

# Transcriptome Analyses on Compound Material Regulating Saline Stress and Alkaline Stress on Cotton

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## Research article

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## Abstract

**Background:** Soil salinization and alkalinization are the main factors that affect the agricultural productivity in the world. Evaluating the persistence of the modifier applied in field soils is an important part of the regulation of saline stress and alkaline stress.

**Result:** To determine the molecular mechanism of cotton's responses to the regulation of saline stress and alkaline stress by the modifier, in this study, cotton was planted in the salinized soil ( $\text{NaCl } 8\text{ g kg}^{-1}$ ) and alkalinized soil ( $\text{Na}_2\text{CO}_3 \text{ 8g kg}^{-1}$ ) after application of the modifier, and ion content, physiological characteristics, and transcription and sequencing of new leaves during the flowering and boll-forming stage of cotton were analyzed. The results showed that compared with saline stress, alkaline stress increased the content of  $\text{Na}^+$ ,  $\text{K}^+$ , SOD, and MDA in leaves, and the application of modifier reduced the content of  $\text{Na}^+$  but increased the  $\text{K}^+/\text{Na}^+$  ratio, the activities of SOD, POD, CAT, and REC. Transcriptome analysis revealed that after the application of the modifier, the  $\text{Na}^+/\text{H}^+$  exchanger gene in cotton leaves was down-regulated, the  $\text{K}^+$  transporter,  $\text{K}^+$  channel and POD genes were up-regulated. Besides, the down-regulation of genes related to lignin synthesis in phenylalanine biosynthesis pathway was consistent with the study results of ion content and physiological characteristics in leaves. The quantitative analysis with PCR proved the reliability of the results of RNA sequencing.

**Conclusion:** These findings indicate that the modifier alleviated saline stress and alkaline stress on cotton by regulating candidate genes in key biological pathways, which improves our understanding of the molecular mechanism of the modifier regulating saline stress and alkaline stress.

## Background

Soil salinization and alkalinization are the main environmental factors that limit crop growth and yield [1]. Salinized soil and alkalinized soils are widely distributed in arid and semi-arid regions around the world [2]. The salinized soil is about 4.34 million hectares and alkalinized soil is about 3.97 million hectares globally [3]. The stress caused by salinized and alkalinized soils directly affects the ion balance of plant cells [4], which in turn affects physiological homeostasis [5, 6]. Many studies have shown that saline stress and alkali stress are two different types of stress for plants [7], and the effect of alkalinized stress on plants is more severe than that of saline stress [8]. Previous studies have shown that saline stress is mainly caused by neutral salt, while alkalinized stress is mainly caused by alkalinized salt [9]. Saline stress generally causes ionic damage and osmotic stress in plants [10], and alkalinized stress not only causes the above-mentioned damage to plants, but also increases the pH in plants [11]. Application of exogenous substances is one of the effective ways to regulate saline stress and alkalinized stress. Gong, et al. [12] found that melatonin regulated the enzyme activity and biosynthesis of polyamines and improved the tolerance of plants to alkalinized stress. Faghah, et al. [13] showed that spraying salicylic acid and methyl jasmonate on the leaves could improve the defense system and antioxidant capacity of strawberry under salt stress. Therefore, it is of great importance to study the response of plants to saline stress and alkalinized stress after the application of the modifier.

Cotton (*Gossypium* spp.) is one of the most important economic crops in the world, among which *Gossypium hirsutum* L. has been widely planted and its planting area accounts for more than 95% of the global cotton production. Although cotton is of salt tolerance, its growth and development still be affected by saline stress and alkaline stress [14]. According to reports, saline stress and alkaline stress reduced seed germination, seedling growth, root growth, flowering, and boll number of cottons, resulting in a great loss of yield [15-17]. Facing the increasing of global demand for cotton, the studies on regulating the damages caused by saline stress and alkaline stress to cotton has increased sharply [18]. Some saline-alkaline response genes in cotton has been selected. For example, ion channels and transporters can mitigate  $\text{Na}^+$  toxicity and  $\text{K}^+/\text{Na}^+$  homeostasis, and overexpression of NHX1 or SOS1 in cotton can improve salt tolerance [19]. *GhSOS3* and *GhCBL10* are involved in saline stress and alkaline stress, and the *GhSOS3/GhCBL10-SOS2* network also plays a central role in *Gossypium hirsutum* L. responses to saline stress and alkaline stress [20]. Besides, by increasing the detoxification capacity of reactive oxygen species, overexpression of the *OSCU/Zn-SOD* gene can improve the detoxification capacity of reactive oxygen species and improve the tolerance of plants under salt stress [21]. However, most of the previous studies were conducted through pot experiments or indoor culture experiments, and few was conducted through field experiments. Field experiments make the growth conditions of crops very close to their natural growth, which can truly reflect the growth law of crops.

In this study, RNA-seq was used to analyze the transcriptional changes of cotton leaves under saline stress and alkaline stress, and to elucidate the molecular mechanism of the modifier regulating the stress and alkaline stress. We analyzed many genes related to plant antioxidant defense,  $\text{K}^+/\text{Na}^+$  transport, and lignin biosynthesis, and these genes may be involved in the regulation of saline stress and alkaline stress by the modifier. The main purposes of this experiment are: (1) to determine the differences in the responses of cotton to saline stress and alkaline stress; (2) to determine the differences in the effects of the modifier regulating saline stress and alkaline stress on  $\text{K}^+$ ,  $\text{Na}^+$ , and physiological characteristics of cotton leaves; (3) to provide insights on the relevant genes in the process of the regulation of saline stress and alkaline stress by the modifier.

## Results

### $\text{K}^+$ , $\text{Na}^+$ and Physiological Characteristics of Cotton Leaves

To make clear the effect of the application of compound material on saline stress and alkaline stress, the contents of  $\text{K}^+$  and  $\text{Na}^+$ ,  $\text{K}^+/\text{Na}^+$ , superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, malondialdehyde (MDA) content, and relative electric conductivity (REC) of cotton leaves were determined (Fig. 1).

The contents of  $\text{K}^+$  and  $\text{Na}^+$  of leaves for  $\text{NaCO}_3$  treatments (CK-J and P-J treatments) were higher than those for  $\text{NaCl}$  treatments (CK-Y and P-Y treatments) (Fig. 1A). The contents of  $\text{K}^+$  and  $\text{Na}^+$  and  $\text{K}^+/\text{Na}^+$  for the CK-J treatment were increased by 30.54% ( $P<0.05$ ), 21.20% ( $P<0.05$ ), and 2.14%, respectively

compared with those for the CK-Y treatment (Fig. 1A). The K<sup>+</sup> content and K<sup>+</sup>/Na<sup>+</sup> for the P-Y and P-J treatments were increased and the Na<sup>+</sup> content was decreased after the application of compound material compared with the controls (CK-Y and CK-J treatments). For the P-Y treatment, the K<sup>+</sup> content was increased by 3.46%, the Na<sup>+</sup> content was decreased by 13.18%, and the K<sup>+</sup>/Na<sup>+</sup> ratio was increased by 18.40%, compared with those of the CK-Y treatment (Fig. 1A). For the P-J treatment, the K<sup>+</sup> content was increased by 6.37%, the Na<sup>+</sup> content was decreased by 18.26%, and the K<sup>+</sup>/Na<sup>+</sup> ratio was increased by 37.11% ( $P<0.05$ ) compared with those for the CK-J treatment (Fig. 1A). Meanwhile, the contents of K<sup>+</sup> and Na<sup>+</sup> and the K<sup>+</sup>/Na<sup>+</sup> ratio for the P-J treatment were increased by 35.14% ( $P<0.05$ ), 14.11%, and 18.27% ( $P<0.05$ ), respectively compared with those for the P-Y treatment (Fig. 1A).

The SOD activity for the CK-J treatment was increased by 46.29% ( $P<0.05$ ), while the POD and CAT activities and REC were decreased by 4.09% ( $P<0.05$ ), 27.60% ( $P<0.05$ ), and 1.67%, respectively compared with those for the CK-Y treatment (Fig. 1B). The antioxidant enzyme activity was increased, the MDA accumulation was decreased, and the cell membrane permeability was enhanced after the application of compound material (P-Y and P-J treatments) compared with the controls (CK-Y and CK-J treatments) (Fig. 1B). The SOD, POD and CAT activities and REC for the P-Y treatment were increased by 45.24%, 44.71%, 29.11%, and 24.02%, respectively ( $P<0.05$ ), while the MDA content was decreased by 0.12% compared with those for the CK-Y treatment. The SOD, POD and CAT activities and REC for the P-J treatment were increased by 18.85%, 30.76% ( $P<0.05$ ), 22.66%, and 2.94%, respectively, and the MDA content was decreased by 0.01% compared with those for the CK-J treatment (Fig. 1B). Meanwhile, the SOD activity for the P-J treatment was increased by 19.72%, and the POD and CAT activities and REC were decreased by 23.72% ( $P<0.05$ ), 31.22% ( $P<0.05$ ), and 18.38%, respectively, compared with those for the P-Y treatment (Fig. 1B).

## Overview of the transcriptomic responses

Transcriptome of each sample was sequenced on Illumina paired-end sequencing platform. The number of generated reads ranged from 39 to 48 million, with a mean of 44 million reads for each sample. The reads were mapped onto the cotton reference transcriptome. The mapping ratio varied from 53.60% to 67.40%, with a mean of 64.11%. The counts of mapped reads were summarized at gene level (Additional file 1: Table S1, Additional file 2: Fig. S1). The principal component analyses (PCA) was performed based on the gene counts (Additional file 3: Fig. S2). The results showed that samples from saline and alkaline treatments were clearly separated on the PC2 dimension, whereas the modified and unmodified samples were separated by at PC1 dimension. To verify the accuracy of RNA-seq data, six genes were randomly selected for quantitative RT-PCR (qRT-PCR) analysis. The expression abundances estimated by qRT-PCR and RNA-seq were highly correlated ( $R^2=0.80$ , Additional file 4: Fig. S3), indicating that the RNA-seq results were robust and suitable for the subsequent analysis.

## Identification of Differentially Expressed Genes

To determine the differences of transcriptional response to the treatments, differentially expressed genes (DEGs) were identified by pair-wise comparisons of the samples. Compared with the CK-Y treatment, 386 genes were up-regulated and 275 genes were down-regulated for CK-J treatment (Fig. 2A). Compared with the corresponding controls, a total of 1937 and 2365 DEGs were identified for the NaCO<sub>3</sub> treatments (CK-J and P-J treatments) and NaCl treatments (CK-Y and P-Y treatments), respectively (Fig. 2A). These results indicated that expression patterns of more genes were altered by NaCl treatment compared with the NaCO<sub>3</sub> treatments. Compared with the CK-Y treatment, NaCl treatments resulted in that 1424 genes were upregulated and 941 genes were downregulated after applied compound material. Compared with the CK-J treatment, 1448 genes were upregulated and 489 genes were downregulated for NaCO<sub>3</sub> treatments after the application of compound material (Fig. 2A). After the application of compound material, compared with the NaCl treatment (P-Y), the NaCO<sub>3</sub> treatment (P-J) resulted in that 1184 genes were upregulated and 373 genes were downregulated. Venn diagram was draw to identify the common and specific DEGs. The results showed that there were 7 common genes differentially expressed for the four treatments (Fig. 2B).

## Enrichment analysis

GO enrichment analysis was performed on the DEGs identified in response to the application of compound material regulating saline stress and alkaline stress. As for NaCl treatment, the top enriched GO terms for BP category were metal ion transport (GO:0030001), signal transduction (GO:0035556), and protein ubiquitination (GO:0016567), those for CC were extracellular region (GO:0005576), nucleosome (GO:0000786), and microtubule (GO:0005874), and those for MF were iron ion binding (GO:0005506), oxidoreductase activity (GO:0016705), and hydrolase activity (GO:0004553). For NaCO<sub>3</sub> treatments (Fig. 3B), the top enriched GO terms for BP were metal DNA replication (GO:0006260), microtubule-based movement (GO:0007018), and metal ion transport (GO:0030001), those for CC were nucleosome (GO:0000786), MCM complex (GO:0042555), and microtubule (GO:0005874), and those for MF were protein heterodimerization activity (GO:0046982), iron ion binding (GO:0005506), and microtubule binding (GO:0008017). For controls (Fig. 3C), the top enriched GO terms for BP were signal transduction (GO:0007165), lipid metabolic process (GO:0006629), and cell wall modification (GO:0042545), those for CC were cytoplasm (GO:0005737), integral component of plasma membrane (GO:0005887), and apoplast (GO:0048046), and those for MF were iron ion binding (GO:0005506), oxidoreductase activity, acting on paired donors (GO:0016705), and sequence-specific DNA binding (GO:0043565). For compound material treatments (Fig. 3D), the top enriched GO terms for BP were signal transduction (GO:0007165), lipid metabolic process (GO:0006629), and metal ion transport (GO:0030001), those for CC were cytoplasm (GO:0005737), apoplast (GO:0048046), and extracellular region (GO:0005576), and those for MF were sequence-specific DNA binding (GO:0043565), calcium ion binding (GO:0005509), and iron ion binding (GO:0005506).

To further understand the molecular interactions among the DEGs, KEGG enrichment analysis was carried out. The results showed phenylpropanoid biosynthesis, pertussis, and brassinosteroid biosynthesis

pathway were significantly enriched in NaCl treatments (CK-Y and P-Y treatments) (Fig. 4A); systemic lupus erythematosus and alcoholism pathway were significantly enriched in NaCO<sub>3</sub> treatments (CK-J and P-J treatments) (Fig. 4B); phenylpropanoid biosynthesis, glycerolipid metabolism, amino sugar and nucleotide sugar metabolism, and pentose and glucuronate interconversions pathway were significantly enriched in controls (CK-J and CK-Y treatments) (Fig. 4C); phenylpropanoid biosynthesis and alpha-Linolenic acid metabolism pathway were significantly enriched in compound material treatments (P-J and P-Y treatments) (Fig. 4D).

### Response of the salt ion transporter in cotton leaves

The transporter-mediated salt ion balance is an important component in cotton leaves when the compound material regulates saline stress and alkaline stress. In NaCl treatments (CK-Y and P-Y treatments), K<sup>+</sup> transporter and K<sup>+</sup> channel genes were significantly regulated by the compound material, and four up-regulated K<sup>+</sup> channel genes (GH\_A13G1568, GH\_D01G0882, GH\_D13G1517, and GH\_A01G0868) were identified. Besides, one K<sup>+</sup> transporter gene (GH\_D08G2294) was up-regulated, one sodium/hydrogen exchanger gene (GH\_A09G0801) was down-regulated (Table 1). For NaCO<sub>3</sub> treatments (CK-J and P-J treatments), the expression levels of genes related to K<sup>+</sup> transporter (GH\_D05G2808) and K<sup>+</sup> channel (GH\_A01G0868, GH\_D01G0882, and GH\_D13G1517) changed markedly, and all were up-regulated (Table 1). For compound material treatments (P-J and P-Y treatments), one K<sup>+</sup> channel gene (GH\_A09G0801) was down-regulated (Table 1).

Table 1  
Expression patterns of DEGs involved in salt ions transport.

	Gene ID	log2FC	Pvalue	Padj	Description
CK-Y vs. P-Y	GH_A09G0801	-1.2	0.04993	0.83714	sodium/hydrogen exchanger 3
	GH_D08G2294	1.31	0.00057	0.15126	potassium transporter 1
	GH_A13G1568	2.49	0.00742	0.55377	two-pore potassium channel 1
	GH_D01G0882	2.67	0.01501	0.69433	two-pore potassium channel 1
	GH_D13G1517	2.85	0.00823	0.57328	two-pore potassium channel 1
	GH_A01G0868	2.99	0.00726	0.55086	two-pore potassium channel 1
CK-J vs. P-J	GH_A01G0868	2.20	0.04683	0.98810	two-pore potassium channel 1
	GH_D01G0882	2.35	0.03191	0.93991	two-pore potassium channel 1
	GH_D13G1517	2.55	0.01693	0.82443	two-pore potassium channel 1
	GH_D05G2808	2.03	0.00451	0.57112	potassium transporter 2
P-J vs. P-Y	GH_A05G1107	-1.20	0.00348	0.47248	potassium channel SKOR

## **Regulation of antioxidative defense in cotton leaves**

Many DEGs in cotton leaves were significantly enriched in oxidoreductase activity Go term. Eight peroxidase genes (GH\_A06G1119, GH\_D11G2319, GH\_D10G1060, GH\_A12G2651, GH\_A05G0628, GH\_D10G1977, GH\_D06G1268, and GH\_A06G1247) were up-regulated for NaCl treatments (CK-Y and P-Y treatments). Three peroxidase genes (GH\_A05G4223, GH\_A06G1247, and GH\_A05G0628) were up-regulated for NaCO<sub>3</sub> treatments (CK-J and P-J treatments). Besides, one peroxidase gene (GH\_A05G1582) was down-regulated, five peroxidase gene (GH\_A03G1283, GH\_D03G1634, GH\_D04G0154, GH\_D03G1633, and GH\_D08G2611) was up-regulated for compound material treatments (P-J and P-Y treatments). One peroxidase gene (GH\_D05G1612) was down-regulated, and one peroxidase gene (GH\_D10G1977) was up-regulated for controls (CK-J and CK-Y treatments) (Table 2).

Table 2  
Expression patterns of DEGs involved in peroxidase.

	Gene ID	log2FC	Pvalue	Padj	Description
CK-Y vs. P-Y	GH_A06G1119	3.29	0.04162	0.81913	peroxidase A2
	GH_D11G2319	17.66	0.00005	0.02426	peroxidase A2
	GH_D10G1060	2.50	0.04006	0.81328	peroxidase 50
	GH_A12G2651	2.57	0.01132	0.63957	peroxidase 5
	GH_A05G0628	4.46	0.03935	0.81251	peroxidase 46
	GH_D10G1977	19.80	0.00000	0.00046	peroxidase 29
	GH_D06G1268	4.54	0.00327	0.39170	peroxidase 12
	GH_A06G1247	3.44	0.00624	0.52647	peroxidase 12
CK-J vs. P-J	GH_A05G4223	3.40	0.01666	0.82443	peroxidase P7
	GH_A06G1247	3.85	0.00266	0.47075	peroxidase 12
	GH_A05G0628	4.35	0.04211	0.97147	peroxidase 46
P-J vs. P-Y	GH_A05G1582	-2.30	0.04592	1.00000	peroxidase 19
	GH_A03G1283	1.42	0.03043	1.00000	peroxidase 3
	GH_D03G1634	1.76	0.02381	0.96048	peroxidase 4
	GH_D04G0154	2.85	0.01578	0.85903	peroxidase P7
	GH_D03G1633	5.85	0.01184	0.78791	peroxidase 4
	GH_D08G2611	8.65	0.01451	0.84244	peroxidase 53
CK-J vs. CK-Y	GH_D05G1612	-1.74	0.01021	1.00000	peroxidase 19
	GH_D10G1977	17.04	0.00002	0.02101	peroxidase 29

### DEGs involved in the phenylpropanoid biosynthesis pathway

The expression of the genes involved in phenylpropanoid biosynthesis of cotton leaves in response to the application of compound material regulating saline stress and alkaline stress was showed in Fig.5. For NaCl treatments (CK-Y and P-Y treatments), the upregulated DEGs were in beta-glucosidase (EC:3.2.1.21), coniferyl-alcohol glucosyltransferase (EC:2.4.1.111), and coniferyl-aldehyde dehydrogenase (EC:1.2.1.68), while DEGs involved in scopoletin glucosyltransferase (EC:2.4.1.128), caffeic acid 3-O-methyltransferase (EC:2.1.1.68), ferulate-5-hydroxylase (EC:1.14.-.-), 4-coumarate-CoA ligase (EC:6.2.1.12), shikimate O-hydroxycinnamoyl transferase (EC:2.3.1.133), cinnamyl-alcohol dehydrogenase (EC:1.1.1.195), and peroxidase (EC:1.11.1.7) were downregulated (Fig. 5A). For NaCO<sub>3</sub> treatments (CK-J and P-J treatments),

the up-regulated DEGs were in ferulate-5-hydroxylase (EC:1.14.-), while DEGs involved in shikimate O-hydroxycinnamoyl transferase (EC:2.3.1.133), and peroxidase (EC:1.11.1.7) were down-regulated (Fig. 5B). For controls (CK-J and CK-Y treatments), the up-regulated DEGs were in phenylalanine ammonia-lyase (EC:4.3.1.24), scopoletin glucosyltransferase (EC:2.4.1.128), and 4-coumarate-CoA ligase (EC:6.2.1.12), while DEGs involved in shikimate O-hydroxycinnamoyl transferase (EC:2.3.1.133), ferulate-5-hydroxylase (EC:1.14.-), coniferyl-aldehyde dehydrogenase (EC:1.2.1.68), and peroxidase (EC:1.11.1.7) were down-regulated (Fig. 5C). For compound material treatments (P-J and P-Y treatments), the up-regulated DEGs were in phenylalanine ammonia-lyase (EC:4.3.1.24), feruloyl-CoA 6-hydroxylase (EC:1.14.11.61), scopoletin glucosyltransferase (EC:2.4.1.128), caffeic acid 3-O-methyltransferase (EC:2.1.1.68), 4-coumarate-CoA ligase (EC:6.2.1.12), shikimate O-hydroxycinnamoyl transferase (EC:2.3.1.133), and caffeic acid 3-O-methyltransferase (EC:2.1.1.68), while DEGs involved in ferulate-5-hydroxylase (EC:1.14.-) and peroxidase (EC:1.11.1.7) were down-regulated (Fig. 5D).

## Discussion

Under saline stress and alkaline stress, excessive  $\text{Na}^+$  will accumulate in plant leaves, inhibiting the transport of  $\text{K}^+$  and causing  $\text{K}^+/\text{Na}^+$  imbalance in plant cells [22]. However, the regulation mechanisms of ion balance under saline stress and alkaline stress are different. Wang, et al. [9] showed that the  $\text{Na}^+$  content under alkaline stress was greater than that under saline stress in pot experiments. In this study, the  $\text{Na}^+$  and  $\text{K}^+$  contents of cotton leaves under alkaline stress were significantly higher than those under saline stress, and there was no significant difference in  $\text{K}^+/\text{Na}^+$ . In order to regulate the saline stress and alkaline stress on cotton, the modifier was applied in field trials. Zhang, et al. [23] found that the sodium/hydrogen exchanger 4 of sesame aerial parts was up-regulated under saline stress through hydroponic culture. Zhao, et al. [24] found 17  $\text{Na}^+/\text{H}^+$  antiporters in the root of chrysanthemum in response to saline stress. Niu, et al. [25] culture experiments found that salinity significantly decreased the expression of NHX4 in leaf veins. In the study, for the treatments treated with the modifier, the  $\text{Na}^+$  content was decreased, which was because the stress signal of  $\text{Na}^+$  could be quickly inhibited by the down-regulation of a  $\text{Na}^+/\text{H}^+$  exchanger 3 gene when the modifier was applied to salinized soil.  $\text{Na}^+/\text{H}^+$  exchanges (NHXs) reduced the accumulation of  $\text{Na}^+$  by fixing  $\text{Na}^+$  and storing it in vacuoles [26, 27]. Besides, Huang, et al. [28] found that the potassium channel KAT1 of the aboveground part of barley was down-regulated under saline stress. In this study, under saline stress and alkaline stress, the  $\text{K}^+$  content and  $\text{K}^+/\text{Na}^+$  ratio for the modifier treatments were increased, which was because the application of the modifier increased the transcription level of certain genes encoding K transporters and K channels in cotton leaves. In particular, under saline stress, several genes of two-pore  $\text{K}^+$  channel 1 and  $\text{K}^+$  transporter 1 were obviously up-regulated for saline treatments, and several genes of  $\text{K}^+$  transporter 2 and two-pore  $\text{K}^+$  channel 1 were also obviously up-regulated for alkaline treatments. Moreover, a  $\text{K}^+$  channel SKOR gene was down-regulated for the alkaline treatments compared with that for the saline treatments, indicating that the application of the modifier had a better effect on the recovery of  $\text{K}^+$  of cottons under saline stress.

The differences in K<sup>+</sup> and Na<sup>+</sup> contents between saline treatments and alkaline treatments were due to the different physiological damages suffered by cotton. In this study, it was found that alkaline stress caused more serious physiological damage to cotton leaves than saline stress. Gong, et al. [12] found that the application of foreign substances could promote the antioxidant system to remove excess free radicals and regulate physiological damage. In this study, we also found that the application of the modifier could regulate the physiological damage suffered by cotton. It has the same effect on the regulation of antioxidant enzymes under saline stress and alkaline stress, but the degrees of the effects are different. For example, no matter under saline stress or alkaline stress, the modifier could increase the SOD, POD, and CAT activity of cotton leaves, which was because a large number of DEGs were related to oxidoreductase activity for CK-Y, P-Y, CK-J and P-J treatments. There were 51 DEGs of oxidoreductase activity Go term were regulated for NaCl treatments (CK-Y and P-Y treatments), and 29 DEGs were regulated for NaCO<sub>3</sub> treatments (CK-J and PJ treatments); moreover, the modifier activated the oxidative stress response under saline stress and alkaline stress. Among them, the modifier significantly increased the activities of SOD and CAT under saline stress, while the modifier did not significantly increase the activities of SOD and CAT under alkaline stress, indicating that the modifier had less effect on the activities of SOD and CAT under alkaline stress, and SOD activity may not be necessary for saline and alkaline tolerances [29]. Studies have shown that POD is the main detoxification enzyme of plants under saline stress and alkaline stress [30]. This study found that the modifier significantly increased the POD activity of cotton leaves under saline stress and alkaline stress, which was because the genes related to peroxidase were up-regulated for CK-Y, P-Y, CK-J, and P-J treatments, and the expression of antioxidant enzyme gene was also increased, leading to the improvement of the tolerance of cotton to saline stress and alkaline stress after applying the modifier; besides, the application of modifier under saline stress increased the expression of a great number of antioxidant enzyme genes. Luo, et al. [31] showed that *SOD1* and *CAT1* genes were involved in the cottons' response to saline stress. G Geng, et al. [32] found that the *POD7* and *SOD [Cu-Zn]* genes of the salt-tolerant varieties of sugar beet were significantly up-regulated, while in our study, the regulation of saline stress and alkaline stress by the application of modifier only significantly regulated the POD A2/50/5/46/29/12/P7 in cotton leaves. This might be because the permeability of the soil in the field enhanced root vitality and promoted plant resistance, so only peroxidase-related genes responded to the reactions to saline stress and alkaline stress. We also noticed that under saline stress and alkaline stress, the REC and MDA in leaves were affected, resulting in membrane damage. The difference of REC under saline stress and alkaline stress was not significant, but the MDA contents under alkali stress was much higher than that under saline stress. Cui, et al. [33] found that the RCE of peanut leaves was increased during saline stress recovery. Gong, et al. [12] found that the leaf MDA content of *Malus hupehensis* Rehd. was decreased by applying melatonin under alkali stress. Our study also found that the application of the modifier to cottons under saline stress and alkaline stress increased the REC content of cotton leaves but decreased the MDA content. Among them, only the increases in REC of cottons under saline stress were significant, indicating that the modifier alleviated the damaged of saline stress and alkaline stress to the stability of the cell membrane and maintained the integrity of the cell membrane; moreover, the effect of the modifier on the cell membrane of cottons under saline stress was better than that under alkaline stress.

The genes for lignin biosynthesis are dynamically regulated at different levels to protect plant cell metabolism from oxidative damage [34]. In the transcription of data, functional analysis of DEGs was performed through KEGG and GO enrichment analysis, and it was found that a large number of genes were involved in the phenylpropanoid biosynthesis pathway. The phenylpropanoid biosynthesis pathway is one of the most important secondary metabolite pathways in plants, and is related to the plant's response to saline stress and alkaline stress. The lignin metabolites produced in this pathway are of great significance for plants to resist abiotic stress [35, 36]. Besides, four lignins (p-hydroxyphenyl lignin, guaiacyl lignin, 5-hydroxy-guaiacyl lignin and syringyl lignin) were aggregated by four monomers (p-coumaryl alcohol, coniferyl alcohol, 5-hydroxy-coniferyl alcohol, and sinapyl alcohol), while four alcohols were catalyzed by peroxidase (EC: 1.11.1.7), leading to the formation of these lignins (Fig. 5). Shen, et al. [37] found that seven genes related to lignin biosynthesis in *Arabidopsis thaliana* were up-regulated under saline stress. This study found that 4CL, HCT, COMT, TOGT1, F5H, CAD, and POD were down-regulated for P-Y treatment compared with those for the CK-Y treatment, indicating that these enzymes might play a vital role in the decrease of lignin synthesis and the protection of cotton from the damage caused by saline stress by modifier. Moreover, 4CL enzyme changes the accumulation of lignin [38], HCT enzyme modifies H lignin [39], COMT enzyme participates in the biosynthesis of S lignin [40], F5H enzyme regulates the composition of S/G lignin (syringyl (S)/guaiacyl (G) lignin) in plants [41], CAD enzymes change the lignin content and structure [42] and POD enzymes participate in lignin biosynthesis and affect plant growth and development [43]. The expression levels of HCT and POD for P-J treatment were down-regulated compared with those for the CK-J treatment, indicating that 5-O-Caffeoylshikimic acid and caffeoyl quinic acid would not be converted into caffeoyl-CoA. However, caffeoyl-CoA is an essential intermediate for lignin biosynthesis [44]. The above indicates that under both saline stress and alkaline stress, the application of modifier will down-regulate the peroxidase (EC: 1.11.1.7), which was because that the modifier may reduce the lignin biosynthesis caused by saline stress and alkaline stress.

## Conclusions

Field test results show that saline stress and alkaline stress are two different stresses. Under salt stress, the contents of  $\text{Na}^+$  and MDA in cotton leaves are high, the activities of POD and CAT are low, and the inhibitory effect of alkaline stress are greater than saline stress. The application of the modifier is mainly to increase cotton  $\text{K}^+/\text{Na}^+$  and POD activity to regulate cotton damages from saline stress and alkaline stress. Through transcription analysis, it was further found that  $\text{K}^+$  transporter genes and peroxidase-related genes were up-regulated during the regulation of saline stress and alkaline stress by the modifier, which improved the saline and alkaline tolerances of cotton; and the enzymes involved in lignin biosynthesis were down-regulated, which protected cotton from the damage caused by saline stress and alkaline stress (Fig.6). Among them, these up-regulated genes and down-regulated enzymes are abundant in cotton leaves during the regulation of saline stress by modifier. Moreover, these differentially expressed genes obtained in field trials have high stability, which deserves to be popularized.

## Materials And Methods

## Experiment Site

Experiments were conducted at the Experimental Station of Grape Research Institute in Shihezi City, Xinjiang Province, China ( $44^{\circ}20' N$ ,  $86^{\circ}03' E$ ). The soil is a desert grey soil. Soil basic characteristics are shown in Table 3.

Table 3  
Soil basic characteristics of the tested soil.

Item	Value	Reference
pH	7.72	Bao, 2000
cation exchange capacity (CEC)	17.32 cmol kg <sup>-1</sup>	
Organic matter contents	12.5 g kg <sup>-1</sup>	
Alkali-hydrolyzable nitrogen	54 mg kg <sup>-1</sup>	
Available phosphorus	11.7 mg kg <sup>-1</sup>	
Available potassium	218 mg kg <sup>-1</sup>	

## Experimental materials and experimental design

The cotton (Xinluzao 62) and a compound material were used as the experimental material in this study. Compound material was a mixture of calcium lignosulfonate, manganese sulfate, ammonium sulfate, ferric sulfate, and boric acid (Mass ratio: 4:4:4:2:1).

The experiment was conducted from April 20th to September 20th, 2018. There were four treatments in total. Two of which were compound material treatments: (1) P-Y treatment (compound material was applied in salinized soil), (2) P-J (compound material was applied in salinized soil); the other two treatments were the controls (CK-Y: no modifier was applied in salinized soil; CK-J: no modifier was applied in alkalinized soil), with three repetitions per treatment and a randomized block design. On April 20th, soils were putted into the plastic barrels (0.5 m in diameter and 0.6 m in height) according to the soil layer of the field, and then barrels were buried back to the field. NaCl and Na<sub>2</sub>CO<sub>3</sub> was added into the barrels and mixed fully in the plough layer. NaCl of 8 g kg<sup>-1</sup> were applied into the NaCl treatments (CK-Y and P-Y treatments), and the pH and EC of the soil were 8.24 and 1.84 s m<sup>-1</sup>, respectively; Na<sub>2</sub>CO<sub>3</sub> of 8 g kg<sup>-1</sup> were applied into the NaCO<sub>3</sub> treatments (CK-J and P-J treatments), and the pH and EC of the soil were 9.78 and 1.03 s m<sup>-1</sup>, respectively. On April 29th, for all treatments, 360 kg hm<sup>-2</sup> urea and 795 kg hm<sup>-2</sup> synthetic fertilizer (formulated for drip irrigation; N: 135 kg hm<sup>-2</sup>; P: 135 kg hm<sup>-2</sup>; K: 135 kg hm<sup>-2</sup>) were applied. On May 4th, cotton was sown; and after emergence, six seedlings were retained in each barrel. On May 6th, compound material was applied after diluting with water. Compound material of 300 kg hm<sup>-2</sup> was applied into the compound material treatments (P-Y and P-J treatments). Both the fertilizer and

compound material were applied to soils with drip irrigation at once. The seedlings were irrigated for the first time on June 25th. The irrigation cycle was 3 days. At the flowering and boll-forming stage (August 19th), new leaves were collected for transcriptome sequencing (three replicates per treatment). All samples were immediately placed in liquid nitrogen and stored at -80 °C until use.

## Plant Physiological Analysis

Leaf samples (0.5 g) were homogenized in 5 mL of potassium phosphate buffer (pH 7.8) in ice. The homogenate was centrifuged at 10,000×g for 20 min at 4 °C, and the supernatant was used for the determination of the enzyme activities with the spectrophotometric method. Superoxide dismutase (SOD) activity was determined following the method of Paoletti, et al. [45] based on the photochemical reduction in the NBT. The reaction mixture (3 mL) was comprised of 0.05 M potassium phosphate buffer (pH 7.8), 130 mM methionine, 750 µM NBT, 20 µM riboflavin, 100 µM EDTA-Na<sub>2</sub>, and 200 µL of enzyme extract. The SOD activity was measured at 560 nm (one unit of SOD activity was denoted by the amount of enzyme required to cause 50% inhibition of the NBT photochemical reduction). The peroxidase (POD) activity was determined as described by Zhou, et al. [46]. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 6.0), guaiacol, H<sub>2</sub>O<sub>2</sub>, and 100 µL enzyme extract. The absorbance caused by guaiacol were measured at 470 nm. Catalase (CAT) activity was determined using H<sub>2</sub>O<sub>2</sub> for 1 min at A240 in 2.5 mL reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.0), and 0.1 M H<sub>2</sub>O<sub>2</sub>, according to Cakmak, et al. [47].

The contents of thiobarbituric acid reactive substances (TBARS) of leaf samples were determined to calculate the malondialdehyde (MDA) content (nmol/g; extinction coefficient: 155 mM cm<sup>-1</sup>) [48]. Fresh cotyledons (0.5 g) were homogenized and extracted in 10 mL of 0.6% TBA (made in 10% trichloroacetic acid). After that, the extract was heated at 100 °C for 30 min and then cooled on ice. Then, the samples were centrifuged at 5000×g for 10 min; the absorbance was measured at 532 nm. Non-specific turbidity was corrected by subtracting the absorbance value at 600 nm. For relative electrical conductivity (REC) [49], 0.1 g of fresh leaves were cut into 1 cm slices, placed in 10 mL of deionized water, and shaking for 24 h at room temperature on a rotary shaker (QL200H, Shanghai, China). Then, electrical conductivity of the solution (L1) was measured using a conductivity meter (EM38, ICT international, Armidale, NSW, Australia). The solution was boiled for 15 min and cooled to room temperature, and electrical conductivity (L2) was again measured. REC was calculated as REC = L1/L2.

The contents of Na<sup>+</sup> and K<sup>+</sup> in the leaf samples were determined according to the method of Bao [50]. Leaf samples were immersed in 98% H<sub>2</sub>SO<sub>4</sub> and 30% H<sub>2</sub>O<sub>2</sub>, and a flame spectrophotometer (AP1200 type, Shanghai, China) was used for the determination.

## Transcriptome Sequencing and Data Analysis

In this study, Oligo (dT) magnetic beads were used to enrich the mRNA with polyA structure in the total RNA, and the RNA was interrupted (300 bp) by ion interruption. After the RNA-Seq library was constructed,

library fragments were enriched by PCR amplification, and then library selection was performed based on the fragment size (450 bp). Then, the quality of the library was tested by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Calif.). After RNA extraction, purification, and library building, the samples were paired-end (PE) based on the Illumina platform using Next-Generation Sequencing (NGS).

Quality of the generated reads was checked using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Fastp was used to remove the adapter and low-quality sequences in the reads [51]. Cotton genome sequence of (*Gossypium hirsutum*, ZJU) were downloaded from Hu, et al. [52] and used as the reference genome. The clean reads were quasi-mapped on to all cotton annotated transcripts using Salmon [53]. Expression abundance at the unit of transcript per million (TPM) was calculated at gene level. DESeq2 was used to identify the differentially expressed genes (DEGs) between samples with the thresholds of adjusted p-value less than 1 and absolute value of log<sub>2</sub>(fold change) larger than 1 [54]. Principal component analysis (PCA) was performed to display the transcriptomic similarity among the samples based on the counts of top 1000 genes. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using clusterProfiler [55].

### Quantitative real-time PCR validation

To validate the RNA-seq data, 11 DEGs from the pathway enrichment analysis were selected for qRT-PCR analysis. Samples of RNA-Seq were reverse transcribed into cDNA for real-time qPCR validation using the PrimeScript™ 1st stand cDNA Synthesis Kit and SYBR Green Master Mixes (Vazyme Biotech, Nanjing, China). qRT-PCR was performed on a fluorescence quantitative system TIB8600 (Taipu, Biotech, Xiamen, China). Each sample was measured with three biological and three technical replicates, and the relative expression levels were calculated using the  $2^{-\Delta\Delta Ct}$  method. The endogenous reference gene used was GhEF1a. The gene-specific primers are listed in Table S2 (Additional file 1).

### Statistical analysis

One-way analysis of variance (ANOVA) was performed for K<sup>+</sup>, Na<sup>+</sup> and physiological characteristics of cotton leaves (Duncan test, P<0.05, SPSS 22.0). All the above analyses were performed in R (Version 3.2.3, <http://www.r-project.org>) using the Vegan, and Origin 8.0.

### Abbreviations

SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; MDA: malondialdehyde; REC: relative electrical conductivity; Go: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: differentially expressed genes (DEGs)

### Declaration

#### Ethics approval and consent to participate

Not applicable.

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests

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### **Authors' contributions**

MJA, XLW, KYW conceived and designed the experiments; MJA and XLW performed experiments and analyzed data; DDC, SW, DSH assisted with the experiments; MJA wrote the manuscript, KYW and HF revised the paper. All authors read and approved the manuscript.

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### **Authors' information**

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## **References**

[1] Li YY, Zhao K, Ren JH, Ding LY, Wu LL. Analysis of the dielectric constant of saline-alkali soils and the effect on radar backscattering coefficient: a case study of soda alkaline saline soils in western Jilin Province using RADARSAT-2 data. *The Scientific World J.* 2014; 56: 30-45.

[2] Ge Y, Li Y, Lv DK, Bai X, Ji W, Cai H, Wang AX, Zhu YM. Alkaline-stress response in *Glycine soja* leaf identifies specific transcription factors and ABA-mediated signaling factors. *Funct Integr Genomic.* 2011; 11: 369-379.

- [3] Wang G, Zhu QG, Meng QW, Wu CA. Transcript profiling during salt stress of young cotton (*Gossypium hirsutum*) seedlings via solexa sequencing. *Acta Physiol Plant.* 2012; 34(1): 107-115.
- [4] Li Q, Yang A, Zhang WH. Comparative studies on tolerance of rice genotypes differing in their tolerance to moderate salt stress. *Bmc Plant Biol.* 2017; 17(1): 141.
- [5] Munns R, Tester M. Mechanisms of salinity tolerance. *Annu Rev of Plant Biol.* 2008; 59: 651-681.
- [6] Guo HJ, Hu ZQ, Zhang HM, Min W, Hou ZA. Comparative Effects of Salt and Alkali Stress on Antioxidant System in Cotton (*Gossypium Hirsutum L.*) Leaves. *Open Chem.* 2019; 17(1): 1352-1360.
- [7] Jia XM, Wang H, Svetla S, Zhu YF, Hu Y, Cheng L, Zhao T, Wang YX. Comparative physiological responses and adaptive strategies of apple *Malus halliana* to salt, alkali and saline-alkali stress. *Sci. Hortic.* 2019; 245: 154-162.
- [8] Ma HX, Meng CM, Zhang KX, Wang KY, Fan H, Li YB. Study on Physiological Mechanism of Using Cottonseed Meal to Improve Salt-Alkali Tolerance of Cotton. *J Plant Growth Regul.* 2020; 1-11.
- [9] Wang XP, Geng SJ, Ri YJ, Cao DH, Liu J, Shi DC, Yang CW. Physiological responses and adaptive strategies of tomato plants to salt and alkali stresses. *Sci. Hortic.* 2011; 130(1): 248-255.
- [10] Kiełkowska A, Grzebelus E, Lis-Krzyścin A, Maćkowska K. Application of the salt stress to the protoplast cultures of the carrot (*Daucus carota L.*) and evaluation of the response of regenerants to soil salinity. *Plant Cell Tiss Org.* 2019; 137: 379-395.
- [11] Mao L, Lu JG, Jiang HY. Mechanisms of plant responses to salt-alkali stress. *Mol Plant Breeding.* 2019; 1: 07–24.
- [12] Gong XQ, Shi ST, Dou F, Ma FW. Exogenous Melatonin Alleviates Alkaline Stress in *Malus hupehensis* Rehd by Regulating the Biosynthesis of Polyamines. *Molecules.* 2017; 22(9): 1542.
- [13] Faghih S, Zarei A, Ghobadi C. Positive effects of plant growth regulators on physiology responses of *Fragaria × ananassa* cv. 'camarosa' under salt stress. *Int J Fruit Sci.* 2018; 19(2): 104-114.
- [14] Ashraf J, Zuo DY, Wang DY, Malik W, Zhang YP, Abid MA, Cheng HL, Yang QH, Song GL. Recent insights into cotton functional genomics: progress and future perspectives. *Plant Biotechnol. J.* 2018; 16(3): 699-713.
- [15] Yin ZP, Zhang H, Zhao Q, Yoo MJ, Zhu N, Yu JL, Yu JJ, Guo SY, Miao YC, Chen SX, et al. Physiological and comparative proteomic analyses of saline-alkali  $\text{NaHCO}_3$ -responses in leaves of halophyte *Puccinellia tenuiflora*. *Plant Soil.* 2019; 437(1-2): 137-158.
- [16] Razzaq A, Ali A, Safdar LB, Zafar MM, Rui Y, Shakeel A, Shaukat A, Ashraf M, Gong WK, Yuan YL. Salt stress induces physiochemical alterations in rice grain composition and quality. *J Food Sci.* 2020; 85(1):

- [17] Wei YY, Xu YC, Lu P, Wang XX, Li ZQ, Cai XY, Zhou ZL, Wang YH, Zhang ZM, Lin ZX, et al. Salt stress responsiveness of a wild cotton species (*Gossypium klotzschianum*) based on transcriptomic analysis. *Plos One*. 2017; 12(5): e0178313.
- [18] Chen L, Liu LT, Lu B, Ma TT, Jiang D, Li J, Zhang K, Sun HC, Zhang YJ, Bai ZY, et al. Exogenous melatonin promotes seed germination and osmotic regulation under salt stress in cotton (*Gossypium hirsutum* L.). *Plos one*. 2020; 15(1): e0228241.
- [19] Guo HJ, Li SN, Min W, Ye J, Hou ZA. Ionomeric and transcriptomic analyses of two cotton cultivars (*Gossypium hirsutum* L.) provide insights into the ion balance mechanism of cotton under salt stress. *PLoS One*. 2019; 14(12): e0226776.
- [20] Xu YC, Magwanga RO, Yang X, Jin DS, Cai XY, Hou YQ, Wei YY, Zhou ZL, Wang KB, Liu F. Genetic regulatory networks for salt-alkali stress in *Gossypium hirsutum* with differing morphological characteristics. *BMC Genomics*. 2020; 21(1): 1-15.
- [21] Lee SB, Kwon HB, Kwon SJ, Park SC, Jeong MJ, Han SE, Byun MO, Daniell H. Accumulation of trehalose within transgenic chloroplasts confers drought tolerance. *Mol Breed*. 2003; 11: 1-13.
- [22] Zhu JK. Abiotic stress signaling and responses in plants. *Cell*. 2016; 167(2): 313-324.
- [23] Zhang YJ, Li DH, Zhou R, Wang X, Dossa K, Wang LH, Zhang YX, Yu JY, Gong HH, Zhang XR, 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): 66-79.
- [24] Zhao Q, He L, Wang B, Liu QL, Pan YZ, Zhang F, Jiang BB, Zhang L, Liu GL, Jia Y. Transcriptome Comparative Analysis of Salt Stress Responsiveness in Chrysanthemum (*Dendranthema grandiflorum*) Roots by Illumina- and Single-Molecule Real-Time-Based RNA Sequencing. *DNA Cell Biol*. 2018; 37(12): 1-15.
- [25] Niu ML, Xie JJ, Chen C, Cao HS, Sun JY, Kong QS, Shabala S, Shabala L, Huang Y, Bie ZL. An early aba-induced stomatal closure,  $\text{Na}^+$  sequestration in leaf vein and  $\text{K}^+$  retention in mesophyll confer salt tissue tolerance in cucurbita species. *J Exp Bot*. 2018; 69(20): 4945-4960.
- [26] Barragán V, Leidi EO, Andrés Z, Rubio L, Luca AD, Fernández JA, Cubero B, Pardo JM. Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis*. *Plant Cell*. 2012; 24: 1127-1142.
- [27] Qiu QS, Guo Y, Quintero FJ, Pardo JM, Schumaker KS, Zhu JK. Regulation of vacuolar  $\text{Na}^+/\text{H}^+$  exchange in *Arabidopsis thaliana* by the salt-overly-sensitive (SOS) pathway. *J Biol Chem*. 2004; 279:

207-215.

- [28] Huang L, Kuang LH, Li X, Wu LY, Wu DZ, Zhang GP. Metabolomic and Transcriptomic Analyses Reveal the Reasons Why *Hordeum marinum* has Higher Salt Tolerance than *Hordeum vulgare*. *Environ Exp Bot.* 2018; 156: 48-61.
- [29] Chakraborty K, Bishi SK, Goswami N, Singh AL, Zala PV. Differential fine-regulation of enzyme driven ROS detoxification network imparts salt tolerance in contrasting peanut genotypes. *Environ. Exp. Bot.* 2016; 128: 79-90.
- [30] Zhang L, Ma HJ, Chen TT, Pen J, Yu SX, Zhao XH. Morphological and physiological responses of cotton (*Gossypium hirsutum* L.) plants to salinity. *PLoS One.* 2014; 9(11): e112807.
- [31] Luo XL, Wu JH, Li YB, Nan ZR, Guo X, Wang YX, Zhang AH, Wang ZA, Xia GX, Tian YC. Synergistic effects of GhSOD1 and GhCAT1 overexpression in cotton chloroplasts on enhancing tolerance to methyl viologen and salt stresses. *Plos One.* 2013; 8(1): e54002.
- [32] Geng G, Lv C, Stevanato P, Li R, Liu H, Yu L, Wang Y. Transcriptome Analysis of Salt-Sensitive and Tolerant Genotypes Reveals Salt-Tolerance Metabolic Pathways in Sugar Beet. *Int J Mol Sci.* 2019; 20(23): E5910.
- [33] Cui F, Sui N, Duan GY, Liu YY, Han Y, Liu SS, Wan SB, Li GW. Identification of Metabolites and Transcripts Involved in Salt Stress and Recovery in Peanut. *Front Plant Sci.* 2018; 9: 217.
- [34] Shafi A, Chauhan R, Gill T, Swarnkar MK, Sreenivasulu Y, Kumar S, Kumar N, Shankar R, Ahuja PS, Singh AK. Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in *Arabidopsis* under salt stress. *Plant Mol. Biol.* 2015; 87(6): 615-631.
- [35] Chun HJ, Baek D, Cho HM, Lee SH, Jin BJ, Yun DJ. Lignin biosynthesis genes play critical roles in the adaptation of *Arabidopsis* plants to high-salt stress. *Plant Signal Behav.* 2019; 14(8): 1625697.
- [36] Dixon RA, Liu CG, Jun JH. Metabolic engineering of anthocyanins and condensed tannins in plants. *Curr Opin Biotechnol.* 2013; 24(2): 329-335.
- [37] Shen X, Wang Z, Song X, Xu J, Jiang C, Zhao Y, Ma C, Zhang H. Transcriptomic profiling revealed an important role of cell wall remodeling and ethylene signaling pathway during salt acclimation in *Arabidopsis*. *Plant Mol Biol.* 2014; 86(3): 303-317.
- [38] Liu H, Guo ZH, Gu FW, Ke SW, Sun DY, Dong SY, Liu W, Huang M, Xiao WM, Yang GL, et al. 4-Coumarate-CoA ligase-like gene OsAAE3 negatively mediates the rice blast resistance, floret development and lignin biosynthesis. *Front. Plant Sci.* 2017; 7: 2041.
- [39] Vanholme B, Cesario I, Goeminne G, Kim H, Marroni F, Van Acker R, Vanholme R, Morreel K, Ivens B, Pinoio S, et al. Breeding with rare defective alleles (BRDA): A natural *Populus nigra* HCT mutant with

modified lignin as a case study. *New Phytol.* 2013; 198(3): 765-776.

[40] Eudes A, Dutta T, Deng K, Jacquet N, Sinha A, Benites VT, Baidoo EEK, Richel A, Sattler SE, Northen TR, et al. SbCOMT (Bmr12) is involved in the biosynthesis of tricin-lignin in sorghum. *PLoS One.* 2017; 12(6): e0178160.

[41] Wagner A, Tobimatsu Y, Phillips L, Flint H, Geddes B, Lu F, Ralph J. Syringyl lignin production in conifers: Proof of concept in a Pine tracheary element system. *Proc. Natl. Acad. Sci.* 2015; 112(19): 6218-6223.

[42] Van Acker R, Déjardin A, Desmet S, Hoengenaert L, Vanholme R, Morreel K, Laurans F, Kim H, Santoro N, Foster C, et al. Different metabolic routes for coniferaldehyde and sinapaldehyde with CINNAMYL ALCOHOL DEHYDROGENASE1 deficiency. *Plant Physiol.* 2017; 175(3): 1018-1039.

[43] Herrero J, Fernández-Pérez F, Yebra T, Novo-Uzal E, Pomar F, Pedreño MÁ, Cuello J, Guéra A, Esteban-Carrasco A, Zapata JM. Bioinformatic and functional characterization of the basic peroxidase 72 from *Arabidopsis thaliana* involved in lignin biosynthesis. *Planta.* 2013; 237(6): 1599-1612.

[44] Liu QQ, Luo L, Zheng LQ. Lignins: Biosynthesis and Biological Functions in Plants. *Int J Mol Sci.* 2018; 19(2): 335.

[45] Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Anal. Biochem.* 1986; 154(2): 536-541.

[46] Zhou W, Leul M. Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. *Plant Growth Regul.* 1999; 27(2): 99-104.

[47] Cakmak I, Marschner H. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol.* 1992; 98(4): 1222-1227.

[48] Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 1968; 125(1): 189-198.

[49] Lutts S, Kinet JM, Bouharmont J. NaCl-induced senescence in leaves of rice (*Oryza sativa*) cultivars differing in salinity resistance. *Ann. Bot-London.* 1996; (3): 389-398.

[50] Bao SD. Soil agrochemical analysis, 3rd edition. China Agriculture Press, Beijing. 2000.

[51] Chen SF, Zhou YQ, Chen YR, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics.* 2018; 34 (17): 884-890.

[52] Hu Y, Chen J, Fang L, Zhang Z, Ma W, Niu Y, Ju L, Deng J, Zhao T, Lian J. *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the origin and evolution of allotetraploid cotton. *Nat*

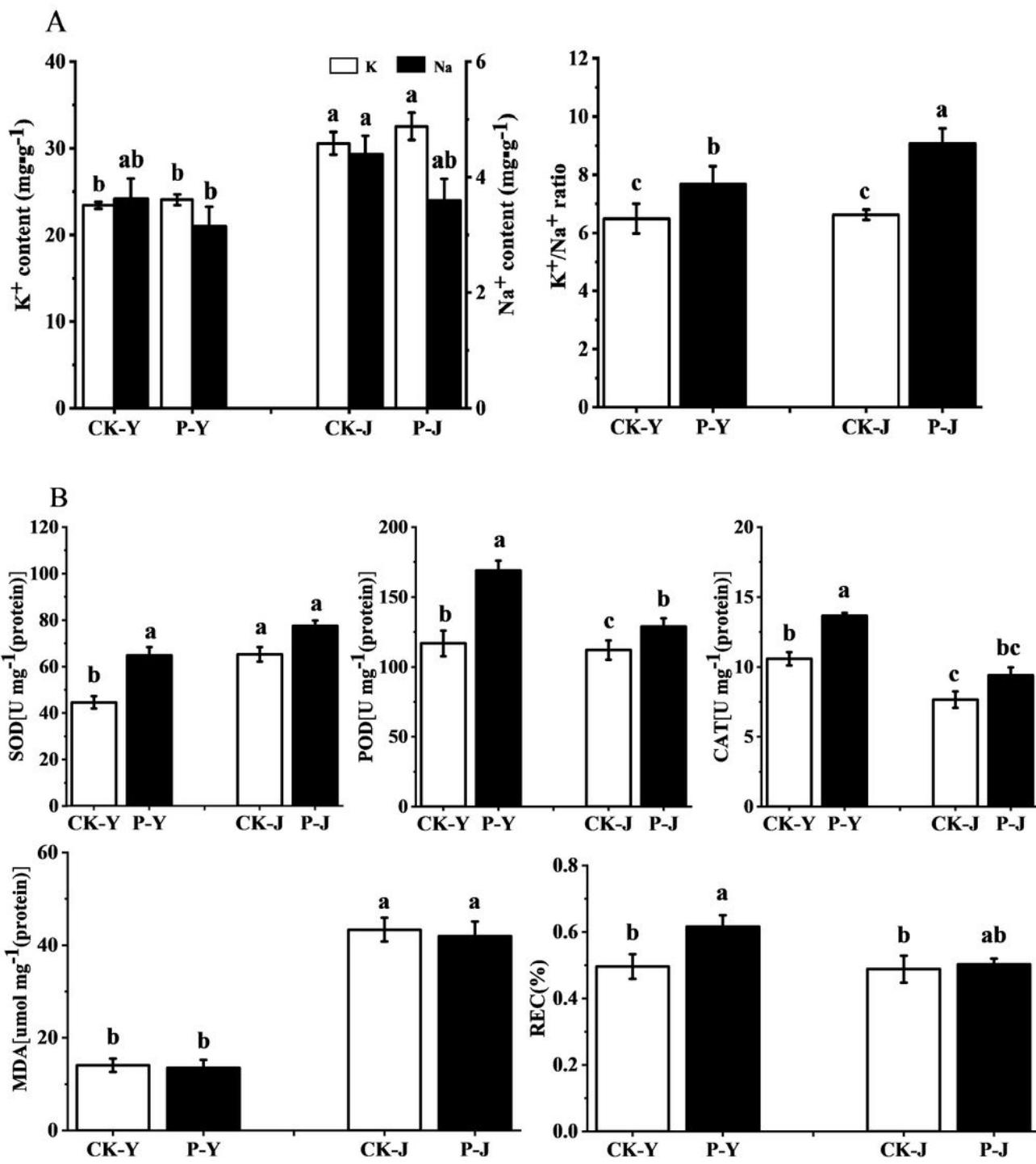
Genet. 2019; 51(4): 739-748.

[53] Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods. 2017; 14(4): 417-419.

[54] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15(12): 550.

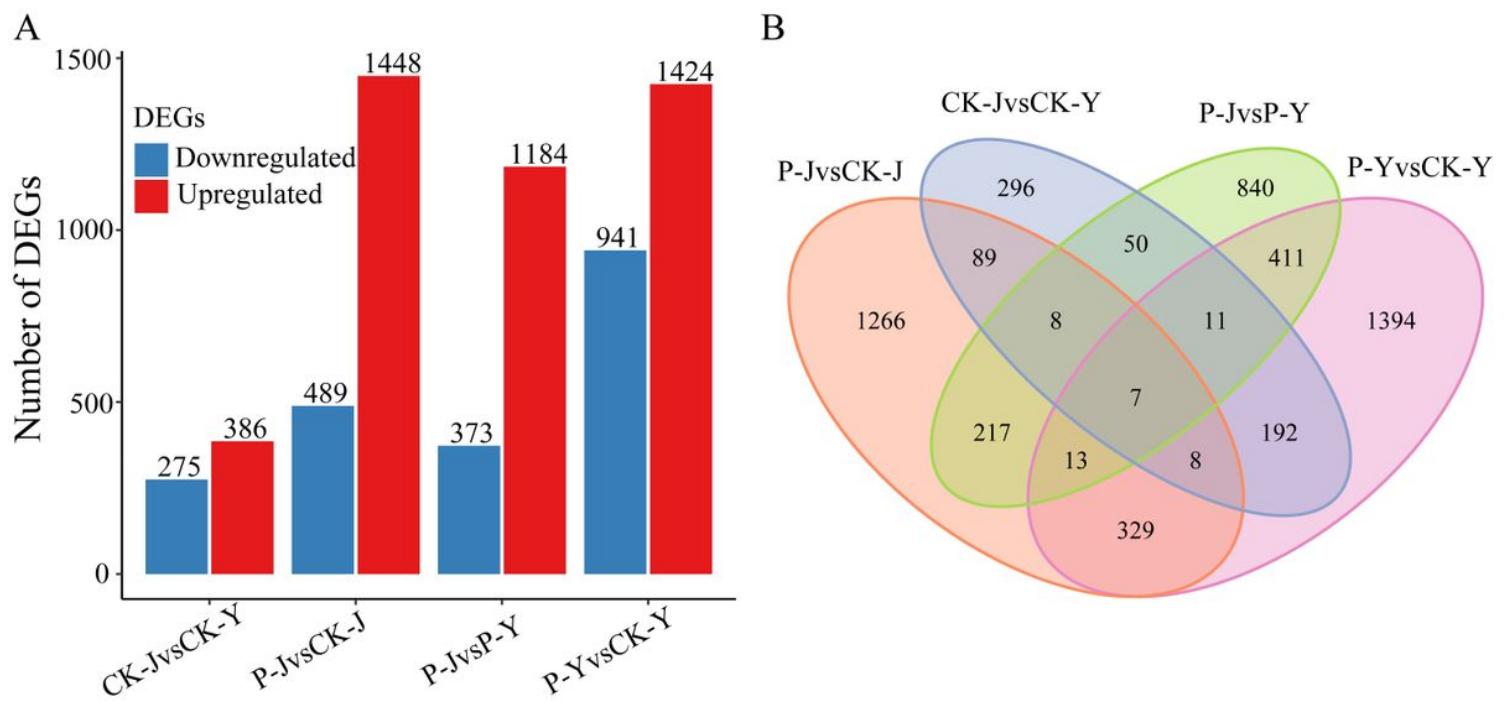
[55] Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012; 16(5): 284-287.

## Figures



**Figure 1**

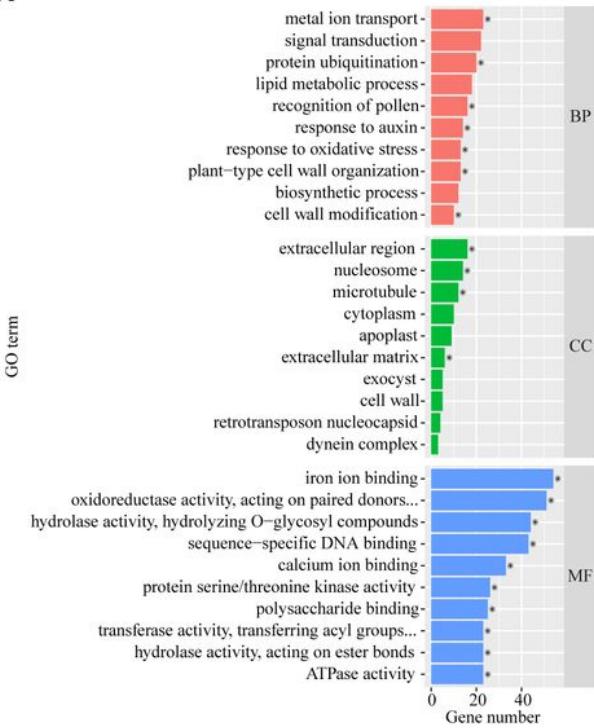
Effect of the application of compound material on the contents of K<sup>+</sup> and Na<sup>+</sup>, K<sup>+</sup>/Na<sup>+</sup> (A), antioxidative enzymes activity, and the contents of MDA and REC (B) in leaves.



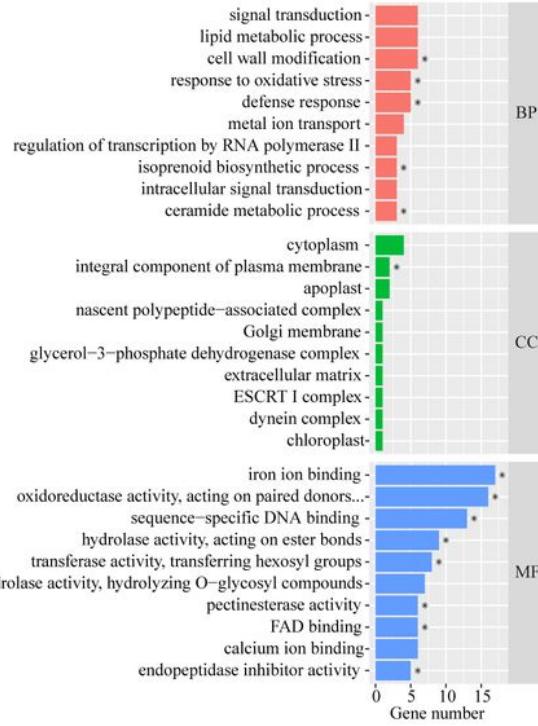
**Figure 2**

Transcriptome analysis of cotton leaves in response to the application of compound material regulating saline stress and alkaline stress. Numbers of DEGs identified in cotton leaves (A). Venn diagram of DEGs (B).

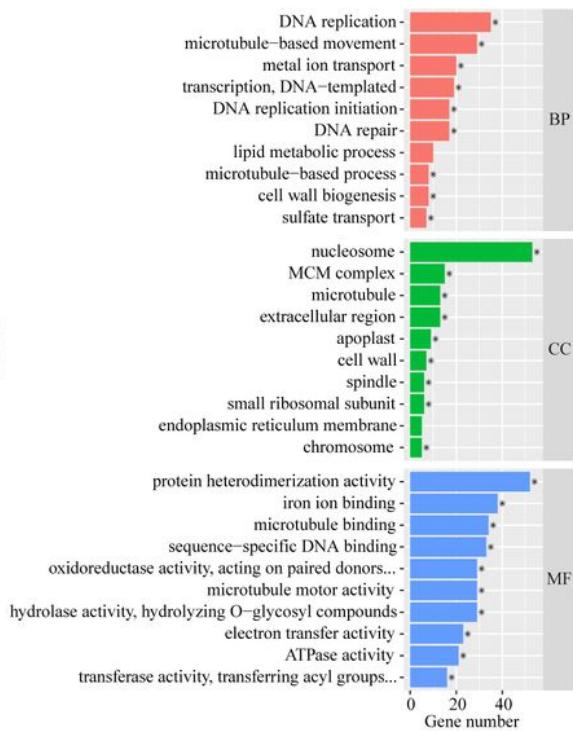
A



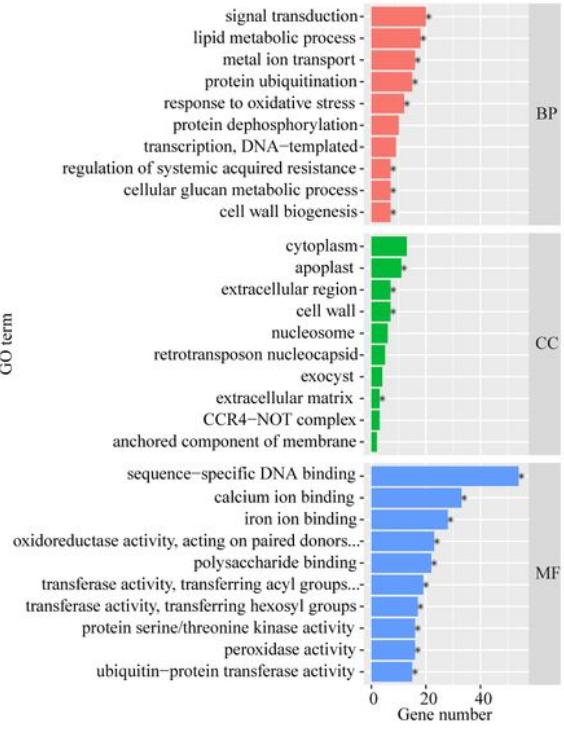
C



B

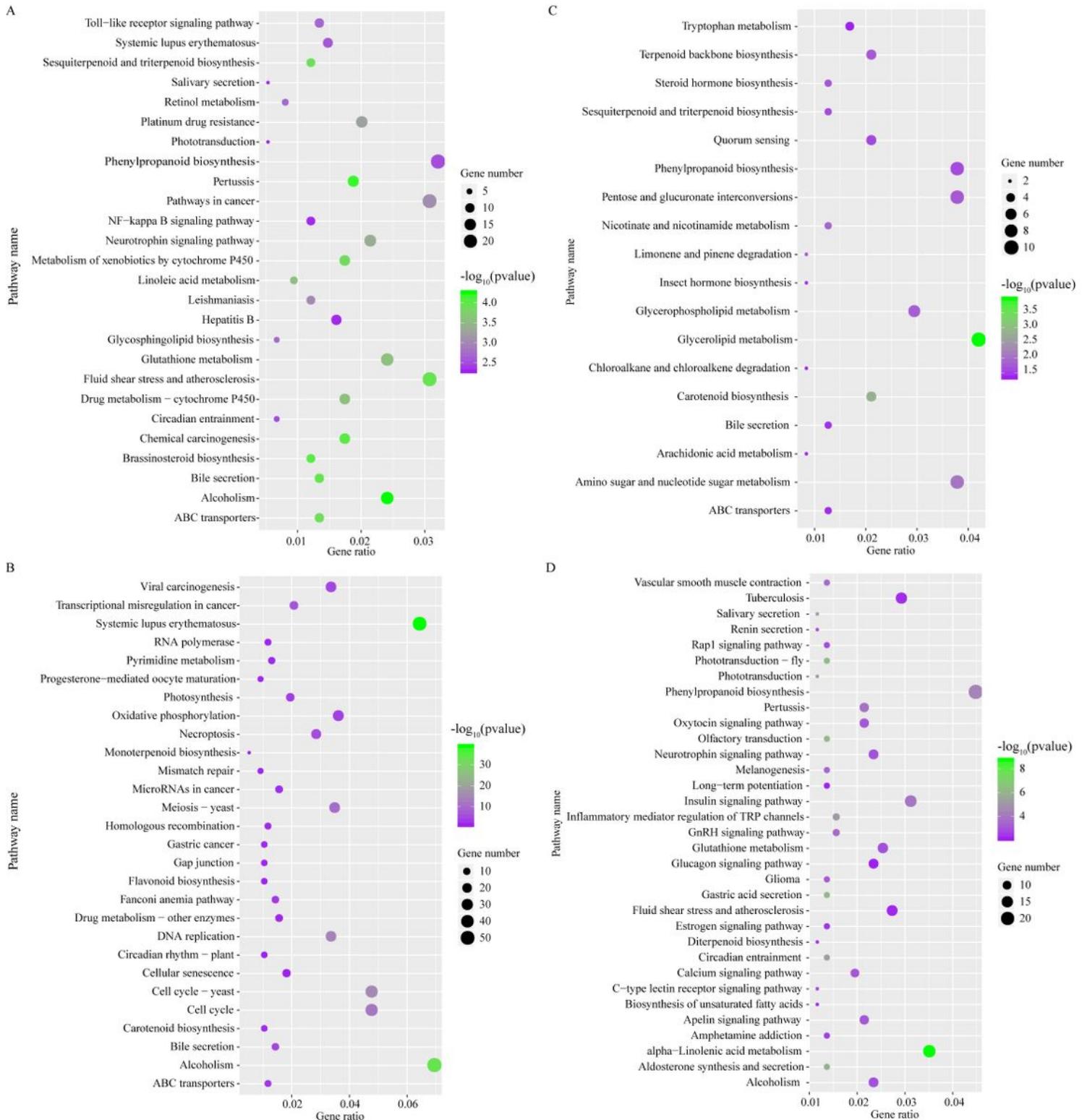


D

**Figure 3**

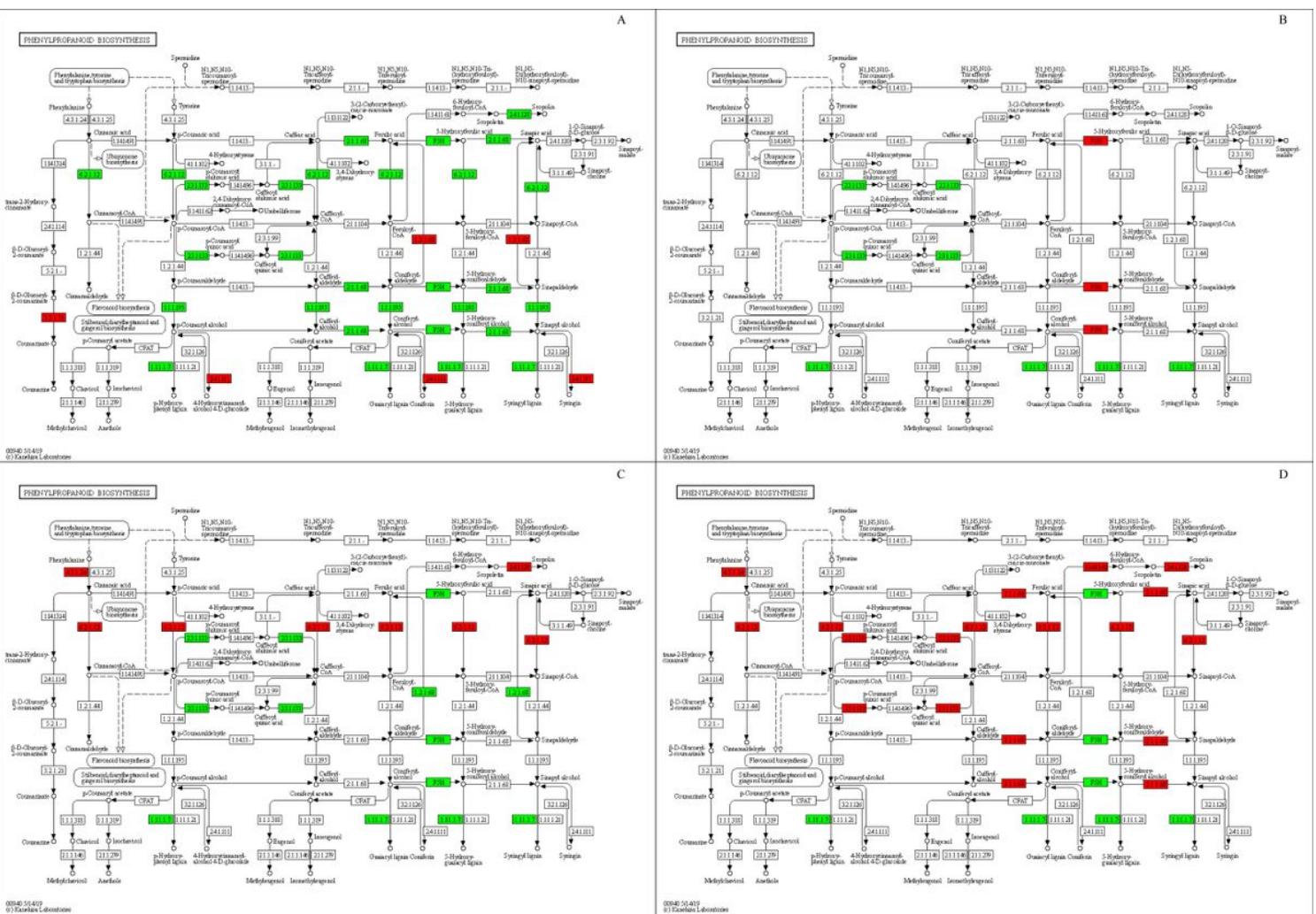
GO enrichment analysis of DEGs. (A) The top 10 enriched GO terms in NaCl treatments (CK-Y and P-Y treatments). (B) The top 10 enriched GO terms in NaCO<sub>3</sub> treatments (CK-J and P-J treatments). (C) The top 10 enriched GO terms in control treatments (CK-J and CK-Y treatments). (D) The top 10 enriched GO terms in compound material treatments (P-J and P-Y treatments). BP, CC, and MF represent biological

process, cellular component, and molecular function, respectively. The asterisks represent the significant level of 0.05.



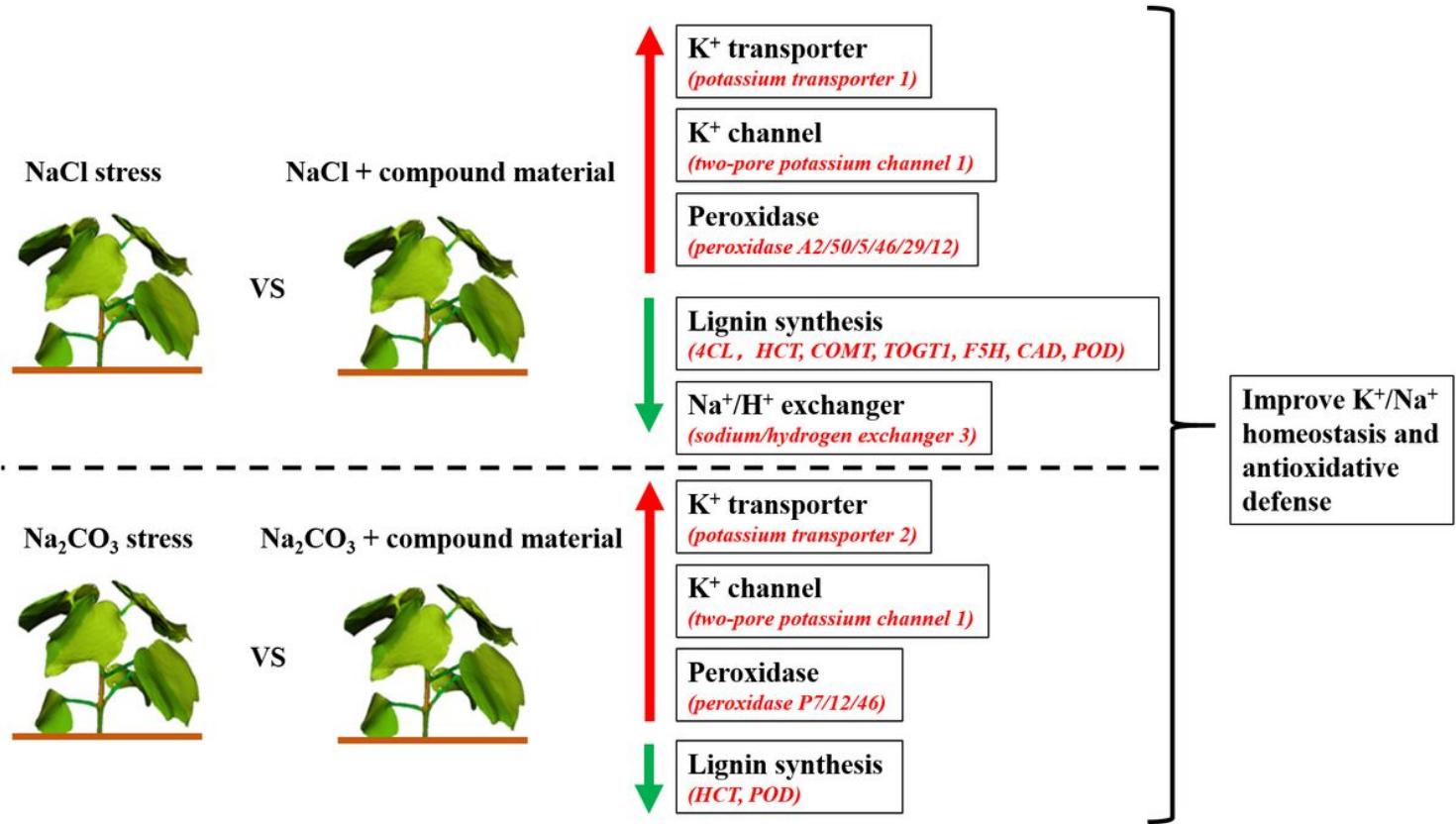
**Figure 4**

KEGG enrichment analysis of DEGs. (A) Pathway in NaCl treatments (CK-Y and P-Y treatments). (B) Pathway in NaCO<sub>3</sub> treatments (CK-J and P-J treatments). (C) Pathway in controls (CK-J and CK-Y treatments). (D) Pathway in compound material treatments (P-J and P-Y treatments).



**Figure 5**

Representation of genes related to phenylpropanoid biosynthesis pathway. The red frames represent up-regulated DEGs, the green frames represent down-regulated DEGs. (A) Pathway in NaCl treatments (CK-Y and P-Y treatments). (B) Pathway in NaCO<sub>3</sub> treatments (CK-J and P-J treatments). (C) Pathway in control treatments (CK-J and CK-Y treatments). (D) Pathway in compound material treatments (P-J and P-Y treatments).



**Figure 6**

Proposed model for the function of the compound material in regulating saline stress and alkaline stress of cotton leaves. The up-pointing red arrows mean that the candidate genes are up-regulated; the down-pointing green arrows mean that the candidate genes are down-regulated.

## Supplementary Files

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