Plant growth promoting *Bipolaris* sp. CSL-1 mitigate salinity stress in soybean via altering endogenous phytohormonal level, antioxidants and genes expression

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Research

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Abstract

Background

Salinity stress is one of the most devastating environmental stress that inhibits plants growth and development. Many strategies including plant growth promoting fungi have been reported to mitigate salt stress.

Results

In this study, we adopted environmental friendly technique and screened different plant growth promoting fungi for different PGP traits and salinity stress. Among these isolate CSL1 were selected based on the basis of plant growth promoting characteristics producing IAA, GAs, organic acid and tolerance to NaCl stress. Furthermore, inoculation of fungal isolate CSL1 significantly increased shoot length (16%), root length (37%), shoot fresh and dry weight (19% and 25%), root fresh and dry weight (47 and 51%) and chlorophyll content (24%) under NaCl stress (200 mM). Endogenous ABA level (0.77 folds) were significantly decreased while SA contents (16%) were increase in CSL1 inoculated plants under NaCl stress. Similarly, higher level of antioxidants such as MDA (2 folds), SOA (29%), POD (8 folds) and PPO (3 folds) was observed in NaCl treated non-inoculated plants. ICP analysis showed an increase in Na^+ (11 folds) and decrease in K^+ content (15%). Furthermore, CSL-1 inoculation improved soybean adaptability against NaCl stress and a significant decrease in *GmFDL19* expression (5 folds) *GmNARK* (4 folds) and *GmSIN1* (3 folds) was observed. However, higher expression of *GmAKT2* (15%) were observed in CSL-1 treated plants.

Conclusion

Fungal isolate CSL-1 have capability to mitigate salinity stress in soybean, increase plant growth and could be used as valuable ecofriendly microorganism resource, low cost based biotechnological approach for sustainable agriculture in salt affected areas.

Introduction

Salinization of soil with sodium chloride is one of the most devastating environmental stress that inhibits plants functions, causing reduction of crops yield and quality [1]. Currently 20% of total cultivated and 33% of irrigated agricultural land is affected by salinity stress worldwide. It has been estimated that 50% of all arable land will be impacted by salinity stress in 2050 globally and results in more than US $12 billion in annual losses due to reduced crop productivity [2, 3]. Salinity is recognized as the main threat to environmental resources in several countries, affecting almost 1 billion hectors worldwide, which represent about 7% of the earth continental area [1, 3]. Soil salinity has been reported to limit crops productivity by impairing the root growth, nutrient uptake and affect metabolic processes [4–6]. Salinity stress affect various physiological, morphological and biochemical process which results in reduction of crops biomass and productivity [6, 7]. Morphological changes observed under salinity stress on all growth stages including germination, seedling, vegetative and maturity stages [8, 9]. Biochemical changes include modulating phytohormones (decrease stress hormone ABA and increase defense hormones SA), change in ion uptakes (accumulation or removal of ions), activating antioxidants enzymes and accumulation of ROS generation, and altering photosynthetic pathways [5]. Salt induce ion toxicity in plant cells through higher influx of Na + ion and efflux of K + ion. Na and K homeostasis play a vital role in the growth and development of crop plants [10], and alters the metabolic process and ion mobilization system [3].

Diverse strategies like development of salt tolerant verities, plant genetic engineering, shifting crop calendars, transgenic plants, leaching of salt from root zone and chemical application to decrease the toxic effects caused by salinity stress [11, 12]. Although the use of these approaches for sustainable management can ameliorate yield reduction under salinity stress and its implementation is often limit because of cost, time taking and availability of resources. Evolving efficient, low cost, environmentally friendly and easily adaptable methods for the mitigation of salinity stress is a major challenge for agricultural scientist. Recently the use of soil borne microbes and plant associated fungi has been extensively used for their capacity to promote plant growth and environmentally friendly method for alleviating the toxic effects of salinity stress [13–16]. Microorganisms have the ability to produce phytohormones, siderophore production and organic acid production [13, 14]. Various phytohormones producing fungi were reported to enhance plant growth under various environmental stress [14, 17].

Soybean growth is affected by various environmental factor including salinity stress [18]. Soybean are rich sources of protein, oil and flavonoids [12]. However the contents of all these materials were reduced in soybean exposed to salinity stress [12]. Many soybean genes have been found to confer salinity stress tolerance (Shuo Li, 2019). *GmFDL19* enhance tolerance to salt stress by reducing Na ion and malondialdehyde content, increase the activity of several antioxidant enzyme and chlorophyll content in soybean [19]. Similarly, *GmNARK* was induce by ABA and NaCl treatment and sensitivity to salt stress. On the other hand, *GmSIN1* exhibited insensitivity to high salinity and had higher activities of antioxidants like SOD and POD. Similarly, salinity stress inhibits K uptake, while *GmAKT2* regulate K transport through electric cell signaling and membrane excitability. Higher expression of *GmAKT2* in soybean under salinity stress regulate potassium gradient that play a vital role in osmotic adjustment, regulation of membrane potential and source of energy in plants. Therefore, in current study, previously reported plant growth promoting fungi from Crop physiology lab, Kyungpook National University, South Korea were grown and screen on PDA plates with different NaCl concentration. Based on their high tolerance, isolate CSL1 were selected and upon inoculation observed the growth attribute, endogenous phytohormones, antioxidant enzyme responds and expression of different salt related genes under NaCl stress in soybean plants.

Results

Isolation, screening and identification

In vitro IAA, GAs and organic acid quantification of CSL-1

The culture filtrate of isolate CSL-1 was quantified for IAA, GAs and organic acid production by using GC/MS and HPLC. IAA results showed that isolate CSL-1 produce a significant amount of IAA (Fig 1A). On the other hand, different both bioactive and non-bioactive GAs were observed in cultural broth of CSL-1 (Fig 1B). Organic acid analysis reviled that the CF of isolate CSL-1 produced citric acid, quinic acid and succinic acid etc. Our results showed that highest amount of quinic acid and succinic acid were produce by CSL-1 in czapek media (Fig 1C).

Bacterial isolate CSL-1regulates soybean growth under salinity stress

Salinity stress adversely affected the growth attributes of soybean plants. However, isolate CSL-1 was found significantly enhanced salinity stress tolerance by regulating plant growth, plant biomass and other biochemical attributes (Fig. 2; Table 1). Under salinity stress, a decrease in shoot length (25%), root length (46%), shoot fresh and dry weight (25% and 37%), root fresh and dry weight (49 and 51%) were observed at 200mM of NaCl stress compared with control plants. However, isolate CSL-1 induced significant increase in shoot length (16%), root length (37%), shoot fresh and dry weight (19% and 25%), root fresh and dry weight (47 and 51%) in salinity stressed plants as

compared to 200mM NaCl treated plants (Table 1). Similarly, chlorophyll analysis results revealed an increase in chlorophyll content (22%), in CSL-1 inoculated soybean plants compared to control plants under normal conditions (Table 1). However, when plants were subjected to NaCl stress, a decreased in chlorophyll content (25%), were observed (Fig.). However, inoculation with halotolerant CSL-1 mitigate NaCl stress and increased the chlorophyll content (24%) (Table 1).

Effect of isolate CSL-1 on plant endogenous phytohormones

Salinity stress induced a significant increase in ABA (160%) contents of soybean plants (Fig 3A). However, in CSL-1 inoculated soybean, a decrease in ABA (133%) contents were observed compared with NaCl stress plants (200mM) (Fig 3A). However, in contrast to endogenous ABA levels, an increase of 2% in the endogenous SA contents under normal and 16% in NaCl stress was observed in isolate CSL-1 inoculated soybean plants compared with NaCl stress plants (Fig 3B).

Antioxidants quantification in soybean plants under salinity stress

In current study, change in different antioxidants were investigated in soybean plant treated with NaCl stress with the inoculation of CSL-1. Malondialdehyde (MDA content were evaluated to assess the extent of lipid peroxidation (LPO). MDA content results showed that higher level of MDA (2 folds) was observed in soybean treated with NaCl stress (200mM) compared with CSL-1 inoculation (0.5 folds) (Fig 4A). Similarly, SOA results showed increase to NaCl treatment (29%). However, the production of SOA was significantly inhabited in CSL-1 inoculated soybean plants (16%) compared with NaCl stress plants (38%-91%) (Fig 4B). A similar trend was also observed in POD, and PPO content that showed lower POD and PPO content in salinity stress soybean plants inoculated with isolates CSL-1 (Fig 4C&D). To further elucidate the salinity stress mitigation, the GSH content in soybean plants were examined that showed a significant enhance in GSH content (56%-179%) in CSL-1 inoculated plants compared with control plants (37%-136%) under varying NaCl level (200mM) (Fig 4E) In contrast to POD, PPO, LPO; total protein content showed a significant decreased (31%) in salinity stress compared with control plants. However, total protein content increased in soybean plant inoculation with halotolerant CSL-1 (18%) compared with control stressed plants (Fig 4F).

Role of bacterial isolates in ion uptake during salinity stress

Inductively-coupled mass spectrometry (ICP) analysis of Na and K content were investigated. Our results showed that soybean plant treated with NaCl (200mM) increased Na content (11 folds) (Fig 5A). However, in CSL-1 inoculated plant a significant decrease in Na content were observed (7 folds) (Fig 5A). Compared with Na, K content showed a significant decrease in salinity stress (15%) compared with control plants (Fig 5B). On the other hand, K uptake content increased (7%) in soybean plants inoculated with halotolerant CSL-1 compared with control stressed plants (Fig 5B).

Gene expression during salinity stress and bacteria inoculation

Gene expression results that *GmFDL19* was highly expressed (8.6 folds) in soybean plant exposed to NaCl stress (200mM) (Fig 6A). However, in CSL-1 inoculation improved soybean adaptability against NaCl stress and a significant decrease in *GmFDL19* expression (5 folds) in soybean plants exposed to NaCl stress (200mM) (Fig 6A). Similarly, *GmNARK* and *GmSIN1* results showed a significant increase in the expression of *GmNARK* and *GmSIN1* was observed in soybean plants exposed to NaCl stress (7folds and 6 folds) (Fig 6B&C). However, CSL-1 inoculation enhanced soybean resistance to NaCl stress and reduced the expression of *GmNARK* and *GmSIN1* (4 folds and to 3 folds) in soybean plants exposed to NaCl stress (200mM) (Fig 6B&C). In contrast to *GmFDL19*, *GmNARK* and

GmSIN1, Gene expression results of *GmAKT2* decrease (24%) in salinity stress compared with control plants. However, inoculation with halotolerant CSL-1 a higher expression of *GmAKT2* (15%) were observed in soybean plant compared with control stressed plants (Fig 6D).

Discussion

Higher salinity stress has a significant negative impact on the productivity of crops [20]. Salinity exhibit a spectrum of brutal effects on morphological, biochemical, physiological and molecular process of plants such as seed germination, growth, nutrient uptake [20]. In current observation, exposure of the experimental plant to NaCl stress inhabit soybean growth, root/shoot length, and biomass (fresh/dry weight) (Fig 2; Table 1). Similarly, beneficial effect of isolate CSL-1 on soybean growth were observed in root/shoot length, and biomass (fresh/dry weight) (Fig 2; Table 1). The result is supported with the finding of [20-22] who reported that NaCl tolerant *Penicillum brevicompactum, P. chrysogenum*, and mycorrhizal fungi augment salinity stress and enhance plant growth attribute in tomato, lettuce and pepper. Chlorophyll content play a vital role in photosynthesis and indicate the relative plant tolerance to salinity stress to some extent [23]. Under salinity stress a decrease in chlorophyll content were observed in soybean plant (Table 1). The reduction in chlorophyll content under salinity stress might be cause by decrease in K absorption [24]. Higher Na uptake has antagonistic effect on K absorption and suppress specific enzymes that are responsible for the synthesis of chlorophyll content [24, 25]. However, an increase in chlorophyll content of CSL-1 inoculated plant were observed suggested that chlorophyll synthesis was less affected by salinity stress. Higher chlorophyll content were reported previously in zucchini, pepper and tomato plants under saline stress treated with mycorrhizal fungi [22, 26, 27]. Higher Na content in soil inhibit the uptake of K and results in nutrient imbalance [28]. K play a vital role in stomatal movement, and enzymes activation [29]. In current investigation, salinity stress significantly decreases K content (Fig 5B). However our results support the previous finding of [30, 31], that reported that upon the inoculation of mycorrhizal fungi enhance K uptake under salinity stress. Similarly a decrease in Na content were also reported in mycorrhizal inoculated plants previous [30, 32-34], that support the results of our current investigation that soybean plants inoculated with CSL-1 decrease Na uptake and enhance plant growth (Fig 5A).

Phytohormones producing plant growth promoting endophytic fungi produce different plant hormones, i.e. gibberellins, indole-3-acetic acid and organic compounds that help the plants to tolerate or avoid abiotic stress including salinity stress [35, 36]. Previously [37] and [38] reported that GAs producing endophytic fungi *Phoma herbarum* and *Penicillium* sp mitigate salinity stress and enhance plant growth in soybean and cucumber. Isolate CSL1 produce different bioactive and inactive GAs (Fig 1B). Similarly IAA is a key phytohormone, play important role in plant growth and tolerate plant to different abiotic stresses by regulating several developmental and physiological process [35, 39]. This also show consistency toward the current finding of our study that isolate CSL1 produce IAA and enhance growth, biomass and tolerate soybean plants to salinity stress upon inoculation (Fig 1A). Other important osmolytes in plants are organic acids that are found in plant vacuoles and regulate crucial role in abiotic stress tolerance including salinity stress [1]. Previously, reported that fungal endophytes release organic compound that assimilate plant growth under saline condition [40, 41]. Isolate CSL1 used in our current study produce different types of organic acid that help soybean tolerance to NaCl stress (Fig 1C).

Under salinity stress, higher ROS (superoxide, singlet oxygen) are generated in different compartment of plant cell and disrupt the normal metabolism of plants [42, 43]. Higher MDA contents in soybean plants under salinity suggest enhance in lipid peroxidation and protein oxidation, which is consistent with the previous finding of [44, 45]. For mitigation of salinity stress and ROS generation, plant activate its antioxidants defense systems such as POD and other non-enzymatic antioxidants (PPO and total protein). SOD mediate detoxification of superoxide radical's and

prevent stress induce cellular damages. Furthermore, non-enzymatic antioxidant such as total protein, glutathione and phenolic compounds in plants are known to be involved in the internal detoxification of NaCl induce toxicity. Higher TP, PPO and GSH were observed in CSL-1 inoculated soybean plants under NaCl stress (Fig 4). Similarly, our results suggest that total protein content decreased in NaCl stress, while inoculation of halotolerant CSL-1 increased TP content in salinity stressed soybean (Fig 4F). These antioxidants play an important role in salinity tolerance and better growth, which were reflected in various morphological and physiological parameter of soybean growth under NaCl stress. Previously several authors reported higher enzymatic antioxidant activities in endophytic fungi inoculated plants compared with non-inoculated plants [32, 33, 46]. To cope with damage caused by salinity stress, plants have regulate hormonal synthesis [47, 48]. Abscisic acid are involved in cell responses to salinity toxicity [48]. It has been shown that salinity stress increase the ABA content in plants [48, 49]. Plant-microbe interaction has been previously reported that mitigate the adverse effects of abiotic stress in plants through reducing ABA levels [50]. The results of our current study showed that the inoculation of NaCl tolerant CSL-1 enhance plant growth parameters and mitigate NaCl stress through the reduction of ABA accumulation (Fig 3A).

Glycine max is a protein and oil rich crop however, salinity adversely affected its growth and development. Where salinity is a prominent soil problem, there is also a natural solution for it in the form of salt stress tolerant fungi which exhibit plant indigenous salt stress. To validate the function of fungi *Bipolaris* sp. in response to salt stress we evaluated the expression of salt induced *G. max* nodule autoregulation receptor kinase (*GmNARK*) gene which is induced by salt stress. The result showed that *GmNARK* expression level was higher in NaCl treated plants while, NaCl treated plants inoculated with fungus showed reduced expression level as shown in the (Fig 6B). This phenomenon indicates that *Bipolaris sp*. is significantly involved in mitigation of salt stress. Previous studies show that NARK is also involved in JA signaling and alter plant defense system [51]. Cheng C, Li C, Wang D, Zhai L and Cai Z [52] reported that overexpression of *GmNARK* in *Arabidopsis* enhanced ABA and NaCl tolerance which shows that this gene is keen candidate for salt tolerance. Further they predicted that identical to calcineurin B-like protein (CBL10), NARK may be involved in fast signal transduction in cell under salinity. CBL10 is function as a calcium sensor in response to salt stress. Our study showed that *GmFDL19* expression pattern was identical to *GmNARK* (Fig 6A). The expression level was reduced in the NaCl treated fungi inoculated plant as compared to the pure NaCl treated plants which shows that *Bipolaris* sp. is functionally involved in salt tolerance. We further predicted that *Bipolaris* sp. is involved in NaCl uptake which protect the plant from salt stress du to prevention of direct contact of NaCl to plant cells. *GmFDL19* is also salt induce gene and express during salt stress by reducing the uptake of Na^+ and enhance expression of stress and ABA responsible genes [19]. The overexpression of *GmFDL19* in soybean positively regulate multiple stresses by activation of antioxidant machinery. *GmAKT2* is a K^+ transporter gene involved in plant stress tolerance. Potassium is a key component of cell which play an important role in growth and development. It is been studied that K^+ alter plant metabolic and hormonal pathway which further enhance plant tolerance to multiple stress [53]. Contradictory to *GmNARK* and *GmFDL19*, *GmAKT2* expression reduced in NaCl treated plants as compared to NaCl treated fungi inoculated plants (Fig 6D). This pattern of expression shows that during the salt stress, AKT2 downregulates which reduces K^+ transportation through the channel. However, the fungi inoculated plants reduces salt stress and shows upregulation of AKT2 compared to NaCl treated plants which also enhance K^+ transportation. To suppress the stress condition, enough concentration of K^+ is needed which could be achieved by the expression of AKT2. *GmSIN1* gene is involved in salt tolerance and root development which is achieved by regulation of ABA and ROS generation [54]. SIN1 gene alter ABA and ROS by induction of their responsible genes such as NCED3s and RbohBs respectively [54]. Our results evaluated that *GmSIN1* expression increased in salt stress while the expression level was less in the plant under stress condition coupled with fungi

inoculation as comparted to pure salt stressed plants (Fig 6C). This indicated that due to the stress mitigation of *Bipolaris* sp., the expression of *GmSIN1* reduced which is evident that SIN induced by salt stress.

Conclusions

In the present study, previously reported plant growth promoting fungi isolate CSL-1 was screened as salinity resistant with PGP traits (IAA, gibberellins and organic acid production). Here, inoculation of CSL-1 showed significant effect on plant growth promotion characteristics by altering plant endogenous hormones, antioxidant system, salt stress related gene expression level and Na^+ and K^+ uptake These observations demonstrated that isolate CSL-1 can mitigate salinity stress and valuable ecofriendly microorganism resource, low cost based biotechnological approach which can be used for sustainable agriculture in salt affected areas.

Materials And Methods

Isolation, screening and identification

Several plant growths promoting endophytic fungi (previously reported [17, 36, 55, 56] were deposited to gene bank in the crop physiology lab, school of applied biosciences, Kyungpook National University. These fungi were screened for NaCl stress resistance by using five concentration of NaCl (0mM, 50 mM, 100 mM, 150 mM, 200 mM and 250 mM) and 0.1% of culture aliquot was inoculated into 100 ml of sterilized czapek media and incubated in a shaking incubator at 30°C. Based on high tolerance during the screening experiments, isolate *Bipolaris* sp. CSL-1 was selected for further experimentation.

Isolate CSL-1produce IAA, GAs and organic acid

CSL-1 was grown in czapek media for seven days, centrifuged (500Xg, 15 mints) and analyzed for IAA, GAs and organic acid content. For IAA analysis, following the detail method of Khan et al., 2020, while for gibberellins the detail method of [56] was used. For organic acid analysis, the culture filtrate was filtered through a Millipore filter and 10 microliters of each sample was injected into high performance liquid chromatography column [waters 600E; Column: RSpak KC-811(.0x300 mm): Eluent: 0.1% H3Po4/H2O; flow rate: 1.0 ml/mint; temperature: 400C; HPLC]. For detecting the presence of organic acids, retention times and peaks area of chromatograms were compared with the standards from Sigma-Aldrich, USA.

Growth condition and treatments

Soybean seeds Vir pungsannamul were sown in trays filled with autoclaved horticultural soil [57, 58]. After 2 weeks of germination, seedlings were transferred to pots (440 x 270 x195mm) and were grown in a growth chamber at a temperature of 28°C ± 0.5°C for 16 hours and 25°C for 8 hours, 55%-65% relative humidity, and light intensity of 200 µmol m^{-2} s^{-1} under long-day conditions (16 h of day time and 8 h of night time). The experimental design includes (a) Control- well watered (b) Fungal treated SCL-1 (c) 200mM NaCl stress (d) 200mM NaCl stress with isolate CSL-1. To test plant protection activity of CSL-1 under salinity stress, 1L of CSL-1 were inoculated via the soil drench method, while distilled water was used for control plants for 2 weeks. After stress completion, growth attributes and biomass were determined, plants were immediately harvested in liquid nitrogen and stored at −80°C until further biochemical analyses. Before the harvest, chlorophyll contents were measured using the chlorophyll meter 300 (ADC BioScientific Ltd., Herts, England). For chlorophyll content SPAD meter was used [58].

Quantification of endogenous Phytohormones

Endogenous ABA measurement was performed following an established protocol [58, 59]. while Plant endogenous SA was extracted from freeze-dried soybean powder samples following the protocol [60] using HPLC.

Quantification of Total Protein and Antioxidants

For protein analysis, frozen fresh plant tissues were ground with ice-cold pestle and mortar, and then added to a solution of 50 mM phosphate buffered saline, 0.1% polyvinylpyrrolidone (PVP), and 1 mM ethylene diamine (EDTA). The homogenate was centrifuged at 10000 × g for 10 min at 4°C. The supernatant was immediately collected and used for protein and antioxidant enzyme quantification. For protein contents; Bradford [61] method was used in accordance with the BSA as a standard. Superoxide dismutase (SOD) was measured according to the detail method of Khan et al. LPO, GSH, POD and PPO was determined in accordance with the method described by [62], and [63] by measuring the absorbance at 290 nm, 470 nm and 42nm using a T60 UV-Vis spectrophotometer.

RNA extraction, cDNA synthesis and qRT-PCR analysis

For RNA extraction, the protocol of Chan et al was used, while for cDNA synthesis qPCRBIO cDNA Synthesis Kit from PCRBIOSYSTEMS was used. qRT-PCR was performed using qPCRBIO SYBER Green Kit from PCRBIOSYSTEM using detail method of Jan et al 2019.

Determination of Na and K uptake in plant

Na, and K content in shoot of bacterial inoculated and non-inoculated plant samples at varying concentration of NaCl was investigated according to the detail method of Khan et al by using inductively coupled plasma mass spectrometry 9ICP-MS; Optima 7900DV, Perkin-Elmer, USA).

Statistical analysis

The results were statistically evaluated by analysis of variance using SAS 9.4 software. All analyses were repeated thrice with 10 plants per replicate. Duncan's multiple range tests were used to determine 95% confidence level.

Declarations

Conflict of interests

The authors declare that they have no conflict of interest.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no competing interest.

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Authors' Contributions

L and MAK conducted the experiments. SA and MW helped in writing of the manuscript. MAK and conduct hormonal and antioxidant analysis. RJ and KMK conduct qRT-PCR analysis. IJL designed, supervised and financed the research. All authors have read and agreed to its content and also that the manuscript conforms to the journal's policies.

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Tables

Table 1: Effect of Isolate CSL-1 on the growth attributes and chlorophyll content of soybean plants under normal and salinity stress. Each value represent mean + SD of three replicates. Values with different letters in columns are

significantly different from each other as evaluated by DMRT.

	SL(cm)	RL (cm)	SFW(g)	RFW(g)	DSW(g)	DRW(g)	CC(SPAD)
Cont.	19.66±0.57	14.66±0.54	15.33±0.41	11.06±0.51	4.60±0.20	1.10±0.10	27.47±1.52
CSL-1	24.33±0.55	19.07±1.00	17.16±1.04	14.10±1.01	6.28±0.30	1.30±0.11	32.33±2.30
200mM NaCl	15.01±2.64	7.67±0.57	10.80±0.72	5.68±0.25	2.75±0.31	0.54±0.02	20.33±1.52
20mM+CSL-1	20.00±1.09	11.01±1.14	13.86±0.23	8.47±0.50	3.35±0.25	0.79±0.08	24.83±2.08

Figures

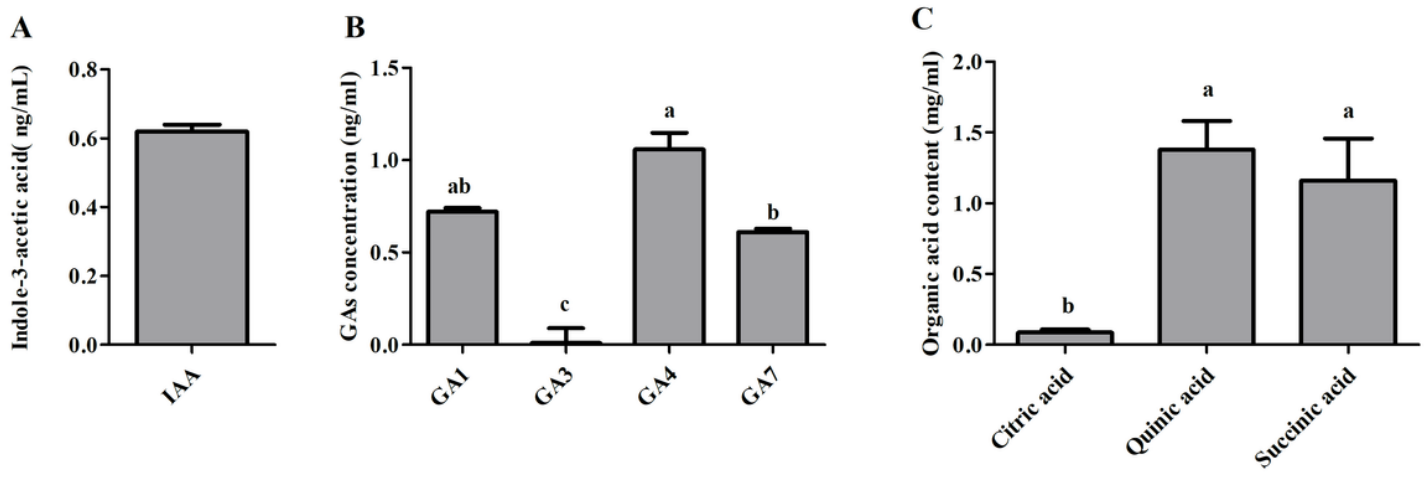
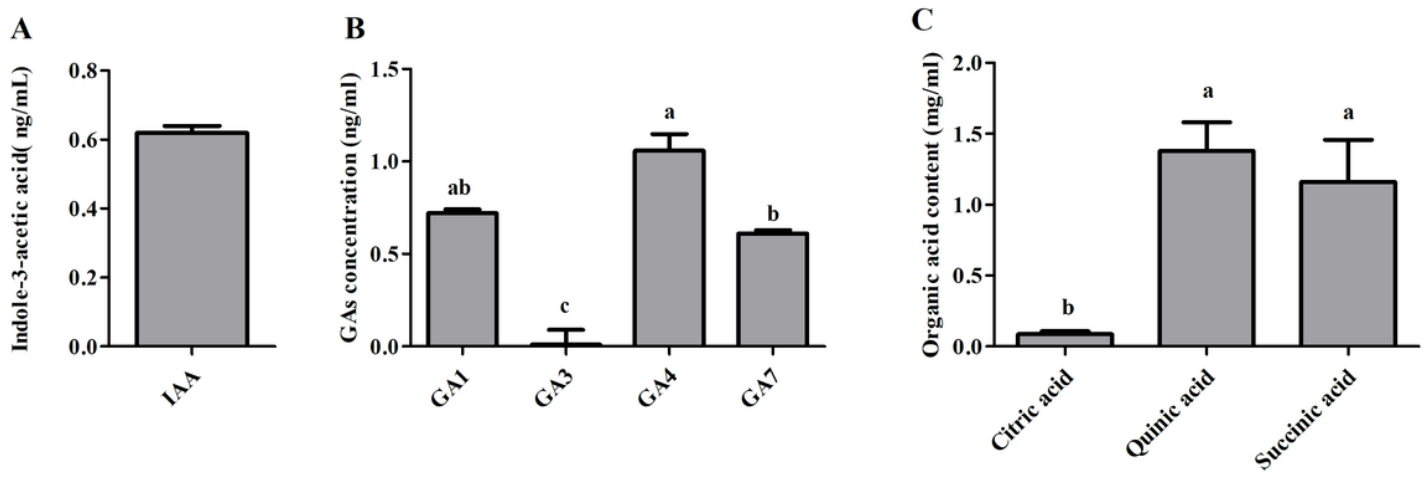


Figure 1

Quantification of indole-3-acetic acid (IAA), Gibberellins (GAs) and organic acid content in the culture broth of isolate CSL-1 through GC/MS-SIM analysis. Each data point is the mean of three replication and error bars representing standard errors. The bars with different letters are significantly different from each other as evaluated by DMRT analysis.



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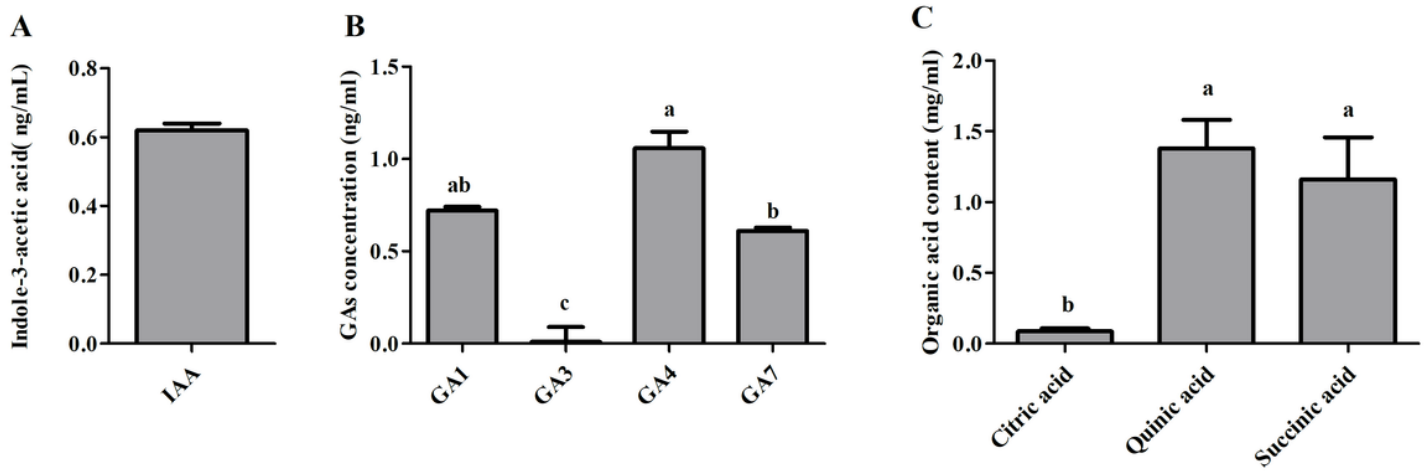

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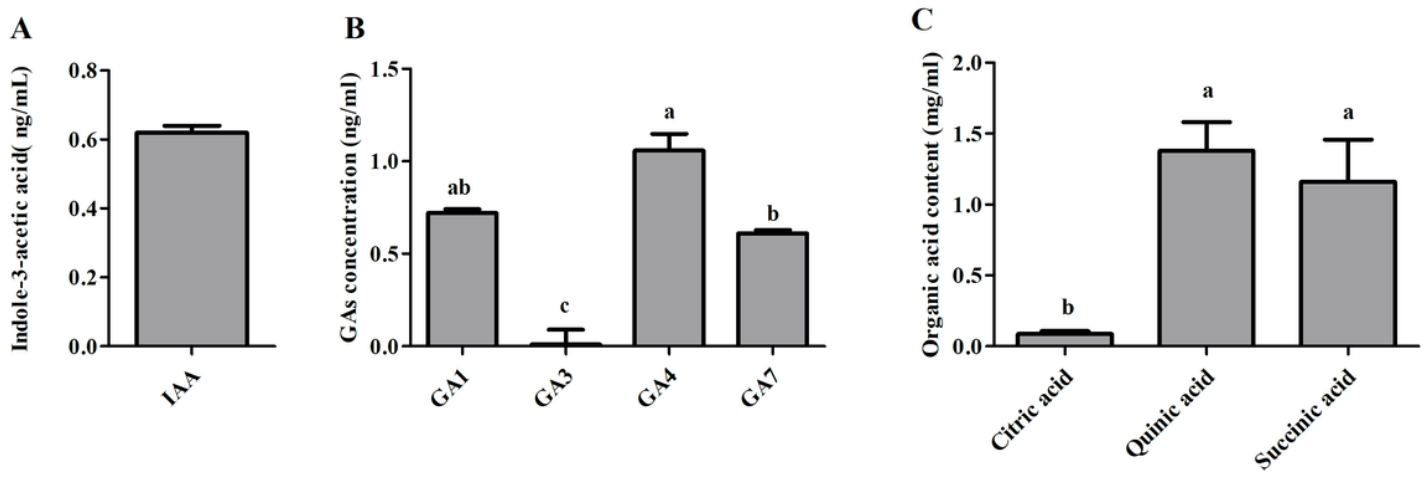

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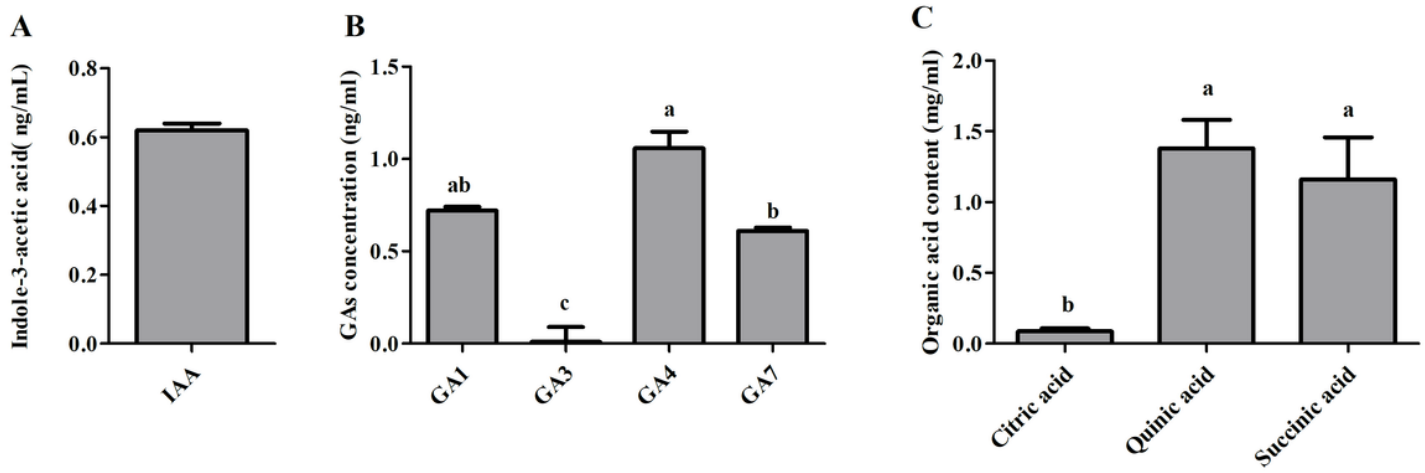

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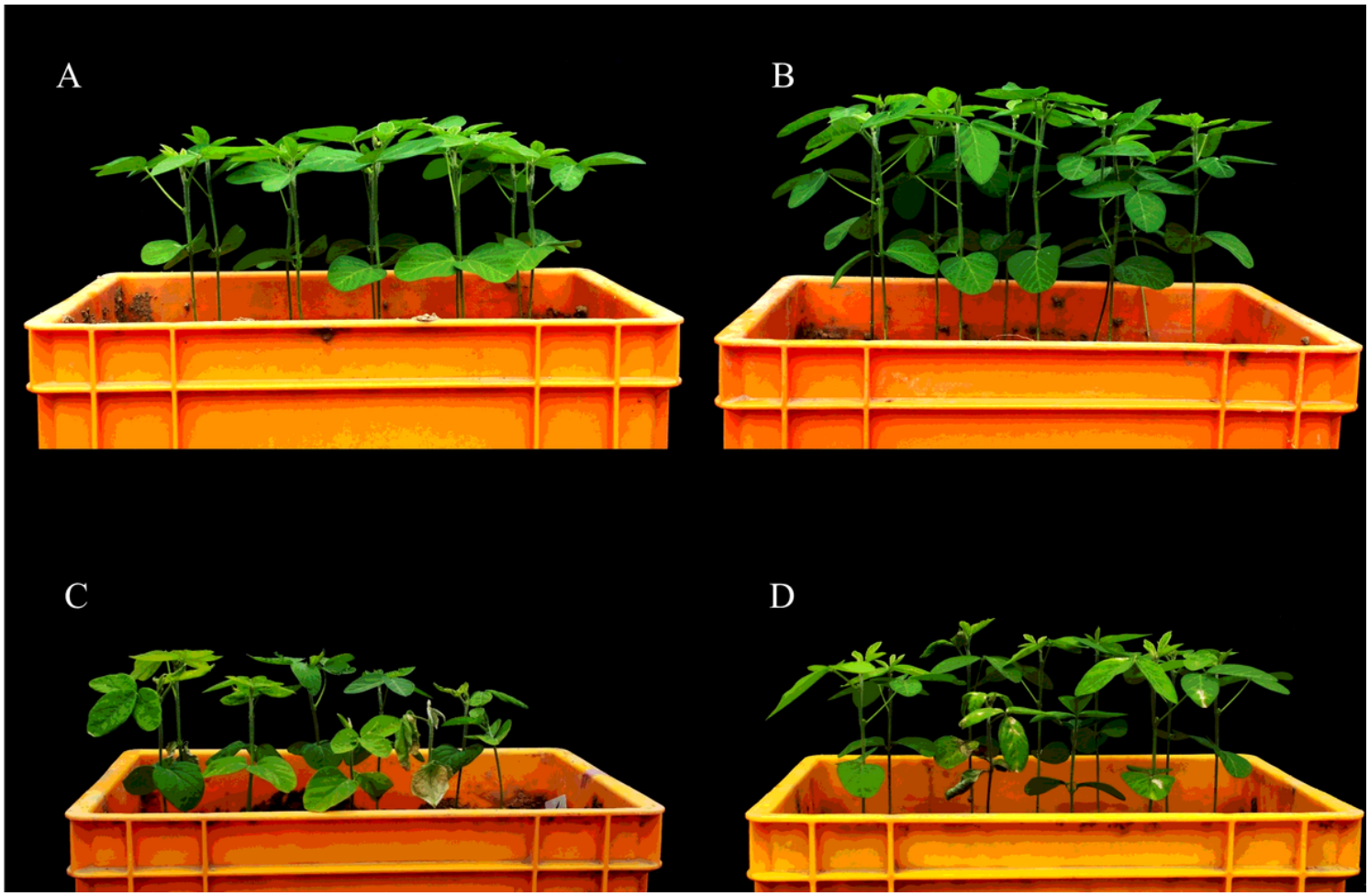
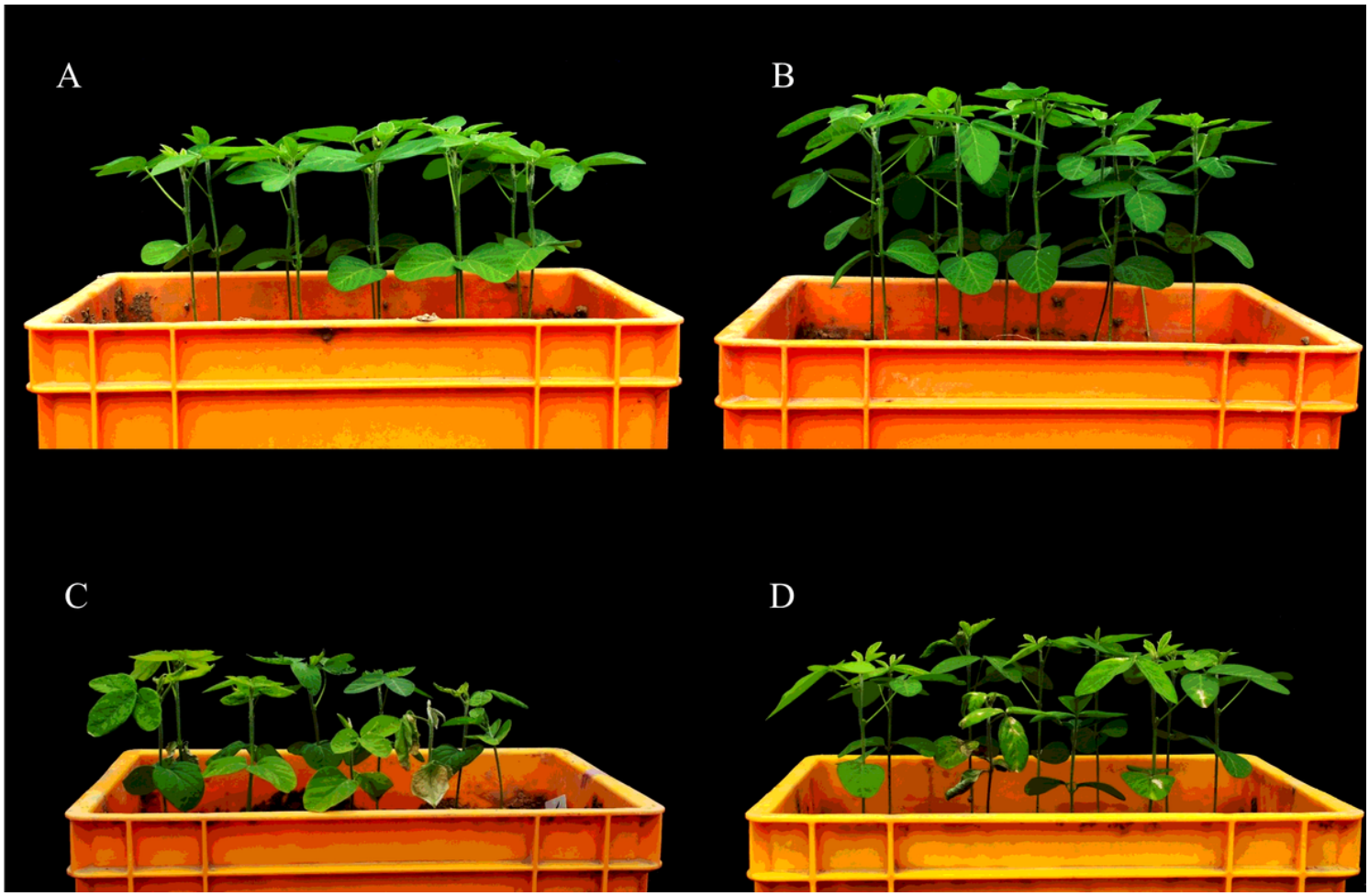


Figure 2

Effects of fungal isolate CSL-1 on the growth of soybean plants under normal and NaCl stress. (A) Control Soybean; (B) CSL-1 treated soybean; (C) NaCl stress (200mM) and (D) NaCl + CSL-1 soybean plants.



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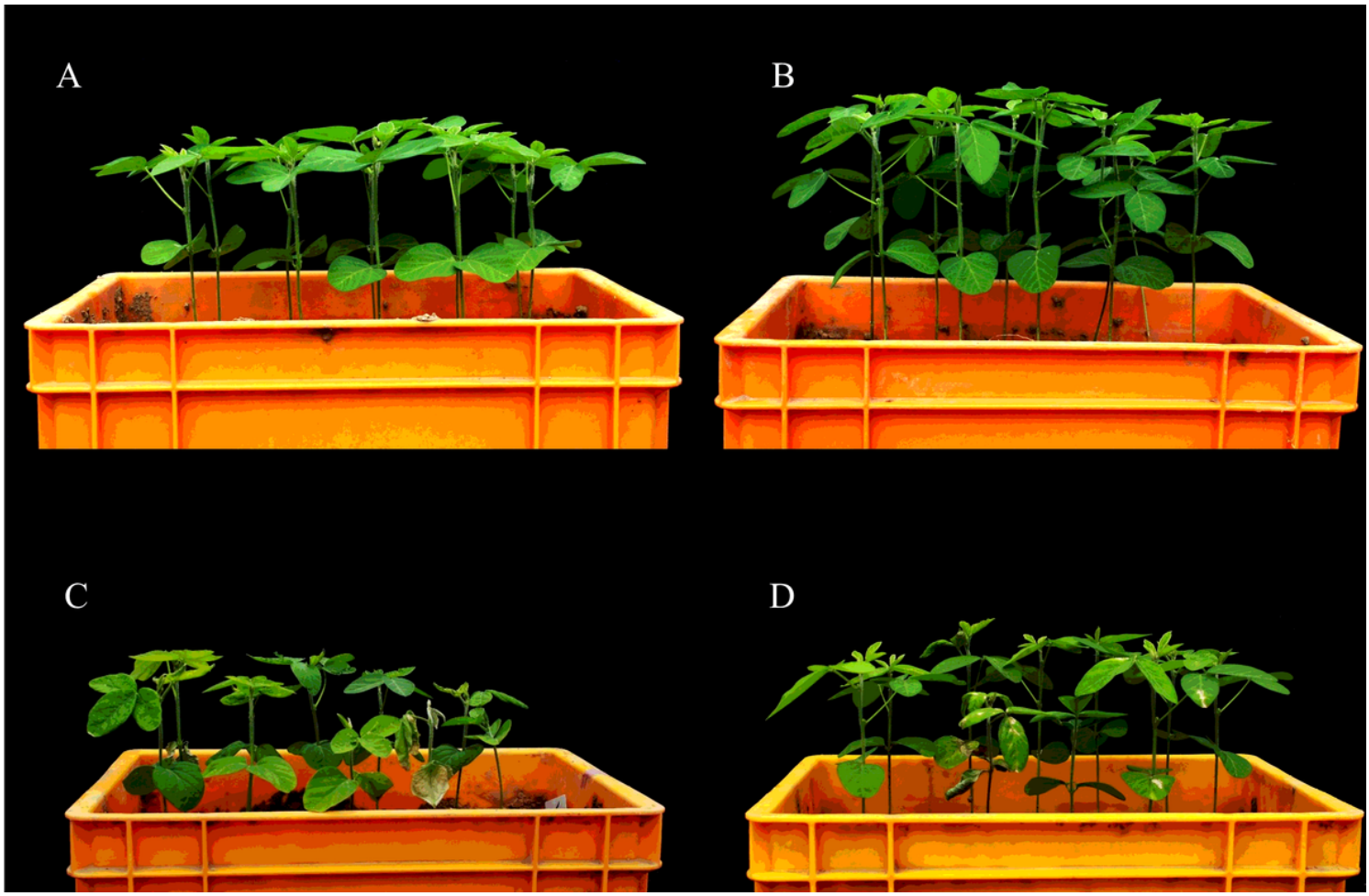

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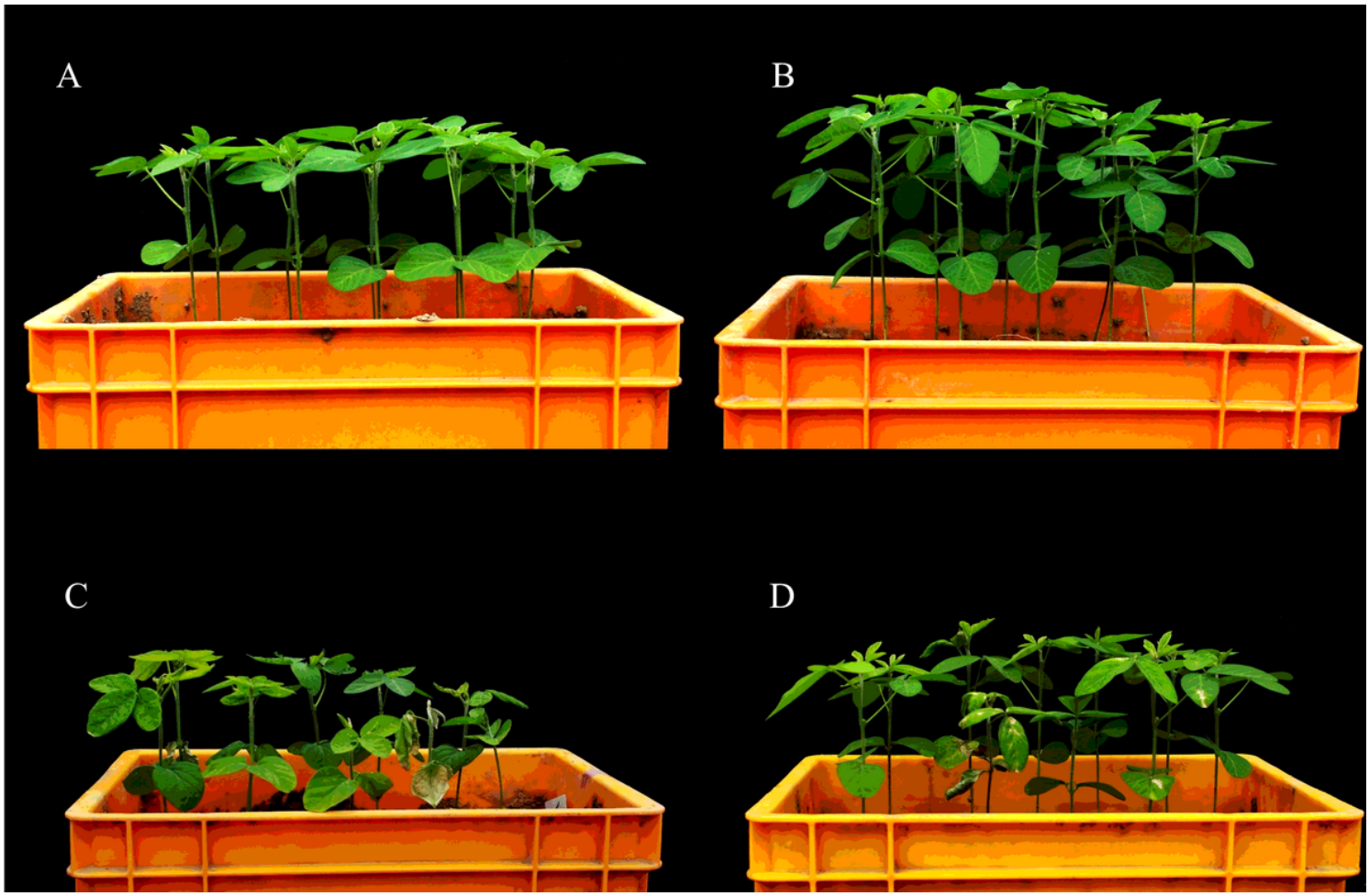

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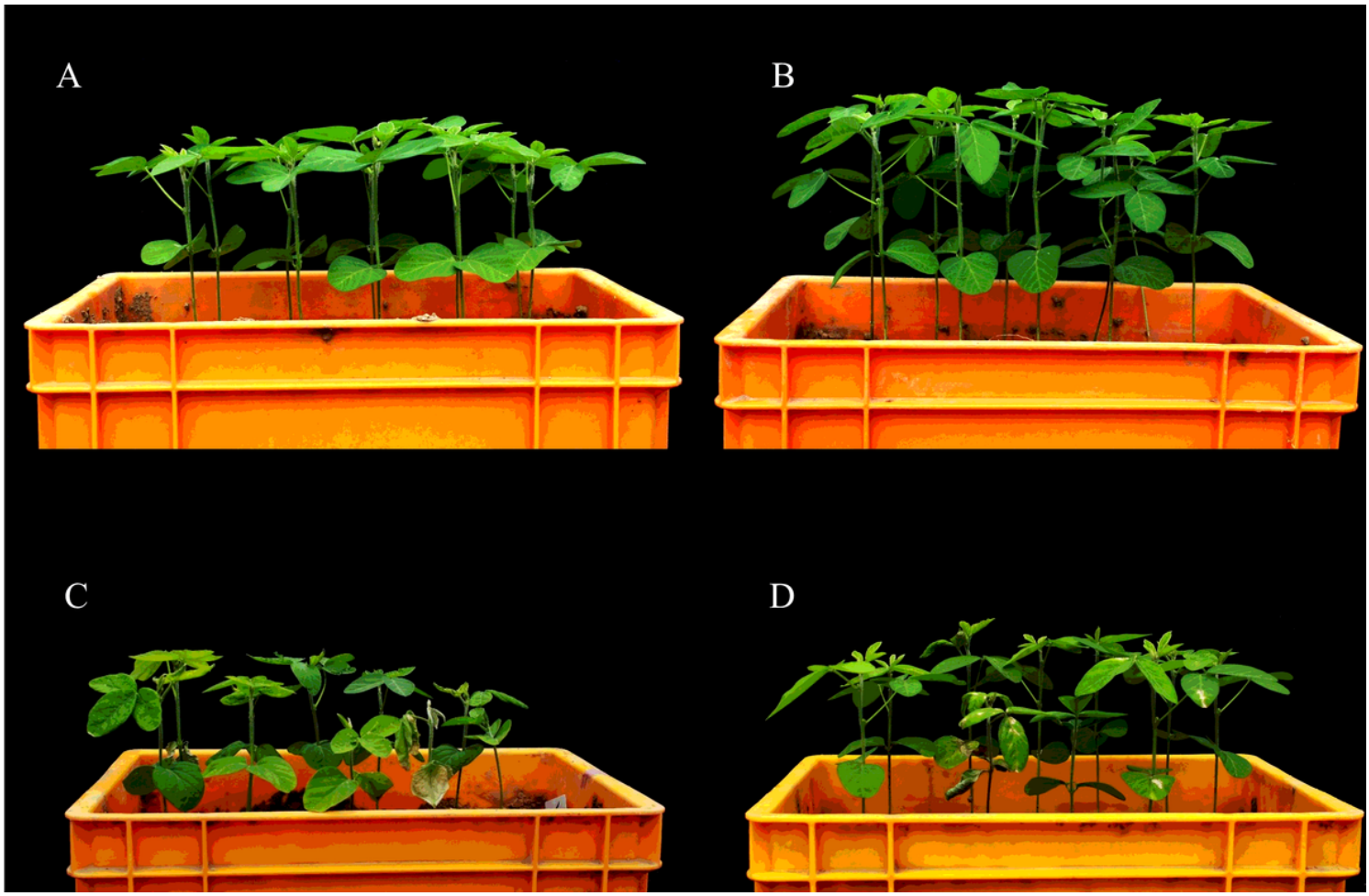

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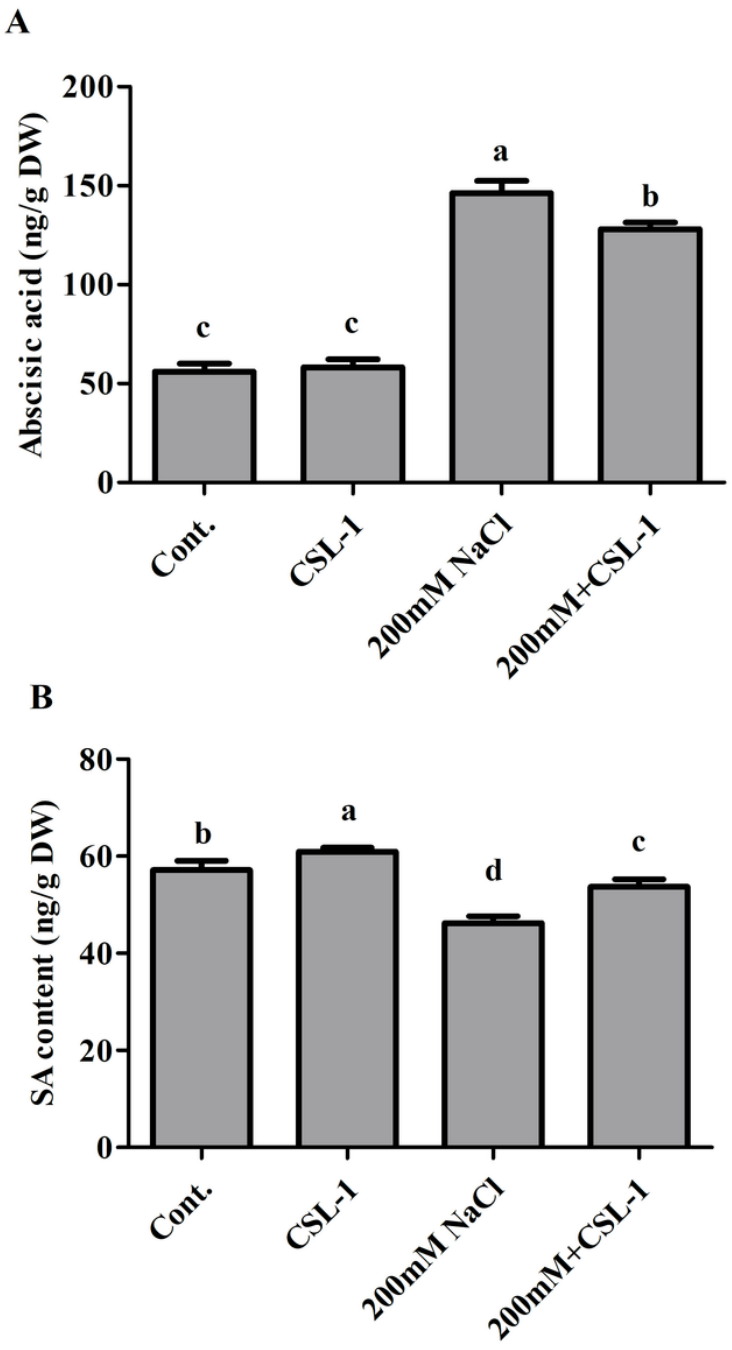
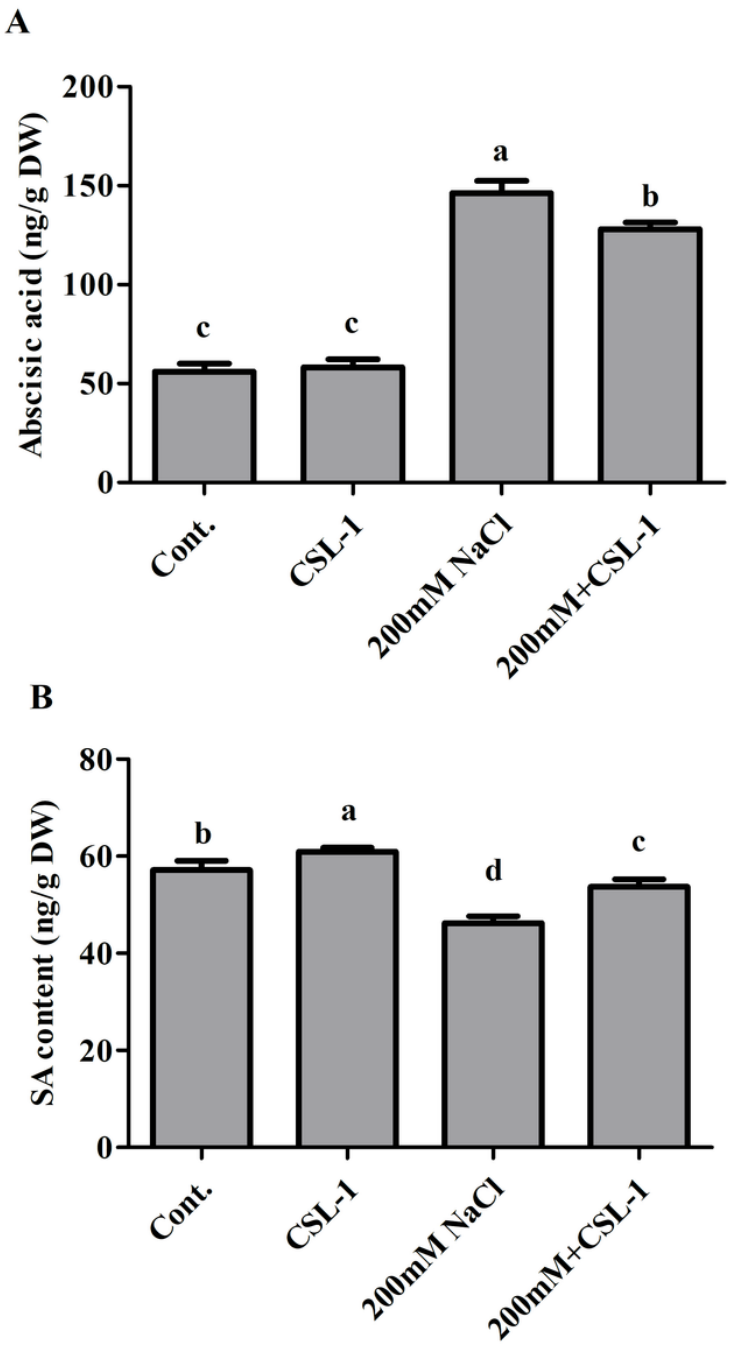


Figure 3

Endogenous abscisic acid (ABA), and salicylic acid (SA) quantification in soybean plants inoculated withCSL-1. (A) Demonstrates ABA, (B) shows the amount of SA under normal and NaCl stress. Each data point is the mean of at least three replicates. Error bars represent standard errors. The bars presented with different letters are significantly different from each other as evaluated by DMRT.



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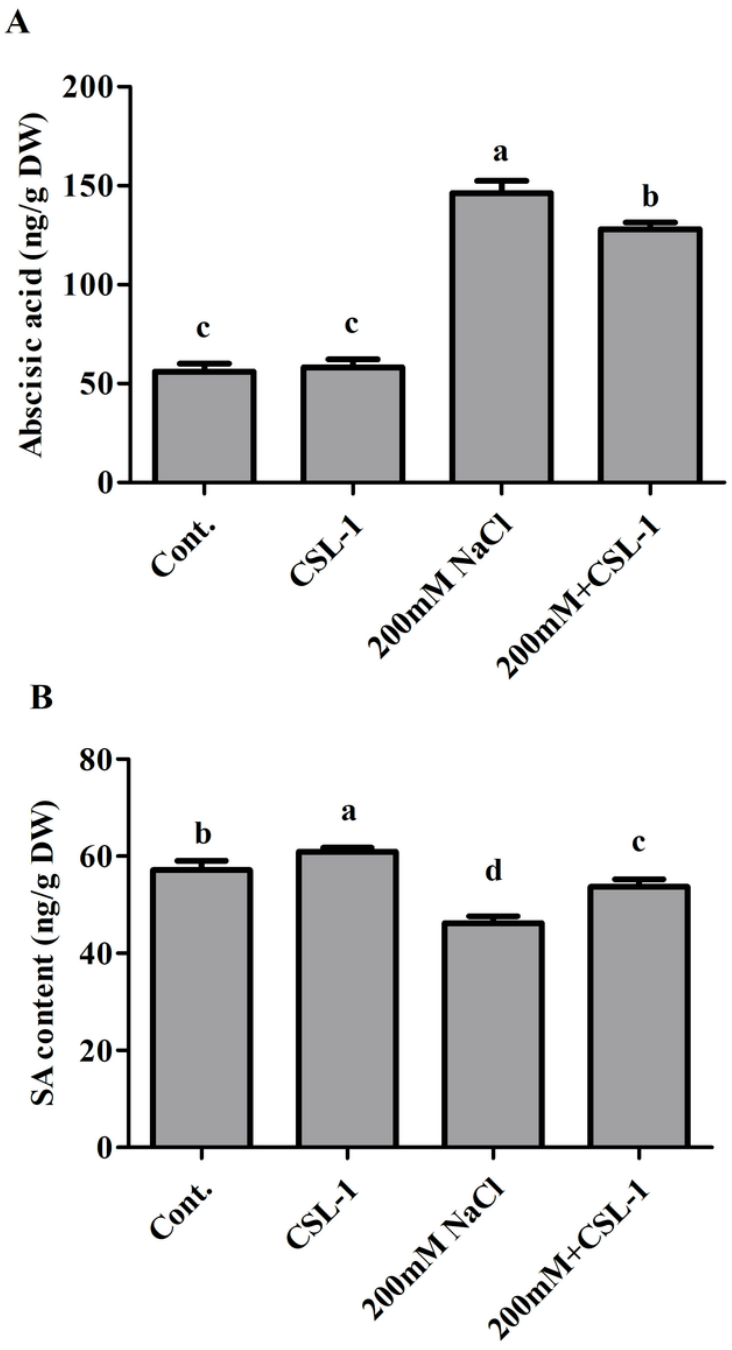

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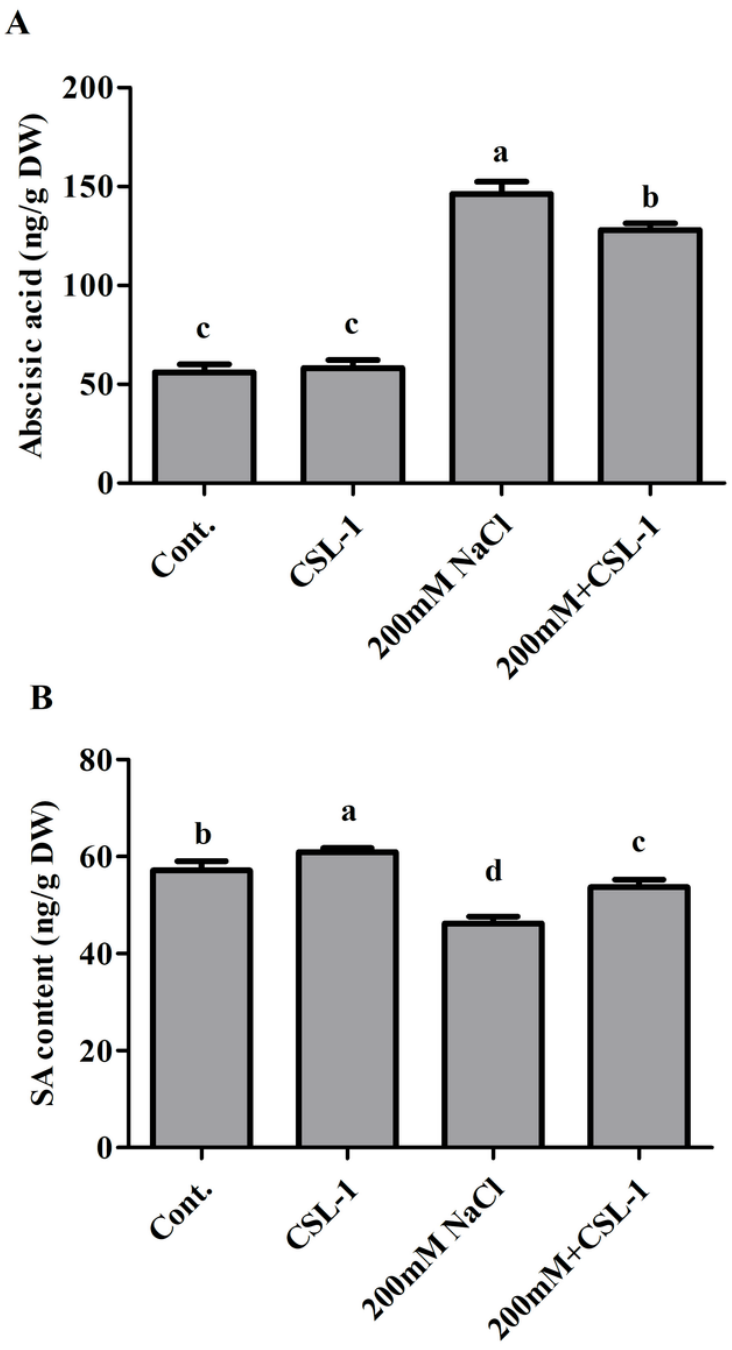

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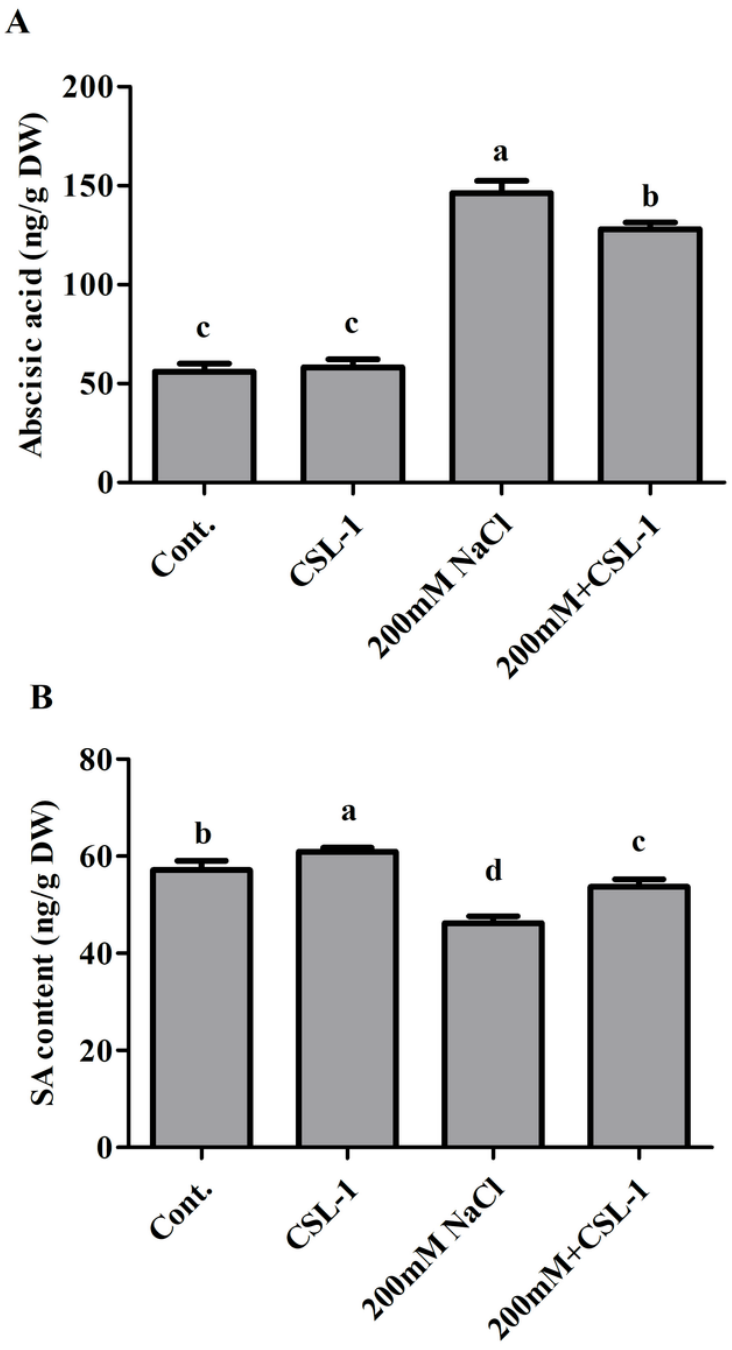

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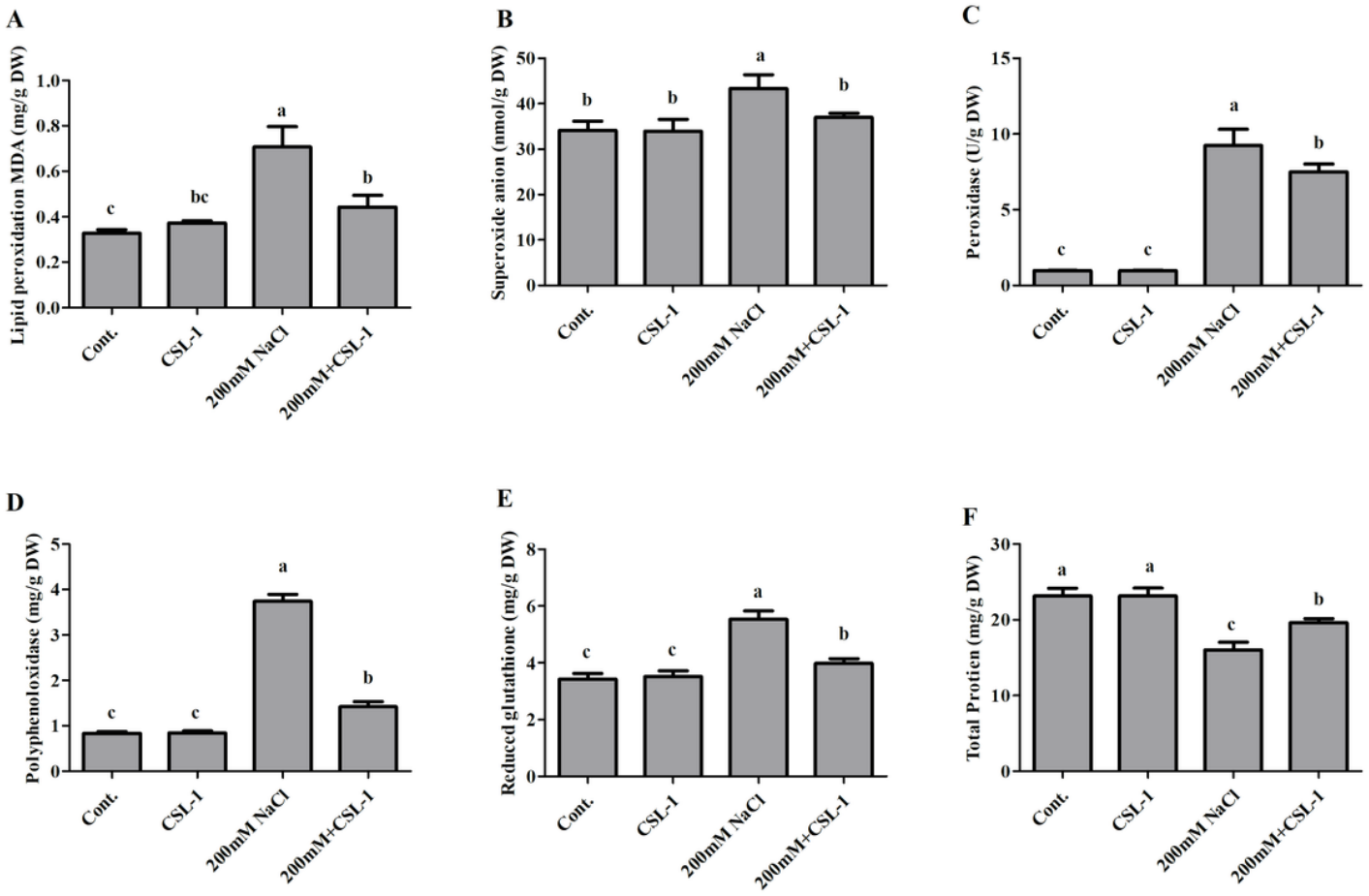
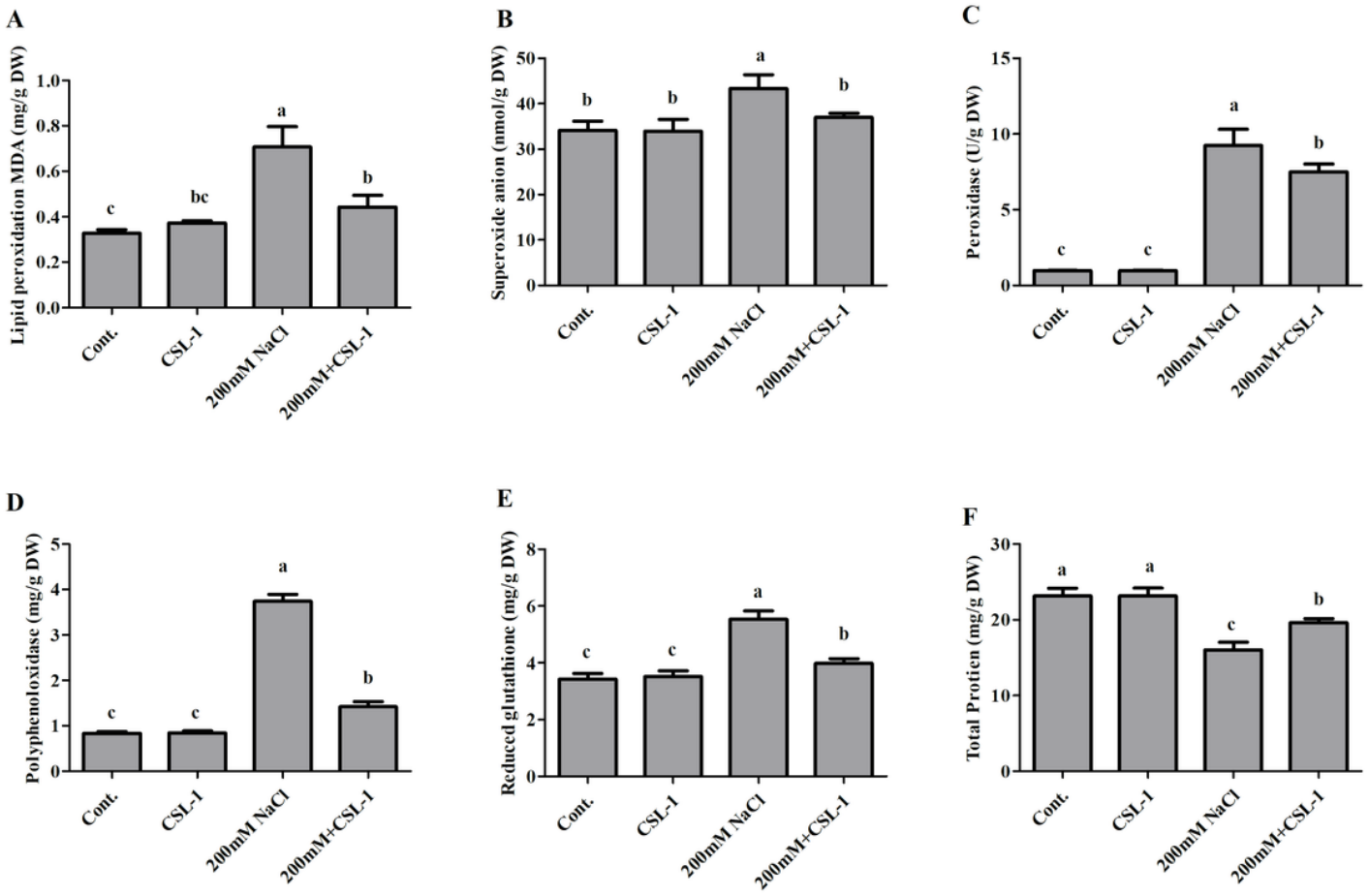


Figure 4

The effect of fungal isolate CSL-1 on different antioxidants. (A) Lipid peroxidation (MDA); (B) Superoxide anions (SOA); (C) Peroxidase (POD); (D) Polyphenol oxidase (PPO); (E) Reduced glutathione (GSH), and (F) Total protein (TP) contents in soybean plants under normal and NaCl stress. Each data point is the mean of three replicates. Error bars represent standard errors. The bars presented with different letters are significantly different from each other as evaluated by DMRT.



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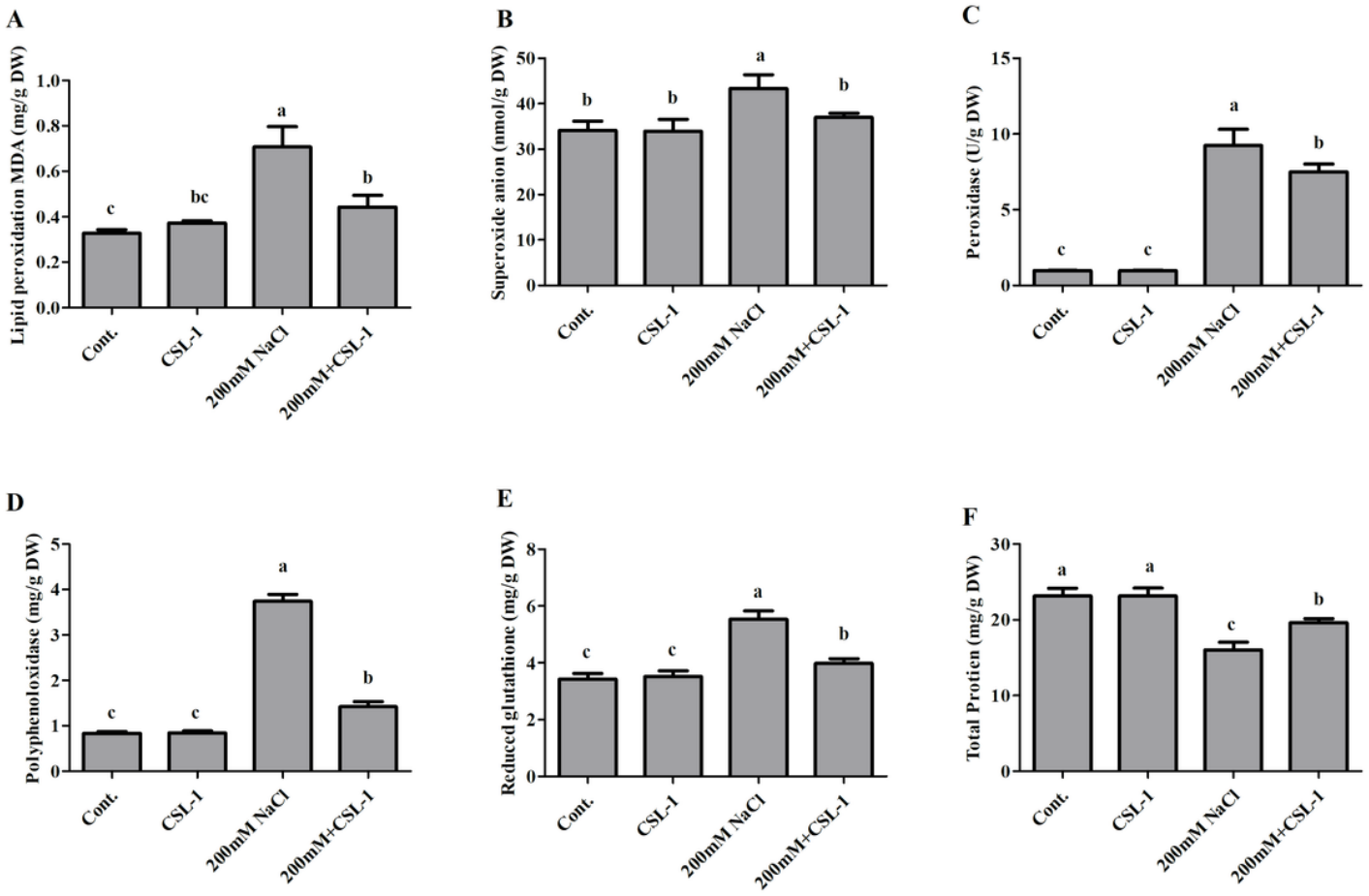

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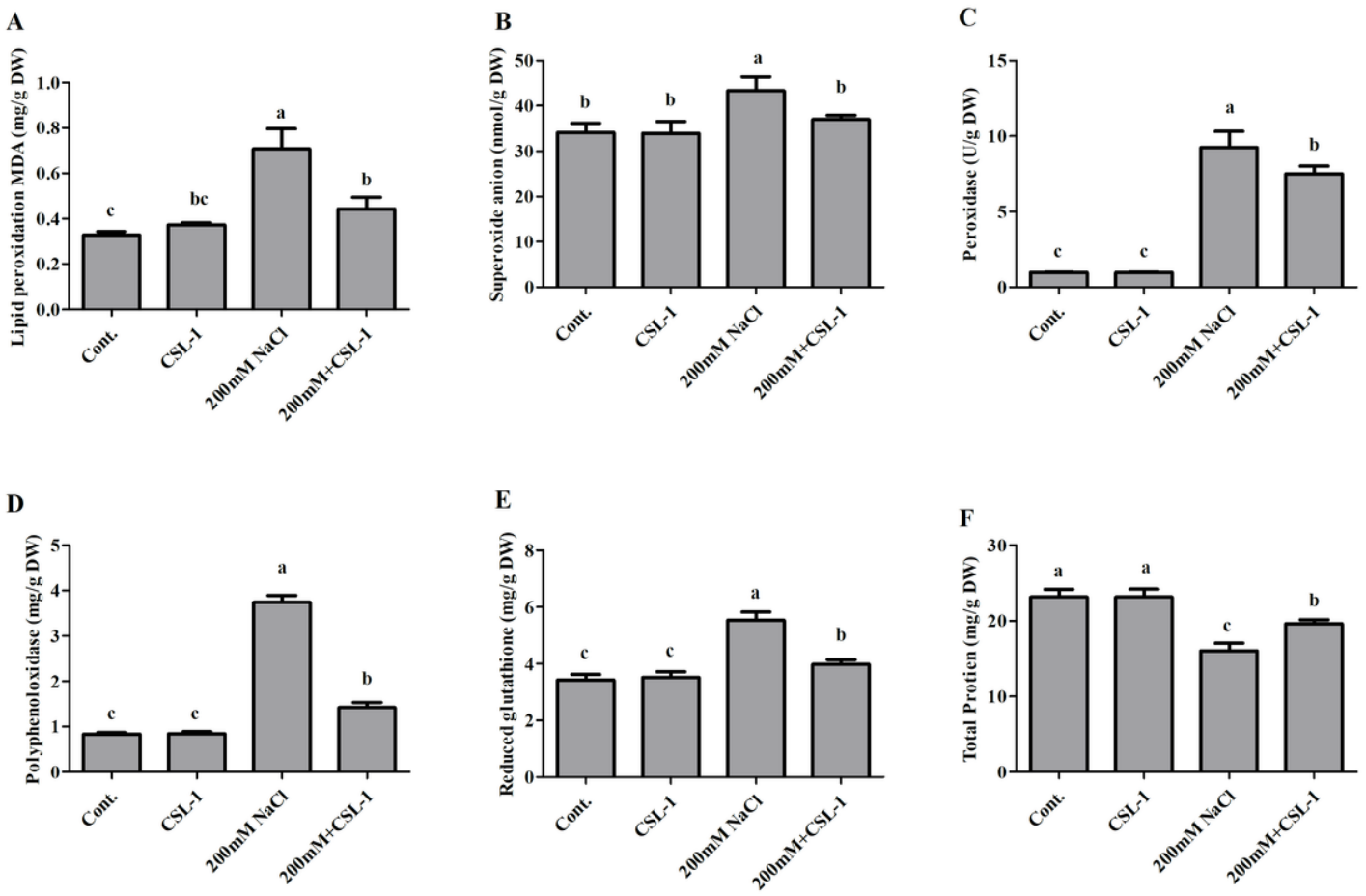

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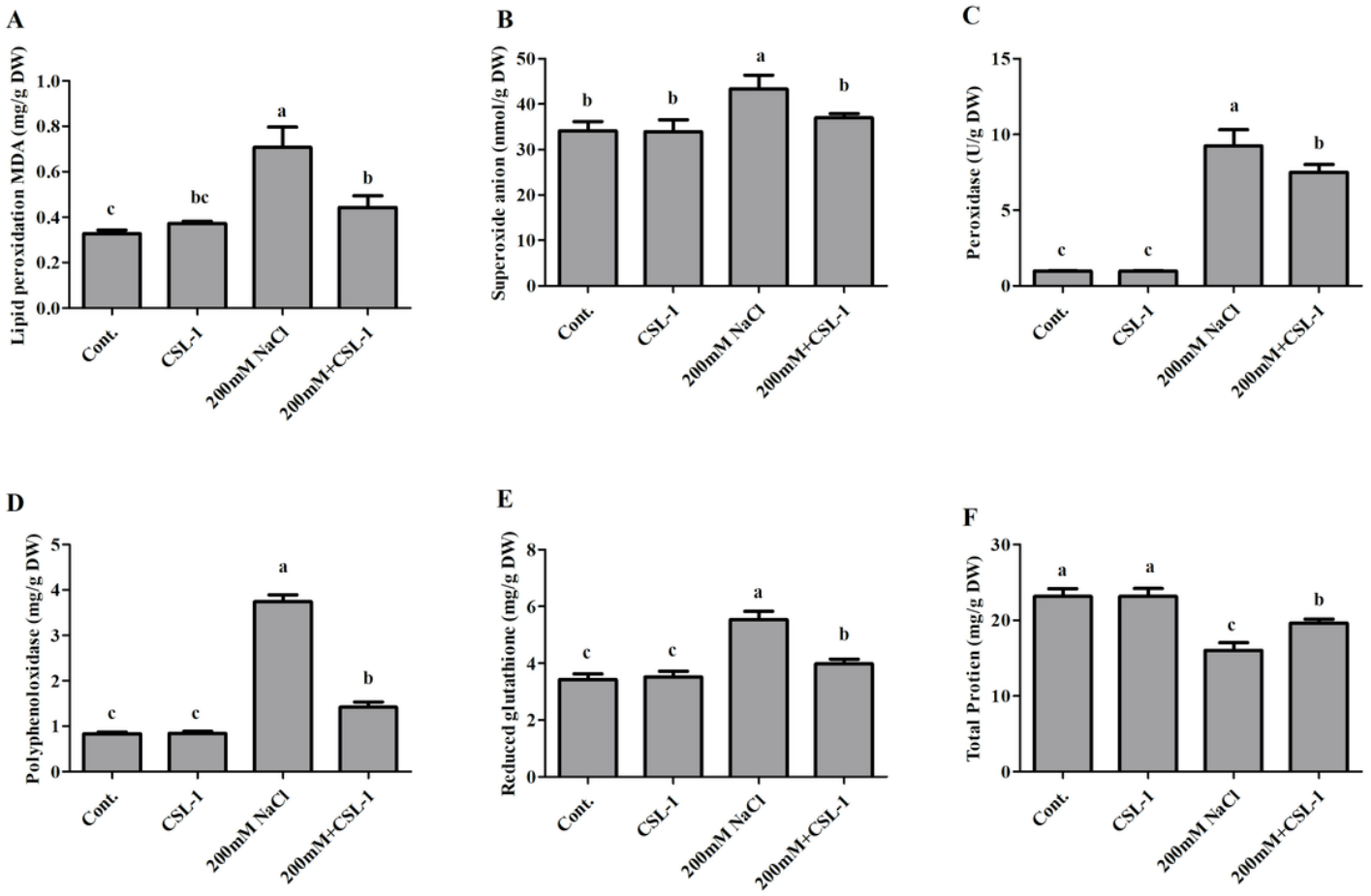

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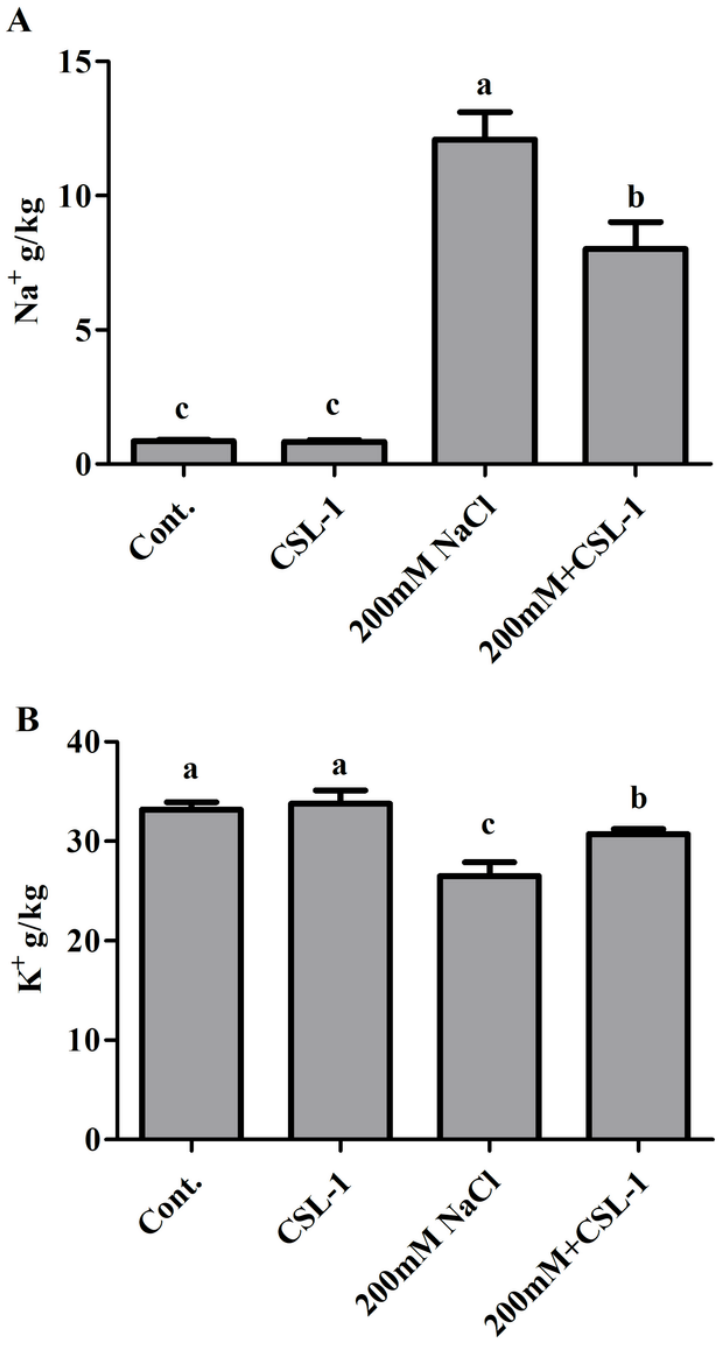

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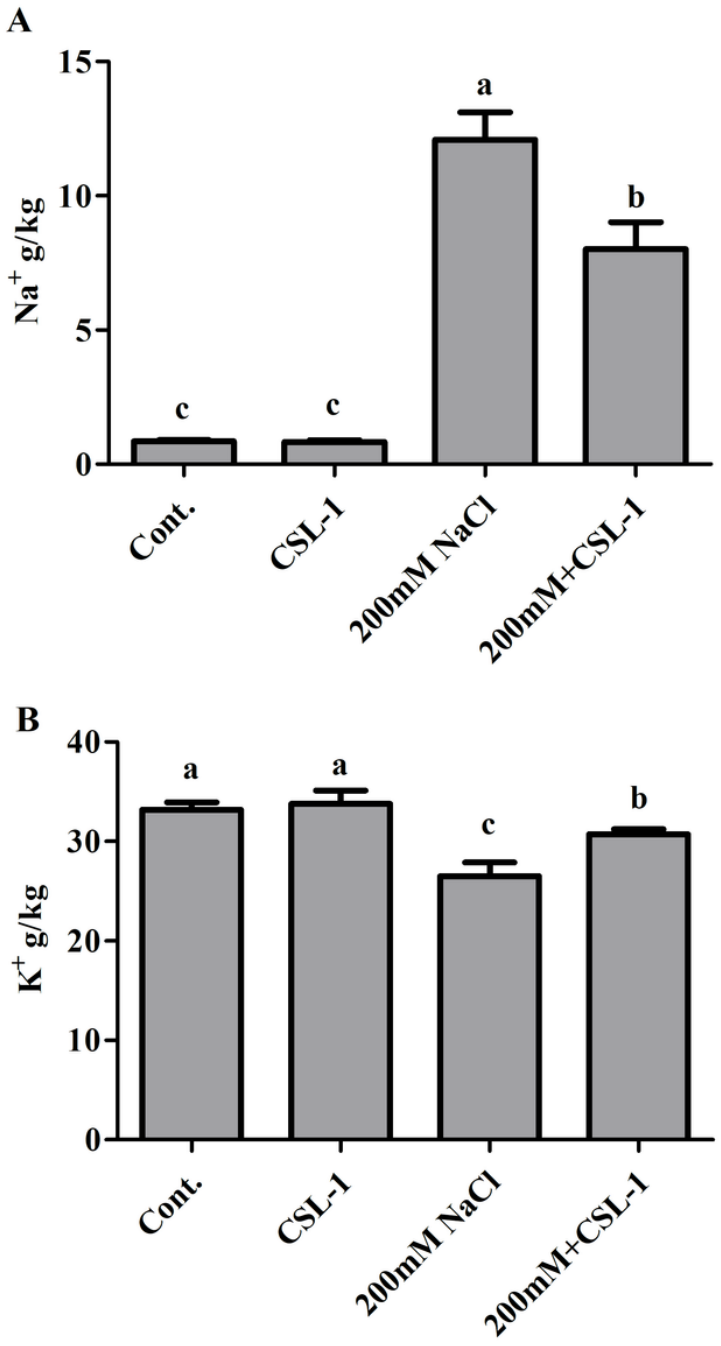

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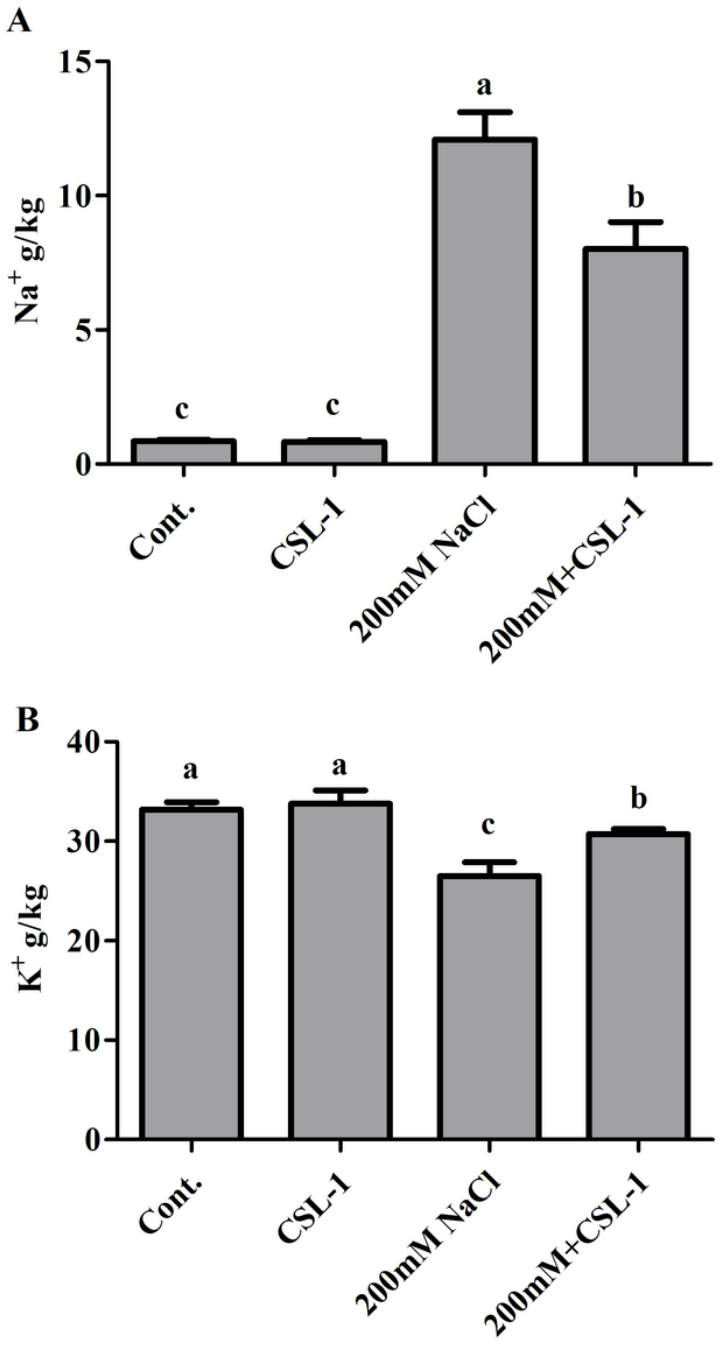

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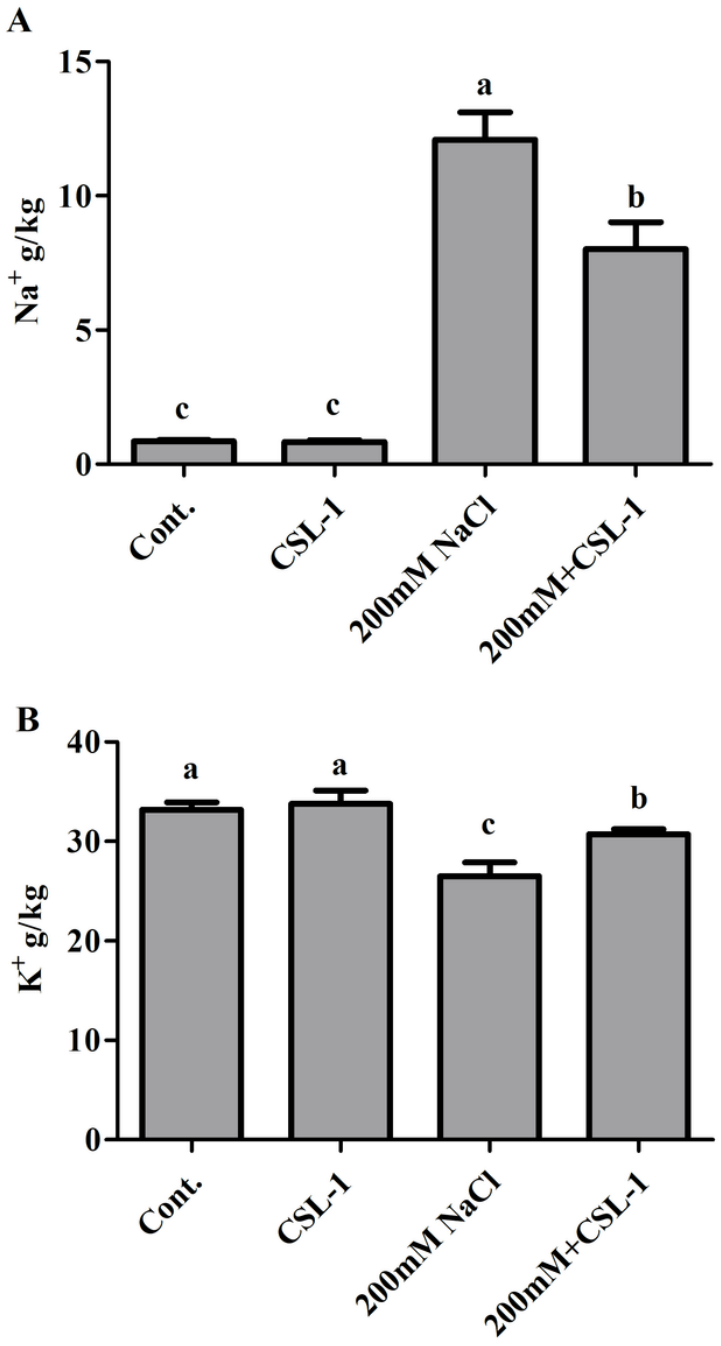

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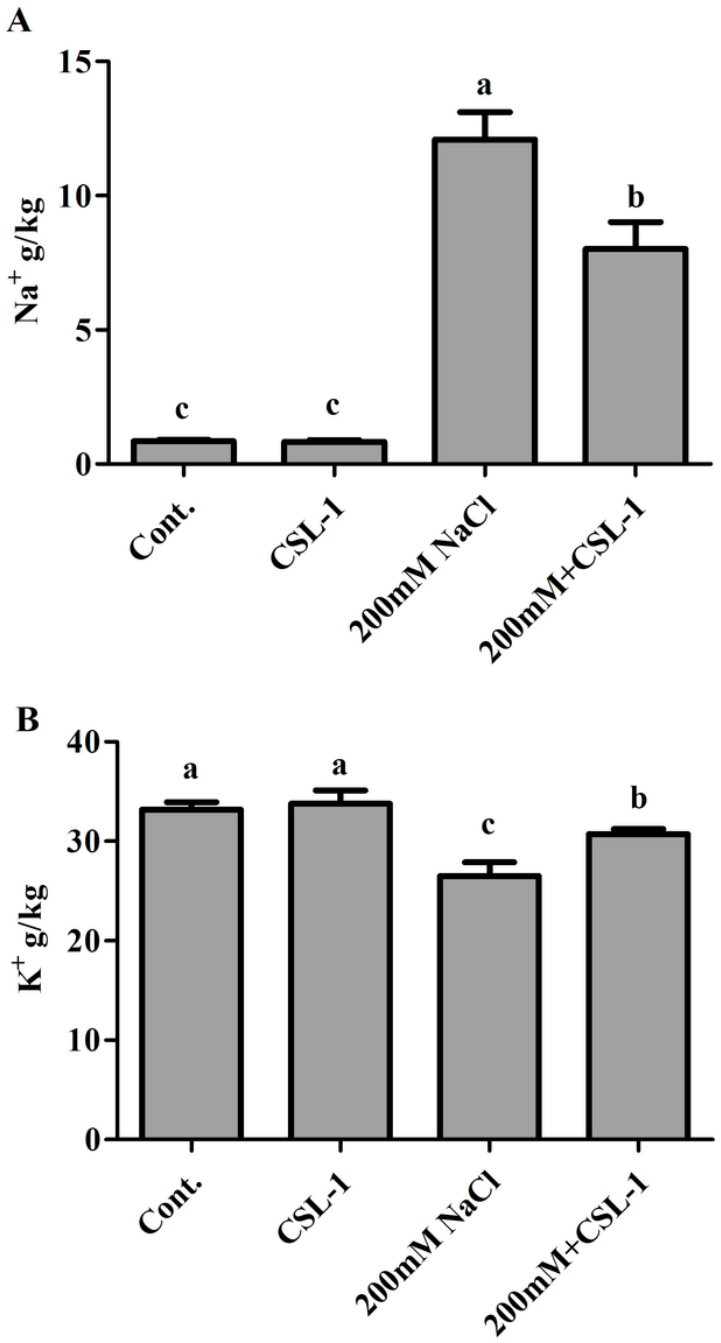

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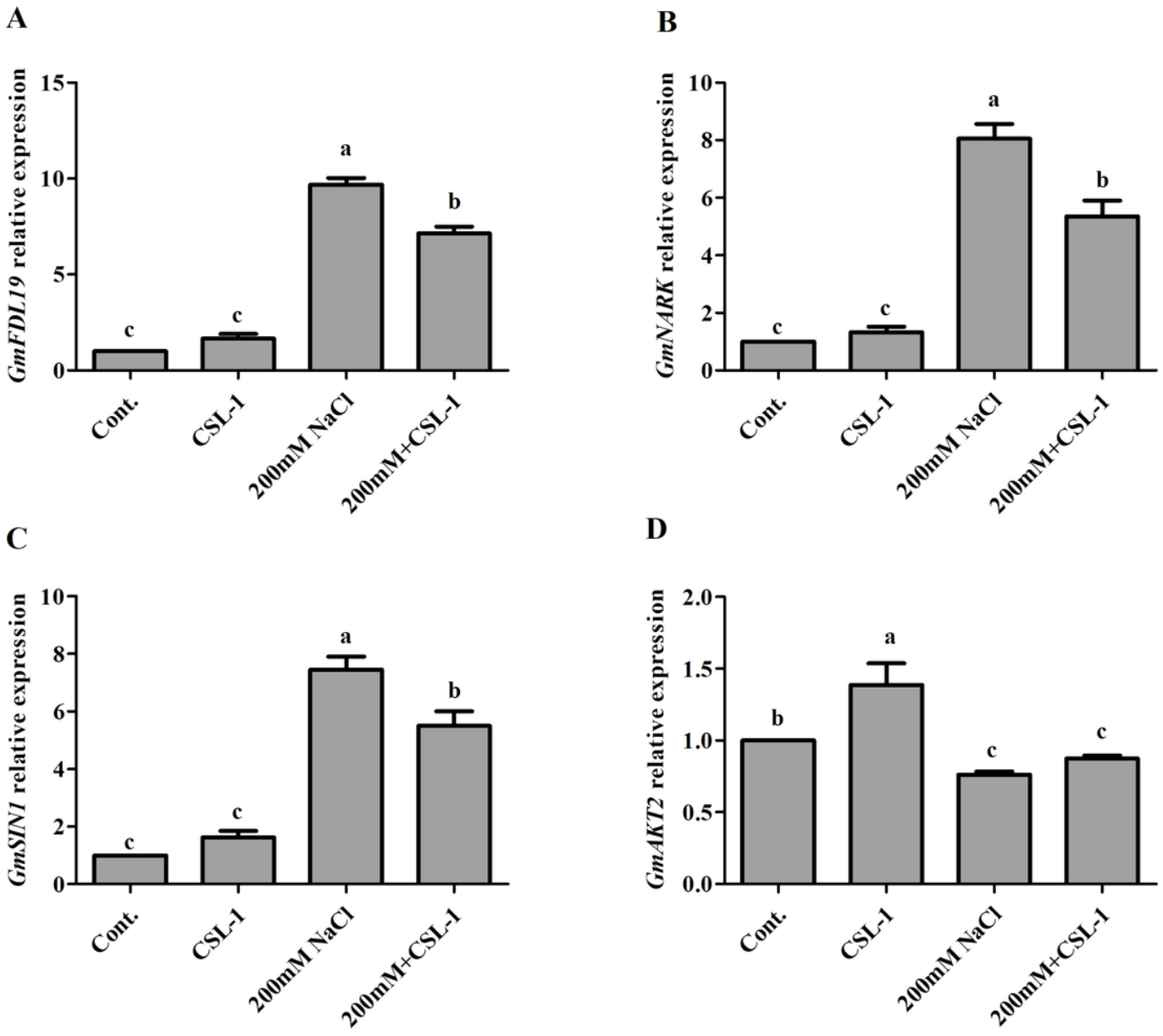
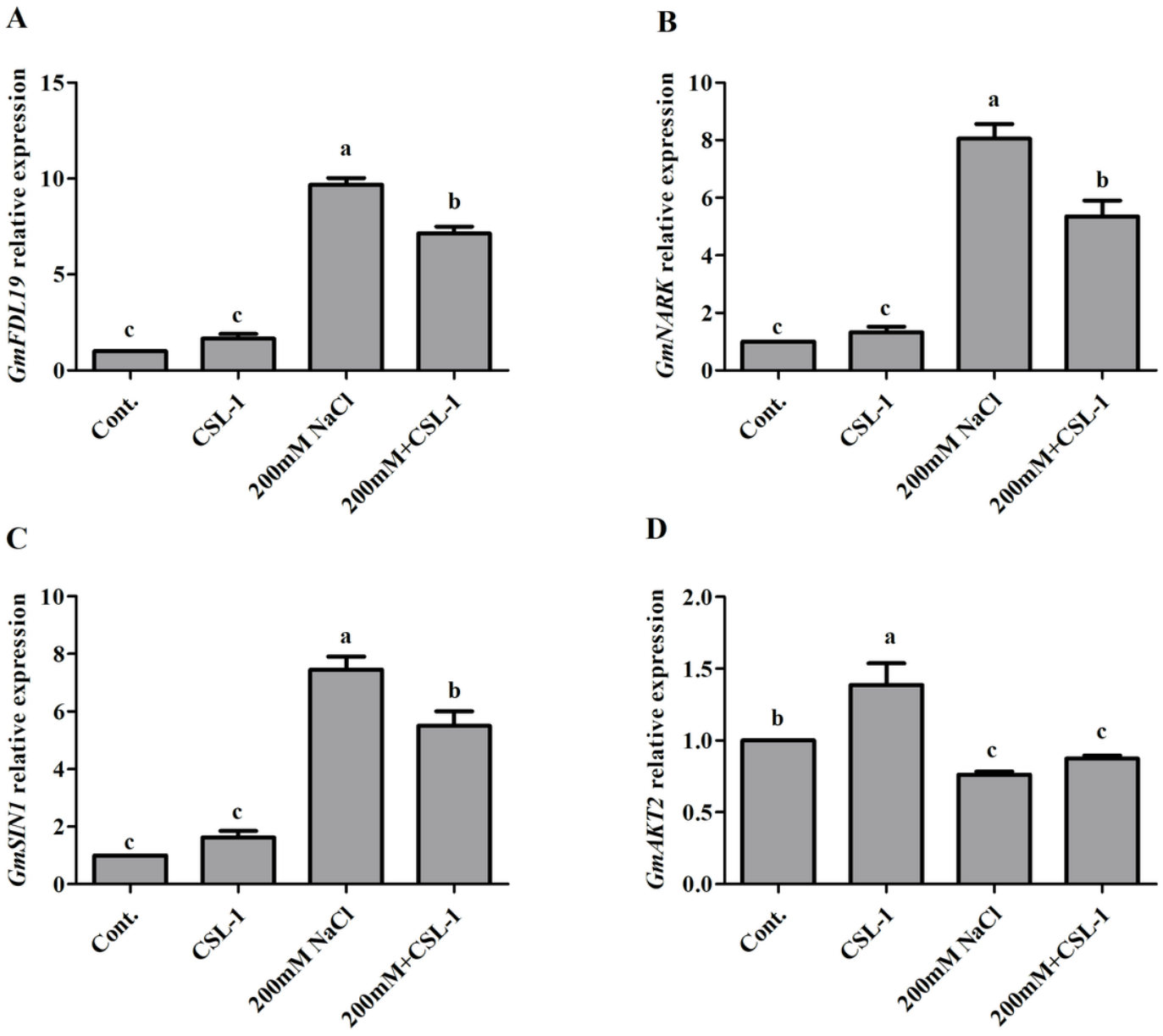


Figure 6

Relative expression of GmFLD19 and GmNARK genes in soybean plants with and without inoculation of ALT29 and ALT 43 under NaCl stress. The values were calculated relative to those of actin gene expression and are means of three replicates. Error bars represent standard errors. The bars represented with different letters are significantly different from each other as evaluated by DMRT analysis.



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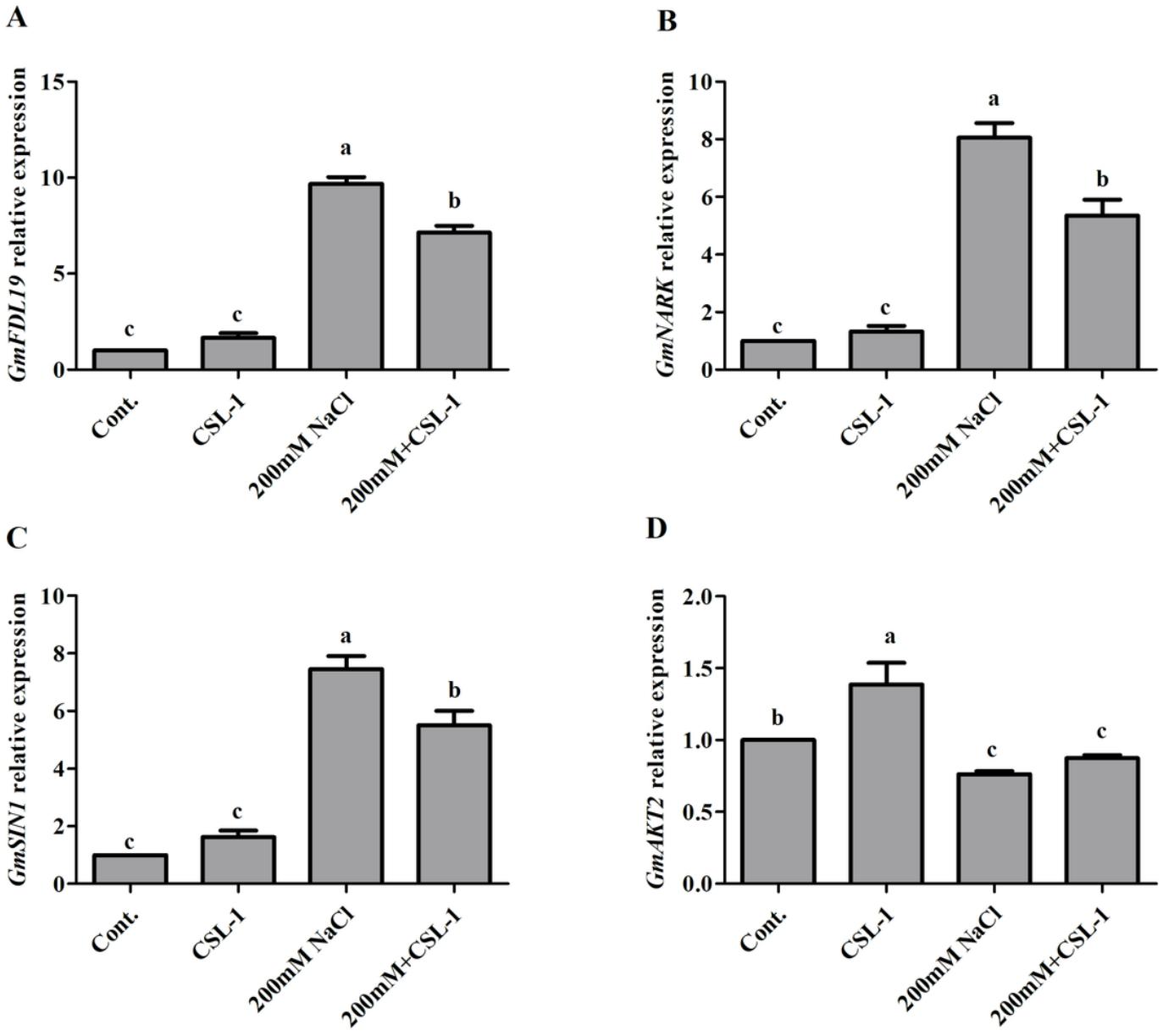

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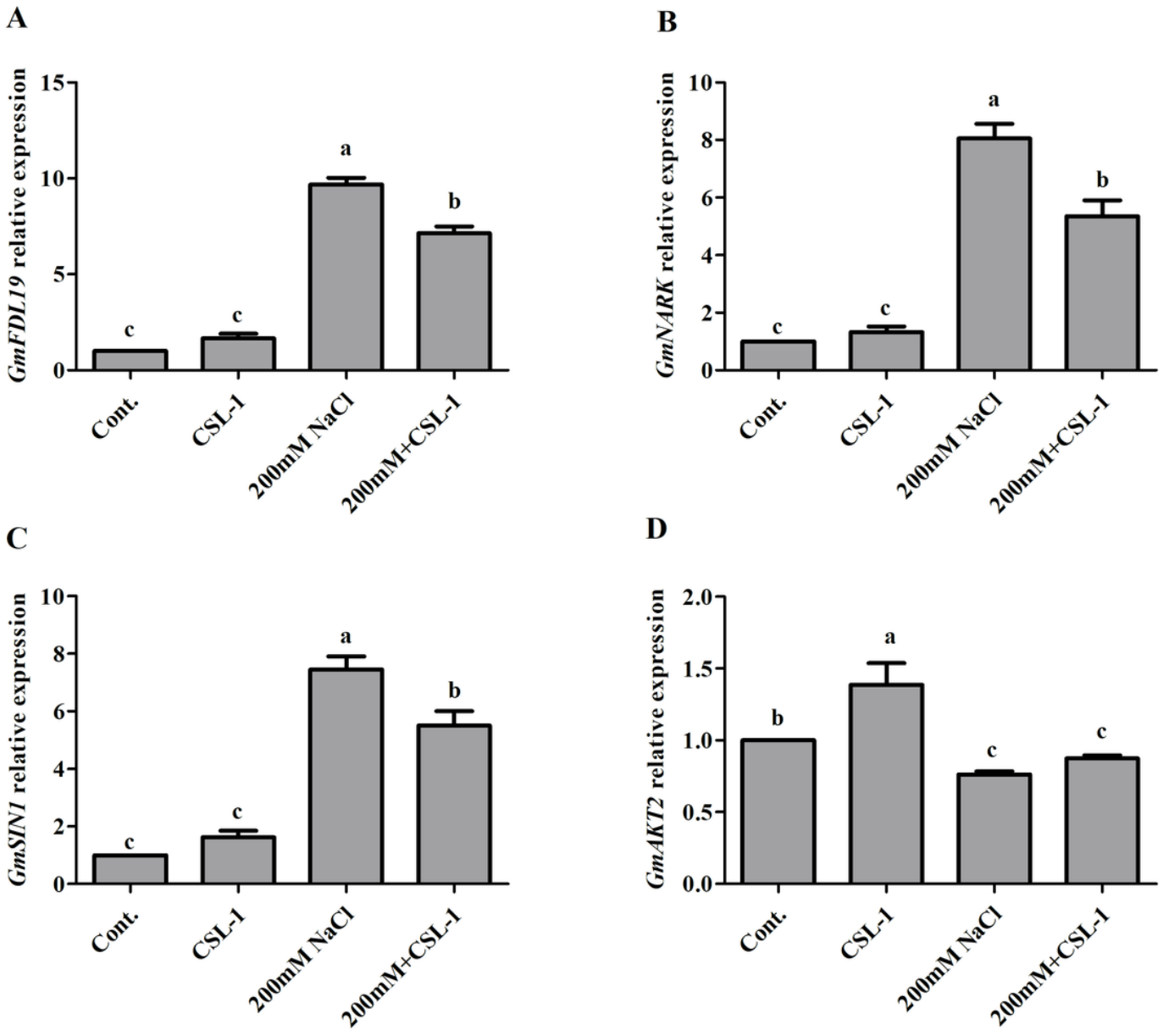

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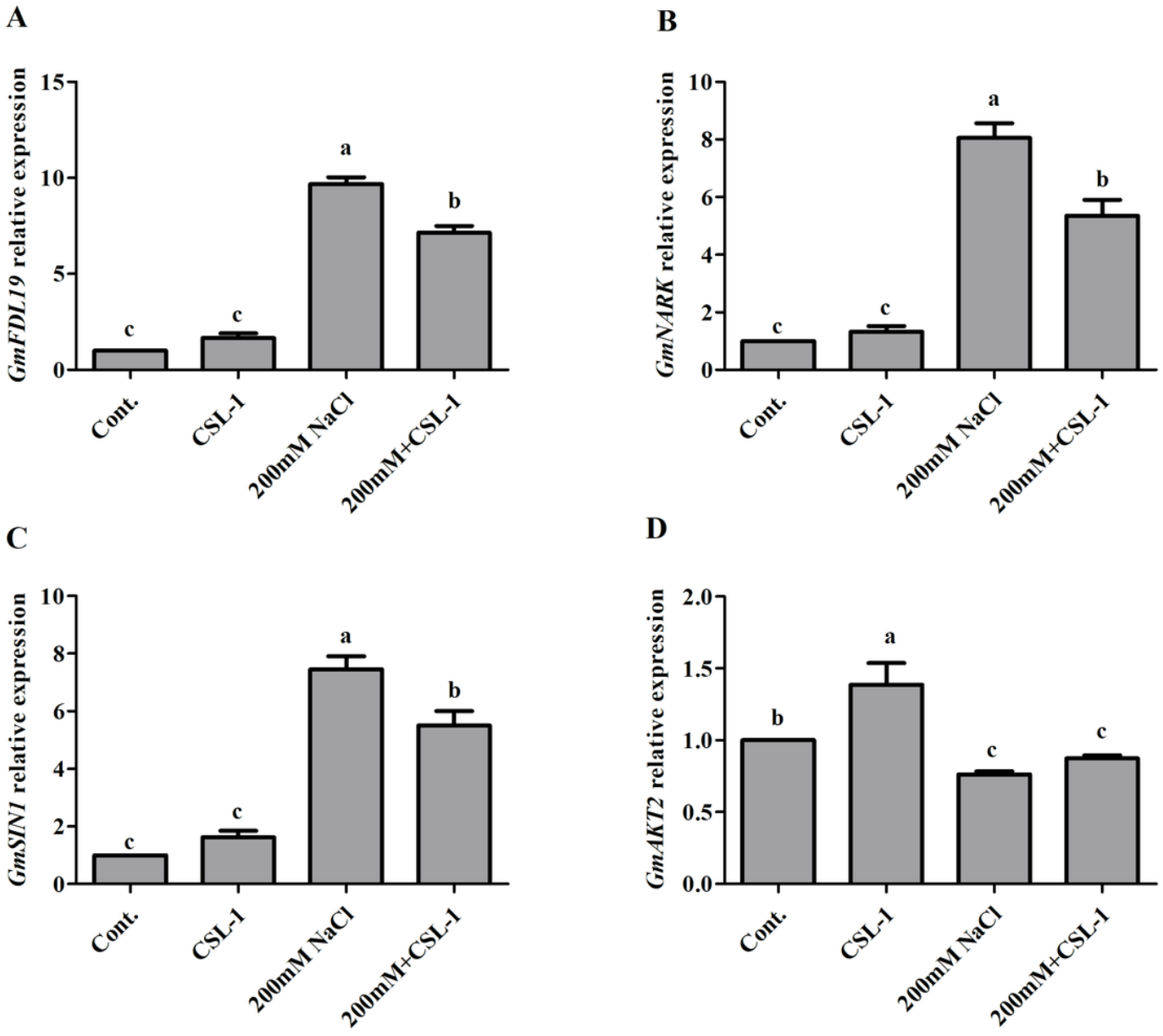

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Supplementary Files

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

- S.Table1.docx
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