1 **Running Title:** QTL mapping of shoot and root trait divergence in *Panicum hallii*

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4 **The genetic architecture of shoot and root trait divergence between upland and lowland ecotypes of**

5 **a perennial grass**

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21 **Introduction**

22 Adaptation to abiotic stress is an important driver of contemporary evolution in plant populations. Abiotic

23 stressors have been implicated as driving factors in ecological speciation (Stebbins, 1952; Lexer & Fay,

24 2005), where populations have diverged across a number of traits, exhibit different niche characteristics,

25 and eventually become reproductively isolated (Clausen, 1951; Lowry, 2012; Yardeni e*t al.*, 2016). Local

26 adaptation to soil water availability is an especially important driver of plant evolution (Stebbins, 1952;

27 Rajakaruna, 2004; Kooyers *et al.,* 2015) and can impose strong natural selection on plant populations that

28 can lead to the formation of ecotypes that are differentially adapted to xeric and mesic habitats (Porter,

29 1966; Joly *et al*., 1989; Kumar *et al.*, 2008). Xeric and mesic ecotypes are often characterized by the

30 divergence of common suites of morphological and phenological traits (Clausen, 1951; Lowry, 2012)

31 related to maintaining water status and avoiding periods of drought (Chapin *et al.*, 1993; Markesteijn &

32 Poorter, 2009; Juenger, 2013).

33 Although leaf and shoot traits are important drivers of adaptation to drought (Tsialtas *et al*., 2004; Carmo-

34 Silva *et al*., 2009; Juenger, 2013), the properties of root systems determine plant water access and can

35 place constraints on shoot water status (Price e*t al.,* 2002; Hund *et al*., 2009). Shoot traits may be related

36 to root traits through genetic correlation (Bouteille *et al*., 2012) or dependent upon root traits though

37 resource allocation tradeoffs (Hammer *et al*., 2009), including changes in carbon allocation between root

38 and shoot systems (Hummel *et al*., 2010). Higher root mass ratio (RMR) increase water foraging

39 capability to maintain plant water status, which can be accomplished by allocating more resources

40 towards roots (Knights *et al*., 2006) or by inhibiting above ground growth (Hendricks *et al*., 2016). Many

41 root and shoot traits show correlated responses to water limitation and varied degrees of morphological

42 and physiological integration. For example, decreased soil moisture in xeric environments is positively

43 associated with high specific leaf area (SLA; Ramírez-Valiente & Cavender-Bares, 2017) and high

44 specific root length (SRL; Comas *et al*, 2013) while increased soil moisture is positively associated with

45 low SLA and low SRL (Price *et al*., 2017). Increased SRL can increase plant water acquisition under

46 drought without increasing carbon allocation per root length by producing longer and thinner roots

47 (Comas *et al*., 2013). Despite strong evidence that root and shoot trait covariance is an important driver of

48 plant adaptation to drought, few studies have documented how combinations of specific shoot and root

49 traits generate locally adapted ecotypes and the genetic basis of such trait complexes is poorly understood.

50 Genetic crosses and quantitative trait analysis are commonly used tools that can lead to a better

51 understanding of genetic architecture (Michael *et al*., 2003; Lowry *et al*., 2014b), and can help to

52 associate genetic variation among and between root and shoot traits with ecotype divergence.

53 Quantitative genetic analyses and the mapping of quantitative trait loci (QTL) permit exploration of the

54 genetic basis of trait correlations and trait divergence (Fishman *et al*., 2002; Lovell *et al.,* 2015; Milano *et*

55   *al*., 2016). Indeed, QTL mapping has been used for identifying the genetic basis of root traits and their

56   function in plant water acquisition (Johnson *et al.*, 2000; Tuberosa *et al*., 2002; Comas *et al*., 2013).

57   Importantly, by simultaneously analyzing multiple traits, QTL mapping can infer the loci that drive

58   ecological trait correlations. Functional traits with a high degree of correlation that underlie divergence

59   can result from pleiotropy or genetic linkage through shared developmental genetics (Lande, 1980; Via &

60   Hawthorne, 2005; Lovell *et al*., 2013) or as a result of correlational selection (Brodie *et al*., 1995). For

61   example, colocalized QTL for root and shoot traits including root biomass, root volume, shoot biomass

62   and plant height have been identified in a wheat recombinant inbred line population (Iannucci *et al*.,

63   2017), which may be driven by pleiotropy and / or multiple physically linked genes. Overall, there is

64   growing evidence for substantial genetic variation in root system architecture and root/shoot relationships.

65   However, the loci driving these trait correlations and the degree to which these patterns impact plant

66   productivity are largely unknown.

67   *Panicum hallii* is a small, self-fertilizing, C_4 perennial bunch grass native to North America. *P. hallii*

68   occurs across a large geographical range with diverse habitats and climates. Average annual precipitation

69   ranges from 127 cm per year on the eastern border of its distribution to 13 cm per year on the west. *P.*

70   *hallii* occurs as two distinct ecotypes (upland and lowland) that are classified as separate subspecies, *P.*

71   *hallii* subsp. *hallii* (hereafter referred to as *hallii*) and *P. hallii* subsp. *filipes* (hereafter referred to as

72   *filipes*). *Hallii* is typically found in upland xeric (habitats with shallow, dry, calcareous and rocky soils in

73   the American southwest and northern Mexico; while *filipes* occurs in lowland mesic areas on clay and silt

74   soils mostly along the Gulf Coast Plain of Texas and Mexico (Gould, 1975; Waller, 1976). The upland

75   ecotype, *hallii*, is smaller in stature and overall size than the lowland ecotype *filipes*: with smaller leaves,

76   fewer tillers, earlier flowering time, less flowers per inflorescence, but larger seed size and seed mass

77   (Waller, 1976; Lowry *et al*., 2013). This is consistent with its polyploid relative, *Panicum virgatum* (an

78   important biofuel candidate)*,* where upland ecotypes are typically smaller, flower earlier (Lowry *et al*.,

79   2014b) and have lower leaf area (McMillan, 1965) than lowland ecotypes. Previous analyses of shoot

80   traits in a F_2 population of *P. hallii* (Lowry *et al.*, 2014a) demonstrated that a few large-effect loci drove

81   multivariate shoot trait divergence between *hallii* and *filipes.* Here, we investigate the genetic architecture

82   of multidimensional root phenotypic traits and their relationship with shoot phenotypic traits to develop a

83   more complete picture of the adaptive differences between these ecotypes.

84   In this study, we develop and analyze a recombinant inbred population derived from a cross between the

85   upland and lowland ecotypes of *P. hallii*. We employ QTL mapping to identify the genetic architecture of

86   root and shoot trait relationships and their divergence among ecotypes. Specifically, we sought to answer

87   three questions. Are shared QTL involved in genetic correlations between root and shoot traits and

88   biomass partitioning? Do allelic effects of individual QTL underscore root and shoot trait divergence

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89   between *hallii* and *filipes*? Will observed QTL and genetic correlations in the RIL population be

90   preserved across two experiments conducted separately under glasshouse and field conditions.

91   **Materials and Methods**

92   *Development of the RIL mapping population*

93   We developed a population of recombinant inbred lines (RILs) in order to evaluate the genetic basis of

94   divergence between *hallii* and *filipes*. The parents of the RIL mapping population were genotypes

95   selected from populations of the upland and lowland ecotypes of *P. hallii*. The upland parent (HAL2-11,

96   hereafter referred to as HAL2) was a one-generation selfed progeny of an individual selected from a

97   glasshouse planting of seed collected from a natural population of *hallii* located at the Lady Bird Johnson

98   Wildflower Center (Austin, TX, USA; 30.16°N, 97.87°W). The lowland parent (FIL2) was selected from

99   a glasshouse planting of seed collected from a natural population of *filipes* located near the coastal city of

100   Corpus Christi, Texas (27.65°N, 97.40°W). FIL2 and HAL2 represent the genome reference genotypes

101   for *filipes* and *hallii* respectively (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Phallii)

102   and are largely homozygous individuals. A cross of these two genotypes, with HAL2 as the maternal

103   parent, yielded an F_1 hybrid and self-fertilized seed obtained from this individual was used to establish a

104   large F_2 population (Lowry, 2012). A number of these F_2 progeny were selected at random and

105   propagated repeatedly via single seed descent until the F_6 generation. DNA was obtained from leaf tissue

106   of F_6 seedlings and submitted for whole genome resequencing at the DOE Joint Genome Institute through

107   the Community Science Program. F_7 seed was subsequently collected from the sequenced F_6 individuals

108   for this experiment.

109   SNPs were called from whole genome resequencing of 356 RIL lines on four Illumina 2x150 runs at 12x

110   coverage. Libraries were quality filtered using the fastx toolkit 'fastq_quality_filter' program with a

111   quality threshold of 33. Filtered reads were mapped to a soft masked *P. hallii* reference genome (FIL-2

112   V2.0) using bwa mem with the default parameters. Mapped reads were filtered by samtools –Shb with a

113   quality of 20. Bam files were indexed, sorted and duplicates were removed with picard. Reads adjacent to

114   insertions / deletions were masked using GATK RealignerTargetCreator and reads were re-sorted and re-

115   indexed prior to SNP calling. SNPs were called via GATK haplotypeCaller independently for each

116   library, producing a gVCF for each. These were merged and re-genotyped by GATK's genotypeGVCF

117   and condensed into a 0/1/2 (alternate allele counts) matrix with vcfTools. Genotype data from 335 RIL

118   lines were included in the output genotype matrix. The resultant matrix was processed in R. SNPs with

119   >10% and <80% homozygotes and <5% NA and <20% heterozygotes were retained.

120   We applied a 3-step sliding window approach for marker calling: 1) The genome was broken into 200

121   marker windows (overlapping by 100 markers) and the proportion of each genotype was calculated. 2)

122   Training data was constructed, retaining the 100 strongest heterozygous sites and a random sampling of

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123   100 of the sites with > the mean proportion of each homozygote; 3) A random forest machine learning

124   model was fit to the training data (the R caret package) and used to predict the genotypes of all sliding

125   window intervals resulting in a 3361 marker matrix.

126   *Genetic map construction*

127   To build the genetic map, we culled the genotype matrix such that no two markers could have a pairwise

128   recombination fraction <0.005. This culling procedure minimized the amount of segregation distortion

129   and missing data within any 0.5 cM window. Linkage groups were formed from the resulting 1278

130   marker matrix. Markers were ordered within linkage groups using a travelling salesperson problem solver

131   as implemented through the concorde program and parsed through the TSPMap function tspOrder

132   (Monroe *et al.*, 2017). We then fine-tuned the resulting genetic map first by culling the genotype matrix to

133   a 711-marker grid where no markers resided <1cM from an adjacent marker, then looking at improving

134   the fine-order of markers using the ripple algorithm. Finally, chromosomes were named and oriented to

135   maximize the similarity with the physical position of markers in the FIL2 genome annotation

136   (phytozome.net).

137   *Morphological shoot and root phenotyping under glasshouse conditions*

138   Seeds of 174 F_7 RILs and the two parental genotypes were scarified with sandpaper and placed on wet

139   sand in round petri dishes on September 5, 2016 and allowed to germinate in a glasshouse located at the

140   University of Texas at Austin, Brackenridge Field Lab (12-h days at 500 μE m^{-2} s^{-1}, 28°C; 12-h nights

141   at 24°C). On the 7th day after sowing, seedlings were transferred to 6 cm x 30 cm cone-tainers (Stuewe

142   and Sons, Tangent, OR). Cone-tainers were lined with 1 mil plastic liners (perforated at the bottom for

143   drainage) to facilitate separation of the plant from the container during harvest. Cone-tainers were filled

144   with Field and Fairway Profile (The Turf Trade, NJ, USA) media. Plants were then assigned to a

145   completely randomized block design within three blocks on a single glasshouse bench. Plants were

146   bottom watered every three days with Grow liquid nutrient solution (DynaGro, Richmond, CA) to

147   promote seedling growth. Plants were harvested within three days of a common developmental stage

148   defined as when a fully expanded flag leaf with a visible ligule was observable on any tiller with an

149   emerging panicle. The plant in its plastic bag was pulled from the pot gently to prevent damage to the root

150   system. Then the bag was cut open and the profile substrate was gently removed by shaking the plant on

151   wire mesh followed by light washing of the root system in a bucket of tap water. Shoot material was

152   separated from root material. The tiller height (from base of the plant to the node of the flag leaf on the

153   tiller with the emergent panicle), leaf length and area of the flag leaf of the main tiller were measured and

154   tiller number was counted at the time of harvest. Total root number was counted and then the root system

155   was spread out in a clear acrylic water filled tray and scanned at a 600 dpi resolution using an EPSON

156   Scanner (Model 12000XL, Epson America, Inc., San Jose, CA, USA) calibrated for use with WinRhizo

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157   Pro 2015 root image analysis software (Regent Instruments Inc., Canada). Leaf, shoot and root tissue was

158   collected separately, dried for 96 hours in an oven at 55°C, and weighed to obtain biomass. Specific leaf

159   area (SLA) was calculated as fresh leaf area divided by dry mass of the leaf ($cm^2 \ g^{-1}$).

160   Root trait data was obtained from scans using WinRhizo Pro 2015 software and included total root length

161   (cm), total root volume (cm^3), and average root diameter (mm). The Lagarde's local threshold parameter

162   in the analysis software was enabled to ensure detection of thin and pale roots and the diameter class size

163   was set to 0.25 mm to ensure accurate calculation of average root diameter. Specific root length (SRL;

164   total root length / root biomass ($cm \ g^{-1}$)), root tissue density (root biomass / total root volume ($g \ cm^{-3}$)),

165   root length density (total root length / soil volume ($cm \ cm^{-3}$)) and root mass ratio (RMR, root biomass /

166   total biomass) were calculated for each plant.

167   *Data and QTL analysis*

168   Data analyses centered on fitting linear mixed models and considered RIL genotype as a fixed effect (proc

169   mixed, SAS) for the measured shoot and root phenotypic traits. Block was also included as a fixed effect

170   covariate when it had a significant impact on measured traits (emergence day, specific root length and

171   root diameter). The SAS procedure PROC CORR was used to calculate genetic correlation coefficients of

172   traits based on RIL line means. Trait heritability was calculated using h2boot software with one-way

173   ANOVA among clonal lines with 1000 bootstrap runs (Phillips & Arnold, 1999). Trait divergence

174   between parental lines was tested with a t-test in SAS.

175   The majority of the measured traits were continuously distributed with relatively strong multivariate

176   structure based on pairwise correlational analyses. As such, we also used genetic principal component

177   analysis (PCA) to obtain a multidimensional overview of shoot and root trait variation and integration.

178   PCA analysis was performed on the trait means of each line for the following phenotypic variables:

179   emergence day, tiller number, root number, root biomass, shoot biomass, root diameter, root tissue

180   density, specific root length, specific leaf area, tiller height, leaf length, root volume and total root length.

181   PCA was completed using SAS with the proc princomp function. The first three principal components

182   that together explained 75% of total variation were retained for QTL analysis.

183   QTL mapping was completed in R using the R/qtl package (Broman & Sen, 2009) on the RIL breeding

184   values as described above (Table S2). When quantitative trait data distributions were not normally

185   distributed, data was log (emergence day, tiller number) or square root (shoot biomass) transformed. Two

186   functions were used to determine the position of QTL and to conduct the calculation of estimates for

187   additive effects and effects of epistasis (an additive-by-additive interaction between quantitative trait loci,

188   script: https://github.com/AlbinaKh/P.hallii_RIL_RootShoot_QTLmapping). The scantwo function with

189   1000 permutations was used to calculate penalties for main effect and interactions for each phenotypic

190   trait, and the stepwise QTL function was used to conduct a forward-backward search and account for

191   epistasis with a maximum of 6 QTL (at least two QTL peaks in addition to those detected with the

192   scanone function) that optimized the penalized LOD score criterion. Threshold values for type 1 error

193   rates were set at alpha = 0.05 for all traits based on permutation. 1.5 LOD drop interval of QTL was

194   calculated using the qtlStats function. QTL analysis was performed on the first three principal

195   components following the above procedure.

196   *Confirming root and shoot biomass QTL in a field study*

197   An important question in root biology is the degree to which genetic effects discovered in controlled

198   conditions (i.e. glasshouse, growth chamber, or artificial media experiments) relate to real world variation

199   in root system architecture in the field (Champoux *et al.*, 1995; Ochoa *et al.*, 2006; Lovell *et al.*, 2016).

200   To further confirm and evaluate major QTL detected in our glasshouse study, we conducted a follow up

201   experiment on a focal QTL during the 2016 growing season. Ten RILs homozygous at the shared QTL

202   region for root and shoot biomass were selected for this experiment (5 with *filipes* alleles and 5 with *hallii*

203   alleles). Seed of selected lines were germinated and established in the glasshouse using the procedure

204   outlined above for the RIL planting and subsequently transplanted into the field at the age of one month.

205   Eight biological replicates of each line and eight replicates of the parental genotypes were planted on May

206   10, 2016 under both restrictive and well-watered irrigation treatments (10 RIL lines x 2 parents x 8

207   biological replicates x 2 irrigation levels = 192 plants). All plants were well-watered for one week after

208   transplant for establishment.

209   The field experiment was conducted at a site located within the Brackenridge Field Laboratory property

210   of the University of Texas in Austin, TX, USA (N 30.2845, W 97.7809). The site elevation is 133 m above

211   sea level and soils are Yazoo sandy loam greater than 1.2 m deep. The mean maximum temperature

212   (August) is ~35.0 °C and the mean minimum temperature (January) is ~ 3.0 °C. This experiment was co-

213   planted in available space within an existing *P. hallii* experiment to take advantage of established

214   irrigation infrastructure. The site contains 32 differentially irrigated 'beds' which are separated

215   underground by 1.2-meter-deep plastic sheeting (Regal Plastics, Austin, TX, USA) to prevent the spread

216   of applied irrigation water. Irrigation was applied by dripline (0.9 GPH, 12" emitter spacing, Rain Bird,

217   Azusa, CA). The treatment period occurred from June through August with the restrictive treatment

218   receiving 4.5 fold less irrigation in both number of irrigation events and total amount of water applied.

219   Plants were harvested towards the end of the summer growing season in August over a three-day period.

220   To account for differences in size of the plants, an equal volume of the soil under each plant was

221   harvested using a 'shovelomics' device that regulated shovel angle and depth while extracting plants from

222   the field soil. Plants with roots attached were rinsed clean of soil over a metal screen and allowed to air

223   dry. Shoot biomass was then separated from root biomass, and both biomass types were dried at 55°C for

224   4 days before weighing. Trait values more extreme than 1.5x the interquartile range were removed as

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225   outliers prior to analysis. For statistical analysis, we used linear mixed models with proc mixed in SAS.

226   The main effect for the model was genotype of the QTL (*filipes* or *hallii* alleles at the marker position),

227   treatment and genotype by treatment interaction. RIL line was used as a random effect to control for

228   background genetic variance.

229   **Results**

230   *Heritable shoot and root trait differences between upland and lowland ecotypes*

231   The RIL parents representing upland and lowland ecotypes of *Panicum hallii* (HAL2 and FIL2) had

232   significantly different shoot trait mean values (Table 1) and also displayed corresponding differences

233   between root traits. The upland genotype, HAL2, had 2.3-fold faster first panicle emergence (*t* values at 5

234   dfs and *P* values; $t=2.87$, $P=0.035$), 3.3-fold less shoot biomass ($t=4.39$, $P=0.007$) and 2.8-fold less root

235   biomass ($t=3.08$, $P=0.028$), 1.8-fold shorter plant height ($t=3.43$, $P=0.018$), 2.2-fold shorter leaf length

236   ($t=6.3$, $P=0.001$), 2-fold shorter total root length ($t=3.29$, $P=0.022$), 2.5-fold lower total root volume

237   ($t=3.41$, $P=0.02$), and 1.3-fold increased specific root length ($t=-2.5$, $P=0.05$) relative to the lowland

238   genotype FIL2 (Table 1).

239   We estimated broad-sense trait heritability (H^2) as the proportion of observed phenotypic variance due to

240   genetic differences among RILs in the population. In the RIL population, all measured traits were

241   heritable, with H^2 ranging from 18% to 66% for shoot traits (bootstrap based significance, in all cases

242    $P<0.001$) and from 34% to 60% for root traits (bootstrap bases significance, in all cases $P<0.001$). The

243   most heritable traits were leaf length (66%), plant height (64%), shoot biomass (60%), root length (60%)

244   and root biomass (58%; Table 1). Transgressive segregation, where the range of recombinant phenotypes

245   extends beyond the range of parental values (Rieseberg *et al*., 1999), was found among the majority of

246   traits except shoot biomass, plant height, leaf length, root biomass and root number. In the parental lines,

247   FIL2 had trait values that were the highest or close to the highest of population wide values, while HAL2

248   values were generally in the middle of the population trait distribution (Table 1).

249   Many shoot and root phenotypic traits also showed remarkably strong genetic correlations in the RIL

250   population (Table 2). For example, shoot and root biomass (r=0.92, $P<0.0001$), tiller and root number

251   (r=0.67, $P<0.001$), shoot biomass and root volume (r=0.91, $P<0.0001$), and shoot biomass and total root

252   length (r=0.90, $P<0.001$). We performed principal component analysis (PCA) to characterize the

253   multivariate structure of our data. The first three PCA axes explained 75% of the overall trait variance.

254   Principal component one (PC1; 45.5% variance explained) was composed of general plant size traits.

255   Principal component two (PC2; 16.5%) was mainly composed of root resource acquisition traits. Principal

256   component three (PC3; 12.6%) was composed of carbon acquisition and allocation traits (Table S1; Fig.

257   1a, b, c).

258   *QTL underscore root and shoot trait divergence between hallii and filipes*

259   Given high H^2 values, it is not surprising that QTL were detected for all measured traits. A total of 31

260   QTL were identified for 13 phenotypic traits: two for one phenological trait, 14 QTL for five shoot traits

261   and 15 QTL for seven root traits (Table 3, Fig. 2). QTL for all traits showed additive effects in the

262   direction of parental divergence, except for one of three QTL for tiller number, one of four QTL for root

263   diameter, and one of three QTL for SRL. *Filipes* alleles increased trait values associated with phenology

264   and plant size, including: emergence day, root number, root tissue density, root biomass, shoot biomass,

265   tiller height, leaf length and root volume. *Hallii* alleles increased trait values associated with resource

266   acquisition and allocation, including: specific root length, root mass ratio, and specific leaf area.

267   The main effects of each QTL explained from 5.25% to 15.4% of phenotype variation for shoot traits, and

268   from 5.9% to 18.6% for root traits (Table 3). Of these 31 QTL, eight QTL occupied unique positions

269   throughout genome: RTD on chr1, leaf length on chr2, tiller number on chr3 and chr8, root number on

270   chr3, SLA on chr5, tiller height and root diameter on chr8. Three of these single QTL were also

271   confirmed by principle component QTL (Table 4, Fig. 2). The confidence intervals of all other QTL are

272   shared with at least one other QTL.

273   *Trait-specific QTL cluster into genomic 'hotspots'*

274   Divergence of correlated traits in natural populations may be driven by pleiotropic genes or linked genes

275   with correlated effects. We identified three major and five minor clusters of root and shoot trait QTL over

276   five different chromosomes (Table 3, Fig. 1). Here we identify clusters (CL) by chromosome number and

277   number from the 0 cM position for each chromosome. As expected, we found that positions of QTL for

278   principle components were highly indicative of the locations of QTL clusters for the traits included in

279   their particular PC axis (Table 4, Fig. 2).

280   PC1 QTL associated with three clusters of QTL for plant size traits. CL9.1 contains shoot biomass and

281   leaf length. CL5.1 contains root biomass, shoot biomass, root volume, and panicle emergence and CL5.3

282   contains root biomass, shoot biomass, root volume, tiller number and root number. Both chr5 clusters

283   overlap the large confidence interval of the QTL for total root length. To test if there are multiple

284   undetected QTL for total root length that could explain the large confidence interval, we lowered the

285   LOD threshold and found two separate QTL associated with each of the two primary chr5 clusters (data

286   not shown). A separate QTL pair of tiller height and root diameter not indicated by PC1 lies between

287   these two large clusters. PC2 QTL associated with of two clusters of QTL for root resource acquisition

288   traits. CL1.1 and 3.1 both contain SRL and root diameter. PC3 associated with a single cluster (CL7.2)

289   related to carbon allocation traits. CL7.2 contains panicle emergence day, leaf length, number of tillers,

290   RMR and SLA. Near this PC3 associated QTL is a minor cluster (CL7.1) of leaf length and SRL (Fig. 2).

291   Four pairwise epistatic interactions, where the effect of one QTL depends on the allelic state of an

292   unlinked QTL, were detected (Table 3, 4; Fig. 3 a-d). Three QTL from cluster CL5.3 (shoot biomass, root

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293   biomass and PC1) interacted with other QTL for these traits located in CL5.2; and the root number QTL

294   from CL5.3 interacted with the root number QTL on chr3. Individuals that possess the *hallii* allele for

295   these QTL at CL5.3 mask the positive effects of their interactive QTL.

296   *A Major Pleotropic Effect QTL is Confirmed in the Field*

297   To confirm the effects of QTL observed in a controlled glasshouse study, we phenotyped two sets of RIL

298   lines homozygous for different parental alleles at the loci for shoot and root biomass (CL5.2) in a field

299   experiment. While the magnitude of increased biomass for lines with *filipes* alleles at the selected QTL

300   observed in the field is 24% less for the root biomass and 11% less for the shoot biomass relative to the

301   glasshouse, the effects are significant and in the same direction as those observed in the glasshouse. Field

302   grown lines with *filipes* parental alleles produced 1.9-fold more root biomass (P=0.0024) and 2.7-fold

303   more shoot biomass (P=0.0002) relative to field grown lines with *hallii* parental alleles (Fig. 4). In

304   addition, the HAL2 parental line showed a 1.8-fold increase trend in RMR (P=0.09) over the FIL2

305   parental line under field conditions compared to the 1.2-fold difference observed in the glasshouse

306   (P=0.018). There were no significant differences between the irrigation treatments or the interaction of

307   treatment by genotype for RIL lines or the parental genotypes. However, root biomass showed a 1.2-fold

308   increase trend under the dry treatment relative to the wet treatment (P=0.08).

309   **Discussion**

310   Previous tests of upland and lowland ecotype divergence have focused primarily on shoot traits and their

311   relationship to plant water status and drought strategies (Maherali *et al.*, 2008; Latta 2009; Olsen *et al.*,

312   2013; Lowry, 2014a). Root traits can also be involved in adaptive differentiation to abiotic stresses by

313   their direct effects on water acquisition, and through correlation, tradeoffs or constraints with shoot traits

314   (Hammer *et al.*, 2009; Mace *et al.*, 2012). Understanding the genetic control of root and shoot trait

315   integration will aid in developing a more complete picture of the process of ecotype divergence.

316   In this study, to examine both above and below ground trait divergence, we assembled a high quality

317   genetic linkage map by resequencing a recombinant inbred population derived from a cross between

318   upland and lowland ecotypes of *Panicum hallii* and conducted a QTL analysis. We mapped at least one

319   QTL for all measured shoot and root traits. QTL for the majority of ecotype differentiating root and shoot

320   size traits were colocalized into several genomic 'hotspots' while QTL for differentiating resource

321   acquisition traits mapped to largely independent genomic regions. We characterized allelic effects of

322   individual QTL for traits involved in ecotype differentiation and found that they underscored trait

323   divergence between the parental populations. A subsequent field study conducted on fully mature

324   individuals at the end of the growing season confirmed the co-localized QTL relationships for shoot and

325   root biomass that were observed in the glasshouse study of plants at first maturity.

326   *Genetic architecture of shoot and root traits underlying ecotype divergence*

327   Ecotypes are often differentiated by suites of correlated root and shoot traits that may share common

328   genetic and developmental architectures as a result of adaptive differentiation. One of our major findings

329   was several genomic 'hotspots' of colocalized QTL for multiple shoot and root traits. This is consistent

330   with the previous study of a *P. hallii* F_2 population covering a suite of ecotype differentiating shoot trait

331   QTL which clustered on chr5 (Lowry et al. 2014a). In addition to confirming this important locus, we

332   discovered additional root traits linked to this region and additional regions of clustered loci for root and

333   shoot traits. This pattern of colocalized QTL that control traits such as root biomass, shoot biomass,

334   among others, has also been shown in a RIL populations of wheat and sorghum (Iannucci *et al.*, 2017;

335   Mace *et al.*, 2012). These findings indicate that specific loci can shape both shoot and root morphological

336   traits, through tight linkage of several genes controlling individual traits or a single pleiotropic gene that

337   controls several traits. All QTL clusters detected in this RIL population were indicated by various QTL

338   for principle components.

339   PC1 indicated three QTL clusters of multiple size related root and shoot traits (shoot biomass, root

340   biomass, root volume, and other). We found that the *hallii* allele had additive effects in the direction of

341   ecotype divergence and contributed to smaller root and shoot phenotypes in every case compared to the

342   *filipes* allele. This finding is consistent with the global pattern observed in angiosperm plants whose shoot

343   and root biomass are positively correlated (Enquist & Niklas, 2002) and with other studies on perennial

344   grasses where total biomass is decreased under water limited conditions (Baruch, 1994; Weißhuhn *et al.*,

345   2011; Tozer *et al.*, 2017).

346   In addition to differences in absolute size, there are expected differences in carbon acquisition and

347   allocation between upland and lowland ecotypes. PC3 indicated one cluster of carbon allocation and

348   phenology related traits (SLA, RMR, tiller number and panicle emergence). Plants with *hallii* alleles had

349   greater SLA, RMR, tiller number, and faster panicle emergence. Thinner leaves (high SLA) are less

350   carbon costly to produce and associated with increased photosynthetic capacity (Reich *et al.*, 1997;

351   Cornelissen *et al.*, 2003). Increased RMR helps to maintain plant water status and productivity under

352   drought (Comas *et al.*, 2013). Faster flowering time along with greater tiller number allows for rapid

353   production of seeds when resources are available for short time periods. These factors combined may

354   indicate that *hallii* employs a fast acquisitive strategy for drought escape; acquiring nutrients rapidly and

355   flowering quickly to enter a dormant state before periods of summer drought. Acquisitive shoot and root

356   strategies have been associated with fast growth strategies and summer dormancy in other perennial

357   grasses (Balachowski *et al.*, 2016). This contrasts with the lower SLA, and RMR of the lowland *filipes*,

358   which may employ a slow strategy of thicker longer lasting leaves, larger more persistent roots, and

359   abundant above ground foliage. This common genetic control of ecotype differentiating traits involving

360   shoot and root organs suggests that these factors evolved in tandem.

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361   SLA and SRL are both important plant traits that are linked to resource acquisition (Reich, 2014; Cheng

362   *et al.*, 2016) and associated with fast growth (Reich, 2014, Pérez-Harguindeguyb *et al.*, 2016). SRL is

363   typically thought of as the below ground analog of SLA (Eissenstat *et al.*, 2000; Reich, 2014), where an

364   acquisitive root strategy (high SRL) can be aided by an acquisitive leaf strategy (high SLA, Perez-Ramos

365   *et al.*, 2013). In some cases, these traits are found to be positively correlated (Withington *et al.*, 2006,

366   Reich, 2014). We found that in the RIL population the genetic correlation between these traits was only

367   25% and each trait had three independent QTL. Thus divergence of these traits is likely due to

368   independent loci which become structured across ecotypes through an accumulation of linkage

369   disequilibrium resulting from strong directional or correlational selection. In this case, our crossing

370   scheme was able to largely decouple these traits through recombination.

371   Observed pairwise epistatic interactions for root biomass, shoot biomass and root number showed that

372   *hallii* alleles mask the effects of *filipes* alleles in all cases. When lines are homozygous for *hallii* parental

373   alleles at CL5.3, it contributes to smaller phenotypes for these traits, regardless of the genotype at their

374   respective interactive QTL. This suggests that the CL5.3 loci could include a pleiotropic gene with major

375   effect that controls the development of multiple shoot and root size related traits. Natural populations of

376   *P. hallii* ecotypes are largely homozygous, thus these linked QTL likely work together in a positive

377   direction and contribute to the phenotypic trait correlations that underlie ecotype divergence. However,

378   the observed epistasis in the RIL population could represent a Dobzhansky–Muller type incompatibility

379   (Coyne & Orr, 2004; Sweigart & Willis, 2012; Bomblies, 2013), where these interactions in hybrid plants

380   could be deleterious and impact survivorship by undermining synergistic trait relationships. The

381   combination of reduced root and shoot size effected by *hallii* alleles is desirable in xeric environments,

382   but maybe deleterious in the higher competition lowland environments *filipes* inhabits.

383   *Glasshouse detected genetic correlations confirmed under field conditions*

384   There is persistent concern that effects observed in glasshouse studies are not representative of plant

385   performance in natural or agronomic environments. Although glasshouses and growth chambers may be

386   able to replicate a wide range of temperature and light conditions, other differences between these

387   artificial and natural environments can be significant. Furthermore, glasshouse studies are often

388   conducted on very young plants and in smaller than optimal pots, which can significantly alter root

389   architectures compared to natural environments. Several recent studies have highlighted how differences

390   in conditions between glasshouse and natural settings can affect the mapping of genetic architectures for

391   various plant traits (Poorter *et.al.*, 2012; reviewed in Lovell *et al.*, 2016).

392   We sought to overcome this concern by confirming the glasshouse detected genetic architecture of two of

393   our chief traits of interest (root biomass and shoot biomass) in selected RIL lines and parental genotypes

394   in a field setting at full plant maturity. In the RIL lines, we found that our glasshouse observed QTL were

395   confirmed, even though the selected lines had differing genetic backgrounds. For the parental lines, we

396   found that root mass ratio differences between the xeric and mesic ecotypes nearly doubled under field

397   conditions as compared to the glasshouse study. This suggests that adaptive allocation of biomass to roots

398   increases with plant age and can also be constrained by pot limitations in the glasshouse. More

399   importantly, these results provide credence to the assumption that our glasshouse study is predictive of

400   plant performance in a natural setting.

401   *Conclusion*

402   In the process of ecotype formation, populations can diverge across many traits and exhibit different niche

403   characteristics, which requires coordination between plant organ systems. Our study sheds light on the

404   genetic architecture underlying the relationships between root and shoot traits involved in ecotype

405   divergence of *Panicum hallii* and demonstrates that some correlated traits are under common genetic

406   control as a result of QTL colocalization while other traits are controlled by independent loci. We found

407   several genomic hotspots relating to multiple root and shoot traits and further insight into the molecular

408   basis of these loci is an important step in understanding the genetic coordination of root and shoot

409   systems involved in ecotype divergence. The RIL population utilized in this study has recently been

410   increased to over 400 lines and should prove a valuable tool in investigating multiple facets of perennial

411   grass biology.

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426   and J.E.B. conducted experiments. J.J. and J.S. conducted the *Panicum hallii* genome assembly. Y.Y.,

427   J.J., and J.S. performed sequencing of the RIL population. J.T.L. created the genomic map.

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625 **Tables**

626 **Table 1** Means, one standard error (SE) and broad-sense heritability (H^2) of root and shoot traits for the *Panicum hallii* RIL population and its parental
627 genotypes.

Phenotypic Trait	FIL2	HAL2	t	P-value	RIL mean	RIL range	$H^2 \pm$SE
Panicle Emergence (day)	9.25±1.19	4.00±1.38	2.87	**0.035**	7.01±1.74	1.00 – 18.33	0.51±0.05
Shoot Biomass (g)	4.74±0.49	1.41±0.57	4.39	**0.007**	1.65±0.33	0.29– 4.74	0.59±0.05
Tiller Number	6.25±0.48	5.00±0.56	1.68	0.150	6.00±0.83	3.00 – 14.50	0.50±0.05
SLA	325.62±18.15	382.77±20.96	-2.06	0.094	381.58±33.17	264.67 – 499.36	0.18±0.08
Plant Height (cm)	21.18±1.82	11.63±2.11	3.43	**0.018**	12.57±1.56	4.30 – 23.65	0.63±0.04
Leaf Length (cm)	30.77±1.72	14.23±1.98	6.30	**0.001**	15.66±1.46	4.75– 24.27	0.66±0.04
Root Biomass (g)	1.38±0.18	0.51±0.21	3.08	**0.028**	0.54±0.10	0.12 – 1.60	0.58±0.06
Root Number	14.00±0.97	8.33±1.11	3.84	**0.012**	8.87±1.39	2.50 – 15.00	0.38±0.05
SRL (cm g^{-1})	10.14±0.85	13.37±0.98	-2.50	0.055	12.27±1.11	6.12 – 17.95	0.43±0.06
RTD (g cm^{-3})	0.06±0.01	0.05±0.01	1.31	0.247	0.05±0.01	0.03 – 0.08	0.39±0.07
Root Diameter (mm)	0.46±0.01	0.44±0.02	1.27	0.259	0.45±0.01	0.37 – 0.55	0.37±0.05
Root Volume (cm^3)	2.43±0.28	0.98±0.32	3.41	**0.019**	1.00±0.17	0.26 – 2.90	0.56±0.05
Root Length (m)	1.37±0.14	0.67±0.16	3.29	**0.022**	0.65±0.11	0.12 – 1.64	0.59±0.04
RMR	0.22±0.01	0.27±0.01	-3.44	**0.018**	0.25±0.02	0.16 – 0.39	0.34±0.09

FIL2, *P. hallii* subsp. *filipes*; HAL2, *P. hallii* subsp. *hallii*. Values are mean ± 1SE. *t*, t-statistics at 5 degrees of freedom in test for divergence

between parental lines. Statistically significant values are indicated in bold text.

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637 **Table 2** Pearson Correlation Coefficients for genetic correlations in the *Panicum hallii* RIL population.

Trait	ED	TN	RTN	SHMASS	RTMASS	SRL	RTD	HEIGHT	LFLG	RMR	SLA	RTDM	RTLG
TN	0.116												
RTN	0.039	**0.669**											
SHMASS	**0.215**	**0.545**	**0.758**										
RTMASS	0.132	**0.615**	**0.789**	**0.921**									
SRL	**-0.192**	**-0.115**	-0.05	-0.021	**-0.15**								
RTD	**0.195**	**0.195**	**0.191**	**0.281**	**0.336**	**-0.544**							
HEIGHT	0.119	**0.285**	**0.598**	**0.824**	**0.719**	0.118	0.136						
LFLG	0.015	**0.2**	**0.573**	**0.759**	**0.678**	0.135	0.109	**0.769**					
RMR	**-0.281**	-0.005	-0.121	**-0.424**	-0.085	**-0.267**	0.029	**-0.495**	**-0.399**				
SLA	**-0.331**	0.079	0.001	-0.223	-0.095	**0.259**	**-0.288**	-0.114	-0.047	**0.388**			
RTDM	0.125	-0.071	**-0.183**	**-0.260**	-0.163	**-0.696**	-0.115	**-0.317**	**-0.338**	**0.290**	-0.135		
RTLG	0.057	**0.566**	**0.772**	**0.905**	**0.925**	**0.185**	**0.198**	**0.762**	**0.734**	**-0.196**	0.003	**-0.450**	
RTVOL	0.120	**0.614**	**0.802**	**0.911**	**0.975**	-0.059	**0.150**	**0.722**	**0.670**	-0.107	-0.045	-0.134	**0.934**

ED, panicle emergence; TN, tiller number; RTN, root number; SHMASS, shoot biomass; RTMASS, root biomass; SRL, specific root length; RTD, root tissue density; HEIGHT, plant height; LFLG, leaf length; RMR, root mass ratio; SLA, specific leaf area; RTDM, root diameter; RTLG, root length. Significant correlations are indicated in bold text.

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643 **Table 3** Main and epistatic effects of QTL for the *Panicum hallii* RIL population.

Phenotype	Chr	Peak (cM)	1.5 Lod Interval	LOD	% var	Effect	SE	Donor of Positive allele	QTL Cluster (CL)
Panicle Emergence	5	52.1	40-59	4.59	9.85	-0.044	0.009	*filipes*	CL5.1
(day)	7	80.0	31-83	4.31	9.2	-0.039	0.008	*filipes*	CL7.2
Shoot Biomass	5	58.6	56-60	7.43	14.8	-0.044	0.007	*filipes*	CL5.1
(g)	5	136.0	128-142	5.08	9.82	-0.031	0.007	*filipes*	CL5.3
	9	66.1	60-71	4.78	9.19	-0.027	0.005	*filipes*	CL9.1
	Epi5:5			2.86	5.36	0.027	0.007		
Tiller Number	3	40.5	38-48	7.23	14.74	-0.054	0.009	*filipes*	
(count)	5	137.0	128-142	3.47	6.73	-0.037	0.009	*filipes*	CL5.3
	7	73.6	46-81	4.84	9.56	0.039	0.008	*hallii*	CL7.2
SLA	5	13.3	0-26	3.15	5.25	9.772	2.543	*hallii*	
(cm^{2}g^{-1})	7	66.0	60-74	8.56	15.37	16.394	2.494	*hallii*	CL7.2
	8	19.8	16-23	8.33	14.90	16.077	2.484	*hallii*	
Tiller Height	5	76.0	74-77	6.16	13.34	-1.765	0.320	*filipes*	CL5.2
(cm)	6	83.9	69-88	3.82	8.05	-1.096	0.256	*filipes*	
Leaf Length	2	89.7	76-96	4.28	8.56	-1.19	0.264	*filipes*	
(cm)	7	43.6	35-64	4.39	8.80	-1.293	0.283	*filipes*	CL7.1
	9	63.4	59-75	3.41	6.76	-0.985	0.246	*filipes*	CL9.1
Root Biomass	5	58.6	56-60	8.81	18.61	-0.012	0.002	*filipes*	CL5.1
(g)	5	136.0	135-142	8	16.71	-0.010	0.002	*filipes*	CL5.3
	Epi5:5			4.61	9.21	0.008	0.002		
Root Number	3	88.0	69-104	6.18	13.9	-1.08	0.199	*filipes*	
(count)	5	125.7	125-130	5.36	11.94	-0.81	0.196	*filipes*	CL5.3
	Epi3:5			2.79	5.99	0.73	0.202		
SRL (cm g^{-1})	1	91.5	82-94	5.3	11.02	0.66	0.131	*hallii*	CL1.1
	3	18.8	17-36	5.16	10.7	0.78	0.156	*hallii*	CL3.1
	7	44.7	34-49	3.16	6.4	-0.55	0.145	*filipes*	CL7.1
RTD (g cm^{-3})	1	6.3	0-20	3.15	7.9	-0.001	0.0004	*filipes*	
Root Diameter	1	86.0	82-94	4.73	8.68	-0.009	0.002	*filipes*	CL1.1
(mm)	3	34.2	30-36	5.36	9.91	-0.011	0.002	*filipes*	CL3.1
	5	71.9	66-75	3.78	6.84	0.010	0.002	*hallii*	CL5.2
	8	47.9	43-52	4.65	8.50	-0.009	0.002	*filipes*	
Root Volume	5	58.6	56-63	3.96	8.85	-0.134	0.030	*filipes*	CL5.1
(cm^3)	5	117.2	109-142	3.07	6.77	-0.119	0.032	*filipes*	CL5.3
Root Length	5	58.6	44-138	3.12	7.85	-0.82	21.29	*filipes*	CL5.1,2,3
RMR (ratio)	7	67.0	62-74	6.36	15.34	0.0137	0.002	*hallii*	CL7.2

Chr, chromosome; Peak, cM (centimorgan) position of the QTL peak; LOD, logarithm of odds; % var, present of variance; SE, one standard error; SLA, specific leaf area; SRL, specific root length; RTD, root tissue density; RMR, root mass ratio; Epi, epistasis.

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645   **Table 4** Main and epistatic effects of the first three principal component QTL for the *Panicum hallii*

646   RIL population.

Principal Component	Chr	Peak (cM)	1.5 Lod Interval	LOD	% var	Effect	SE	Donor of Positive allele	QTL Cluster (CL)
PC1	5	58.6	56-60	7.19	14.42	-1.215	0.209	*filipes*	CL5.1
	5	136.0	135-142	6.34	12.67	-1.049	0.206	*filipes*	CL5.3
	9	66.1	58-84	3.51	6.7	-0.664	0.164	*filipes*	CL9.1
	Epi5:5			3.14	6.0	0.812	0.212		
PC2	1	88.7	83-93	4.51	8.26	-0.458	0.099	*filipes*	CL1.1
	3	34.2	18-36	4.97	9.14	-0.533	0.109	*filipes*	CL3.1
	5	1.1	0-4	4.13	7.52	0.457	0.103	*filipes*	
	8	58.0	42-74	3.45	6.24	0.392	0.097	*filipes*	
PC3	7	67.0	65-72	12.14	25.05	0.676	0.084	*hallii*	CL7.2
	8	18.5	16-26	3.59	6.60	0.354	0.085	*hallii*	CL8.1

Chr, chromosome; Peak, cM (centimorgan) position of the QTL peak; LOD, logarithm of odds; % var, present of variance explained; SE, one standard error; PC1, principal component 1; PC2, principal component 2; PC3, principal component 3; Epi, epistasis.

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667 **Figures**

668 **Fig. 1** Principle component analysis of shoot and root traits for the *Panicum hallii* RIL population.

669 Traits: PC, principal component; RMR, root mass ratio; SLA, specific leaf area; SRL, specific root

670 length; RTLRNGTH, root length; LFLG, leaf length; HEIGHT, plant height; SHMASS, shoot

671 biomass; RTMASS, root biomass; RTVOL, root volume; RTN, root number; TN, tiller number; RTD,

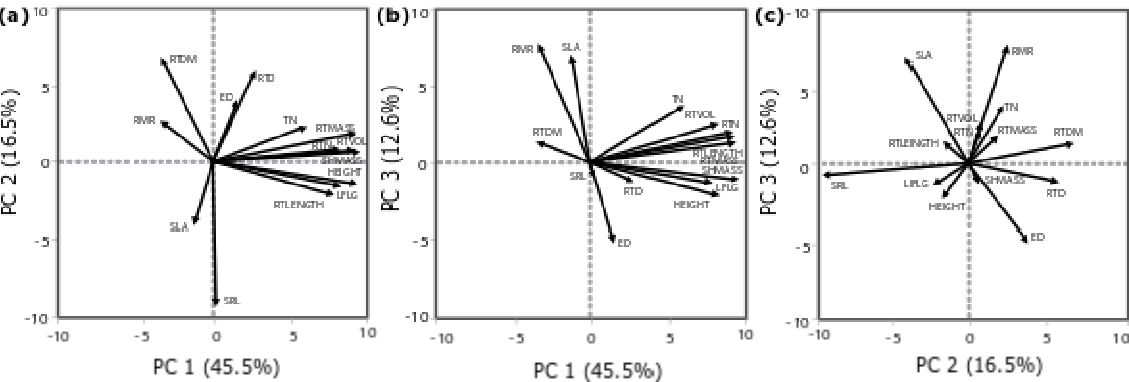672 root tissue density; ED, emergence day; RTDM, root diameter.

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695 **Fig. 2** Genetic map of the *Panicum hallii* RIL population with location of trait QTL. Colored bars

696 indicate 1.5-LOD drop confidence intervals. Location of dots within the bars is the location of QTL

697 peaks. Arrow is the direction of additive effect, with up or down arrow indicating the HAL2 allele

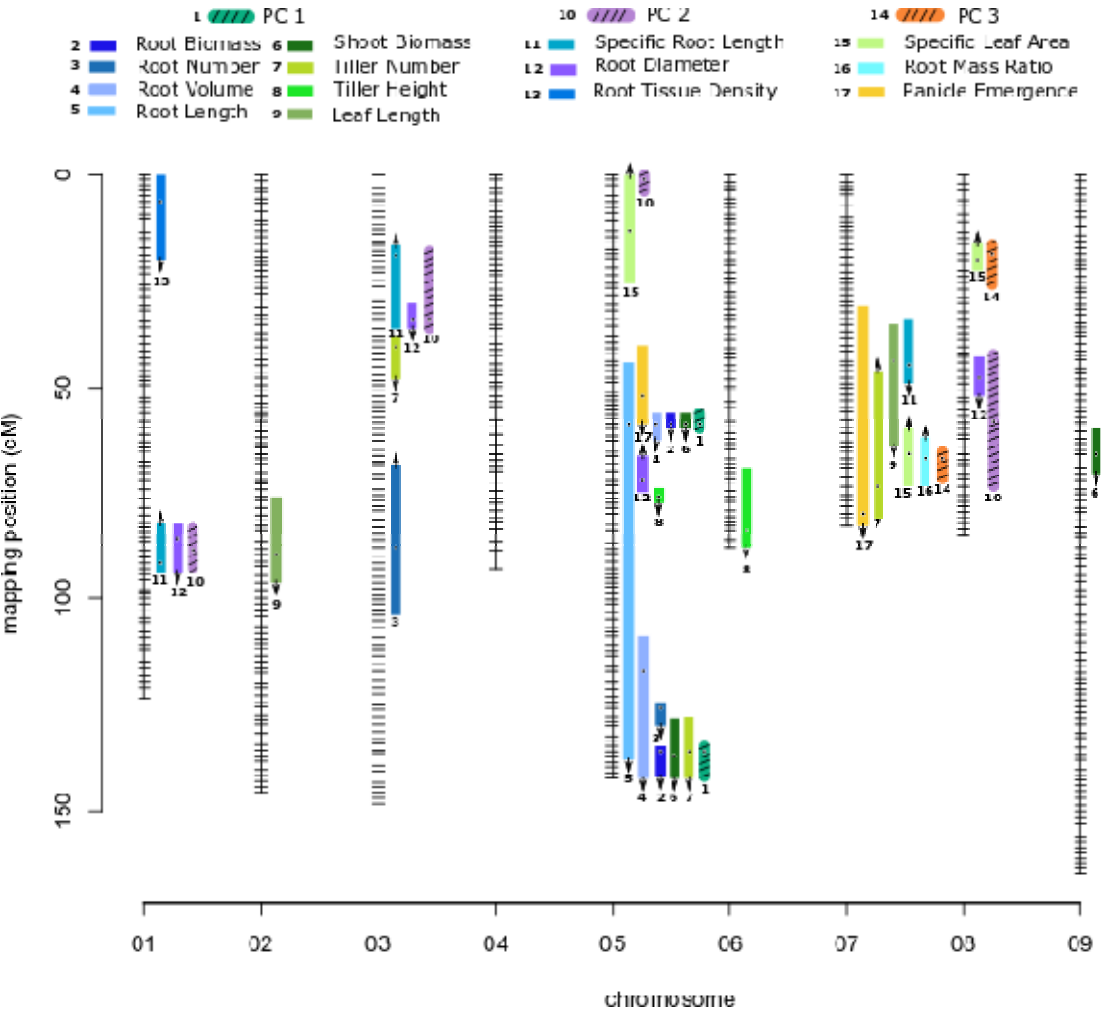698 increases or decreases the trait value.

710 **Fig. 3** Pairwise epistatic QTL in the *Panicum hallii* RIL population. Plotted points indicate two-locus

711 genotype means ± 1SE for the two loci containing root biomass (a), shoot biomass (b), root number

712 (c) and PC1 (d).

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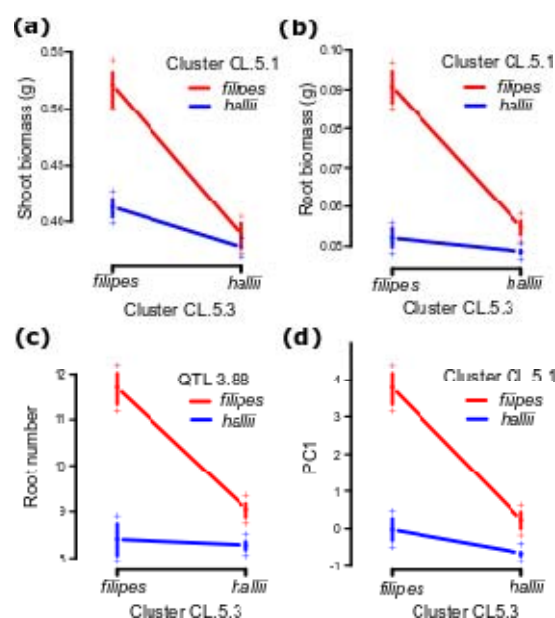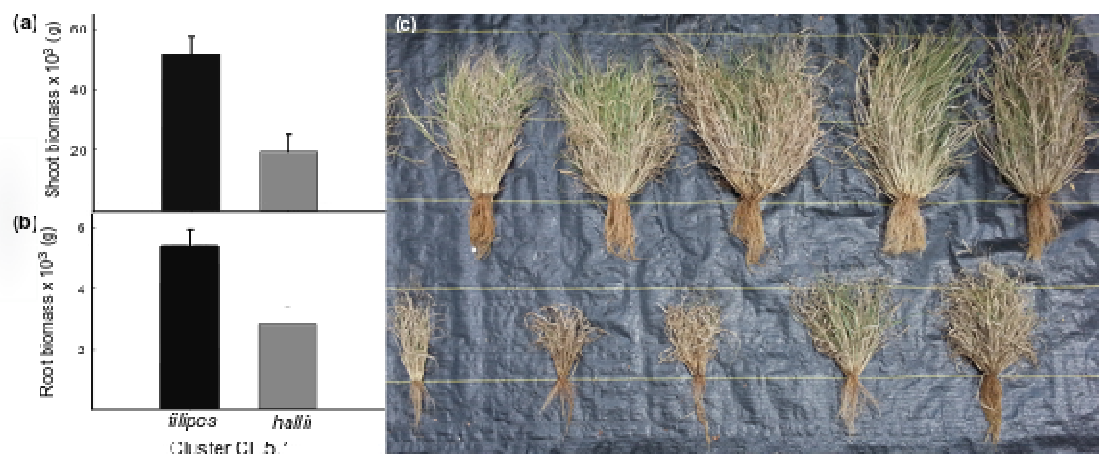726
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734   **Fig. 4** Mean ± 1SE of shoot biomass (a) and root biomass (b) for field grown *Panicum hallii* RIL lines

735   homozygous for either *filipes* or *hallii* parental alleles at shoot and root biomass QTL located in

736   cluster CL5.2. Picture of field grown RIL lines homozygous at CL5.2 for *filipes* allele (top row) and

737   *hallii* allele (bottom row) (c).

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762   **Supportive information Legends**

763   **Table S1** Principal component loadings of measured traits in the *Panicum hallii* RIL population.

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765   **Table S2** R/qtl input file for QTL mapping of measured traits in the *Panicum hallii* RIL population.

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