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RESEARCH ARTICLE

Interaction of phytohormones with the antioxidant and pro-oxidant systems of cotton under salt stress

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Abstract

Despite extensive studies on the physiological effects of salinity in cotton, the combined regulatory roles of phytohormones in modulating the plant antioxidant and pro-oxidant systems under salt stress remain poorly understood. The biochemical responses of cotton plant varieties with different salt tolerances were studied through the exogenous application of phytohormones. The study used cotton plants of *Gossypium hirsutum* L. species developed by classical cotton breeding (Gulistan and C-4727), two gene knockout cultivars (Porlok-1 and Porlok-4) and two cultivars developed through DNA marker-assisted introgression into local varities (Ravnak-1 and Ravnak-2). Based on the results obtained, the exogenous application of abscisic acid (ABA) was found to alleviate the negative effects of salinity in all varieties. Under laboratory conditions, in saline models with 1 % and 4 % NaCl (sodium chloride), significant biochemical indicators including antioxidant enzyme activities, as well as concentrations of endogenous ABA and proline were observed to increase respectively in Porlok-1, Porlok-4 and Gulistan cotton cultivars. The biochemical resistance of gene knockout cotton varieties such as Porlock varieties to abiotic stresses has been confirmed by molecular studies. When these varieties were compared with unmodified cotton varieties, the modified lines exhibited higher antioxidant enzyme activity and greater synthesis of free proline, reducing sugars and phytohormones, indicating enhanced stress adaptation.

Keywords: abscisic acid; antioxidant enzyme; gene-knockout cotton; *Gossypium hirsutum* L.; indole-acetic acid; lipid peroxidation; phytohormones; proline

Introduction

Soil salinization poses a serious global environmental challenge, impacting around 1.1 billion hectares of land, which accounts for nearly 7 % of the planet's terrestrial area (1). This stressor has intensified under the dual pressures of global climate change and the adoption of high-input agricultural systems. However, plants possess intricate defense mechanisms at cellular, organ-specific and systemic levels to cope with such environmental challenges (2).

In cotton (*Gossypium* spp.), salinity stress leads to profound biochemical adjustments in leaf tissues. These include modifications in antioxidant enzyme activity and elevated concentrations of stress markers such as proline, malondialdehyde (MDA) and water-soluble sugars, which serve as biochemical indicators of stress response (3, 4). These biomarkers are widely utilized by breeders as physiological indicators for selecting salt-tolerant genotypes.

Among emerging mitigation strategies, the exogenous application of phytohormones such as abscisic acid (ABA), ethylene (ET), indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins (CKs), salicylic acid (SA), jasmonates (JA), brassinosteroids (BRs) and strigolactones (SLs) has shown considerable efficacy in alleviating the negative impacts of salt and other abiotic stresses (5). Phytohormones not only directly modulate stress responses but also act as critical signaling molecules involved in plant adaptive responses. Understanding the hormonal regulation of stress adaptation is thus a central theme in plant biochemistry and molecular biology. These hormones govern multiple physiological processes and contribute to the enhancement of plant tolerance mechanisms, offering a strategic framework for sustaining productivity under environmental stress (6).

Abscisic acid functions as a central phytohormone in plant responses to salinity stress by activating defense pathways related to osmotic regulation, ion homeostasis and oxidative stress mitigation (7). Through modulation of gene expression, ABA enhances root water uptake, stabilizes cellular membranes and reduces transpirational water loss, thereby maintaining internal water balance under saline conditions (8). In addition to its role in abiotic stress signaling, ABA promotes hydrogen peroxide (H_2O_2) accumulation and triggers the expression of antioxidant defense genes, contributing to enhanced stress tolerance (9). Hydrogen peroxide plays pivotal role as a potent regulator in signaling pathways when the plant is under stress. The oxidative damage triggered by the various reactive oxygen species (ROS) generated in response to various stresses continuously decreasing the productivity plant. Hydrogen peroxide plays an important role in cell signaling at low concentrations. For quite a long time, ROS were thought to play essentially negative role in cells causing oxidative stress. But findings from many recent studies demonstrated high physiological value of ROS in ontogenesis of plant organisms (10).

Indole-3-acetic acid, the principal natural auxin, regulates key developmental processes such as cell elongation, vascular tissue differentiation and apical dominance. Indole-3-acetic acid mediated control of shoot architecture and directional growth responses, including phototropism and gravitropism, enables plants to optimize spatial orientation and resource acquisition (11). Collectively, ABA and IAA coordinate physiological and molecular responses that strengthen plant adaptability and performance under abiotic stress conditions, particularly salinity.

Therefore, the present study aimed to investigate the biochemical responses of cotton cultivars with different levels of salt tolerance to exogenous applications of ABA and IAA under saline conditions. Specifically, the study sought to evaluate changes in antioxidant enzyme activities, proline, reducing sugars and MDA concentrations, as well as endogenous hormone levels, in order to clarify the role of phytohormones in modulating the antioxidant and pro-oxidant systems of cotton under salt stress.

Materials and Methods

Plant materials

For this study, 6 cultivars (*Gossypium hirsutum* L.) developed through gene knockout, marker-assisted selection (MAS) and conventional breeding methods were selected. The gene-knockout and MAS-based cultivars Porlok-1, Porlok-4, Ravnak-1 and Ravnak-2, were provided by the Center for Genomics and Bioinformatics of the Academy of Sciences of the Republic of Uzbekistan. For comparison, conventionally bred cultivars Guliston and C-4727 were also included in the study. Seeds of these cultivars were obtained from the Cotton Breeding, Seed Production and Agrotechnology Research Institute under the Ministry of Agriculture and Water Resources of the Republic of Uzbekistan. Guliston and C-4727 are widely utilized in national cotton breeding programs and represent genetic resources with varying degrees of tolerance to environmental stress factors.

Stress treatment

The cotton seeds were treated with 96 % concentrated sulfuric acid for 20-30 sec, followed by rinsing in cold running water for 15 min and soaking in distilled water for 12 hr to promote swelling. Afterward, the seeds were germinated in a dark, humid chamber at 30 °C for 7 days (12). Once the germination period ended, half of the seedlings were kept in distilled water as a control, while the remaining seedlings were subjected to six different saline treatments: 1 % NaCl, 1 % NaCl with 10^{-7} M ABA, 1 % NaCl with 10^{-7} M IAA, 4 % NaCl, 4 % NaCl with 10^{-7} M ABA and 4 % NaCl with 10^{-7} M IAA. After treatment, the seedlings were frozen in liquid nitrogen for biochemical analysis.

Biochemical study

The extraction of enzymes was performed according to the method described previously (13). The activity of ascorbate peroxidase (APX) was determined following the procedure established elsewhere (14). Total superoxide dismutase (SOD) activity was determined as described previously (15). The total protein of the samples was quantified (16), with bovine serum albumin (BSA; Sigma-Aldrich, USA) as the standard. The reducing sugars content was determined (17). The MDA content was measured (18). The proline content was measured using ninhydrin reagent (19) with some modifications (20). The concentration of ABA and IAA were performed using Agilent Technologies 1200 series for high-performance liquid chromatography (HPLC) (21).

Statistical analysis

The experiments were repeated thrice, each of which was independently reproduced thrice. Statistical analysis was performed using GraphPad Prism version 8 and data are presented as the mean ± standard deviation (SD). A *t* test was applied with a significance threshold of $p \leq 0.05$.

Results and Discussion

Two *G. hirsutum* cultivars, Gulistan and C-4727, developed through classical cotton breeding, were selected for this study due to their differing responses to salinity, with Gulistan being tolerant (22) and C-4727 sensitive (12).

In addition to conventionally bred cultivars, two gene-knockout lines of *G. hirsutum* (Porlok-1 and Porlok-4), generated by RNA interference targeting the *PHYA1* phytochrome gene, were included to evaluate how targeted genetic modification influences phytohormone-mediated antioxidant and pro-oxidant responses under salinity stress (23, 24). The RNA interference of *PHYA1* gene in cotton is known to result both in suppression of *PHYA1* target gene and in the increase of expression of other genes of phytochromes, in its turn changing plant architecture, activating vegetative growth, early flowering and maturating of cotton bolls. In addition, two cultivars of *G. hirsutum* species generated by marker-associated selection were used for experiments. Ravnak-1 was generated by introgression to Andijan -35, a local cultivar, of loci associated with length and strength of fibers of Line L-141 by means of DNA markers, to name BNL1604 and BNL1122. Ravnak-2 was generated by introgression to Mekhnat, a local cotton cultivar, of loci impacting the length and strength of fiber in L-N1 cotton line by means of DNA markers, to name BNL1604 and BNL1122 (25).

First, we studied concentrations of phytohormones. After exposure to salinity stress, ABA was intensively synthesized in the control samples of Porlok-1 and Porlok-4, as compared to the other cultivar control samples (Fig. 1). In particular, in 1 % NaCl, the highest concentrations of ABA could be seen in Porlok-1 and Porlok-4 (184.0 and 198.0 ng/g ABA per 1 g tissue, respectively), while the lowest ABA concentration was found in C-4727 (75.0 ng/g). In Guliston, Ravnak-1 and Ravnak-2, concentrations of the synthetized ABA were 97.0, 125.0 and 132.0 ng/g, respectively. In 4 % NaCl, all cotton cultivars showed an increase in the biosynthesis of endogenous ABA with increasing salinity.

Previous studies have shown that, ABA is biosynthesized in plants under conditions of water shortage; the expression of several genes sensitive to ABA is realized to stimulate the development and growth of roots under stress conditions, increasing their hydraulic activity and influencing the opening of stomata (26). Therefore, the rapid increase in the amount of ABA is a non-specific protective and adaptive response, which is important for the adaptation processes of plants.

These results are consistent with those obtained by other scientists. For example, salinity increases the amount of ABA in cucumber leaves; as a result, cold and heat tolerance of cucumber seedlings increases (27). In the halophyte *Suaeda maritima*, known by the common names herbaceous seepweed and annual seablite, ABA concentrations were found to vary by the extent of salinity (28). It was observed that ABA reduced adverse effects of NaCl and increases the resistance of tobacco callus (29) and sorghum (30) to ionic stress.

Salinity increased the concentrations of ABA in young and mature iris leaves (31). Studies have also demonstrated an increase in endogenous ABA levels under salt stress conditions in alfalfa seedlings and tobacco cells (32).

After exposure to salinity stress, Porlok-1 and Porlok-4 had higher concentrations of IAA (95.0 and 108.0 ng/g, respectively); its lowest concentrations were found in the seedlings of C-4727 (60.0 ng/g) (Fig. 1).

In the medium with 1 % NaCl, the highest IAA concentrations, that is 126.0 ng/g and 135 ng/g were registered in Porlok-1 and Porlok-4, respectively, while IAA lowest concentration was found in C-4727 (72.0 ng/g). In Guliston, Ravnak-1 and Ravnak-2 varieties, IAA concentrations were 95.0 ng/g, 106.0 ng/g and 112 ng/g, respectively.

A decrease in the IAA concentrations leads to the inhibition of growth processes, as a result of which energy resources are spent not on growth, but on the maintenance of vital reactions, including the change in the balance of phytohormones, which ensures the activation of normal development programs of cells, to name growth and ontogenesis, which occurs in the initial period of the stressor's effect (33).

The enzymatic activity of principal antioxidant components, namely SOD and APX, was determined in the stem tissues of the selected cotton cultivars as an indicator of antioxidant defense system performance. The results can be seen in Table 1 & 2. After 1 hr of exposure to stress, SOD showed high activity in control samples of Porlok-1 and Porlok-4 (8.06 and 6.48 U/mg protein, respectively); C-4727 demonstrated low activity (1.5 U/mg protein) (Fig. 1).

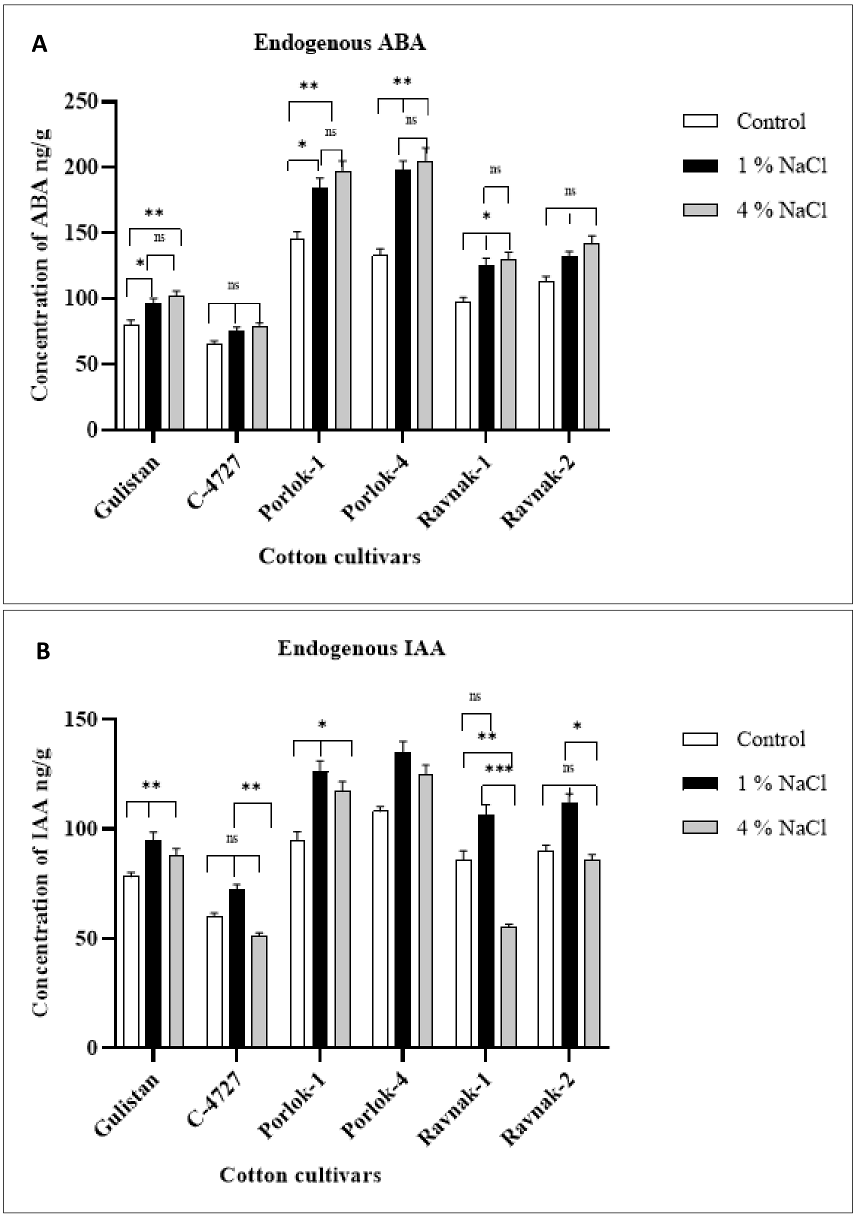

Fig. 1. Concentrations of endogenous ABA (A) and IAA (B) in seedlings of various cotton cultivars under salinity stress. Vertical bars represent ± standard error (n = 3). *, **, *** Significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively. NS: not significant.

Table 1. Exogenous effects of ABA and IAA on the activity of SOD in seedlings of various cotton cultivars under salinity stress

Samples	SOD activity, U/mg protein					
	Cotton varieties					
	Guliston	C-4727	Porlok-1	Porlok-4	Ravnak-1	Ravnak-2
Control	2±0.9^{d}	1.5±0.05^{d}	8.06±0.3^{b}	6.48±0.25^{b}	5.46±0.3^{c}	5.84±0.32^{c}
1 % NaCl	2.5±0.12^{d}	1.75±0.11^{d}	10.2±0.45^{a}	8.05±0.3^{b}	5.85±0.25^{c}	7.5±0.55^{b}
1 % NaCl + ABA	3.1±0.14^{c}	1.9±0.20^{d}	12.75±0.52^{a}	10.06±0.45^{a}	7.00±0.36^{b}	8.33±0.62^{b}
1 % NaCl + IAA	2±0.85^{d}	1.17±0.13^{d}	8.72±0.35^{b}	7.5±0.25^{b}	4.98±0.42^{c}	6.2±0.50^{b}
4 % NaCl	3.5±0.18^{c}	1.5±0.18^{d}	15.00±0.4^{a}	10.47±0.35^{a}	6.84±0.25^{b}	10±0.45^{a}
4 % NaCl + ABA	5.1±0.14^{c}	1.8±0.12^{d}	19.86±0.30^{a}	14.8±0.67^{a}	8.39±0.30^{b}	12.6±0.10^{a}
4 % NaCl + IAA	2.5±0.09^{d}	1.5±0.17^{d}	1.51±0.05^{d}	3.4±0.15^{c}	6.22±0.40^{b}	4.6±0.20^{c}

Note: Data are presented as mean ± standard deviation (M ± SD, n = 3). Different superscript letters indicate values that differ significantly at $p \leq 0.05$ according to Student's *t* test. ABA – abscisic acid; IAA – indole-3-acetic acid; SOD – superoxide dismutase; NaCl – sodium chloride.

As compared to control samples, in 1 % NaCl medium, SOD activity in Porlok-1 and Porlok-4 cultivars increased by 26 % and 24 %, respectively and in high salinity medium with 4 % NaCl by 86 % and 61 %, respectively. These results further confirm that Porlok-1 and Porlok-4 demonstrated their resistance even under high salinity condition. In addition, the SOD activity was found to increase by 25 %, 7 % and 28 % in Guliston, Ravnak-1 and Ravnak-2 in 1 % NaCl medium, respectively, when compared to the controls.

Table 2. Exogenous effects of ABA and IAA on APX activity in the seedlings of various cotton cultivars under chloride salinity stress conditions

Samples	The activity of APX U/ mg protein					
	Cotton varieties					
	Guliston	C-4727	Porlok-1	Porlok-4	Ravnak-1	Ravnak-2
Control	17.6±0.75^{d}	10.6±0.40^{d}	23.74±1.00^{c}	34.35±1.60^{b}	24.48±0.65^{c}	25.42±0.28^{c}
1 % NaCl	18.7±0.50^{d}	8.04±0.35^{d}	39.27±1.10^{b}	48.28±1.20^{b}	30.26±0.45^{b}	29.22±0.85^{c}
1 % NaCl + ABA	23.3±1.00^{c}	10.00±0.25^{d}	57.62±1.20^{a}	61.56±1.75^{a}	36.3±0.75^{b}	35.47±1.13^{b}
1 % NaCl + IAA	12.4±0.50^{d}	7.5±0.15^{d}	18.6±0.85^{d}	25.00±1.10^{c}	13.81±0.55^{d}	17.5±0.48^{d}
4 % NaCl	26.9±1.00^{c}	15.11±0.65^{d}	50.78±0.95^{a}	69.45±0.85^{a}	41.54±1.30^{b}	32.35±0.92^{b}
4 % NaCl + ABA	29.00±1.20^{c}	13.1±0.50^{d}	54.7±1.12^{a}	75.86±2.50^{a}	55.65±0.68^{a}	35.57±0.25^{b}
4 % NaCl + IAA	24.7±1.10^{c}	11.66±0.30^{d}	22.5±1.10^{c}	15.2±0.55^{d}	13.82±0.53^{d}	10±0.46^{d}

Note: Data are presented as mean ± standard deviation (M ± SD, n = 3). Different superscript letters indicate values that differ significantly at $p \leq 0.05$ according to Student's *t* test. ABA – abscisic acid; IAA – indole-3-acetic acid; SOD – superoxide dismutase; NaCl – sodium chloride.

It was observed that SOD enzyme activity increased in all varieties of cotton with increasing salinity level. In addition, it was observed that in all varieties of cotton, the exogenous ABA increased the activity of SOD by several times, as compared to IAA (in the medium with 1 % NaCl+ABA, in Porlok1 and Porlok-4, it was the maximum one). As compared to the controls under 4 % NaCl+ABA condition, the SOD activity was found to be 46 %, 32 % and 41 % higher in Guliston, Porlok-1 and Porlok-4 varieties, respectively. Under 1 % and 4 % NaCl + IAA treatments, SOD activity sharply declined in all varieties: Gulistan (28 %), C-4727 (31 %), Porlok-1 (81 %), Porlok-4 (67 %), Ravnak-1 (15 %) and Ravnak-2 (54 %).

The observed increase in SOD activity may result from either the activation of latent isoforms or the de novo synthesis of the enzyme. For example, it was shown that the activation of SOD in soybean, cabbage, chickpea and rosemary plants under salinity is associated with the accumulation of transcripts of the genes encoding it (33). These results are in agreement with previous studies, which also reported higher SOD activity in the leaves of the salt-tolerant cotton cultivar CCRI-44 compared to the salt-sensitive Sumian 12 variety. This shows that the salt-resistant variety has the ability to quench O_2^- radicals (34). Similar results were reported in other cotton varieties (34).

After the 1 hr exposure to salinity stress, high APX activity in Porlok-4 (34.35 (U/mg protein) and low one was found in C-4727 (10.6 U/mg protein) (Table 2). In particular, in 1 % NaCl media, APX activity in Porlok-1 and Porlok-4 increased by 65 % and 40 %, respectively and by 47 % and 27 % under the effect of exogenous ABA, respectively. It was found that the enzyme activity decreased by 24 % in the C-4727.

As it can be seen, as compared to IAA, ABA consistently enhanced enzyme activity across all cultivars (Table 2). Enzyme activity also increased with increasing salinity in all cotton cultivars under study. In high salinity medium with 4 % NaCl in Guliston, C-4727, Porlok-1, Porlok-4, Ravnak-1 and Ravnak-2, an increase by 53 %, 42 %, 114 %, 102 %, 70 % and 27 %, respectively, was demonstrated. Even under high salinity, ABA increased the enzyme activity by maximum 34 % in Ravnak-1 and minimum by 7.8 % in Guliston, while IAA reduced enzyme activity in all varieties (by 56 % and 78 % in Porlok-1 and Porlok-4, respectively).

Under saline conditions, APX is a key component of the plant antioxidant defense system; however, the extent and pattern of its activity may vary depending on the plant species. For example, the APX activity in the leaves of the halophyte *Sosuvium portulacastrum* commonly known as shoreline purslane increased transiently in response to salinity, while the enzyme activity was found to be unchanged in the glycophyte *Brassica juncea* known by common name Chinese mustard (35). Ascorbate peroxidase activity increased in maize roots when exposed to salt stress (36). In bean plants, enzyme activity also rose with the salinity level, with a similar increase seen in both resistant and susceptible varieties (34).

Proline, a non-toxic osmoprotectant, accumulates in many plants under stress conditions, including salinity and water deficiency (36). Consequently, the impact of ABA and IAA on proline concentrations in cotton leaves was explored. There were significant differences in proline content between various cotton varieties, which correlated with their salinity tolerance and the presence of specific genomic elements (Fig. 2). The lowest levels of proline were found in the C-4727 variety, whereas Porlok-1 and Porlok-4 exhibited the highest concentrations. After 1 hr of exposure to salinity stress, the control samples of Porlok-1 and Porlok-4 had the highest concentrations of proline (60.0 and 53.0 µmol/g fresh weight, respectively), while in C-4727, lower concentrations of proline were biosynthesized (22.0 µmol/g) (Fig. 2). In 1 % NaCl, proline biosynthesis in Guliston, C-4727, Porlok-1, Porlok-4, Ravnak-1 and Ravnak-2 demonstrated an increase by 39 %, 27 %, 45 %, 41 %, 24 % and 24 %, 21 % respectively, as compared to the controls. Exogenous ABA and increasing salinity caused sharp increase in proline synthesis.

In particular, the maximum proline biosynthesis was found in the medium with 1 % NaCl + ABA in Porlok-1 (43 %) and C -4727 (36 %), as compared to the controls in the medium with 1 % NaCl. Under the influence of the exogenous IAA, it was found that proline synthesis was sharply reduced in all cotton cultivars under study, as compared to the control with 1 % NaCl (especially by 43 % in the C-4727). Therefore, it was found that exogenous ABA had a positive effect on the increase of proline concentrations in all cotton cultivars.

Previous studies have shown that free proline has been widely studied in different plant species. Previously, under the influence of salinity stress, in *Pisum sativum* (peas) (37), *Brassica juncea* (brown mustard) (38) and *Triticum aestivum* (common wheat) (39), accumulation of free proline was observed. Accumulation of proline can occur due to an increase in synthesizing enzymes or a decrease in its oxidizing enzymes. ABA was found to be directly involved in proline accumulation and mobilization in the leaves of *Brassica rapa* (turnip) (40). Many amino acids, including proline, alanine, arginine, glycine, serine, leucine, valine, citrulline, ornithine, glutamine and asparagine, accumulate in plants in a saline environment (40). Proline, a key osmolyte, accumulates in plants under stress conditions. Apart from serving as an osmolyte, it also aids in scavenging ROS, stabilizing subcellular structures, balancing cellular redox status,

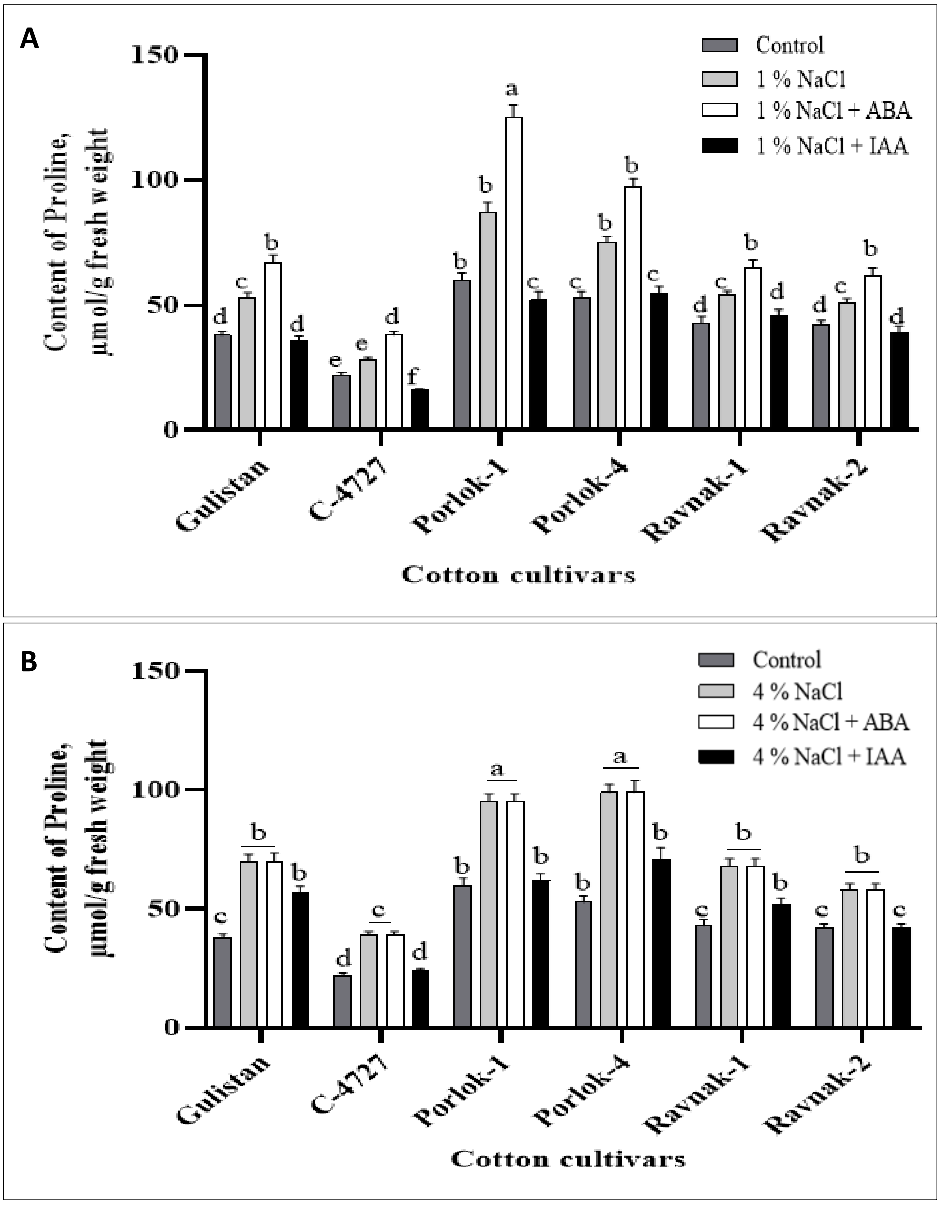

Fig. 2. Exogenous effects of ABA and IAA on the proline concentrations in seedlings of various cotton cultivars under salinity conditions, (**A**: 1 % NaCl; **B**: 4 % NaCl). Vertical bars represent ± standard error (n = 3).

providing energy and functioning as a signalling molecule (41). Although the accumulation of proline is a typical plant response to salt stress, the degree of accumulation varies between salt-tolerant and salt-sensitive varieties. Our findings demonstrate that proline levels were significantly higher in the leaves of the tolerant cultivar compared to the sensitive cultivar. As the primary products of photosynthesis in cotton, reducing sugars are also sensitive to abiotic stresses (4). Given that Porlok cotton varieties exhibit greater tolerance to abiotic stress (23), we studied the levels of reducing sugars in different cotton genotypes.

The levels of reducing sugars in cotton leaves were found to vary based on the presence of the vector construct in the plant's genome (Table 3). Specifically, the knockout gene-containing cultivars (Porlok-1 and Porlok-4) showed elevated reducing sugar concentrations when compared to the non-transformed cotton cultivars. These results align with previous studies regarding sugar content in different cotton genotypes under salt stress conditions (4).

After 1 hr exposure to salinity stress, most reducing sugars in the control samples of Ravnak-2, Porlok-4 and Porlok-1 were found to be synthetized. In plants, under salinity stress conditions, accumulation of reducing sugars is reported which allowed the plants to adjust osmotically. Plants have been attributed an adaptation by increase in carbohydrate level in response to stresses. In addition to osmoregulators reducing sugars may act as osmoprotectants for protein under stressed condition (42).

As compared to the control samples, biosynthesis of reducing sugars in Guliston, C-4727, Porlok-1, Porlok-4, Ravnak-1 and Ravnak-2 cotton cultivars in 1 % NaCl medium increased by 0.76, 0.56, 1.05, 1.16, 0.96 and 1.33 mg/mL respectively. As a result of the exogenous ABA effect, as compared to the controls, it was found to increase by 0.96 (Guliston), 0.59 (C-4727), 1.35 (Porlok-1), 1.4 (Porlok-4), 1.14 (Ravnak-1) and 1.20 (Ravnak-2) mg/mL respectively. When compared to the control of the values in 1 % NaCl under the effect of exogenous IAA, it was found that the concentrations of reducing sugars decreased sharply by a maximum of 54 % in the Guliston variety and a minimum of 4 % in the Ravnak-1 variety with increasing salinity level, under 4 % NaCl conditions; the synthesis of reducing sugars increased in Porlok-1, Guliston and Porlok-4, as compared to control samples by 1.25, 0.96 and 1.28 mg/mL, respectively. As a result of using the 4 % NaCl + ABA medium, the highest increase in reducing sugar content compared to the 4 % NaCl control group was observed in the cotton cultivars Ravnaq-1 (22.6 %) and C-4727 (17.3 %).

Table 3. Exogenous effects of ABA and IAA on the concentrations of reducing sugars in seedlings of various cotton cultivars under salinity stress

Samples	Concentrations of reducing sugars, mg/mL					
	Cotton varieties					
	Gulistan	**C-4727**	**Porlok-1**	**Porlok-4**	**Ravnak-1**	**Ravnak-2**
Control	0.60±0.02^{b}	0.43±0.01^{c}	0.76±0.04^{b}	0.86±0.02^{b}	0.71±0.03^{b}	0.93±0.03^{a}
1 % NaCl	0.76±0.03^{b}	0.56±0.02^{b}	1.05±0.03^{a}	1.16±0.01^{a}	0.96±0.04^{a}	1.33±0.05^{a}
1 % NaCl + ABA	0.96±0.03^{a}	0.59±0.02^{b}	1.35±0.03^{a}	1.4±0.03^{a}	1.14±0.04^{a}	1.20±0.07^{a}
1 % NaCl + IAA	0.35±0.01^{c}	0.40±0.01^{b}	0.88±0.01^{b}	0.61±0.02^{b}	1.00±0.03^{a}	0.93±0.06^{a}
4 % NaCl	0.96±0.03^{a}	0.52±0.01^{b}	1.25±0.02^{a}	1.28±0.03^{a}	1.06±0.05^{a}	1.33±0.04^{a}
4 % NaCl + ABA	1.06±0.04^{a}	0.61±0.02^{b}	1.4±0.03^{a}	1.50±0.02^{a}	1.30±0.04^{a}	1.43±0.02^{a}
4 % NaCl + IAA	0.26±0.01^{c}	0.29±0.01^{c}	0.96±0.01^{a}	0.88±0.01^{b}	0.41±0.02^{b}	0.50±0.02^{b}

Note: Data are presented as mean ± standard deviation (M ± SD, n = 3). Different superscript letters indicate values that differ significantly at *p* ≤ 0.05 according to Student's *t* test. ABA – abscisic acid; IAA – indole-3-acetic acid; SOD – superoxide dismutase; NaCl – sodium chloride.

In addition, it was found that under the influence of exogenous IAA, concentrations of reducing sugars were significantly reduced in all cotton varieties, as compared to the controls in 4 % NaCl medium, especially in Gulistan cotton variety (by 72 %).

Malondialdehyde concentrations were estimated in the seedlings of cotton varieties studied under stressful conditions (Fig. 3). Our findings demonstrated that the MDA concentrations measured after the 1 hr exposure to stress in the control samples of Porlok-1, Porlok-4, Guliston and Ravnak-2 were low (respectively 17.3; 20.8; 25 and 29 × 10^{-5} mM/g); the highest concentrations could be seen in C-4727 (55.8 × 10^{-5} mM/g). MDA biosynthesis in Guliston, C-4727, Porlok-1, Porlok-4, Ravnak-1 and Ravnak-2 in 1 % NaCl medium was shown to increase by 15.2 %, 17 %, 6.9 %, 26 %, 17 % and 14 %, respectively. In 1 % NaCl + ABA and 4 % NaCl + ABA conditions, the synthesis of MDA as a result of the exogenous effect in C-4727 was sharply reduced by 40 % and 50 %, respectively, when compared to the control samples with 1 % NaCl and 4 % NaCl. Therefore, it was shown that concentrations of reducing sugars in all cotton varieties sharply decreased under the influence of exogenous IAA; on the contrary, they increased under the influence of ABA. In addition, MDA biosynthesis increased dramatically in all cotton cultivars with increasing salinity. It was observed that MDA biosynthesis decreased by 25 %, 32 %, 36 %, 20 % and 35 %, respectively, in Guliston, Porlok-1, Porlok-4, Ravnak-1 and Ravnak-2 in 4 % NaCl + ABA.

The reduction in MDA under ABA treatment indicates a mitigation of oxidative stress, suggesting enhanced stress tolerance. Thus, the MDA concentrations were found to increase in all cultivars under study with increasing salinity. In addition, it was found that MDA was accumulated in low concentrations in Porlok-1 and Porlok-4. On the contrary, it was shown that the highest MDA concentrations were synthesized in C-4727. As a result of the exogenous ABA effect, the MDA concentrations sharply reduced and this is one of the important indicators of plant cell resistance to stress conditions.

Conclusion

Due to the higher endogenous levels of ABA, proline and reducing sugars in the Porlok-1, Porlok-4 and Gulistan cotton cultivars, as well as the high activities of important antioxidant enzymes such as SOD and APX, low concentrations of synthesized MDA are observed in these cotton varieties. Additionally, it can be concluded that a sharp decrease in the amount of MDA has been caused by less damage to the biological membranes of these cultivars compared to other

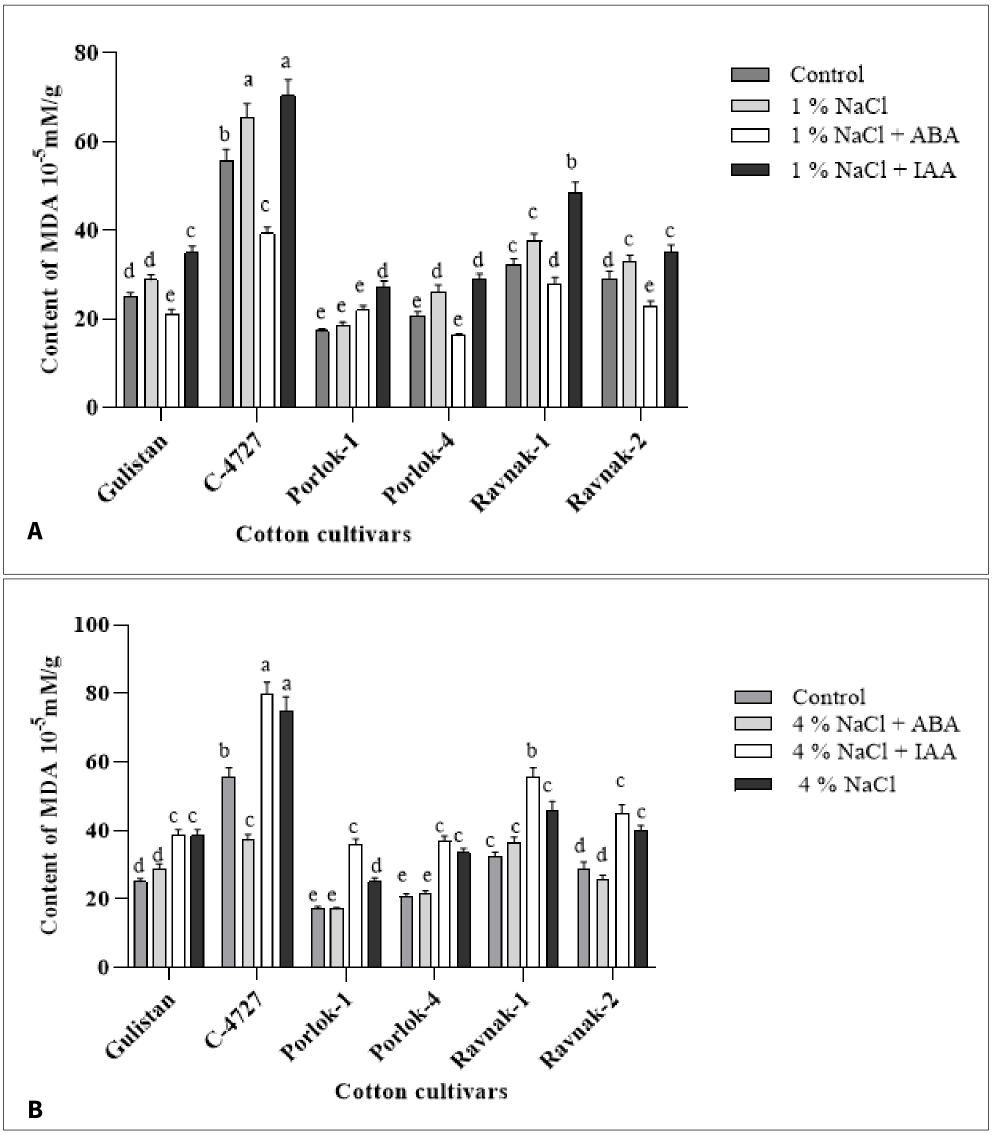

Fig. 3. Exogenous effects of ABA and IAA on the MDA concentrations in seedlings of various cotton cultivars under salt stress. Vertical bars represent ± standard error (n = 3). (**A**: 1 % NaCl; **B**: 4 % NaCl). Means with the same letter(s) on top of the columns do not differ significantly.

varieties. Higher concentrations of free proline, reducing sugars and phytohormones have been detected in these varieties, along with correspondingly higher activities of antioxidant enzymes, as compared to the unmodified cotton varieties.

Future studies should focus on elucidating the molecular mechanisms underlying phytohormone-mediated regulation of the antioxidant and pro-oxidant balance in cotton under combined abiotic stresses such as salinity and heat. Moreover, transcriptomic and metabolomic analyses of gene-knockout and marker-assisted cultivars could provide deeper insight into stress signalling pathways and identify key genes responsible for enhanced stress tolerance. Field-based experiments across different environmental conditions are also recommended to validate the laboratory findings and assess the practical applicability of phytohormone treatments in cotton production.

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Authors' contributions

KK and SN completed the literature review, designed and performed the experiments, AA provided overall supervision of the project, contributed to conceptualization and critically reviewed the manuscript. NK wrote, edited and finally reviewed the manuscript. All authors read and approved the final manuscript.

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Only minimal assistance was used from QuillBot for paraphrasing selected sentences. All scientific content, interpretation and conclusions were developed independently by the authors. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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