The extended day length promotes earlier flowering of bermudagrass

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Day length is a very critical environmental factor affecting plant growth and development. The extended light application time have been shown to promote flowering in the long-day plant and to shorten breeding time in some crops. However, previous research on the regulation of bermudagrass flowering by light application time is scarce. Therefore, this study investigated the effect of day length on the growth and flowering of bermudagrass by prolonging the light application time in a controlled greenhouse. Three different light application times were set up in the experiment: 22/2 h (22 hours light/2 hours dark), 18/6 h (18 hours light/6 hours dark) , 14/10 h (14 hours light/10 hours dark). Results showed that extending the light application time not only promoted the growth of bermudagrass (plant height, fresh weight, dry weight) but also its nutrient uptake (nitrogen (N) and phosphorous (P) content)**.** In addition, daily light integrals were different when flowering under different light application times. Most importantly, under 22/2 h condition, flowering time was successfully reduced to 44 days for common bermudagrass (*Cynodon dactylon* [L.] pers) genotype, A12359 and 36 days for African bermudagrass (*Cynodon transvaalensis* Burtt-Davy) genotype, ABD11. This study demonstrated a successful method of bermudagrass flowering earlier than usual time by manipulating light application time which might provide useful insight for bermudagrass breeding.

1   **Title page**

3   **The extended day length promotes earlier flowering of bermudagrass**

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23 **Abstract**

24 Day length is a very critical environmental factor affecting plant growth and development. The

25 extended light application time have been shown to promote flowering in the long-day plant and

26 to shorten breeding time in some crops. However, previous research on the regulation of

27 bermudagrass flowering by light application time is scarce. Therefore, this study investigated the

28 effect of day length on the growth and flowering of bermudagrass by prolonging the light

29 application time in a controlled greenhouse. Three different light application times were set up in

30 the experiment: 22/2 h (22 hours light/2 hours dark), 18/6 h (18 hours light/6 hours dark), 14/10 h

31 (14 hours light/10 hours dark). Results showed that extending the light application time not only

32 promoted the growth of bermudagrass (plant height, fresh weight, dry weight) but also its nutrient

33 uptake (nitrogen (N) and phosphorus (P) content). In addition, daily light integrals were different

34 when flowering under different light application times. Most importantly, under 22/2 h condition,

35 flowering time was successfully reduced to 44 days for common bermudagrass (*Cynodon dactylon*

36 [L.] pers) genotype, A12359 and 36 days for African bermudagrass (*Cynodon transvaalensis*

37 Burtt-Davy) genotype, ABD11. This study demonstrated a successful method of bermudagrass

38 flowering earlier than usual time by manipulating light application time which might provide

39 useful insight for bermudagrass breeding.

40 **Key Words**: Bermudagrass, Light application time, Flowering, Rapid Breeding

41 **Introduction**

42 Light is not only a source of energy, but it is also one of the most important environmental

43 factors for plants growth and development (Fukuda et al, 2008). Light intensity, light quality

44 (spectral qualities of light), and day length are three major factors that have significant influences

45 on plants (Nadav & Nirit, 2021). Photosynthesis is directly affected by light intensity, with

46   increased photon flux density at selective wavelengths enabling higher rates of carbon fixation.

47   Light quality influences the synthesis and storage of photosynthetic pigments in leaves, as well

48   as the metabolism of nutrients, and nitrogen (Shafiq et al,2021). It also regulates plant

49   development, photo-morphogenesis, and material metabolism (primary and secondary

50   metabolism) (Monostori et al, 2018). Day length is the amount of time that plants are exposed to

51   a predefined light and dark state each day, which influences plant physiological and biochemical

52   reactions to enhance fast growth, and development (Adams & Langton, 2005; Zha & Liu, 2018).

53   Day length not only plays an important role in inducing external growth of the crop, but also has

54   significant effects on flowering (Munir et al, 2001; Martín et al, 2018). Plants must shift from

55   vegetative to reproductive development in order to secure their own reproduction in a variety of

56   uncertain surroundings. It depends mainly on the interaction of internal regulation and external

57   factors. The photoperiodic pathway, vernalization pathway, autonomous pathway, and

58   gibberellin pathway are all involved in the regulation of plant blooming.

59   The purpose of plant breeding is to produce plants with specific characteristics (Godwin et al,

60   2019). Turfgrass breeding is a method to cultivate new varieties and strains of turfgrass with

61   specific traits (such as disease resistance, insect resistance, trampling tolerance, herbicide

62   resistance and stress resistance) and stable inheritance according to the needs of people's

63   production and life. The development of the turfgrass industry is closely related to turfgrass

64   breeding, which is based on traditional breeding methods with the application of newly

65   developed biotechnology. In terms of the remarkable results of breeding, traditional breeding

66   methods usually play a very important role whether in crop breeding, forage breeding or

67   turfgrass breeding, the breeding of breakthrough varieties almost always depends on the repeated

68   hybridization of superior germplasm resources (Hanna et al, 2013). However, breeding new,

69 advanced varieties for most crops and grasses will take years. The efficacy of agricultural genetic

70 improvement is substantially determined by cycle time, and various ways have been explored to

71 decrease the duration of plant reproductive cycles (Voss-Fels et al, 2019). Speed breeding, which

72 involves using regulated environmental conditions and extended light application time for early

73 flowering, can shorten the fertility period of turfgrasses and forages and accelerate research

74 through rapid generational progress. Watson et al. reduced the generation time of long-cycle

75 crops such as spring wheat (*Triticum aestivum*) and oilseed rape (*Brassica napus*) to 6 and 4

76 generations per year, respectively, rather than 2-3 generations under normal glasshouse

77 conditions. Ghosh et al. promoted rapid growth of spring bread wheat (*Triticum aestivum*),

78 durum wheat (*T. durum*), barley (*Hordeum vulgare*), pea (*Pisum sativum*), the model grass,

79 *Brachypodium distachyon* and the model legume, *Medicago truncatula* through speed breeding.

80 This non-transgenic strategy cuts generation time in half, speeds up breeding and other cutting-

81 edge plant breeding procedures, and can meet the genetic gain targets required for our future

82 crops (Li et al, 2018; Wanga et al, 2021). Turfgrass and forage have a promising future, but there

83 is still a gap in research on rapid breeding of turfgrass and forage.

84   Bermudagrass has better ornamental color, high density, drought endurance, salt tolerance,

85 wear resistance, and quick reproduction, making it one of the most valued grasses among other

86 warm-season turfgrasses (Pang et al, 2011). It's not just one of world's most popular turfgrasses,

87 but it's also a great plant for soil consolidation and slope protection (Shi et al, 2014; Zheng et al,

88 2017). It is also an excellent forage grass due to its rich foliage, soft texture, and good

89 palatability (Taliaferro, 1995). Light duration has a great influence on the development of

90 grasses and grains. The growth and development of tropical forage grasses are influenced by

91 several environmental factors. Sinclair et al. (2003) found that extending light application time

92   can promote the growth of 'Tifton 85' bermudagrass. Somayeh Esmail et al. (2012) found that the

93   fresh weight, tiller density, relative water content and reducing sugar content of shoot and root of

94   bermudagrass decreased with the decrease of light duration. In all seed crops, the floral transition

95   is a key developmental switch that determines dry matter production, and flowering time, plant

96   requirements to vernalization are major factors in the climatic adaptation of regions of good

97   germplasm. As turfgrass, it is induced to flower for the purpose of breeding and seed production.

98   Crossbreeding (C. *dactylon* ×C. *transvaalensis*) was used to create popular varieties as Tifway,

99   Latitude 36, Northbridge, Tahoma 31, and TifTuf (Reasor et al, 2016). Furthermore, because

100   developing genetically stable bermudagrass variants takes a long time, quick breeding is required

101   to enhance the breeding of outstanding kinds. The objectives of this work were to shorten the

102   flowering time by analyzing the growth and flowering characteristics of bermudagrass under

103   long light application time, in order to accelerate the purification of hybrid progeny and

104   transgenic plants, and to provide a theoretical basis for rapid breeding of bermudagrass.

105   **Materials and methods**

106   **Plant materials and growth conditions**

107   The plant materials used in this investigation were common bermudagrass (*Cynodon dactylon*)

108   and African bermudagrass (*Cynodon transvaalensis*), designated as A12359 (2n=36) and

109   ABD11(2n=18), respectively (The germplasm resources collected by our research group and

110   named A12359 and ABD11). The experimental materials were separated into two identical

111   portions for monitoring growth and blooming. The medium at the experiment was commercial

112   peat soil, and whole bermudagrass stolons were extracted and planted in cylindrical flowerpots

113   (Peilei, Zhenjiang, China). The same number of bermudagrass stolons were planted in each

114   cylindrical flowerpot (six stolons per pot). To remove seeding disparities between individuals,

115   stolons from the mother plant were evenly propagated. Half-strength Hoagland nutrient solution

116   (1/2 HS) was irrigated once a week.

117   **Treatment**

118   The experiment was carried out in the greenhouse of coastal grass germplasm resources and

119   breeding center of Ludong University on September 2020. The location is 127 meters above sea

120   level, with geographical coordinates of N37.53° (latitude) and E121.36° (longitude). The entire

121   experiment lasted three months, from September to December 2020. All experimental materials

122   were grown on plant shelves (The wire beneath the shelves was breathable and temperature-

123   permeable) in the controlled greenhouse and were supplemented by sodium metal halide lights

124   (RVP350; PHILIP Shanghai China) positioned at a height of 1.8 m from the shelves. Three light

125   application times, namely 22/2 (22 hours light/2 hours dark), 18/6 (18 hours light/6 hours dark),

126   and 14/10 (14 hours light/10 hours dark) were used. Each light application condition had the

127   same bermudagrass (three replicates per material, one replicate per pot). The supplementary light

128   was set to automatically turn off for two hours from 9pm to 11pm to achieve a 22/2 light

129   application time. Under the same conditions, 18/6 and 14/10 were covered by paper boxes at

130   regular intervals and guaranteed complete darkness. The 18/6 light application time was covered

131   at 5 pm and uncovered at 9 pm; 14/10 light application time was covered at 9 pm and uncovered

132   at 7 am the next day **(Fig. 1)**. The greenhouse had a fixed relative humidity and atmospheric

133   pressure of 60% and 101kpa respectively. In the experiment, three light application times were

134   treated under the same temperature and light intensity. A temperature and light recorder

135   (WS1PROG; ubibot, Dalian, China) was used to record the temperature and light intensity in

136   greenhouse **(Fig. S1)**.

137   **Recording inflorescence number and growth indicator**

138 Every two weeks, growth indices such as plant height, branch number, and biomass (fresh

139 weight, dry weight) were assessed under various light application times. The growing material

140 was clipped every two weeks. At the specified times, each pot was harvested manually using a

141 hand shear at a consistent height of 10 cm above the soil surface. Every two weeks, the plant

142 height in each pot was measured from the soil surface to the plant's topmost tip (using the

143 Standard scale), and the branches were counted. After mowing, the fresh weight was weighed

144 using an electronic balance. The samples were then dried for 30 minutes at 105°C in a forced-

145 draft oven (DHG-9140A; Shanghai, China) before being oven-dried for 72 hours at 75°C until

146 they reached a consistent weight. After drying the samples, the dry weight (DW) was weighted.

147 The above-ground (shoots) and subsurface sections (roots) of bermudagrass were separated at the

148 end of the experimental treatment. After carefully washing the tissues with deionized water, the

149 fresh weight (FW) of the shoots and roots was weighted, and the dry weight (DW) was weighted

150 after drying the samples. We counted the number of headings and flowering every two days after

151 the first inflorescence developed until the number of headings and flowering were steady.

152 **Determination of nitrogen and phosphorus content**

153 To assess the nutritional value, shoots were pulverized and weighed around to 0.1g, placed in a

154 desiccating tube, mixed with 5 ml of 95% concentrated sulfuric acid (H_2SO_4) and digested in a

155 graphite digestion apparatus (SH220N; Jinan Hanon, Shandong, China). Then, using a chemical

156 automated analyzer, the nitrogen (N) and phosphorus (P) content was determined (SmartChem

157 200; AMS Alliance, Guidonia, Rome, Italy).

158 **The average photosynthetic photon flux density (PPFD) and daily light integral (DLI)**

159 Based on the temperature and light intensity recorded in Supplementary Fig. 1, we calculated the

160 average photosynthetic photon flux density (PPFD) for each treatment and temperature, and the

161 average daily PPFD for 22/2, 18/6, and 14/10 h application times were: 98.93μmol m^{-2}s^{-1},

162 83.54μmol m^{-2}s^{-1}, 66.29μmol m^{-2}s^{-1}, respectively. The average temperature was 20℃.

163 Furthermore, we integrated the total light energy received by the material under three light

164 application times. The interval of DLI for different light application times was one week.

165 **Data analysis**

166 For data processing, Microsoft Excel was utilized. Whereas, for date visualization,

167 Sigmaplot12.3 was used. Originlab was used for greenhouse temperature and light mapping.

168 SPSS ver. 22.0 software was used to conduct statistical analysis (Microsoft Corp). The

169 noteworthy differences between various light application times were analyzed using One-way

170 analysis of variance (ANOVA) (P＜0.05). In this study, two ploidy bermudagrass were chosen

171 as the representative to reflect their response characteristics to photoperiod, so only the effect of

172 photoperiod on them was emphasized and statistically analyzed, and compared under the same

173 conditions of the same species in the same week. The differences caused by light application

174 time were marked by letters (a, b, c). Otherwise, Two-way analysis of variance (ANOVA) was

175 employed to examine the effects of 'Light application time', 'Week' and 'Light application time

176 * Week' (p<0.05) **(Table. S1)**.

177 **RESULTS**

178 **Effect of different light application times on the growth of bermudagrass**

179 The result was presented in Figure 2, where the charts depicted the change in plant height during

180 grass mowing. For common bermudagrass (A12359), the interaction between Light application

181 time and week was significant **(Table. S1)**, and different light application times had a substantial

182 effect on plant height, under longer light application times 22/2 and 18/6 h being higher than that

183   under shorter light application time 14/10 h **(Fig. 2a,2b)**. In addition, the plant height of common

184   bermudagrass, A12359 tended to rise in early stages (2-4 weeks) before got stabilized in the later

185   stages **(Fig. 2a)**. For African bermudagrass (ABD11), there was significant interaction between

186   light application time and week **(Table. S1)**. Moreover, plants with longer light application times

187   (22/2 and 18/6 h) were taller than those with shorter (14/10 h), and the difference was significant.

188   It was worth noticing that the plant height of African bermudagrass, ABD11 did, however, show

189   a consistent upward tendency **(Fig. 2b)**. The pattern of plant height variation suggested that

190   frequent mowing contributes to the growth of African bermudagrass.

191   The biomass (fresh weight) was measured in every two weeks, as illustrated in Figure 2c,2d.

192   There were considerable changes in fresh weight while grown under different light application

193   times. And plants grown under longer light application times *viz* 22/2 and 18/6 h were found

194   with higher fresh weight compared to plants under shorter light application time 14/10 h **(Fig.**

195   **2c,2d)**. For common bermudagrass (A12359), the interaction between light application time and

196   week was significant **(Table. S1)**, and A12359's fresh weight in the 22/2 and 18/6 h conditions

197   differed significantly from that in the 14/10 h condition **(Fig. 2c)**. Further, the maximal biomass

198   for A12359 was attained in week twelve **(Fig. 2d)**. For African bermudagrass (ABD11), the

199   interaction between light application time and week was significant **(Table. S1)**, however, there

200   was no significant difference in fresh weight between 18/6 and 14/10 h treatments, while 22/2 h

201   was considerably different from the other two groups **(Fig. 2d)**. The maximal biomass for

202   ABD11 was attained in week eight **(Fig. 2d)**.

203   The study discovered that variations in dry and fresh weights of A12359 and ABD11 essentially

204   followed the same pattern. The dry weight exhibited considerable changes under different light

205   application times. Dry weight was greater when plants were grown at longer light application

206   times (22/2 and 18/6 h) compared to shorter light application time (14/10 h) **(Fig. 2e,2f)**.

207   The fresh weight and dry weight of the bermudagrass shoots and roots were evaluated in order

208   to determine the influence of light application time on their biomass. As predicted, longer light

209   application times resulted in higher biomass of both shoots and roots than shorter light

210   application times **(Fig. 2g-2j)**. The fresh weight of common bermudagrass (A12359) shoots and

211   roots exhibited significant variations between the three light application times **(Fig. 2g)**, while

212   the dry weight at longer light application time 22/2 h showed significant differences from the

213   other two groups **(Fig. 2h)**. Furthermore, the fresh weight of shoots of African bermudagrass

214   (ABD11) at 22/2 h was significantly different from the other two groups, but the fresh weight of

215   roots was not significantly different among the three light application times **(Fig. 2i)**.

216   Nevertheless, dry weight of its roots, at 22/2h was significantly different from the other two light

217   application times **(Fig. 2j)**.

218   **Effect of different light application times on nutrient absorption of bermudagrass**

219   For A12359, the effect of different light application times on N and P content was significant,

220   and the interaction between light application time and week was significant **(Table. S1)**. And

221   plants at longer light application time, N and P content was higher than the shorter **(Fig. 3a,3c).**

222   Under the light application time 22/2 h, A12359 achieved their maximum N and P at week 12th.

223   For ABD11, the effect of different light application times on N and P content was significant,

224   and the interaction between light application time and week was significant **(Table. S1)**.

225   Moreover, the N and P content at longer light application time 22/2 h showed significant

226   differences from the other two groups **(Fig. 3b,3d)**. Under the 22/2 h, ABD11 reached their

227   maximum N and P at week 8th **(Fig.3b,3d)**.

Effect of different light application times on flowering of bermudagrass

Branch numbers were greater when plants were grown at longer light application times (22/2 and

18/6 h) compared to shorter (14/10 h) **(Fig. 4a,4b)**. For common bermudagrass (A12359), the

effect of different light application times on branch number was significant, and the interaction

between light application time and week was significant **(Table. S1)**. And branch numbers were

greater when plants were grown at longer light application times (22/2 and 18/6 h) compared to

shorter (14/10 h) **(Fig. 4a)**. The common bermudagrass (A12359) did not have a propensity to

increase its branching number at 14/10 h **(Fig. 4a)**, indicating that A12359 could only branch

when it reached a particular daylight level, and the daylight length 14/10 h was insufficient for

its branching **(Fig.4a)**. However, the similar pattern of branching number expansion was seen in

both 18/6 and 22/2 h light application times **(Fig.4a)**, suggesting that 18/6 could be adequate for

branching for A12359. Furthermore, by week 12th, the common bermudagrass (A12359) reached

its maximum number of branches **(Fig. 4a)**. For African bermudagrass (ABD11), the effect of

different light application times on branch number was significant, and the interaction between

light application time and week was significant **(Table. S1)**. The number of branches with a light

application time of 22/2 h was greater than that of 18/6 and 14/10 h, and the difference was

significant. In African bermudagrass (ABD11) branching number increased as daylight duration

rose, and the link between daylight length and branching number showed a linear change **(Fig.**

4b).

The blooming number of bermudagrass displayed an inverse curve trend in response to

different light application times **(Fig. 4c,4d)**. For common bermudagrass (A12359), the first to

flower at the lengthy light application time, 22/2h. It took 44 days under longer light application

time 22/2 h, 63 days under 18/6 h, and 85 days under short light application time 14/10 h from

251   planting to flowering, *i.e.* the longer light application time shortened common bermudagrass

252   flowering time by 41 days. For African bermudagrass (ABD11), the first to flower was produced

253   at longer light application times, 22/2h and 18/16h. ABD11, on the other hand, took 36 days

254   from planting to blooming under light application time 22/2 h, 36 days under 18/6 h, and 95 days

255   under short light application time 14/10 h. These results suggested that extending light

256   application time might promote flowering in common bermudagrass and African bermudagrass.

257   **Daily light integral for different light application times**

258   The daily light integral curves for the three light application times almost linearly increased with

259   the extension of the treatment time, as shown in Figure 5. Moreover, the daily light integral of

260   22/2 h was the most in the whole experiment. For material A12359, the long light application

261   time 22/2 h flowered at week 6.43, the accumulated daily light integral was approximately 382

262   mol m^{-2} d^{-1}, the 18/6 h treatment flowered at week 9.14, the accumulated daily light integral was

263   approximately 482 mol m^{-2} d^{-1}, and the 14/10 h treatment flowered at week 12.43, the

264   accumulated daily light integral was approximately 582 mol m^{-2} d^{-1}. The longer the light

265   application time, the earlier the flowering, however, the longer light application time

266   accumulates the least amount of daily light integral at the first flowering. A12359 showed

267   different daylight integrals when flowering under different light application times, while ABD11

268   showed roughly similar daylight integrals when flowering under 22/2 and 18/6 h.

269   **Discussion**

270   Light duration has a significant effect on the developmental pattern of grasses and cereals. It has

271   been discovered that increasing light application time might encourage the growth of

272   bermudagrass biomass. The fresh and dry weights of bermudagrass, for example, rose

273   considerably in light application times 22/2 and 18/6 h as compared to the shorter light

274   application time 14/10 h **(Fig. 2c-2f)**. This observation was consistent with previous studies of

275   Hay (1990) in case of dry-matter production of grass species like high-latitude types of *Phleum*

276   *pratense* (both vegetative and reproductive), *Poa pratensis* (vegetative), and Bromus inermis

277   (vegetative). Thomas et al. (2001) found that the yields of the three species (*Paspalum notatum*

278   Flugge; *Cynodon dactylon* L.; *Cynodon nlemfuensis* Vanderyst) under long light application time

279   were higher than those under natural daylight. But the growth of Florakirk bermudagrass and

280   Florona stargrass was not as pronounced as Tifton 85 bermudagrass or Pensacola bahiagrass.

281   Grasses that were not sensitive to daylight also showed decreased yields under short light

282   application time. Trenholm et al. (1998) found that the shoot growth of the 'Flora Dwarf'

283   bermudagrass had a 41% decrease in short light application time compared to long light

284   application time. However, Marousky et al. (1992) reported that the shoot growth of 'Tif Dwarf'

285   bermudagrass did not increase significantly under long light application time. It is common that

286   long light application times can promote plant growth, but the effects vary among species.

287   Flowering transition is a critical feature in plant growth that signifies the end of the vegetative

288   phase and the start of the reproductive state (Quiroz et al, 2021). Prior to the flowering transition,

289   plants undergo a transition from juvenile to adult as they reach their flowering capacity under

290   adaptive induced environmental conditions. And light application time is one of the main

291   environmental stimuli. Most plants have their own biorhythm clock that can sense changes in the

292   external light environment, so the duration of light can significantly affect the flowering of

293   plants. Flowering of plants can be advanced or delayed by artificially changing the length of the

294   light application time. In our investigation, it has found that prolonged light application time in

295   the controlled greenhouse might encourage the bermudagrass to bloom earlier, with the

296   bermudagrass blooming first under the circumstance of a longer light application time 22/2 h

297   **(Fig 4c,4d)**. This finding was congruent with that of *Arabidopsis thaliana*, a characteristic long-

298   day plant that blooms sooner on long days and slows blooming on short days (Samach, 2000;

299   Suárez-López et al, 2001; Fornara, Montaigu & Coupland, 2010; Ye et al, 2021). The correlation

300   between the response to light application time and climatic conditions of forage origin appears to

301   be complex. Heide et al. (1994). found that the requirements of light application time of timothy

302   varied according to the geographic origin of the cultivar. Therefore, it needs to be further verified

303   whether other germplasm of bermudagrass can promote flowering under long light application

304   time. However, light intensity seems to be particularly important during the transition from

305   vegetative to reproductive. In general, an increase in the light intensity or daily light integral

306   increases biomass and flower developmental rate. The extension of light application time also

307   increased the total light energy irradiated on the plant, and the daily light integrals were different

308   when flowering under different light application times **(Fig. 5)**. Exposure to high levels of light

309   intensity can hasten time to flowering. For example, *Achillea millefolium* flowered after 57, 45,

310   and 37 days when grown under 100, 200, or 300 μmol m^{-2}s^{-1} respectively (Zhang et al,1996).

311   *Arabidopsis* plants also flower earlier under high light intensity. The use of longer light

312   application time to boost plant development has long been investigated; Sysoeva et al. (2010)

313   undertook a thorough survey of the literature on this issue published in the previous 90 years

314   (Sysoeva et al, 2010), including spring wheat (*Triticum aestivum*), barley (*Hordeum vulgare* L.),

315   peas (*Pisum sativum* L.), radish (*Raphanus sativus*), alfalfa (*Medicago sativa*), flax (*Linum*

316   *usitatissimum*), and arabidopsis (*Arabidopsis thaliana*). The current attention to the molecular

317   mechanism of flowering regulation is mainly focused on model plants such as Arabidopsis, rice

318   and wheat, and there are few studies on flowering in forage grasses and turfgrasses, so the

319 applicability of the current flowering mechanism in the plant world needs to be further explored.

320   In addition, for plant introduction and breeding, extending the light in the controlled

321 greenhouse and artificially changing the light application time are crucial. The technology of

322 speed breeding, which allows for rapid generational growth, has been tweaked to generate up to

323 six generations of wheat each year. As a result, it's a useful tool for cutting the breeding cycle

324 (Alahmad et al, 2018). It takes years of thorough screening and testing of warm-season turfgrass

325 varieties during seed propagation to uncover superior genotypes that persist and are considerably

326 superior to the present varieties (Hanna et al, 2013). Several molecular techniques have been

327 developed to expose the diversity of crop natural germplasm resources. Rapid generation

328 evolution to purity following crossing in a breeding setting will boost genetic gain of essential

329 features and enable for more rapid development of better varieties through the breeding program

330 (Stacy et al, 2006). As a result, we proposed a rapid generation system for bermudagrass in the

331 greenhouse with additional supplemental light, extending the daylight length to 22/2 h, to

332 accelerate plant development and thus shorten the breeding time, and it was successful in

333 reducing the flowering time of common bermudagrass to 44 days and that of African

334 bermudagrass to 36 days. It has been demonstrated that African bermudagrass has a high genetic

335 diversity and may be utilized to improve intraspecific and interspecific breeding (Kenworthy et

336 al, 2006). To speed progress, it is critical to identify the flowering thresholds of African

337 bermudagrass and common bermudagrass.

338   This study shortened the flowering time by increasing the light application time and provided

339 a theoretical basis for speed breeding of bermudagrass. Furthermore, fast breeding may be

340 integrated with molecular biology, such as genomics, high-throughput sequencing, and genome

341 editing, to increase the speed and precision of developing excellent varieties.

342 **Conclusion**

343 This study was carried out in the controlled greenhouse in order to explore the influence of

344 enhanced daylight length on the development and flowering of bermudagrass. The changes of

345 plant height, fresh weight, dry weight and nitrogen and phosphorus content were analyzed and

346 concluded that long light application time promoted the growth and nutrient uptake (N and P

347 content) of bermudagrass. In addition, daily light integrals were different when flowering under

348 different light application times. Longer daylight length 22/2 h (22 hours light/2 hours dark)

349 clearly enhanced the development and flowering and eventually reduced the flowering period to

350 44 days for common bermudagrass and 36 days for African bermudagrass. Therefore, the

351 breeding time can be shortened by increasing the light application time. This study provides a

352 theoretical basis for speed breeding of bermudagrass.

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355 **Additional information and declarations**

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360 **Competing Interests**

361 The authors declare there are no competing interests.

362 **Author Contributions**

363 G.W. and J.F. designed and coordinated the study. M.J wrote the manuscript and performed the

364 data analysis. All authors approved the manuscript.

365 **Data Availability**

366 The following information was supplied regarding data availability: The raw data are available in

367 the Supplemental Files.

368 **References**

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579   **Figure titles and legends**

580   **Figure 1 Schematic diagram of three artificially designed light application times**

581   The control of light application time was achieved in controlled greenhouses by supplementary

582   lights. The supplementary light was automatically turned off for two hours from 9 PM to 11PM

583   every day to achieve a 22/2 Light application time; 18/6 Light application time was covered by

584   paper boxes at 5pm and uncovered at 9pm; 14/10 Light application time was covered at 9 pm

585   and uncovered at 7 am the next day. Black square shows cover with box (complete darkness).

586   **Figure 2 Plant height (cm), fresh weight (g) and dry weight (g) of Common bermudagrass**

587   **(A12359) and African Bermudagrass (ABD11) grown in three light application times (22/2,**

588   **18/6 and 14/10 hrs light/dark)**

589   The graph depicts the plant height, fresh weight and dry weight, of A12359 and ABD11. (a)

590   A12359 and (b) ABD11 Changes in plant height every two weeks under 3 light application

591   times. (c) (d) Biomass (fresh weight) of A12359 and ABD11 measured every two weeks at 3

592   light application times. (e) (f) Dry weight of A12359 and ABD11 measured every two weeks at 3

593   light application times. (g) (h) At the end of the experiment, the fresh weights of shoot and root

594   of A12359 and ABD11 were measured under 3 light application times. (i) (j) At the end of the

595   experiment, the dry weights of shoot and root of A12359 and ABD11 were measured under 3

596   light application times. Fresh and dry weights are in grams per pot. Each material is designed to

597   be replicated three times. According to the SNK test at $P < 0.05$, different lowercase letters

598   indicate significant differences between the three light application times at the same time.

599   **Figure 3 Nitrogen (N) and phosphorus (P) content of common Bermudagrass (A12359) and**

600   **African Bermudagrass (ABD11) grown in three light application times (22/2, 18/6 and**

601   **14/10 hrs light/dark)**

602   The graph depicts the N and P content of A12359 and ABD11. Fresh samples from each mowing

603   were dried and utilized to determine the nitrogen (N) and phosphorus (P) levels. The N and P

604   content measured was accumulated over two weeks. (a) (b) Changes of P content in A12359 and

605   ABD11 under different light application times. (c) (d) Changes of N content in A12359 and

606   ABD11 under different light application times. Each material is programmed to have three

607   duplicates. According to the SNK test at $P < 0.05$, different lowercase letters indicate significant

608   differences between the three light application times at the same time.

609   **Figure 4 Branching and flowering of common bermudagrass (A12359) and African**

610   **bermudagrass (ABD11) grown in three light application times (22/2, 18/6 and 14/10 hrs**

611   **light/dark)**

612   The graph depicts the blooming and branching of A12359 and ABD11. Every two weeks, the

613   number of branches was tallied, and every two days, the number of blossoms was counted. (a)

614   Branch number variation of A12359 under different light application times. (b) Branch number

615   variation of ABD11 under different light application times. (c) Changes of flowering number of

616   A12359 under different light application times. (d) Changes of flowering number of ABD11

617 under different light application times. Each material is designed to be replicated three times.

618 According to the SNK test at P< 0.05, different lowercase letters indicate significant differences

619 between the three light application times at the same time.

620 **Figure 5 Daily light integral (DLI) for three Light application times**

621 This graph depicts the daily light integral for 3 different light application times. We integrated

622 the total light energy received by the material under three light application times. The interval of

623 DLI for different light application times was one week.

624 **Supplementary Figure 1 Temperature and illumination intensity of the greenhouse**

625 The temperature and light intensity of the greenhouse were recorded during the experiment (a)

626 illumination intensity (b) temperature.

627 **Supplementary Figure 2 Hydrogen peroxide content of common bermudagrass (A12359)**

628 **and African bermudagrass (ABD11) grown in three light application times (22/2, 18/6 and**

629 **14/10 hrs light/dark)**

630 To verify the feasibility of the experimental design we measured the hydrogen peroxide

631 content at the end of the experiment. The concent of H_2O_2 was measured using a comm

632 ercially available kit ((Nanjing Jiancheng Bioengineering Institute). For the determination

633 of H_2O_2, the 0.1g of leaves of bermudagrass were ground in liquid nitrogen, dissolved in

634 phosphate buffered solution (pH: 7.4, 0.1 mol/L), and then centrifuged for 10 min at 100

635 00 g. The A405 was determined and the content of H_2O_2 was then calculated. Bar plots

636 depicts the hydrogen peroxide content of the leaves at the end of the experiment. (a) (b)

637 Hydrogen peroxide content of leaves of A12359 and ABD11 under different application t

638 imes (22/2, 18/6, 14/10). Each material is designed to be replicated three times. Accordin

639 g to the SNK test at P< 0.05, different lowercase letters indicate significant differences b

640   etween the three light application times at the same time. Whether it was A12359 or A

641   BD11, there was no significant difference in hydrogen peroxide content between the long

642   er and shorter light application times. These results suggested that prolonged light applica

643   tion time had no effect on ROS levels in bermudagrass.

644   **Supplementary Table 1 Two-way ANOVA to examine the effects of 'Light applicatio**

645   **ntime', 'Week' and 'Light application time * Week'.**

646

647

Figure 1

Figure 1 Schematic diagram of three artificially designed light application times

The control of Light application time was achieved in controlled greenhouses by supplementary lights. The supplementary light was automatically turned off for two hours from 9 PM to 11PM every day to achieve a 22/2 Light application time; 18/6 Light application time was covered by paper boxes at 5pm and uncovered at 9pm; 14/10 Light application time was covered at 9 pm and uncovered at 7 am the next day. Black square shows cover with box (complete darkness).
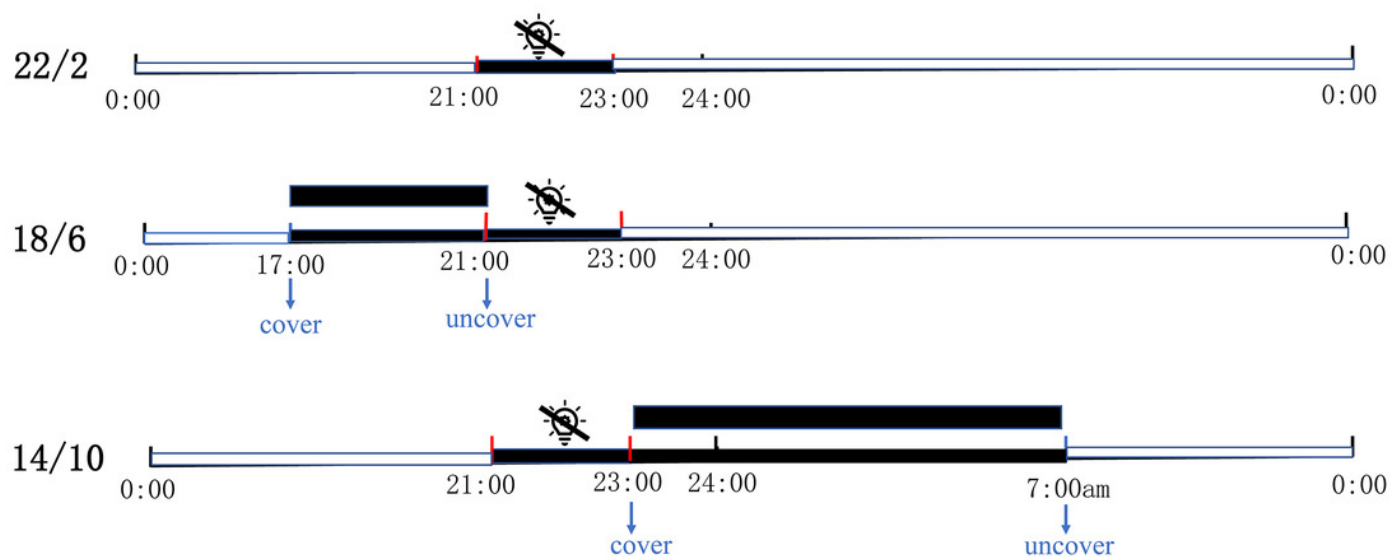
Figure 2

Figure 2 Plant height (cm), fresh weight (g) and dry weight (g) of Common bermudagrass (A12359) and African Bermudagrass (ABD11) grown in three light application times (22/2, 18/6 and 14/10 hrs light/dark)

The graph depicts the plant height, fresh weight and dry weight, of A12359 and ABD11. (a) A12359 and (b) ABD11 Changes in plant height every two weeks under 3 light application times . (c) (d) Biomass (fresh weight) of A12359 and ABD11 measured every two weeks at 3 light application times . (e) (f) Dry weight of A12359 and ABD11 measured every two weeks at 3 light application times. (g) (h) At the end of the experiment, the fresh weights of shoot and root of A12359 and ABD11 were measured under 3 light application times . (i) (j) At the end of the experiment, the dry weights of shoot and root of A12359 and ABD11 were measured under 3 light application times . Fresh and dry weights are in grams per pot. Each material is designed to be replicated three times. According to the SNK test at $P< 0.05$, different lowercase letters indicate significant differences between the three light application times at the same time.

Figure 3

Figure 3 Nitrogen (N) and phosphorus (P) concent of common Bermudagrass (A12359) and African Bermudagrass (ABD11) grown in three light application times (22/2, 18/6 and 14/10 hrs light/dark)

The graph depicts the N and P content of A12359 and ABD11. Fresh samples from each mowing were dried and utilized to determine the nitrogen (N) and phosphorus (P) levels. The N and P content measured was accumulated over two weeks. (a) (b) Changes of P content in A12359 and ABD11 under different light application times. (c) (d) Changes of N content in A12359 and ABD11 under different light application times. Each material is programmed to have three duplicates. According to the SNK test at P< 0.05, different lowercase letters indicate significant differences between the three light application times at the same time.
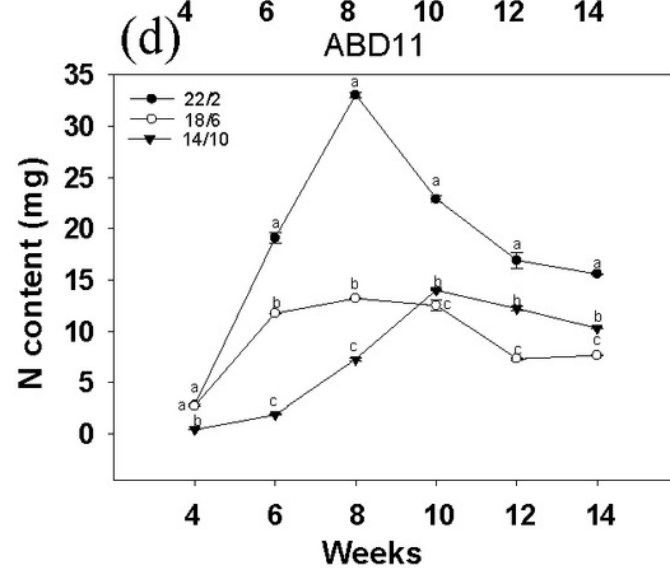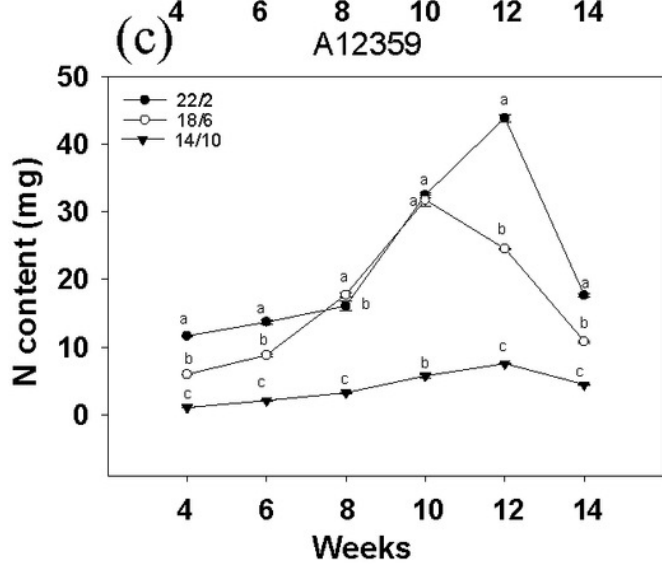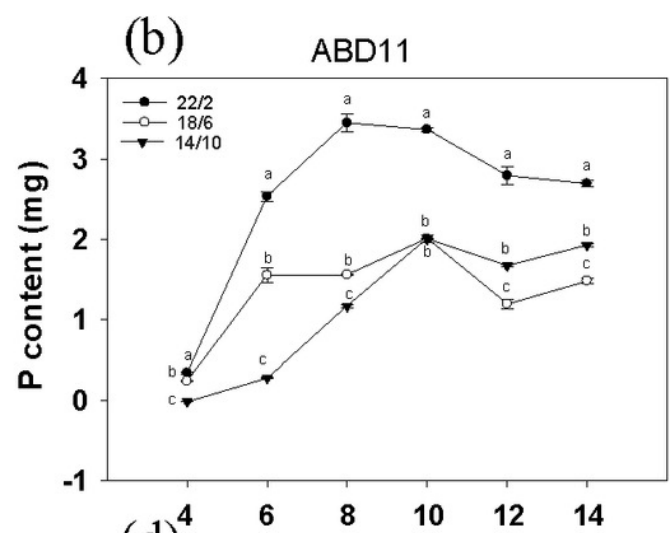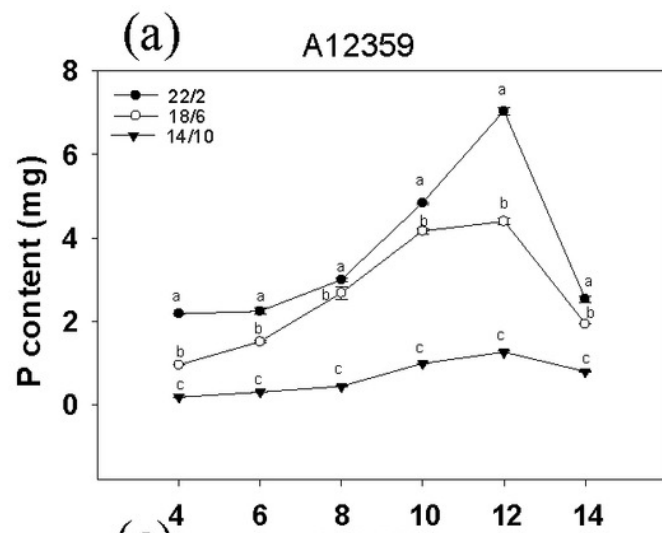
Figure 4

Figure 4 Branching and flowering of common bermudagrass (A12359) and African bermudagrass (ABD11) grown in three light application times (22/2, 18/6 and 14/10 hrs light/dark)

The graph depicts the blooming and branching of A12359 and ABD11. Every two weeks, the number of branches was tallied, and every two days, the number of blossoms was counted. (a) Branch number variation of A12359 under different light application times. (b) Branch number variation of ABD11 under different light application times. (c) Changes of flowering number of A12359 under different light application times. (d) Changes of flowering number of ABD11 under different light application times. Each material is designed to be replicated three times. According to the SNK test at $P< 0.05$, different lowercase letters indicate significant differences between the three light application times at the same time.
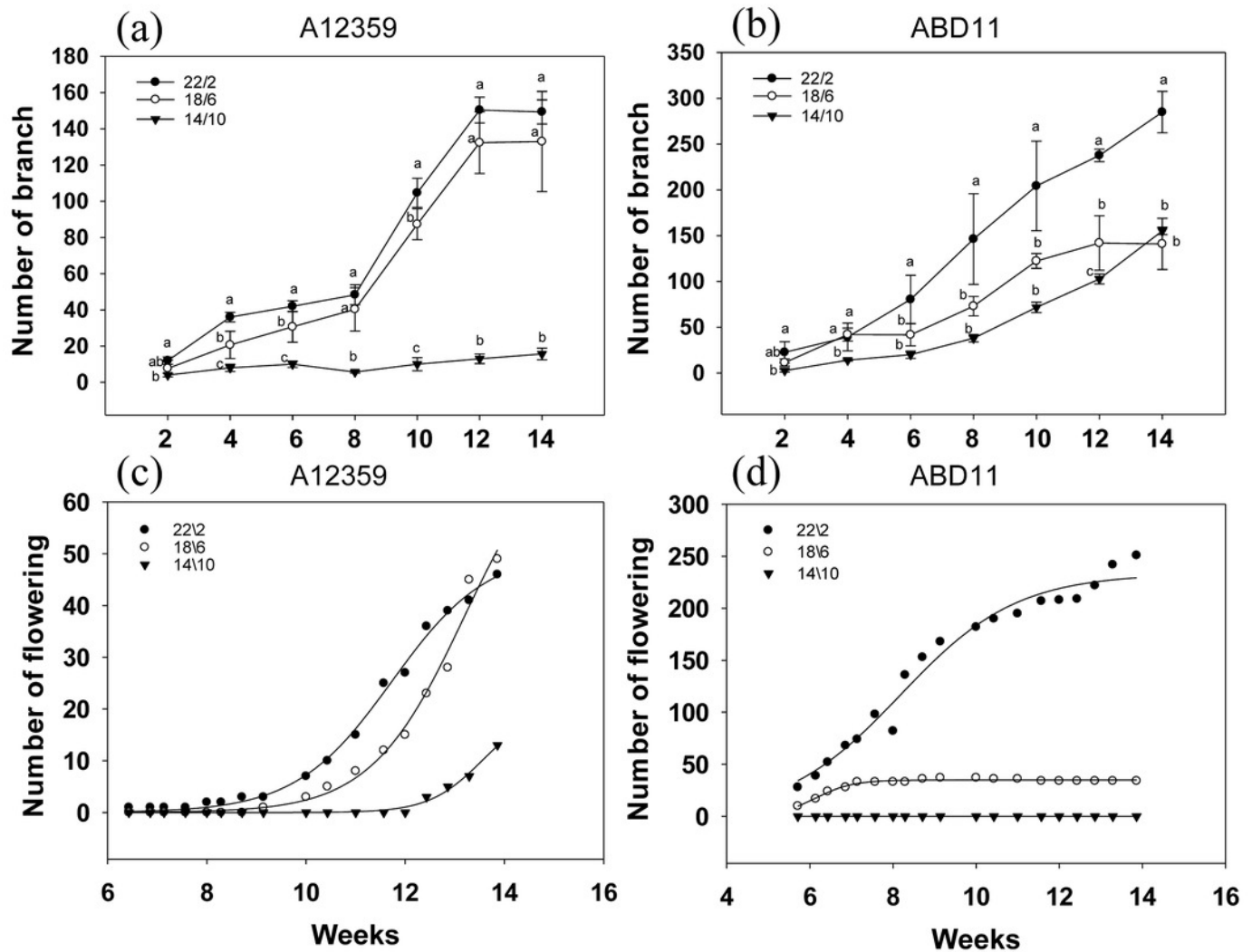
Figure 5

Figure 5 Daily light integral (DLI) for different Light application times

This graph depicts the daily light integral for 3 different light application times. We integrated the total light energy received by the material under three light application times. The interval of DLI for different light application times was one week.