# Melatonin improves the germination rate of cotton seeds under drought stress by opening pores in the seed coat

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The germination of cotton (*Gossypium hirsutum* L.) seeds is affected by drought stress; however, little is known about the physiological mechanism affecting germination and the effect of melatonin (MT) on cotton seed germination under drought stress. Therefore, we studied the effects of exogenous MT on the antioxidant capacity and epidermal microstructure of cotton under drought stress. The results demonstrated a retarded water absorption capacity of testa under drought stress, significantly inhibiting germination and growth in cotton seeds. Drought stress led to the accumulation of reactive oxygen species (ROS), malondialdehyde (MDA), and osmoregulatory substances (e.g., proline, soluble protein, and soluble sugars); it also decreased the activity of antioxidant enzymes and $\alpha$-amylase. Drought stress inhibited gibberellin acid (GA) synthesis and increased abscisic acid (ABA) content, seriously affecting seed germination. However, seeds pre-soaked with MT (100 $\mu$M) showed a positive regulation in the number and opening of stomata in cotton testa. The exogenous application of MT increased the germination rate, germination potential, radical length, and fresh weight, as well as the activities of superoxide dismutase (SOD), peroxidase (POD), and $\alpha$-amylase. In addition, MT application increased the contents of organic osmotic substances by decreasing the hydrogen peroxide ($H_2O_2$),

superoxide anion ($O_2^-$), and MDA levels under drought stress. Further analysis demonstrated that seeds pre-soaked with MT alleviated drought stress by affecting the ABA and GA contents. Our findings show that MT plays a positive role in protecting cotton seeds from drought stress.

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18 **ABSTRACT**

19 The germination of cotton (*Gossypium hirsutum* L.) seeds is affected by drought stress; however,

20 little is known about the physiological mechanism affecting germination and the effect of

21 melatonin (MT) on cotton seed germination under drought stress. Therefore, we studied the

22 effects of exogenous MT on the antioxidant capacity and epidermal microstructure of cotton

23 under drought stress. The results demonstrated a retarded water absorption capacity of testa

24 under drought stress, significantly inhibiting germination and growth in cotton seeds. Drought

25 stress led to the accumulation of reactive oxygen species (ROS), malondialdehyde (MDA), and

26 osmoregulatory substances (e.g., proline, soluble protein, and soluble sugars); it also decreased

27 the activity of antioxidant enzymes and α-amylase. Drought stress inhibited gibberellin acid

28 (GA) synthesis and increased abscisic acid (ABA) content, seriously affecting seed germination.

29 However, seeds pre-soaked with MT (100 μM) showed a positive regulation in the number and

30 opening of stomata in cotton testa. The exogenous application of MT increased the germination

31  rate, germination potential, radical length, and fresh weight, as well as the activities of

32  superoxide dismutase (SOD), peroxidase (POD), and α-amylase. In addition, MT application

33  increased the contents of organic osmotic substances by decreasing the hydrogen peroxide

34  ($H_2O_2$), superoxide anion ($O_2^-$), and MDA levels under drought stress. Further analysis

35  demonstrated that seeds pre-soaked with MT alleviated drought stress by affecting the ABA and

36  GA contents. Our findings show that MT plays a positive role in protecting cotton seeds from

37  drought stress.

38  **Subjects** Agricultural Science; Plant Science

39  **Keywords** Cotton, Melatonin, Seed germination, Drought stress, Testa, Physiology

## INTRODUCTION

42  Drought is a global challenge because of climate warming and increasingly constrained water

43  resources (Jury & Vaux, 2005). Drought is also one of the main abiotic stress factors limiting the

44  growth and yield of plants (Farooq et al., 2009a), and the changing global climate is making this

45  situation more extreme (Farooq et al., 2013). The response of plants to drought stress are

46  interconnected (Huang et al., 2019); specifically, drought influences many morphological,

47  physiological, and biochemical processes such as stomatal closure, cellular dehydration, the

48  peroxidation of membrane lipids, and reduced antioxidant capacity, affecting plant survival and

49  development (Ahammed et al., 2019a; Huang et al., 2019; Gao et al., 2018). However, plants

50  have evolved complex stress-resistant mechanisms to counteract damage due to drought, which

51  may allow plants to complete their life cycles (Ahammed et al., 2019a).

52  Germination is a complex process regulated by a series of physical and metabolic events in the

53  life cycle of plants (Leónie & Maarten, 2008; Xiao et al., 2019). As the initial stage of plant life

54  cycles and the critical stage of seedling establishment, seed germination is extremely sensitive to

55  drought stress (Ibrahim & Ehab, 2016). A previous study has shown that the mechanical barrier

56  provided by the seed coat increases under stress conditions, indicating that drought stress inhibits

57  seed germination (Debeaujon, Léon-Kloosterziel & Koornneef, 2000). Drought stress leads to

58  the over-production of reactive oxygen species (ROS) in plant cells, which in turn triggers

59  oxidative damage and may lead to cell death (Farooq et al., 2009b; Nahar et al., 2015; Xiao et al.,

60  2019). However, plants have evolved efficient antioxidative defense mechanisms, including the

61  activities of antioxidant enzymes and the regulation of non-enzymatic antioxidants to reduce

62   ROS induced oxidative damages (Posmyk et al., 2009). Plant hormones are important signaling

63   molecules that respond to environmental changes in seed germination (Yamaguchi, Kamiya &

64   Nambara, 2007). Gibberellic acid (GA) and abscisic acid (ABA) are well-known phytohormones

65   that play crucial roles in seed germination and early seedling establishment (Shu et al., 2018;

66   Ahammed et al., 2020a). ABA, a universal abiotic stress hormone, responded positively to

67   abiotic stress, while GA acts as a plant growth regulator to promote seed germination in response

68   to stress (Shu et al., 2018). Drought stress adversely affects seed germination by disrupting the

69   dynamic balance of ABA catabolism and GA biosynthesis (Vishal & Kumar, 2018; Ahammed et

70   al., 2020a). A previous study reported the effects of GA and salinity on physiological

71   characteristics in maize, and their findings showed that GA could reduce proline accumulation

72   and electrolyte leakage, alleviating the damage to plants caused by salt stress by delaying the

73   decrease of superoxide dismutase (SOD) and peroxidase (POD) (Tuna et al., 2008). Previous

74   studies have shown that the accumulation of ABA is closely related to the generation of ROS;

75   specifically, when the accumulation of ABA was lower, the formation of hydrogen peroxide

76   ($H_2O_2$) reduced accordingly (Liu et al., 2010; Ye et al., 2011). Drought stress negatively

77   regulates metabolism due to low α-amylase activity, leading to a significant inhibition of seed

78   germination and seedling growth (Farooq et al., 2020). The accumulation of osmoregulatory

79   substances, such as proline, soluble sugar, and soluble protein, which can reduce the water

80   potential and enhance the water absorption capacity of cells, ensures normal plant metabolism

81   and improves drought resistance (Zhang et al., 2015a; Zhang et al., 2015b).

82       Cotton, the world's major fiber and oil crop, is also severely affected by drought. Drought

83   weakens seedling growth by limiting seed germination, which adversely affects cotton

84   productivity (Farah et al., 2012; Luo et al., 2018). Therefore, it is urgent to improve the rate of

85   cotton seed germination under drought conditions.

86       Melatonin (N-acetyl-5-methoxytryptamine, MT), a regulatory molecule with various

87   biological functions, has been detected in regards to the regulation of seed germination, root

88   development, leaf senescence, and fruit maturation in plants (Ahammed et al., 2020b; Liang et

89   al., 2018). In addition to its physiological functions, MT can also enhance plant tolerance against

90   multiple adverse environmental stressors (Hardeland et al., 2011; Posmyk et al., 2009; Li et al.,

91   2018; Ahammed et al., 2020b; Li et al., 2020). Numerous studies have shown that MT plays an

92   important role in seed germination and plant growth may be associated with MT-induced

93   changes in physiological mechanisms (Posmyk et al., 2009; Zhao et al., 2011; Kang et al., 2010).

94   Evidence has suggested that the pre-treatment of seeds with MT could improve the effectiveness

95   of pores under drought stress (Khan et al., 2019). A previous study conducted on cucumber

96   (*Cucumis sativus* L.) illustrated the positive influences of MT treatment in plants suffering from

97   PEG stress including the promotion of seed germination (Zhang et al., 2012). As a broad-

98   spectrum antioxidant, MT can enhance the activity of antioxidant enzymes, such as SOD and

99   POD, by lowering ROS levels, which protects the plants from stress-induced damage (Gao et al.,

100  2018; Wang, Reiter & Chan, 2018). Previous results have shown that the appropriate

101  concentration of MT can reduce the malondialdehyde (MDA) content in soybean seedlings,

102  alleviating the damage to the membrane system and improving resistance to abiotic stress (Wei

103  et al., 2014). Treatment with exogenous MT significantly increased the content of proline,

104  soluble sugar, and soluble protein in plants, while increasing cell fluid concentrations and

105  reducing the MDA content in plants under abiotic stress (Zhang et al., 2012; Turk et al., 2014).

106  Similarly, the exogenous application of MT has been shown to enhance the activity of α-amylase

107  in maize seeds under chilling stress (Cao et al., 2019). MT may be a plant growth hormone that

108  is closely related to auxin (IAA) in its structural components (Arnao & Hernandez-Ruiz, 2017).

109  Phytohormones are involved in seed germination and dormancy, and the exogenous application

110  of MT can alleviate damage to seed germination caused by environmental stress by regulating

111  the synthesis of GA and the decomposition of ABA (Zhang et al., 2014).

112      Despite the several previously proposed hypotheses suggesting that the exogenous application

113  of MT may promote seed germination under drought stress (Meng et al., 2014; Zhang et al.,

114  2012), a description of the morphological and physiological mechanisms by which MT plays a

115  role in alleviating drought stress is limited. Furthermore, little is known about the effects of MT

116  on the germination of cotton seeds and its physiology and epidermal microstructure under

117  drought stress. The objectives of our study were to (1) investigate the morphological and

118  physiological changes in cotton seeds induced by soaking the seed in MT and then treating with

119  drought stress and (2) examine the effects of soaking the seed in MT on improving the

120  germination of cotton seeds grown under drought stress.

121  **MATERIALS & METHODS**

122  **Reagent, plant material, and experimental conditions**

123    MT (N-acetyl-5-methoxytryptamine) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

124    The experiment was conducted in the Key Laboratory of Crop Growth Regulation of Hebei

125    Agricultural University, Hebei Province, China. Seeds of the widely cultivated cotton cultivar,

126    Guoxin NO. 9 were obtained from Guoxin Agricultural Research Association of Hejian, Hebei

127    Province, China.

128    **Determination of cotton drought resistance**

129    Seeds of cotton were surface sterilized with 75% ethanol for 30 min and then rinsed with

130    distilled water five times. Sterilized seeds were placed in Petri dishes (each with 50 seeds and

131    five replicates) containing three layers of filter paper and 10 mL of the following treatment

132    solutions: 5, 10, 15, or 20% PEG. Distilled water was used as the control. Then, seeds in Petri

133    dishes were left to germinate in an incubator at $25 \pm 1°C$ in the dark. To determine the suitable

134    concentration of PEG-6000, the seed germination rate (GR) was measured at 7 days.

135    **Determination of melatonin concentration**

136    Selected sterile seeds were soaked in different concentrations of MT solutions (10, 50, 100, 200,

137    and 500 μM MT) for 24 h at 25°C in a darkened incubator. For the control, the sterilized seeds

138    were soaked in distilled water. Then, 50 seeds were transferred to Petri dishes containing three

139    layers of filter paper moistened with 10 mL of PEG-6000 solution to simulate drought stress and

140    incubated at 25°C in a darkened growth chamber for 7 days. The selected 10% PEG-6000

141    concentration was established based on the trial described above. The treatments were replicated

142    five times and each replicate contained 50 seeds. The number of germinated seeds was observed

143    daily and the GR, germination potential (GP), radical length (RL), and fresh weight (FW) were

144    determined to select the effective MT concentration for cotton seed germination under drought

145    stress.

146        The seeds were pre-soaked with 100 μM MT for 24 h at 25°C in a darkened incubator and then

147    allowed to germinate following the same methods as described above. Distilled water was used

148    as the control. Approximately 10 g of embryo and radicle were harvested from each treatment at

149    2, 4, and 6 days after germination, and then stored at -80°C for subsequent analyses of SOD and

150    POD activities, osmoregulation, ROS, and hormone content. Four treatments were applied as

151    follows: [1] water soaking + no stress (W); [2] water soaking + 10% PEG-6000 (W+DS); [3] 100

152    μM MT soaking + no stress (MT); [4] 100 μM MT soaking + 10% PEG-6000 (MT+DS).

153    Seed coat microstructures were observed in dry, water-soaked, and 100 μM MT-soaked cotton

154    seeds for 24 hours at 25°C using a scanning electron microscope (SEM).

155    **Assessment of seed germination**

156    The number of seeds germinated was recorded daily. The length of the radicle and hypocotyl was

157    measured with a straightedge ruler. Seeds were considered germinated when their radicle and

158    hypocotyl length exceeded half of the seed length.

159    The numbers of germinated seeds were calculated as GP and GR at 3 and 7 days after

160    germination, respectively. GP=germinated seeds on day 3 / total seeds × 100%; GR= germinated

161    seeds on day 7 / total seeds × 100% (Li et al., 2007; Thabet et al., 2018). Germination index (GI)

162    and seed vigor index (VI) were calculated as follows: $GI = \sum (Gi / i)$, where Gi is the number of

163    germinated seeds on day i. VI = GI × RL of germinated seeds on day 7 (Li, Yu & Yin, 2017). In

164    addition, the RL and FW of seeds were determined on day 7 after germination. The weight of 50

165    seeds was recorded using an analytical balance (0.01 g).

166    **Measurement of SOD and POD activities and the content of MDA and ROS**

167    The activities of SOD and POD were estimated using the assay kits (A001-1 and A084-3,

168    Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Crushed frozen samples (0.3 g)

169    were homogenized with 3 mL 50 mM phosphate buffer (PH 7.8) containing 1%

170    polyvinylpyrrolidone and 0.1% mercaptoethanol (w/v), followed by centrifugation at 4°C for 20

171    min at 10,000 rpm. The SOD and POD activity was determined in the supernatant using a

172    spectrophotometer at 560 nm and 470 nm based on the manufacturer's instructions, respectively.

173    The activity of SOD and POD was presented as protein content.

174    The MDA content was tested using the Malondialdehyde assay kit (Nanjing Jiancheng

175    Bioengineering Institute, China). Cotton seed tissues were fully homogenized with pH 7.8

176    phosphate buffer, after centrifugation at 6,000 rpm for 10 min. Two milliliters of supernatant

177    were added to a test tube contained 3 mL of 5% trichloroacetic acid and 1 mL of 0.5%

178    thiobarbituric acid solution. Then the mixture was boiled for 10 min, cooled rapidly, and

179    centrifuged at 6,000 rpm for 10 min. The light absorption value was measured in the resulting

180    supernatant using a spectrophotometer at 532 nm and 600 nm.

181    The $H_2O_2$ and $O_2^-$ content were tested using a Hydrogen Peroxide Assay kit and Superoxide

182    Anion Assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the

183    manufacturer's protocols.

184 **Determination of soluble sugar, soluble protein, proline, and amylase contents**

185 The method described by Zhang et al. (2006) was adapted for the estimation of soluble sugar

186 contents with some slight modification. Briefly, 0.3 g seed samples were added to 9 mL of

187 distilled water in a boiling water bath for 30 min. One milliliter of the supernatant was mixed

188 with 5 mL of sulfuric acid-anthrone reagent, followed by boiling for 10 min and cooling. The

189 absorption value was measured in a spectrophotometer at 620 nm.

190 The proline content was estimated following the method described by Subramanyam, Du &

191 Van (2019). Briefly, approximately 0.3 g frozen samples were homogenized with 3 mL of 3%

192 aqueous sulfosalicylic acid. After centrifugation, 2 mL of supernatant was mixed with 2 mL of

193 glacial acetic acid and 2 mL of ninhydrin reagent, and then heated in boiling water for 30 min.

194 After cooling, the mixture was centrifuged at 10,000 rpm for 5 min. The light absorption values

195 were recorded at 520 nm using a spectrophotometer.

196 E

197 **Quantification of GA and ABA**

198 GA and ABA concentrations were determined using a previously described indirect ELISA

199 technique (He, 1993). To assay GA and ABA, 0.3 g frozen tissue were ground in liquid nitrogen.

200 After homogenization, 4 mL of 80% methanol was added to the samples. The solution was then

201 incubated for 4 h at 4°C in the dark. After centrifugation at 10,000 g for 20 min, the supernatant

202 was eluted using a Sep-Pak C18 cartridge and then dried under a stream of $N_2$. The dried samples

203 were re-dissolved with 5 mL of eluting buffer containing 1 mM $MgCl_2$, 150 mM NaCl, and 10%

204 (v/v) methanol in 50 mM Tris. GA and ABA were determined with an immunosorbent assay

205 according to the manufacturer's instructions, and the antibodies used in this assay were all

206 monoclonal antibodies provided by China Agricultural University.

207 **Scanning electron microscopy (SEM) analysis**

208 Parts of the testa were collected from healthy cotton seeds and observed using SEM (Hitachi

209 SU8020, Japan). The collected samples were fixed using 3% glutaraldehyde for 12 h at 4°C, and

210 then washed using 0.1 M sodium phosphate buffer. Samples were fixed with 1% osmium for 1h

211 at 4°C and washed twice with 0.1 M sodium phosphate buffer. Samples were dehydrated in

212 serially diluted ethanol and critical point dried using carbon dioxide. The samples were firmly

213 mounted using double-sided adhesive tape on stubs and then sprayed with gold using a vacuum

214 plating instrument.

215   **Statistical analysis**

216   We conducted five independent replicates for germination tests. Physiological parameters were

217   assessed using three replicates. The data were statistically analyzed using IBM SPSS Statistics

218   17.0 software. A least significant difference test was determined using Duncan's test to compare

219   the mean value. All data were expressed as mean ± standard deviation (SD). Differences were

220   considered significant at $P < 0.05$. The figures were developed using the GraphPad Prism 8.0

221   software.

222   **RESULTS**

223   **Selection of PEG concentration**

224   Cotton seeds treated with different concentrations of PEG-6000 (0–20%) were used to determine

225   the optimum concentration of PEG-6000. Fig. 1 illustrates the daily germination rate of cotton

226   seeds treated with varying concentrations of PEG-6000 within 7 days of seed germination. Fig. 2

227   shows the effects of different degrees of drought stress on the seed germination rate. The

228   increase in PEG concentration resulted in a significant delay in the germination rate of seeds.

229   Treatment with 5, 10, 15, and 20% PEG-6000 significantly reduced the germination rate by

230   3.36%, 20.17%, 37.82%, and 57.14%, respectively, compared with the control treatment. The

231   inhibitory effect of 5% PEG on seed germination was weak, whereas the degree of stress of 15%

232   and 20% PEG severely inhibited seed germination; treatment with 10% PEG created a moderate

233   level of stress on seed germination. Therefore, in subsequent experiments, 10% PEG was

234   selected for the drought stress treatment.

235

236   **Selection of MT concentrations for seed treatment**

237   We conducted seed germination tests to determine whether different concentrations of MT affect

238   seed germination under drought stress. In addition, we measured the radical length and fresh

239   weight of the seeds 7 days after germination. As shown in Table 1, treatment with different

240   concentrations of MT limited the negative effects of PEG-induced drought stress on germination.

241   Further analysis showed that a low concentration of MT (10–50 μM) had a limited effect on GP,

242   GR, RL, and FW under drought stress conditions. Pre-soaking seeds with 100 μM MT was the

243   most effective at relieving drought stress, and the results showed that GP, GR, RL, and FW

244   increased by 8.99%, 10.42%, 7.62%, and 53.37% respectively, compared to the 0 μM MT

245   treatment. Fewer seeds germinated when the seeds were pre-soaked in 200 μM or 500 μM MT.

246    Overall, the results showed that pre-treatment with MT at an appropriate concentration could

247    promote seed germination under drought stress. Based on these results, we selected 100 µM MT

248    for subsequent germination tests.

249    **Effect of MT on germination indices under drought stress conditions**

250    The seeds pre-soaked in100 µM MT showed no significant change for germination indices under

251    the well-watered condition (Table 2). MT+DS treatment resulted in a significantly higher GP

252    (8.9%) and GR (9.8%) when the seeds were germinated under drought stress conditions

253    compared with the W+DS treatment. At 10% PEG-6000, MT acted as a good regulator,

254    significantly improving GI and VI under drought stress. Further analysis showed that the

255    treatment of MT+DS increased GI and VI by 13.4% and 16.2%, respectively, compared with

256    W+DS. Therefore, pre-treatment with MT could positively affect seed vigor.

257    **Effects of melatonin on SOD and POD activities of cotton under drought stress**

258    We determined the changes of SOD and POD activities at different stages of germination. Fig.

259    3A shows that the SOD activity of each treatment increased first and then decreased, and the

260    highest SOD activity was found 4 days after germination. Two days after seed germination under

261    well-watered conditions, the activity of SOD significantly increased by 3.3% in MT compared

262    with that in W. In the seeds pre-soaked with MT, when cotton seeds were grown in normal

263    moisture conditions for 4 and 6 days, the SOD activity increased by 2.6% and 6.3%,

264    respectively, compared to the W treatment, but there was no statistical difference 4 days after

265    germination. Similarly, the activity of SOD was enhanced by the pre-treatment of seeds with MT

266    when the seeds were germinated under drought stress conditions. Compared with the W+DS

267    treatment, the SOD activity of the MT+DS treatment significantly increased by 5.69%, 2.91%,

268    and 6.03% at days 2, 4, and 6 after germination, respectively.

269        The results of the POD activity analysis were consistent with that of the SOD activity data

270    (Fig. 3B). In all cases, POD activity was substantially improved by pre-soaking seeds with MT

271    compared to seeds pre-treated with distilled water. Under well-watered conditions, POD activity

272    was increased 1.5 times by seeds pre-soaked with MT compared with the W treatment at 2 days

273    after germination. Compared with W, the POD activity of the MT treatment improved by

274    38.74% and 17.9% at 4 and 6 days after germination, respectively. Soaking seeds in MT

275    enhanced seed vigor; therefore, the POD activity of MT+DS on days 2 and 4 of germination

276    under drought stress significantly increased by 28.28% and 36.23% respectively, compared with

277     the W+DS treatment. Six days after seed germination, the POD activity in the MT+DS treatment

278     increased by 0.6%, but this increase was not statistically significant.

279

280     **Effects of melatonin pre-treatment on ROS in seeds under drought stress conditions**

281     The accumulation of ROS significantly increased under drought stress. On the contrary, pre-

282     soaking seeds with MT resulted in relatively low $H_2O_2$ and $O_2^-$ content (Fig. 4). The $H_2O_2$

283     content in the seeds pre-soaked with MT under no stress conditions significantly decreased by

284     24.4%, 4.3%, and 31.6%, respectively at 2, 4, and 6 days, compared with W treatment. Under

285     drought stress conditions, the MT+DS treatment caused a decrease by 37.91%, 2.85%, and

286     30.31% at 2, 4, and 6 days after germination, respectively compared with the W+DS treatment

287     (Fig. 4A).

288        The $O_2^-$ content showed an upward trend over the course of seed germination. Under normal

289     growth conditions, the $O_2^-$ content increased in the W treatment. However, the increased $O_2^-$ was

290     alleviated by pre-soaking seeds with MT. On 2, 4, and 6 days after seed germination, the content

291     of $O_2^-$ in the MT treatment decreased by 43.1%, 0.3%, and 8.1%, respectively, compared with

292     W. A similar effect was observed under drought stress conditions at 2, 4, and 6 days after

293     germination; specifically, the MT+DS treatment reduced $O_2^-$ content by 6.16%, 6.81%, and

294     2.82%, respectively, compared to the W+DS treatment. These data indicate that MT can regulate

295     ROS during germination in the drought stress treatment (Fig. 4B).

296

297     **Effects of melatonin treatment on the MDA content of cotton under drought stress**

298     MDA is an important indicator reflecting the degree of membrane lipid peroxidation. The data

299     showed that drought stress induces the increase in MDA content, which was mitigated by pre-

300     soaking seeds with MT. Under drought stress, the MT+DS treatment resulted in 29.07%,

301     15.58%, and 16.83% lower MDA content compared with the W+DS treatment at 2, 4, and 6 days

302     after seed germination, respectively, which further suggests the effects of MT as an antioxidant

303     (Fig. 5).

304     **Effect of melatonin treatment on amylase activity of seeds under drought stress**

305     In our experiment, α-amylase activity was analyzed during seed germination. The activity of α-

306     amylase under drought stress was decreased (Fig. 6). In contrast, seed soaking with 100 μM MT

307     significantly enhanced the activities of α-amylase. Two days after germination, the activity of α-

308  amylase on the MT+DS treatment significantly increased by 14.5% compared with W+DS under

309  drought stress. MT showed higher α-amylase activity compared with W and was increased by

310  5.9% and 3.5% at 4 and 6 days after germination under normal conditions, respectively (Fig. 6).

311  Similarly, under drought stress conditions, the α-amylase activity in the MT+DS treatment

312  increased by 5.2% and 5.8% on the fourth and sixth day after germination, respectively,

313  compared to the W+DS treatment. Therefore, under drought stress conditions, the α-amylase

314  activity was effectively improved in seeds pre-soaked with 100 μM MT.

315  **Effects of melatonin treatment on osmoregulatory substances in seeds under drought stress**

316  As shown in Fig.7A, drought stress enhanced the accumulation of proline content in seeds.

317  Under well-watered conditions, seeds pre-soaked with MT displayed a negligible effect on

318  proline content. While the MT+DS treatment significantly improved the proline content

319  compared with the W+DS treatment under drought stress. Our data showed that the proline

320  content of the MT+DS treatment significantly increased by 7.11%, 9.92%, and 3.08% compared

321  with the W+DS treatment at 2, 4, and 6 days after seed germination, respectively.

322      Drought stress also increased the soluble sugar content. The soluble sugar content in seeds

323  pre-soaked with MT was higher than seeds not pre-treated with MT. Under normal conditions,

324  the soluble sugar content increased by 16.37%, 2.85%, and 28.22% in the MT treatment at 2, 4,

325  and 6 days after germination, respectively, compared with W treatment. Under drought stress,

326  the soluble sugar content in the MT+DS treatment was 6.91%, 6.82%, and 6.89% higher at 2, 4,

327  and 6 days after germination, respectively, compared with the W+DS treatment (Fig. 7B).

328      The soluble protein content also responded positively to pre-soaking seeds with MT. Pre-

329  treatment with MT considerably inhibited the decrease of soluble protein content under all

330  conditions. The soluble protein content under the MT treatment was increased by 2.16%, 5.03%,

331  and 7.98% at 2, 4, and 6 days after seed germination, respectively, compared to the W treatment.

332  Under drought stress conditions, the soluble protein content in the MT+DS treatment increased

333  by 6.36% and 7.95%, respectively, at 2 and 4 days after seed germination compared with the

334  W+DS treatment. However, 6 days after germination, the effect of the MT treatment on the

335  protein content was more effective under drought stress than under the water treatment; the

336  protein content in the MT+DS treated cotton seeds increased by 5.22% compared with the

337  W+DS treatment. Consequently, we can infer that MT may accelerate the germination of cotton

338  seeds under drought stress conditions (Fig. 7C).

339 **Effects of the melatonin treatment on endogenous hormones under drought stress**

340 As shown in Fig. 8A, GA continuously increased over the course of germination; however,

341 drought stress conditions negatively affected GA biosynthesis. The GA content was greater in

342 the seeds pre-soaked with MT compared with those not pre-soaked with MT under drought stress

343 conditions. The GA content increased by 10.81% in the MT+DS treatment, compared with the

344 W+DS treatment 2 days after germination under drought stress conditions; however, this

345 difference was not significant. Four days after germination, the GA accumulation significantly

346 increased by 16.09% in the MT+DS treatment compared with the W+DS. Thus, this treatment

347 alleviated the inhibitory effects of drought stress. At the end of the germination experiment, pre-

348 treatment with MT further promoted GA synthesis in seeds, but there was no significant

349 difference compared with the W+DS treatment. The effect of MT on drought stress during

350 germination could be via enhancing GA biosynthesis.

351 Similar to GA, the ABA content increased over the course of germination (Fig. 8B). In this

352 study, MT accelerated ABA catabolism during germination in all cases. In response to drought

353 induced by treatment with PEG-6000, ABA catabolism was limited during seed germination.

354 Additionally, the ABA content in the seeds pre-soaked with MT was lower compared to the W

355 treatment under normal conditions. Under drought stress conditions, the ABA content of the

356 MT+DS treatment decreased by 6.22%, 4.2%, and 6.42% 2, 4, and 6 days after germination,

357 respectively, compared with the W+DS. These results indicated that the mechanism by which

358 MT reduced PEG-6000 stress was by promoting the degradation of ABA.

359 **Microstructural observation of testa**

360 Fig. 9A shows that the seed coat stomata were rare on the surface of dry seeds. Only a small

361 number of pores were observed in seeds soaked in water (Fig. 9B). However, seeds soaked in

362 MT showed a greater number of pores (Fig. 9C). The stomata were located on the surface of the

363 epidermis between the ridges of the irregular epidermal cells, but the inner side of the stomata

364 was not surrounded by epidermis. The pores were oval and not indented on the surface of the dry

365 seeds (Fig. 9D). The periphery of the stomata was elliptic and ranged from oval to round toward

366 the center, but the inner guard cells were covered with cuticular remnants in seeds soaked in

367 water (Fig. 9E). However, soaking seeds with MT played a significant role in the opening of

368 pores in the testa. Compared with dry seeds and seeds soaked in water, the pores of seeds soaked

369 in MT were completely open and contained large guard cells (Fig. 9F).

370 **DISCUSSION**

371 Drought stress markedly impairs plant growth via different mechanisms and influences seed

372 germination and metabolism (Zhang et al., 2012). The immediate response of a plant to drought

373 stress can be determined by the inhibition of the germination rate. The damage caused by

374 drought stress to seed germination is mainly due to decrease in water intake and reduced energy

375 supply to seeds, inducing a series of changes in metabolism, including ROS accumulation,

376 antioxidant defense system, hormone signal transduction, and the production of osmotic

377 regulatory substances (Hussain, Farooq & Lee, 2017; Shu et al., 2018; Sharma & Zheng, 2019).

378 Numerous studies have demonstrated that drought stress significantly inhibits seed germination

379 in various plant species (Liu et al., 2018; Luo et al., 2017; Liu et al., 2016; Liu et al., 2019). Our

380 experiments showed complementary evidence that the germination rate of cotton seeds was

381 inhibited by the application of 10% PEG-6000 (Fig. 2) because it caused a negative regulation in

382 physiological mechanisms and inhibited water absorption by seeds, suggesting that drought

383 stress affected cotton seed germination.

384     Recently, plant growth regulators have been widely used to treat seeds before sowing, and can

385 improve their resistance to abiotic stressors and promote seed germination (Liang et al., 2018;

386 Zheng et al., 2016). In the present study, seed germination inhibition caused by PEG-6000 was

387 alleviated by soaking cotton seeds with MT. Under the same drought stress treatment, seeds

388 soaked with 100 μM MT showed an accelerated seed germination rate and improved radical

389 length and fresh weight compared with seeds not pre-treated with MT. In addition, 10–50 μM

390 MT treatments had little effect on cotton seed germination. However, 200–500 μM MT exerted a

391 significantly negative effect on cotton seed germination (Table 1). These results demonstrated

392 that the effect of MT on cotton seeds is closely associated with its concentration and 100 μM MT

393 was defined as the optimum concentration to promote cotton seed germination under drought

394 stress. These findings are in accordance with Simlat et al. (2018), who demonstrated that a low

395 concentration of exogenous MT promoted seed germination, while treatments with a high dose

396 of MT inhibited seed germination. Previous studies reported that soaking seeds in exogenous MT

397 (20 and 100 μM) alleviated drought-induced germination inhibition by advancing the antioxidant

398 defense system (Ding, Liu & Zhang, 2017). The germination test indicated that treatment with

399 100 μM MT (MT+DS) increased the GP, GR, GI, and VI compared with the seeds only pre-

400 soaked with water (W+DS) under drought stress conditions (Table 2). Our findings are consistent

401 with previous results showing that the application of exogenous MT could alleviate the inhibition

402 of PEG stress on cucumber seed germination (Zhang et al., 2014).

403     The decisive factors for seed germination are water and oxygen, which are absorbed and

404 transmitted through the seed coat (Leónie & Maarten, 2008; Kebede, Smith & Ray, 2014). The

405 increase in seed coat stomata promotes the intake of water and oxygen from the environment,

406 resulting in seed germination (López-Ribera & Carlos, 2017; Wang & Hasenstein, 2016);

407 therefore, the seed coat stomata play a vital role in seed germination and growth. The stomata

408 consist of two guard cells and subsidiary cells (Wang & Hasenstein, 2016). Previous studies have

409 shown that the absorption rate of water is highly related to pore density (Paiva, Lemos &

410 Oliveiva, 2006). The number of seed stomata is not necessarily species-specific and may depend

411 on environmental conditions (Wang & Hasenstein, 2016). In the current study, few stomata were

412 observed in dry seeds, suggesting that the lack of stomata may be due to dehydration in maturing

413 seeds. The variability in the number and shapes of pores indicates that the number of stomata is

414 fluid and may have some physiological function (Wang & Hasenstein, 2016). The results of the

415 SEM showed that pre-treatment with MT increased the number of stomata and accelerated the

416 opening of pores in testa, confirming that exogenous MT stimulated the physiological function of

417 stomata and accelerated the water absorption rate of seeds (Fig. 9). Therefore, a vital effect of the

418 MT treatment is the induction of the opening of stomata in the cotton seed coat. While a

419 quantitative analysis of stomata in testa was not carried out, our results do suggest that the

420 function of MT on the regulation mechanism of seed coat stomata requires further study.

421     Seed germination is controlled by physiological factors and ROS concentration is closely

422 correlated with water uptake by seeds during the process of germination (Moothoo et al., 2016).

423 The balance between the production and elimination of ROS is disturbed by drought stress,

424 which leads to the accumulation of ROS, including $O_2^-$ and $H_2O_2$, and leads to the instability of

425 cell membranes (Kaur et al., 2015; Yang, Yao & Zhang, 2010; Li et al., 2015; Sohag et al.,

426 2020). $H_2O_2$ content is associated with embryo elongation during seed germination (Zhang et al.,

427 2014). MT is a free radical scavenger, which may directly or indirectly eliminate ROS (Reiter et

428 al., 2007; Tan et al., 2000). Previous research has suggested that pre-treatment with MT

429 effectively inhibited the accumulation of $H_2O_2$ in corn seeds under stress conditions (Cao et al.,

430 2019). Similarly, the application of MT reduced the accumulation of ROS and alleviated

431 membrane damage of tomato seeds and cucumber seedlings under the drought stress (Zhang et

432   al., 2012; Liu et al., 2015). It is necessary to determine whether MT can promote the germination

433   of cotton seeds by inhibiting the accumulation of ROS. We found that seeds pre-soaked in

434   distilled water and treated with normal and dry conditions had an increase in $H_2O_2$ and $O_2^-$

435   contents; however, the $O_2^-$ and $H_2O_2$ contents were decreased in seeds pre-soaked with MT (Fig.

436   4). This may be because MT was instrumental in accelerating water absorption by the seed and

437   maintaining the balance between ROS production and clearance. Furthermore, this may have led

438   to repair of the cell membrane even under drought stress conditions. These results provided

439   evidence that exogenous MT played an important role in removing excessive ROS and

440   alleviating the membrane lipid peroxidation in cotton seeds faced with drought stress.

441       To inhibit excessive generation of ROS, plants possess a complex enzymatic defense system

442   to combat oxidative damage (Souza et al., 2014; Ahammad et al., 2020c). SOD and POD act as

443   important antioxidant enzymes that can eliminate redundant ROS from plant tissues, protecting

444   the plasma membrane from peroxidation (Xiao et al., 2019; Ramachandra et al., 2004). In plant

445   cells, SOD can rapidly convert $O_2^-$ to form $H_2O_2$ and $O_2$, while $H_2O_2$ can be further scavenged by

446   other antioxidant enzymes (Li et al., 2017). Many studies have shown that MT plays a vital role

447   in enhancing the activity of antioxidant enzymes and scavenging radicals in response to stress-

448   induced ROS damage (Zhang et al., 2014; Sharif et al., 2018; Jiang et al., 2016; Shi et al., 2015).

449   In the present study, the activity of SOD and POD were low at 2 d of germination, peaked at 4 d,

450   and gradually decreased by 6 d, indicating that the antioxidant enzyme system was active in the

451   middle of seed germination. Drought stress led to the inhibition of SOD and POD activities in

452   cotton seeds, which may be because drought stress induced the excessive generation of ROS,

453   negatively affecting antioxidant enzyme activities. We treated cotton seeds with exogenous MT

454   to enhance the tolerance of cotton seeds to drought stress. We found that seeds pre-soaked with

455   MT enhanced the activities of the SOD and POD both under normal water and drought stress

456   conditions by inhibiting the accumulation of ROS (Fig. 3). These results were in consistent with

457   a previous study that described that treatment with exogenous MT promoted SOD and POD

458   activities in grape exposed to drought conditions (Meng et al., 2014).

459       The accumulation of ROS induces lipid peroxidation and the degradation of unsaturated fatty

460   acids, producing MDA, which causes damage to the seed structure and reduces seed germination

461   (Rajjou & Debeaujon 2008; Xiao et al., 2019). Previous studies have shown that the application

462   of exogenous MT significantly suppressed MDA accumulation under abiotic stressors in several

463  plant species (Ahammed et al., 2019b; Hasan et al., 2019). Zhang et al. (2014) demonstrated that

464  treatment with 300 μM MT, which increased the activity of antioxidant enzymes, could

465  effectively reduce the MDA content in cucumber seeds under drought stress. In the present

466  study, the MDA content gradually increased during the seed germination assay. Drought stress

467  increased the MDA content and induced lipid membrane peroxidation. However, pre-soaking

468  seeds with 100 μM MT decreased the accumulation of MDA compared with seeds pre-soaked in

469  water under both normal and drought conditions (Fig. 5), suggesting that MT enhanced the

470  activity of antioxidant enzymes potentially by eliminating ROS and reducing membrane lipid

471  peroxidation in cotton seeds (Ahammed et al., 2020b).

472      Seed germination is regulated by the presence of several storage products (Waterworth et al.,

473  2016; Fleming, Richards & Walters, 2017). Amylase and osmotic regulators (e.g., proline,

474  sugars, and protein) are compatible solutes that provide necessary energy for seed

475  morphogenesis. Furthermore, amylase protects plants from stress conditions (Sadura &

476  Janeczko, 2018) by accumulating in the form of an inactive polymer and storing in the

477  endosperm of dry seeds as storage protein, which is released and activated gradually during

478  germination; therefore, amylase plays an important role in seed germination (Swanston &

479  Molina, 2001). Pre-treatment with exogenous MT has been revealed to increase α-amylase

480  activity in *Limonium bicolor* seeds and accelerate seed germination under salt stress (Li et al.,

481  2019). In the present study, under normal conditions, MT treatment was not positively correlated

482  with α-amylase activity at 2 d of germination. However, the α-amylase activity increased in

483  response to MT treatment by days 4 and 6, confirming that the effectiveness of exogenous MT

484  on amylase activity may not be apparent at the beginning of germination under normal

485  conditions. The MT+DS treatment enhanced the activity of α-amylase at all germination time

486  points under drought stress, compared with the W+DS treatment (Fig. 6), suggesting that MT

487  may promote starch hydrolysis and seed respiration, improving seed germination and growth.

488      Proline is an important osmotic regulator and free radical scavenger that can alleviate stress

489  damage by reducing water potential (Hayat et al., 2012; Bala, 2000). We found that the proline

490  content of each treatment increased gradually throughout the process of seed germination. The

491  effect of the MT treatment was similar to that of W treatment and did not show a positive role in

492  increasing proline content. However, the MT+DS treatment performed better under drought

493  stress compared with the W+DS treatment, as evident from the increased proline content (Fig.

494   7A). These results indicate that MT may regulate the metabolism of osmotic substances under

495   drought stress and improve drought tolerance. Pre-treatment with 100 μM MT can improve the

496   drought resistance of cotton seeds, which is in agreement with a previous study that found that

497   pre-treatment with MT increases the proline content in *Brassica napus* exposed to Se-stress

498   (Ulhassan et al., 2019). Soluble sugar and soluble protein, which play a role in osmoregulation

499   and cell metabolism, are the main components of organelles (Wang et al., 2004; Zhang et al.,

500   2015a). This study found that the content of soluble sugar and protein first increased and then

501   decreased during the process of seed germination. The contents of soluble sugar and soluble

502   protein in the MT+DS treatment were higher than in the W+DS treatment, and the exogenous

503   application of MT may be a particularly important for the accumulation of osmotic regulators

504   under drought stress conditions (Fig. 7). These findings indicate that MT could reduce the

505   damage to plant cells caused by drought stress, reduce excessive water loss, and enhance the

506   drought resistance of plants. These findings are similar to those presented by Jiang et al. (2016).

507   Plant hormones play vital roles in the life cycle of plants, including seed germination,

508   dormancy, and response to abiotic stress (Zhou et al., 2019; Liu et al., 2016; Shu et al., 2016). It

509   is generally recognized that ABA and GA regulate seed germination antagonistically (Okamoto

510   et al., 2006); GA promotes seed germination by breaking seed dormancy, while ABA inhibits

511   seed germination (Zhou et al., 2019; Yang et al., 2014; Okamoto et al., 2006; Toh et al., 2008).

512   Pre-treatment with MT enhanced the stress resistance of plants by regulating the changes of

513   endogenous hormones (Jiang et al., 2016). Zhang et al. (2014) showed that the exogenous

514   application of MT significantly increased the GA content and accelerated ABA degradation in

515   cucumber seeds under salt stress. Our results showed that ABA content was increased, whereas

516   GA content was decreased under drought stress conditions. Compared with the W+DS treatment,

517   the MT+DS treatment increased the GA content and inhibited the production of ABA content in

518   cotton seeds grown under drought stress conditions (Fig. 8), which is indicative of the role of MT

519   on regulating the endogenous hormones in cotton under drought stress. Similarly, the pre-

520   treatment of cotton seeds with MT can also promote the accumulation of GA and decrease the

521   ABA content under normal water conditions; however, the effect of the MT treatment on the GA

522   content was not obvious at day 6. This may be because the effect of MT on GA biosynthesis was

523   inconspicuous at the later stage of germination in the normal water treatment. This confirmed

524  that the application of MT can maintain the balance of GA and ABA, effectively accelerating the

525  germination of cotton seeds under drought stress.

526  **CONCLUSIONS**

527  Pre-treatment of cotton seeds with MT improves germination under drought stress conditions.

528  The results of our ultrastructure analysis of seed coats demonstrated that pre-treatment with MT

529  increased the number of stomata and promoted the opening of pores. Likely, this enhanced

530  antioxidant enzyme activities and reduced drought-induced ROS accumulation. Pre-treatment

531  with MT also increased starch metabolism and the content of osmotic substances, overall

532  enhancing the germination rate of cotton seeds. Pre-treatment with 100 μM MT may promote

533  germination by regulating the hormone content; seeds pre-treated with MT reduced ABA content

534  and increased GA content. Therefore, cotton seeds soaked with MT showed a significant

535  improvement in the ability of seeds to germinate under PEG-6000 simulated drought stress. Our

536  study highlights the beneficial effects of pre-treatment with MT on the germination of cotton

537  seeds, as shown by multiple perspectives including morphological and physiological traits.

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842

843

844 **Figure and table legends**

845

846 **Figure 1 The daily germination rate of seeds treated with different concentrations of PEG-6000.**

847

848 **Figure 2 Effects of drought stress on the germination rate of cotton seeds.** PEG at concentrations of 0

849 (control), 5, 10, 15, and 20% were used at the seed germination stage. Different lowercase letters indicate

850 a significant difference at the 0.05 probability level ($P < 0.05$). Error bars indicate standard errors

851 calculated for five replicates.

852

853 **Figure 3 Effect of melatonin treatment on (A) superoxide dismutase (SOD) and (B) peroxidase**

854 **(POD) activities of cotton seeds under drought stress.** Cotton seeds were pre-soaked in the solutions of

855 0 (control) and 100 μM MT for 24h and germination under 0 (control) and 10% PEG stress. Different

856 lowercase letters indicate a significant difference at the 0.05 probability level ($P < 0.05$). Error bars

857 indicate standard errors calculated for three replicates.

858

859 **Figure 4 Effects of pre-treatment with melatonin on $H_2O_2$ (A) and $O_2^-$ (B) content of cotton grown**

860 **under drought stress conditions.** Cotton seeds were pre-soaked in the solutions of 0 (control) and 100

861   μM MT for 24h and germination under 0 (control) and 10% PEG stress. Different lowercase letters

862   indicate a significant difference at the 0.05 probability level ($P < 0.05$). Error bars indicate standard errors

863   calculated for three replicates.

864

865   **Figure 5 Effects of melatonin treatment on MDA content of cotton under drought stress.** Cotton

866   seeds were pre-soaked in the solutions of 0 (control) and 100 μM MT for 24h and germination under 0

867   (control) and 10% PEG stress. Different lowercase letters indicate a significant difference at the 0.05

868   probability level ($P < 0.05$). Error bars indicate standard errors calculated for three replicates.

869

870   **Figure 6 Effects of melatonin treatment on α-amylase activity of cotton under drought stress.** Cotton

871   seeds were pre-soaked in the solutions of 0 (control) and 100 μM MT for 24h and germination under 0

872   (control) and 10% PEG stress. Different lowercase letters indicate a significant difference at the 0.05

873   probability level ($P < 0.05$). Error bars indicate standard errors calculated for three replicates.

874

875   **Figure 7 Effects of melatonin treatment on proline (A), soluble sugar (B), and soluble protein (C)**

876   **content of cotton seeds under drought stress.** Cotton seeds were pre-soaked in the solutions of 0

877   (control) and 100 μM MT for 24h and germination under 0 (control) and 10% PEG stress. Different

878   lowercase letters indicate a significant difference at the 0.05 probability level ($P < 0.05$). Error bars

879   indicate standard errors calculated for three replicates.

880

881   **Figure 8. Effects of melatonin treatment on contents of gibberellin (GA) (A) and abscisic acid**

882   **(ABA) (B) under drought stress.** Cotton seeds were pre-soaked in the solutions of 0 (control) and100

883   μM MT for 24h and germination under 0 (control) and 10% PEG stress. Different lowercase letters

884   indicate a significant difference at the 0.05 probability level ($P < 0.05$). Error bars indicate standard errors

885   calculated for three replicates.

886

887   **Figure 9 Scanning electron microscopic of the testa of cotton seeds.** A, D dry seed; B, E seed soaked

888   in water for 24 hours at 25 ˚C; C, F seed soaked in MT solution for 24 hours at 25 ˚C

889

890   **Table 1 Effects of different concentrations of melatonin on the germination and growth of seeds**

891   **under drought stress.** Cotton seeds were pre-soaked in MT solutions of 0 (control), 10, 50, 100, 200, and

892   500 μM for 24h and germination under 10% PEG stress. Different lowercase letters indicate a significant

893   difference at the 0.05 probability level ($P < 0.05$). Error bars indicate standard errors calculated for five

894   replications.

895

896    **Table 2 Effect of melatonin treatment on GP, GR, GI, and VI of cotton under drought stress.** Cotton

897    seeds were pre-soaked in the solutions of 0 (control) and 100 μM MT for 24h and germination under 0

898    (control) and 10% PEG stress. Different lowercase letters indicate a significant difference at the 0.05

899    probability level ($P < 0.05$). Error bars indicate standard errors calculated for five replicates.

Table 1 Effects of different concentrations of melatonin on the germination and growth of seeds under drought stress.

1 **Table 1 Effects of different concentrations of melatonin on the germination and growth of seeds**

2 **under drought stress.** Cotton seeds were pre-soaked in MT solutions of 0 (control), 10, 50, 100, 200, and

3 500 μM for 24h and germination under 10% PEG stress. Different lowercase letters indicate a significant

4 difference at the 0.05 probability level ($P < 0.05$). Error bars indicate standard errors calculated for five

5 replications.

| Melatonin concentration (μM) | GP (%) | GR (%) | RL (cm) | FW (g) |
|---|---|---|---|---|
| 0 | 44.5 ± 1 b | 72 ± 3.65 c | 6.3 ± 0.16 b | 11.49 ± 1.06 c |
| 10 | 43.2 ± 2.68 b | 76 ± 1.41 b | 6.15 ± 0.11b | 13.84 ± 0.89 b |
| 50 | 43.2 ± 2.28 b | 73.5 ± 1.91 bc | 6.6 ± 0.08 a | 13.32 ± 0.69 b |
| 100 | 48.5 ± 1.91 a | 79.5 ± 1.91 a | 6.78 ± 0.19 a | 17.63 ± 0.94 a |
| 200 | 33.2 ± 2.28 c | 63.2 ± 1.09 d | 5 ± 0.12 c | 14.21 ± 0.98 b |
| 500 | 28.4 ± 2.61 d | 50.5 ± 1 e | 2.46 ± 0.36 d | 13.72 ± 0.88 b |

Table 2 Effect of melatonin treatment on GP, GR, GI, and VI of cotton under drought stress.

1 **Table 2 Effect of melatonin treatment on GP, GR, GI, and VI of cotton under drought stress.** Cotton

2 seeds were pre-soaked in the solutions of 0 (control) and 100 μM MT for 24h and germination under 0

3 (control) and 10% PEG stress. Different lowercase letters indicate a significant difference at the 0.05

4 probability level ($P < 0.05$). Error bars indicate standard errors calculated for five replicates.

| Treatments | GP (%) | GR (%) | GI (%) | VI (%) |
|---|---|---|---|---|
| W | 51.5 ± 1 a | 90 ± 1 a | 18.29 ± 1.11 a | 137.55 ± 5.29 a |
| W+DS | 44.5 ± 1 c | 72.25 ± 4.03 c | 15.37 ± 0.64 c | 103.44 ± 1.83 c |
| MT | 52.67 ± 0.58 a | 87.67 ± 1.53 a | 19.36 ± 0.98 a | 121.97 ± 6.16 b |
| MT+DS | 48.51 ± 0.57 b | 79.33 ± 2.31 b | 17.44 ± 0.61 b | 120.15 ± 4.31 b |

# Figure 1

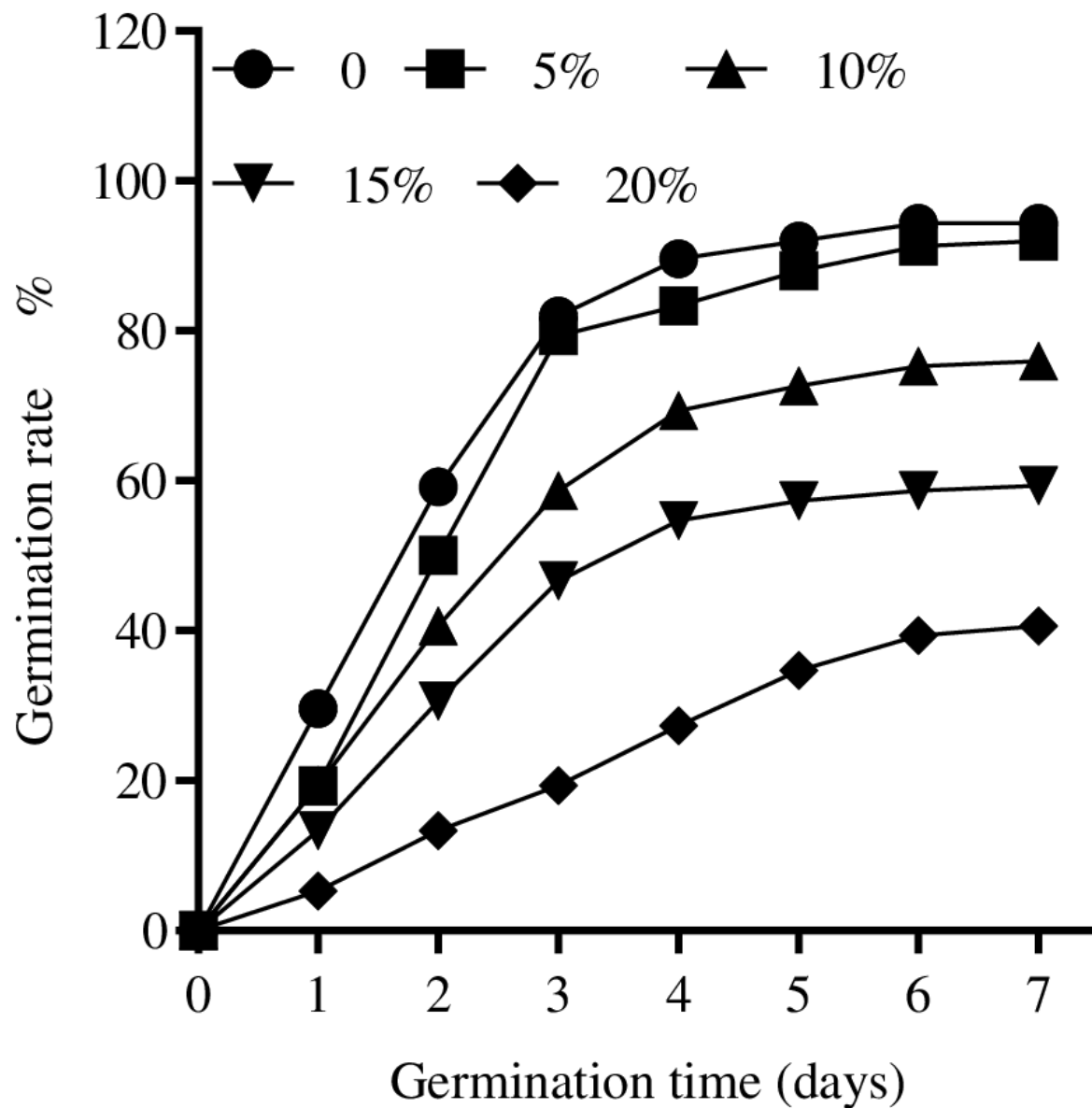
Figure 1 The daily germination rate of seeds treated with different concentrations of PEG-6000

# Figure 2

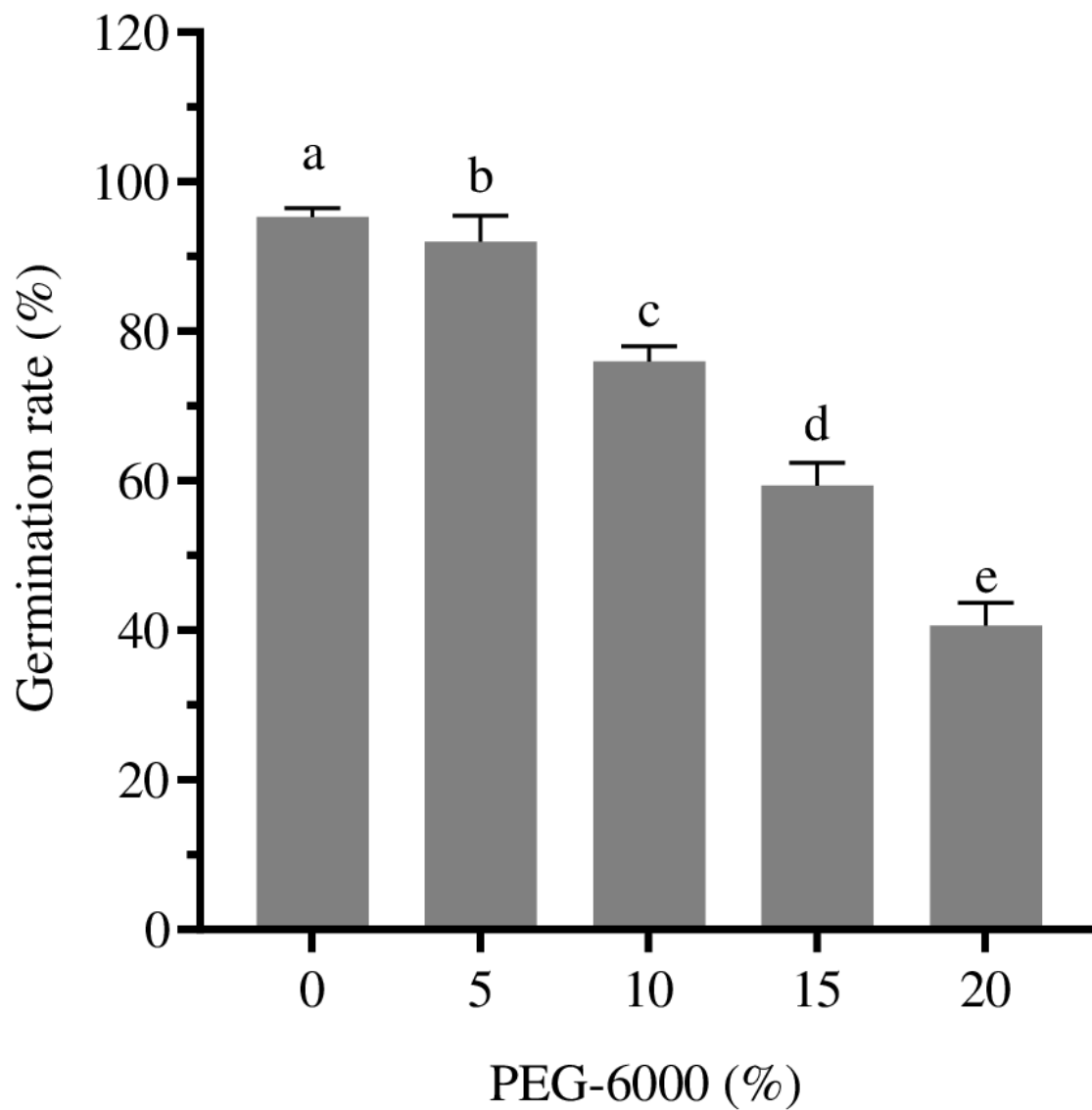
Figure 2 Effects of drought stress on the germination rate of cotton seeds.

# Figure 3

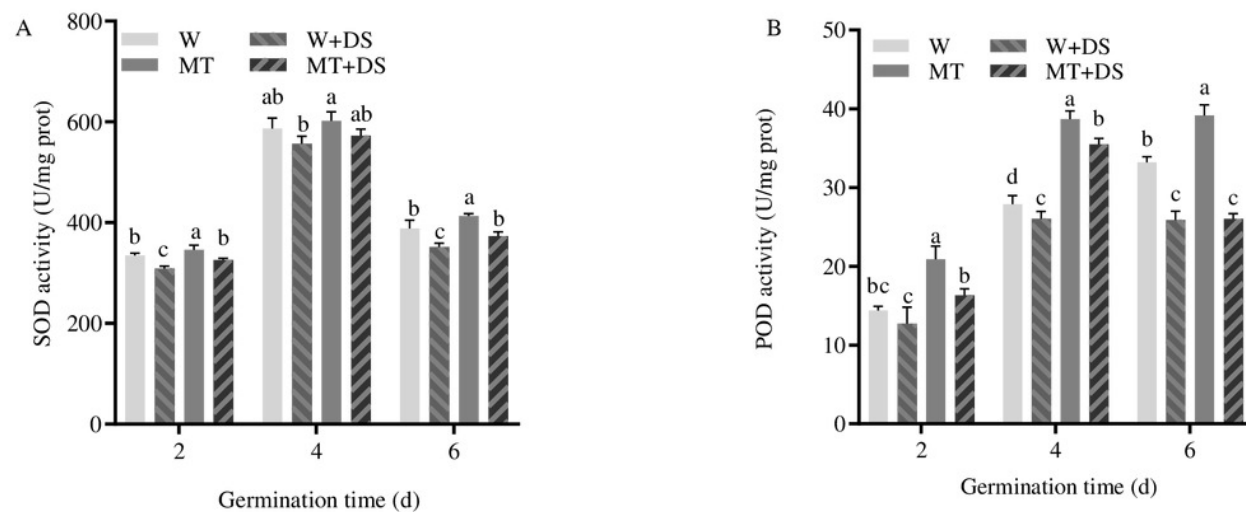
Figure 3 Effect of melatonin treatment on (A) superoxide dismutase (SOD) and (B) peroxidase (POD) activities of cotton seeds under drought stress.

# Figure 4

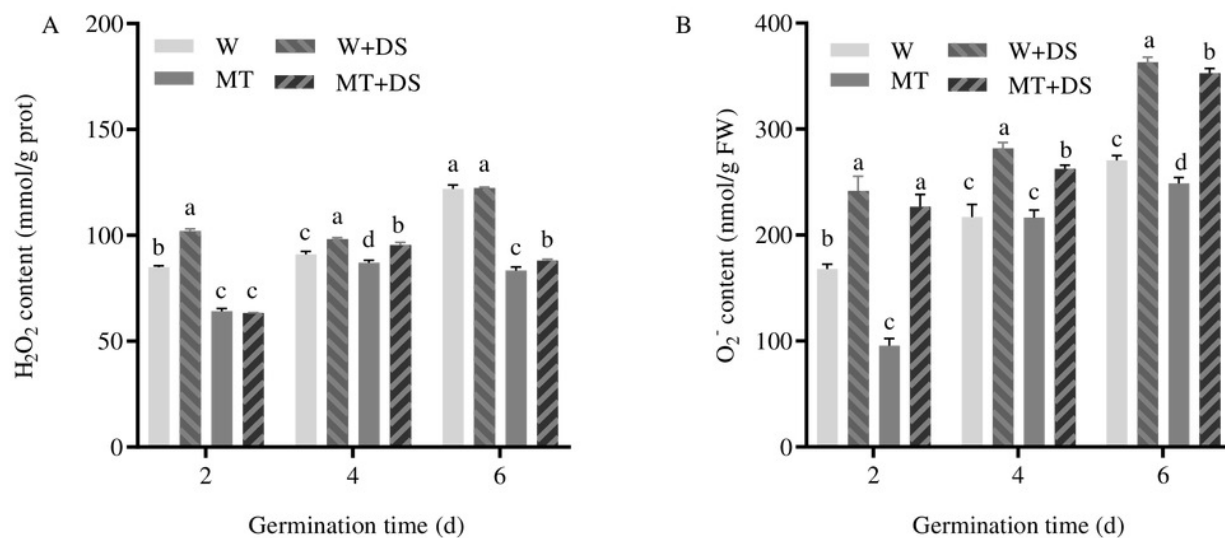
Figure 4 Effects of pre-treatment with melatonin on $H_2O_2$ (A) and $O_2^-$ (B) content of cotton grown under drought stress conditions.

# Figure 5

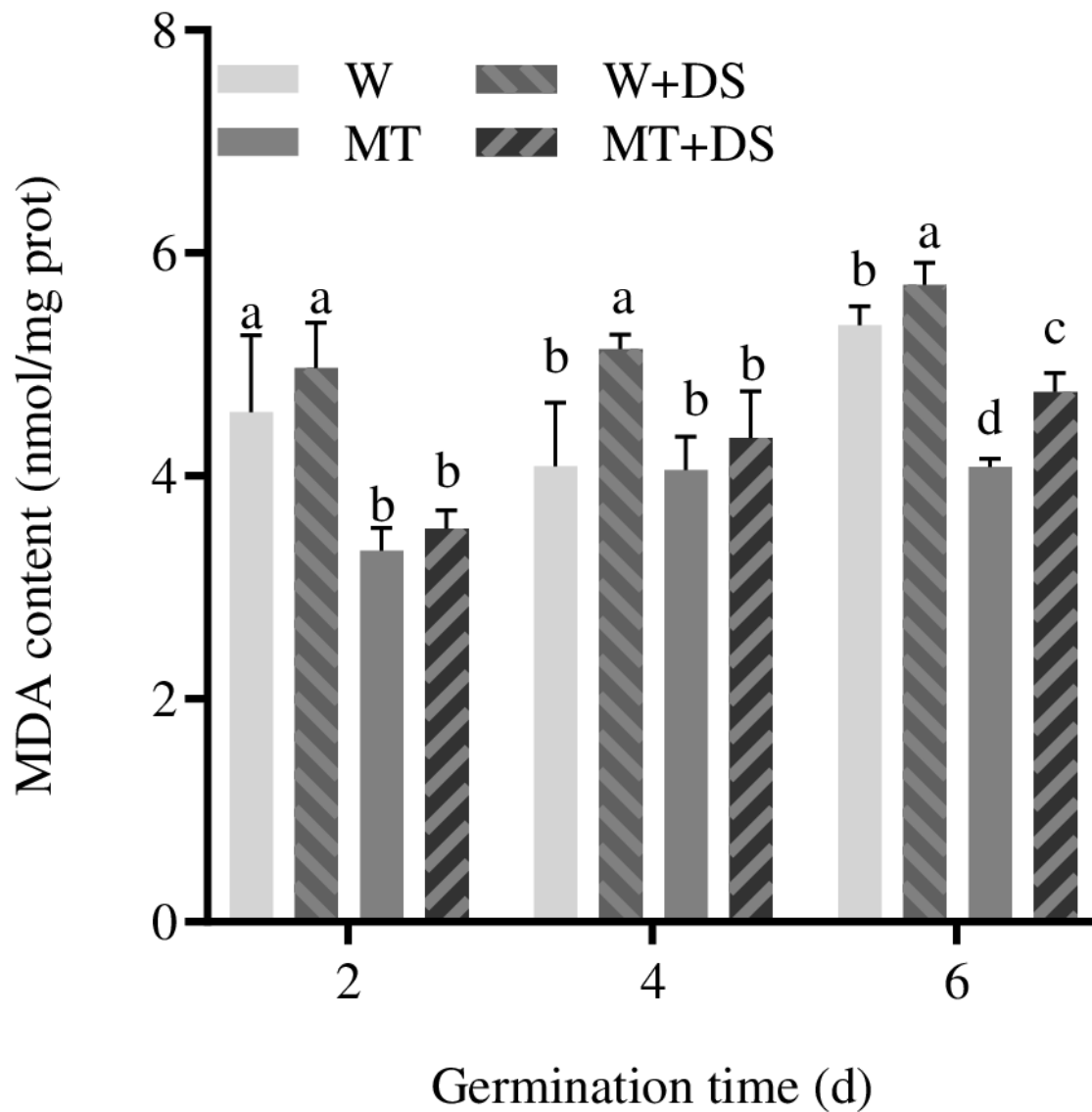
Figure 5 Effects of melatonin treatment on MDA content of cotton under drought stress.

# Figure 6

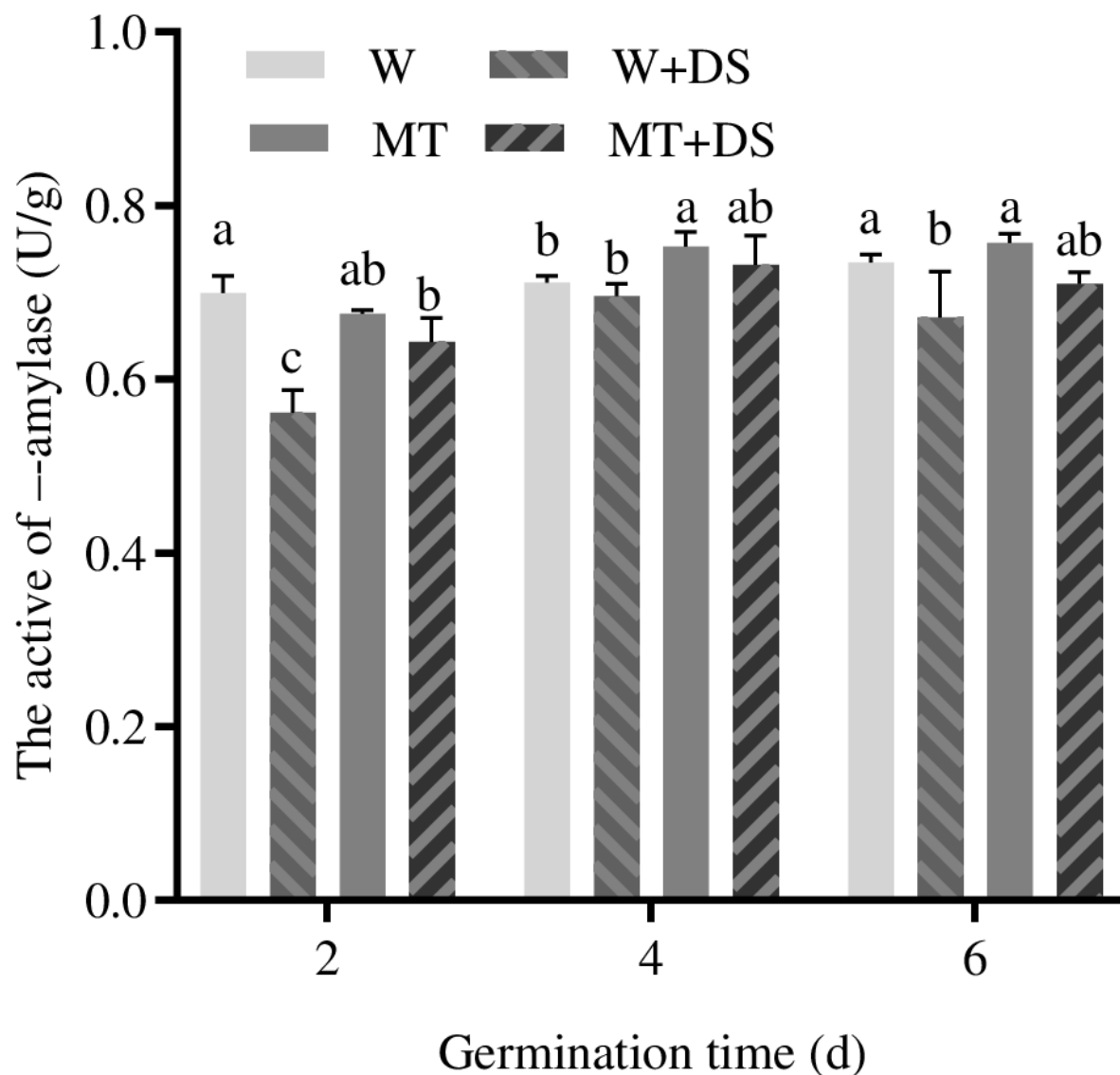
Figure 6 Effects of melatonin treatment on α-amylase activity of cotton under drought stress.

# Figure 7

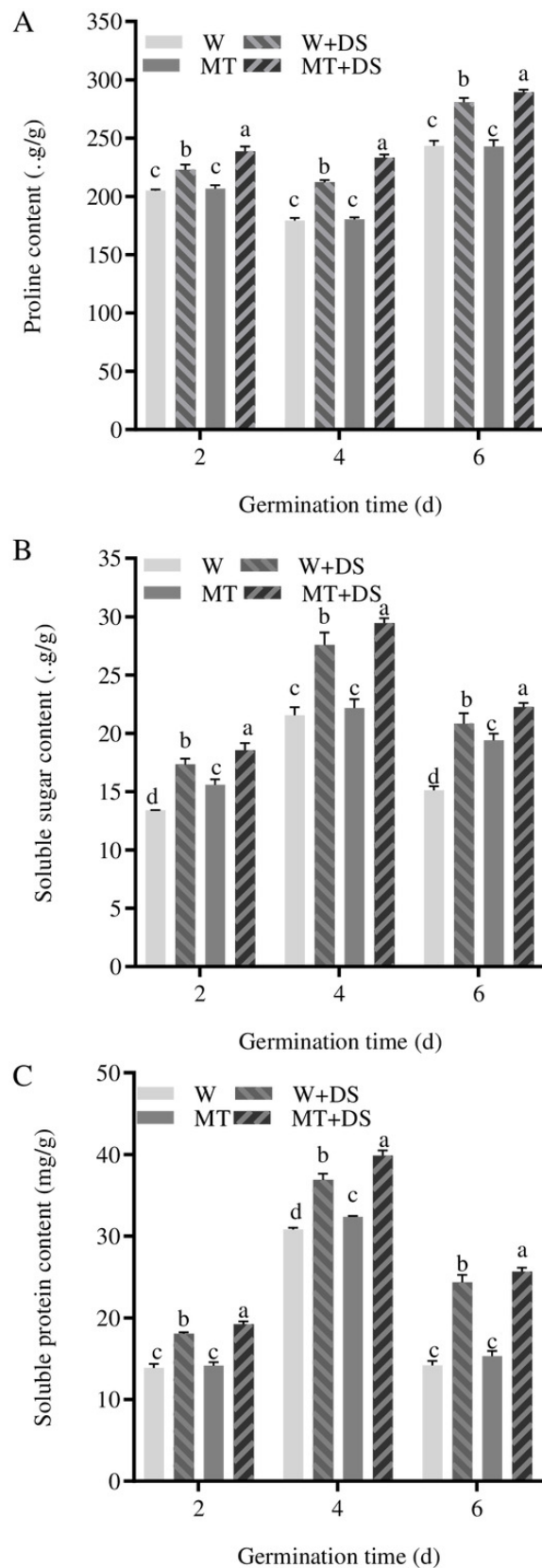
Figure 7 Effects of melatonin treatment on proline (A), soluble sugar (B), and soluble protein (C) content of cotton seeds under drought stress.

# Figure 8

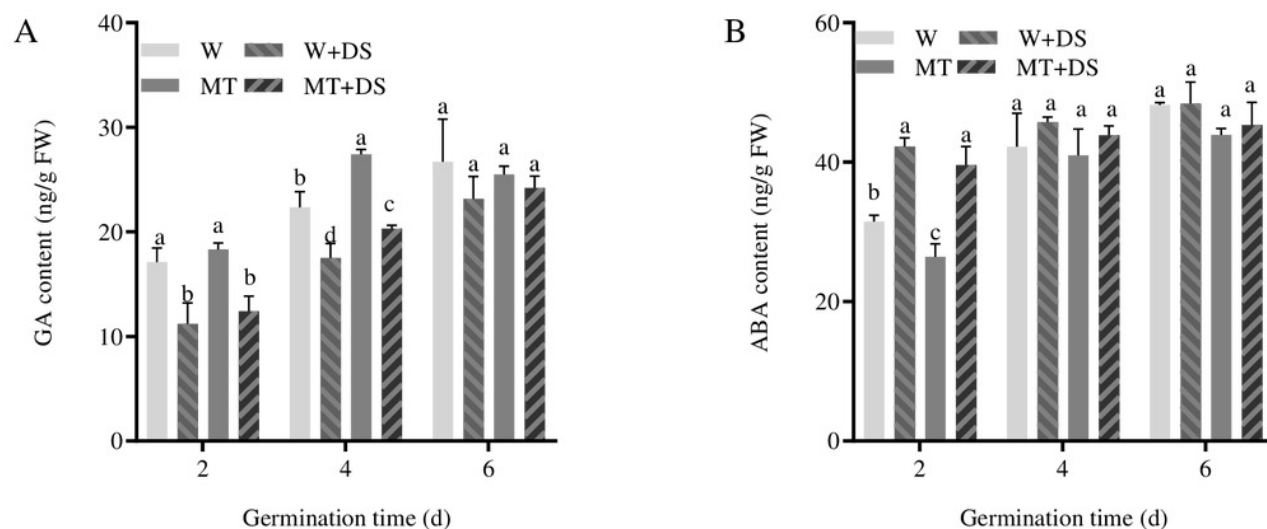
Figure 8. Effects of melatonin treatment on contents of gibberellin (GA) (A) and abscisic acid (ABA) (B) under drought stress.

# Figure 9

Figure 9 Scanning electron microscopic of the testa of cotton seeds.