Non-linear genetic diversity and notable population differentiation caused by low gene flow of Bermudagrass [*Cynodon dactylon* (L.) Pers.] along longitude gradients

Jing-Xue Zhang [1, 2], **Miaoli Wang** [2], **Jibiao Fan** [1], **Zhi-Peng Guo** [2], **Yongzhuo Guan** [2], **Gen Qu** [2], **Chuan-Jie Zhang** [1], **Yu-Xia Guo** [Corresp., 2], **Xuebing Yan** [Corresp. 1]

[1] College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China

[2] College of Animal and Veterinary Science, Henan Agricultural University, Zhengzhou, Henan, China

Corresponding Authors: Yu-Xia Guo, Xuebing Yan
Email address: guoyuxia@henau.edu.cn, yxbbjzz@163.com

Background. Environmental variation related to ecological habitat is the main driver of plant adaptive divergence. Longitude plays an important role in the formation of plant population structure, indicating that environmental differentiation can significantly shape population structure.

Methods. Genetic diversity and population genetic structure were estimated using 105 expressed sequence tag-derived simple sequence repeat (EST-SSR) loci. A total of 249 *C. dactylon* (L.) Pers. (common bermudagrass) individuals were sampled from 13 geographic sites along the longitude (105°57′34″ -119°27′06″ E).

Results. There was no obvious linear trend of intra-population genetic diversity along longitude and the intra-population genetic diversity was not related to climate in this study. Low gene flow (Nm = 0.7701) meant a rich genetic differentiation among populations of *C. dactylon* along longitude gradients. Significantly positive Mantel correlation (r = 0.438, *P* = 0.001) was found between genetic distance and geographical interval while no significant partial Mantel correlation after controlling the effect of mean annual precipitation, which indicated geographic distance correlated with mean annual precipitation affect genetic distance. The genetic diversity of *C. dactylon* with higher ploidy level was higher than that with lower ploidy level and groups of individuals with higher ploidy level were separated further away by genetic distance from the lower ploidy levels. Understanding the different genetic bases of local adaptation comparatively between latitude and longitude is one of the core findings in the adaptive evolution of plants.

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Jing-Xue Zhang[1, 2], Miao-Li Wang[2], Ji-Biao Fan[1], Zhi-Peng Guo[2], Yong-Zhuo Guan[2], Gen Qu[2], Chuan-Jie Zhang[1], Yu-Xia Guo[2*] and Xue-Bing Yan[1*]

[1.] College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China

[2.] College of Animal and Veterinary Science, Henan Agricultural University, Zhengzhou, Henan, China

Corresponding Author:

Yu-Xia Guo[2] and Xue-Bing Yan[1]

63 Nongye Road, Zhengzhou, Henan, 450002, China

NO 48, Wenhui east road, Yangzhou, Jiangsu, 225000, China

Email address: guoyuxia@henau.edu.cn and yxbbjzz@163.com

Abstract

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35 correlated with mean annual precipitation affect genetic distance. The genetic diversity of *C.*
36 *dactylon* with higher ploidy level was higher than that with lower ploidy level and groups of
37 individuals with higher ploidy level were separated further away by genetic distance from the
38 lower ploidy levels. Understanding the different genetic bases of local adaptation comparatively
39 between latitude and longitude is one of the core findings in the adaptive evolution of plants.

40

41 **Keywords:** *Cynodon dactylon*; Landscape genetics; Genetic diversity; Longitude; Polyploidy.

42

Introduction

44 Plants will undergo adaptive genomic and phenotypic changes in response to environmental
45 heterogeneity on a spatial scale (Yang et al., 2017; Rellstab et al., 2015). Identification of adaptive
46 genetic diversity and population structure of various populations of different environments is of
47 great significance for improving our understanding of the evolutionary process and exploring the
48 adaptive potential of plants (Li et al., 2017). Landscape genetics is helpful for assessing the ability
49 to produce and transmit adaptive genes (Jordan et al., 2017). Genetic differentiation and landscape
50 genetic structure are the results of evolutionary processes including gene migration, random
51 genetic drift and natural selection related to environmental variation due to latitude and longitude.
52 However, gene flow among different populations can effectively prevent genetic difference caused
53 by genetic drift (Schoville et al., 2012; Rundle and Nosil, 2005). Further study of the spatial
54 patterns of genetic diversity is valuable in estimating evolutionary process and predicting the
55 ability of populations to respond to climate change. Studying the genetic structure of more widely
56 distributed plant populations would provide insights into genetic differentiation and evolution.
57 Morphological plasticity and stress tolerances of utilized germplasm should match the
58 environmental characteristics of a specific geographical region (Casler et al., 2004; Boe and
59 Casler, 2005). Population distributions in a large scale could be shaped by regional climate and
60 geographic factors such as latitude and longitude (Peters et al., 2012; Sánchez-gonzález and López-
61 mata, 2005). Longitude is correlated with some environmental variables, and environmental
62 factors (like temperature and precipitation) also play important roles in shaping these assemblages
63 along the longitudes. Longitude was the driving factor behind the distribution of some study
64 species, and the distribution of Carex physodes was controlled by longitude (Zhang et al., 2016).
65 Decreasing MAT (mean annual temperature) and MAP (mean annual precipitation), along with

66 increasing altitude, appear from the east to west along the longitude. Genetic isolation along

67 longitude gradients caused by mountainous environments has been found in other widely

68 distributed Europe-Asian plants (Bartha et al., 2015). Plants in eastern and western Greenland have

69 been genetically different because the Greenlandic ice cap blocks gene flow (Eidesen et al., 2013;

70 Alsos et al., 2009).

71 Bermudagrass (*Cynodon dactylon* (L.) Pers.) is a genus of the tribe Chlorideae (Poaceae),

72 which inhabits most countries and islands, usually in between about 45°N and 45°S and penetrates

73 to approximately latitude 53°N in Europe (Burton, 1947; Casler et al., 2003). The warm season

74 grass is widely used for turf and pasture because of its drought and heat tolerance and low

75 maintenance requirements (Harlan, 1970; Beard, 1972). *C. dactylon* is an enormously variable and

76 cosmopolitan warm-season turfgrass used for lawns, parks, and sports fields (Shi et al., 2013),

77 which justifies new and innovative approaches toward evaluating and understanding the genetic

78 relationships among different populations at different longitudes. Some studies have been used to

79 analyze genetic diversity and population structure of bermudagrass because of its efficient

80 dispersal and establishment abilities, rapid population growth, and phenotypic plasticity (Linder

81 et al., 2018; Zheng et al., 2017). Identifying the correlation between simple sequence repeat (SSR)

82 markers and turf-quality-related traits among different bermudagrass populations can efficiently

83 help to select high turf quality for bermudagrass. Different ploidy levels including diploid, triploid,

84 tetraploid, pentaploid and hexaploid have been reported for *C. dactylon* (Harlan et al., 1970; Wu

85 and Taliaferro, 2009). Polyploidy can improve competitive ability to adapt to new environments,

86 which is vital for the worldwide distribution of grasses (Te Beest et al., 2012; Linder and Barker,

87 2014). There have been multiple polyploidy events during plant evolution (Wendel, 2015), so

88 evolutionary significance of polyploidy on wild plant populations is being studied. Polyploidy has

89 been the subject of many studies for the genetic and genomic consequences of WGDs (whole-

90 genome duplication) (Soltis et al., 2016).

91 The lack of genomic information makes genotyping difficult, but molecular markers enhance

92 the study of plant genetic diversity (Govindaraj et al., 2015). SSRs are characterized by high

93 reproducibility, high polymorphism, co-dominant inheritance, and relative abundance across the

94 entire genome (Vasemägi et al., 2005), and are better selective markers for genetic population

95 analysis of some orphan species (Carneiro Vieira et al., 2016; Hodel et al., 2016). EST-SSRs may

96 reveal some different genetic patterns, because selective processes may affect their polymorphism

97   (Defaveri et al., 2013). These molecular markers have great potential in genetic diversity and

98   population genetic structure evaluation. Genetic diversity and population structure of 690 *C.*

99   *dactylon* accessions have been analyzed using expressed sequence tag-simple sequence repeats

100   (EST-SSRs), which is a crucial step of studying genetic evolution (Vlachou et al., 1997; Jewell et

101   al., 2010). Although *C. dactylon* has important research value, the understanding of its population

102   genetic structure and geographic variation is limited. Genetic diversity and genetic structure of *C.*

103   *dactylon* along a latitudinal gradient across China and the relationship between genetic diversity

104   and ploidy levels were also studied in our previous study (Zhang et al., 2019). Sampling includes

105   maximum geographical variation from flooded east to arid west along the longitude to quantify

106   the relative importance of longitude for adaptive genetic divergence of different bermudagrass

107   populations. The genetic diversity of *C. dactylon* along longitude gradients may be correlated to

108   complex geographic conditions and its biological characteristics including perennial growth, wind-

109   pollination, polyploidy, sexual and asexual reproduction. Different environment factors along

110   longitude gradients can also promote genetic variation and complex genetic structure of *C.*

111   *dactylon*. Therefore, this study has four objectives (1) quantify population genetic diversity of 249

112   individuals from 13 populations ranging from different longitudes using EST-SSRs; (2) estimate

113   the spatial population genetic structure and gene flow along longitude; (3) test the relationship

114   between ploidy levels and genetic diversity; (4) compare genetic pattern between longitude and

115   latitude. Studying genetic variation of the wild *C. dactylon* along longitude gradients can promote

116   broadening the genetic resources, understanding the genetic process of adaptation to

117   environmental variation along with longitude and providing some valuable information for the

118   better utilization of wild germplasms of different ploidy levels in breeding.

119

Materials & Methods

Plant materials and DNA extraction

122   Plant materials consisting of 249 *C. dactylon* individuals were sampled at 13 different geographic

123   origins locating 105°57′34″ - 119°27′06″ E, China (Fig. 1). Mean annual precipitation and

124   temperature at the collection sites were provided by the China Meteorological Administration

125   (Table 1). Total genomic DNA was extracted using a Zymo Research Plant DNA Extraction Kit

126   (Xinmaijie, Zhengzhou, China) and the quality and concentration of DNA were checked by

127   electrophoresis on 1% agarose gels.

EST-SSR amplification

129 The 20 EST-SSR primer pairs from previous research (Zhang et al., 2019) produced clear and
130 polymorphic bands and were subsequently used to evaluate genetic diversity for this study. 25 μL
131 PCR amplifications reaction solution contains 1 μL of template DNA, 2.5 μL 10× PCR buffer, 2
132 μL of 25 mM $MgCl_2$, 0.5 μL of 10 mM deoxy nucleoside triphos phate (dNTP), 0.5 μL each of
133 forward and reverse primers, 0.2 μL of Taq DNA polymerase, and 17.8 μL of nuclease-free water.
134 The amplifications were performed with the following PCR protocol used in the previous
135 research (Zhang et al., 2019): First 1 cycle of 3 min at 95℃, followed by 10 cycles at 95℃, 60℃
136 and 72℃ respectively for 30 s, finally terminating with 1 cycle of 6 min at 72℃. Next, the samples
137 were subjected to 1 cycle at 95 ℃ for 3 min, then 20 cycles of 30 s at 95℃, 55℃, and 72℃
138 respectively, and finally 1 cycle at 72 ℃ for 6 min. PCR amplified fragments labelled with one
139 fluorescent tag (6-FAM (6-carboxyfluorescein) (blue) and HEX (green)) were detected on a
140 3730XL Genetic Analyzer (ABI, USA) with the size standard GS500 LIZ. Capillary
141 electrophoresis analysis was made by using GeneMapper Software Version 4.0 (Applied
142 Biosystems, Foster City, CA, USA).

Analysis of genetic diversity

144 Genetic diversity for all populations and ploidy levels was calculated using POPGENE V. 1.32
145 Software (Yeh et al., 1997). These indicators included Nei's gene diversity index (He), Shannon
146 diversity index (I) and polymorphic information content (PIC) (Smith et al., 1997; Yeh et al.,
147 1999). He was generated according to genetic distance to analysis genetic diversity. I was
148 computed based on the obtained allele frequencies, and PIC reflected the degree of variation of
149 microsatellite DNA. Ploidy level of each sampled individual was determined by using a flow
150 cytometer (Cube8, Partec, Germany) based on the method outlined by Zhang et al. (2020). Linear
151 association between ploidy levels and longitude was investigated under a regression analysis using
152 the software package SPSS13.0 for Windows (SPSS Inc. Chicago, USA) considering correlation
153 coefficient values and significance of variables in the model. All individuals of the same ploidy
154 level were treated as a population to compare the genetic diversity among different ploidy levels.
155 Significant differences in genetic diversity parameters among different populations and ploidy
156 levels were evaluated under a one-way analysis by Duncan's test using SAS 9.3 software (Institute
157 Inc., Cary, NC, USA) (Duncan, 1955). We divided all the individuals into four groups by longitude

158 from east to west to calculate the genetic diversity parameters for the four groups with four

159 populations within one group as described above.

Analysis of genetic relationship among all populations of *C. dactylon*

161 Distance matrices of intra-population genetic diversity indicators, environmental distance

162 matrices, genetic distance matrices and geographical distance matrices based on the dataset for 13

163 populations were calculated using GenAlEx 6.1 (Peakall and Smouse, 2006). Mantel correlation

164 tests (Mantel, 1967) between intra-population genetic diversity indexes and environmental factors

165 (temperature and precipitation) were conducted to examine the effects of environments on the

166 intra-population genetic diversity. Mantel correlation coefficient between genetic distances and

167 longitude distances for *C. dactylon* at different spatial scales was also investigated using NTSYS

168 pc version 2.0 (Khan et al., 2002) and the probabilities for the significance were assessed using

169 Bonferroni inequality adjustment. Partial Mantel test was conducted in R (ver. 4.0.5; R Core

170 Development Team 2011) to estimate correlation between genetic distances and geographic

171 distance, while controlling for mean annual precipitation Analyses of molecular variance

172 (AMOVA) and population differentiation index (FST) were estimated using ARLEQUIN v3.5

173 (Excoffier and Lischer, 2010). The significance of FST was determined by random resampling of

174 the genotypic data through 1000 permutations. Principal coordinates (PCoA) analysis of genetic

175 variations at different longitudes was performed using GenAlEx Version 6.1. With the help of

176 clustering in NTSYSpc-v.2.1 software, UPGMA (unweighted pair-group method with arithmetic

177 means) was used for cluster analysis to generate a dendrogram for genetic variation along longitude

178 gradients (Rohlf, 2000).

Population structure analysis

180 The population structure of the 249 individuals was constructed using a Bayesian clustering

181 method implemented in STRUCTURE 2.3 (Hubisz et al., 2009) as previously described in Zhang

182 et al. (2019). The model-based approach implemented was used to subdivide the individuals into

183 different subgroups. Specifically, twenty independent runs were performed for each simulated

184 value of K ranging from 2 to 13. For each run, 10,000 iterations were set as the burn-in time

185 following 100,000 iterations of Markov Chain Monte Carlo (MCMC). Outputs were processed

186 with CLUMPP V1.1.2 (Jakobsson and Rosenberg, 2007) and STRUCTURE barplots were

187 displayed using DISTRUCT 1.1 (Rosenberg, 2004). The best K value was determined from the

188   modal value of the run with the delta K. Gene flow was calculated using POPGENE V. 1.32

189   Software (Yeh et al., 1997) based on the formula of Nm = 0.5(1- Gst)/Gst.

190   Mismatch distribution analysis (MDA; Rogers and Harpending, 1992) was used to investigate

191   whether *C. dactylon* had undergone recent population expansion. Goodness-of-fit was tested with

192   the sum of squared deviations (SSD) between observed and expected mismatch distributions

193   (Librado and Rozas, 2009) and Harpending's raggedness index (Harpending, 1994) (HRag), using

194   1000 parametric bootstrap replicates. The formula $t=\tau/2u$ was used to estimate the time since

195   expansion began for the expanding population. The value u was calculated as $u=\mu kg$, where μ is

196   the substitution rate (6.5×10^{-9} synonymous substitutions per synonymous site per year (Gaut et

197   al., 1996)), k is the average sequence length used for analysis (105 bp) and g is the generation time

198   in years (1 year was used as an approximation for g according to age of first reproduction of *C.*

199   *dactylon*). Fu's FS (Fu, 1997) and Tajima's D (Tajima, 1989) neutrality test were used to infer

200   demographic history of each population. Statistical significance was estimated by performing 1000

201   random permutations. Demographic analyses were performed in ARLEQUIN.

202

203   Results

204   Genetic diversity and differentiation along longitude gradient

205   The genetic diversity parameters including the PIC value, He and I were calculated for EST-SSR

206   markers in 13 populations (Table 2). The PIC values ranged from a high of 0.68 and a low of 0.40.

207   Highest He value of 0.19 and lowest of 0.06 were calculated. The value of I ranged from 0.11 to

208   0.34 respectively for EST-SSR markers. Non-significant difference in genetic diversity parameters

209   among groups (group 1 consisted of populations 1-3; group 2 consisted of populations 4-6; group

210   3 consisted of populations 7-9; group 4 consisted of populations 10-13) was observed along

211   longitude gradients (He among populations: F = 0.027, *P* = 0.974). However, populations 4, 5, 6

212   and 8 at mid-longitude and populations 1, 12, 13 at lowest and highest longitude had higher genetic

213   diversity than other populations according to I and He (Fig. 2). In general, there was also no

214   significant correlation (*P* > 0.05) between intra-population genetic diversity and climate (mean

215   annual temperature and precipitation), suggesting that temperature and precipitation showed little

216   influence on intra-population genetic diversity along longitudes.

217   AMOVA results indicated that 38.23% of the genetic variation was attributed to difference

218   among populations. About 61.77% of the genetic variation showed within population, which

219   accounted for a greater part of the whole genetic variation (Table 3).Mantel's test revealed

220   significantly positive correlation between genetic distance and longitudinal distance (r = 0.276, P

221   = 0.022). Environmental measures along longitude gradients (geographical distance (r = 0.438, P

222   = 0.001); mean annual precipitation (r = 0.305, P = 0.033)) were correlated with genetic distance,

223   while mean annual temperature was not (r = 0.006, P = 0.418). However, a partial Mantel test

224   accounting for mean annual precipitation was used to estimate that genetic distance was not

225   independently correlated with geographic distance (P = 0.146). Gene flow (Nm) among these $C.$

226    $dactylon$ populations along longitude gradients was 0.7701 (< 1), which mainly reflected low

227   communication frequency of gene among populations.

228   **Genetic separation by UPGMA dendrogram and principal coordinate analysis**

229   A UPGMA dendrogram dependent on Nei's genetic distance showed that 13 different populations

230   were grouped into three distinct clusters (Fig. 3). The first cluster consisted of populations from

231   Lianyungang and Tancheng that located at high-longitude in the east of China near the Yellow

232   Ocean. Most populations along longitude gradient assigned to the second cluster, so a certain

233   degree of kinship was identified among populations of $C.$ $dactylon$ located at different regions

234   along longitude gradient. Populations 7, 9, 10 clustered together and population 3 was close to

235   population 5. The third mixture cluster contained plants coming exclusively from interval locations

236   along the longitude, such as Shanxian (4), Zhengzhou (6), Sanmenxia (8), and Baoji (12). In

237   addition, each of four populations in the mixture cluster had higher intra-population genetic

238   diversity. The Principal coordinate analysis (PCoA) (Fig. 4) revealed a similar pattern as inferred

239   with UPGMA dendrogram. The first, second and third principal coordinates respectively explained

240   31.57%, 17.78% and 11.90%, altogether 61.25% of the variability. At axis one, 13 populations

241   could be divided into two parts with one of cluster 2 and others of clusters 1, 3. At axis two, cluster

242   1 consisting of populations 1 and 2 could be clearly separated from cluster 3. Additionally,

243   populations of cluster 1 were clearly far from the other populations. Populations 3, 5, 7, 9 and 10

244   clustered together in the cluster 2. Populations 4, 6, 8 and 12 with relatively higher genetic diversity

245   formed the cluster 3.

246   **Population genetic structure of *C. dactylon***

247   Bayesian analysis of the population structure of $C.$ $dactylon$ was used to conduct separate

248   calculation from K = 2 to K = 20. The results clearly demonstrated that the appropriate K values

249   were obtained when the 13 populations were subdivided into 4 or 8 groups (Fig. S1). The results

250 of 13 populations from K = 2–13 strongly indicated different clusters in populations of *C. dactylon*

251 (Fig. 5; Fig. S2). When K=4, population 1 and 2 clustered together (blue) and the cluster with

252 green consisted of populations 4, 6, 8, 12. Populations 5, 7, 10, 13 were differentiated from

253 populations 3, 9, 11 compared to K=3. And when K=8, one group included populations 3, 5, 7, 9,

254 10, and another group consisted of the populations 8 and 12. Each of the other six groups consisted

255 of only one population respectively, which indicated a rich genetic differentiation along longitude

256 gradients. Admixture among populations of medium longitude (populations 5, 6, 8, 9) could be

257 observed from the structure of *C. dactylon* populations (K = 2 to 13), the clustering of populations

258 at low and high longitude were relatively stable except for the populations 1, 12, 13.

259 **Mismatch analysis and neutral test of *C. dactylon***

260 The mismatch distribution for each population of *C. dactylon* (Table 4) suggested populations 1-

261 11, 13 possibly underwent recent population expansion. The result was also proved by significantly

262 negative values of Fu's FS among the 13 populations of *C. dactylon*. Significant SSD values and

263 non-significantly positive Tajima's D values of the population 12 might suggest relatively stable

264 population size in Baoji. In addition, the demographic expansion time of each population was

265 calculated based on the value of τ.

266 **Genetic variation and differentiation among different ploidy levels**

267 Genetic diversity (He and I) results of *C. dactylon* with five ploidy levels showed that triploid had

268 significantly lower genetic diversity than pentaploid ($P = 0.019$ for He; $P = 0.008$ for I) and

269 hexaploid ($P = 0.049$ for He; $P = 0.020$ for I) (Table S1). The genetic diversity of the *C. dactylon*

270 individuals with higher ploidy level was significantly higher than that of individuals with lower

271 ploidy level except for diploid. Significant genetic diversity exists among different ploidy level,

272 but there is no significant difference in genetic diversity between pentaploid and hexaploid based

273 on trend lines of PIC ($P > 0.05$). Diploid and triploid respectively had significantly lower genetic

274 diversity than other polyploidy. All the individuals of *C. dactylon* at different ploidy levels fell

275 into three major clusters based on UPGMA. The first and second clusters contained diploid and

276 triploid individuals, respectively. Tetraploid, pentaploid, and hexaploid individuals formed the

277 third cluster (Fig. 6). The genetic distance between two groups among different ploidy levels

278 increased with the increase of ploidy level. In addition, there was no obvious growth trend of the

279 distribution of ploidy levels, and regression analysis indicated that there was no significant linear

280 association between ploidy level and longitude (r = 0.071; $P > 0.05$ – Fig. 7).

281

Discussion

Genetic diversity and structure among populations along longitude gradients

284 Identifying relative roles of spatial and environmental factors in shaping patterns of population
285 genetic differentiation is of interest for understanding landscape genetics. Each of 13 populations
286 of *C. dactylon* along longitude gradients had a remarkable genetic variation within population
287 based on the study. Genetic diversity can be improved by sexual propagation, and clonal
288 propagation by rhizome and stolon can promote stable inheritance not being easily affected by
289 genetic drift (Loveless and Hamrick, 1984). Intra-population genetic diversity had no obvious
290 linear relationship with longitude in this study. In addition, mean annual temperature and
291 precipitation at different longitudes had no significant influence on the intra-population genetic
292 diversity. However, according to our previously published paper, higher within-population genetic
293 diversity appeared at lower latitude with higher temperature (> 25℃), while relatively low
294 temperature at higher latitude might have little effect on the intra-population genetic diversity
295 (Zhang et al., 2019). So higher temperature (> 25℃) may be associated with higher genetic
296 variation of *C. dactylon*. From sampling regions, there was a little higher genetic diversity of
297 populations at mid-longitudes (4, 5, 6, 8) than other populations at low- and high-longitudes,
298 because outcrossing and self-incompatibility of *C. dactylon* could produce low communication
299 among different populations of the east-west transitional locations. Genetic differences among
300 distant geographical sites with unique alleles can increase genetic diversity (Brochmann et al.,
301 2003; Westergaard et al., 2011; Parducci et al., 2012; Eidesen et al., 2013).

302 Landscape heterogeneity may lead to the formation of local adaptive populations, and different
303 genotypes may be selected to adapt to different environments (Felsenstein, 1976; Rieseberg and
304 Willis, 2007; Anderson et al., 2011). Environmental distance matrices were also calculated to
305 estimate the influences of environmental distance matrices (IBE) on the genetic distance. No
306 significant correlation was observed between genetic distance and geographic distance after
307 controlling the effect of mean annual precipitation, which might be due to a correlation between
308 geographic distance and mean annual precipitation. *P. arundinacea* (Phalaris) also exhibits a
309 certain broad-scale population genetic structure in east-to-west gradients (Perdereau et al., 2017).
310 The adaptive differentiation among populations is influenced by the natural selection of
311 heterogeneous landscape and gene flow (Dionne et al., 2008; Poelchau and Hamrick, 2012). Low

312   east-to-west inter-population gene flow (Nm = 0.7701) indicated that there was little adaptive

313   selective pressure mediated evolutionary dynamics among different populations along longitude

314   gradients (Casler et al., 2007). In addition, these factors to hinder further communication among

315   populations include the transmission mechanism of pollen and seeds of species and the barrier

316   caused by environmental conditions. In our study, restricted seed dispersal may exist among

317   populations at different longitudes exhibiting significant IBD, and mountains in the Henan–Shanxi

318   region may hinder further genetic communication among populations as natural barriers. Alpine

319   plants from several mountain ranges in Europe usually have a strong patterns of longitudinal

320   differentiation (Ronikier et al., 2008; 2012). Geographical isolation along an ecological gradient

321   or environmental isolation can lead to strong genetic differentiation for widespread plants (Hoskin

322   et al., 2005).

323   The result of AMOVA indicated 61.77% of the genetic variation was attributed to differences

324   within populations and FST was 0.3823. Higher FST along with increasing distance along

325   longitude than latitude gradients may indicate local genetic differentiation among populations

326   along longitude gradients (Edillo et al., 2009). Both spatial and environmental factors interweave

327   in the formation of population differentiation pattern. In our case, low gene flow among

328   neighboring populations may show the isolation-by-distance pattern among two neighboring

329   populations. Several reproductive barriers in different environments within the same geographical

330   area can block gene flow (Lowry, 2012; Fishman et al., 2013). Interestingly, some populations

331   separated by 200 km in distance are close in genetic distance. A probable explanation is that

332   human-mediated introduction allow contact among these populations. The mismatch distribution

333   test and neutrality test both indicated expansion within population along longitude gradients, with

334   an estimated time around 12.5 Ma (under the demographic expansion model), which may be

335   related to forests shrink and grasslands spread during Miocene time. The dramatic expansion of

336   C4 grasslands in India is a part of increasing tropical C4 savannah vegetation worldwide in the

337   late Miocene, which has been variously ascribed to uplift of the Tibetan plateau (Polissar et al.,

338   2015). In contrast, the population in Baoji with non-significant SSD and HRag values was

339   interpreted as reflecting past demographic stability.

340   The low number of admixed genotypes at mid-longitude can be explained by the geographical

341   distribution of the collection sites. Natural or human-mediated transmission may have increased

342   genetic diversity and affected the genetic structure of several grass species (Balfourier et al., 2000;

343 McGrath et al., 2007; Petit et al., 2003; Taberlet et al., 1998). Hybridization at population

344 boundaries and gene migration appear to promote homogenization. Populations at low and high-

345 longitude may evolve much earlier than populations from sites of mid-longitude based on the

346 result. Therefore, populations of *C. dactylon* at mid-longitude may evolve from complex

347 introgressions between populations at low and high-longitude with close genetic affiliation.

348 Historic changes in landscape configuration, spatial isolation and related gene flow changes may

349 gradually lead to intraspecific genetic variation between eastern and western populations of *C.*

350 *dactylon* in different geographical areas. The admixture among populations determines their

351 potential to adapt to new environmental conditions (Lee, 2002).

Relationship between ploidy levels and genetic diversity

353 The higher values of He, I and PIC were observed in populations with pentaploid and hexaploid

354 than triploid. Polyploid retains most of the population's gene pool in the form of fixed

355 heterozygosity (Brochmann et al., 2004). Hybridization of polyploid progeny from different

356 parents of the same species can increase the genetic diversity. Multiple introductions from different

357 origins and recombination between different genomes led to genetic mixture. Moreover, groups of

358 individuals with the same ploidy level were separated by genetic distance along with the increasing

359 ploidy levels. The higher genetic distance between two different ploidy levels along with the

360 increasing ploidy levels was observed in the study, which indicated that each chromosome

361 replication process might gradually increase the genetic dissimilarity. Diploid plants can

362 participate in the formation of many polyploid (Ramsey and Schemske, 2002; Otto, 2007), and

363 polyploid are often separated from their progenitors to produce new genotypes (Čertner et al.,

364 2017; Alsayied et al., 2015; Sweigart et al., 2008). Different chromosome numbers could lead to

365 reproductive segregation or sterility, which limited gene flow between hybrids and their parents

366 (Tate et al., 2005). Polyploidy not only produce direct genetic redundancy, but also promote

367 emergence of new gene functions and drive diversification and evolution (Alix et al., 2017). The

368 hybrid genome composition of two related heterotetraploid species was analyzed using molecular

369 cytogenetics to elucidate the role of heteropolyploid in the realization of *Hordeum* diversity

370 (Cuadrado et al., 2017). From the facts above, polyploidization can lead to rich genetic variation

371 and diversification for probable speciation of *C. dactylon.*

Comparison of genetic pattern between longitude and latitude

373   No significant difference exists in genetic diversity parameters within-population along longitude
374   gradients, while higher within-population genetic diversity appeares at low latitude with higher
375   temperature along latitudinal gradients (> 25℃) (Zhang et al., 2019). The result showed higher
376   temperature might promote higher within-population genetic variation of *C. dactylon.* The barrier
377   of hybridization of rice mainly focused on the environmental variation, just as temperature
378   influenced cellularization of the endosperm to promote hybrid (Folsom et al., 2014). Higher
379   temperatures may have affected the genetic similarity of *Culex pipiens* populations sampled early
380   and later in the breeding season (Edillo et al., 2009).

381   A relatively low gene flow (Nm = 0.7701) means a rich genetic differentiation along longitude
382   gradients (Nm < 1) (Wright, 1965). In our previous study on the genetic diversity and genetic
383   structure of *C. dactylon* along a latitudinal gradient, low genetic differentiation and notable
384   admixture structure existed among populations along latitudes (Zhang et al., 2019). Low genetic
385   differentiation caused by different latitudes may be attributed to the wind-pollination outcrossing
386   system and vegetative materials through human activities, which indicated that the latitude interval
387   could not be an obstacle to genetic exchange. In addition, stronger admixture could be observed
388   along with the latitude in another study (Zhang et al., 2019) than that along with longitude in this
389   study, which indicated relatively lower gene flow among populations of *C. dactylon* occurred
390   along the longitude gradients. North-south direction gene flow has existed between the eastern
391   *Lemus chinense* populations and the western ones (Long et al., 2019). The eastern Eurasian
392   populations of some species with global distribution pattern are a little different from-the western
393   Eurasian populations, which occurs between late Miocene and Pleistocene (Tabata et al., 2018;
394   Hardouin et al., 2015; Rodrigues et al., 2014). East Asia was less extensively glaciated and had a
395   relatively mild glacial climate. The Qinghai-Tibetan Plateau uplifted rapidly in Pleistocene and the
396   Asian summer monsoon intensified, so the formation of the inland arid belt of Eurasia resulted in
397   long-term ecological isolation (Clark and Mix, 2002; López-Pujol et al., 2011). A probable
398   explanation is that the genetic differentiation along longitude gradients was little and the gene flow
399   was frequent before the uplift of Tibet Plateau, but the situation reversed after that because
400   populations were ecologically isolated. Therefore making a comparison between latitude and
401   longitude, a stronger gene flow from the north-south than east-west direction is characteristic of
402   genetic structure of *C. dactylon* in China. Distinct genetic divergence of *C. dactylon* presented
403   between the lower and the higher ploidy level along both longitude and latitude gradients. In

404 addition, widespread polyploidization may cause significantly increasing genetic diversity with
405 the ploidy level.

406

Conclusions

408 A large-scale population genetic survey of genetic diversity of *C. dactylon* has been conducted to
409 evaluate genetic variation and population structure along a longitude gradient. Landscape
410 heterogeneity and geographical isolation along several mountain ranges can lead to formation of
411 locally adapted populations with reduced gene flow between habitats. Geographic distance
412 correlated with precipitation may influenced genetic diversity among populations along longitude
413 gradients. A higher level of genetic differentiation among populations of *C. dactylon* along
414 longitude gradients than those along latitudinal gradients indicated a stronger gene flow from the
415 north-south than east-west direction. Decreased genetic diversity is estimated for individuals with
416 lower ploidy level compared to higher ploidy level, and relatively rich genetic differentiation exists
417 among different ploidy levels. Identifying genetic diversity along longitude gradients in contrast
418 with latitudinal gradients provides important genetic information and improves the ability to
419 protect biodiversity.

420

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Competing interests

425 The authors declare that they have no competing interests.

Authors Contributions

427 • Jing-Xue Zhang conceived and designed the experiments, performed the experiments, analyzed
428 the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved
429 the final draft.

430 • Xue-Bing Yan and Yu-Xia Guo conceived and designed the experiments, authored or reviewed
431 drafts of the paper, and approved the final draft.

432 • Miao-Li Wang, Biao-Ji Fan, Zhi-Peng Guo, Yong-Zhuo Guan, Gen Qu performed the
433 experiments and approved the final draft. Chuan-Jie Zhang checked linguistic accuracy and
434 approved the final draft.

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652

653   **Figure legends**

654   Fig. 1 Locations of sampled populations of *C. dactylon*. The blue dots represent the sampling sites
655   along longitude gradients.

656

657   Fig. 2 Summary statistics of within-population genetic variation of 13 *C. dactylon* populations
658   from estern to western. He: Nei's gene diversity index; I: Shannon diversity index.

659

660   Fig. 3 The UPGMA (unweight pair-group method with arithmetic means) dendrogram generated
661   cluster analysis based on EST-SSR data of 13 *C. dactylon* populations. The *C. dactylon*
662   populations in this study were grouped into three clusters (blue, red, green).

663

664   Fig. 4 Principal component analysis of the 13 populations based on the genetic distance derived
665   from EST-SSR markers. Populations in this study were grouped into three clusters (black circles).

666

667   Fig. 5 Population structure analysis of 249 *C. dactylon* individuals sampled from 13 populations
668   using the model-based program STRUCTURE as follows: **A**-K = 4 and **B**-K = 8. Each color shows
669   one Bayesian ancestry group (K) and the length of the colored segment represents the estimated
670   membership proportions in different Bayesian ancestry groups (K).

671

672   Fig. 6 The UPGMA (unweight pair-group method with arithmetic means) dendrogram of
673   bermudagrass groups with di□erent ploidy level.

674

675   Fig. 7 Regression analysis between ploidy level of bermudagrass (*C. dactylon*) and longitude.

Figure 1

Locations of sampled populations of *C. dactylon*.

Fig. 1 Locations of sampled populations of *C. dactylon*. The blue dot represents the sampling sites along longitudinal gradient.
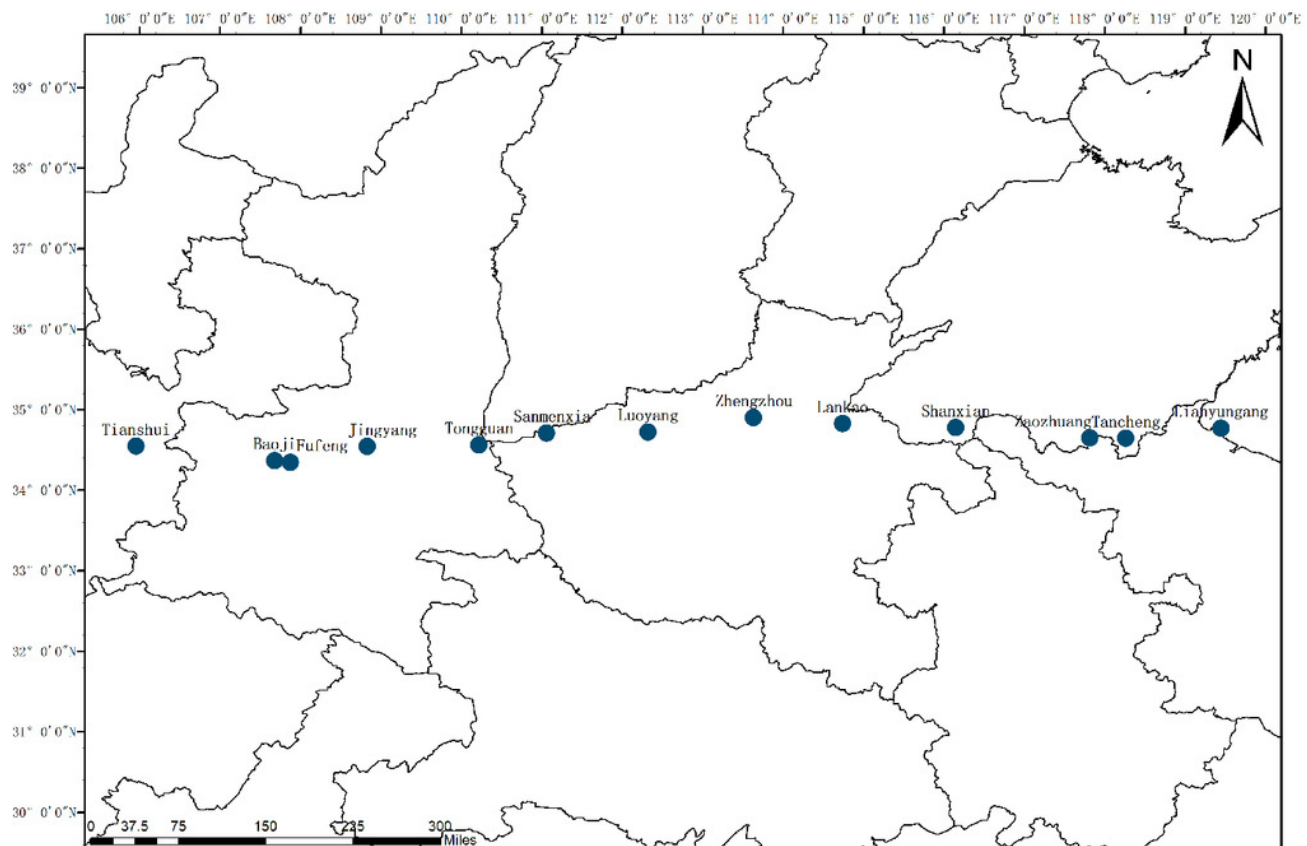
Figure 2

Summary statistics of within-population genetic variation of 13 *C. dactylon* populations from estern to western.

Fig. 2 Summary statistics of within-population genetic variation of 13 *C. dactylon* populations from estern to western. He: Nei's gene diversity index; I: Shannon diversity index.
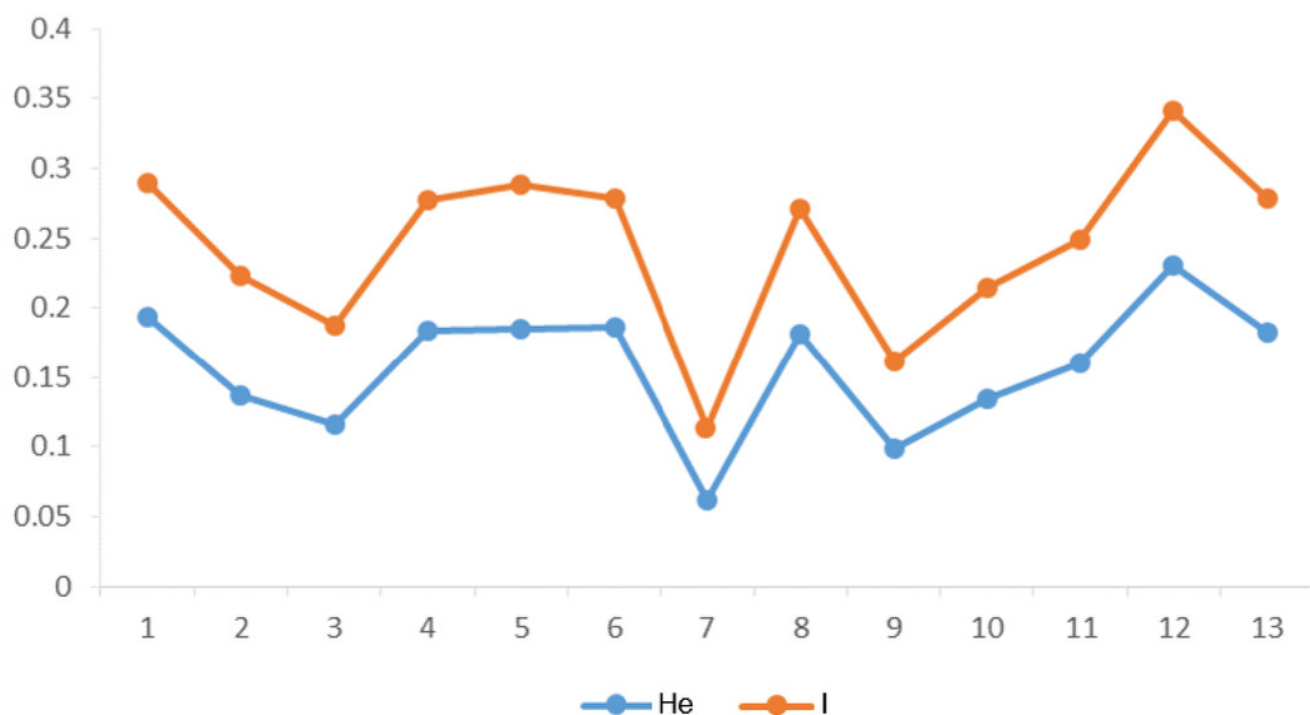
Figure 3

The UPGMA (unweight pair-group method with arithmetic means) dendrogram generated cluster analysis based on EST-SSR data of 13 *C. dactylon* populations.

Fig. 3 The UPGMA (unweight pair-group method with arithmetic means) dendrogram generated cluster analysis based on EST-SSR data of 13 *C. dactylon* populations. The *C. dactylon* populations in this study were grouped into three clusters (blue, red, green).
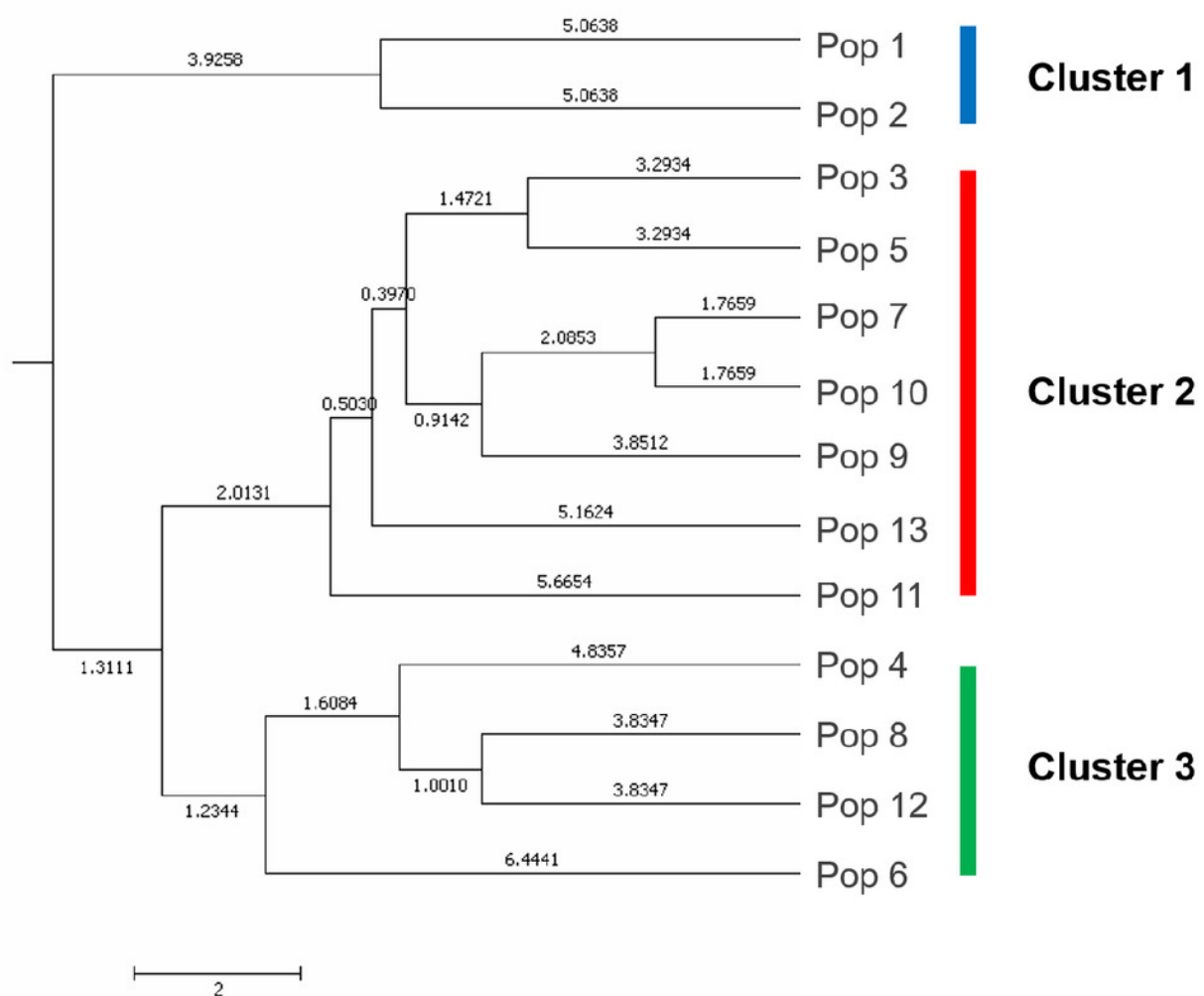
Figure 4

Principal component analysis of the 13 populations based on the genetic distance derived from EST-SSR markers.

Fig. 4 Principal component analysis of the 13 populations based on the genetic distance derived from EST-SSR markers. Populations in this study were grouped into three clusters (black circles).
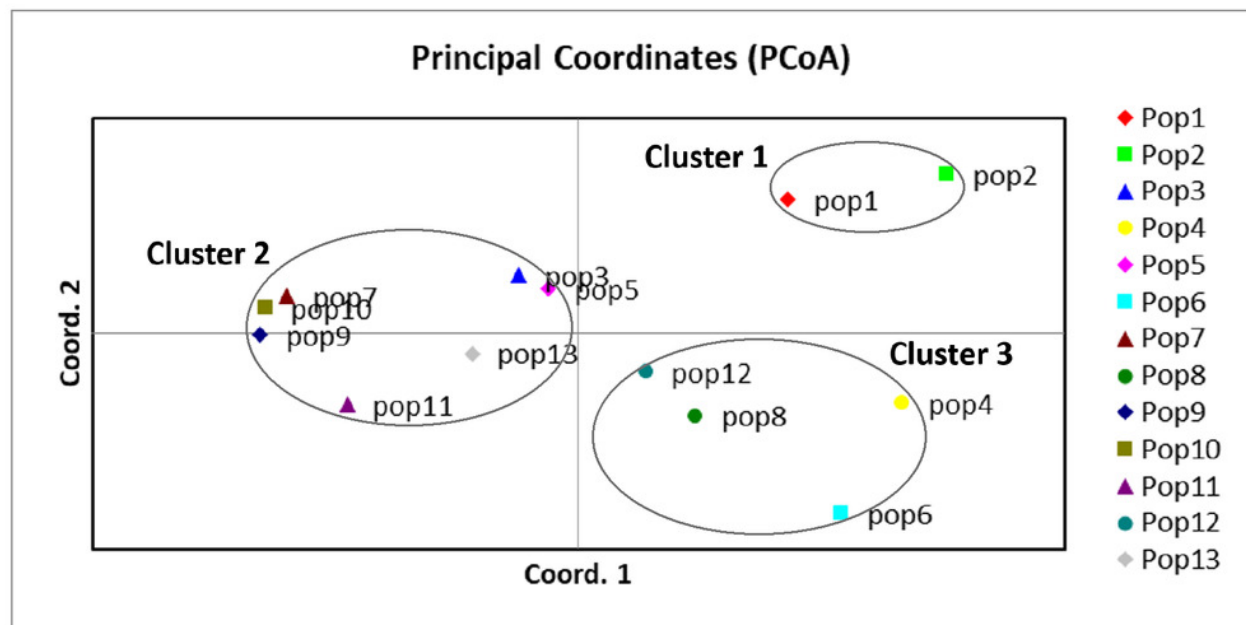
Figure 5

Population structure analysis of 249 *C. dactylon* plants sampled from 13 populations using the model-based program STRUCTUREas follows: A-K = 4 and B-K = 8.

Fig. 5 Population structure analysis of 249 *C. dactylon* plants sampled from 13 populations using the model-based program STRUCTUREas follows: **A**-K = 4 and **B**-K = 8. Each color shows one Bayesian ancestry group (K) and the length of the colored segment represents the estimated membership proportions in different Bayesian ancestry groups (K).
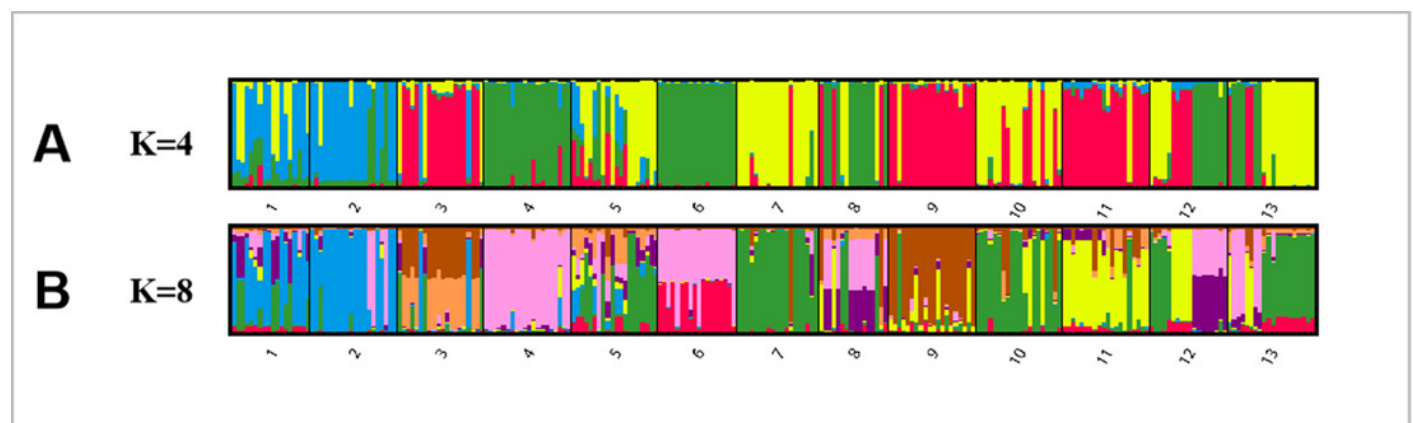
Figure 6

The UPGMA (unweight pair-group method with arithmetic means) dendrogram of bermudagrass groups with different ploidy level.

Fig. 6 The UPGMA (unweight pair-group method with arithmetic means) dendrogram of bermudagrass groups with different ploidy level.
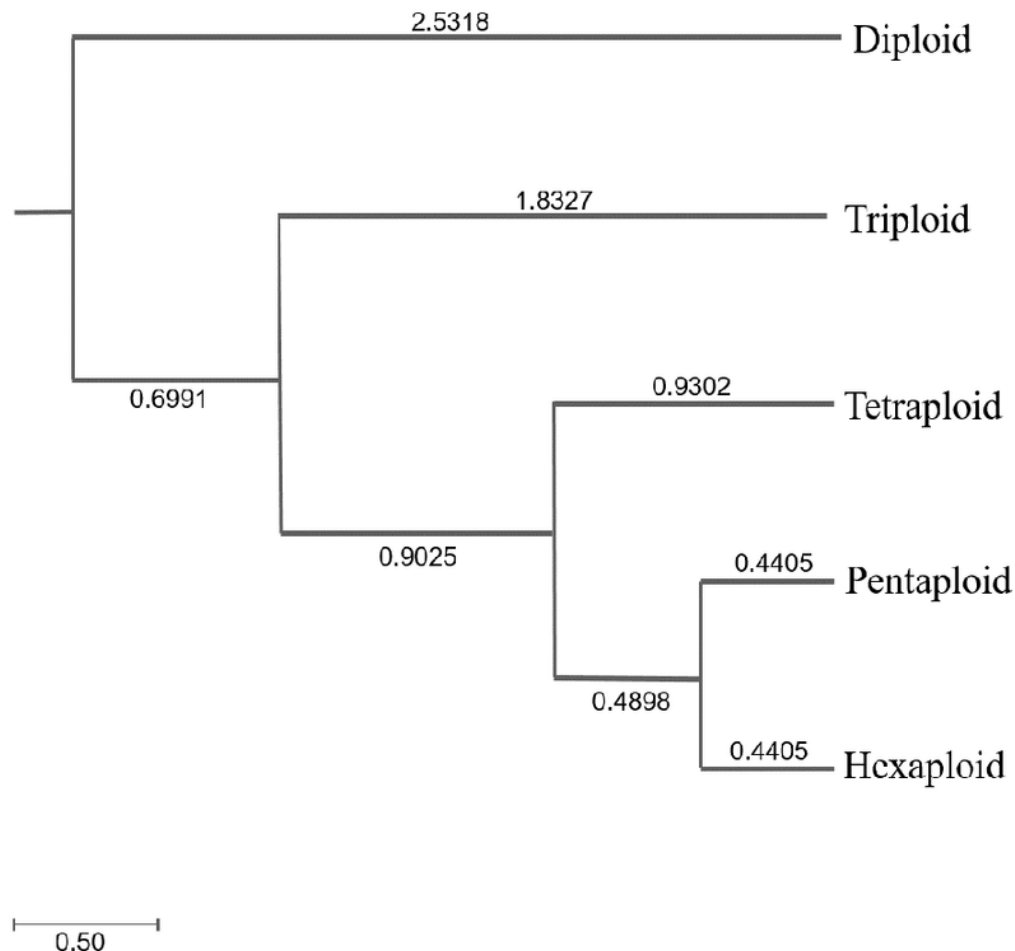
Figure 7

Regression analysis between ploidy level of bermudagrass (*C. dactylon*) and longitude.
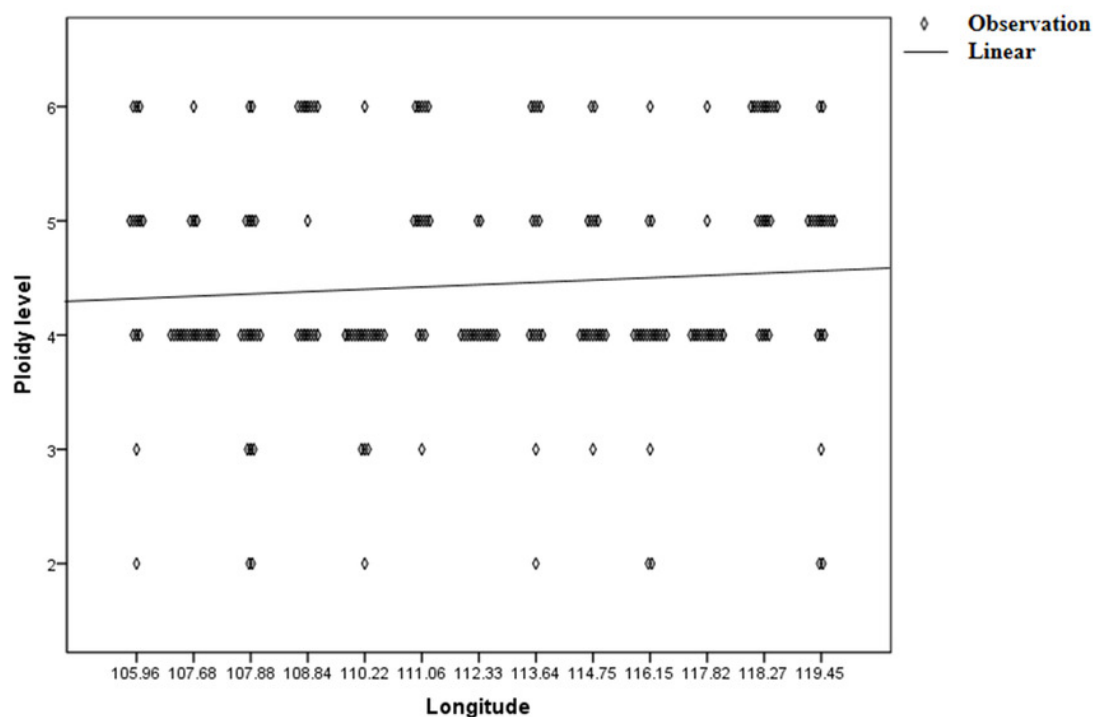
Fig.7 Regression analysis between ploidy level of bermudagrass (*C. dactylon*) and longitude.

Information on geographical sites and climate of *C. dactylon* populations collected from different longitudes in China.

1   Table 1 Information on geographical sites and climate of *C. dactylon* populations collected from

2   different longitudes in China.

Code	Region	Habitat	Longitude (E)	Latitude (N)	Altitude (m)	MAT (℃)	MAP (mm)
1	Lianyungang	Island	119°27′06″	34°46′09″	50	14.5	883.9
2	Tancheng	Roadside	118°16′08″	34°38′37″	30	13.8	832.9
3	Zaozhuang	Roadside	117°49′20″	34°38′48″	89	14.4	820.3
4	Shanxian	Roadside	116°09′11″	34°46′31″	30	14.2	621.4
5	Lankao	Roadside	114°44′55″	34°49′32″	60	14.3	631.1
6	Zhengzhou	Swamp	113°38′20″	34°54′04″	90	14.7	640.8
7	Luoyang	Roadside	112°19′30″	34°43′20″	210	14.4	637.2
8	Sanmenxia	Roadside	111°03′49″	34°42′29″	340	14	558.1
9	Tongguan	Roadside	110°13′18″	34°33′41″	540	13.1	602.9
10	Jingyang	Roadside	108°50′07″	34°32′32″	410	13.5	504.1
11	Fufeng	Roadside	107°52′41″	34°20′35″	570	12.8	569.9
12	Baoji	Ditch side	107°41′03″	34°21′54″	630	13.5	645.9
13	Tianshui	Roadside	105°57′34″	34°32′43″	1050	11.4	500.7

3   MAT, mean annual temperature; MAR, mean annual precipitation

Within-population genetic diversity of 13 *C. dactylon* populations at different longitudes.

1   Table 2. Within-population genetic diversity of 13 *C. dactylon* populations at different

2   longitudes.

Pop	N	He	I	PIC
1	18	0.1939	0.2895	0.55
2	20	0.1367	0.2231	0.56
3	20	0.1157	0.1872	0.47
4	20	0.1837	0.2769	0.57
5	20	0.1843	0.2878	0.68
6	18	0.1859	0.279	0.53
7	19	0.0619	0.1131	0.40
8	16	0.1809	0.271	0.52
9	20	0.0984	0.1617	0.43
10	20	0.1338	0.2142	0.51
11	20	0.1603	0.2493	0.57
12	18	0.231	0.3408	0.62
13	20	0.1818	0.2779	0.57
Total	249	0.2587	0.4029	1.00

3   He, Nei's gene diversity index; I, Shannon's diversity index; PIC, Polymorphic information

4   content

Analysis of molecular variance (AMOVA) of based on 249 individuals from the 13 populations of bermudagrass.

1   Table 3. Analysis of molecular variance (AMOVA) of bermudagrass of different population.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	12	1336.521	5.364	38.23%
Within populations	236	2045.455	8.667	61.77%
Total	248	3381.976	14.031	

2   Note: df, Degree of freedom

Results of the mismatch distribution analysis and neutrality tests of *C. dactylon* of each population along longitudinal gradients.

Results of the mismatch distribution analysis and neutrality tests of *C. dactylon* of each population along longitudinal gradients. SSD, sum of squared deviation under expansion model; HRI, raggedness; *Significant ($P \leq 0.05$); **highly significant ($P \leq 0.01$).

1   Table 4 Results of the mismatch distribution analysis and neutrality tests of *C. dactylon* of each population along longitude gradients.

Populations	Tau (τ)	Time since expansion began(t, Ma)	SSD	P-value	HRI	P-value	Fu's FS	P-value	Tajima's D	P-value
1	29.500	21.612	0.006	0.960	0.013	0.820	-5.704	0.017*	1.152	0.916
2	0.000	0.000	0.013	1.000	0.017	0.940	-9.177	0.000**	-0.370	0.377
3	2.000	1.465	0.008	0.870	0.011	0.810	-10.399	0.001**	-0.299	0.435
4	16.000	11.722	0.005	0.390	0.009	0.610	-7.288	0.002**	0.813	0.838
5	24.500	17.949	0.010	0.650	0.020	0.280	-7.270	0.007**	0.072	0.559
6	21.200	15.531	0.008	0.540	0.011	0.880	-5.904	0.013*	1.116	0.888
7	0.400	0.293	0.012	0.700	0.019	0.800	-14.636	0.000**	-1.731	0.033*
8	30.400	22.271	0.023	0.340	0.020	0.670	-4.805	0.017*	0.944	0.881
9	2.600	1.905	0.011	0.390	0.009	0.830	-11.688	0.000**	-0.570	0.301
10	8.700	6.374	0.008	0.540	0.011	0.680	-9.330	0.002**	-0.116	0.495
11	21.500	15.751	0.004	0.900	0.007	0.960	-8.116	0.004**	0.194	0.619
12	35.300	25.861	0.021	0.000	0.020	0.500	-4.927	0.020*	1.493	0.940
13	28.900	21.172	0.009	0.780	0.016	0.560	-7.349	0.002**	0.762	0.840
Mean	17.003	12.457	0.011	0.620	0.014	0.718	-8.199	0.007**	0.266	0.625

3   SSD, sum of squared deviation under expansion model; HRI, raggedness; *Significant (P≤0.05); **highly significant (P≤0.01).