Melatonin Promotes Seed Germination Under Salt Stress by Regulating ABA and GA3 in Cotton (Gossypium Hirsutum L.)

Li Chen
Hebei Agricultural University https://orcid.org/0000-0001-7216-1469
Bin Lu
Hebei Agricultural University
Liantao Liu
Hebei Agricultural University
Wenjing Duan
Hebei Agricultural University
Dan Jiang
Hebei Agricultural University
Jin Li
Hebei Agricultural University
Ke Zhang
Hebei Agricultural University
Hongchun Sun
Hebei Agricultural University
Yongjiang Zhang
Hebei Agricultural University
Cundong Li
Hebei Agricultural University
Zhiying Bai (zhiyingbai@126.com)

Research article

Keywords: abscisic acid, cotton seed, germination, gibberellin acid, melatonin, salt stress

DOI: https://doi.org/10.21203/rs.3.rs-27842/v1

Abstract

Background: Although previous studies have found that melatonin can promote seed germination, the phytohormone regulation mechanism by which exogenous melatonin mediates salt tolerance during cotton seed germination is still largely unknown. We investigated the effect of melatonin on the germination traits and physiological parameters of GXM9 cotton seeds (*Gossypium hirsutum* L.) under three salt stress treatments (CK, germination of seeds pretreated with water alone; S, germination of seeds pretreated in 150 mM NaCl under salt stress; SM, germination of seeds pretreated in 20 μM melatonin under 150 mM NaCl solution) in the laboratory.

Results: We found that salt stress (150 mM) inhibited cotton seed germination and endogenous melatonin accumulation, and pretreatment with 20 μM exogenous melatonin enhanced the cotton germination rate and hypocotyl length as well as the content of endogenous melatonin during seed germination. This suggests that exogenous melatonin promotes seed germination from a morphological perspective. The contents of starch, α-amylase (EC3.3.1.1), β-galactosidase (EC3.2.1.23), abscisic acid (ABA), and gibberellin (GA) were determined simultaneously. The results showed that the α-amylase and β-galactosidase contents in the cotton seeds decreased by 56.97% and 20.18%, respectively, under salt stress compared with the control, while the starch content increased by 11.53% compared with the control at day 7. The ABA content increased by 25.18% and GA content decreased by 27.99% under salt stress compared with the control at 24 h. When exogenous melatonin was applied to the cotton seeds, the content of α-amylase and β-galactosidase increased by 121.77% and 32.76%, respectively, whereas the starch contents decreased by 13.55% compared with the S treatment at day 7. Similarly, the ABA content increased by 12.20% and the GA content increased by 4.77% at 24 h. To elucidate the molecular mechanism by which melatonin promotes seed germination under salt stress, the effects of ABA- and GA-related genes on plant hormone signal transduction were analyzed by quantitative real-time PCR and RNA sequencing. The results indicated that melatonin regulated the expression of ABA and GA genes in the plant signal transduction pathway, induced embryo root development and seed germination, and alleviated dormancy. We found that the expression of the ABA signaling gene *GhABF2* was up-regulated and *GhDPBF2* was down-regulated, and the expression of GA signaling genes (e.g., *GhGID1C* and *GhGID1B*) was up-regulated by melatonin.

Conclusions: We discovered that melatonin enhances salt tolerance in cotton seeds by regulating ABA and GA and by mediating the expression of hormone-related genes in plant hormone signal transduction. This should help us to explore the regulatory mechanisms of cotton resistance and provide a foundation for the cultivation of new varieties.

Background

In recent years, abiotic stresses, such as salinity, low temperature, drought, and cold, have affected plant growth and quality, which has significantly reduced agricultural production and crop yield [1–2]. Arid and semi-arid climates are characterized by high soil salinity, and anthropogenic factors such as poor

irrigation practices also contribute to increased salinity in agricultural land [3]. Salt stress is still a major factor affecting crop production and plant development in rice, cabbage, *Bixa orellana*, and other crops around the world [4–6]. Under salt stress, the seed germination, lateral root formation and biomass are significantly inhibited [7–8]. High salinity has negative effects on various plant processes, causing cell membrane damage and reducing antioxidant activity and osmotic regulation [9–13].

Plant hormones play a critical and even decisive role in regulating plant growth and development. Different hormones interact with each other to exert their respective biological functions. Abscisic acid (ABA) is a hormone that mediates the plant response to abiotic stress and is antagonistic to gibberellin (GA). Salt stress may cause changes in ABA and GA by activating catabolism enzymes in seeds [14]. The fundamental means by which stress affects plant morphology and physiology is by inducing gene changes at the molecular level. The expression of salt tolerance genes is regulated by transcription factors in the alkaline leucine zipper (bZIP), WRKY, MYB (v-myb avian myeloblastosis viral oncogene homolog), and NAC families [15]. *ABF*2 is a key gene in the ABA signal transduction pathway and plays an essential role in regulating downstream expression by reducing stress-responsive genes and improving stress tolerance [16].

Cotton (*Gossypium hirsutum* L.) is an economic crop that has an important role in global economic development [17]. Eighty-seven percent of the world's cotton growing areas are in developing countries, and China's cotton planting area has reached 5.9 million hectares [18]. Seeds in the germination stage are susceptible to abiotic (e.g., salinity stress, temperature stress) and biotic stresses (e.g., seed predators, pathogens). When the root tips of the seeds appear through the seed coats during the germination process, they are stimulated by the external environmental and initiate a series of rapid morphological and physiological changes [19]. Research on cotton seed germination under stress is thus of great significance to farming.

Melatonin (*N*-acetyl-5-methoxytryptamine) is widely found in plants and animals [12, 20, 21]. In 1958, melatonin was first discovered in the pineal gland [22]. The physiological functions of melatonin in humans are widely understood and include the scavenging of reactive oxygen, regulation of circadian rhythms, and the control of metastatic diseases [22–23]. In the following years, melatonin was first discovered in tomato fruits and ivy-leaved morning glory (*Convolvulaceae*) [24–27]. Over the years, the application of melatonin in plants has become a pertinent topic in plant stress resistance research. Recently, the alleviatory effect of melatonin on plants under vanadium stress [28], drought stress [29], nano zinc oxide stress [30] and other conditions has been demonstrated. Melatonin may be a lipophilic and hydrophilic compound that reacts with hydroxyl radicals and peroxyl radicals [31–32]. Melatonin mediates seed germination, root length, antioxidant enzyme activity, leaf senescence, phytohormone synthesis, and metabolism under stress [33–37]. Sharif et al [26] reported that melatonin serves as an antioxidant, protecting cells and reducing ROS by repairing the mitochondria [26, 38]. With the growing research on melatonin in plants, researchers have revealed the function of melatonin from different aspects under stress, including at the phenotypic, physiological, and molecular levels.

Under salt stress, exogenous melatonin treatment was found to significantly increase rice germination index (GI), germination potential (GP), vital index (VI), and root vigor, and reduce relative electrolytic leakage [39]. Similar results have been obtained in various plants, including in watermelon, tomato, and rapeseed seedlings under different stresses [34, 35, 28]. Melatonin can promote the activity of α-amylase and β-amylase in cotton seeds and reduce the inhibitory effect of sodium chloride stress on seed germination [33]. The ABA level increased in tomato under drought stress through the high expression of *SlNCED* in the roots and leaves [40], but an exogenous melatonin treatment down-regulated the expression of ABA biosynthesis genes to avoid heat-induced leaf senescence in perennial ryegrass [41]. Melatonin treatment upregulated the expression of key genes involved in GA biosynthesis and downregulated key genes involved in ABA biosynthesis to promote nutrient utilization, synthesize new proteins, and enhance *Limonium bicolor* seed germination under salt stress [42]. Additionally, Ge et al reported that *GID1C* had positive effects on the GA signaling pathway during seed germination [43].

Understanding the regulatory effects of melatonin on plants under stress is important. However, little is known regarding the regulatory effect of melatonin on the germination of cotton seeds under salt stress, and the molecular regulatory mechanism of melatonin on ABA and GA signal transduction during cotton seed germination remains unclear. Therefore, in the present study, we analyzed the role of exogenous melatonin in the salinity tolerance of cotton seeds by evaluating the germination traits, physiological parameters, and associated molecular mechanisms. Our aims were to explore the regulatory effects of melatonin on salt resistance in cotton and provide a foundation for the cultivation of new resistant varieties.

Results

Melatonin pre-treatment promotes GR, GP, and GI in cotton under salt stress

High concentrations of salt inhibit seed germination or promote dormancy [44]. Our results demonstrated that melatonin has an unexpected role in cotton seed germination. As shown in Table 2, compared with CK (control), GR was decreased significantly by 18.00% under salt stress, while pre-soaking with 20 μM melatonin alleviated the inhibitory effects of salt stress. According to our data, the GR of cotton seed pretreated with melatonin increased by 11.23% compared with that of salt stress after germination. The seed GP under salt stress was 69.67%, which was 17.19% lower than CK. The GP of the seeds increased significantly after adding melatonin. In the germination experiment, the number of germinated seedlings was recorded daily to calculate GI. Similarly, significant differences between salt stress and pre-melatonin were observed. Melatonin may promote seed germination when cotton seeds suffer from salt stress.

Treatment	Germination rate (%)	Germination potential (%)	Germination index
CK	89.63 ± 4.02[a]	84.13 ± 3.83[a]	1048.50 ± 41.26[a]
S	73.30 ± 1.86[c]	69.67 ± 2.30[c]	883.75 ± 20.03[c]
SM	81.53 ± 2.07[b]	75.53 ± 2.17[b]	959.23 ± 20.69[b]

Significant difference between different treatments at $P < 0.05$ based on Tukey's multiple range test. Different lowercase letters indicate significant differences at the 0.05 probability level ($P < 0.05$) according to and Tukey's multiple range tests. Values are the mean ± SEs, calculated for six replications. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated with 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

Table 2
Effect of different treatments on the GR, GP, and GI of cotton seed.

Melatonin pre-treatment promotes the hypocotyl length of the cotton seeds under salt Stress

The radicles of seeds that germinated at the same time were scanned at 2 d, 4 d, and 6 d. As shown in Fig. 1A, melatonin pre-treatment significantly increased hypocotyl elongation. After treatment for 2 d, the hypocotyl length in the CK group was significantly higher than that in the S and SM group, and the hypocotyl diameter was slightly longer than that of the S group. On the fourth day of seed germination following melatonin treatment, the cotyledons broke through the seed coat, while no cotyledons emerged in CK and S. On day 6 of seed development in the S group, the cotyledons had broken through the seed coat, but the hypocotyl was short and small, and the root tips were damaged. In contrast, pre-treatment with melatonin promoted radicle growth, which was not observed in the S group. To further evaluate this effect, the hypocotyl length at 7 d of seed germination was measured with a Vernier caliper (Fig. 1B). The hypocotyl length under salt stress was 40.81% shorter than that of the control, whereas the hypocotyl length of the melatonin pre-treatment group was 33.58% longer than that under salt stress. The hypocotyl length and phenotype thus confirm the positive effect of melatonin on cotton seed germination under salt stress.

Melatonin pre-treatment improves starch content, α-AMS, and β-GAL in cotton under salt stress

As the main carbohydrate in plant seeds, starch provides significant nutrients for seed germination. To investigate the effect of melatonin on the starch content of the cotton seeds under salt stress, various cotton seed germination parameters were determined at 7 d. As indicated in Fig. 2A, the starch contents of the seeds under the salt treatment were 11.53% higher than that of the control. During seed

germination under salt stress, starch hydrolysis is inhibited, which makes it difficult to provide large amounts of nutrients for the seeds, thus ultimately inhibiting seed germination. The starch content of the melatonin treatment group decreased by 13.55% compared with the S treatment. This result indicated that melatonin promoted starch hydrolysis, thus providing nutrients for the seeds and delaying the inhibitory effect of salt stress on the cotton seeds.

α-AMS is the most important hydrolytic enzyme in the early stage of seed germination and directly determines the germination rate of the seeds. β-GAL is an enzyme involved in cell wall degradation and provides an essential energy source for seed germination. Therefore, we further determined the α-AMS and β-GAL contents of the cotton seeds under the different treatments (Fig. 2B). The data showed that the α-AMS content of the cotton seeds was significantly decreased by 56.97% under salt stress compared with that of CK, while melatonin treatment significantly improved the content of α-AMS, although the quantity was less than under normal conditions. The β-GAL content was significantly decreased by 20.18% under salt stress compared with that of the control. Melatonin pretreatment greatly increased the content of β-GAL by 5.96% and 32.76% in comparison to CK and S, respectively (Fig. 2C). This demonstrates that melatonin plays a key role in nutrient promotion in cotton seed metabolism.

Melatonin is involved in cotton seed germination

To investigate the effect of salt stress on melatonin concentration in cotton seeds during germination, we analyzed the melatonin content in cotton seeds at different time points (0, 6, 12, 24 h) under different treatments. As indicated in Fig. 3, under normal conditions, the melatonin content peaked (42.56 ng g^{-1}) at 12 h and then tended to decline. Under salt stress, the melatonin content was significantly decreased by 17.81%, 39.96%, 31.34%, and 16.63% at 0, 6, 12, and 24 h, respectively. This may be due to the degradation of endogenous melatonin under salt stress. The melatonin content of the cotton seeds pretreated with melatonin was significantly increased compared with CK and S. The melatonin content of the seeds pretreated with 20 μM melatonin for 0 h (63.76 ng g^{-1}) was three times higher than that in the control group. As the germination time of the cotton seeds was extended, melatonin was consumed continuously to resist the inhibitory effect of the salt stress. Notably, the content of melatonin was obviously increased at the early stage of seed germination under CK and S, especially at 12 h. The melatonin content under CK and S reached 42.56 ng g^{-1} and 32.41 ng g^{-1}, respectively, which is about twice that at 0 h. This suggests that the metabolism of each treatment is very vigorous at the beginning of germination and then tends to eventually stabilize. Similarly, at 6 h after germination, the melatonin content of the seeds pre-treated with melatonin was significantly reduced, indicating that exogenous melatonin played an additional role in seed germination.

Melatonin pre-treatment increases phytohormones under salt stress in cotton

The phytohormones ABA and GA are necessary regulators of seed germination. To further study the effect of melatonin on plant hormones, we measured the content of ABA and GA at 0, 6, 12, and 24 h under different treatments. As shown in Fig. 4A, under control conditions, the ABA content gradually decreased with the seed germination process, indicating that ABA compounds are inhibited during seed germination and that seed dormancy is also broken. However, salt stress led to an increase in ABA content. The ABA content under salt stress increased by 6.93%, 65.08%, 49.81%, and 25.13% at 0, 6, 12, and 24 h, respectively, compared with CK, indicating that the synthesis pathway of ABA was activated, or its degradation pathway was inhibited under salt stress. It was also observed that ABA content peaked at 6 h after germination under salt stress, at which point it was 65.06% higher than that of the control. Exogenous melatonin decreased the ABA content in the cotton seeds, exhibiting a trend of first rising and then falling at the germination stage. ABA content also peaked at 6 h and decreased at 12 h after germination, indicating that cotton seed dormancy was broken, and seed germination was promoted by melatonin pretreatment.

ABA and GA are classical plant hormones that have antagonistic effects on seed germination. With the extension of the seed germination time, the GA content increased constantly under the different treatments, showing that GA level is a key factor affecting seed germination (Fig. 4B). Salt treatment significantly inhibited GA_3 synthesis during the seed germination phase. GA_3 content was significantly decreased by 32.38%, 33.40%, and 27.99% at 6, 12, and 24 h, respectively, compared to that of the control. As expected, melatonin treatment significantly increased the GA_3 content during cotton seed germination. The GA_3 content was 23.80%, 13.70%, 78.39%, and 4.77% higher, respectively, compared with that of the S treatments seeds. In addition, the content of GA_3 increased sharply at 12 h after germination, showing that seed germination was accelerated by melatonin. Meanwhile, only melatonin pretreatment had a 33.43% higher GA_3 content than CK at 0 h. Therefore, melatonin can promote GA_3 accumulation and reduce salt damage to cotton seed.

Melatonin pre-treatment regulates phytohormone signal transduction genes under salt stress

To further prove that melatonin promotes germination under salt stress by regulating the expression of hormone signal transduction genes, we used *GhHIS* as the housekeeping gene and analyzed the expression of key genes using qRT-PCR, including the ABA genes *GhABF2* (Gh-A03G2095), *GhDPBF2* (Gh-A09G0901) and GA genes *GhGID1C* (Gh-A13G1518) and *GhGID1B* (Gh-D11G1242). As indicated in Fig. 5A, *GhABF*2 was generally down-regulated in the control and up-regulated under salt stress and pre-melatonin during germination; however, *GhDPBF*2 was generally down-regulated in all treatments during germination. When salt stress was administered, the expression level of *GhABF*2 and *GhDPBF*2 was significantly increased at 6, 12, and 24 h of seed germination. These changes in *GhABF*2 and *GhDPBF*2 expression levels may be related to the increased content of ABA caused by salt stress. Melatonin-pretreated seeds had lower expression levels of *GhABF*2 at 6 h and higher expression levels of *GhABF*2 at

12 and 24 h than those of S at 1.70- and 1.56-fold, respectively. Melatonin-pretreated seeds had lower expression levels of *GhDPBF*2 at 6, 12, and 24 h than those of S at 1.30-, 1.06-, and 1.19-fold, respectively. This suggests that melatonin up-regulated the expression of *GhABF*2 and down-regulated the expression of *GhDPBF*2 in the ABA signaling pathway under salt stress, which may be related to the decreased ABA levels caused by melatonin (Fig. 5A-B).

We again analyzed the expression of key genes in the GA signaling pathway during seed germination. An upward trend in the expression levels of *GhGID1C* and *GhGID1B* in the GA signaling pathway was observed during seed germination. When salt stress was administered, the expression levels of *GhGID1C* and *GhGID1B* decreased during seed germination, except at 24 h. These changes in *GhGID1C* and *GhGID1B* expression levels may be related to the down-regulation of GA biosynthesis. Melatonin-pretreated seeds had higher expression levels of *GhGID1C* and *GhGID1B* at 12 h (38.54% and 25.73%) and at 24 h (140.08% and 84.22%), respectively, than S. This suggests that melatonin increased the content of GA and mediated the up-regulated expression of *GhGID1B* and *GhGID1C* genes in the GA signaling pathway under salt stress during seed germination (Fig. 5C-D).

In addition, the *GhABF2*, *GhDPBF2*, *GhGID1C*, and *GhGID1B* genes were verified by RNA-Seq (Fig. 6). The changes in gene expression at different germination time points were consistent with those of the qRT-PCR data. The results indicated that all changes were related to melatonin, which regulated the expression of related genes at the molecular level.

Discussion

In addition to being a critical stage in the initiation of the plant life cycle, seed germination is also the stage most sensitive to environmental factors during the plant growth process [45]. Salt stress has been found to reduce the GP, GI, and antioxidant enzyme activity in seedlings [46, 47, 48, 35]. Pre-treatment with melatonin not only promotes germination but also significantly improves the growth of young roots and the accumulation of nutrients [41]. Suitable melatonin concentrations can effectively alleviate the impact of salt stress on seed germination [49]. In this study, we found that melatonin enhanced cotton seed germination under typical salinity stress conditions. The data are consistent with previous reports on cucumber seedlings [49, 33].

Arnao et al. [50] determined the endogenous melatonin level under different stresses in lupin plants, and the results showed that the endogenous melatonin levels were the product of environmental stress. High levels of melatonin are also essential components for cucumber seed germination [49]. During the cotton seed germination process in the present study, the melatonin content in the seedlings pre-treated with melatonin showed a decreased trend. This may be due to the degradation of melatonin under salt stress, as melatonin plays a key role in the perception of external stimuli by the cells and the adaptation to salt stress [51, 52].

It is well known that starch provides energy for seed germination and is the main carbohydrate storage organ for seed growth. Starch biosynthesis is closely related to photosynthesis, and α-AMS and β-GAL are

important hydrolytic enzymes in seed germination that can induce the body to hydrolyze starch and provide the energy required for seed germination, playing an important role in plant growth [53, 54]. Salt stress stimulates electron transport in photosynthesis, which significantly increases the number of chloroplasts and starch accumulation in the chloroplasts [55–57]. The activity of α-AMS and β-GAL decreased under salt stress, thus reducing the hydrolysis of starch, as verified in our cotton experiment. Previous results have revealed that melatonin is closely structurally associated with indole-3-acetic acid [58], as are their metabolic pathways, promoting the activity of various enzymes and the expansion of cells, as also determined in *Lupinus micranthus* [59–60]. Proteomics data suggest that melatonin upregulates enzyme (α-amylase) activity in related metabolic pathways and promotes starch metabolism to produce ATP [33]. Our results showed that melatonin pre-treatment enhanced the activity of α-AMS and β-GAL and promoted starch hydrolysis, thus providing nutrients for seed germination and promoting cotton seed germination under salt stress. However, no further complex regulatory mechanism associated with synthesis and metabolism was observed.

ABA and GA are classic plant hormones that regulate plant growth and abiotic stress and have antagonistic effects on seed germination and dormancy [61]. The content of GA and ABA in cucumber seeds changed significantly within 24 h after germination [52], which was consistent with our results (Fig. 5). The roles of ABA and GA in plant hormone signal transduction were analyzed using RNA-Seq (Fig. 6). The receptor signal in the ABA-activated membrane is transferred to the receptor protein PYR/PYL (Fig. 7A). ABA first binds to PYR1 and then binds to PP2C to inhibit PP2C. PP2C maintains the activity of the SnRK2 receptor protein through dephosphorylation, and the active SnRK2 further activates the downstream transcription factor ABF, thereby initiating gene expression and leading to seed dormancy and stomatal closure [62–63]. In addition, under the same conditions, GAs sense external signals and enter the nuclear membrane through the cell membrane. Previous studies have proved that the GID1 protein is a soluble GA receptor [64]. After the activation of the GID1 protein, the N-terminal domain of the DELLA protein interacts with the GID1 protein [65–66]. GID1 mediates DELLA protein degradation, and the DELLA protein acts as a repressor and inhibits plant development. Finally, GA promotes seed germination and plant growth by degrading the DELLA protein [67] (Fig. 7B).

Through comprehensive analysis, we found that there were significant differences in the expression of genes related to the ABA and GA signaling pathways (Fig. 8). The changes in gene expression, including *GhABF*2, *GhDPBF*2, *GhGID1C*, and *GhGID1B*, in the cotton seeds were verified by qRT-PCR, the results of which were consistent with the RNA-Seq data at the different germination times. Salt stress significantly upregulated the expression of the *GhDPBF*2 gene. Melatonin thus inhibits the expression of the *GhDPBF2* gene and induces the expression of the *GhGID1B* gene in response to salt stress. Therefore, melatonin may interact with other plant hormones to regulate gene expression (Fig. 8). Melatonin plays a key role via two aspects (Fig. 9). Firstly, it induces a decrease in ABA content during seed germination, which downregulates the expression of GhDPBF2 and upregulates the expression of the GhGID1B and GhGID1C genes in the GID1 receptor protein of the GA signaling pathway. It is also involved in the regulation of genes and proteins for improving seed germination and salt tolerance [68].

Conclusions

We comprehensively assessed the influence of melatonin on cotton seed germination under salinity stress in an economically valuable crop plant. Exogenous melatonin promoted seed germination by mediating the expression of plant hormone signal transduction genes, thus inducing metabolic changes (e.g., starch, α-AMS, and β-GAL). As indicated by the phenotypic data, melatonin can reduce salt stress damage to the cotton seeds and promote seed germination. Our study is the first to explore the effects of melatonin on cotton germination and has implications for the breeding and engineering of resilient cotton seeds. Future research should explore the mechanism by which melatonin reduces salt stress and promotes plant growth. Our findings provide a foundation for the breeding of new and high-yield cotton varieties.

Methods

Reagents

All chemicals used in the experiments were of analytical grade. Melatonin (*N*-acetyl-5-methoxytryptamine) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd, (Beijing, China).

Plant materials

The following experiments were conducted in the laboratory at Hebei Agricultural University, Baoding (38.85°N, 115.30°E), China. GXM9 (*Gossypium hirsutum* L.), a commercial cotton cultivar used locally, was used in this study. This cultivar was developed by the Guoxin Rural Technical Service Association of Hejian Co., Ltd, (Hebei, China). Briefly, similar seeds were screened according to their size and mass.

Germination tests

A NaCl concentration of 150 mM and melatonin concentration of 20 μM were selected for seed germination [69]. Five hundred cotton seeds were sterilized with 75% ethanol for 17 min and rinsed in distilled water four times. They were then divided into three treatment groups: CK (germination of seeds pretreated with just water); S (germination of seeds pretreated in 150 mM NaCl under salt stress); and SM (germination of seeds pretreated in 20 μM melatonin under 150 mM NaCl solution). Each group was soaked in the different solutions for 24 h and then placed onto an ultra-clean table to dry. One hundred cotton seeds were spread across a total of five Petri dishes (15 cm × 15 cm) with filter paper (Whatman International Ltd.) and cultured at 25 °C and 50% humidity for 7 d in an incubator in the dark. Seed germination was characterized by the emergence of the germinating root tip through the seed coat and the appearance of a visible radicle. Seed germination potential and germination rate were recorded on the

third and seventh day, respectively. All phenotypic measurements included six independent biological repeats.

Germination rate (%) = (number of germinated seeds by day 7/ total number of test seeds) x100

Germination potential (%) = (number of germinated seeds by day 3/ total number of test seeds) x100

Germination index = $\sum(G_i / T_i)$, where G_i is the germination percentage of the i^{th} day, and T_i is the day of the germination test.

Morphological observation and determination of hypocotyl length

Cotton seeds from the three treatments (CK, S, SM) were placed in incubators (25 °C and 50% humidity) for 7 d. Cotton seed morphology was observed at 2, 4, and 6 d. The hypocotyl length of 20 cotton seeds was measured on day 7 with a Vernier caliper. Six independent biological repeats were measured.

Determination of α-amylase, β-galactosidase, and starch content

Cotton seeds from the three treatments (CK, S, SM) were placed in incubators for 7 d. The activity of α-amylase (α-AMS) and β-galactosidase (β-GAL) in the cotton seeds was measured on the 7th day of cotton seed germination according to the manufacturer's protocol of the kit from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Six independent biological repeats were measured. All samples were rapidly frozen in liquid nitrogen and stored at − 80 °C before analysis.

Fresh germinating seed samples of 0.3 g were weighed and combined with 2.7 mL of phosphate buffer. The mixture was ground thoroughly at a low temperature (0–4 °C), swirled, and mixed for 3 min, following which it was centrifuged at 3500 *g* for 10 min. The supernatant was collected and stored at − 80 °C until analysis by the Nanjing Jiancheng Bioengineering Institute. The starch content was measured on the 7th day of cotton seed germination using a starch kit from the Nanjing Jiancheng Bioengineering Institute.

Extraction and determination of melatonin

Melatonin was extracted from 20 cotton seeds at four time points (0, 6, 12, 24 h) from the beginning of the seed germination trial according to Pothinuch et al. [70], with slight modifications. Three independent biological repeats were assessed. All samples were rapidly frozen in liquid nitrogen and stored at − 80 °C before analysis.

Cotton seeds (0.3 g) were ground in liquid nitrogen, combined with 2 mL sample extract, ground into a homogenate in an ice bath, and extracted for 4 h. The solution was then centrifuged at 1000 *g* at 4 °C for 15 min, following which the supernatant was transferred to a 10 mL Eppendorf tube for storage at 4 °C. This process was repeated three times. The supernatants were applied to C-18 cartridges (Sep-Pak Vac 3 cc, C18 Cartridges, Ireland), and the eluate was collected. The collected samples were dried under a nitrogen stream to remove the methanol, and a certain volume of diluent was added for sample determination. The melatonin content was determined by enzyme linked immunosorbent assay (ELISA) using the Plant MT ELISA KIT produced by Shanghai MLBIO Biotechnology Co. Ltd (Shanghai, China). Data were analyzed using a microplate reader (Bio Tek Instruments, Inc, USA) following the manufacturer's instructions.

Extraction and assay of phytohormone ABA and gibberellin (GA_3)

ABA and GA in the seeds were determined at different time points (0, 6, 12, 24 h) during the germination phase using an ELISA kit provided by China Agricultural University and included three independent biological repeats. All samples were rapidly frozen in liquid nitrogen and stored at − 80 °C before analysis.

Cotton seeds weighing 0.3 g were combined with 2 mL of sample extract, ground into a homogenate in an ice bath, and transferred to a 10-mL test tube. The mortar was rinsed with 2 mL of extract, and the solution was transferred to a test tube and shaken well, following which it was placed into a refrigerator at 4 °C. The extract was extracted at 4 °C for 4 h and then centrifuged at 1000 *g* for 15 min. The supernatant was obtained, and 1 mL of the extract was added to the pellet, stirred evenly, and then extracted at 4 °C for 1 h. The mixture was then centrifuged for 15 min, mixed with the supernatant, and the volume recorded. The supernatant was passed through C-18 (Sep-Pak Vac 3 cc, C18 Cartridges, Ireland) solid-phase extraction columns and then transferred to a 5-mL plastic centrifuge tube, concentrated under vacuum or dried under nitrogen to remove the methanol, and then diluted to a constant volume in accordance with the protocol of the China Agricultural University Kit. The samples were incubated with antibodies and measured at 492 nm with a microplate reader (Bio Tek Instruments Inc., USA).

RNA isolation and quantitative real-Time PCR (qRT-PCR) analysis

The total RNA of the cotton seeds at different time points (0, 6, 12, 24 h) was extracted using an EASYspin Plus Kit (Alidlab company USA) according to the manufacturer's protocol. About 100 mg of each sample was extracted after germination by the liquid nitrogen grinding method. The RNA concentration and purity were assessed on a NanoDrop 2000 spectrophotometer, and only RNA samples with A260/A280 > 1.8 and A260/A230 > 2.0 were used for cDNA synthesis. Total RNA of 2 μg was reverse

transcribed using a PrimeScript 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). The primer sequences used for the qRT-PCR analysis were designed by Primer Premier 6.0 software (Primer Premier, Canada). The qRT-PCR reaction mixture was composed of 10 μL of AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China), 8.5 μL of deionized water, 1 μL of five-fold diluted template, and 0.5 μL of each amplification primer. The cycling conditions were set as follows: initial denaturation of 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing at 60 °C for 30 s. Relative expression levels were calculated according to the $2^{-\triangle\triangle CT}$ method [71]. *GhHIS* was used as an internal reference gene. The primer sequences for qRT-PCR of the signal transduction genes in cotton are shown in Table 1.

gene name	Primer sequences (5′-3′)
GhHIS	F: ATACCGTCCTGGAACTGTTGCTCT
	R: TTCAAAAAGACCCACAAGGTATGC
GhABF2	F: CCAATTTGCTGGGAAGGATAA
	R: GGTAACTGGTGCTGGTTTGATT
GhDPBF2	F: CAAACTTCGGGATGGGACA
	R: TCTAACAGGCGGTTGGTGC
GhGID1C	F: TCGCAAAGTCCCTGCTAATG
	R: AGCTTCCACCGTGAAAGAAAA
GhGID1B	F: GTTCGGTGGGCAGATGAGA
	R: TGAGACCTTCGAGGGTTCG

Table 1
Primers designed by qRT-PCR.

RNA-Seq quantitative analysis

Quantitative RNA-Seq analysis of the cotton seeds at different time points (0, 6, 12, 24 h) was performed by Beijing Nuohe Zhiyuan Tech Co. Ltd. (Beijing, China). Total RNA was extracted with TRIzol and the concentration of RNA was accurately determined using a Qubit2.0 fluorometer. The integrity of the RNA was detected by an Agilent 2100 bioanalyzer.

The library was constructed using the NEBNNext RNA Library Prep Kit from Illumina, and the starting RNA of the library was total RNA. After the library was constructed, a preliminary quantification was performed using a Qubit 2.0 fluorometer. The insert size of the library was then detected by an Agilent 2100 bioanalyzer, and the effective concentration of the library was quantified by qRT-PCR.

Differential expression analysis of the two comparisons was performed using DESeq2 software (1.16.1). Cluster profiler software was used to perform Gene Ontology (GO) enrichment analysis of the differentially expressed genes and to statistically analyze the differentially expressed genes in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Statistical analysis

The experiment was conducted according to a completely randomized design with six replications per treatment group. The data were expr essed as the mean ± standard error, and statistical analysis was conducted with SPSS software 22.0 (IBM Corp, Armonk, NY, USA). Tukey's post-hoc tests were used to determine which means differed significantly. A *P*-value of < 0.05 indicated a significant difference. Graphs were drawn using GraphPad Prism 5.0 (GraphPad Software, Inc. USA).

Abbreviations

ABA: abscisic acid; GA: gibberellins; α-AMS: α-amylase; β-GAL: β-galactosidase; bZIP: basic region-leucine zipper; qRT-PCR: quantitative real-time PCR; ROS: reactive oxygen species; GI: germination index; GP: germination potential; VI: vital index; ATP: adenosine triphosphate.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Availability of data and materials

All data and materials are presented in the main manuscript.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 31871569, 31900623), the China Agricultural Research System (CARS-18-21), the Modern Technology System of Agricultural Industry in Hebei (HBCT2018040201, CL), and the Fund of Research Group Construction for Crop Science in Hebei Agricultural University (TD2016C318). LL conceived and designed the experiments, KZ contributed reagents, materials, and analysis tools.

Authors' contributions

BZ, LL, LC conceived and designed the experiments. BL, LC, DW performed the experiments. JL and DJ analyzed the data. KZ, ZY and SH contributed reagents, materials, and analysis tools. LC, BL and LL wrote the paper. All authors read and approved the final the manuscript.

Acknowledgements

The authors thank the anonymous reviewers for their valuable comments and suggestions. We also thank our laboratory members for their help and efforts. Li Chen, Bin Lu, and Liantao Liu contributed equally to this research.

References

1. Nguyen H C, Lin K H, Ho S L, Chiang C M, Yang C M. Enhancing the abiotic stress tolerance of plants: From chemical treatment to biotechnological approaches. Physiologia plantarum. 2018, 164(4): 452-466.
2. You J, Chan Z L. ROS regulation during abiotic stress responses in crop plants. Front Plant Sci. 2015, 6: 1092.
3. Liang W, Ma X, Wan P, Liu P. Plant salt-tolerance mechanism: A review. Biochem Biophys Res Commun. 2018, 495(1): 286-291.
4. Wang N. B. L, Kim B K, Yoon I S, Kim K H, Kwon T R. Salt tolerance in rice: Focus on mechanisms and approaches. Rice Science. 2017, 24(3): 123-144.
5. Wang J, Qiu N, Wang P, Zhang W R, Yang X Y, Chen M, et al. Na^+ compartmentation strategy of Chinese cabbage in response to salt stress. Plant Physiol Biochem. 2019, 140:151-157.
6. Sankari M, Hridya H, Sneha P, George Priya Doss C, Godwin Christopher J, Mathew J. Implication of salt stress induces changes in pigment production, antioxidant enzyme activity, and qRT-PCR expression of genes involved in the biosynthetic pathway of *Bixa orellana* Funct Integr Genomics. 2019, 19(4): 565-574.
7. Zhang N, Zhao B, Zhang H J, Weeda S, Yang C, Yang Z C, et al. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus*). J Pineal Res. 2013, 54(1): 15-23.

8. Zhang Y J, Li D H, Zhou R, Wang X, Dossa K, Wang L H, et al. Transcriptome and metabolome analyses of two contrasting sesame genotypes reveal the crucial biological pathways involved in rapid adaptive response to salt stress. BMC Plant Biol. 2019, 19(1): 66.
9. Zhu J K. Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol. 2003, 6(5): 441-445.
10. Hahm M S, Son J S, Hwang Y J, Kwon D K, Ghim S Y. Alleviation of Salt Stress in Pepper (*Capsicum annum*) Plants by Plant Growth-Promoting Rhizobacteria. J Microbiol Biotechnol. 2017, 27(10):1790-1797.
11. Liang W J, Cui W N, Ma X L, Wang G, Huang Z J, et al. Function of wheat Ta-UnP gene in enhancing salt tolerance in transgenic Arabidopsis and rice. Biochem Biophys Res Commun. 2014, 450(1): 794-801.
12. Farouk S, Al-Amri S M. Exogenous melatonin-mediated modulation of arsenic tolerance with improved accretion of secondary metabolite production, activating antioxidant capacity and improved chloroplast ultrastructure in rosemary herb. Ecotoxicol Environ Saf. 2019, 180: 333-347.
13. Bose J, Rodrigo-Moreno A, Shabala S. ROS homeostasis in halophytes in the context of salinity stress tolerance. J Exp Bot. 2014, 65(5): 1241-57.
14. Atia A, Debez A, Barhoumi Z, Smaoui A, Abdelly C. ABA, GA_3, and nitrate may control seed germination of Crithmum maritimum (Apiaceae) under saline conditions. C. R. Biologies. 2009, 332: 704-710.
15. Deinlein U, Stephan A B, Horie T, Luo W, Xu G H, Schroeder J I. Plant salt-tolerance mechanisms. Trends in Plant Science. 2014, 19, 6.
16. Zhao B Y, Hu Y F, Li J J, Yao X, Liu K D. BnaABF2, a bZIP transcription factor from rapeseed (*Brassica napus*), enhances drought and salt tolerance in transgenic *Arabidopsis*. Bot Stud. 2016, 57(1): 1-12.
17. Shah A N, Iqbal J, Tanveer M, Yang G Z, Hassan W, Fahad S, et al. Nitrogen fertilization and conservation tillage: a review on growth, yield, and greenhouse gas emissions in cotton. Environ Sci Pollut Res Int. 2017, 24(3): 2261-2272.
18. Nix A, Paull C, Colgrave M. Flavonoid profile of the cotton plant, *Gossypium hirsutum*: A review. Plants (Basel). 2017, 6(4): 43.
19. Footitt S, Douterelo-Soler I, Clay H, Finch-Savage W E. Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. Proc Natl Acad Sci U S A. 2011, 108(50): 20236-20241.
20. Reiter R J, Mayo J C, Tan D X, Sainz R M, Moises A J, Qin L L. Melatonin as an antioxidant: Under promises but over delivers. J Pineal Res. 2016, 61(3): 253-78.
21. Arnao M B, Hernández-Ruiz J. Melatonin: A new plant hormone and/or a plant master regulator? Trends Plant Sci. 2019, 24(1): 38-48.
22. Claustrat B, Leston J. Melatonin: Physiological effects in humans. Neuro chirurgie. 2015, 61(2): 77-84.

23. Tan D X, Chen L D, Poeggeler B, Manchester L C, Reiter R J. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocrine Journal. 1993, 1: 57-60.
24. Jung-Hynes B, Reiter R J, Ahmad N. Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. J Pineal Res. 2010, 48(1): 9-19.
25. Su S C, Hsieh M J, Yang W E, Chung W H, Reiter R J, Yang S F. Cancer metastasis: Mechanisms of inhibition by melatonin. J Pineal Res. 2017, 62(1).
26. Sharif R, Xie C, Zhang H Q, Arnao M B, Ali M, Ali Q. Melatonin and its effects on plant systems. Molecules. 2018, 23(9).
27. Dubbels R, Reiter R J, Klenke E, Goebel A, Schnakenberg E, Ehlers C, et al. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. J Pineal Res. 1995, 18(1):28-31.
28. Nawaz M A, Jiao Y Y, Chen C, Shireen F, Zheng Z H, Imtiaz M, et al. Melatonin pretreatment improves vanadium stress tolerance of watermelon seedlings by reducing vanadium concentration in the leaves and regulating melatonin biosynthesis and antioxidant-related gene expression. J Plant Physiol. 2018, 220: 115-127.
29. Gao W Y, Zhang Y J, Feng Z, Bai Q Q, He J J, Wang Y J. Effects of melatonin on antioxidant capacity in naked oat seedlings under drought stress. Molecules. 2018, 23(7): 1580.
30. Zuo Z Y, Sun L Y, Wang T Y, Miao P, Zhu X C, Liu S Q, et al. Melatonin improves the photosynthetic carbon assimilation and antioxidant capacity in wheat exposed to nano-ZnO stress. Molecules. 2017, 22(10): 1727.
31. Gbahou F, Cecon E, Viault G, Gerbier R, Jean-Alphonse F, Karamitri A, et al. Design and validation of the first cell-impermeant melatonin receptor agonist. Br J Pharmacol. 2017, 174(14): 2409-2421.
32. Vishnoi S, Raisuddin S, Parvez S. Glutamate excitotoxicity and oxidative stress in epilepsy: Modulatory role of melatonin. J Environ Pathol Toxicol Oncol. 2016, 35(4): 365-374.
33. Zhang N, Zhang H J, Sun Q Q, Cao Y Y, Li X S, Zhao B, et al. Proteomic analysis reveals a role of melatonin in promoting cucumber seed germination under high salinity by regulating energy production. Sci Rep. 2017, 7(1): 503.
34. Liu J L, Wang W X, Wang L Y, Sun Y. Exogenous melatonin improves seedling health index and drought tolerance in tomato. Plant Growth Regulation. 2015, 77(3): 317-326.
35. Zeng L, Cai J S, Li J J, Lu G Y, Li C S, Fu G P, et al. Exogenous application of a low concentration of melatonin enhances salt stress in rapeseed (*Brassica napus*) seedlings. Journal of Integrative Agriculture. 2018, 17(2): 328-335.
36. Wang P, Sun X, Li C, Wei Z W, Liang D, Ma F G. Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. J Pineal Res. 2013, 54(3): 292-302.
37. Zhao H L, Zhang K, Zhou X T, Xi L J, Wang Y P, Xu H J, et al. Melatonin alleviates chilling stress in cucumber seedlings by up-regulation of CsZat12 and modulation of polyamine and abscisic acid metabolism. Sci Rep. 2017, 7(1): 4998.

38. Rudiger Hardeland. Melatonin in plants-diversity of level and multiplicity of functions. Frontiers in Plant Science. 2016, 7: 198.
39. Li X J, Yu B J, Cui Y Q, Yin Y F. Melatonin application confers enhanced salt tolerance by regulating Na^+ and Cl^- accumulation in rice. Plant Growth Regul. 2017, 83(3): 441-454.
40. Thompson A J, Andrews J, Mulholland B J, Mckee J M T, Hilton H W, Horridge J S, et al. Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. Plant Physiol. 2007, 143(4): 1905-1917.
41. Zhang J, Shi Y, Zhang X Z, Du H M, Xu B, Huang B R. Melatonin suppression of heat-induced leaf senescence involves changes in abscisic acid and cytokinin biosynthesis and signaling pathways in perennial ryegrass (*Lolium perenne*). Environmental and Experimental Botany. 2017, 138: 36-45.
42. Li J, Zhao C, Zhang M, Yuan F, Chen M. Exogenous melatonin improves seed germination in *Limonium bicolor* under salt stress. Plant Signal Behav. 2019, 14(11): 1-10.
43. Ge W J, Steber C M. Positive and negative regulation of seed germination by the Arabidopsis GA hormone receptors, *GID1a*, *b*, and *c*. Plant Direct, 2018, 2(9): e00083.
44. Li W, Phan Tran L S. Effects of ethylene on seed germination of halophyte plants under salt stress. Methods Mol Biol. 2017, 1573: 253-259.
45. Weitbrecht K, Muller K, Leunbner-Metzger G. First off the mark: early seed germination. Journal of Experiental Botany. 2011, 62(10): 3289-3309.
46. Gao W Y, Feng Z, Bai Q Q, He J J, Wang Y J. Melatonin-mediated regulation of growth and antioxidant capacity in salt-tolerant naked oat under salt stress. Int J Mol Sci. 2019, 7; 20(5): 1176.
47. Yu Y, Wang J, Shi H, Gu J, Deng X W, et al. Salt stress and ethylene antagonistically regulate nucleocytoplasmic partitioning of COP1 to control seed germination. Plant Physiol. 2016, 170(4): 2340-2350.
48. Castañares J L, Bouzo C A. Effect of exogenous melatonin on seed germination and seedling growth in melon (*Cucumis melo*) under salt stress. Horticultural Plant Journal. 2018, 5(2): 79-87.
49. Zhang H J, Zhang N, Yang R C, Wang L, Sun Q Q, Li D B, et al. Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA_4 interaction in cucumber (*Cucumis sativu*s L.). J Pineal Res. 2014, 57(3): 269-79.
50. Arnao M B, Hernández-Ruiz J. Growth conditions determine different melatonin levels in Lupinus albus L. J Pineal Res. 2013, 55(2): 149-55.
51. Wang L, Feng C, Zheng X, Guo Y, Zhou F, Shan D, et al. Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. J Pineal Res. 2017, 63(3): e12429.
52. Tan D X, Manchester L C, Liu X, Rosales-Corral S A, Acuna-Castroviejo D, Reiter R J. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. J Pineal Res. 2013, 54(2): 127-38.
53. Yue C, Cao H, Lin H, Hu J, Ye Y, Li J, et al. Expression patterns of alpha-amylase and beta-amylase genes provide insights into the molecular mechanisms underlying the responses of tea plants

(*Camellia sinensis*) to stress and postharvest processing treatments. Planta. 2019, 250(1): 281-298.

54. Sulpice R, Pyl E T, Ishihara H, Trenkamp S, Steinfath M, Witucka-Wall H, et al. Starch as a major integrator in the regulation of plant growth. Proc Natl Acad Sci U S A. 2009, 106(25): 10348-53.
55. Wang X, Chang L, Wang B, Wang D, Li P, Wang L, et al. Comparative proteomics of *Thellungiella halophila* leaves from plants subjected to salinity reveals the importance of chloroplastic starch and soluble sugars in halophyte salt tolerance. Mol Cell Proteomics. 2013, 12(8): 2174-2195.
56. Zanella M, Borghi G L, Pirone C, Thalmann M, Pazmino D, Costa A, et al. β-amylase 1 (BAM1) degrades transitory starch to sustain proline biosynthesis during drought stress. J Exp Bot. 2016, 67(6): 1819-1826.
57. Lv S, Zhang K, Gao Q, Lian L, Song Y, Zhang J. Overexpression of an H^+-PPase gene from *Thellungiella halophila* in cotton enhances salt tolerance and improves growth and photosynthetic performance. Plant Cell Physiol. 2008, 49(8): 1150-64.
58. Arnao M B, Hernandez-Ruiz J. Melatonin and its relationship to plant hormones. Annals of Botany. 2018, 121(2): 195-207.
59. Hernández-Ruiz J, Cano A, Arnao M B. Melatonin: A growth-stimulating compound present in lupin tissues. Planta. 2004, 220(1): 140-1444.
60. Arnao M B, Hernández-Ruiz J. Functions of melatonin in plants: A review. J Pineal Res. 2015, 59(2): 133-150.
61. Wang M, Qu H, Zhang H, Liu S, Li Y, Zhang C. Hormone and RNA-seq analyses reveal the mechanisms underlying differences in seed vigour at different maize ear positions. Plant Mol Biol. 2019, 99(5): 461-476.
62. Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park S Y, et al. *In vitro* reconstitution of an abscisic acid signalling pathway. Nature. 2009, 462(7273): 660-4.
63. Park S Y, Fung P, Nishimura N, Jensen D R, Fujii H, Zhao Y, et al. Abscisic acid inhibits PP2Cs via the PYR/PYL family of ABA-binding START proteins. Science. 2009, 324(5930): 1068-71.
64. Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, et al. GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature. 2005, 437(7059): 693-8.
65. Dill A, Jung H S, Sun T P. The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc Natl Acad Sci U S A. 2001, 98(24): 14162-7.
66. Hirano K, Asano K, Tsuji H, Kawamura M, Mori H, Kitano H, et al. Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. Plant Cell. 2010, 22(8): 2680-96.
67. Kanako Okada, Takeshi Ito, Jutarou Fukazawa, et al. Gibberellin induces an increase in cytosolic Ca2+ via a DELLA-Independent signaling pathway. Plant Physiol. 2017, 175(4): 1536-1542.
68. Wang Y, Reiter R J, Chan Z. Phytomelatonin: a universal abiotic stress regulator. J Exp Bot. 2018, 69(5): 963-974.

69. Chen L, Liu L T, Ma T T, Jiang D, Sun H C, Zhang Y J, et al. Effects of melatonin on the antioxidant enzyme activities and seed germination of cotton (*Gossypium hirsutum*) under salt-stress conditions. Cotton Science. 2019, 31(5): 438-447.
70. Pothinuch P, Tongchitpakdee S. Melatonin contents in mulberry (*Morus*) leaves: Effects of sample preparation, cultivar, leaf age and tea processing. Food Chem. 2011, 128(2): 415-9.
71. Livak K J, Schmittgen T D. Analysis of relative gene expression data using real-time quantitative PCR and $2^{-\triangle\triangle CT}$ method. Methods. 2011, 25(4): 402-8.

Figures

Figure 1

Effects of melatonin on seed morphology under salt stress in cotton. (A) Phenotypes of the different treatments at 2, 4, and 6 d. (B) Hypocotyl length of the cotton seedlings under different treatments at 7 d. Different lowercase letters indicate significant differences at the 0.05 probability level ($P < 0.05$) according to Tukey's multiple range tests. Vertical bars indicate the mean ± standard errors (SEs) calculated for six replications. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated with 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

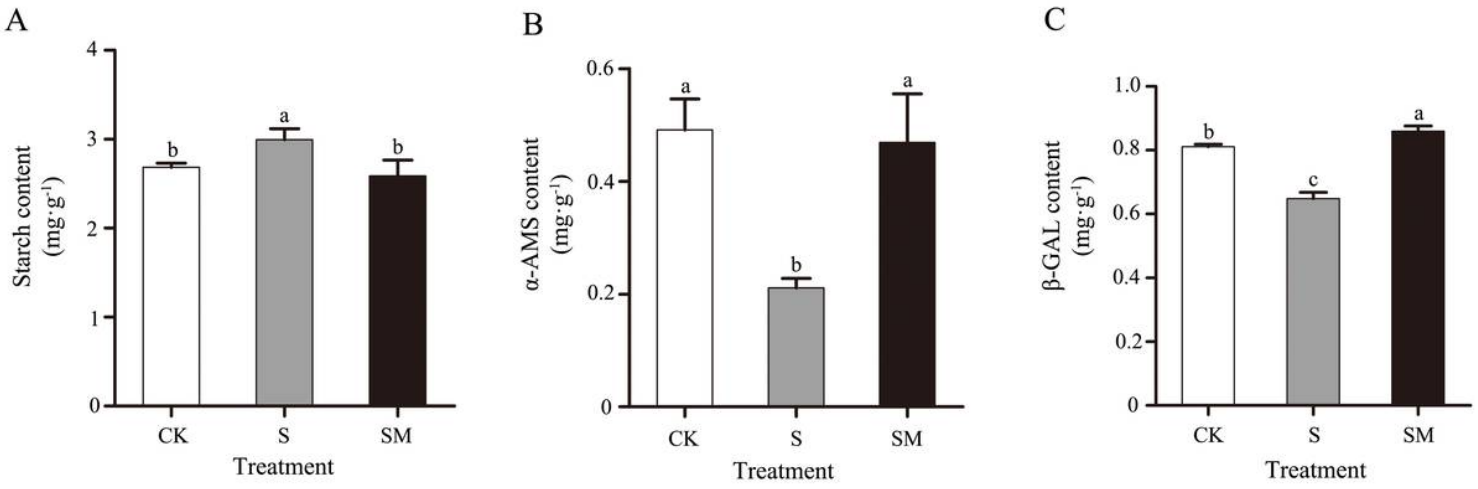

Figure 2

Effects of melatonin on starch content (A), α-amylase (α-AMS) content (B), and β-galactosidase (β-GAL) content (C) at 7 d under salt stress in cotton seeds. Different lowercase letters indicate significant differences at the 0.05 probability level ($P < 0.05$) according to Tukey's multiple range tests. Vertical bars indicate the mean ± SEs calculated for six replications. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated with 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

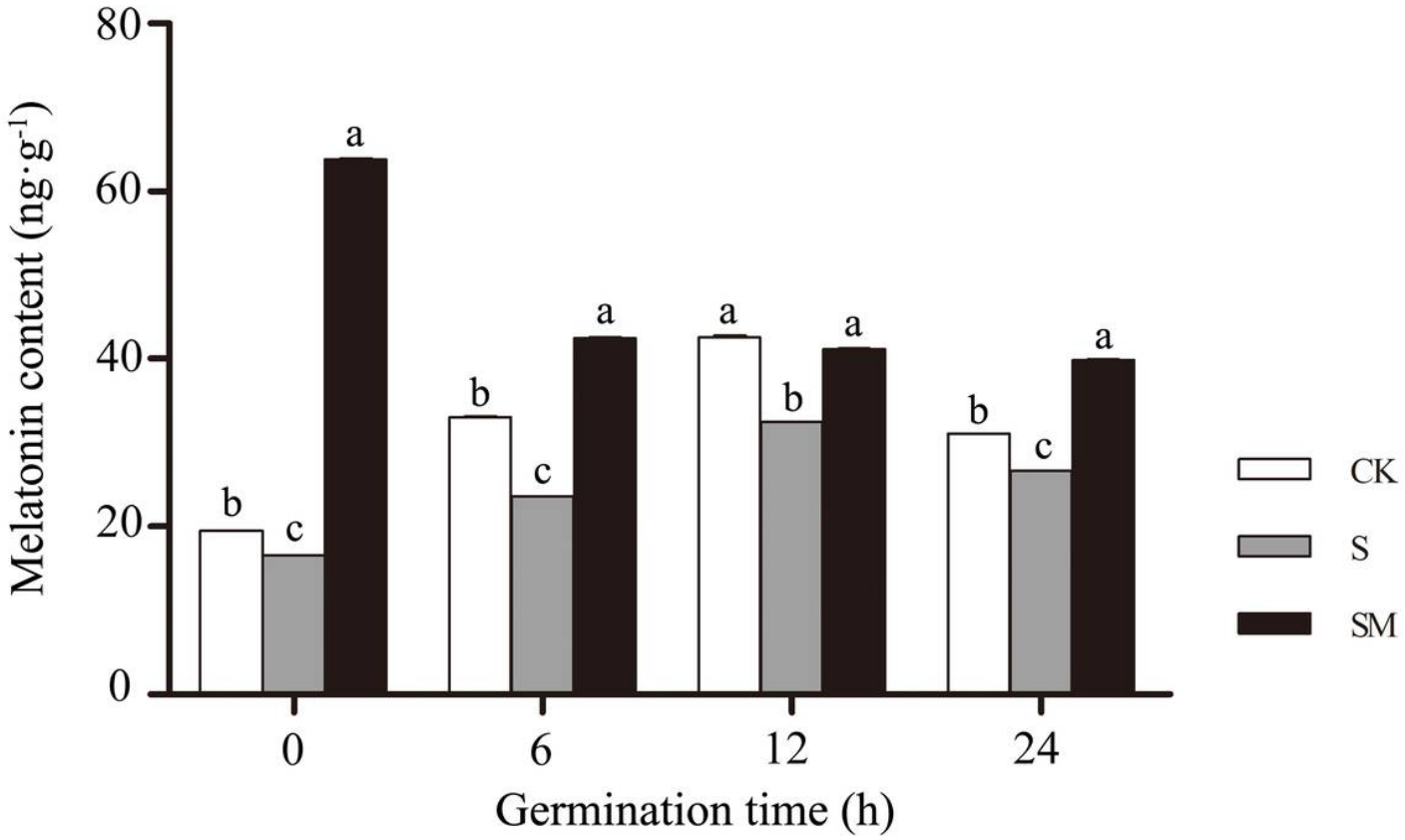

Figure 3

The melatonin content of the cotton seeds at different germination times under salt stress. Different lowercase letters indicate significant differences at the 0.05 probability level ($P < 0.05$) according to Tukey's multiple range tests. Vertical bars indicate the mean ± SEs calculated for three replications. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated with 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

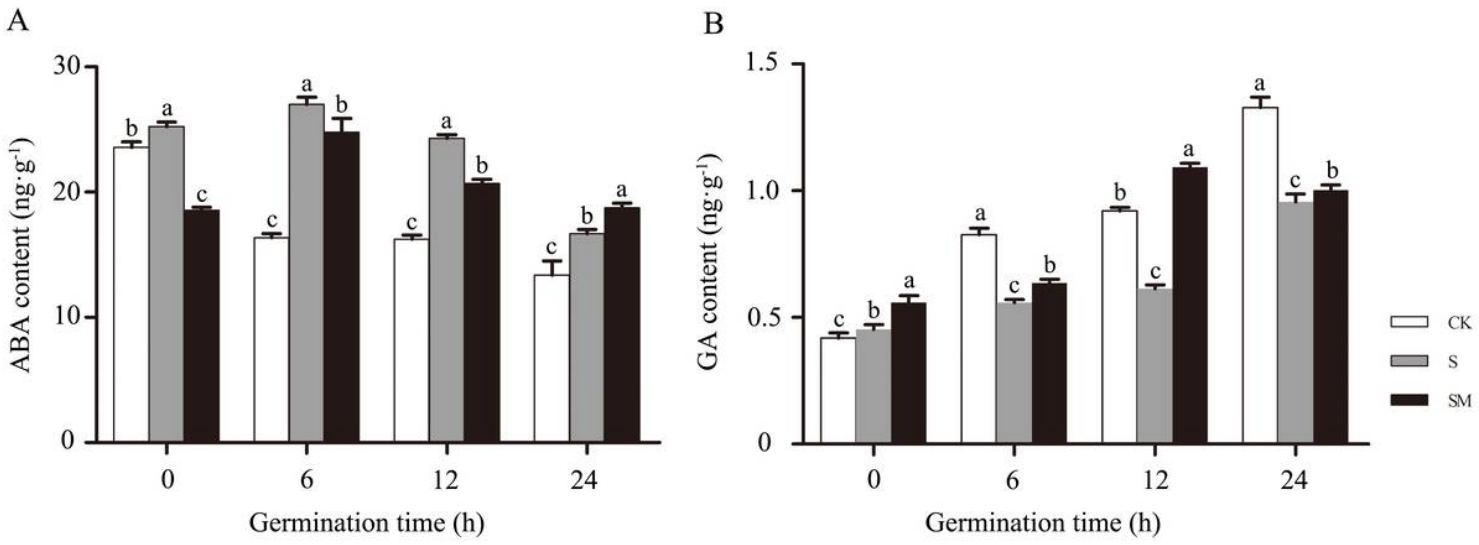

Figure 4

Effects of melatonin on phytohormones under salt stress in cotton seeds. (A) Abscisic acid content (ABA). (B) Gibberellin (GA) content. Different lowercase letters indicate significant differences at the 0.05 probability level ($P < 0.05$) according to Tukey's multiple range tests. Vertical bars indicate the mean ± SEs calculated for three replications. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated with 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

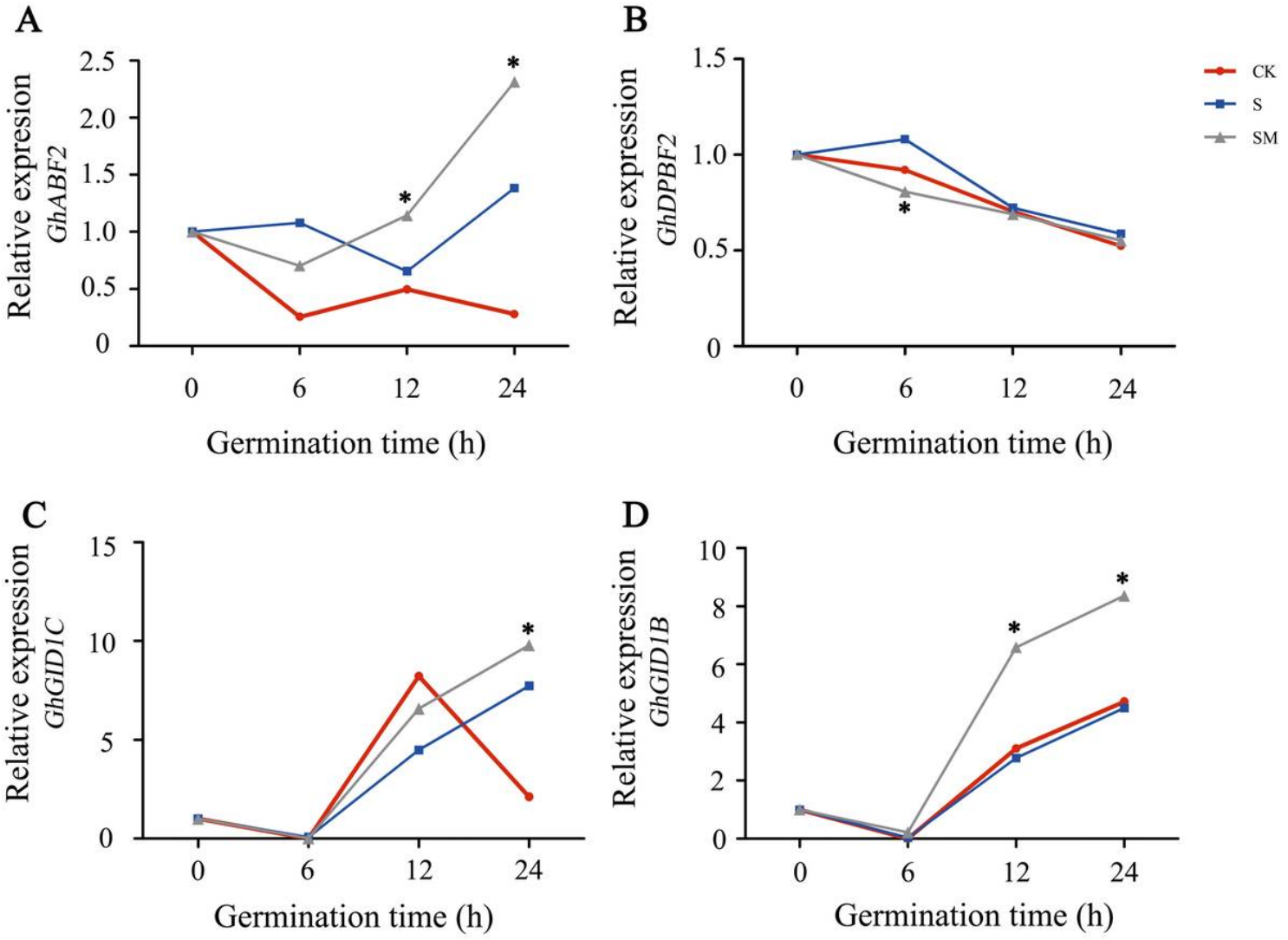

Figure 5

Effects of melatonin on the expression levels of key genes involved in the signaling of ABA and GA plant hormones under salt stress in cotton seed by qRT-PCR. Single (*P < 0.05) asterisks denote statistically significant differences between CK and S. (A) GhABF2, (B) GhDPBF2, (C) GhGID1C, and (D) GhGID1B. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated with 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

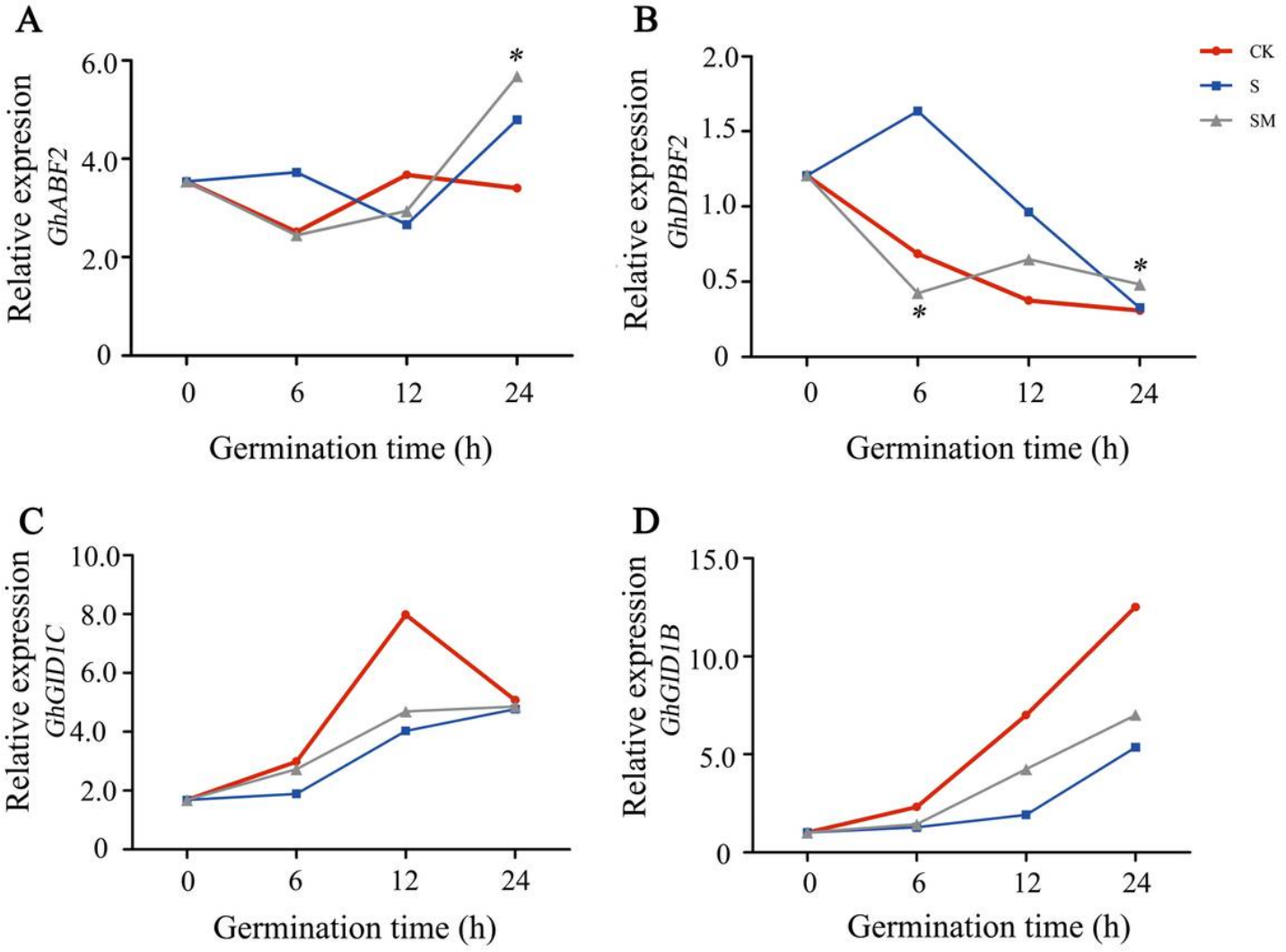

Figure 6

Effects of melatonin on the transcript levels of ABA and GA genes in cotton seeds under salt stress. Single (*P < 0.05) asterisks denote statistically significant differences between CK and S. (A) GhABF2, (B) GhDPBF2, (C) GhGID1C, and (D) GhGID1B. CK: germination of seeds pretreated with water alone; S: germination of seeds pretreated in 150 mM NaCl under salt stress; SM: germination of seeds pretreated with 20 μM melatonin under 150 mM NaCl solution.

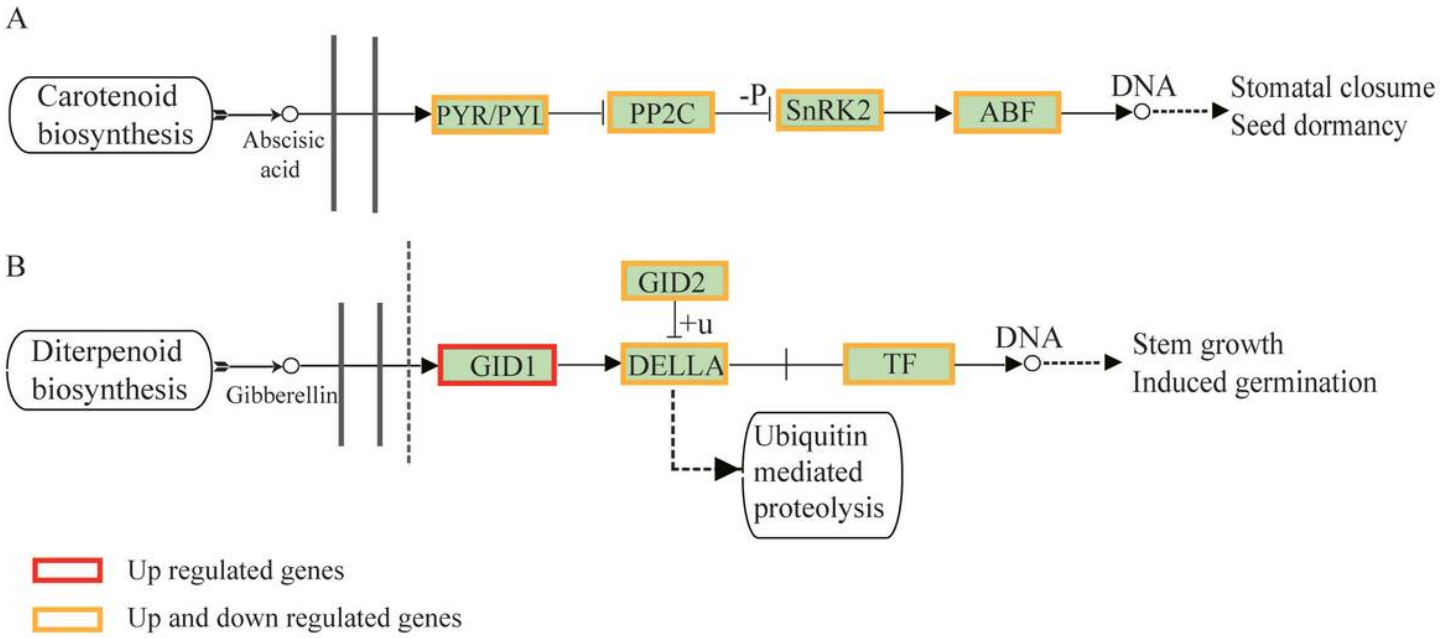

Figure 7

Plant hormone signaling pathway-related gene expression. (A) Represents the change in genes involved in the ABA signaling pathway. (B) Represents the change in genes involved in the GA signaling pathway. The red frame represents up-regulated genes in the receptor, and the yellow frame represents down-regulated genes in the receptor.

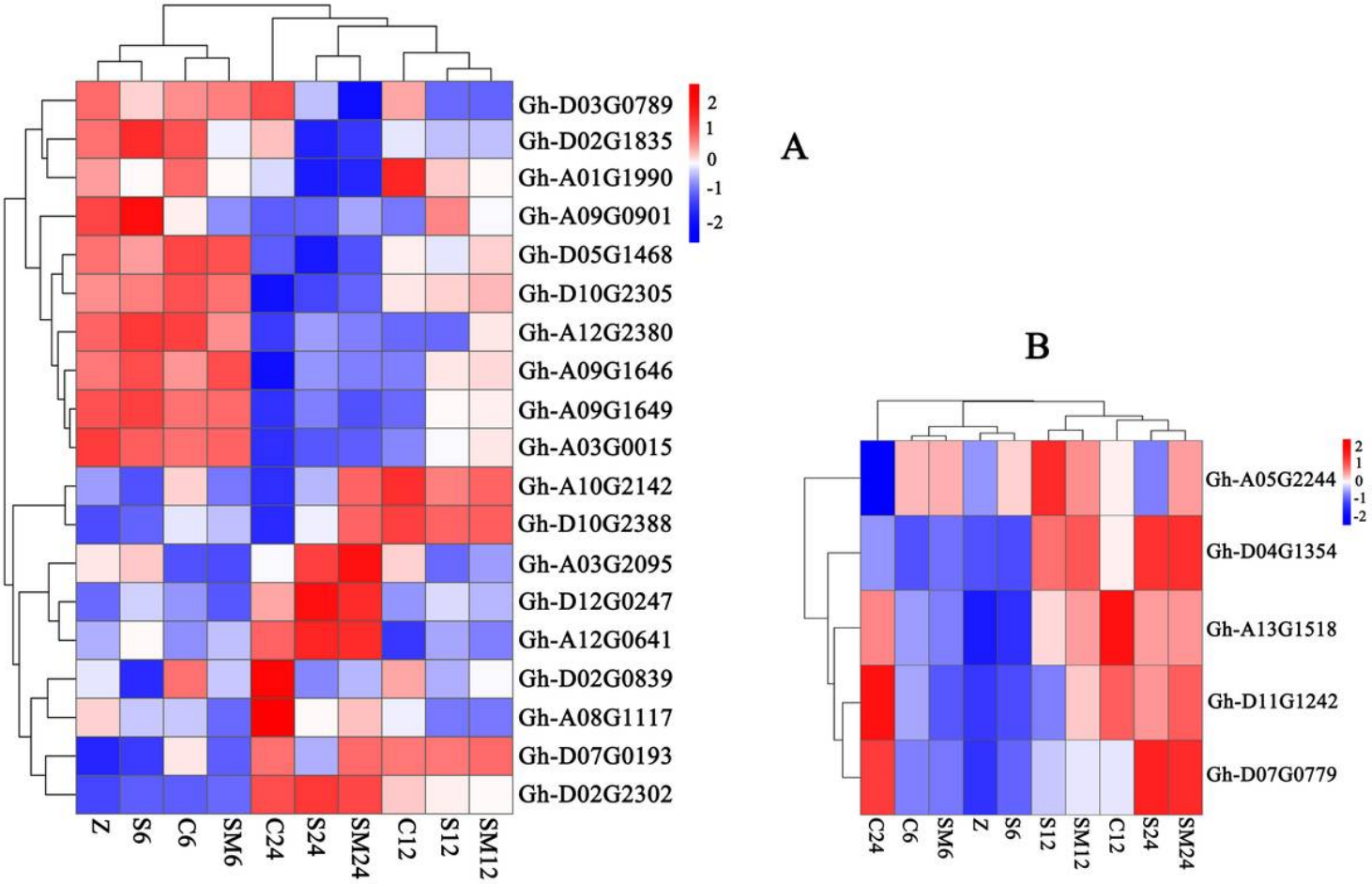

Figure 8

Heatmap showing the DEGs involved in ABA (A) and GA (B) signaling pathways in cotton seeds under salt stress. The colored bars represent the value [log2 (fold change)] of the change in gene expression of the different stress treatments. Red represents up-regulated DEGs, and blue represents down-regulated DEGs.

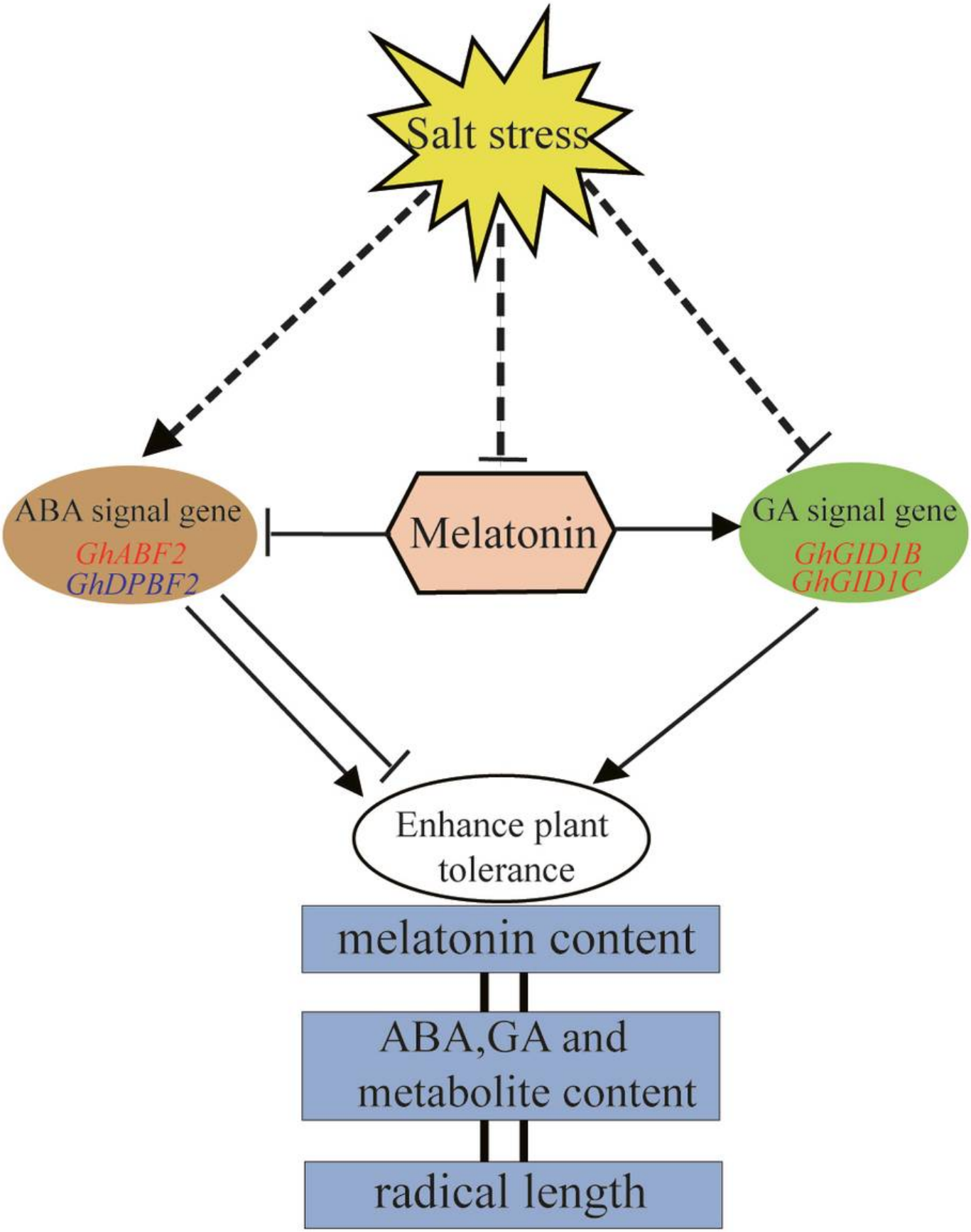
Salt stress
ABA signal gene
GhABF2
GhDPBF2
Melatonin
GA signal gene
GhGID1B
GhGID1C
Enhance plant tolerance
melatonin content
ABA,GA and metabolite content
radical length

Figure 9

Schematic diagram of salt tolerance in melatonin-pretreated cotton seed. Salt stress regulates the expression of plant signaling genes and reduces melatonin levels. Additionally, the signal gene induces plant tolerance, mainly in terms of radical length, metabolite content, and phytohormone content. The genes in red and blue are up-and down-regulated by melatonin, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Rawdata.xlsx