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

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Abstract: Although phosphorus supplementation (SP) has been shown to improve salinity tolerance in plants, crosstalk between hydrogen sulfide (H₂S) or L-cysteine desulphhydrase (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 H₂S, 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 KH₂PO₄ 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 H₂S, 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 H₂S and proline, and LDES activity. However, HT significantly reversed the levels of H₂S, but did not reduce LDES activity. The application of PAG reversed both H₂S 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 H₂S 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 (H₂S), 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 H₂S 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 desulphhydrase is known as a crucial enzyme in H₂S biosynthesis, which is responsible for H₂S synthesis (Guo et al., 2017).

Although H₂S 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 H₂S and LDES in P-induced salinity tolerance. In the current investigation, we intended to appraise the putative role of H₂S and LDES in P-induced salinity tolerance by using a scavenger of H₂S, 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
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 KH₂PO₄ 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 H₂S, 0.1 mM hypotaurine (HT), or the inhibitor of LDES, 0.3 mM propargylglycine (PAG), for 12 h to confirm if H₂S 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.

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 retaning 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).

Electrolyte leakage (EL) was used as a standard.

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 sulfsalicylic 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.

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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:

\[ \text{LRWC} (%) = \frac{[(FM - DM)]}{(TM - DM)} \times 100 \]

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 sulfsalicylic 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 retaning 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 H₂SO₄ 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 H₂SO₄ (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% sulfsalicylic 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 desulphhydrase activity (LDES) and hydrogen sulfide (H₂S)

The LDES activity was estimated by the release of H₂S 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 FeCl₃ (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 H₂S was determined by the methylene blue formation from dimethyl-p-phenylenediamine in H₂SO₄, 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 H₂O₂, potassium phosphate buffer (50 mM, pH 7.0), and Na₄ 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 H₂O₂, 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 (H₂O₂) and malondialdehyde (MDA)

Leaf H₂O₂ 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, Ca²⁺, 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⁺, Ca²⁺, 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 H₂S, reversed the improved phenotypic appearance induced by SP (Figure 1).

3.2. SP-induced H₂S and L-cysteine desulphhydrase (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 ≤ 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-propargyglycine (PAG; 0.3 mM), or the scavenger of H₂S, 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 (Fᵥ/Fₒ) 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-stressed maize plants.
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$_2$S or LDES was blocked within plants, SP treatment remained ineffective in alleviating salinity stress. Thus, H$_2$S and LDES jointly participated in SP-induced salinity tolerance in maize plants.

A considerable (P ≤ 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$_2$S relates to SP-induced SS tolerance of maize plants, alteration in endogenous H$_2$S 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$_2$S 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$_2$S accumulation in the maize plants subjected to SS. These results reveal that supplemented P might enhance the synthesis of endogenous H$_2$S by triggering LDES activity in the maize plants. The application of PAG or HT along with SP under salinity stress totally eliminated H$_2$S 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$_2$S synthesis, but HT can only be effective in reducing H$_2$S alone. Thus, the positive effect of SP under salinity stress might have been due to activation of total LDES and the H$_2$S synthesis. These findings suggest that H$_2$S could be a downstream signal molecule involved in the regulation of SP-induced salinity tolerance in maize plants. Under control conditions, H$_2$S synthesis was reduced by both HT and PAG, but total LDES activity was significantly (P ≤ 0.001) reduced by PAG alone.

Ascorbic acid (AsA) and glutathione (GSH) contents were both reduced significantly (P ≤ 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$_2$S 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, supplemented P significantly (P ≤ 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 increase in Na$^+$ in the maize plants subjected to salinity stress. These nutrient contents under control conditions were not changed by different treatments.
3.4. SP-induced LDES activity and the H$_2$S 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$_2$O$_2$ and MDA contents as well as electrolyte leakage (EL) in the maize plants (Figures 6A–6C). Supplemented P effectively reduced the accumulation of H$_2$O$_2$ and MDA as well as EL in the maize plants under salinity stress. However, H$_2$O$_2$ 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 of 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.
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 pepper (Daei-hassani et al., 2016) and maize (Sacala et al., 2016).

To obtain evidence that LDES activity and H$_2$S participate in the SP-induced SS tolerance of maize plants, an inhibitor of LDES (PAG) or scavenger of H$_2$S (HT) was sprayed on the leaves of SS plants to observe if SP could trigger LDES activity and H$_2$S 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$_2$S; 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$_2$S to induce salinity tolerance; thus, H$_2$S could be a downstream signal molecule involved in the mechanism of SP-induced salinity tolerance. The positive effect of exogenously applied H$_2$S 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$_2$S 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$. 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 PSI 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...
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 Ca²⁺ (Rahman et al., 2016). Phosphorus is one of the essential mineral nutrients that has been tested in various

Figure 4. L-cysteine desulphhydrase [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, L-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.
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\(_2\)S synthesis to induce tolerance to salinity stress. Exogenously applied H\(_2\)S 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\(_2\)S reduced Na\(^+\) content in wheat plants under SS (Deng et al., 2016).

Salinity stress increased H\(_2\)S 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\(_2\)S 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\(_2\)S generation in SS plants. There seems to be no report on SP-induced endogenous H\(_2\)S synthesis in the literature; thus, it is reasonable to suggest that SP could trigger endogenous H\(_2\)S 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\(_2\)S 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\(_2\)O\(_2\) 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\(_2\)S synthesis, suggesting that LDES activity and endogenous H\(_2\)S accumulation are involved in P-induced SS tolerance of maize plants. Furthermore, it has been reported that

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**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\(_2\)S. Mean ± S.E; Mean values carrying different letters within each parameter differ significantly (P ≤ 0.001) based on the Duncan's multiple range test.
exogenously applied H\textsubscript{2}S can reduce oxidative damage and improve the antioxidant defence system (Wei et al., 2019).

5. Conclusions
Overall, SP activated LDES and H\textsubscript{2}S synthesis, which improved SS tolerance of maize plants, suggesting that H\textsubscript{2}S 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\textsubscript{2}S jointly participated in improving SS tolerance of maize plants. Furthermore, LDES and H\textsubscript{2}S 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.

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