TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2020) 44: 36-46

doi:10.3906/bot-1907-27

The endogenous L-cysteine desulfhydrase and hydrogen sulfide participate in supplemented phosphorus-induced tolerance to salinity stress in maize (*Zea mays*) plants

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Received: 16.07.2019 • **Accepted/Published Online:** 11.10.2019 • **Final Version:** 07.01.2020

Abstract: Although phosphorus supplementation (SP) has been shown to improve salinity tolerance in plants, crosstalk between hydrogen sulfide (H_2S) or L-cysteine desulfhydrase (LDES) and SP-induced salinity tolerance needs to be elucidated. Thus, prior to initiation of stress treatment, young seedlings were transferred to aqueous solution containing the scavenger of H_2S, 0.1 mM hypotaurine (HT), or the inhibitor of LDES, 0.3 mM DL-propargylglycine (PAG), for 12 h. The plants grown under control (no NaCl added) or salinity stress (SS; 100 mM NaCl) were then supplemented with SP (2.0 mM in total) as KH_2PO_4 for 5 weeks. SS caused a significant decrease in plant growth, PSII maximum efficiency, chlorophyll a and b, leaf water potential, relative water content, mineral element contents, ascorbate, and glutathione, but a significant increase in hydrogen peroxide, malondialdehyde, electron leakage, proline, the activities of different key antioxidant enzymes, Na^+ contents, endogenous H_2S, and LDES activity. The SP-induced tolerance to salinity stress of maize plants was found to be due to reduced leaf Na^+ content and oxidative stress, as well as improved antioxidant defence system, leaf mineral nutrient contents, plant growth and photosynthetic traits, levels of H_2S and proline, and LDES activity. However, HT significantly reversed the levels of H_2S, but did not reduce LDES activity. The application of PAG reversed both H_2S and LDES in the salinity-stressed maize plants. Both HT and PAG reversed the positive effects of SP on oxidative stress and the antioxidant defence system, suggesting that H_2S and LDES both jointly participated in P-induced salinity tolerance of maize plants.

Key words: Phosphorus, salinity stress, reactive oxygen species, oxidative stress, hydrogen sulfide, maize, antioxidant enzymes

1. Introduction

Salinity stress is a premier impediment for plant growth and development (Negrão et al., 2017). Salinity is known to hamper plant growth and development through water stress, cytotoxicity because of high acquisition of ions, e.g. sodium (Na^+) and chloride (Cl^-), and deficiency of mineral elements (Isayenkov and Maathuis, 2019). Additionally, salinity leads to oxidative stress in plants because of overaccumulation of reactive oxygen species (Verma et al., 2019).

Phosphorus (P) is one of the limited nutrients in natural and agricultural ecosystems (Scheible and Rojas-Triana, 2018). Phosphorus deficiency leads to reduced biomass and grain yield of plants (Vandamme et al., 2016). Salinity stress causes P deficiency in plants; hence, application of supplementary P positively affects growth and yield of plants under salinity stress by inducing physiological alterations, including changes in osmolytes and antioxidants (Bargaz et al., 2016).

Hydrogen sulfide (H_2S), a gaseous molecule, is biologically very active because it is involved in plant responses to biotic and abiotic stresses at low levels (Chen et al., 2013). Some investigations have aimed to assess the physiological role of H_2S in plants, such as increased tolerance of sweet potato to copper stress (Zhang et al., 2008), cucumber to boron toxicity (Wang et al., 2010), alfalfa to salinity stress (Wang et al., 2012), and wheat and barley to aluminum toxicity (Chen et al., 2013). L-cysteine desulfhydrase is known as a crucial enzyme in H_2S biosynthesis, which is responsible for H_2S synthesis (Guo et al., 2017).

Although H_2S is believed to be a key signaling molecule involved in alleviation of salinity stress (Wang et al., 2012), there seem to be no reports in the literature relating to the contribution of H_2S and LDES in P-induced salinity tolerance. In the current investigation, we intended to appraise the putative role of H_2S and LDES in P-induced salinity tolerance by using a scavenger of H_2S, hypotaurine (HT), or an inhibitor of LDES, DL-propargylglycine (PAG).

2. Materials and methods

2.1. Plant growth conditions and treatments

This experiment was set up under glasshouse conditions with maize (*Zea mays*) cv. DK5783. Prior to sowing, the

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seeds were sterilized with NaOCl solution (1% v/v). Initially, 5 seeds were sown in each plastic pot with 8.5 L of perlite. Following germination, 2 seedlings were uprooted and 3 seedlings per pot retained to grow further. The photoperiod was 11 h light period and 13 h dark period during the entire growing season.

Plants were grown for 5 weeks under control (complete nutrient solution including 1.0 mM P and no NaCl added) or salinity stress (SS) as 100 mM NaCl provided to the growth medium 2 weeks after germination. Supplemented phosphorus (2.0 mM in total) as KH_2PO_4 was combined with these treatment solutions. Moreover, before the initiation of the stress treatment, young seedlings were also transferred to aqueous solution containing the scavenger of H_2S, 0.1 mM hypotaurine (HT), or the inhibitor of LDES, 0.3 mM $_{DL}$-propargylglycine (PAG), for 12 h to confirm if H_2S and LDES jointly participated in P-induced tolerance to salinity during stress treatments.

The nutrient solution (NS) contained all macro- and microelements, and its detail is described elsewhere (Kaya and Ashraf, 2015); the pH of the complete NS was kept at 5.5. A range of 0.1–1.0 L of water was provided to the growth medium of plants every 2 days over the course of the experimentation.

Each treatment had 3 replicates and there were 15 plants in each replicate; i.e. there were 45 plants per treatment. The excess applied NS was allowed to flow through the containers so that salinity stress could be sustained at the desired level in the growth medium throughout the course of the experimentation.

Five weeks after the initiation of different treatments, 6 plants from each replication (18 plants from each treatment) were taken and divided into roots and shoots to determine dry weights. Shoot and root samples were placed in an oven at 75 °C for 3 days for dry weight determination. The remaining 9 plants from each replication (27 plants from each treatment) were taken and the attributes given below determined.

2.2. Chlorophyll contents and chlorophyll fluorescence

The procedure described by Strain and Svec (1966) was employed to determine chlorophyll content. Leaf tissue (1 g) was homogenized using 90% acetone (5 mL). The extraction solution was then filtered prior to storing in light-tight tubes. The sample solutions were read at 663.5 and 645 nm for the quantification of chlorophyll a and chlorophyll b, respectively.

A leaf that had been dark-adapted for 30 min was used for the determination of chlorophyll fluorescence. A Mini-PAM chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) was used to measure fluorescence attributes.

2.3. Determination of leaf relative water content (RWC) and leaf water potential

The procedure outlined by Yamasaki and Dillenburg (1999) was followed to appraise RWC. After the leaves were removed from the stem, they were weighed to obtain fresh mass (FM). To measure the turgid mass (TM), the leaves were kept in distilled water in a closed Petri dish. Maximum turgidity was measured by weighing the leaves till no more weight increase observed. The leaf samples were then kept in an oven at 80 °C for 48 h to obtain dry mass (DM). The measurements so obtained were used to calculate LRWC by employing the following formula:

$$LRWC\ (\%) = [(FM - DM)/ (TM - DM)] \times 100$$

Leaf water potential measurements were carried out early in the morning using a newly expanded leaf of a plant and subjecting it to a water potential measurement system (PMS model 600, PMS Instrument Company, Albany, OR, USA).

2.4. Leaf free proline content

Free proline content was determined in fresh leaf samples following the procedure of Bates et al. (1973).

A mixture containing ninhydrin (1.25 g) and glacial acetic acid (30 mL) was dissolved in phosphoric acid (6 M, 20 mL). A sample comprising 1 g of leaves was extracted in sulfosalicylic acid (10 mL, 3%). After filtering the extract through a Whatman No. 2 filter paper, the extraction solution was boiled for 1 h at 100 °C. Absorbance readings of the cooled samples were recorded at 515 nm against toluene control. The formerly prepared L-proline solution was used as a standard.

2.5. Electrolyte leakage (EL)

A fresh leaf sample of 0.2 g was cut into equal pieces and placed in a test tube containing distilled water (10 mL). After retaining in a water bath for 2 h, the first electrical conductivity (EC1) was measured with an EC meter. The samples were then incubated at 121 °C for 20 min and cooled down to room temperature to measure the second electrical conductivity (EC2). The EL was calculated using the equation outlined by Dionisio-Sese and Tobita (1998).

2.6. Assay of ascorbate (AsA)

Assay of ascorbate was appraised according to Mukherjee and Choudhuri (1983). Leaf tissues were powdered in liquid nitrogen and then homogenized in a mixture consisting of 2% dinitrophenyl–hydrazine and 6% trichloroacetic acid prepared in half-strength H_2SO_4 and 10% thiourea dissolved in 70% ethanol. The mixture was boiled for 15 min in a water bath, and its temperature was then lowered down to 25 °C. Following centrifugation for 10 min at 1000 *g* at 4 °C, the resultant pellet was liquefied in H_2SO_4 (80%). The optical densities of all samples were noted at 530 nm.

2.7. Glutathione (GSH) assay

The total GSH was analysed following the procedure outlined by Ellman (1959). An aliquot of 3 mL of 4% sulfosalicylic acid was added to the tissue extracted (0.5 mL) in phosphate buffer. The mixture was subjected to

centrifugation at 3000 *g*, and an aliquot of the supernatant (0.5 mL) was treated with Ellman's reagent. After a lapse of 10 min, the optical densities (ODs) were noted spectrophotometrically at 412 nm.

2.8. L-cysteine desulfhydrase activity (LDES) and hydrogen sulfide (H_2S)

The LDES activity was estimated by the release of H_2S from L-cysteine in the existence of dithiothreitol (DTT) following the procedure outlined by Li et al. (2012). Leaf tissue (5.0 g) was powdered in a mortar and pestle with liquid nitrogen, and the soluble protein was homogenized by adding Tris–HCl (20 mM, 5 mL, pH 8.0). To attain an equivalent amount of protein in each sample, the content of protein in the extract was adjusted to 100 μg mL^{-1}. A 1.0-mL sample of the of the extraction consisted of 0.8 mM L-cysteine, 100 mM Tris–HCl, 2.5 mM DTT at pH 9.0, and 10 μg protein solution. L-cysteine was added to start the reaction; it was then subjected to 37 °C for 15 min. An aliquot of 0.1 mL of $FeCl_3$ (30 mM) was dissolved in 1.2 M HCl, and 0.1 mL of 20 mM N,N-dimethyl-p-phenylenediamine dihydrochloride was dissolved in 7.2 M HCl; this was then added to the extract as a last reaction. The absorbance of the treated solutions was read at 667 nm after keeping it at room temperature for 15 min.

Leaf H_2S was determined by the methylene blue formation from dimethyl-*p*-phenylenediamine in H_2SO_4, based on the procedure reported by Li et al. (2012).

2.9. Antioxidant enzymes and soluble protein

The activity of SOD was assessed by measuring the SOD ability to thwart the photochemical inhibition of nitroblue tetrazolium (NBT) (Van Rossum et al., 1997). A half-gram of fresh leaf material was homogenized in 50 mM Na-P buffer which contained 1% of soluble polyvinyl pyrrolidine. The mixture was centrifuged at 20,000 *g* at 4 °C for 15 min; the supernatant was then separated and used for appraisal of SOD enzyme activity. One SOD unit was termed as the enzyme amount that blocked 50% reduction of cytochrome C.

The procedure outlined by Milosevic and Slusarenko (1996) was followed to determine the activity of catalase. Following the procedure, protein extract (50 μL) was added to the reaction mixture consisting of 2.95 mL of 10 mM H_2O_2, potassium phosphate buffer (50 mM, pH 7.0), and Na_2 EDTA (4 mM). The absorbance readings were recorded at 240 nm at 25 °C for 30 sec.

The activity of peroxidase was measured based on the procedure outlined by Chance and Maehly (1955). Fresh leaf tissue was extracted as described earlier. The supernatant of leaf extract (100 μL) was mixed with 3 mL of reaction mixture [13 mM guaiacol, 5 mM H_2O_2, and 50 mM Na-phosphate (pH 6.5)]. The absorbance readings were recorded at 470 nm for 1 min at 25 °C.

2.10. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)

Leaf H_2O_2 was determined following the protocol outlined by Velikova et al. (2000). The leaf MDA was quantified following the procedure outlined by Weisany et al. (2012)

2.11. Mineral nutrient analysis

Well-macerated leaf samples were subjected to a high temperature of 550 °C in a muffle furnace to determine Na^+, P, Ca^{2+}, and K^+. Hot HCl (5 mL, 2M) was added to each sample ash, and the final volume of each sample was raised to 50 mL with the addition of distilled deionized water. A vanadate–molybdate method was followed to quantify P using a spectrophotometer (Chapman and Pratt, 1982). An ICP was used to analyze Na^+, Ca^{2+}, and K^+ in the digested samples.

2.12. Statistical analysis

The data for all parameters were analysed using analysis of variance. Mean data along with standard errors are presented in all figures at $P < 0.0$.

3. Results

3.1. SP improved phenotypic appearance of maize plants

Salinity stress (SS) caused yellowing, which was apparent on the leaves of the maize plants. Furthermore, SS led to reduced plant height and leaf size. Supplementary P mitigated the leaf chlorosis and yellowing induced by SS. However, application of HT, the scavenger of H_2S, reversed the improved phenotypic appearance induced by SP (Figure 1).

3.2. SP-induced H_2S and L-cysteine desulfhydrase (L-DES) generation enhanced key physiological parameters in salinity-stressed maize plants

Salinity stress (100 mM NaCl) significantly depressed the shoot, root, and total plant dry mass of maize plants by 48.2%, 38.9%, and 45.8%, respectively, compared to those of the control plants ($P \leq 0.001$). Conversely, supplemented P (SP) partly enhanced the shoot, root, and total plant dry mass by 37.9%, 57.5%, and 41.6%, respectively, compared to those under salinity stress conditions alone. Application of the inhibitor of L-DES, $_{DL}$-propargylglycine (PAG; 0.3 mM), or the scavenger of H_2S, hypotaurine (HT; 0.1 mM), along with SP, fully reversed the positive effects of SP on the earlier-mentioned attributes in plants under salinity stress. These parameters did not differ in control plants with different treatments (Figures 2A–2C).

A substantial decrease was observed in chlorophyll a and b contents as well as efficiency of photosystem II (F_v/F_m) by 29.1%, 25.2%, and 22.1%, respectively, in maize plants subjected to salinity stress compared to those in control plants (Figures 2D–2F). These traits were not changed by different treatments in control plants, but SP successfully improved these parameters in salinity-

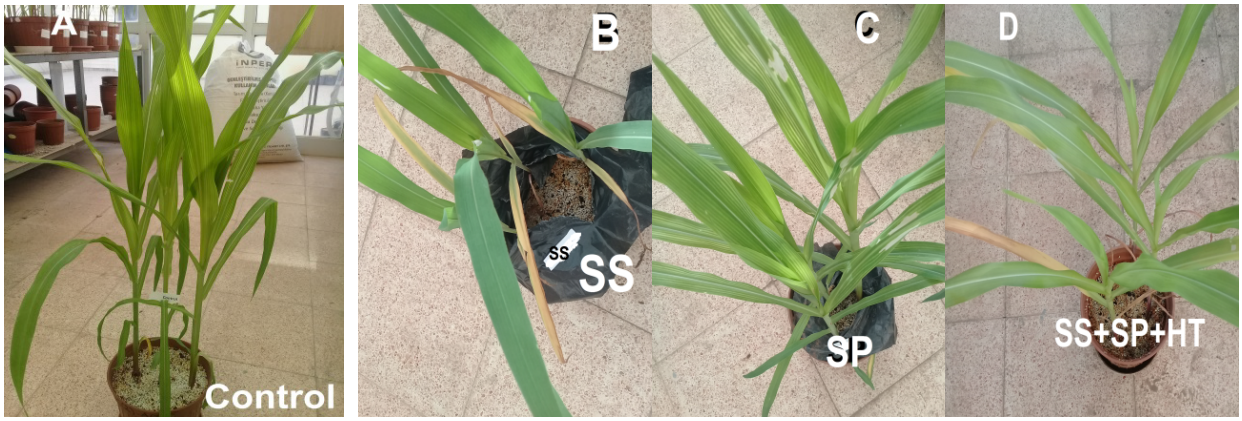

Figure 1. Phenotypic effects of supplementary phosphorus (SP) and the scavenger of hydrogen sulphide (H_2S), hypotaurine (HT), on maize plants subjected to salinity stress (SS) in comparison with controls. (A) control treatment: half strength Hoagland's nutrient solution; (B) SS treatment: 100 mM NaCl; (C) SS + SP treatment: 100 mM NaCl + 2.0 mM P; (D) SS + SA + HT treatment: 100 mM NaCl + 2.0 mM SP + 0.1 mM HT. Photographs were taken 21 days after the initiation of treatments.

stressed plants by 38.3%, 32.2%, and 27.2%, respectively, in comparison to those in plants under salinity stress alone. These enhancements induced by the SP treatment in all of these parameters were completely eliminated by HT or PAG application. These findings show that when either H_2S or LDES was blocked within plants, SP treatment remained ineffective in alleviating salinity stress. Thus, H_2S and LDES jointly participated in SP-induced salinity tolerance in maize plants.

A considerable ($P \leq 0.001$) decrease was observed in leaf relative water contents (RWC) and water potential (ΨI) of the maize plants, but proline content increased in the plants under salinity stress (Figures 3A–3C). SP enhanced RWC, leaf ΨI, and proline in the maize plants exposed to SS. However, these attributes did not differ in the control plants due to different treatments. The positive effects of SP on these parameters were completely masked by the supply of HT or PAG.

In order to determine if H_2S relates to SP-induced SS tolerance of maize plants, alteration in endogenous H_2S content and activity of LDES was additionally measured in the maize plants. Salinity stress led to an increase in both total LDES activity and H_2S synthesis by 35.7% and 48.2%. respectively. compared to those in the control treatment (Figures 4A and 4B). SP led to a further increase in LDES activity and H_2S accumulation in the maize plants subjected to SS. These results reveal that supplemented P might enhance the synthesis of endogenous H_2S by triggering LDES activity in the maize plants. The application of PAG or HT along with SP under salinity stress totally eliminated H_2S synthesis, while total LDES activity was reduced by PAG alone. In view of these results, it can be inferred that PAG can terminate the activity of LDES, thereby inactivating H_2S synthesis, but HT can only be effective in reducing H_2S alone. Thus, the positive effect of SP under salinity stress might have been due to activation of total LDES and the H_2S synthesis. These findings suggest that H_2S could be a downstream signal molecule involved in the regulation of SP-induced salinity tolerance in maize plants. Under control conditions, H_2S synthesis was reduced by both HT and PAG, but total LDES activity was significantly ($P \leq 0.001$) reduced by PAG alone.

Ascorbic acid (AsA) and glutathione (GSH) contents were both reduced significantly ($P \leq 0.001$) due to SS compared to those in the control plants (Figures 4C and 4D). Supplemented P led to an increase in the contents of AsA and GSH by 67.3% and 106.3%, respectively, compared to those in the maize plants subjected to salinity stress alone. These increments in AsA and GSH due to SP under salinity stress were totally eliminated by both PAG and HT, while these metabolites were not changed by different treatments under control conditions.

3.3. SP-induced LDES activity and H_2S synthesis reduced leaf Na^+ content and improved leaf P, Ca^{2+}, and K^+ in salinity-stressed maize plants

As expected, SS caused a significant increase in leaf Na^+ content in salt-stressed maize plants (Figure 5A). However, SP significantly ($P \leq 0.001$) lowered the leaf Na^+ accumulation by 30.1% in the maize plants under saline stress. PAG or HT plus SP treatment completely eliminated the reduction in Na^+ level in the maize plants subjected to salinity stress.

Salinity stress noticeably ($P \leq 0.001$) reduced Ca^{2+}, K^+, and P contents in the maize plants (Figures 5B–5D). However, supplemented P improved leaf Ca^{2+}, K^+, and P contents in salinity-stressed maize plants. PAG or HT plus P treatment completely eliminated the increase in Ca^{2+}, K^+, and P in the maize plants subjected to salinity stress. These nutrient contents under control conditions were not changed by different treatments.

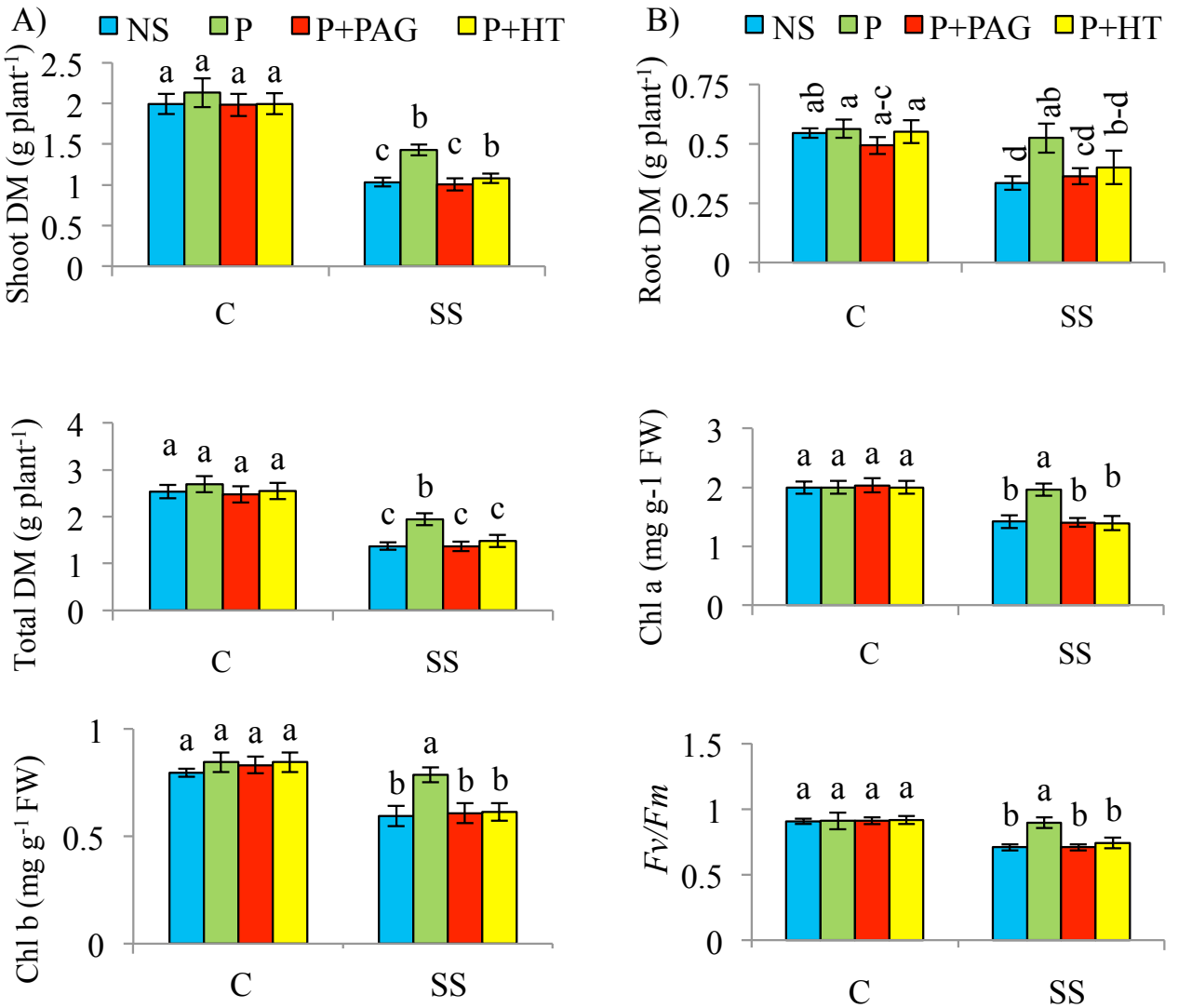

Figure 2. Shoot (A), root (B), and total (C) dry matter (DM), chlorophyll a (D), chlorophyll b (E) on fresh weight (FW) basis and chlorophyll fluorescence parameters [*Fv*/*F*m (F)] in maize plants supplied with 1 mM additional phosphorus (P) or not supplied (NS) into nutrient solution under control (C) and 100 mM salt stress (SS) combined with 0.3 mM inhibitor of L-DES, $_{DL}$-propargylglycine (PAG), and 0.1 mM hypotaurine (HT), the scavenger of H_2S. Mean ± S.E.; mean values carrying different letters within each parameter differ significantly (P ≤ 0.001) based on the Duncan's multiple range test.

3.4. SP-induced LDES activity and the H_2S synthesis reversed oxidative stress and improved antioxidant defence system in maize plants under salinity stress

Salinity stress considerably (P ≤ 0.001) elevated the accumulation of H_2O_2 and MDA contents as well as electrolyte leakage (EL) in the maize plants (Figures 6A–6C). Supplemented P effectively reduced the accumulation of H_2O_2 and MDA as well as EL in the maize plants under salinity stress. However, H_2O_2 and MDA contents and EL in the control plants were not changed under different treatments. SP plus PAG or HT totally eliminated the decrease in oxidative-stress–related traits to the levels of those found in plants under salinity stress alone.

Salinity stress considerably improved (P ≤ 0.001) SOD, CAT, and POD activities in comparison to those in the unstressed plants. SP further increased the activities of these enzymes. Application of PAG or HT fully overturned (P ≤ 0.001) the positive effect of supplemented P on all pf these enzymes (Figures 7A–7C).

4. Discussion

As shown in the present investigation, reduced plant growth due to SS has been reported in a range of plant species, e.g. rice (Tian et al., 2016) and pepper (Al-Taey, 2018). Reduced plant growth due to salinity stress might be due to impairment in key physiological events

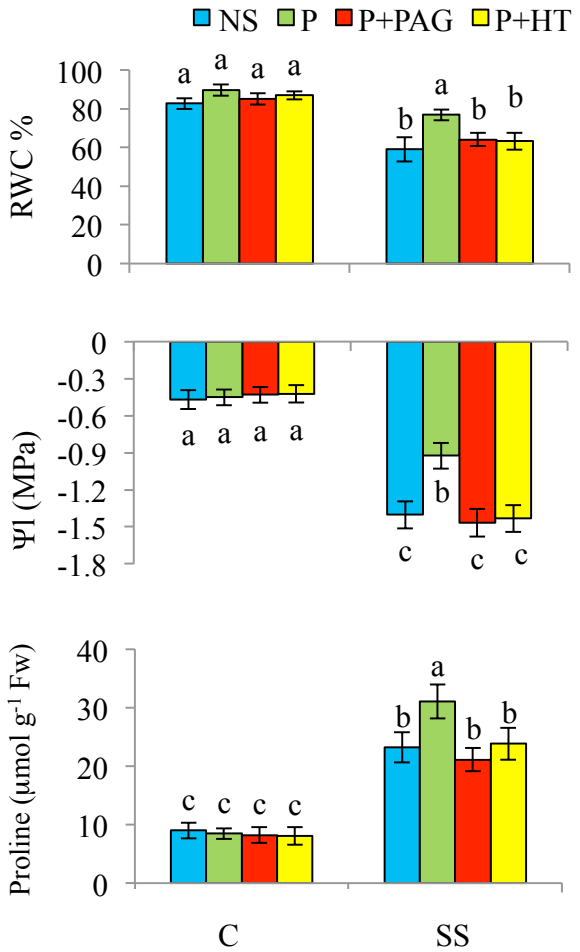

Figure 3. Leaf relative water content [RWC (A)], leaf water potential [Ψl (B)], and proline (C) content in maize plants supplied with 1 mM additional phosphorus (P) or not supplied (NS) into nutrient solution under control (C) and 100 mM salt stress (SS) combined with 0.3 mM inhibitor of L-DES, $_{DL}$-propargylglycine (PAG) and 0.1 mM hypotaurine (HT), the scavenger of H_2S. Mean ± S.E.; mean values carrying different letters within each parameter differ significantly ($P \leq 0.001$) based on the Duncan's multiple range test.

and restriction of nutrient uptake in plants (Zeng et al., 2018). To ascertain if supplemented P can alleviate the decrease in the growth of SS plants, SP was supplied into the nutrient solution of SS plants. Our findings showed that SP partly alleviated the decrease in plant growth induced by SS. Hence, SP could be an effective fertiliser in maize plant's response to SS, as has been previously reported in tomato by Çimrin et al. (2010) and in bean by Bargaz et al. (2016). Similarly, the beneficial effect of SP has previously been reported in some plant species under various stresses, e.g. boron toxicity in tomato (Kaya et al., 2009), cadmium stress in wheat (Arshad et al., 2016), and water stress in maize (Ahmad et al., 2017). The mitigation effect of SP on salt-induced reduced plant growth may be due to increased chlorophyll content and *Fv*/*F*m as well as nutrient elements, e.g. P, Ca^{2+}, and K^+, which were in fact reduced by SS. These results suggest that SP may be involved in the response of plants to SS, as has been previously reported in pepper (Daei-hassani et al., 2016) and maize (Sacala et al., 2016).

To obtain evidence that LDES activity and H_2S participate in the SP-induced SS tolerance of maize plants, an inhibitor of LDES (PAG) or scavenger of H_2S (HT) was sprayed on the leaves of SS plants to observe if SP could trigger LDES activity and H_2S content. Our results showed that when PAG or HT was applied along with SP, SP did not mitigate the decrease in plant growth induced by SS. The PAG or HT eliminated the increase in LDES activity and H_2S; hence, SP did not successfully maintain salinity tolerance of the maize plants. Our findings show that SP activated LDES in the leaves of maize plants under SS, and LDES enzyme triggered H_2S to induce salinity tolerance; thus, H_2S could be a downstream signal molecule involved in the mechanism of SP-induced salinity tolerance. The positive effect of exogenously applied H_2S has been reported in various plants under SS such as alfalfa, rice, and *Vicia faba* (Wang et al., 2012; Mostofa et al., 2015; Ma et al., 2019, respectively), as well as under other stresses, e.g. water stress in tobacco (Zhang et al., 2018) and iron deficiency in wheat (Zhang et al., 2017) and strawberry (Kaya and Ashraf, 2019). Conversely, no report could be found in the available literature on the role of SP-induced H_2S and LDES activity in enhancing SS tolerance. Hence, this seems to be the first report providing insight into the underlying mechanism of SP-induced SS tolerance.

Both chlorophyll content and maximum photochemical efficiency (*Fv/Fm*) of plants have been reported to be negatively affected by stress conditions (Poudyal et al., 2019), as has been found in the present experiment in which SS considerably decreased these attributes. In the former studies, the likely reason for decrease in both attributes under stress conditions could be generally connected to the overgeneration of H_2O_2 in plants (Alyemeni et al., 2018), as has been shown in the present experiment. Furthermore, SP improved chlorophyll content and *Fv/Fm* as well as reduced H_2O_2 levels in SS plants, suggesting that SP plays a key role in mitigating the negative effects of SS on chlorophyll content and *Fv/Fm*, probably by decreasing the accumulation of H_2O_2. Similarly, the stimulation of biosynthesis of chlorophyll molecules is encouraged by the uptake of phosphorus at optimum levels (Arshad et al., 2016). Moreover, SP might have enhanced the energy reallocation between PSII and PSI to enable plants to endure stress conditions (Carstensen et al., 2018). It has also been reported that SP enhanced *Fv/Fm* in the stressed plants. As an example, Rady et al. (2018) stated

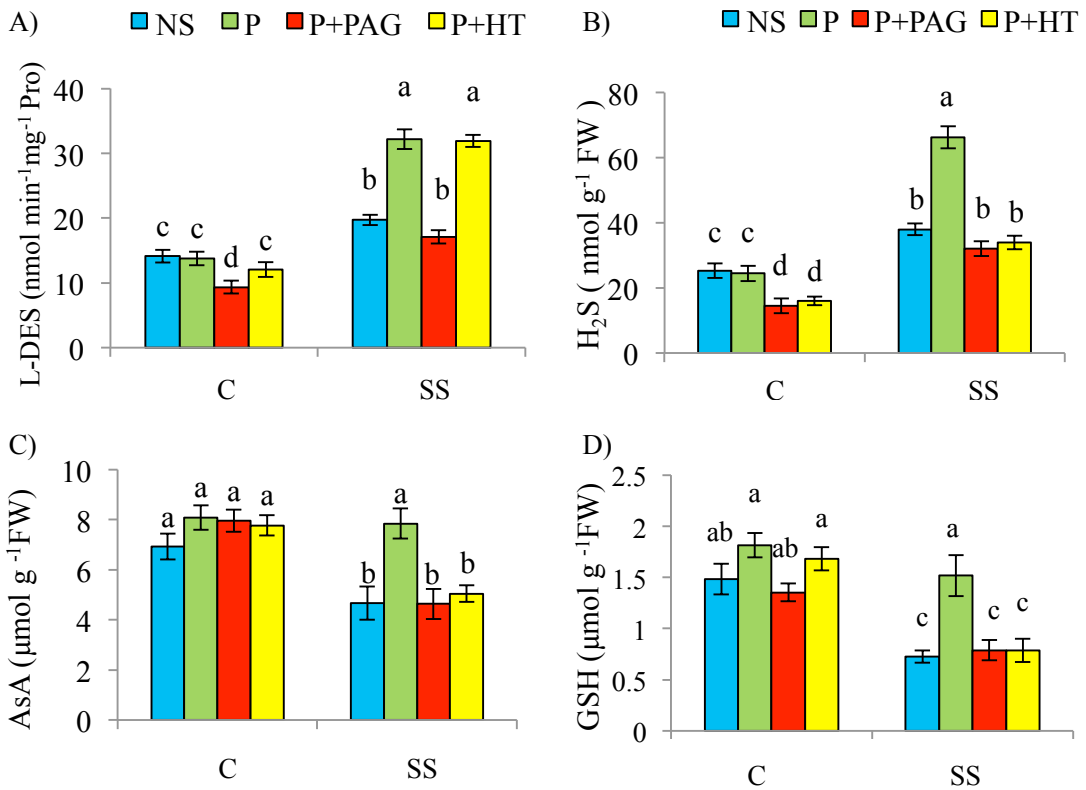

Figure 4. L-cysteine desulfhydrase [L-DES (A)], hydrogen sulfide [H_2S (B)], ascorbate [AsA; (C)] and glutathione [GSH; (D)] on fresh weight (FW) basis in maize plants supplied with 1 mM additional phosphorus (P) or not supplied (NS) into nutrient solution under control (C) and 100 mM salt stress (SS) combined with 2 mM inhibitor of L-DES, $_{DL}$-propargylglycine (PAG) and 0.1 mM hypotaurine (HT), the scavenger of H_2S. Mean ± S.E.; mean values carrying different letters within each parameter differ significantly ($P \leq 0.001$) based on the Duncan's multiple range test.

that SP amended *Fv/Fm* in common bean plants exposed to salinity stress. In addition, SP enhanced *Fv/Fm* in *Eucalyptus grandis* seedlings under drought stress (Tariq et al., 2018). Both LDES activity and endogenous H_2S content increased with SP in plants under SS. However, application of HT or PAG reversed the increase in LDES activity and endogenous H_2S, and eliminated an increase in chlorophyll content and *Fv/Fm,* suggesting that LDES activity and H_2S might play vital roles in SP-enhanced chlorophyll content and *Fv/Fm*. It has been reported that exogenously applied H_2S improved chlorophyll content in pepper plants subjected to zinc toxicity stress (Kaya et al., 2018), in cucumber stressed with cadmium (Kabała et al., 2018) and strawberry plants subjected to iron deficiency (Kaya and Ashraf, 2019).

Salinity stress affects both water status (Gandonou et al., 2018) and nutrient uptake of plants (Rahneshan et al., 2018), as shown in our experiment. However, SP improved these attributes under SS conditions. SP has been reported to improve RWC in wheat plants under water stress (Zhang et al., 2018). Enhanced water status of plants by SP in salinity-stressed plants might be due to enhanced proline accumulation. Similar to the present data, accumulation of proline at a reasonable level is known to improve plant water status under SS (Ghani et al., 2018).

One more crucial mechanism operative in plants is adaptation to salinity stress by regulating the antioxidant defence system by inducing ascorbate (AsA) and glutathione (GSH), as they play a key role in removing ROS and sustaining cellular redox potential (Das et al., 2018). Our results also reveal that AsA and GSH contents increased against the accumulation of H_2O_2 to reverse oxidative damage in SS plants.

Mineral nutrients are required for a range of key metabolic processes. They must be accumulated adequately in plants to sustain stability of plant structure and key physiological processes; plant metabolism can markedly be disrupted in the case of any deficiency of these elements (Liang et al., 2018). However, one of the detrimental effects induced by elevated accumulation of Na^+ due to salinity is nutritional imbalance such as of P (Bargaz et al., 2016; Sacala et al., 2016), K^+ (Ahanger and Agarwal, 2017), and Ca^{2+} (Rahman et al., 2016). Phosphorus is one of the essential mineral nutrients that has been tested in various

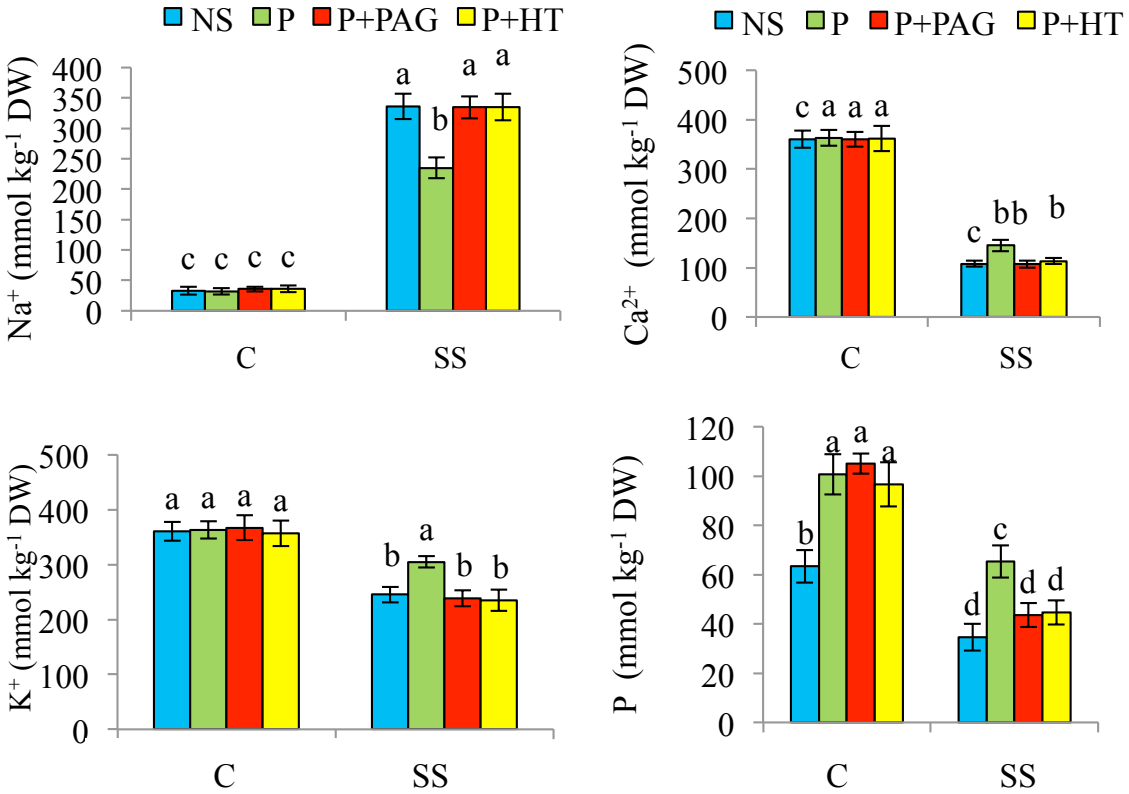

Figure 5. Leaf sodium [Na (A)], calcium [Ca^{2+} (B)], potassium [K^+ (C)] and phosphorus [P (D)], on dry weigh (DW) basis in maize plants supplied with 1 mM additional phosphorus (P) or not supplied (NS) into nutrient solution under control (C) and 100 mM salt stress (SS) combined with 0.3 mM inhibitor of L-DES, $_{DL}$-propargylglycine (PAG) and 0.1 mM hypotaurine (HT), the scavenger of H_2S. Mean ± S.E; Mean values carrying different letters within each parameter differ significantly ($P \leq 0.001$) based on the Duncan's multiple range test.

plant species against salinity stress (Bargaz et al., 2016; Sacala et al., 2016). Furthermore, it has been reported that SP increased leaf K^+ and Ca^{2+} contents in the plants under SS (Rady et al., 2018). The present results reveal that SP enhanced tolerance to salinity stress of maize plants by enhancing water status as well as leaf P, Ca^{2+}, and K^+ contents and reducing Na^+ content in plants.

The treatment of PAG or HT combined with SP reduced leaf water status and nutrient element contents, suggesting that SP activated LDES activity and H_2S synthesis to induce tolerance to salinity stress. Exogenously applied H_2S has been observed to improve RWC and leaf Ca^{2+} and K^+ levels in rice plants under salinity stress (Mostofa et al., 2015). It has also been reported that exogenous H_2S reduced Na^+ content in wheat plants under SS (Deng et al., 2016).

Salinity stress increased H_2S content in tomato (da-Silva et al., 2018) and rice (Mostofa et al., 2015), as has been shown in our experiment. Hence, these results suggest that H_2S might act as a signal molecule in some crucial metabolic processes of salt-stressed plants. Furthermore, supplemented P led to a further increase in H_2S generation in SS plants. There seems to be no report on SP-induced endogenous H_2S synthesis in the literature; thus, it is reasonable to suggest that SP could trigger endogenous H_2S synthesis, and it may also activate LDES, a specific enzyme involved in some vital processes of plants (Qiao et al., 2015), but application of PAG or HT along with SP treatment reversed the positive effect of SP by lowering both LDES activity and endogenous H_2S accumulation.

The present results show that SP reversed oxidative stress and upregulated the activities of antioxidant enzymes such as SOD, CAT, and POD in maize plants under salinity stress. Similar findings have been reported in other studies, e.g. Daei-hassani et al. (2016) in pepper and Sadji-Ait Kaci et al. (2017) in *Cicer arietinum* plants under salinity stress. One of the strategies developed by plants to reverse oxidative stress is to generate antioxidant enzymes, including CAT and POD, to eliminate H_2O_2 under saline stress (Wang et al., 2016). However, application of PAG or HT along with SP treatment inverted these improvements by blocking LDES activity and endogenous H_2S synthesis, suggesting that LDES activity and endogenous H_2S accumulation are involved in P-induced SS tolerance of maize plants. Furthermore, it has been reported that

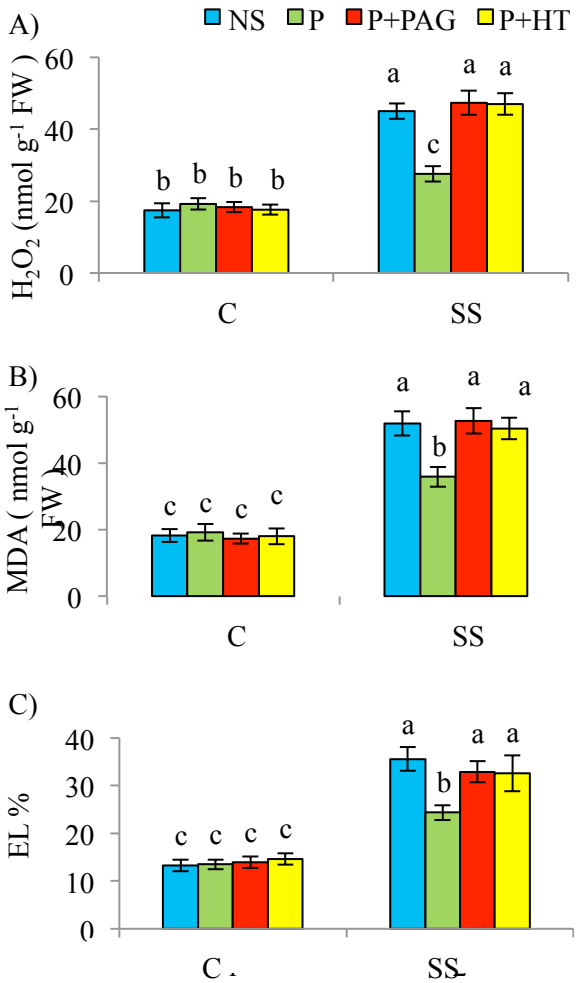

Figure 6. Hydrogen peroxide [H_2O_2 (A)], malondialdehyde [MDA (B)] and electrolyte leakage [EL (C)] in maize plants supplied with 1 mM additional phosphorus (P) or not supplied (NS) into nutrient solution under control (C) and 100 mM salt stress (SS) combined with 0.3 mM inhibitor of L-DES, $_{DL}$-propargylglycine (PAG) and 0.1 mM hypotaurine (HT), the scavenger of H_2S. Mean ± S.E.; mean values carrying different letters within each parameter differ significantly ($P \leq 0.001$) based on the Duncan's multiple range test.

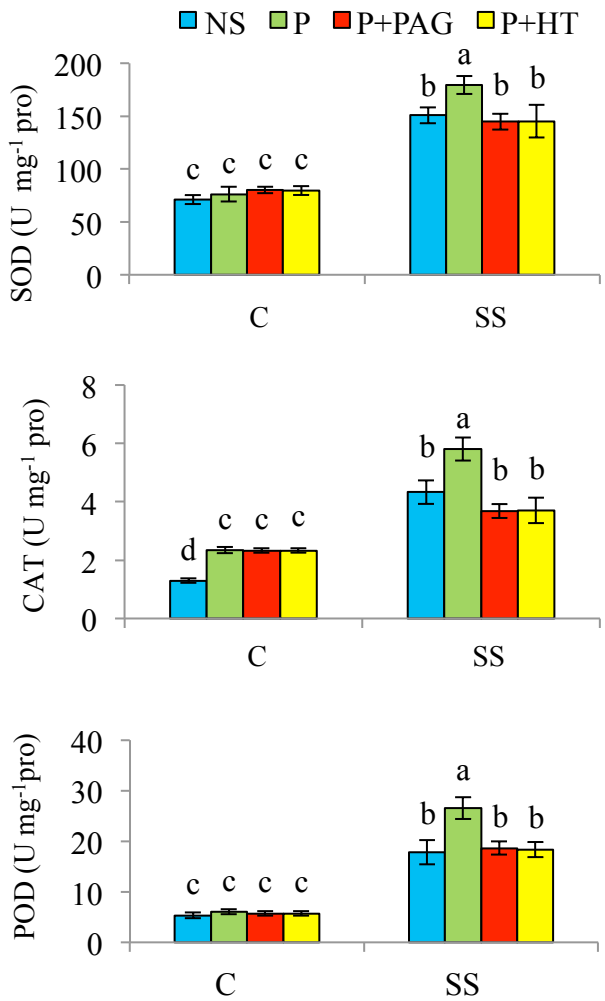

Figure 7. Activities of superoxide dismutase [SOD (A)], catalase [CAT (B)] and peroxidase [POD (C)] in maize plants supplied with 1 mM additional phosphorus (P) or not supplied (NS) into nutrient solution under control (C) and 100 mM salt stress (SS) combined with 0.3 mM inhibitor of L-DES, $_{DL}$-propargylglycine (PAG) and 0.1 mM hypotaurine (HT), the scavenger of H_2S. Mean ± S.E.; mean values carrying different letters within each parameter differ significantly ($F \leq 0.001$) based on the Duncan's multiple range test.

exogenously applied H_2S can reduce oxidative damage and improve the antioxidant defence system (Wei et al., 2019).

5. Conclusions

Overall, SP activated LDES and H_2S synthesis, which improved SS tolerance of maize plants, suggesting that H_2S could be a downstream signal molecule involved in the mechanism of SP-induced enhanced salinity tolerance. The treatment of PAG or HT plus SP reversed the alleviating effects of SP on plants under SS and made the SP ineffective in improving SS tolerance. Hence, SP, LDES, and H_2S jointly participated in improving SS tolerance of maize plants. Furthermore, LDES and H_2S induced by SP might be a key enzyme and a signalling molecule, respectively, in maintaining the antioxidant defence enzymes to eliminate oxidative stress. As a future prospect, the roles of other signalling molecules in SP-induced response of plants under SS also need to be uncovered.

Acknowledgment

The authors wish to thank the University of Harran (HUBAK-18227) for supporting the present study.

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