such as  
832 genomic prediction models would be needed to identify minor genes that are associated with  
833 agronomic and physiological traits. The admixed genotypes identified in this study offer  
834 potential as genetic resources in drought tolerance and biofortification breeding programmes,  
835 especially within the sugar, red mottled and navy bean market classes.

836

## 837 **Acknowledgements**

838 The authors would like to thank the Pan Africa Bean Research Alliance (PABRA) for the  
839 technical support received during the field and laboratory work. The authors are grateful to the  
840 Department of Research and Specialist Services (DR&SS) for providing their experimental  
841 sites for the field work. We would also like to thank PABRA for providing the germplasm.

842

## 843 **Author Contributions**

844 **Conceptualization:** Bruce Mutari, Julia Sibiya, Edmore Gasura

845 **Data Curation:** Bruce Mutari, Admire Shayanowako, Charity Chidzanga

846 **Formal analysis:** Bruce Mutari, Admire Shayanowako, Charity Chidzanga

847 **Investigation:** Bruce Mutari

848 **Funding acquisition:** Bruce Mutari

849 **Methodology:** Bruce Mutari, Julia Sibiya, Edmore Gasura, Prince Matova

850 **Software:** Admire Shayanowako, Charity Chidzanga

851 **Resources:** Bruce Mutari

852 **Supervision:** Julia Sibiya, Edmore Gasura

853 **Validation:** Bruce Mutari, Julia Sibiya, Edmore Gasura, Admire Shayanowako, Charity  
854 Chidzanga

855 **Writing – original draft:** Bruce Mutari, Julia Sibiya

856 **Writing – review & editing:** Bruce Mutari, Julia Sibiya, Edmore Gasura, Admire  
857 Shayanowako, Charity Chidzanga, Prince Matova  
858

## 859 **References**

- 860 1. Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, et al. A reference  
861 genome for common bean and genome-wide analysis of dual domestications. *Nat Genet.*  
862 2014; 46(7): 707–13. <https://doi.org/10.1038/ng.3008>.
- 863 2. Welch RM, House WA, Beebe S, Cheng Z. Genetic selection for enhanced bioavailable  
864 levels of iron in bean (*Phaseolus vulgaris* L.) seeds. *J Agric Food Chem.* 2000; 48(8):  
865 3576–80.
- 866 3. Kamfwa K, Cichy KA, Kelly JD. Genome-Wide Association Study of Agronomic Traits  
867 in Common Bean. *Plant Genome.* 2015; 8(2): 1–12.  
868 <https://doi.org/10.3835/plantgenome2014.09.0059>.
- 869 4. Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA. Phenotyping common beans for  
870 adaptation to drought. *Front Physiol.* 2013; 4: 1–20.  
871 <http://doi.org/10.3389/fphys.2013.00035>.
- 872 5. Sauer J. *Historical Geography of Crop Plants: A selected Roster.* Boca Raton, USA:  
873 CRC Press; 1993.
- 874 6. Keller B, Ariza-Suarez D, Portilla-Benavides AE, Buendia HF, Aparicio JS, Amongi  
875 W, et al. Improving Association Studies and Genomic Predictions for Climbing Beans  
876 With Data From Bush Bean Populations. *Front Plant Sci.* 2022; 13.  
877 <https://doi.org/10.3389/fpls.2022.830896>.
- 878 7. Singh SP, Gepts P, Debouck DG. Races of common bean (*Phaseolus vulgaris*,  
879 Fabaceae). *Econ Bot.* 1991; 45(3): 379–96. <https://doi.org/10.1007/BF02887079>.
- 880 8. Bitocchi E, Bellucci E, Giardini A, Rau D, Rodriguez M, Biagetti E, et al. Molecular  
881 analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in  
882 Mesoamerica and the Andes. *New Phytol.* 2013; 197(1): 300–13.  
883 <https://doi.org/10.1111/j.1469-8137.2012.04377.x>.
- 884 9. Rainey K, Griffiths P. Evaluation of common bean yield components under heat stress.  
885 *HortScience.* 2003; 38(5): 682.
- 886 10. Rainey KM, Griffiths PD. Differential response of common bean genotypes to high  
887 temperature. *J Am Soc Hortic Sci.* 2005; 130(1): 18–23.

- 888 <https://doi.org/10.21273/JASHS.130.1.18>.
- 889 11. Katungi E, Farrow A, Chianu J, Sperling L, Beebe S. Common bean in eastern and  
890 southern Africa: A situation and outlook analysis. Cali, Colombia; 2009; 61: 1-44.
- 891 12. Beebe S. Common bean breeding in the tropics. In: Janick J, editor. Plant breeding  
892 reviews. 36th ed. New Jersey, USA: John Wiley & Sons, Ltd; 2012. pp. 357–412.
- 893 13. Katungi E, Farrow A, Mutuoki T, Gebeyehu S, Karanja D, Alemayehu F, Sperling L,  
894 Beebe S, Rubyogo JC, Buruchara R. Improving common bean productivity: An Analysis  
895 of socioeconomic factors in Ethiopia and Eastern Kenya: Baseline Report Tropical  
896 legumes II. Cali, Colombia; 2010; 126.
- 897 14. Hoyos-Villegas V, Song Q, Kelly JD. Genome-wide Association Analysis for Drought  
898 Tolerance and Associated Traits in Common Bean. Plant Genome. 2017; 10(1): 1–17.  
899 <https://doi.org/10.3835/plantgenome2015.12.0122>.
- 900 15. Valdisser PAMR, Müller BSF, de Almeida Filho JE, Morais Júnior OP, Guimarães CM,  
901 Borba TCO, et al. Genome-Wide Association Studies Detect Multiple QTLs for  
902 Productivity in Mesoamerican Diversity Panel of Common Bean Under Drought Stress.  
903 Front Plant Sci. 2020; 11. <https://doi.org/10.3389/fpls.2020.574674>.
- 904 16. Kotir JH. Climate change and variability in Sub-Saharan Africa: a review of current and  
905 future trends and impacts on agriculture and food security. Environ Dev Sustain. 2011;  
906 13(3): 587–605. <https://doi.org/10.1007/s10668-010-9278-0>.
- 907 17. Tar'an B, Michaels TE, Pauls KP. Genetic Mapping of Agronomic Traits in Common  
908 Bean. Crop Sci. 2002; 42(2): 544–56. <https://doi.org/10.2135/cropsci2002.5440>.
- 909 18. Barrios AN, Hoogenboom G, Nesmith DS. Drought stress and the distribution of  
910 vegetative and reproductive traits of a bean cultivar. Sci Agric. 2005; 62(1): 18–22.  
911 <https://doi.org/10.1590/S0103-9016200500010000>.
- 912 19. Manjeru, P., T. Madanzi, B. Makedredza, A. Nciizah and MS. Effects of water stress at  
913 different growth stages on components and grain yield of common bean (*Phaseolus*  
914 *vulgaris* L.). African Crop Sci Conf Proc. 2007; 8: 299–303.
- 915 20. Beebe SE, Rao IM, Cajiao C, Grajales M. Selection for Drought Resistance in Common  
916 Bean Also Improves Yield in Phosphorus Limited and Favorable Environments. Crop  
917 Sci. 2008; 48(2): 582–92. <http://doi.org/10.2135/cropsci2007.07.0404>.
- 918 21. Beebe, S.E, Rao, I.M, Blair, M, Acosta-Gallegos J. Phenotyping common beans for  
919 adaptation to drought. In: Ribaut JM, Monneveux P, editors. Drought Phenotyping in  
920 Crops: From Theory to Practice. Mexico City, Mexico: Generation Challenge Program;  
921 2010. pp. 311–34.



- 922 22. Darkwa K, Ambachew D, Mohammed H, Asfaw A, Blair MW. Evaluation of common  
923 bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. *Crop*  
924 *J.* 2016; 4(5): 367–76. <https://doi.org/10.1016/j.cj.2016.06.007>.
- 925 23. Asfaw A, Blair MW, Struik PC. Multi-environment quantitative trait Loci analysis for  
926 photosynthate acquisition, accumulation, and remobilization traits in common bean  
927 under drought stress. *G3* (Bethesda). 2012; 2(5): 579–95.  
928 <https://doi.org/10.1534/g3.112.002303>.
- 929 24. Mutari B, Sibiya J, Gasura E, Matova PM, Simango K, Kondwakwenda A. Genetic  
930 analysis of grain yield and yield-attributing traits in navy bean (*Phaseolus vulgaris* L.)  
931 under drought stress. *Euphytica*. 2022; 218(5): 51. [https://doi.org/10.1007/s10681-022-](https://doi.org/10.1007/s10681-022-03001-3)  
932 [03001-3](https://doi.org/10.1007/s10681-022-03001-3).
- 933 25. Mutari B, Sibiya J, Bogweh Nchanji E, Simango K, Gasura E. Farmers' perceptions of  
934 navy bean (*Phaseolus vulgaris* L.) production constraints, preferred traits and farming  
935 systems and their implications on bean breeding: a case study from South East Lowveld  
936 region of Zimbabwe. *J Ethnobiol Ethnomed*. 2021; 17(1): 13.  
937 <https://doi.org/10.1186/s13002-021-00442-3>.
- 938 26. Builes VHR, Porch TG, Harmsen EW. Genotypic Differences in Water Use Efficiency  
939 of Common Bean under Drought Stress. *Agron J.* 2011; 103(4): 1206–15.  
940 <https://doi.org/10.2134/agronj2010.0370>.
- 941 27. Asfaw A, Blair MW, Almekinders C. Genetic diversity and population structure of  
942 common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. *Theor*  
943 *Appl Genet*. 2009; 120(1): 1–12. <https://doi.org/10.1007/s00122-009-1154-7>.
- 944 28. Pérez-Vega E, Pañeda A, Rodríguez-Suárez C, Campa A, Giraldez R, Ferreira JJ.  
945 Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL population of  
946 common bean (*Phaseolus vulgaris* L.). *Theor Appl Genet*. 2010; 120(7): 1367–80.  
947 <https://doi.org/10.1007/s00122-010-1261-5>.
- 948 29. Pereira HS, Mota APS, Rodrigues LA, de Souza TLPO, Melo LC. Genetic diversity  
949 among common bean cultivars based on agronomic traits and molecular markers and  
950 application to recommendation of parent lines. *Euphytica*. 2019; 215(2): 38.  
951 <https://doi.org/10.1007/s10681-018-2324-y>.
- 952 30. Papa R, Gepts P. Asymmetry of gene flow and differential geographical structure of  
953 molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.)  
954 from Mesoamerica. *Theor Appl Genet*. 2003; 106(2): 239–50.  
955 <https://doi.org/10.1007/s00122-002-1085-z>.

- 956 31. Cichy KA, Porch TG, Beaver JS, Cregan P, Fourie D, Glahn RP, et al. A *Phaseolus*  
957 *vulgaris* Diversity Panel for Andean Bean Improvement. *Crop Sci.* 2015; 55(5): 2149–  
958 60. <https://doi.org/10.2135/cropsci2014.09.0653>.
- 959 32. Perseguini JM KC, Oblessuc PR, Rosa JR BF, Gomes KA, Chiorato AF, Carbonell SAM,  
960 et al. Genome-Wide Association Studies of Anthracnose and Angular Leaf Spot  
961 Resistance in Common Bean (*Phaseolus vulgaris* L.). *PLoS One.* 2016; 11(3):  
962 e0150506. <https://doi.org/10.1371/journal.pone.0150506>.
- 963 33. Katuramu DN, Hart JP, Porch TG, Grusak MA, Glahn RP, Cichy KA. Genome-wide  
964 association analysis of nutritional composition-related traits and iron bioavailability in  
965 cooked dry beans (*Phaseolus vulgaris* L.). *Mol Breed.* 2018; 38(4): 44.  
966 <https://doi.org/10.1007/s11032-018-0798-x>.
- 967 34. Dramadri IO, Nkalubo ST, Kelly JD. Identification of QTL Associated with Drought  
968 Tolerance in Andean Common Bean. *Crop Sci.* 2019; 59(3): 1007–20.  
969 <https://doi.org/10.2135/cropsci2018.10.0604>.
- 970 35. Cichy KA, Fernandez A, Kilian A, Kelly JD, Galeano CH, Shaw S, et al. QTL analysis  
971 of canning quality and color retention in black beans (*Phaseolus vulgaris* L.). *Mol*  
972 *Breed.* 2014; 33(1): 139–54. <https://doi.org/10.1007/s11032-013-9940-y>.
- 973 36. Cortés AJ, Chavarro MC, Blair MW. SNP marker diversity in common bean (*Phaseolus*  
974 *vulgaris* L.). *Theor Appl Genet.* 2011; 123(5): 827–45. [https://doi.org/10.1007/s00122-](https://doi.org/10.1007/s00122-011-1630-8)  
975 011-1630-8.
- 976 37. Zhu C, Gore M, Buckler ES, Yu J. Status and Prospects of Association Mapping in  
977 Plants. *Plant Genome.* 2008; 1(1): 5–20.  
978 <https://doi.org/10.3835/plantgenome2008.02.0089>.
- 979 38. Ingvarsson PK, Street NR. Association genetics of complex traits in plants. *New Phytol.*  
980 2011; 189(4): 909–22. <https://doi.org/10.1111/j.1469-8137.2010.03593.x>.
- 981 39. Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q, et al. Genome-wide association study  
982 of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat*  
983 *Genet.* 2012; 44(1): 32–9. <https://doi.org/10.1038/ng.1018>.
- 984 40. Li H, Peng Z, Yang X, Wang W, Fu J, Wang J, et al. Genome-wide association study  
985 dissects the genetic architecture of oil biosynthesis in maize kernels. *Nat Genet.* 2013;  
986 45(1): 43–50. <https://doi.org/10.1038/ng.2484>.
- 987 41. Li N, Shi J, Wang X, Liu G, Wang H. A combined linkage and regional association  
988 mapping validation and fine mapping of two major pleiotropic QTLs for seed weight  
989 and silique length in rapeseed (*Brassica napus* L.). *BMC Plant Biol.* 2014; 14(1): 114.

- 990 <https://doi.org/10.1186/1471-2229-14-114>.
- 991 42. Nordborg M, Tavaré S. Linkage disequilibrium: what history has to tell us. *Trends*  
992 *Genet.* 2002; 18(2): 83–90. [https://doi.org/10.1016/S0168-9525\(02\)02557-X](https://doi.org/10.1016/S0168-9525(02)02557-X).
- 993 43. Huang X, Han B. Natural variations and genome-wide association studies in crop plants.  
994 *Annu Rev Plant Biol.* 2014; 65: 531–51. [https://doi.org/10.1146/annurev-arplant-](https://doi.org/10.1146/annurev-arplant-050213-035715)  
995 [050213-035715](https://doi.org/10.1146/annurev-arplant-050213-035715).
- 996 44. Sukumaran S, Yu J. Association mapping of genetic resources: achievements and future  
997 perspectives. In: Tuberosa R, Graner A, Frison E, editors. *Genomics of plant genetic*  
998 *resources*. Dordrecht, Netherlands: Springer; 2014. pp. 207–35.
- 999 45. Gizaw SA, Godoy JGV, Garland-Campbell K, Carter AH. Genome-Wide Association  
1000 Study of Yield and Component Traits in Pacific Northwest Winter Wheat. *Crop Sci.*  
1001 2018; 58(6): 2315–30. <https://doi.org/10.2135/cropsci2017.12.0740>.
- 1002 46. Zuiderveen GH, Padder BA, Kamfwa K, Song Q, Kelly JD. Genome-Wide Association  
1003 Study of Anthracnose Resistance in Andean Beans (*Phaseolus vulgaris*). *PLoS One.*  
1004 2016; 11(6): e0156391 <https://doi.org/10.1371/journal.pone.0156391>.
- 1005 47. Tock AJ, Fourie D, Walley PG, Holub EB, Soler A, Cichy KA, et al. Genome-Wide  
1006 Linkage and Association Mapping of Halo Blight Resistance in Common Bean to Race  
1007 6 of the Globally Important Bacterial Pathogen. *Front Plant Sci.* 2017; 8: 1170.  
1008 <https://doi.org/10.3389/fpls.2017.01170>.
- 1009 48. Tigist SG, Melis R, Sibiya J, Amelework AB, Keneni G, Tegene A. Population Structure  
1010 and Genome-Wide Association Analysis of Bruchid Resistance in Ethiopian Common  
1011 Bean Genotypes. *Crop Sci.* 2019; 59(4): 1504–15.  
1012 <https://doi.org/10.2135/cropsci2018.09.0559>.
- 1013 49. Nkhata W, Shimelis H, Melis R, Chirwa R, Mzengeza T, Mathew I, et al. Combining  
1014 ability analysis of common bean (*Phaseolus vulgaris* L.) genotypes for resistance to  
1015 bean fly (*Ophiomyia* spp.), and grain yield and component traits. *Euphytica.* 2021;  
1016 217(5): 93. <https://doi.org/10.1007/s10681-021-02833-9>.
- 1017 50. Zia B, Shi A, Olaoye D, Xiong H, Ravelombola W, Gepts P, et al. Genome-Wide  
1018 Association Study and Genomic Prediction for Bacterial Wilt Resistance in Common  
1019 Bean (*Phaseolus vulgaris*) Core Collection. *Front Genet.* 2022; 13: 853114.  
1020 <https://doi.org/10.3389/fgene.2022.853114>.
- 1021 51. Mukeshimana G, Butare L, Cregan PB, Blair MW, Kelly JD. Quantitative Trait Loci  
1022 Associated with Drought Tolerance in Common Bean. *Crop Sci.* 2014; 54(3): 923–38.  
1023 <https://doi.org/10.2135/cropsci2013.06.0427>.

- 1024 52. Trapp JJ, Urrea CA, Cregan PB, Miklas PN. Quantitative Trait Loci for Yield under  
1025 Multiple Stress and Drought Conditions in a Dry Bean Population. *Crop Sci.* 2015;  
1026 55(4): 1596–607. <https://doi.org/10.2135/cropsci2014.11.0792>.
- 1027 53. Briñez B, Perseguinti JM, Rosa JS, Bassi D, Gonçalves JGR, Almeida C, et al.  
1028 Mapping QTLs for drought tolerance in a SEA 5 x AND 277 common bean cross with  
1029 SSRs and SNP markers. *Genet Mol Biol.* 2017; 40(4): 813–23.  
1030 <https://doi.org/10.1590/1678-4685-GMB-2016-0222>.
- 1031 54. Kamfwa K, Cichy KA, Kelly JD. Genome-wide association analysis of symbiotic  
1032 nitrogen fixation in common bean. *Theor Appl Genet.* 2015; 128(10): 1999–2017.  
1033 <https://doi.org/10.1007/s00122-015-2562-5>.
- 1034 55. Cichy KA, Wiesinger JA, Mendoza FA. Genetic diversity and genome-wide association  
1035 analysis of cooking time in dry bean (*Phaseolus vulgaris* L.). *Theor Appl Genet.* 2015;  
1036 128(8): 1555–67. <https://doi.org/10.1007/s00122-015-2531-z>.
- 1037 56. Makunde G. Quantification of genetic diversity for drought adaptation in a reference  
1038 collection of common bean (*Phaseolus vulgaris* L.). Doctoral Thesis, University of Free  
1039 State. 2013. Available from: <https://scholar.ufs.ac.za:8080/handle/11660/8210>.
- 1040 57. Moghaddam SM, Mamidi S, Osorno JM, Lee R, Brick M, Kelly J, et al. Genome-Wide  
1041 Association Study Identifies Candidate Loci Underlying Agronomic Traits in a Middle  
1042 American Diversity Panel of Common Bean. *Plant Genome.* 2016; 9(3): 1–21.  
1043 <https://doi.org/10.3835/plantgenome2016.02.0012>.
- 1044 58. Beattie AD, Larsen J, Michaels TE, Pauls KP. Mapping quantitative trait loci for a  
1045 common bean (*Phaseolus vulgaris* L.) ideotype. *Genome.* 2003; 46(3): 411–22.  
1046 <https://doi.org/10.1139/g03-015>.
- 1047 59. Blair MW, Iriarte G, Beebe S. QTL analysis of yield traits in an advanced backcross  
1048 population derived from a cultivated Andean x wild common bean (*Phaseolus vulgaris*  
1049 L.) cross. *Theor Appl Genet.* 2006; 112(6): 1149–63. <https://doi.org/10.1007/s00122-006-0217-2>.
- 1050
- 1051 60. Wright EM, Kelly JD. Mapping QTL for seed yield and canning quality following  
1052 processing of black bean (*Phaseolus vulgaris* L.). *Euphytica.* 2011; 179(3): 471–84.  
1053 <https://doi.org/10.1007/s10681-011-0369-2>.
- 1054 61. Checa OE, Blair MW. Inheritance of Yield-Related Traits in Climbing Beans  
1055 (*Phaseolus vulgaris* L.). *Crop Sci.* 2012; 52(5): 1998–2013.  
1056 <https://doi.org/10.2135/cropsci2011.07.0368>.
- 1057 62. Payne RW, Murray DA, Harding S. An introduction to GenStat command language.

- 1058 Hampstead, UK: VSN International; 2018.
- 1059 63. Cullis BR, Smith AB, Coombes NE. On the design of early generation variety trials with  
1060 correlated data. *J Agric Biol Environ Stat.* 2006; 11(4): 381–93.  
1061 <https://doi.org/10.1198/108571106X154443>.
- 1062 64. Johnson HW, Robinson HF, Comstock RE. Estimates of Genetic and Environmental  
1063 Variability in Soybeans. *Agron J.* 1955; 47(7): 314–8.  
1064 <https://doi.org/10.2134/agronj1955.00021962004700070009x>.
- 1065 65. DArT. Extraction protocol for DArT. Camberra, Australia; 2014.
- 1066 66. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL:  
1067 software for association mapping of complex traits in diverse samples. *Bioinformatics.*  
1068 2007; 23(19): 2633–5. <https://doi.org/10.1093/bioinformatics/btm308>.
- 1069 67. Qin P, Lin Y, Hu Y, Liu K, Mao S, Li Z, et al. Genome-wide association study of  
1070 drought-related resistance traits in *Aegilops tauschii*. *Genet Mol Biol.* 2016; 39(3): 398–  
1071 407. <https://doi.org/10.1590/1678-4685-GMB-2015-0232>.
- 1072 68. Pritchard J, Wen X, Falush D. Documentation for Structure Software: Version 2.3.  
1073 University of Chicago, Chicago, 2010.
- 1074 69. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using  
1075 the software STRUCTURE: a simulation study. *Mol Ecol.* 2005; 14(8): 2611–20.  
1076 <https://doi.org/10.1111/j.1365-294X.2005.02553.x>.
- 1077 70. Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for  
1078 visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet*  
1079 *Resour.* 2012; 4(2): 359–61. <https://doi.org/10.1007/s12686-011-9548-7>.
- 1080 71. Ojwang PPO, Eldridge T, Corredor-Moreno P, Njung'e V. Structure of genetic diversity  
1081 and genome-wide association studies of bean fly (*Ophiomyia spencerella*) resistance in  
1082 common bean. *Euphytica.* 2021; 217(12): 216. <https://doi.org/10.1007/s10681-021-02949-y>.
- 1084 72. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal  
1085 components analysis corrects for stratification in genome-wide association studies. *Nat*  
1086 *Genet.* 2006; 38(8): 904–9. <https://doi.org/10.1038/ng1847>.
- 1087 73. Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, et al. GAPIT: genome  
1088 association and prediction integrated tool. *Bioinformatics.* 2012; 28(18): 2397–9.  
1089 <https://doi.org/10.1093/bioinformatics/bts444>.
- 1090 74. Raggi L, Caproni L, Carboni A, Negri V. Genome-Wide Association Study Reveals  
1091 Candidate Genes for Flowering Time Variation in Common Bean (*Phaseolus vulgaris*

- 1092 L.). *Front Plant Sci.* 2019; 10. <https://doi.org/10.3389/fpls.2019.00962>.
- 1093 75. Liu P, Jin Y, Liu J, Liu C, Yao H, Luo F, et al. Genome-wide association mapping of  
1094 root system architecture traits in common wheat (*Triticum aestivum* L.). *Euphytica*.  
1095 2019; 215(7): 121. <https://doi.org/10.1007/s10681-019-2452-z>.
- 1096 76. Yin L. R package “CMPlots.” 2016. [cited 2022 February 11]. Available from:  
1097 <https://github.com/YinLiLin/R-CMplot>.
- 1098 77. Shin J-H, Blay S, McNeney B, Graham J. LDheatmap: An R Function for Graphical  
1099 Display of Pairwise Linkage Disequilibria Between Single Nucleotide Polymorphisms.  
1100 *J Stat Software, Code Snippets.* 2006; 16(3): 1–9. <https://doi.org/10.18637/jss.v016.c03>.
- 1101 78. R. Core Team. Vienna: R Foundation for Statistical Computing. 2016.
- 1102 79. Blair MW, Cortés AJ, Farmer AD, Huang W, Ambachew D, Penmetza RV, et al. Uneven  
1103 recombination rate and linkage disequilibrium across a reference SNP map for common  
1104 bean (*Phaseolus vulgaris* L.). *PLoS One.* 2018; 13(3): e0189597.  
1105 <https://doi.org/10.1371/journal.pone.0189597>.
- 1106 80. Assefa T, Beebe SE, Rao IM, Cuasquer JB, Duque MC, Rivera M, et al. Pod harvest  
1107 index as a selection criterion to improve drought resistance in white pea bean. *Field*  
1108 *Crops Res.* 2013; 148: 24–33. <https://doi.org/10.1016/j.fcr.2013.04.008>.
- 1109 81. Assefa T, Rao IM, Cannon SB, Wu J, Gutema Z, Blair M, et al. Improving adaptation  
1110 to drought stress in white pea bean (*Phaseolus vulgaris* L.): Genotypic effects on grain  
1111 yield, yield components and pod harvest index. *Plant Breed.* 2017; 136(4): 548–61.  
1112 <https://doi.org/10.1111/pbr.12496>.
- 1113 82. Mathobo R, Marais D, Steyn JM. The effect of drought stress on yield, leaf gaseous  
1114 exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.). *Agric*  
1115 *Water Manag.* 2017; 180: 118–25. <https://doi.org/10.1016/j.agwat.2016.11.005>.
- 1116 83. Schneider KA, Rosales-Serna R, Ibarra-Perez F, Cazares-Enriquez B, Acosta-Gallegos  
1117 JA, Ramirez-Vallejo P, et al. Improving Common Bean Performance under Drought  
1118 Stress. *Crop Sci.* 1997; 37(1): 43–50.  
1119 <https://doi.org/10.2135/cropsci1997.0011183X003700010007x>.
- 1120 84. Beebe S. Biofortification of Common Bean for Higher Iron Concentration. *Front Sustain*  
1121 *Food Syst.* 2020; 4: 206. <https://doi.org/10.3389/fsufs.2020.573449>.
- 1122 85. Liu Z, Gao S, Zhang H, Xu Z, Qian W. Genome-Wide Association Study Reveals That  
1123 PvGUX1\_1 Is Associated with Pod Stringlessness in Snap Bean (*Phaseolus vulgaris*  
1124 L.). *Biology (Basel).* 2022; 11(4): 611. <https://doi.org/10.3390/biology11040611>.
- 1125 86. Crop Breeding Institute. Release proposal for a drought tolerant bean line: Sweet

- 1126 William. Harare, Zimbabwe; 2017.
- 1127 87. Crop Breeding Institute. Release proposal for a biofortified bean line: NUA674. Harare,  
1128 Zimbabwe; 2018.
- 1129 88. Crop Breeding Institute. Release proposal for a biofortified bean line: SMC16. Harare,  
1130 Zimbabwe; 2019.
- 1131 89. Kondwakwenda A, Mutari B, Simango K, Nchanji EB, Chirwa R, Rubyogo JC, et al.  
1132 Decades of Cultivar Development: A Reconciliation of Maize and Bean Breeding  
1133 Projects and Their Impacts on Food, Nutrition Security, and Income of Smallholder  
1134 Farmers in Sub-Saharan Africa. In: Mupambwa HA, Nciizah AD, Nyambo P, Muchara  
1135 B, Gabriel NN, editors. Food Security for African Smallholder Farmers. Singapore:  
1136 Springer Nature Singapore; 2022. pp. 3–26.
- 1137 90. Singh SP. Selection for Water-Stress Tolerance in Interracial Populations of Common  
1138 Bean. *Crop Sci.* 1995; 35(1): 118–24.  
1139 <https://doi.org/10.2135/cropsci1995.0011183X003500010022x>.
- 1140 91. Islam FMA, Beebe S, Muñoz M, Tohme J, Redden RJ, Basford KE. Using molecular  
1141 markers to assess the effect of introgression on quantitative attributes of common bean  
1142 in the Andean gene pool. *Theor Appl Genet.* 2004; 108(2): 243–52.  
1143 <https://doi.org/10.1007/s00122-003-1437-3>.
- 1144 92. Vos PG, Paulo MJ, Voorrips RE, Visser RGF, van Eck HJ, van Eeuwijk FA. Evaluation  
1145 of LD decay and various LD-decay estimators in simulated and SNP-array data of  
1146 tetraploid potato. *Theor Appl Genet.* 2017; 130(1): 123–35.  
1147 <https://doi.org/10.1007/s00122-016-2798-8>.
- 1148 93. Mwadzingeni L, Shimelis H, Rees DJG, Tsilo TJ. Genome-wide association analysis of  
1149 agronomic traits in wheat under drought-stressed and non-stressed conditions. *PLoS*  
1150 *One.* 2017; 12(2): e0171692. <https://doi.org/10.1371/journal.pone.0171692>.
- 1151 94. El Gataa Z, El Hanafi S, Basheer F, Kehel Z, bouhouch Y, El Messoadi K, et al. Genome  
1152 wide association study of grain yield and yield related traits in spring bread wheat  
1153 (*Triticum aestivum* L.) under drought and heat conditions in three different locations. *J*  
1154 *Crop Sci Biotechnol.* 2021; 24(4): 361–73. [https://doi.org/10.1007/s12892-021-00084-](https://doi.org/10.1007/s12892-021-00084-7)  
1155 [7](https://doi.org/10.1007/s12892-021-00084-7).
- 1156 95. Sukumaran S, Reynolds MP, Sansaloni C. Genome-Wide Association Analyses Identify  
1157 QTL Hotspots for Yield and Component Traits in Durum Wheat Grown under Yield  
1158 Potential, Drought, and Heat Stress Environments. *Front Plant Sci.* 2018; 9.  
1159 <https://doi.org/10.3389/fpls.2018.00081>.

- 1160 96. Oladzad A, Porch T, Rosas JC, Moghaddam SM, Beaver J, Beebe SE, et al. Single and  
1161 Multi-trait GWAS Identify Genetic Factors Associated with Production Traits in  
1162 Common Bean Under Abiotic Stress Environments. *G3 Genes|Genomes|Genetics*.  
1163 2019; 9(6): 1881–92. <https://doi.org/10.1534/g3.119.400072>.
- 1164 97. N'Diaye A, Haile JK, Cory AT, Clarke FR, Clarke JM, Knox RE, et al. Single Marker  
1165 and Haplotype-Based Association Analysis of Semolina and Pasta Colour in Elite  
1166 Durum Wheat Breeding Lines Using a High-Density Consensus Map. *PLoS One*. 2017;  
1167 12(1): e0170941. <https://doi.org/10.1371/journal.pone.0170941>.
- 1168 98. Pi B, He X, Ruan Y, Jang J-C, Huang Y. Genome-wide analysis and stress-responsive  
1169 expression of CCCH zinc finger family genes in *Brassica rapa*. *BMC Plant Biol*. 2018;  
1170 18(1): 373. <https://doi.org/10.1186/s12870-018-1608-7>.
- 1171 99. Han G, Lu C, Guo J, Qiao Z, Sui N, Qiu N, et al. C2H2 Zinc Finger Proteins: Master  
1172 Regulators of Abiotic Stress Responses in Plants. *Front Plant Sci*. 2020; 11: 115.  
1173 <https://doi.org/10.3389/fpls.2020.00115>.
- 1174 100. Han G, Qiao Z, Li Y, Wang C, Wang B. The Roles of CCCH Zinc-Finger Proteins in  
1175 Plant Abiotic Stress Tolerance. *Int J Mol Sci*. 2021; 22(15): 8327.  
1176 <https://doi.org/10.3390/ijms22158327>.
- 1177 101. Ai Q, Pan W, Zeng Y, Li Y, Cui L. CCCH Zinc finger genes in Barley: genome-wide  
1178 identification, evolution, expression and haplotype analysis. *BMC Plant Biol*. 2022;  
1179 22(117): 1–20. <https://doi.org/10.1186/s12870-022-03500-4>.
- 1180 102. Wang W, Liu B, Xu M, Jamil M, Wang G. ABA-induced CCCH tandem zinc finger  
1181 protein OsC3H47 decreases ABA sensitivity and promotes drought tolerance in *Oryza*  
1182 *sativa*. *Biochem Biophys Res Commun*. 2015; 464(1): 33–7.  
1183 <https://doi.org/10.1016/j.bbrc.2015.05.087>.
- 1184 103. Seong SY, Shim JS, Bang SW, Kim J-K. Overexpression of OsC3H10, a CCCH-Zinc  
1185 Finger, Improves Drought Tolerance in Rice by Regulating Stress-Related Genes.  
1186 *Plants*. 2020; 9(10): 1298. <https://doi.org/10.3390/plants9101298>.
- 1187 104. Selvaraj MG, Jan A, Ishizaki T, Valencia M, Dedicova B, Maruyama K, et al.  
1188 Expression of the CCCH-tandem zinc finger protein gene OsTZF5 under a stress-  
1189 inducible promoter mitigates the effect of drought stress on rice grain yield under field  
1190 conditions. *Plant Biotechnol J*. 2020; 18(8): 1711–21.  
1191 <https://doi.org/10.1111/pbi.13334>.
- 1192 105. Lin P-C, Pomeranz MC, Jikumaru Y, Kang SG, Hah C, Fujioka S, et al. The Arabidopsis  
1193 tandem zinc finger protein AtTZF1 affects ABA- and GA-mediated growth, stress and



- 1194 gene expression responses. *Plant J.* 2011; 65(2): 253–68. <https://doi.org/10.1111/j.1365->  
1195 313X.2010.04419.x.
- 1196 106. Muthusamy M, Kim J-H, Kim JA, Lee S-I. Plant RNA Binding Proteins as Critical  
1197 Modulators in Drought, High Salinity, Heat, and Cold Stress Responses: An Updated  
1198 Overview. *Int J Mol Sci.* 2021; 22(13): 6731. <https://doi.org/10.3390/ijms22136731>.
- 1199 107. Zhou C, Chen R-J, Gao X-L, Li L-H, Xu Z-J. Heterologous expression of a rice RNA-  
1200 recognition motif gene OsCBP20 in *Escherichia coli* confers abiotic stress tolerance.  
1201 *Plant Omics.* 2014; 7(1): 28–34.
- 1202 108. Corpas FJ, Barroso JB. NADPH-generating dehydrogenases: Their role in the  
1203 mechanism of protection against nitro-oxidative stress induced by adverse  
1204 environmental conditions. *Front Environ Sci.* 2014; 2:55.  
1205 <https://doi.org/10.3389/fenvs.2014.00055>.
- 1206 109. Berny Mier y Teran JC, Konzen ER, Palkovic A, Tsai SM, Rao IM, Beebe S, et al. Effect  
1207 of drought stress on the genetic architecture of photosynthate allocation and  
1208 remobilization in pods of common bean (*Phaseolus vulgaris* L.), a key species for food  
1209 security. *BMC Plant Biol.* 2019; 19(1): 171. <https://doi.org/10.1186/s12870-019-1774->  
1210 2.
- 1211 110. Wang A-H, Yang L, Yao X-Z, Wen X-P. Overexpression of the pitaya  
1212 phosphoethanolamine N-methyltransferase gene (HpPEAMT) enhanced simulated  
1213 drought stress in tobacco. *Plant Cell, Tissue Organ Cult.* 2021; 146(1): 29–40.  
1214 <https://doi.org/10.1007/s11240-021-02040-3>.
- 1215 111. Singh A, Kanwar P, Yadav AK, Mishra M, Jha SK, Baranwal V, et al. Genome-wide  
1216 expressional and functional analysis of calcium transport elements during abiotic stress  
1217 and development in rice. *FEBS J.* 2014; 281(3): 894–915.  
1218 <https://doi.org/10.1111/febs.12656>.
- 1219 112. Zhang Y, Xiao W, Luo L, Pang J, Rong W, He C. Downregulation of OsPK1, a cytosolic  
1220 pyruvate kinase, by T-DNA insertion causes dwarfism and panicle enclosure in rice.  
1221 *Planta.* 2012; 235(1): 25–38. <https://doi.org/10.1007/s00425-011-1471-3>.
- 1222 113. Urano K, Maruyama K, Koyama T, Gonzalez N, Inzé D, Yamaguchi-Shinozaki K, et al.  
1223 CIN-like TCP13 is essential for plant growth regulation under dehydration stress. *Plant*  
1224 *Mol Biol.* 2022; 108(3): 257–75. <https://doi.org/10.1007/s11103-021-01238-5>.
- 1225 114. Kim M, Lee U, Small I, des Francs-Small CC, Vierling E. Mutations in an Arabidopsis  
1226 mitochondrial transcription termination factor-related protein enhance thermotolerance  
1227 in the absence of the major molecular chaperone HSP101. *Plant Cell.* 2012; 24(8): 3349–

1228 65. <https://doi.org/10.1105/tpc.112.101006>.

1229 **Supporting information**

1230

1231 **S1 Table. List of common bean genotypes used in the study, their sources and**  
1232 **structure membership coefficient (K2) for K = 2.**

1233

1234 **S2 Table. Grain yield (across environments), drought susceptibility index, geometric**  
1235 **mean productivity, drought tolerance index and percent grain yield reduction of the 185**  
1236 **Andean-Mesoamerican Diversity Panel.**

1237

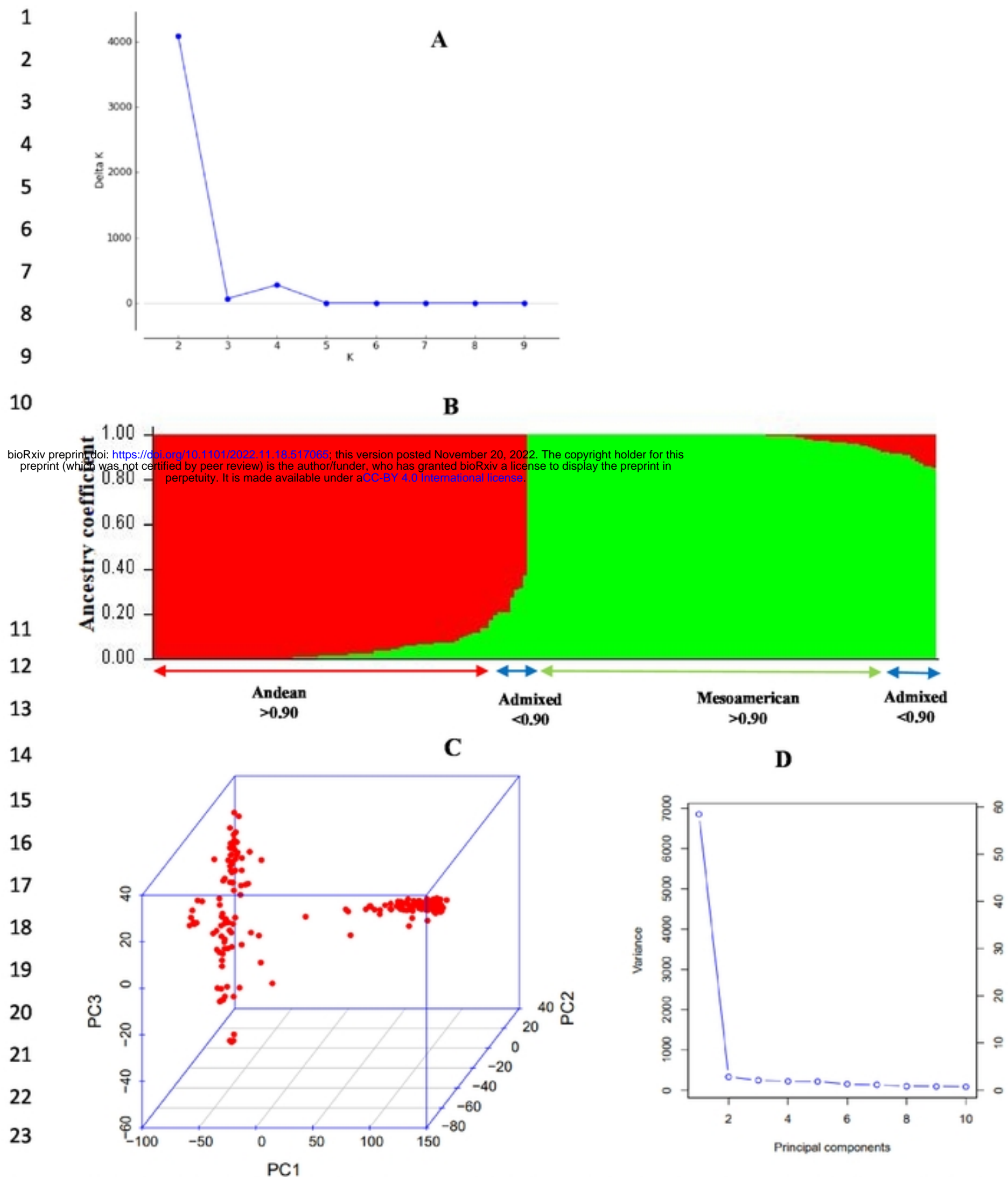
1238 **S3 Fig. Quantile –Quantile (QQ) of the p- values observed and the expected from the**  
1239 **genome-wide association study under drought stressed conditions.** Note A = Leaf  
1240 temperature, B = Days to 50% flowering, C = Grain yield, D = Plant height, E = Seed size, F  
1241 = Stomatal conductance.

1242

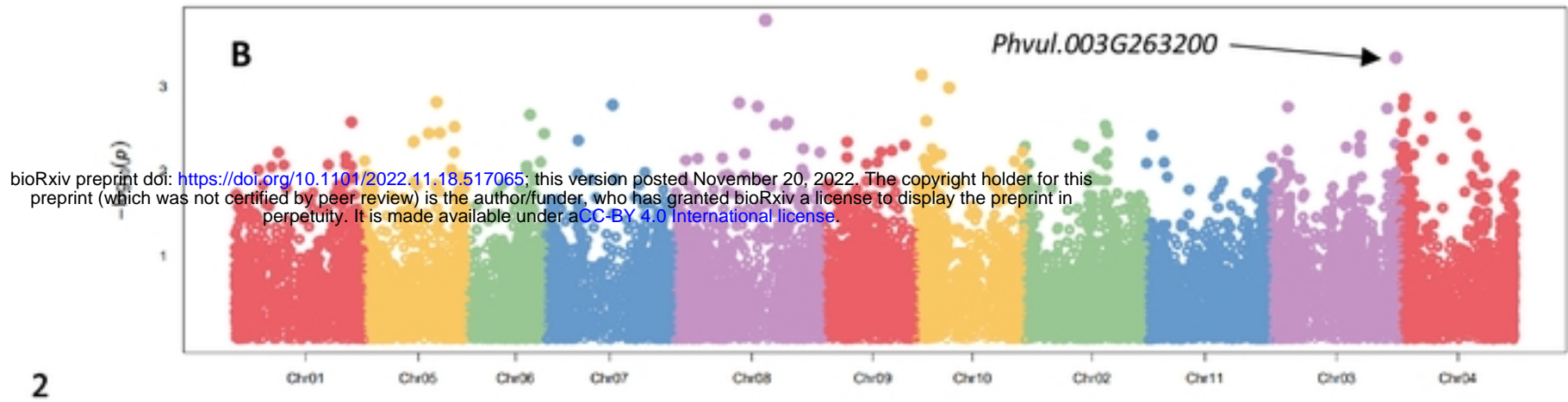
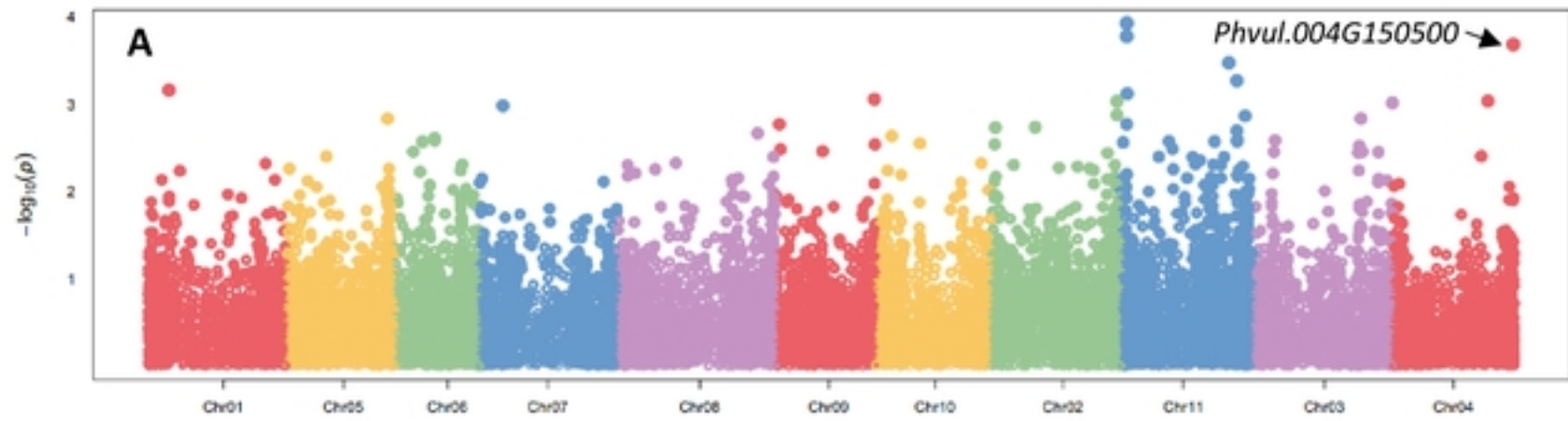
1243 **S4 Fig. Quantile –Quantile (QQ) of the p- values observed and the expected from the**  
1244 **genome-wide association study under well-watered conditions.** Note A = Days to 50%  
1245 flowering, B = Days to physiological maturity, C = Grain yield, D = Leaf chlorophyll  
1246 content, E = Plant height, F = Leaf temperature.

1247

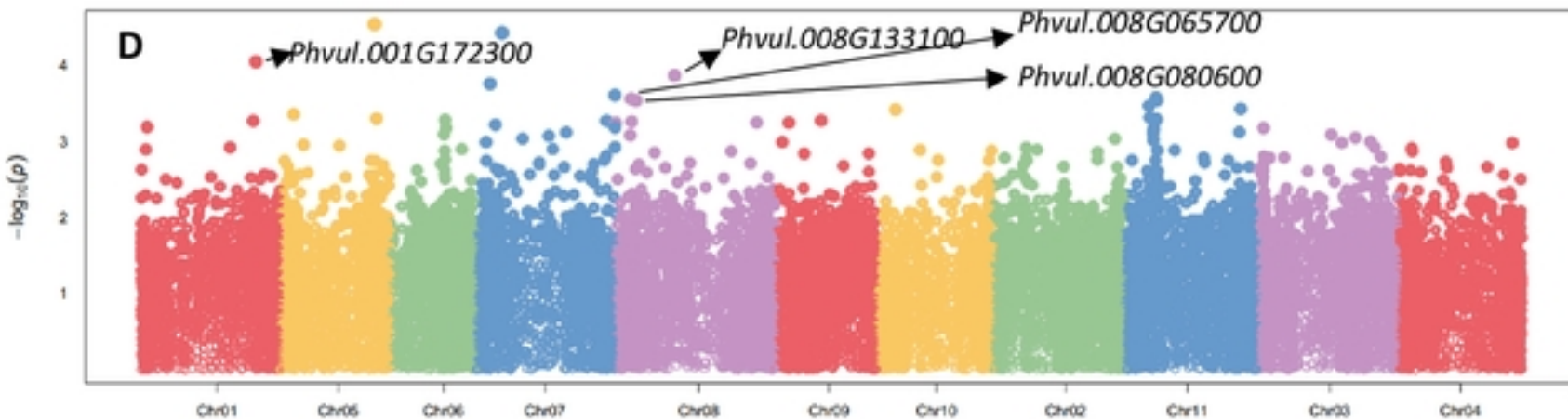
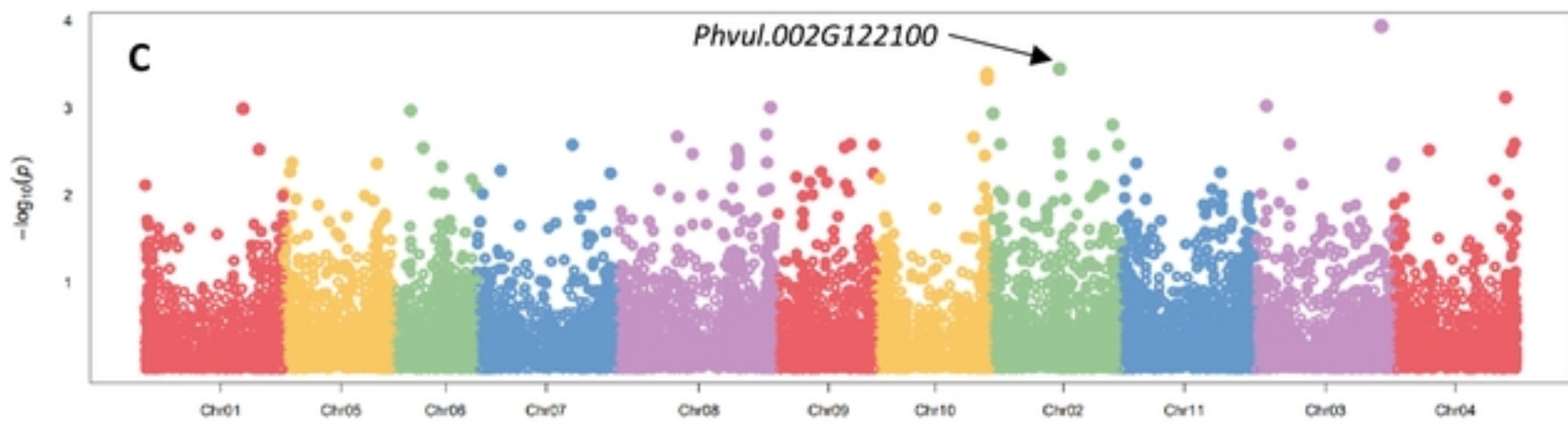
1248



24 **Fig 1. Population structure of 185 Andean and Mesoamerican Diversity Panel (AMDP) from different**  
 25 **models.** Note: A = The  $\Delta K$  determined by the Evanno method showing the stratification of the 185 AMDP into  
 26 two main sub-populations. The cluster with the largest  $\Delta K$  ( $K = 2$ ) was used to determine the number of sub-  
 27 populations in the AMDP of dry bean and the existence of two-sub-populations was inferred; B = Population  
 28 structure of 185 AMDP of dry bean genotypes based on 4095 SNP markers ( $K = 2$  gives the best separation) as  
 29 determined from STRUCTURE analysis. Red and green represents Andean and Mesoamerican sub-populations,  
 30 respectively; C = Three dimensional principal component analysis (PCA) scatter plot illustrating the population  
 31 structure of 185 AMDP of dry bean genotypes based on 9370 SNP markers; D = Screen plot showing the  
 32 percentage of variation explained by the different principal components.



bioRxiv preprint doi: <https://doi.org/10.1101/2022.11.18.517065>; this version posted November 20, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



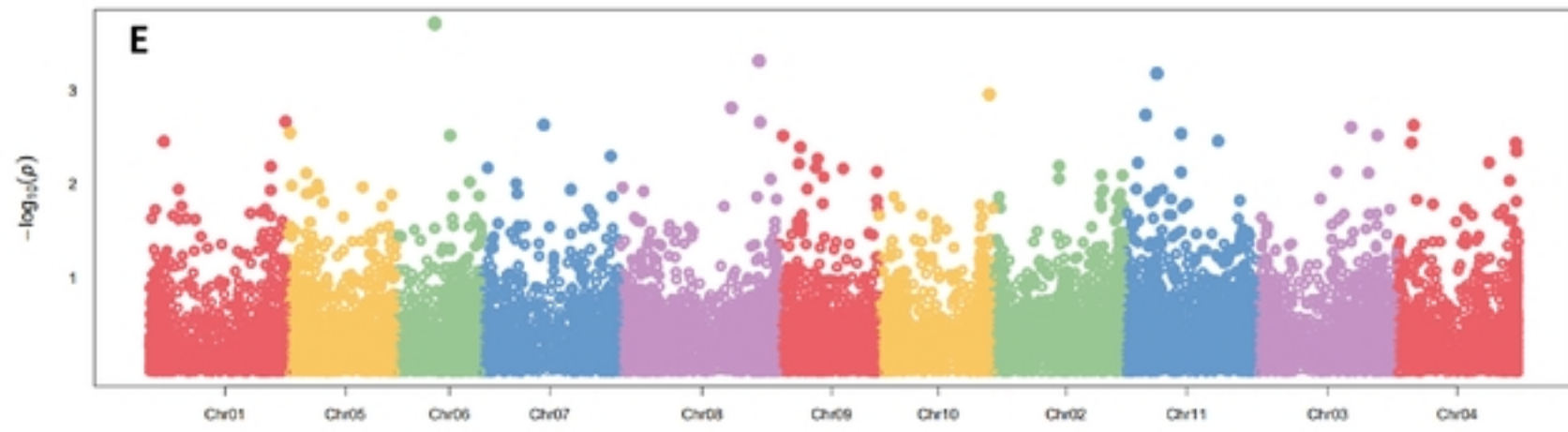
5

6 **Fig 1. Manhattan plots indicating the significant marker-trait associations, their p-values and candidate**  
 7 **genes for agronomic and physiological traits in 185 dry bean genotypes evaluated under drought stressed**  
 8 **conditions. Note: A = Grain yield, B = Seed size, C = Days to 50% flowering, D = Plant height, E = Leaf**  
 9 **temperature, F = Stomatal conductance. \*Chr represents Chromosome, x-axis represents the physical map**  
 10 **locations of the SNPs on each chromosome and the y-axis ( $-\log_{10} p$ -values) represents the degree to which a**  
 11 **SNP is associated with a trait.**

12

13

14



15

16

17

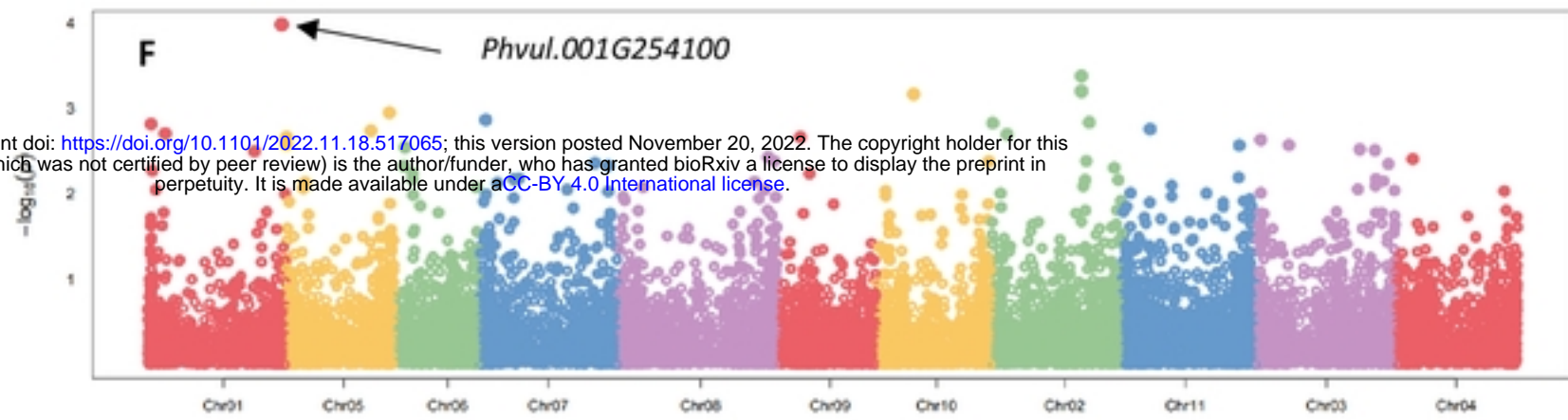
18

bioRxiv preprint doi: <https://doi.org/10.1101/2022.11.18.517065>; this version posted November 20, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

19

20

21

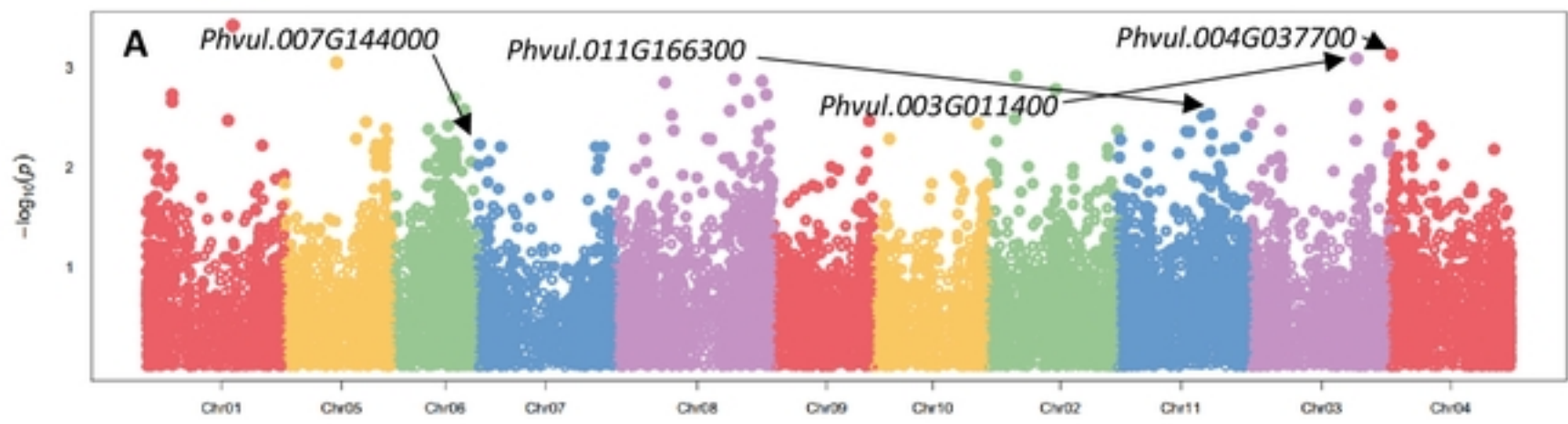


22

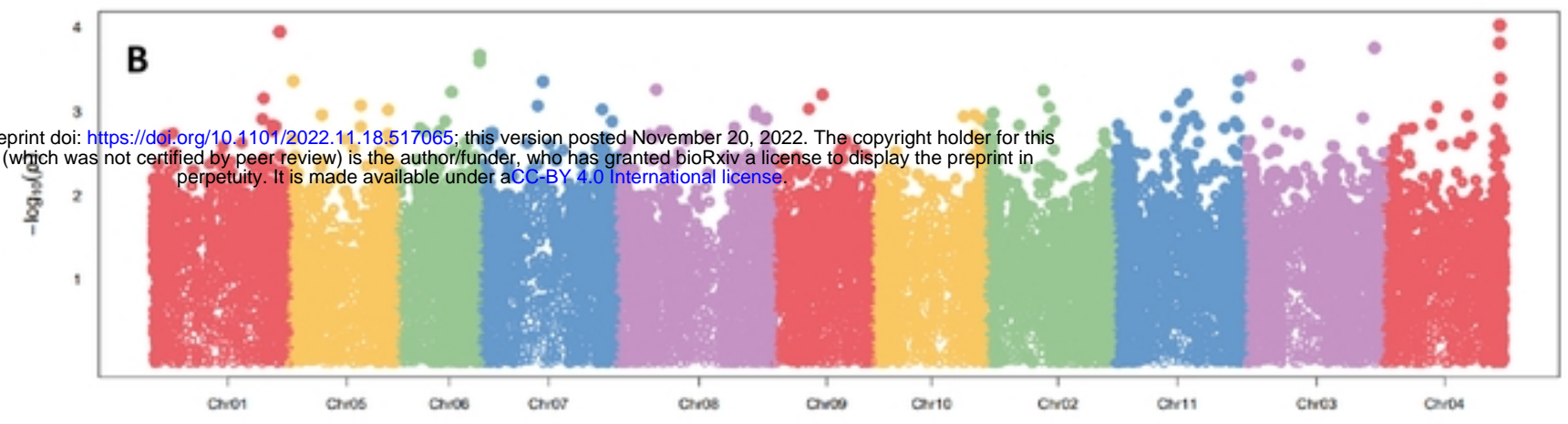
23 **Fig 2 (Continued).**

24

25

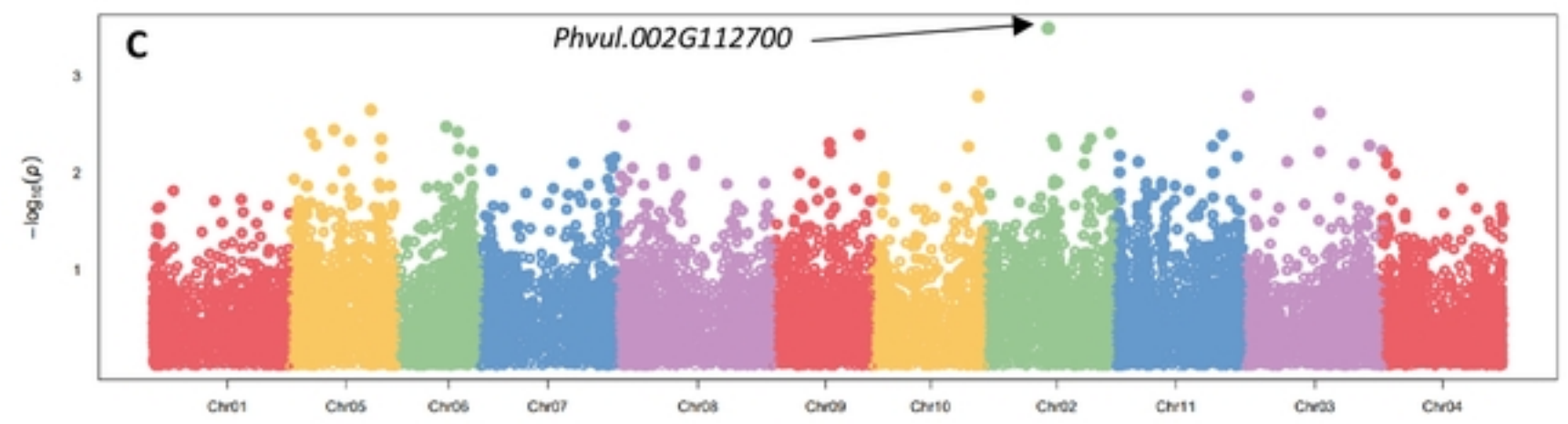


1  
2

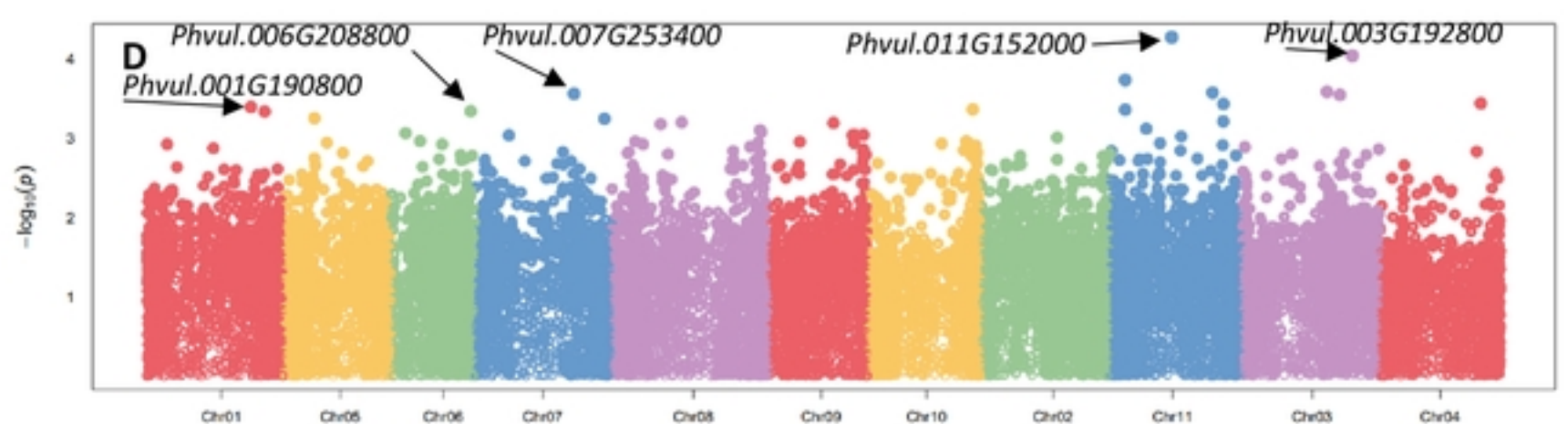


bioRxiv preprint doi: <https://doi.org/10.1101/2022.11.18.517065>; this version posted November 20, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

3  
4

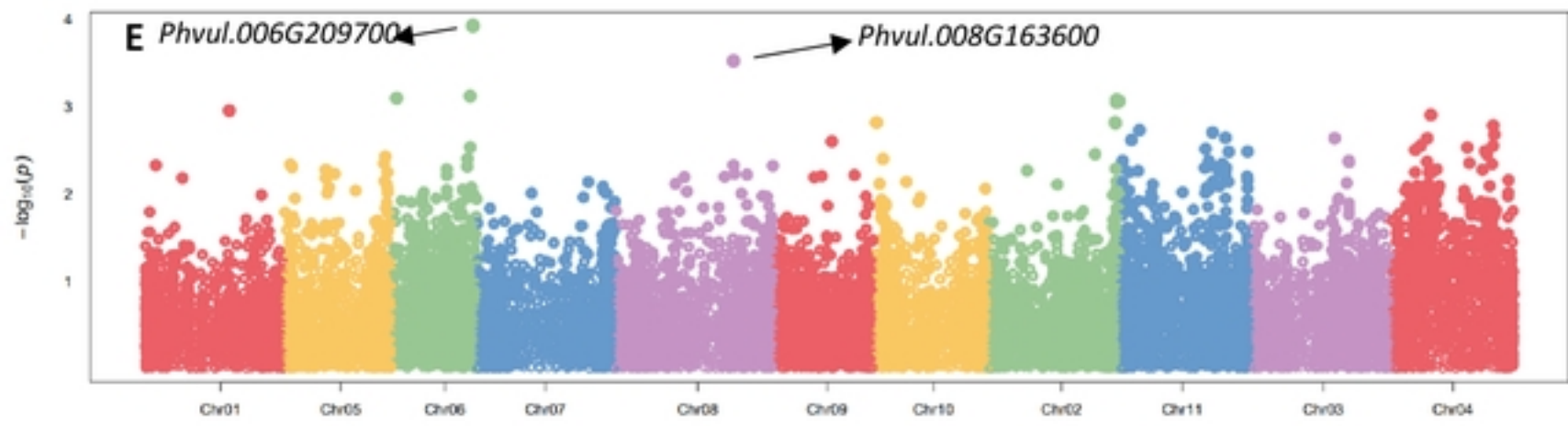


5  
6



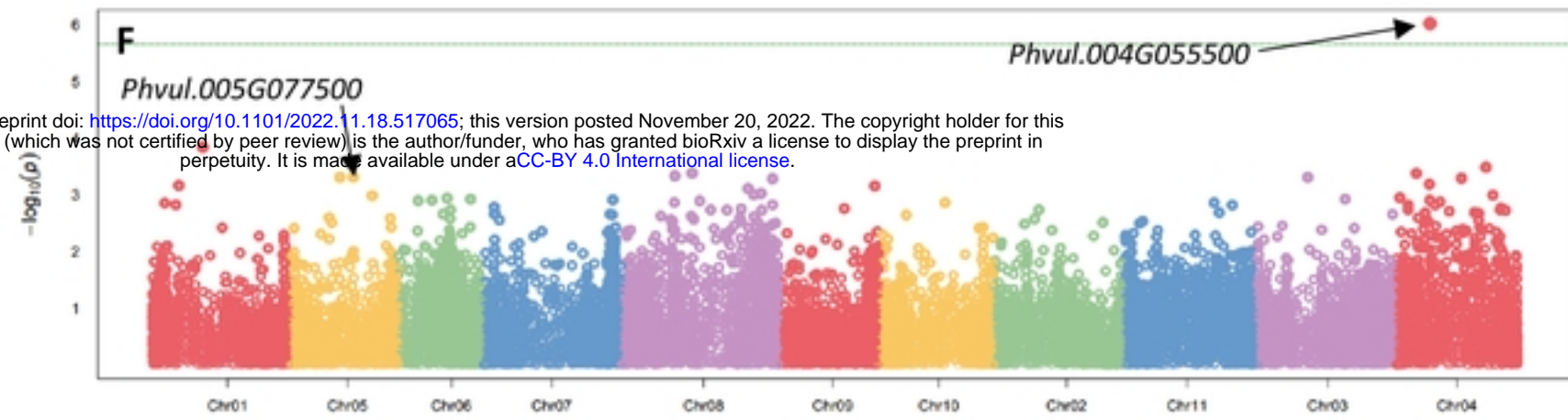
7

8 **Fig 1. Manhattan plots showing significant marker-trait associations, their p-values and candidate genes**  
 9 **for agronomic and physiological traits under well-watered conditions.** Note: A = Days to 50% flowering, B  
 10 = Grain Yield, C = Days to physiological maturity, D = Plant height, E = Leaf chlorophyll content, F = Leaf  
 11 temperature. \*Chr represents Chromosome, x-axis represents the physical map locations of the SNPs and the y-  
 12 axis ( $-\log_{10}$  p-values) represents the degree to which a SNP is associated with a trait.



13

14



bioRxiv preprint doi: <https://doi.org/10.1101/2022.11.18.517065>; this version posted November 20, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

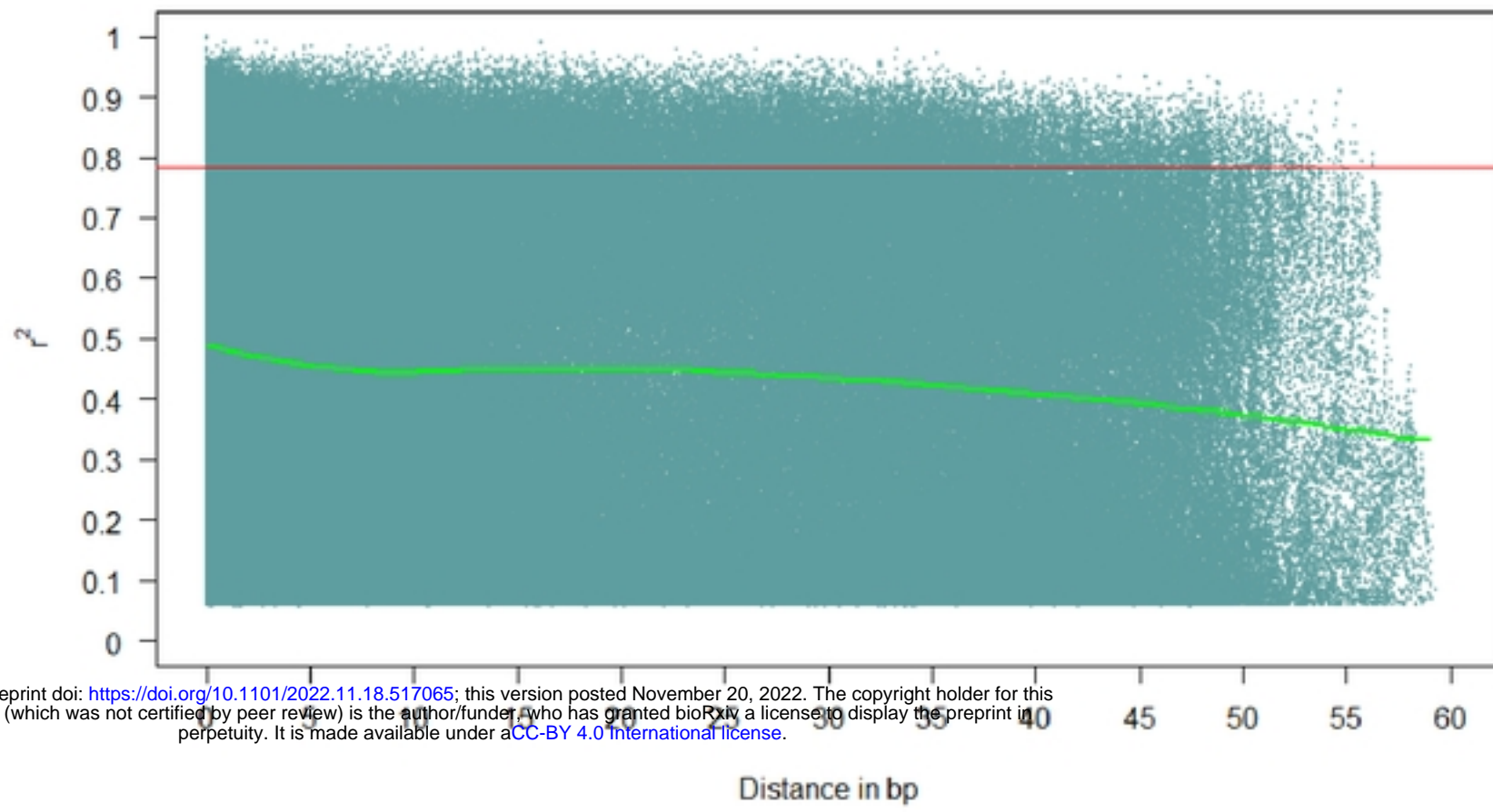
15

16 **Fig 3 (Continued).**

17

18

1



bioRxiv preprint doi: <https://doi.org/10.1101/2022.11.18.517065>; this version posted November 20, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

2

3 **Fig 1. Linkage disequilibrium (LD,  $r^2$ ) decay plot in genome of dry beans based on 9370 single nucleotide**  
4 **polymorphisms (SNPs) in 185 diverse genotypes.**

5