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# Exogenous hydrogen sulfide enhanced Al stress tolerance in tea plant *Camellia sinensis*

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## Abstract

Al is an essential element for the growth of tea plants roots, but excessive Al affects growth and development of *Camellia sinensis*. The underlying mechanism, particularly regulation of gas signaling molecule $H_2S$, remains unclear. This study aims to uncover the function of $H_2S$ on *C. sinensis* under Al stress by treating hydroponic tea seedlings with different Al concentration, $Na_2S$ ($H_2S$ donor) and DL-propargylglycine (PAG, synthesis inhibitor). High concentration of Al inhibits growth of tea roots, while $H_2S$ significantly improves the effects caused by Al stress. Whether it is 2 mM $Al^{3+}$ or 4 mM $Al^{3+}$, $H_2S$ reduces content of Al in the entire plant and roots, increases root activity, further promotes root growth, increases fresh and dry weight, regulates ion homeostasis, improves cell structure, increases chlorophyll content, and thus reduces the damage of Al toxicity in *C. sinensis*. Moreover, in response to the stress of 2 mM $Al^{3+}$, $H_2S$ simultaneously alleviates Al stress by regulating substances related to antioxidant pathways, increasing content of GSH and GSSG, enhancing activity of GST, GR, LCD, and key components of tea, in order to alleviate Al stress. These approaches have effectively improved Al tolerance of *C. sinensis*, providing a new perspective for the study of $H_2S$ enhancing Al tolerance.

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## Introduction

Tea plant [*Camellia sinensis* (L.) O. Kuntze], is suitable for growing in acidic soil with pH 4.5–6.5. Aluminum (Al) toxicity is currently a crucial factor limiting plant growth in acidic environments, because when the pH of the soil is less than 5, Al can be transformed into phytotoxic trivalent cation ($Al^{3+}$) that are readily absorbed by plants, thereby affecting plant growth[1]. As an Al hyperaccumulating plant, *C. sinensis* can contain up to 30,000 mg·kg$^{-1}$ of Al in its mature leaves without showing symptoms of Al toxicity[2]. Appropriate Al concentration promotes the growth and development of tea plants. Once it exceeds 1 mmol·L$^{-1}$, *C. sinensis* suffer from a negative effect on its normal growth[3].

Various strategies for plants to cope with Al toxicity include external exclusion mechanisms such as increasing Al chelation and reducing Al uptake by plants, as well as increasing antioxidant enzyme activity and reducing toxic substances caused by reactive oxygen species and free radicals, among other internal detoxification mechanisms[4]. Meanwhile, excessive Al also has a certain impact on the tea quality components of tea polyphenols, catechins, amino acids, caffeine and other substances[5]. Not only that, tea consumption also increases dietary intake of Al, which is thought to be associated with Alzheimer's disease[6]. Therefore, exploration of measures to reduce content of Al in *C. sinensis* is of great significance in alleviating Al stress and improving tea quality.

Hydrogen sulfide ($H_2S$) is regarded as a poisonous gas and atmospheric pollutant, but it was subsequently found to be the third gaseous signaling molecule after nitric oxide (NO) and carbon monoxide (CO)[7]. And synthesizes endogenous $H_2S$ mainly through L-cysteine desulphydrase (LCD), which is widely present in plants[8]. Recently, research on $H_2S$ has begun to reveal the role of these molecules in regulating plant abiotic and biotic stress resistance responses. Through the exogenous application of $H_2S$ donors, $H_2S$ has been proven to regulate plant growth and increase plant tolerance to drought, salt, temperature, and metal stress. It can be seen that $H_2S$ plays vital roles in facilitating plant with tolerance to environmental stresses[9]. However, the role of $H_2S$ in alleviating Al stress of *C. sinensis* is still unclear.

There are many studies on Al enrichment in tea plants, but currently there is a lack of research on $H_2S$ signaling molecules for Al tolerance in tea plants. Now, through different hydroponic treatments (0.4Al, $H_2S$ + 0.4Al, PAG + 0.4Al, 2Al, $H_2S$ + 2Al, PAG + 2Al, 4Al, $H_2S$ + 4Al, PAG + 4Al), we investigated the effects of $H_2S$ preapplication on the biomass, the content and transfer rate of Al and other elements in different tissues, the content of chlorophyll, photosynthetic indexes, the ultrastructure, the antioxidant enzyme activity and tea quality

components under Al stress. Preliminary exploration of the role of exogenous $H_2S$ in the physiological response of tea plants to Al stress provides new ideas for further research on alleviating Al stress and reducing Al accumulation in tea plants.

## Materials and methods

### Plant material and experimental treatments

For the experiment, annual cutting seedlings of *C. sinensis* cv. 'Zhongcha 108' were obtained from the Nanjing (Ya Run Tea Co., Ltd., Jiangsu Province, China). The tea seedlings were firstly pre-cultured in water for 5 d, then transferred to 1/8, 1/4, and 1/2 total nutrient solutions to culture for 5 d in each strength nutrient solution, and finally transferred to total nutrient solutions for 10 d (culture medium was replaced every 5 d)[10]. The seedlings with consistent growth were used to carry out the subsequent treatment assays with $H_2S$ or PAG and $Al^{3+}$ as shown in Table 1. For treatments, $Al_2(SO_4)_3{\cdot}18H_2O$, $Na_2S{\cdot}9H_2O$ and DL-propargylglycine (PAG) were the $Al^{3+}$ donor[11], $H_2S$ donor[12] and L-cysteine desulfurase (LCD) inhibitor[13], respectively. And solution pH was adjusted to 4.5 ± 0.1 with 1.0 mol·L$^{-1}$ NaOH or 1.0 mol·L$^{-1}$ HCl. The experiments were performed in the Intelligent Greenhouse of Nanjing Agricultural University (China), controlled growth room at 25 °C/22 °C with 16 h light/8 h dark cycle, 30,000 l x light intensity and a relative humidity of 75%.

### Fresh and dry weight analysis

Plants were collected and separated into young leaf (the first and second leaf from the top of plants), mature leaf (remaining leaves), stem and root. Fresh weight (FW) of seedlings were weighed instantly after harvesting and then placed into an oven at 105 °C for 30 min and then baked at 80 °C until biomass became stable. The dry weight (DW) immediately weighed after removal from the oven.

### Root activity assessments

Root activity was measured using the 2,3,5-tripheyl tetrazolium chloride (TTC) method[14]. About 0.5 g of fresh root tips were placed in a mixture of 5 mL 1% TTC and 5 mL phosphate buffer for 1 h at 37 °C in the dark. The assays were terminated by adding 2 mL 1.0 mol·L$^{-1}$ $H_2SO_4$ to the reaction mixture. The reduced TTC was extracted with 3-4 mL ethyl acetate, then ethyl acetate was added to the 10 mL level, and absorbance was read at 485 nm.

### Transmission electron microscopy

Leaf fragments without veins were collected from randomly selected plants, then fixed 24 h in 2.5 % glutaraldehyde solution and stored at 4 °C. Samples were rinsed three times with the same phosphate-buffered saline (PBS, pH 7.2), and post-fixed in 1% osmium oxide for 1 h, washed three times with distilled water. The samples were dehydrated in a graded series of ethanol (50%, 70%, 80%, and 100 %) and at the end treated with absolute acetone for 24 h. Ultra-thin sections (≤ 100 nm) of specimens were prepared for viewing.

**Table 1.** Description of nine experimental treatments.

| Treatments | Days 1–15 | Days 15–30 |
|---|---|---|
| 0.4Al (control) | 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 0.4 mmol·L$^{-1}$ $Al^{3+}$ |
| $H_2S$ + 0.4Al | 100 μmol·L$^{-1}$ $H_2S$ + 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 0.4 mmol·L$^{-1}$ $Al^{3+}$ |
| PAG + 0.4Al | 1 mmol·L$^{-1}$ PAG + 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 0.4 mmol·L$^{-1}$ $Al^{3+}$ |
| 2Al | 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 2 mmol·L$^{-1}$ $Al^{3+}$ |
| $H_2S$ + 2Al | 100 μmol·L$^{-1}$ $H_2S$ + 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 2 mmol·L$^{-1}$ $Al^{3+}$ |
| PAG + 2Al | 1 mmol·L$^{-1}$ PAG + 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 2 mmol·L$^{-1}$ $Al^{3+}$ |
| 4Al | 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 4 mmol·L$^{-1}$ $Al^{3+}$ |
| $H_2S$ + 4Al | 100 μmol·L$^{-1}$ $H_2S$ + 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 4 mmol·L$^{-1}$ $Al^{3+}$ |
| PAG + 4Al | 1 mmol·L$^{-1}$ PAG + 0.4 mmol·L$^{-1}$ $Al^{3+}$ | 4 mmol·L$^{-1}$ $Al^{3+}$ |

### Measurement of chlorophyll content and photosynthesis parameters

Chlorophyll a and chlorophyll b of randomly selected mature leaves per treatment were measured as described previously[15]. Samples were completely immersed with 10.0 mL mixture of acetone-95% ethanol-water (9:9:2, v:v) and transferred into tubes placed in a dark place until the leaves turn completely white. The $OD_{665}$ and $OD_{649}$ values were used to calculate chlorophyll content. A LI-6400XT portable photosynthesis system (Li-Cor Biosciences, Lincoln, Nebraska, USA) was used to measure net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular $CO_2$ concentration (Ci) with 1,200 μmol·m$^{-2}$·s$^{-1}$ illuminance and 500 μmol·mol$^{-1}$ flow rate.

### Assay of Al and other elemental concentrations

The plant samples with 0.2 g were placed into the digestion vessels, mixed with $HNO_3$ : $HClO_4$, (4:1, v:v) and digested in microwave digestion system. The concentrations of Al, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe) and zinc (Zn) in the filtrate were determined using inductively coupled plasma optical emission (ICP-OES, PerkinElmer Inc.) following a standard procedure.

### Analysis of lipid peroxidation and proline content

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content according to Alatawi et al.[16]. Fresh leaves (0.1 g) were ground and extracted in 1 mL of 10% trichloroacetic acid (TCA), then the supernatant was collected by centrifuging at 5,000 rpm for 10 min. 0.5 mL supernatant (0.5 mL distilled water as control) were homogenized in 0.5 ml of 0.6 % 2-thiobarbituric acid (TBA) and heat in boiling water for 15 min, then cooled until room temperature. The absorbance of the supernatant was measured at 532, 600, and 450 nm.

Proline content was determined using the acid ninhydrin method[17]. First, 0.1 g of leaf samples was added to 1 ml of 3% sulfosalicylic acid solution and extracted in boiling water for 10 min, then centrifuged at 5,000 rpm for 10 min. Next, 0.2 mL of supernatant was homogenized and mixed with 0.2 mL of acetic acid and 0.2 mL of 2.5% acid ninhydrin and kept at boiling point for 30 min, then cooled until room temperature, 0.4 mL of toluene treated and then oscillated by vortex for 30 s. After 10 min, supernatant centrifuged at 3,000 rpm for 5 min. Finally, the absorbance was scored at 520 nm.

### Determination of GSH, GSSG and enzyme activity

The glutathione (GSH) and oxidized glutathione (GSSG) were measured by GSH and GSSG kit (NO. BC1170, NO. BC1180; Beijing Solarbio Science & Technology Co., Ltd., China). LCD enzyme was detected by referring to the LCD kit (NO. MBE21193; Nanjing Maibo Biotechnology Co., Ltd., China). The activities of glutathiones-transferase (GST) and glutathione reductase (GR) was determined following the description by kit (NO. BC0350, NO. BC1160; Beijing Solarbio Science & Technology Co., Ltd., China). Superoxide dismutase (SOD), peroxidase

(POD) and catalase (CAT) was performed according to instructions of the kits (NO. R22262, NO. R3031, NO. R22072; Shanghai Yuanye Biotechnology Co., Ltd., China), respectively.

### Determination of tea components content

The contents assay viz. tea polyphenols, catechins, amino acids and caffeine was measured according to GB/T 8313-2018[18], GB/T 8314-2013[19], and GB/T 8312-2013[20], respectively.

### Data statistics and analysis

All the data were from three independent experiments with three biological repeats. The experimental data were statistically processed using Excel 2016, GraphPad Prism 8.0 and variance analysis software SPSS 20.0 (SPSS Inc. version 22.0, Chicago, IL, USA). Different lowercase letters on the graphs indicate that the mean values among different $H_2S$ conditions under the same Al concentration treatment were statistically different at $p < 0.05$ level, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition at $p < 0.05$ level.

## Results

### Effects of different treatments of $H_2S$ and Al on *C. sinensis*

As expected, new root of *C. sinensis* treated with 2Al and 4Al was less than that of normal 0.4Al culture, but early application of $H_2S$ compared to lone Al treatment effectively promoted the root development, while PAG + Al significantly inhibited root growth (Fig. 1). Moreover, application of PAG not only inhibited normal development of root system, but also inhibited the growth of leaves (Fig. 1c, f & i). Chlorosis, even leaf abscission symptoms in leaves have also occurred (Fig. 1c, f & i).

To further clarify whether $H_2S$ is beneficial for tea root growth under different Al conditions, we explored root activity. We observed higher concentrations (2Al and 4Al) resulted in a greatly decrease in root activity (Fig. 1j). And an increase of 37.59%, 58.42%, and 19.55% in root activity under $H_2S$ pretreatment compared to the separate 0.4Al, 2Al and 4Al treatments, respectively (Fig. 1j). However, exogenous PAG treatment significantly inhibited root activity compared to various Al concentrations (Fig. 1j).

### Effects different treatments on fresh and dry weight

Overall, the total fresh weight (FW) and dry weight (DW) of tea plants were both increased by early application of $H_2S$, while the use of PAG reduced the FW and DW of *C. sinensis* (Fig. 2e, j). Moreover, the results showed that, except for $H_2S$ + 4Al, which did not increase FW in the leaves compared to 4Al, the FW of other different tissues under $H_2S$ + Al treatments showed an increase in FW compared to the single Al treatment (Fig. 2a–d). In addition, the DW of other tissues increased under $H_2S$ + Al treatments compared to single Al treatment for tea seedlings, except for $H_2S$ + 4Al which showed decrease in DW of old leaves compared to 4Al (Fig. 2f–i).

### Effect of $H_2S$ on accumulation and translocation factor of Al in *C. sinensis*

There was no significant decrease in content of Al between pre-applied $H_2S$ treatment and single Al treatment in young leaves (Table 2). Nevertheless, compared with 0.4Al treatment,

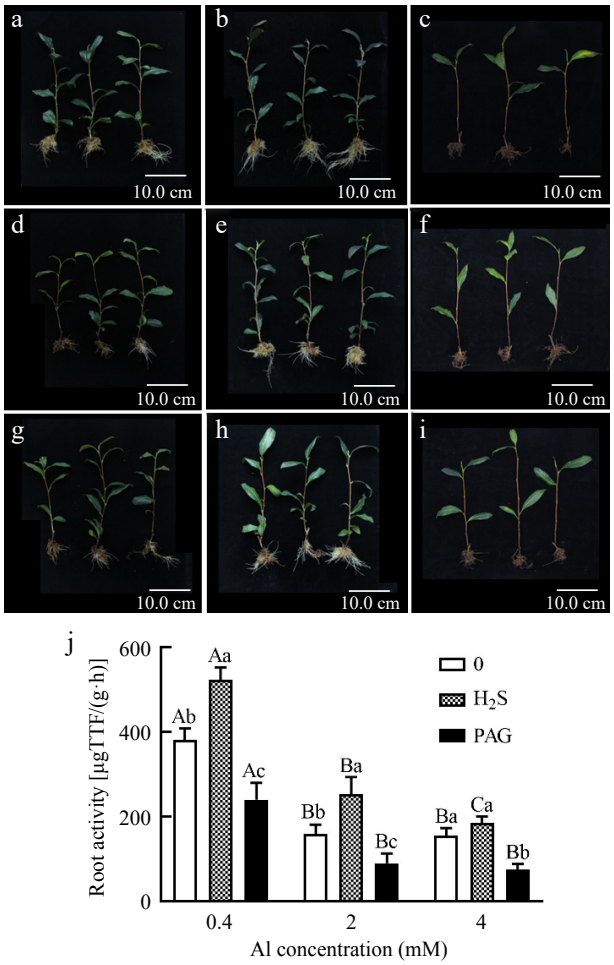


**Fig. 1** Effect of different treatments on symptoms, (a) 0.4Al, (b) $H_2S$ + 0.4Al, (c) PAG + 0.4Al, (d) 2Al, (e) $H_2S$ + 2Al, (f) PAG + 2Al, (g) 4Al, (h) $H_2S$ + 4Al, (i) PAG + 4Al, and (j) root activity in *C. sinensis*. Different lowercase letters in (j) represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

content of Al in roots markedly increased when $H_2S$ was applied in advance, while accumulation of Al in roots was dramatically reduced when $H_2S$ was applied in advance to the 2 mM $Al^{3+}$ and 4 mM $Al^{3+}$ treatments (Table 2). Meanwhile, compared to other treatments within the group, content of Al was the highest in roots when PAG-pretreated was applied in advance, with similar performance in total Al content (Table 2). Unusually, pretreatment with $H_2S$ increased content of Al in mature leaves compared to Al treatment alone, and there was a similar trend of Al accumulation in stems (Table 2). Under normal Al concentration, the translocation factor (TF) of Al of 0.4Al is the highest, which is 1.7 times that of $H_2S$ + 0.4Al and 10.625 times that of PAG + 0.4Al (Table 2). Whereas, TF of Al demonstrated $H_2S$ + Al > Al > PAG + Al after 2Al and 4Al treatment (Table 2).

### $H_2S$ affects ion homeostasis of *C. sinensis* after different treatments

Content of Ca increased in the solution with $H_2S$ or PAG pre-applied, and this increase was more elevated in 0.4Al than 2Al,

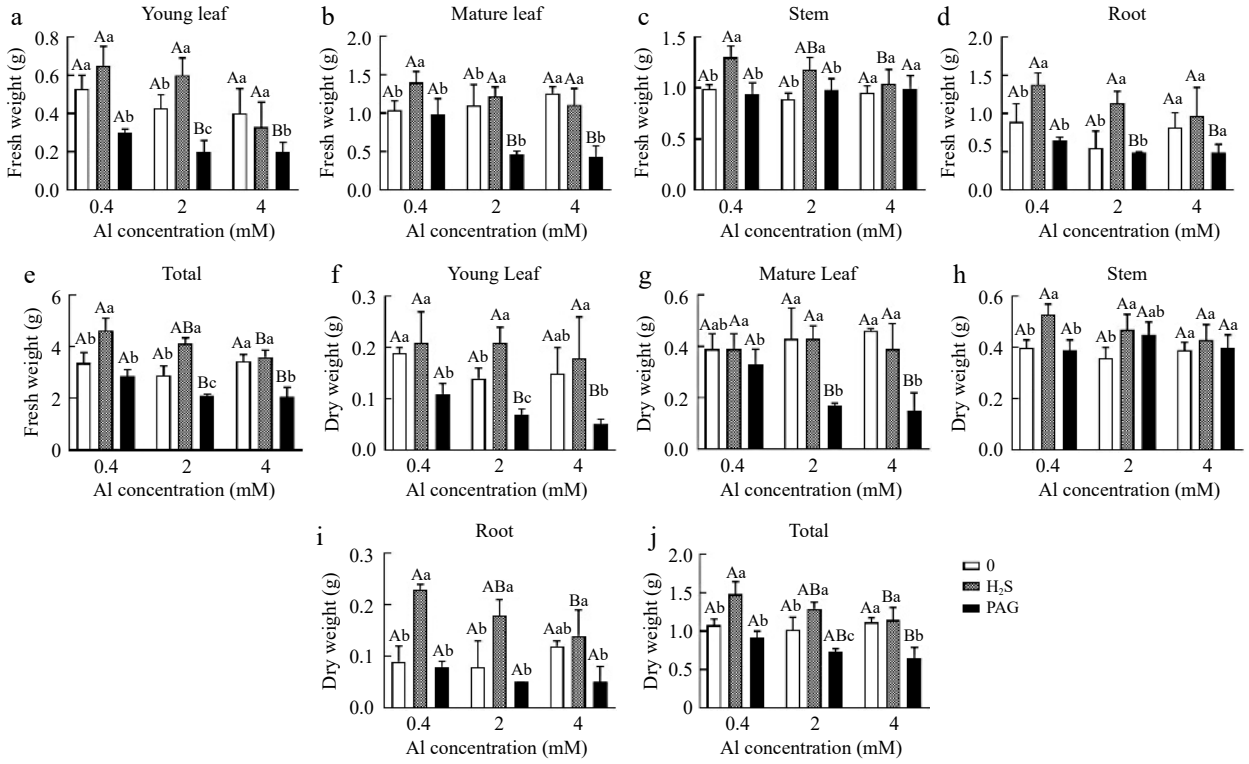


**Fig. 2** Fresh and dry weight in (a, f) young leaves, (b, g) matures leaves, (c, h) stems, (d, i) roots, and (e, j) total content of Al of *C. sinensis* cultured with different treatments. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

**Table 2.** Effects on content and translocation factor (TF) of Al in *C. sinensis* under different treatments.

| Elements | Treatment | YL (mg·kg$^{-1}$) | ML (mg·kg$^{-1}$) | S (mg·kg$^{-1}$) | R (mg·kg$^{-1}$) | Total content (mg·kg$^{-1}$) | TF (%) |
|---|---|---|---|---|---|---|---|
| Al | 0.4Al | 551.30 ± 25.52Ba | 1204.96 ± 324.00Aa | 427.13 ± 12.20Ba | 2566.89 ± 96.52Bc | 4750.29 ± 207.35Bc | 0.85 ± 0.14Aa |
| | 0.4Al + $H_2S$ | 474.49 ± 57.74Ba | 1605.65 ± 30.85Ba | 558.06 ± 69.96Ca | 5323.33 ± 506.90Cb | 7961.52 ± 573.30Bb | 0.50 ± 0.04Bb |
| | 0.4Al + PAG | 291.67 ± 33.05Cb | 1125.82 ± 345.62Aa | 537.35 ± 118.21Aa | 23523.28 ± 1412.99Ba | 25478.12 ± 1623.32Ba | 0.08 ± 0.01Bc |
| | 2Al | 700.68 ± 14.51Ab | 1232.58 ± 102.65Ab | 735.12 ± 40.15Ab | 15651.00 ± 387.50Ab | 18319.37 ± 478.25Ab | 0.17 ± 0.01Bb |
| | 2Al + $H_2S$ | 758.33 ± 51.39Ab | 2000.13 ± 209.09Aa | 976.59 ± 29.70Aa | 8574.85 ± 700.31Bc | 12309.90 ± 638.39Ac | 0.44 ± 0.05Ba |
| | 2Al + PAG | 999.06 ± 45.47Ba | 1101.78 ± 48.02Ab | 669.60 ± 131.31Ab | 28361.41 ± 199.73Aa | 31131.86 ± 296.48Aa | 0.10 ± 0.01ABc |
| | 4Al | 771.52 ± 123.22Ab | 1342.59 ± 60.73Aa | 675.39 ± 120.29Aa | 14741.91 ± 2122.85Ab | 17531.41 ± 2218.98Ab | 0.19 ± 0.03Bb |
| | 4Al + $H_2S$ | 819.06 ± 20.35Ab | 1475.13 ± 107.29Ba | 732.93 ± 62.82Ba | 10066.06 ± 835.49Ac | 13093.18 ± 954.89Ab | 0.30 ± 0.02Aa |
| | 4Al + PAG | 1285.44 ± 106.37Aa | 1086.46 ± 135.20Ab | 756.13 ± 104.12Aa | 26682.34 ± 3130.59ABc | 29810.37 ± 3150.15Aa | 0.12 ± 0.02AAc |

Values are the mean ± SD (n = 3). Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

4Al in young leaves, while more increased in 2Al and 4Al than 0.4Al in mature leaves (Table 3). In stems, application of PAG remarkably enhanced the concentrations of Ca under 0.4Al, but decreased content of Ca in 2Al and 4Al (Table 3). Moreover, results in roots showed that content of Ca under $H_2S$ + 0.4Al was 2.71 times that of 0.4 Al, and content of Ca in PAG + 0.4Al was 4.5 times that of 0.4 Al, but $H_2S$ + 4Al and PAG + 4Al inhibited content of Ca compared to 4Al, and changes in content of Ca between 2Al, $H_2S$ + 2Al, and PAG + 2Al groups were relatively small (Table 3). After $H_2S$ combined 2Al significantly improved content of total Ca, while a little effect on 0.4Al and 4Al (Table 3). In addition, the TF of Ca exhibited 0.4Al > $H_2S$ + 0.4Al > PAG + 0.4Al, while $H_2S$ +2 Al and PAG + 2Al have no significant effect on the TF of Ca compared to 2Al, only $H_2S$ + 4Al significantly promoted TF of Ca compared to 4Al (Table 3).

Content of Mg in young leaves were found to significantly inhibited only in $H_2S$ + 4Al and PAG + 4Al compared to 4Al, but there was no significant change in content of Mg between treatments at only 4Al in mature leaves (Table 3). The application of exogenous PAG contributed to increase in content of Mg in stems, but $H_2S$ had a small effect on the level of Mg compared to Al alone in stems (Table 3). However, $H_2S$ significantly increased Mg levels in roots (Table 3). It was found that the change in total content of Mg was not significant under $H_2S$ + 4Al compared to 4Al, while content of Mg under other $H_2S$ + Al treatments significantly increased compared to Al alone (Table 3). However, the TF of Mg was inhibited by 73.66% under $H_2S$ + 0.4Al compared to 0.4Al, 23.82% under $H_2S$ + 2Al compared to 2Al, and 30.84% under $H_2S$ + 4Al compared to 4Al (Table 3).

**Table 3.** Effects on content and translocation factor (TF) of Ca, Mg, Zn, Mn, Fe in *C. sinensis* under different treatments.

| Elements | Treatment | YL (mg·kg$^{-1}$) | ML (mg·kg$^{-1}$) | S (mg·kg$^{-1}$) | R (mg·kg$^{-1}$) | Total content (mg·kg$^{-1}$) | TF (%) |
|---|---|---|---|---|---|---|---|
| Ca | 0.4Al | 6168.53 ± 606.30Bb | 13894.03 ± 340.79Aab | 2746.77 ± 24.59Bb | 476.37 ± 41.02Cc | 23285.69 ± 1696.58Bb | 47.92 ± 0.76Aa |
| | 0.4Al + $H_2S$ | 8064.69 ± 66.16Ca | 13027.66 ± 1043.18Bb | 2781.84 ± 61.89Cb | 1289.49 ± 60.48Bb | 25163.67 ± 436.51Cb | 18.53 ± 0.58Ab |
| | 0.4Al + PAG | 9322.85 ± 1413.64Aa | 14978.35 ± 230.29Aa | 3606.75 ± 523.74Aa | 2144.36 ± 58.02Aa | 30052.32 ± 1938.42Aa | 13.03 ± 1.24Ac |
| | 2Al | 11223.02 ± 223.36Aa | 12447.40 ± 1926.26Ab | 3559.11 ± 198.46Aa | 1573.00 ± 74.02Ba | 28802.53 ± 598.30Ac | 17.33 ± 0.62Ba |
| | 2Al + $H_2S$ | 11693.96 ± 749.31Aa | 15968.96 ± 553.09Aa | 3955.32 ± 217.77Aa | 1695.65 ± 273.89Aa | 33313.87 ± 1007.07Aa | 19.05 ± 3.82Aa |
| | 2Al + PAG | 10558.09 ± 739.25Aa | 15748.44 ± 629.97Ba | 3026.63 ± 321.80Ab | 1905.82 ± 193.23ABa | 31238.99 ± 388.96Ab | 15.51 ± 1.78Aa |
| | 4Al | 10986.74 ± 1426.59Aa | 12852.33 ± 968.51Ab | 3355.19 ± 272.64Aab | 1878.07 ± 191.42Aa | 29072.34 ± 1204.10Aa | 14.57 ± 1.35Cb |
| | 4Al + $H_2S$ | 10405.55 ± 102.64Ba | 14656.58 ± 769.17ABa | 3566.27 ± 65.69Ba | 1511.59 ± 84.73ABb | 30140.00 ± 1162.14Ba | 18.96 ± 0.69Aa |
| | 4Al + PAG | 10806.80 ± 1205.83Aa | 12881.85 ± 15.75Bb | 3227.25 ± 1.88Ab | 1713.41 ± 164.33Bab | 28629.31 ± 1365.33Aa | 15.77 ± 0.97Ab |
| Mg | 0.4Al | 2161.53 ± 297.57Ba | 2987.80 ± 291.75Aab | 1388.72 ± 43.03Bb | 346.52 ± 15.37Bc | 6884.56 ± 597.72Bb | 18.87 ± 1.45Aa |
| | 0.4Al + $H_2S$ | 2679.11 ± 76.29Ca | 2869.31 ± 12.76Ab | 1326.99 ± 29.76Bb | 1384.29 ± 37.80Aa | 8259.70 ± 93.16Ca | 4.97 ± 0.21Bc |
| | 0.4Al + PAG | 2551.51 ± 378.60Ba | 3266.05 ± 76.24Aa | 2152.37 ± 144.18Aa | 497.68 ± 22.78Ab | 8467.61 ± 526.12ABa | 16.04 ± 1.39Ab |
| | 2Al | 3203.55 ± 83.10Aa | 2612.19 ± 216.71Ab | 1578.27 ± 51.43Ab | 924.90 ± 98.81Ab | 8318.92 ± 253.97Ac | 8.06 ± 0.95Bb |
| | 2Al + $H_2S$ | 3307.60 ± 162.06Aa | 3142.00 ± 338.93Aa | 1660.00 ± 103.48Ab | 1322.66 ± 58.80Aa | 9432.27 ± 159.11Aa | 6.14 ± 0.43Ab |
| | 2Al + PAG | 3089.27 ± 163.42Aa | 3290.93 ± 108.91Aa | 1908.25 ± 31.93Ba | 498.33 ± 39.52Ac | 8786.78 ± 71.55Ab | 16.70 ± 1.37Aa |
| | 4Al | 3610.66 ± 446.66Aa | 2809.35 ± 171.88Aa | 1442.35 ± 119.96ABb | 954.41 ± 122.46Ab | 8816.77 ± 562.41Aa | 8.30 ± 0.78Bb |
| | 4Al + $H_2S$ | 3059.80 ± 57.45Bb | 2752.72 ± 222.97Aa | 1561.74 ± 53.55Ab | 1284.43 ± 54.96Aa | 8658.69 ± 280.14Ba | 5.74 ± 0.13Ab |
| | 4Al + PAG | 2707.61 ± 5.02ABb | 2584.47 ± 406.13Ba | 2072.19 ± 16.34ABa | 387.73 ± 33.22Bc | 7752.01 ± 387.21Bb | 19.12 ± 2.39Aa |
| Zn | 0.4Al | 8.70 ± 1.12Ca | 12.22 ± 1.13Ba | 18.92 ± 1.49Bb | 23.29 ± 1.72Bc | 63.12 ± 2.34Bc | 1.72 ± 0.14Aa |
| | 0.4Al + $H_2S$ | 11.58 ± 3.00Ca | 14.66 ± 2.74Aa | 20.42 ± 2.36Bb | 82.47 ± 4.76Ca | 129.13 ± 5.16Ba | 0.57 ± 0.04Bc |
| | 0.4Al + PAG | 9.33 ± 1.22Ca | 12.43 ± 0.50Ba | 25.11 ± 0.62Ba | 59.10 ± 2.76Aa | 105.96 ± 1.00Cb | 0.80 ± 0.07Cb |
| | 2Al | 13.68 ± 1.25Bab | 14.98 ± 1.59ABa | 27.60 ± 2.46Aa | 74.61 ± 5.32Aa | 130.86 ± 3.45Ab | 0.76 ± 0.11Bb |
| | 2Al + $H_2S$ | 15.49 ± 0.95Ba | 16.72 ± 1.98Aa | 26.31 ± 3.59ABa | 123.25 ± 4.99Aa | 181.77 ± 5.41Aa | 0.48 ± 0.02Cc |
| | 2Al + PAG | 11.80 ± 0.93Bb | 20.59 ± 4.06Aa | 30.27 ± 1.78Aa | 55.57 ± 2.46Aa | 118.24 ± 3.15Bc | 1.13 ± 0.07Aa |
| | 4Al | 17.09 ± 2.08Ab | 18.44 ± 4.07Aa | 29.14 ± 2.30Aa | 82.54 ± 7.89Aa | 147.20 ± 14.19Ab | 0.78 ± 0.09Ba |
| | 4Al + $H_2S$ | 21.37 ± 0.92Aa | 17.90 ± 0.30Aa | 31.47 ± 5.39Aa | 101.06 ± 5.07Aa | 171.80 ± 9.69Aa | 0.70 ± 0.02Aa |
| | 4Al + PAG | 14.61 ± 1.07Ab | 13.16 ± 0.38Bb | 33.98 ± 3.70Aa | 67.89 ± 9.40Aa | 129.63 ± 7.39Ab | 0.93 ± 0.19ABa |
| Mn | 0.4Al | 434.54 ± 49.93Bb | 545.22 ± 57.44Ab | 166.17 ± 11.21Ab | 134.39 ± 12.51Cc | 1280.32 ± 34.67Cc | 8.60 ± 1.20Aa |
| | 0.4Al + $H_2S$ | 331.68 ± 11.06Cb | 877.57 ± 9.52Aa | 344.66 ± 11.87Ba | 754.57 ± 31.78Ab | 2308.49 ± 11.48Bb | 2.06 ± 0.12Bb |
| | 0.4Al + PAG | 834.44 ± 114.52Aa | 550.37 ± 95.76Ab | 133.36 ± 16.66Ac | 4346.17 ± 226.23ABa | 5864.34 ± 188.71Aa | 0.35 ± 0.03Bc |
| | 2Al | 580.86 ± 12.29Ac | 493.94 ± 29.55Ac | 140.13 ± 9.00Bb | 331.60 ± 27.71Bc | 1546.53 ± 53.41Bc | 3.68 ± 0.26Ba |
| | 2Al + $H_2S$ | 725.34 ± 47.40Ab | 812.18 ± 82.33Aa | 441.12 ± 40.94Aa | 700.79 ± 23.40Bb | 2679.43 ± 33.48Ab | 2.83 ± 0.08Ab |
| | 2Al + PAG | 928.92 ± 45.21Aa | 624.51 ± 22.29Ab | 98.97 ± 8.63Ab | 3967.98 ± 23.31Ba | 5620.38 ± 56.40Aa | 0.42 ± 0.01Ac |
| | 4Al | 460.62 ± 66.33Bb | 555.60 ± 64.32Ac | 177.93 ± 12.21Ab | 556.44 ± 62.27Ab | 1750.59 ± 105.01Ab | 2.16 ± 0.17Cb |
| | 4Al + $H_2S$ | 628.13 ± 14.12Ba | 689.53 ± 39.43Ba | 276.52 ± 2.49Ca | 555.42 ± 21.57Cb | 2149.60 ± 47.85Cb | 2.87 ± 0.16Aa |
| | 4Al + PAG | 435.88 ± 72.96Bb | 601.91 ± 11.98Aab | 107.04 ± 23.12Ac | 4802.02 ± 610.68Aa | 5946.86 ± 651.79Aa | 0.24 ± 0.02Cc |
| Fe | 0.4Al | 83.08 ± 8.24Bc | 228.98 ± 29.41Ab | 116.78 ± 11.68Ab | 212.67 ± 18.38Cc | 641.51 ± 8.04Bc | 2.03 ± 0.29Aa |
| | 0.4Al + $H_2S$ | 142.21 ± 15.79Aa | 399.33 ± 0.52Aa | 292.37 ± 11.60Aa | 401.46 ± 66.03Ab | 1235.37 ± 78.04Ab | 2.11 ± 0.34Aa |
| | 0.4Al + PAG | 111.92 ± 8.22Bb | 230.35 ± 33.40ABb | 110.94 ± 14.25Ab | 956.68 ± 72.57Aa | 1409.89 ± 74.53Ba | 0.48 ± 0.04Ab |
| | 2Al | 147.45 ± 5.50Ab | 207.06 ± 16.44Aa | 122.89 ± 29.28Aa | 702.60 ± 109.64Ab | 1180.00 ± 150.22Ab | 0.69 ± 0.07Ba |
| | 2Al + $H_2S$ | 130.75 ± 8.31Ab | 257.56 ± 31.45Ba | 136.29 ± 7.22Ca | 521.81 ± 202.62Ab | 1046.41 ± 198.89Ab | 1.13 ± 0.50Ba |
| | 2Al + PAG | 216.42 ± 11.66Aa | 266.43 ± 43.53Aa | 110.46 ± 8.78Aa | 1110.40 ± 18.95Aa | 1703.70 ± 19.91Aa | 0.53 ± 0.04Aa |
| | 4Al | 134.64 ± 13.60Ab | 208.35 ± 12.59Ab | 105.13 ± 5.55Ab | 534.53 ± 88.15Bb | 982.65 ± 96.52Ab | 0.85 ± 0.16Bb |
| | 4Al + $H_2S$ | 145.29 ± 2.86Ab | 270.95 ± 21.28Ba | 201.43 ± 16.05Ba | 368.04 ± 37.29Ab | 985.70 ± 44.68Ab | 1.69 ± 0.16ABa |
| | 4Al + PAG | 230.61 ± 38.35Aa | 163.31 ± 45.36Bb | 103.44 ± 3.47Ab | 944.38 ± 142.73Aa | 1441.74 ± 137.29Ba | 0.54 ± 0.14Ac |

Values are the mean ± SD (n = 3). Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

It was demonstrated that $H_2S$ promoted an increase in content of Zn in different tissues (Table 3). Meanwhile, the total content of Zn also showed that $H_2S$-pretreated significantly promoted the accumulation of Zn in *C.sinensis*. The effects of $H_2S$ and PAG on TF of Zn under different Al concentrations were also inconsistent. Significantly inhibited TF of Zn was observed in exogenous $H_2S$ or PAG followed by 0.4Al, however, TF of Zn showed significant performance as PAG + 2Al > 2Al > $H_2S$ + 2Al, but there was no significant difference in the effect of exogenous $H_2S$ or PAG on TF of Zn at 4Al (Table 3).

Content of Mn further increased after applying $H_2S$ + 2Al and $H_2S$ + 4Al to young leaves, while content of Mn was decreased but not significant in $H_2S$ + 0.4 Al compared with simple Al treatment (Table 3). It was $H_2S$ + Al that dramatically increased Mn levels compared to Al in mature leaves, consistent with the performance in stems (Table 3). In roots, it was PAG + Al that showed a significant increase in content of Mn compared to Al, and $H_2S$ significantly promoted an increase in Mn only at 0.4Al and 2Al (Table 3). It is interesting to note that total content of Mn was similar to the content of Mn under each treatment in roots (Table 3). The early application of PAG increased the accumulation of total Mn in tea plants compared to Al alone, but its TF was significantly inhibited, with TF of Mn at 0.4Al was 24.57 times that of $H_2S$ + 0.4Al, 2Al was 8.76 times that of $H_2S$ + 2Al, and 4Al was nine times that of $H_2S$ + 4Al.

Exogenous $H_2S$ followed by 0.4Al resulted in a remarkable increase in the content of Fe in young leaves, but the effect of $H_2S$ on content of Fe was not significant at 2Al and 4Al, whereas PAG showed a significant increase in content of Fe (Table 3). $H_2S$ also increased content of Fe in mature leaves at various Al concentrations, as well as in stems (Table 3). Whereas, exogenous PAG significantly increased content of Fe in roots, and the total content of Fe was also significantly affected by exogenous PAG (Table 3). Under different Al concentrations, $H_2S$ + Al exhibited a promotion of Fe-TF, while PAG + Al inhibited TF of Fe (Table 3).

## Observation of ultrastructure under different treatments

A clear cell membrane could be seen in normal Al concentration, and the well-developed chloroplast having regular arrangements of thylakoid membranes could also be observed (Fig. 3a). At the same time, no osmiophilic granules (OG) were present in chloroplasts under $H_2S$ + 0.4Al (Fig. 3b), but application of PAG + 0.4Al led to appearance of OG in chloroplasts (Fig. 3c). Under stress of 2Al, the chloroplast membranes (PE) were still visible, but OG appeared (Fig. 3d), $H_2S$ reduced OG (Fig. 3e), and the early application of PAG generated more OG (Fig. 3f). Although, chloroplast structure was relatively intact, cells with scattered stromal lamellae under 4Al stress, and the stromal lamellar structure of $H_2S$ + 4Al loosened even more (Fig. 3g, h), with solubilization and even vacuolation occurring in PAG + 4Al (Fig. 3i).

## Chlorophyll content and photosynthetic parameters analysis

An increase was observed in chl a content under $H_2S$ as compared to Al treatment alone, however, reduction of chl a showed in exogenous PAG, and chl b content has the same performance (Fig. 4a, b). Furthermore, total chlorophyll content

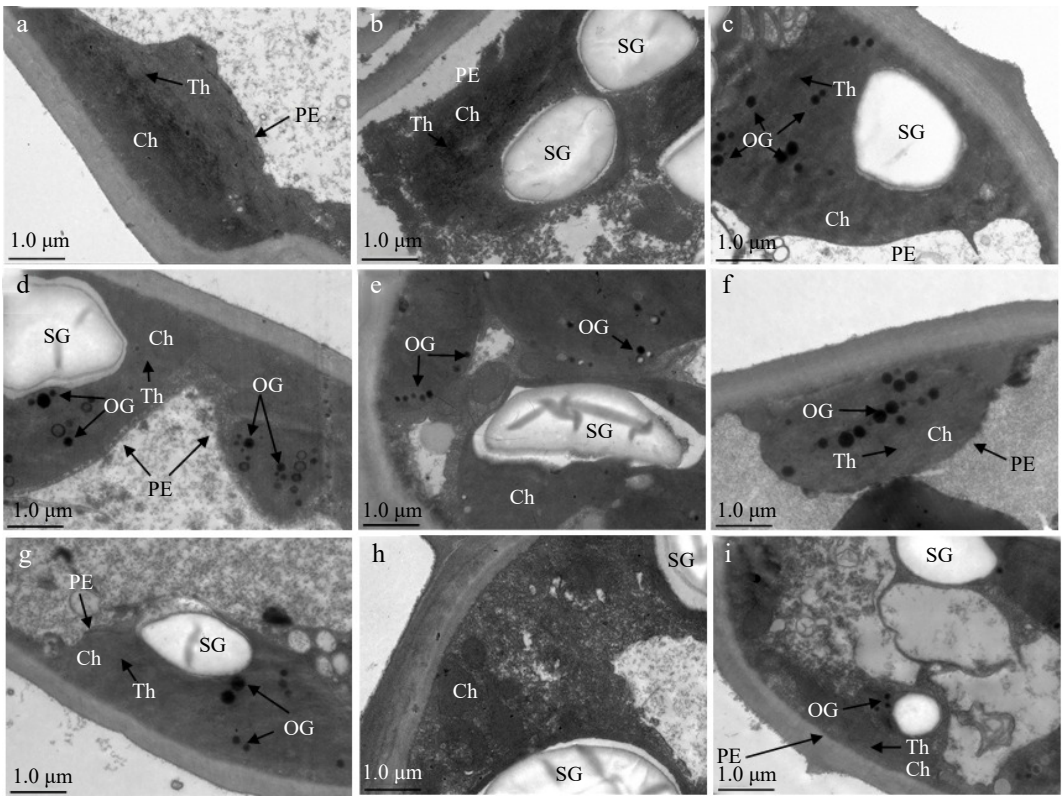


**Fig. 3** Different changes in ultrastructure of *C. sinensis* after different treatments. (a) 0.4Al, (b) $H_2S$ + 0.4Al, (c) PAG + 0.4Al, (d) 2Al, (e) $H_2S$ + 2Al, (f) PAG + 2Al, (g) 4Al, h: $H_2S$ + 4Al, (i) PAG + 4Al. PE: chloroplast membrane, Ch: chloroplast, SG: starch granules, Th: matrix lamellae, OG: osmiophilic granule. Scale bar = 1.0 μm.

also has the same trend, and with the increase of Al concentration, the total chlorophyll content of $H_2S$ + Al increases by 21.15%, 11.59%, and 17.64% compared to Al, respectively (Fig. 4c). Nevertheless, the results of chl a/chl b showed the opposite, namely PAG + Al > Al > $H_2S$ + Al (Fig. 4d).

Pn under 0.4Al was significantly promoted by application of $H_2S$, but pretreatment with PAG significantly decreased Pn (Fig. 5a). Differently, the effect of applying $H_2S$ and PAG on Pn showed an opposite trend at 2Al, and exogenous application of $H_2S$ and PAG showed significant inhibition compared to 4Al alone (Fig. 5a). Gs showed a consistent trend at 0.4Al and 4Al, with $H_2S$ + Al > Al > PAG + Al (Fig. 5b). Ci were different under different treatments with different Al concentrations, namely $H_2S$ + 0.4Al > 0.4Al > PAG + 0.4Al, 2Al > $H_2S$ + 2Al > PAG + 2Al, and PAG + 4Al > 4Al > $H_2S$ + 4Al (Fig. 5c). The results of Tr under normal Al concentration were consistent with those of Pn, Gs and Ci, but at high concentrations of Al, they showed 2Al > PAG + 2Al > $H_2S$ + 2Al, 4Al > $H_2S$ + 4Al > PAG + 4Al, respectively

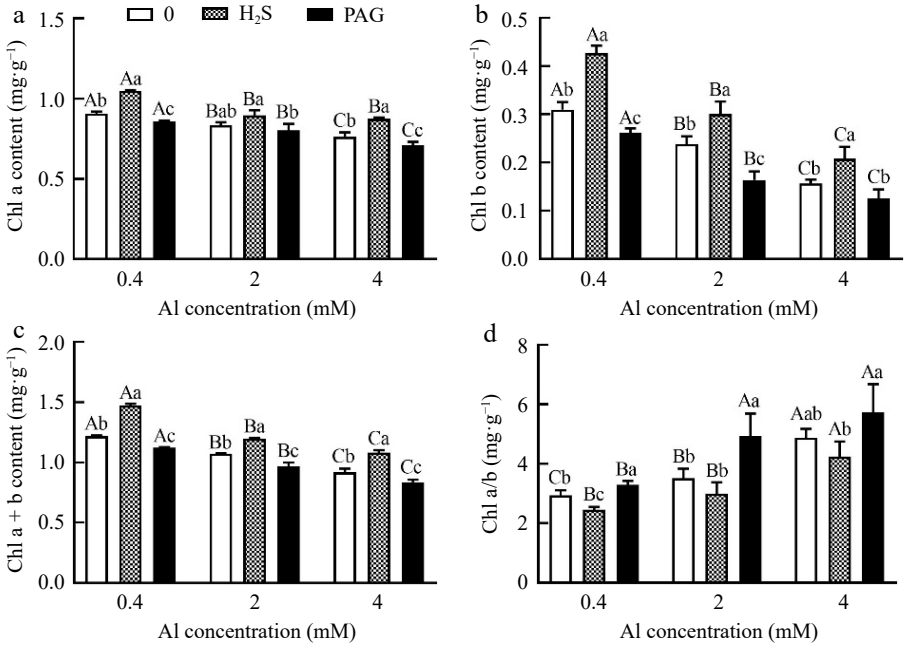


**Fig. 4** Changes in chlorophyll content of *C. sinensis* after different treatments. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

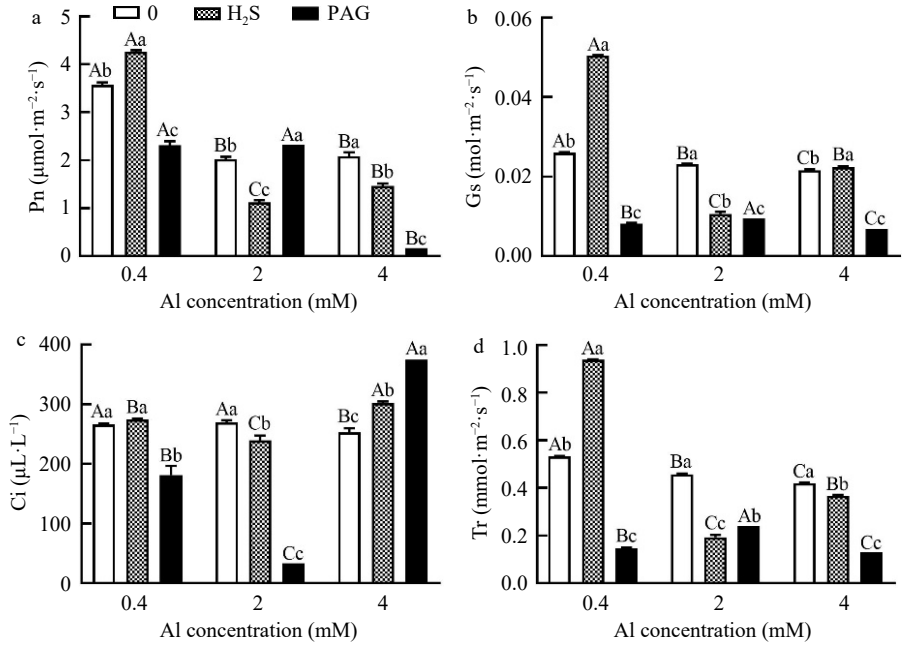


**Fig. 5** Changes in photosynthetic parameters of *C. sinensis* under different treatments. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

(Fig. 5d). Tr agreed with Pn, Gs, Ci results at normal Al concentration, but exhibited 2Al > PAG + 2Al > $H_2S$ + 2Al and 4Al > $H_2S$ + 4Al > PAG + 4Al, respectively, at Al stress concentrations (Fig. 5).

## Effects of $H_2S$ on Proline, MDA content and enzyme activity under Al conditions

Interestingly, MDA content in leaves of $H_2S$ pretreatment was inhibited by 3.61% compared to 2Al, whereas preincubation of PAG significantly increased MDA content (Fig. 6a). Proline content significantly accumulated in Al stress compared to 0.4Al, and its content increases by 2.82% under $H_2S$ + 2Al compared to 2Al, while pretreatment with $H_2S$ before 4Al treatment did not inhibit lipid peroxidation through proline content (Fig. 6b).

Similar tendency was observed in roots and leaves under normal Al, with $H_2S$ + 0.4Al compared to 0.4Al not significantly increasing CAT activity by 15% and 16.67%, respectively (Fig. 7a & b). CAT showed the highest activity of $H_2S$ + 2Al in leaves, but the lowest activity in roots under $H_2S$ + 2Al (Fig. 7a & b). And CAT activity of leaves at 4Al was higher than that of $H_2S$ + 4Al at 4Al and PAG + 4Al, while the CAT activity in roots treated with PAG + 4Al was higher than that of 4Al and $H_2S$ + 4Al (Fig. 7a & b).

Similarly, POD activity showed the same trend in roots and leaves only under normal Al, with POD activity in PAG + 0.4Al greater than that in 0.4Al and $H_2S$ + 0.4Al (Fig. 7c & d). Meanwhile, it is noteworthy that POD activity after $H_2S$ + 2Al is 3.56 times compared to 2Al in leaves, while the lowest POD activity was observed in the roots at $H_2S$ + 2Al, and the same was showed PAG + Al > Al > $H_2S$ + Al at 4 Al (Fig. 7c & d). However, there was no significant difference between the treatments at 4Al for the leaves (Fig. 7c).

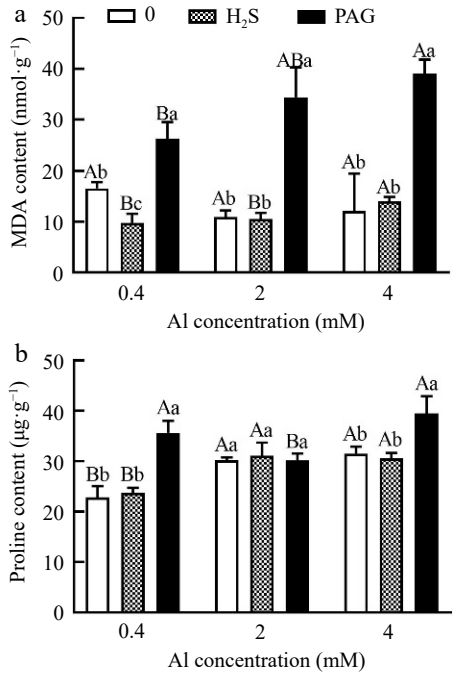


**Fig. 6** The effect of different treatments on (a) MDA and (b) proline content in tea leaves. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

Compared with 0.4Al treatment, $H_2S$ + 0.4Al treatment increased SOD activity in leaves and roots (Fig. 7e & f). However, there was no significant difference in SOD activity after applying $H_2S$ at 4 mM $Al^{3+}$ in the roots and leaves (Fig. 7e & f).

But the application of $H_2S$ and PAG under 2Al conditions in leaves failed to stimulate the activity of SOD, and pretreatment with $H_2S$ or PAG in roots dramatically decreased the activity of SOD (Fig. 7e & f). Furthermore, there was no significant difference in SOD activity among different treatments at 4Al in leaves and roots (Fig. 7e & f).

GSH content in leaves under normal Al and 2Al all exhibited $H_2S$ + Al > Al > PAG + Al, but GSH exhibited Al > $H_2S$ + Al > PAG + Al in 4Al (Fig. 8a). The content of GSSG was decreased in PAG-treated at 0.4Al and 2Al, but was increased in PAG + 4Al cultures (Fig. 8b). Noteworthy, no significant change of GSH/GSSG was discovered when $H_2S$ or PAG was added together with Al treatment (Fig. 8c).

It was found that GST activity in leaves was higher in $H_2S$ + 0.4Al than under 0.4Al and PAG + 0 .4Al treatments (Fig. 8d). And GST activity exhibited the highest in $H_2S$ + Al, followed by 2Al, and the lowest in PAG + 2Al. Unlike under 4Al where the activity of GST was inhibited by 4Al treatment with $H_2S$ and PAG, although the level of decrease was not significant (Fig. 8d).

Tea leaves exposed to $H_2S$ + 0.4Al treatment exhibited a significant increase of GR activity in comparison with 0.4Al alone and PAG + 0.4Al samples (Fig. 8e). PAG + 2Al and $H_2S$ + 4Al treatments had the lowest GR activity compared with 2Al and 4Al, respectively (Fig. 8e).

LCD activity only showed $H_2S$ + 0.4Al > 0.4Al > PAG + 0.4Al under normal Al concentration in leaves, and there was a significant difference among different treatments (Fig. 9a). However, the application of high concentration Al showed no significant difference under early application of $H_2S$ or PAG (Fig. 9a). What is different in root is that except for the insignificant difference in LCD activity between $H_2S$ + 2Al, 2Al and PAG + 2Al, all other groups showed significant differences, and LCD activity showed $H_2S$ + Al > Al > PAG + Al (Fig. 9b).

## Response of tea components to different treatments

The synthesis of tea polyphenols was drastically promoted by $H_2S$ + 0.4Al, but slightly deduced by PAG + 0.4Al (Fig. 10a). Compared to 2Al, $H_2S$ + 2Al increased tea polyphenol content, while PAG + 2Al decreased tea polyphenol content, both of which were not significant (Fig. 10a). Similarly, the effects of various treatments based on 4Al on tea polyphenols were not significant (Fig. 10a).

$H_2S$ + 0.4Al treatment induced the highest content of amino acids after treatment, significantly higher than both Al and PAG + 0.4Al (Fig. 10b). $H_2S$ + 2Al and $H_2S$ + 4Al did not significantly affect the amino acid content when compared to 2Al and 4Al, respectively (Fig. 10b). With PAG + 0.4Al treatment, amino acid content increased compared to 0.4Al, but amino acid content inhibited in PAG + 4Al, and no significant difference between PAG + 2Al and 2Al (Fig. 10b).

Caffeine content at normal Al concentration showed no significant difference in caffeine content among $H_2S$ + 0.4Al, 0.4Al, PAG + 0.4Al. And $H_2S$ + 2Al, 2Al, PAG + 2Al were the same

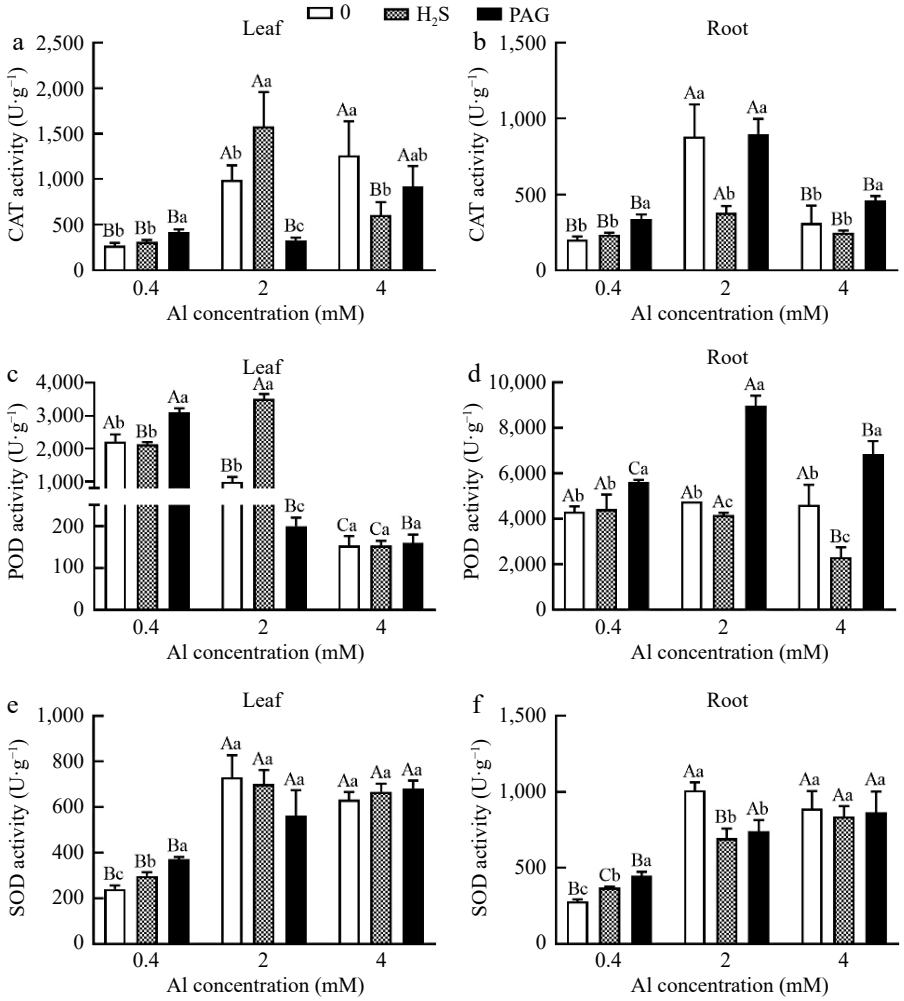


**Fig. 7** *C. sinensis* on antioxidant enzyme activities in (a), (c), (e) leaves and (b), (d), (f) roots with different treatments. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

(Fig. 10c). The caffeine content only after being subjected to PAG + 4Al was greater than that of $H_2S$ + 4Al and 4Al (Fig. 10c).

Results showed that the most abundant one was epicatechin (EC), along with of epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin (GC), gallocatechin gallate (GCG), epicatechin gallate (ECG) and catechin (C) detected in tea leaves (Table 4). Compared to 0.4Al, $H_2S$ + 0.4Al increased the total catechin content by 9.48%, while $H_2S$ + 4Al has a 14.45% increase in total catechin content compared to 4Al (Table 4). In each component, GC and EGC under $H_2S$ + 2Al were increased compared to 2Al. C and EC contents can be generally stimulated under $H_2S$ + 0.4Al and PAG + 0.4Al, while C and EC contents were reduced by $H_2S$ + 4Al (Table 4). Although the contents of EGCG, ECG and GCG of ester catechins were relatively low, early application of $H_2S$ was still sufficient to stimulate an increase in EGCG, ECG, and GCG at 0.4Al and 2Al (Table 4). It was found that $H_2S$ + 0.4Al increased EGCG by 19.35% compared to 0.4Al, and $H_2S$ + 2Al increased EGCG by 8.70% compared to 2Al. Interestingly, even with early application of $H_2S$, EGCG, ECG, and GCG were still repressed by 4Al (Table 4).

## Discussion

### $H_2S$ induced a well-developed *C. sinensis* and improved root activity

It is easy to accumulate too much availability $Al^{3+}$ in the rhizosphere environment of *C. sinensis* suitable for planting in acid soil. Al actually has been regarded as an essential element with dose - dependent effect, which is first reflected in root growth and development[3]. Root growth is stimulated in low concentrations of Al, while in high concentrations of Al, growth of the root and the plant is delayed[21]. In the present study, we also demonstrated that the effects on root development was strongly dependent on the Al concentration, the root system was damaged and new roots failed to generate by Al stress concentration (Fig. 1a–i). At the same time, it showed that $H_2S$ broke the restriction of Al stress on root development, but PAG promoted the root development hindered by Al stress (Fig. 1a–i). Moreover, pre-treatment with $H_2S$ increased total FW, total DW and root activity of *C. sinensis* to cope with excessive Al inhibition (Fig. 1j & 2). Recent research has demonstrated that $H_2S$ alleviates the inhibition of plant growth under metal stress in various crop plant species, including mungbean[22],

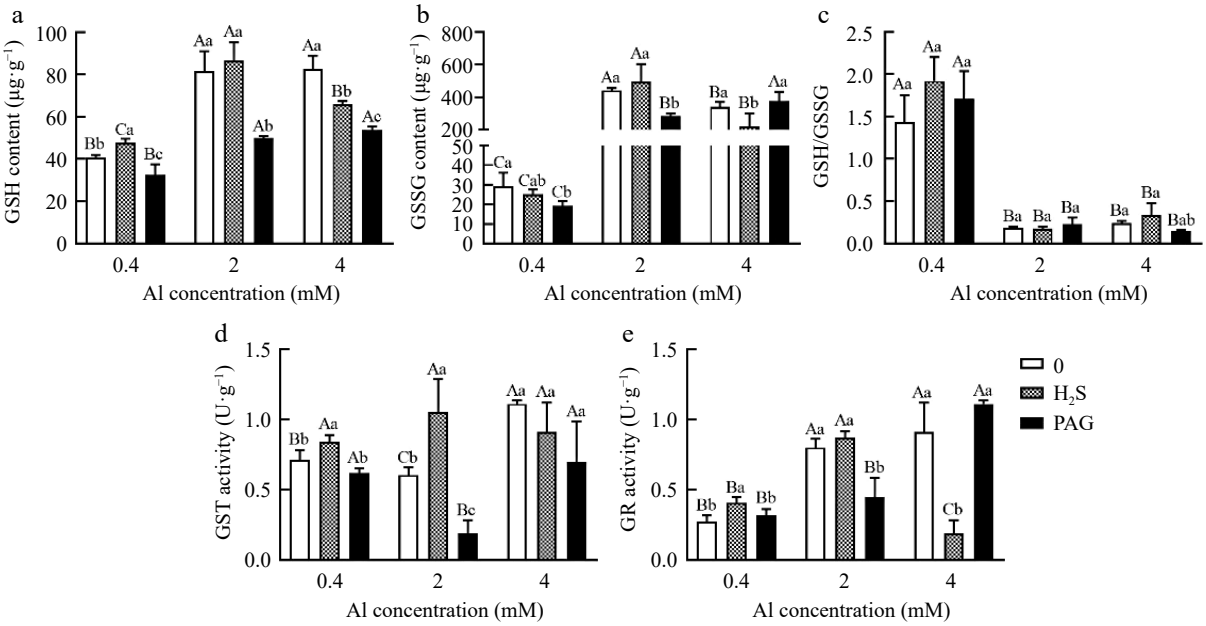


**Fig. 8** Effect of different treatments on (a) GSH content, (b) GSSG content, (c) GSH/GSSG, (d) GST activity and (e) GR activity in tea leaves. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

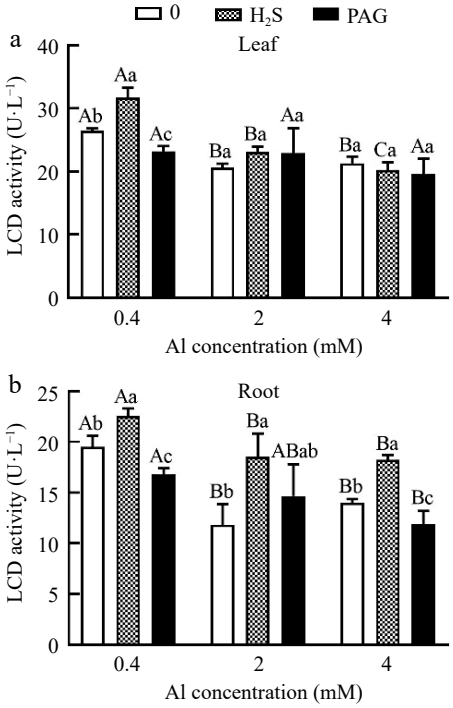


**Fig. 9** *C. sinensis* on LCD activities in (a) leaves and (b) roots with different treatments. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

soybean[23] and *Miscanthus sacchariflorus*[24]. These results indicated that $H_2S$ can effectively alleviate the growth and development of *C. sinensis* under Al stress.

## $H_2S$ promotes plant ion absorption of *C. sinensis* under Al stress

Maintaining constant intracellular ion homeostasis is crucial for plants adapting to stress environments. Most of Al in *C. sinensis* was contained in root after Al stress (Table 2), affecting the root growth attributes more than the shoot growth attributes, which ultimately limited the growth and development of plants. Similar results were also observed in previous studies[25,26]. $H_2S$ alleviated the enrichment of Al in roots and promoted the TF of Al under Al stress, while PAG increased the accumulation of Al and inhibited the TF of Al (Table 2). Moreover, $H_2S$ application helped to maintain ion homeostasis by accumulating Ca in mature leaves, Mg, Zn in root and Mn in above-ground parts and increasing the TF of Fe under Al stress (Table 3). It has also been reported that $H_2S$ improves nutrients uptake under Al stress[27]. The results showed that $H_2S$ directly mitigated inhibitory effect of Al toxicity on root growth by decreasing content of Al in root systems, thus pre-application of $H_2S$ promoted the root growth and development of *C. sinensis*. Therefore, an increased uptake of Ca, Mg, Zn and Mn has been explained as a consequence of the stimulation of root growth under $H_2S$.

## $H_2S$ enhances chlorophyll synthesis and ultrastructural stability under Al stress

We confirmed that excessive accumulation of Al disrupted ultrastructural and inhibited several processes, such as chlorophyll content and photosynthesis. Meanwhile, application of exogenous $H_2S$ enhanced chlorophyll content under Al stress conditions (Fig. 4), which was also reported by Ali et al.[27], who determined that $H_2S$ increased chlorophyll a and chlorophyll b by reducing damage to thylakoids in the chloroplast of *Brassica napus*. It is well known that the chlorophyll content and photosynthetic rate are closely correlated in plants. However, this result indicates that $H_2S$ failed to promote photosynthesis in *C.*

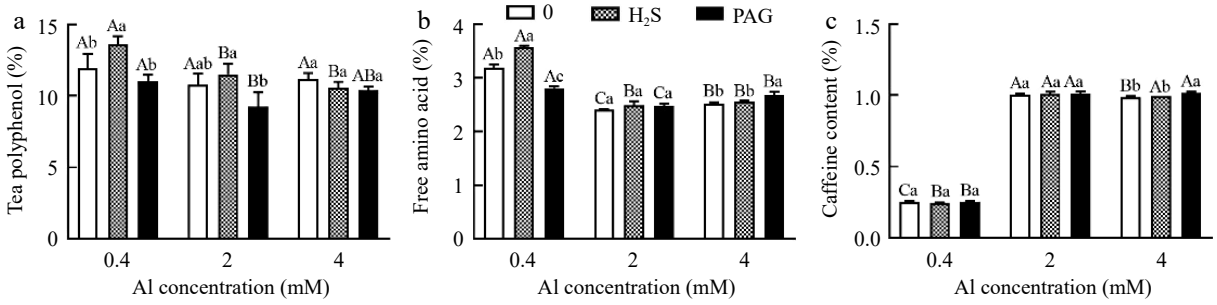


**Fig. 10** The performance of (a) tea polyphenol, (b) free amino acid and (c) caffeine content in different treatments. Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

**Table 4.** Effect of different treatments on catechins in *C. sinensis*.

| Treatment | GC (%) | EGC (%) | C (%) | EC (%) | EGCG (%) | ECG (%) | GCG (%) | Total catechins (%) |
|---|---|---|---|---|---|---|---|---|
| 0.4Al | 0.81 ± 0.03Aa | 2.92 ± 0.41Aa | 0.24 ± 0.00Ab | 3.68 ± 0.42Ab | 0.93 ± 0.09Ab | 0.38 ± 0.01Aa | 0.62 ± 0.06Ab | 9.81 ± 1.00Aa |
| 0.4Al + $H_2S$ | 0.80 ± 0.01Aa | 2.91 ± 0.06Aa | 0.248 ± 0.00Aa | 4.30 ± 0.17Aa | 1.11 ± 0.04Aa | 0.39 ± 0.00Aa | 0.73 ± 0.01Aa | 10.74 ± 0.16Aa |
| 0.4Al + PAG | 0.82 ± 0.01Aa | 2.44 ± 0.22Aa | 0.25 ± 0.00Aa | 3.95 ± 0.13Aab | 0.94 ± 0.05Ab | 0.37 ± 0.01Ab | 0.59 ± 0.04Ab | 9.61 ± 0.23Aa |
| 2Al | 0.76 ± 0.01Ba | 2.30 ± 0.35Aa | 0.24 ± 0.00Aa | 3.44 ± 0.29Aa | 0.92 ± 0.05Aa | 0.37 ± 0.01Aa | 0.60 ± 0.04Aa | 9.41 ± 1.30Aa |
| 2Al + $H_2S$ | 0.79 ± 0.02Aa | 2.80 ± 0.40Aa | 0.24 ± 0.01Ba | 3.35 ± 0.64Ba | 1.00 ± 0.15ABa | 0.37 ± 0.01Ba | 0.62 ± 0.07Ba | 8.91 ± 0.76ABa |
| 2Al + PAG | 0.76 ± 0.04Ba | 2.58 ± 0.31Aa | 0.24 ± 0.00Ba | 3.76 ± 0.62Aa | 1.06 ± 0.17Aa | 0.38 ± 0.01Aa | 0.67 ± 0.09Aa | 9.91 ± 1.19Aa |
| 4Al | 0.75 ± 0.02Ba | 2.31 ± 0.45Aab | 0.24 ± 0.01Aa | 3.59 ± 0.81Aab | 0.96 ± 0.11Ab | 0.37 ± 0.01Aa | 0.57 ± 0.06Ab | 7.82 ± 0.03Aab |
| 4Al + $H_2S$ | 0.77 ± 0.02Aa | 2.11 ± 0.09Bb | 0.23 ± 0.00Bb | 2.75 ± 0.04Bb | 0.82 ± 0.03Bb | 0.36 ± 0.00Ca | 0.53 ± 0.01Bb | 8.95 ± 1.57Bb |
| 4Al + PAG | 0.76 ± 0.01Ba | 2.80 ± 0.32Aa | 0.25 ± 0.00Aa | 4.11 ± 0.51Aa | 1.16 ± 0.11Aa | 0.33 ± 0.00Bb | 0.70 ± 0.04Aa | 10.26 ± 0.99Aa |

Data are mean values ± SD (n = 3). Different lowercase letters represent significant differences among different $H_2S$ conditions under the same Al concentration treatment, and different uppercase letters represent significant differences among different Al concentration treatments under the same $H_2S$ condition ($p < 0.05$), as determined by the Duncan test.

*sinensis* under Al stress (Fig. 5), suggesting that $H_2S$ mitigates Al toxicity mainly through the increase of chlorophyll content and ultrastructural stabilization rather than regulating photosynthetic parameters.

## $H_2S$ regulates the antioxidant system of *C. sinensis* to resist Al stress

Plants suffering from Al toxicity often exhibit symptoms associated with membrane lipid peroxidation, which result in accumulation of MDA[28]. As previously studied[29], the present results indicated that $H_2S$ reduce accumulation of MDA in leaves at 2Al (Fig. 6a). Proline participates in removal membrane lipid peroxidation under stress conditions[30]. However, using exogenous $H_2S$ at 2Al concentration only increases proline content in tea leaves by 2.82% compared to 2Al alone (Fig. 6b). CAT, POD and SOD are the main antioxidant enzymes in plants, all of which are involved in inhibition of oxidative stress and lipid peroxidation[31] in plants under excessive Al conditions, thus mitigating Al toxicity in plants[32]. CAT and POD played a role in the leaves under $H_2S$ + 2Al, because the activities of CAT and POD in $H_2S$ + 2Al were significantly higher than those in 2Al (Fig. 7a and 7c). There is also evidence indicating that $H_2S$-induced alleviation in Al toxicity is attributed to elevated CAT and POD activities, but in barley roots[33]. At the same time, $H_2S$ + 2Al and $H_2S$ + 4Al reduced CAT, POD and SOD activities in roots, compared with 2Al and 4Al, respectively (Fig. 7b, d & f). When concerning reactive oxygen species scavenging systems, it is speculated that $H_2S$ may alleviate Al toxicity through elevated CAT and POD activities in leaves, while the root system mainly alleviates Al injury through other ways, thus the activities of CAT, POD and SOD decreased. Taken together this data supports the idea that $H_2S$ reduces MDA and increases proline levels by regulating antioxidant enzyme activity to alleviate stress in 2Al treatment in leaves.

GSH, the major non-enzymatic antioxidants in the ASA-GSH cycle contribute to plant antioxidant defense[34]. Consistent with previous research results[35], the GSH content in leaves significantly increased after exposure to Al stress. Although exogenous $H_2S$ reduced the GSH content in barley leaves[35], it did not decrease GSH content in tea leaves under $H_2S$ + 2Al, and only decreased the GSH content under $H_2S$ + 4Al (Fig. 8a), indicating that $H_2S$ responds to 4Al toxicity by altering GSH content in leaves, triggering the AsA-GSH cycle and improving antioxidant capacity. Consistently, levels of GSSG, which is reduced to GSH, enhanced in leaves during Al stress exposure, and $H_2S$ reduce the content of GSSG only in 4Al (Fig. 8b). The GSH/GSSG ratio is also an important indicator of intracellular redox homeostasis within cells. Exogenous $H_2S$ modulated the GSH/GSSG ratios by altering GSH and GSSG to varying levels, but resulting in a little change in GSH/GSSG compared to Al stress alone (Fig. 8c). These outcomes are consistent with the findings of previous studies on bermudagrass[36] and rice[37]. GST has been found to catalyze the chelation of GSH with metals and reduce the toxicity of metals to plants[38]. The GST activity under $H_2S$ + 2Al not $H_2S$ + 4Al stress was significantly enhanced (Fig. 8d), plants rely on the binding to minimize damage, which was consistent with the study of *Miscanthus sacchariflorus*[24].

GR regulates the redox state of glutathione by converting GSSG into GSH, and also responsible for combating a large amount of reactive oxygen species in plants[39]. The GR activity

in this study shown an increase under Al stress which is similar to the observations made by Devi et al.[40]. Higher GR activity after $H_2S$ + 2Al and lower GR activity under $H_2S$ + 4Al were observed, respectively in comparison to 2Al and 4Al (Fig. 8e). The above results confirmed that $H_2S$ alleviates 2Al stress by regulating substances derived from antioxidant system, whereas the mechanism was complex, resulting in a small pattern of changes in $H_2S$ + 4Al compared with 4Al stress alone.

LCD is primarily responsible for catalyses the decomposition of cysteine to $H_2S$. Further enzyme analysis indicated that the externally applied $H_2S$ enhanced the activity of LCD relative to Al alone stress, which was especially significant in roots. In *Spinacia oleracea* also clearly showed an increase in LCD activity with application $H_2S$[41], and an early $H_2S$ signal might promoted higher LCD activity than Al stress after 3 h[42]. Taken together, LCD activity regulates the internal $H_2S$ pathway in *C. sinensis* and plays a more effective role in roots rather than leaves

### $H_2S$ altered tea components during Al stress

Various components of the tea plant, including tea polyphenols, amino acids, caffeine, catechins, are not only closely related to the flavor of the tea plant, but also have an effect when *C. sinensis* is exposed to stress. The synthesis of amino acids, caffeine, and catechins is regulated by Al[43]. In this study, compared with normal Al concentration, the changes in tea polyphenol content under Al stress were not significant, while the content of free amino acids was significantly reduced and the content of caffeine was significantly increased (Fig. 9). At normal Al concentration, early application of $H_2S$ increases the content of these substances (Fig. 9), which may be related to the promotion of tea roots growth by $H_2S$[44]. As a major component of the ester type catechins, EGCG has been reported to chelate Al, thus conferring Al tolerance to plants[45]. It was found that the EGCG content increased by 8.70% under $H_2S$ + 2Al compared to 2Al, and excessive stress of 4Al may lead to a decrease in EGCG content, and even with the addition of exogenous $H_2S$, the changes in content remains small under 4Al stress (Table 4). Combined with the above results, it providing further evidence that the part of $H_2S$ that promotes the increase of components may have chelated with too much Al at $H_2S$ + Al, resulting in a decrease in the final content, or may be caused by severe stress at 4Al.

## Conclusions

Our results indicate that $H_2S$ may be pivotal actor in enhancing the resistance of *C. sinensis* to Al stress. Increasing biomass, promoting root activity, reducing accumulation of Al in roots and increasing TF of Al, regulating the content of Ca, Mg, Zn, Mn and Fe and their TF in different tissues, increasing chlorophyll content, maintaining ultrastructural homeostasis, regulating substances related to antioxidant pathways and tea plant components all play key roles in the ameliorating effect. Moreover, compared to 4Al, $H_2S$ can better alleviate the stress caused by 2Al.

## Author contributions

The authors confirm contribution to the paper as follows: conceptualization: Shu Z, Sui X, Wang Y; investigation: Shu Z, Huang P, Wan S; data curation: Zhang Y, Xing A; project administration: Shu Z, Wang Y; supervision: Wang Y; resources: Li X; formal analysis, visualization, writing—original draft preparation: Xing A; writing—review and editing: Xing A, Liu S, Chen X, Li X, Wang Y; funding acquisition: Chen X, Wang Y. All authors read and approved the final manuscript.

## Data availability

All data generated or analyzed during this study are available within the article.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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