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Improving salt tolerance of bean (*Phaseolus vulgaris* L.) with hydrogen sulfide

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Abstract

The current study examined the H_2S applications on growth, biochemical and physiological parameters of bean seedlings under saline conditions. The findings of the study indicated that salt stress decreased plant growth and development, photosynthetic activity, and mineral and hormone content [excluding abscisic acid (ABA)] in bean seedlings. Plant and root fresh mass and dry mass with H_2S applications increased as compared to the control treatment at the same salinity level. Both salinity and H_2S treatments significantly affected the net assimilation rate, stomatal conductance, transpiration rate, and intercellular CO_2 content of bean seedlings. Significant increases occurred in H_2O_2, malondialdehyde (MDA), proline, sucrose content, enzyme activity, and ABA content with salt stress. However, H_2S applications inhibited the effects of salinity on plant growth, photosynthetic activity, and mineral content in beans. H_2S applications reduced H_2O_2, MDA, proline, sucrose content, enzyme activity, and ABA content in beans. As a result, exogenous H_2S applications could mitigate the negative impacts of salinity in beans.

Keywords: bean; hormone; physiology; plant growth; salinity.

Introduction

Soil salinity can be considered an important problem that reduces agricultural productivity in the world. Lack of precipitation and drainage together with high evaporation and undesirable soil properties are the main causes of salinity in arid and semi-arid regions. Especially in recent years, the salinity problem in the world has gained more importance with the effect of global climate change. Salinity causes lots of ravages at the morphological, cellular, physiological, and molecular levels as well as various developmental processes in plants (Kalaji *et al.* 2011, Al-Zubaidi 2018). Plants exposed to salt stress may develop different tolerance strategies in response.

Highlights

- Salinity decreased growth, photosynthesis, and carboxylation efficiency in bean
- Salinity reduced mineral content and altered hormone content
- Hydrogen sulfide alleviated salinity-induced reduction of growth

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Abbreviations: ABA – abscisic acid; CAT – catalase; Chl – chlorophyll; C_i – intercellular CO_2 content; CO – carbon monoxide; CRV – chlorophyll-reading value; DM – dry mass; E – transpiration rate; FM – fresh mass; GA – gibberellic acid; g_s – stomatal conductance; H_2O_2 – hydrogen peroxide; H_2S – hydrogen sulfide; IAA – indole acetic acid; MDA – malondialdehyde; NO – nitric oxide; P_N – net assimilation rate; POD – peroxidase; ROS – reactive oxygen species; RWC – relative water content; SOD – superoxide dismutase; TEC – tissue electrical conductivity.

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Depending on the type and amount of salt compounds to which the plant is exposed, they become harmful to the plant when they exceed a certain concentration. Soil salinity can have a toxic impact on crops by disrupting nutrition and metabolism. In addition, with the elevation in the salt concentration in the soil solution, it becomes difficult for the plant to take water from the soil, the structure of the soil deteriorates, and the plant growth slows down or even stops (Tuteja 2007, Munns and Tester 2008).

All aspects of plant growth and development such as germination, vegetative growth, and yield are negatively affected by soil salinity (Isayenkov and Maathuis 2019). Apart from being toxic to plants, the high salt concentration in the soil creates an osmotic imbalance resulting in the drying of the plant (Zhao *et al*. 2017, Choudhury *et al*. 2021).

Bean is a legume crop that is widely consumed all over the world and is very sensitive to salt stress. Studies have proved that salt stress has negative effects on metabolic, physiological, and biochemical properties in beans, and therefore decreases plant growth and yield (Stoeva and Kaymakanova 2008, Doganlar *et al.* 2010, Gharsallah *et al.* 2016, Zhang *et al.* 2017).

Breeding of salt-tolerant cultivars is time-consuming, expensive, and complex. Previous studies have shown that applications of plant growth regulators, such as biostimulants, hormones, *etc*., improved salt tolerance in plants. H_2S is a colorless and foul-smelling toxic gas produced by industrial processes (Ding *et al.* 2019). Plants can synthesize and secrete H_2S, which is considered another signaling molecule after NO and CO in plants (Hancock 2017). Hydrogen sulfide is produced in plants by both enzymatic and nonenzymatic means. However, production by nonenzymatic means accounts for only a small part of H_2S production. H_2S has been shown to play an important role in many physiological and metabolic processes (Christou *et al.* 2014, Shen *et al.* 2015, Liu *et al.* 2021). Seed germination rates increased with exogenous H_2S applications (Jin and Pei 2015). Leaves of old plants contained higher H_2S concentrations than young plants. Consequently, the importance of H_2S in plant production and aging has become an important subject to investigate. It is known that H_2S alleviates the effect of stress in plants by increasing photosynthetic activity in saline conditions, changing antioxidant enzyme activity to increase stress tolerance, and modulating signal-transmission pathways (Ding *et al.* 2019). Hydrogen sulfide is also effective in increasing plant resistance to environmental stress factors through the development of antioxidant systems in case of osmotic stress, hypoxia, and temperature stress (Li *et al.* 2016, 2022; da-Silva and Modolo 2018). Previous studies showed that H_2S at low concentrations is crucial in the plant life cycle, from germination to growth as well as against environmental stress responses (Liu *et al.* 2021). Lately, it was recognized that H_2S under salt stress makes an important contribution to cell signaling (Li *et al.* 2022). However, studies examining the effect of H_2S in beans under salt stress are limited. Therefore, this study was carried out to determine the plant growth and physiological and biochemical properties of H_2S applications in beans grown under salt stress.

Materials and methods

Plant material: The experiment was carried out in the greenhouses of Atatürk University Crop Production Application and Research Center. In the study, beans (*Phaseolus vulgaris* L., cv. Gina) were used as plant material. The study was carried out in pots under controlled greenhouse conditions (temperature of 25 ± 2°C/18 ± 2°C day/night, humidity of 40 ± 5%).

Three seeds were planted in one pot filled with a mixture of garden soil:peat:sand (3:1:1) and left to one seedling after emergence. Fertilization was made as 100 kg(N) ha^{-1}, 100 kg(P_2O_5) ha^{-1}, and 100 kg(K_2O) ha^{-1} (Esiyok 2012). Some properties of the medium used in the study are as follows: pH of 7.28, EC of 90.05 µS cm^{-1}, lime of 0.75%, organic matter of 1.55%, total N of 0.08%, P of 35.21 ppm, K of 34.30 mmol kg^{-1}, Ca of 200.50 mmol kg^{-1}, Mg of 167.40 mmol kg^{-1}, Na of 28.80 mmol kg^{-1}, B of 0.04 ppm, Cu of 0.35 ppm, Fe of 1.09 ppm, Zn of 0.23 ppm, and Mn of 0.15 ppm.

H_2S treatments: NaHS (H_2S donor) of 0, 25, 50, 75, and 100 µM were foliar applied 10 d after the emergence of bean seedlings. *Tween-20* (0.2%) was added to the solutions prepared with distilled water. Three applications were made at one-week intervals.

Salinity treatments: Irrigation waters with 0, 50, 75, and 100 mM NaCl were used to create salt stress in the root zone. Salinity treatments started one day after the H_2S application. Salt stress in the medium was gradually increased starting with 25 mM initially and finalized at the determined salt concentrations of treatments. A soil moisture meter (*WET* sensor, *Delta-T Devices*, UK) was used to calculate irrigation water amounts.

Morphological, physiological, and biochemical analysis: The experiment was carried out with 240 plants in a randomized plot design with three replications and four plants in each replication. The study was terminated 30 d after the first salt application.

Relative water content (RWC), tissue electrical conductivity (TEC), and chlorophyll reading value (CRV) were determined after 40 d from sowing. Plant height, plant diameter, shoot and root FM, and shoot and root DM were determined. Samples from roots and shoots were taken for different analyses.

The leaf area was determined by a leaf area meter (*CI-202 Portable Laser Leaf Area Meter*, *CID Bio-Science*, USA). Gas-exchange parameters, such as g_s, P_N, C_i, and E were measured one week before the harvest with *Li-Cor 6400* (*LI-COR*, Lincoln, USA). Photosynthetically active radiation (PAR) in the leaf chamber was 1,100 µmol(photon) m^{-2} s^{-1}, leaf to air vapour deficit pressure was 1.7–2.6 kPa, leaf temperature was 20–22°C, and chamber CO_2 was 400 µmol mol^{-1}.

H_2O_2, MDA, and proline content of leaf tissues were assayed according to Ozden *et al.* (2009). Sucrose concentration was measured by a method given by Liu and Huang (2000). Superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) activity was analyzed with spectrophotometer (*Multiskan GO Microplate Spectrophotometer*, *Thermo Fisher Scientific*, Finland) according to Abedi and Pakniyat (2010). SOD activity unit was measured at 560 nm and CAT activity unit was measured at 240 nm wavelength. Peroxidase (POD, EC 1.11.1.7) activity was measured according to Angelini *et al.* (1990). For the assay of TEC and RWC, the methods of Shams *et al.* (2019) were employed.

Chlorophyll (Chl) *a*, *b*, and total Chl content were analyzed according to Lichtenthaler and Buschmann (2001). For the determination of mineral nutrition content, bean leaves were ground after being dried at 68°C for 48 h in an oven. Determination of the total N was achieved by the Kjeldahl method using a *Vapodest 10 Rapid Kjeldahl Distillation Unit* (*Gerhardt*, Germany). An inductively coupled plasma spectrophotometer (*Optima 2100* DV, ICP/OES; *Perkin-Elmer*, Shelton, CT) was used to determine tissue K, P, Ca, Mg, S, Na, Cl, Fe, Zn, Mn, Cu, and B (Mertens 2005a,b).

Indole acetic acid (IAA), abscisic acid (ABA), and gibberellic acid (GA) analyses were conducted according to the method of Battal and Tileklioğlu (2001).

Statistical analysis: The experiment was designed according to a completely randomized factorial design. A two-way analysis of variance (*ANOVA*) was made using *SPSS*. Means were compared according to *Duncan*'s multiple ranges.

Results

Growth parameters: Results from analyzed data showed a significant impact from salt stress, exogenous H_2S application, and their interactions on bean seedlings. Salt stress negatively affected the plant growth of beans, but H_2S applications ameliorated the negative effect of salt (Fig. 1, Table 1). Shoot FM, shoot DM, root FM, and root DM decreased by 17-27-33%, 25-38-54%, 28-38-47%, and 39-46-61% at 50, 75, and 100 mM NaCl, respectively, compared to the control (0 mM NaCl).

The impact of the salinity and H_2S applications on the growth parameters of the bean were determined as statistically significant. Plant height, leaf area, stem diameter, CRV, shoot and root FM, and shoot and root DM of bean were reduced with increased salt stress. H_2S applications alleviated the negative effect of salinity on the investigated parameters (Table 1, Fig. 1). With H_2S application at 100 mM NaCl, plant height, stem diameter, leaf area, and CRV increased by 31% (100 µM H_2S), 23% (50 µM H_2S), 32% (50 µM H_2S), and 26% (25 µM H_2S), respectively, compared to untreated plants (0 µM H_2S). H_2S at 25 µM increased the shoot FM by 11%, 100 µM H_2S increased the shoot DM and root FM by 23 and 54%, respectively, at 100 mM NaCl compared to untreated plants treatment while 50 µM H_2S treatment was more effective in improving root DM (Figs. 1, 2).

Salt-stressed plants had greater TEC values but lower RWC. All doses of H_2S used in the study alleviated the salt stress effects on RWC and TEC (Fig. 3). Salt stress conditions caused a decrease in Chl *a*, Chl *b*, and total Chl content while exogenous H_2S treatments enhanced Chl *a*, Chl *b*, and total Chl under salt stress (Table 1). Furthermore, the interaction between salt stress and H_2S had a significant impact on Chl *a*, *b*, and total Chl content. In 100 mM NaCl, 25 µM H_2S increased Chl *a*, *b*, and total Chl content by 41, 50, and 95%, respectively, compared to 0 µM H_2S (Fig. 2).

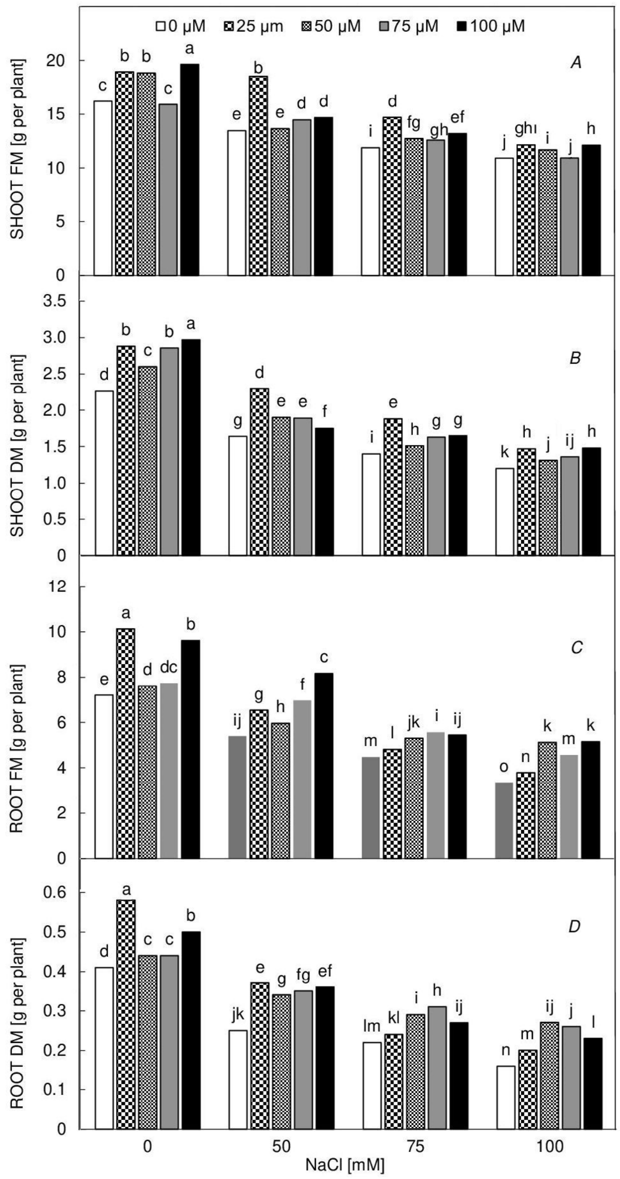

Fig. 1. The effects of H_2S applications on shoot fresh mass (FM) (*A*), shoot dry mass (DM) (*B*), root fresh mass (FM) (*C*), and root dry mass (DM) (*D*) in salt-stressed beans. There is no statistical difference between *the same letters* given in each bar (p<0.001).

Table 1. The effects of H_2S applications on plant height, stem diameter, leaf area, chlorophyll reading value (CRV), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total chlorophyll amount in salt stressed beans. There is no statistical difference between *the same letters* given in each column ($p<0.001$).

NaCl [mM]	H_2S [μM]	Plant height [cm]	Stem diameter [mm]	Leaf area [cm^2 per plant]	CRV [SPAD]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Total Chl [mg g^{-1}(FM)]
0	0	59.89^{bc}	3.59^{c}	361.32^{d}	39.97^{a}	2.30^{f}	2.14^{bc}	4.44$^{c–g}$
	25	58.22^{bc}	4.02^{a}	399.96^{c}	39.60^{a}	4.13^{a}	2.13^{bc}	6.26^{a}
	50	62.94^{a}	4.01^{a}	374.83^{cd}	37.33^{bc}	2.46^{e}	1.81^{de}	4.27$^{e–h}$
	75	56.33^{c}	3.88^{b}	522.86^{a}	37.83^{abc}	2.43^{e}	2.30^{b}	4.77^{c}
	100	62.06^{ab}	3.88^{b}	461.85^{b}	38.90^{ab}	2.58^{d}	2.46^{a}	5.16^{b}
50	0	35.25^{f}	3.45^{d}	306.03^{f}	34.70^{def}	2.65^{cd}	1.48^{fgh}	4.13^{gh}
	25	51.42^{d}	3.52^{cd}	389.29^{c}	34.10^{ef}	2.84^{b}	1.71^{def}	4.56^{cde}
	50	45.17^{e}	3.52^{cd}	299.25^{fg}	34.13^{ef}	2.47^{e}	1.56$^{e–h}$	4.03^{hi}
	75	41.83^{e}	3.51^{cd}	377.76^{cd}	34.00^{ef}	2.59^{cd}	1.91^{cd}	4.50$^{c–f}$
	100	35.00^{f}	3.48^{cd}	332.80^{e}	36.43^{cd}	2.69^{c}	1.95^{cd}	4.63^{cd}
75	0	33.42^{fg}	3.10^{fg}	265.38^{hi}	29.73^{h}	2.42^{e}	1.34^{ghi}	3.76^{ij}
	25	31.83^{fg}	3.32^{e}	301.52^{fg}	35.07^{de}	2.82^{b}	1.52$^{e–h}$	4.34$^{d–h}$
	50	31.47^{fg}	3.32^{e}	277.91^{gh}	29.50^{h}	2.85^{b}	1.32^{ghi}	4.17^{fgh}
	75	32.50^{fg}	3.18^{f}	306.90^{f}	31.60^{gh}	2.49^{e}	1.59^{efg}	4.08^{hi}
	100	33.00^{fg}	3.20^{ef}	278.74^{gh}	33.57^{efg}	1.70^{g}	1.49^{fgh}	3.19^{k}
100	0	26.17^{hi}	2.61^{i}	186.08^{m}	27.43^{i}	0.95^{i}	0.94^{j}	1.89^{m}
	25	29.33^{gh}	2.97^{h}	213.07^{kl}	34.63^{def}	2.29^{f}	1.41$^{f–i}$	3.70^{j}
	50	24.17^{i}	3.22^{ef}	246.29^{ij}	29.93^{h}	1.47^{h}	1.27^{hi}	2.75^{l}
	75	32.31^{fg}	3.03^{gh}	231.89^{jk}	30.20^{h}	1.63^{g}	1.14^{ij}	2.78^{l}
	100	34.33^{f}	3.15^{f}	203.03^{lm}	32.60^{fg}	1.38^{h}	1.31^{ghi}	2.69^{l}

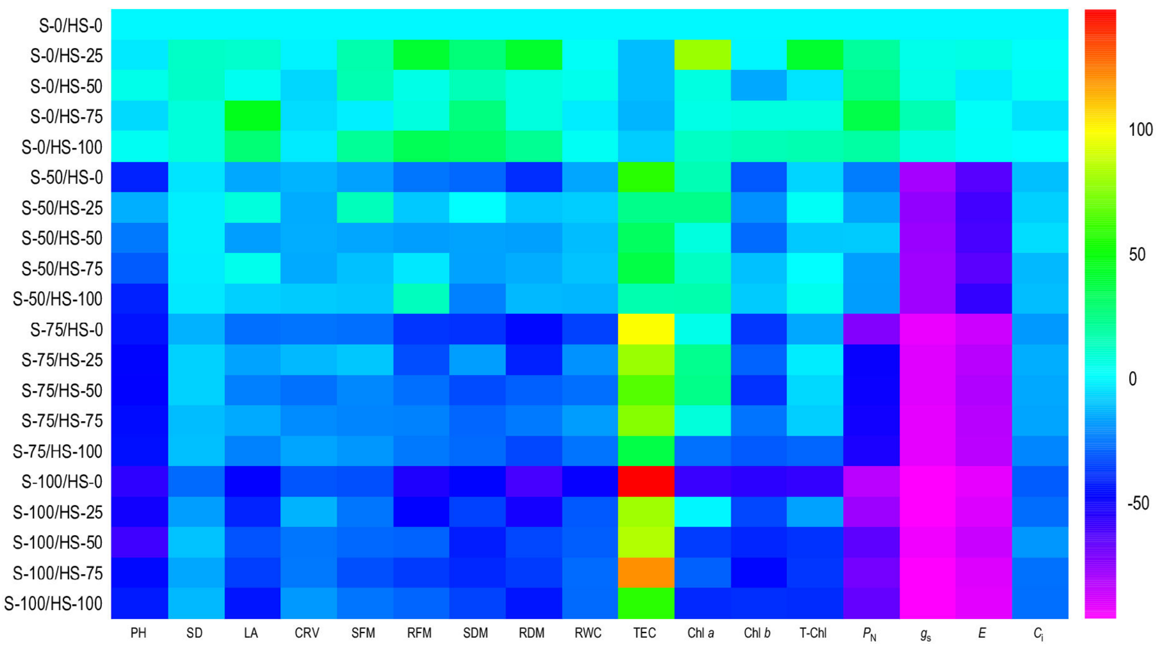

Fig. 2. Heatmap analysis for percentage change [%] of the growth parameters, chlorophyll contents, and photosynthetic activity of bean with different treatments compared to the control. PH – plant height; SD – stem diameter; LA – leaf area; CRV – chlorophyll reading value; SFM – shoot fresh mass; RFM – root fresh mass; SDM – shoot dry mass; RDM – root dry mass; RWC – relative water content; TEC – tissue electrical conductivity; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; T-Chl – total chlorophyll; P_N – photosynthetic rate; g_s – stomatal conductance; *E* – transpiration rate; C_i – intercellular CO_2 content.

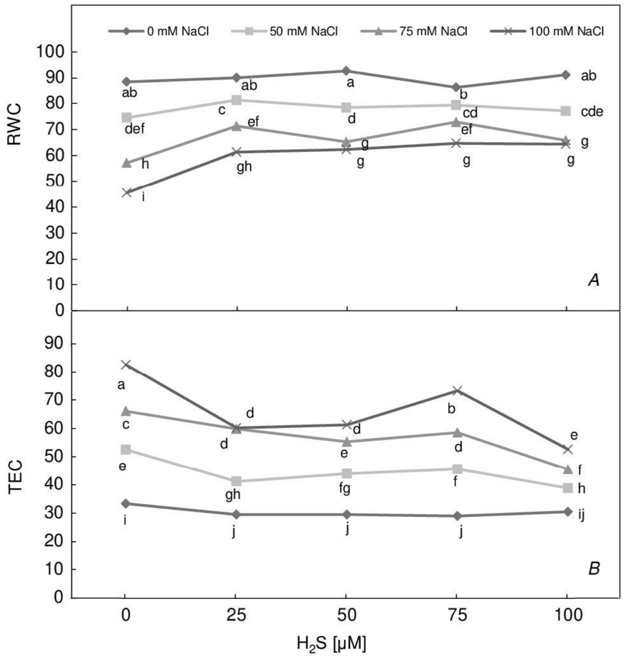

Fig. 3. The effects of H_2S applications on relative water content (RWC) (*A*) and tissue electrical conductivity (TEC) (*B*) in salt stressed beans. There is no statistical difference between *the same letters* given in each line ($p<0.001$).

Photosynthetic characteristics: Table 2 presents the impact of H_2S on the photosynthetic characteristics of bean seedlings under different salinity levels. Both salinity and H_2S treatments significantly affected the P_N, g_s, E, and C_i. These parameters decreased under salt stress, however, exogenous H_2S application mitigated the negative impacts of salt on photosynthetic parameters in bean seedlings. The most effective dose of H_2S in improving P_N, g_s, E, and C_i at 100 mM NaCl was 50 µM H_2S application with the increase ratio of 40, 67, 71, and 16%, respectively, as compared to untreated plants at the same salt stress level (Fig. 2).

Physiological characteristics: In this study, salt-stressed plants had more proline and higher sugar content than nonstressed plants. Exogenous H_2S application lowered the proline and sugar content of bean seedlings under salinity conditions (Table 3). A notable accumulation of MDA and H_2O_2 was observed in bean seedlings under salt stress conditions, whereas these increases were inhibited by exogenous H_2S application at the same salt stress levels. We observed that treatments of 75 and 100 µM H_2S generally had a greater effect in decreasing MDA and H_2O_2 content under salt stress than the other application doses of H_2S (Table 3).

Antioxidant enzyme activity: The activities of POD, CAT, and SOD enzymes in bean leaves significantly increased under saline conditions. We found that H_2S lowered ROS content. All application doses of H_2S decreased the CAT, POD, and SOD activities at different ratios varying between 25–61%, 20–63%, and 16–66%, respectively, in bean seedlings at 100 mM NaCl as compared to untreated plants at same salt stress level (0 µM H_2S and 100 mM NaCl) (Table 3).

Hormone content: ABA content in bean leaves increased with increased salt stress levels in all treatments. The contents of IAA and GA in leaves decreased under salinity. However, exogenous H_2S applications reduced ABA but increased IAA and GA content in bean seedlings (Fig. 4).

Mineral element content: The mineral content in bean seedlings under different salt levels is shown in Table 4. In all salinity treatments, the mineral content (except for Na and Cl) in leaves decreased. However, H_2S treatments enhanced the ion content of the bean under salinity. Application of exogenous H_2S reduced Na and Cl contents in the seedlings under salinity as well (Table 4).

Discussion

Salt stress effects on bean seedlings: Salt stress causes osmotic stress and ionic toxicity, which affects all major plant development processes, such as photosynthesis, cellular metabolism, and plant nutrition (Safdar *et al.* 2019). The results obtained from our study indicated that salinity negatively affected bean growth and decreased the amount of Chl and photosynthetic activity of the plant at examined salt stress levels (Tables 1, 2; Fig. 2). Similarly, previous reports have indicated that salinity negatively influences growth in beans (Vieira *et al.* 2019, Arteaga *et al.* 2020). Scholberg and Locascio (1999) determined that the growth of beans decreased linearly with the increase in the electrical conductivity (EC) of irrigation water. It has been reported earlier that salinity conditions trigger the change of lipid composition in the membrane structure and cause membrane damage (Munns and Tester 2008). Similarly, in our experiment with bean seedlings, RWC decreased with increased salt stress, however, TEC values increased significantly (Fig. 3). Chlorophyll content significantly decreased with increased salt concentrations. Decreased RWC and chlorophyll content and increased electrical leakage under salt stress have been reported earlier in different vegetables including beans (Azimychetabi and Sabokdast 2021, Kul *et al.* 2021). Salt-stressed bean plants had lower P_N, g_s, E, and C_i values than those of nonstressed plants (Table 2). The decrease in photosynthetic characteristics of beans under salt stress can be explained by the effect of NaCl that causes aggregation in adjacent grana membranes, shrinkage of thylakoids, and degradation of chlorophylls. It has been reported earlier that salinity decreases the net photosynthetic rate, transpiration rate, and stomatal conductivity and increases stomatal resistance (Ashraf 2004).

Salt stress is a complex issue for plants and it affects many metabolic activities. In our experiment, salt stress in bean seedlings resulted in a substantial elevation in the concentration of proline, sugar, H_2O_2, and a rise in

Table 2. The effects of H_2S applications on the net assimilation rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 content (C_i) in salt-stressed beans. There is no statistical difference between *the same letters* given in each column ($p<0.001$).

NaCl [mM]	H_2S [µM]	P_N [µmol $m^{-2}s^{-1}$]	g_s [mmol $m^{-2}s^{-1}$]	E [mmol $m^{-2}s^{-1}$]	C_i [µmol mol^{-1}]
0	0	8.25^{d}	0.82^{d}	9.50^{bc}	342.33^{b}
	25	9.89^{c}	0.86^{c}	10.08^{a}	348.67^{ab}
	50	10.23^{b}	0.87^{c}	9.28^{c}	353.00^{a}
	75	11.23^{a}	0.95^{a}	9.73^{b}	327.67^{c}
	100	9.80^{c}	0.88^{b}	9.76^{b}	347.00^{ab}
50	0	6.27^{g}	0.17^{gh}	3.39^{f}	305.00^{e}
	25	6.90^{f}	0.20^{e}	3.79^{de}	315.33^{d}
	50	7.52^{e}	0.19^{ef}	3.66^{ef}	324.00^{cd}
	75	6.83^{f}	0.18^{gh}	3.36^{f}	300.33^{ef}
	100	6.79^{f}	0.18^{gh}	4.04^{d}	304.00^{e}
75	0	2.25^{k}	0.06^{jk}	1.29^{hi}	279.67^{hi}
	25	4.20^{h}	0.08^{i}	1.61^{gh}	293.00^{fg}
	50	4.16^{h}	0.08^{i}	1.75^{g}	289.33^{gh}
	75	4.05^{h}	0.07^{ij}	1.63^{gh}	287.33^{ghi}
	100	3.89^{h}	0.07^{ij}	1.57^{gh}	267.00^{j}
100	0	1.39^{m}	0.03^{l}	0.77^{j}	239.33^{l}
	25	1.82^{l}	0.03^{l}	0.99^{ij}	250.33^{k}
	50	2.84^{i}	0.05^{k}	1.32^{h}	278.33^{i}
	75	2.48^{jk}	0.03^{l}	0.93^{j}	252.33^{k}
	100	2.74^{ij}	0.03^{l}	0.85^{j}	251.33^{k}

Table 3. The effects of H_2S applications on hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline, and sucrose content and catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activity in salt-stressed beans. There is no statistical difference between *the same letters* given in each column ($p<0.001$).

NaCl [mM]	H_2S [µM]	H_2O_2 [mmol kg^{-1}]	MDA [mmol kg^{-1}]	Prolin [mmol kg^{-1}]	Sucrose [%]	CAT [U g^{-1}(FM)]	POD [U g^{-1}(FM)]	SOD [U g^{-1}(FM)]
0	0	42.17^{ij}	28.57^{g}	0.17^{l}	2.30^{d}	222.26^{ghi}	$31,122.59^{g}$	$2,229.16^{ef}$
	25	18.79^{l}	19.60^{ij}	0.19^{l}	2.61^{c}	219.66^{ghi}	$25,921.24^{h}$	$2,374.72^{ef}$
	50	15.86^{l}	12.89^{l}	0.17^{l}	1.42^{jk}	205.19^{hi}	$17,780.02^{k}$	$1,232.18^{jk}$
	75	9.75^{m}	14.29^{kl}	0.21^{l}	2.18^{def}	202.60^{hi}	$13,340.76^{l}$	862.61^{l}
	100	8.87^{m}	48.49^{d}	0.18^{l}	3.53^{b}	198.17^{i}	$10,664.74^{m}$	396.49^{m}
50	0	61.26^{g}	35.30^{f}	0.21^{l}	2.82^{c}	376.76^{e}	$35,157.95^{f}$	$2,539.35^{de}$
	25	45.87^{i}	30.09^{g}	0.26^{k}	2.23^{de}	349.51^{e}	$34,345.07^{f}$	$2,769.84^{d}$
	50	39.74^{j}	17.21^{jk}	0.33^{j}	1.16^{k}	240.25^{gh}	$23,798.86^{i}$	$1,646.28^{hi}$
	75	27.26^{k}	22.42^{i}	0.41^{i}	1.62^{hij}	184.49^{i}	$18,430.35^{k}$	926.06^{kl}
	100	18.50^{l}	19.07^{j}	0.34^{j}	2.05^{d-g}	216.59^{ghi}	$12,568.65^{lm}$	971.93^{kl}
75	0	107.95^{c}	54.30^{c}	0.61^{d}	3.28^{b}	533.82^{bc}	$52,500.66^{c}$	$3,466.26^{c}$
	25	84.30^{e}	45.68^{d}	0.56^{e}	1.96^{efg}	452.55^{d}	$42,422.73^{d}$	$3,400.39^{c}$
	50	55.24^{h}	19.21^{j}	0.47^{gh}	1.48^{j}	436.95^{d}	$37,470.00^{e}$	$1,895.55^{gh}$
	75	38.48^{j}	31.60^{g}	0.51^{fg}	1.47^{j}	295.41^{f}	$20,600.97^{j}$	$1,449.91^{ij}$
	100	41.26^{ij}	25.47^{h}	0.52^{ef}	1.88^{fgh}	251.05^{g}	$23,491.28^{i}$	$1,539.35^{ij}$
100	0	169.31^{a}	86.16^{a}	0.95^{a}	4.45^{a}	751.05^{a}	$70,181.01^{a}$	$4,789.48^{a}$
	25	131.95^{b}	66.92^{b}	0.76^{b}	1.54^{ij}	564.48^{b}	$55,843.57^{b}$	$4,006.86^{b}$
	50	96.34^{d}	42.33^{e}	0.44^{hi}	2.83^{c}	513.15^{c}	$42,281.10^{d}$	$2,455.51^{e}$
	75	46.95^{i}	37.92^{f}	0.68^{c}	1.82^{ghi}	383.17^{e}	$36,273.74^{ef}$	$2,068.31^{fg}$
	100	69.60^{f}	35.40^{f}	0.60^{d}	0.78^{l}	296.51^{f}	$26,011.74^{i}$	$1,636.50^{hi}$

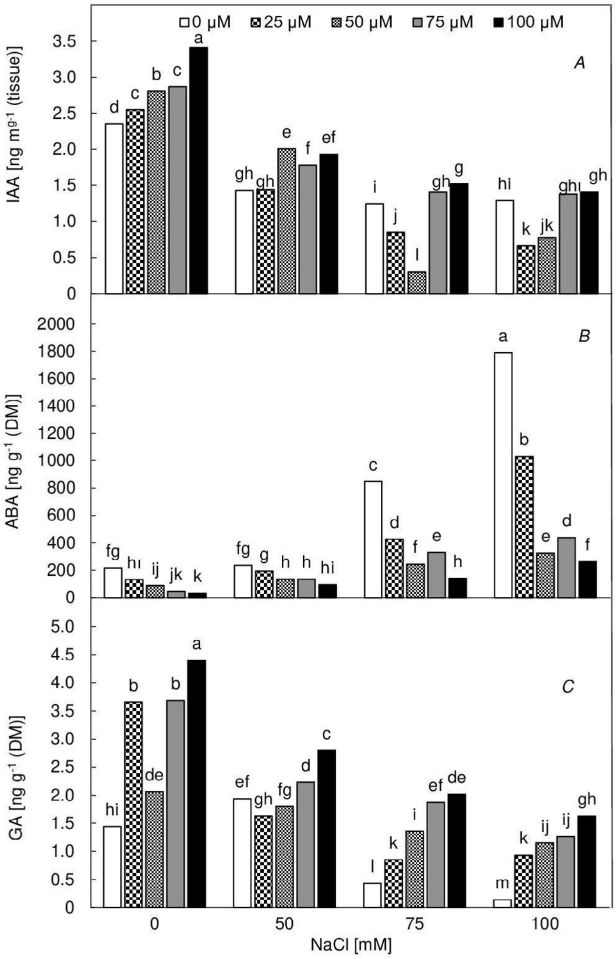

Fig. 4. The effects of H_2S applications on the indole acetic acid (IAA) (*A*), abscisic acid (ABA) (*B*), and gibberellic acid (GA) (*C*) content in salt-stressed beans. There is no statistical difference between *the same letters* given in each bar (p<0.001).

lipid peroxidation (MDA). It is known that under osmotic stress, the availability of water decreases, resulting in the formation of various ROS, such as superoxide ($O_2^{\bullet -}$), H_2O_2, hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) (Ahluwalia *et al.* 2021, Ekinci *et al.* 2021). It is known that increased ROS has a detrimental effect on the cell membrane by lipid peroxidation. Lipid peroxidation, which leads to the destruction of the cell membrane, produces MDA (Ekinci *et al.* 2021, Yildirim *et al.* 2022). Ensuring osmotic balance and preserving cellular structures under stress conditions is achieved by the functions of osmolytes. Proline and sugars are important osmoprotectants that increase the osmotic potential of cells under stress, thereby stabilizing the osmotic potential of internally increased osmotic pressure and stabilizing the structures of membranes and macromolecules (Shafi *et al.* 2019). In our experiment, increased contents of proline, sugar, and MDA under salt stress are a result of the defense mechanism of the bean to adapt to stress conditions.

The results of our study pointed out that salt stress caused an increase in the CAT, SOD, and POD activities of bean plants (Table 3). Plants can reduce and repair oxidative damage with a complex antioxidant system. The activities of CAT, SOD, and POD as antioxidant enzymes are known as extremely effective ROS-scavenging mechanisms (Kusvuran *et al.* 2016).

The hormone content of bean seedlings was significantly affected by salt stress. As represented in Fig. 4, IAA and GA decreased under salt stress as compared to the control treatment. Conversely, ABA content was elevated by increased NaCl concentration. Similarly, it has been also reported that the amount of ABA increased in plants grown under abiotic stress conditions, but the amount of IAA and GA decreased (Samancioglu *et al.* 2016). It has been understood that IAA shows a response mechanism in agricultural plants under salt stress. However, information on the relationship between salt stress and auxin content in plants is limited (Kaya *et al.* 2009).

H_2S application effects on beans under salt stress: The results of the study indicated that exogenous H_2S treatments mitigated the negative effects of salt stress on the growth of the bean. As it was discussed above, all measured parameters of bean seedlings were significantly inhibited under salt stress, whereas the H_2S applications efficiently improved the salt tolerance of the plant. The optimum dose of H_2S in reducing the negative effects of salt on plant growth was 25 µM. Similarly, the mitigating effect of H_2S applications on plant production under salt stress was determined earlier in previous studies (Deng *et al.* 2016, Ekinci *et al.* 2021, Liu *et al.* 2021). In addition, it has been known that plants increase the internal synthesis of H_2S to improve their tolerance to many abiotic stress conditions (Yavaş and Ünay 2018). Therefore, the foliar application of H_2S is supposed to be improving the salt resistance of plants by scavenging ROS accumulation and modulating transcription in multiple defense-related pathways (Zhang *et al*. 2010a).

We observed better growth parameters, such as shoot FM and DM and root FM and DM with H_2S applications under salt stress. Better root growth with different doses of H_2S was announced earlier by Liu *et al*. (2021). Zhang *et al.* (2010a) also reported that exogenous H_2S application stimulated root organogenesis in soybean. In another study, H_2S application with a concentration of 0.4 mM NaHS elevated the progress of lateral roots in wheat. The number and length of lateral roots increased in number as well as their density (Li *et al.* 2022). Li *et al.* (2022) also reported that H_2S application could improve root growth with its inhibitory effect on abiotic stress in many plants.

Foliar H_2S applications caused a significant increase in RWC, Chl *a*, Chl *b*, and total Chl content under salt stress while TEC values decreased with H_2S treatments (Table 1, Fig. 3). Increased membrane permeability and decreased RWC with H_2S application with salinity has been reported earlier with corn (Shan *et al.* 2014) and

Table 4. Effects of H_2S treatments on mineral contents in salt-stressed beans. There is no statistical difference between *the same letters* given in each column ($p<0.001$).

NaCl [mM]	H_2S [μM]	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	S [%]	Na [mg kg^{-1}]	Zn [mg kg^{-1}]	Fe [mg kg^{-1}]	Mn [mg kg^{-1}]	Cu [mg kg^{-1}]	B [mg kg^{-1}]	Cl [mg kg^{-1}]
0	0	3.26^{b}	0.34^{c}	2.61^{c}	1.45^{cd}	0.36^{c}	0.23^{c}	549.00^{l}	43.45^{c}	106.96^{c}	33.71^{c}	35.03^{d}	12.73^{b}	4.93^{j}
	25	3.01^{c}	0.33^{d}	2.60^{c}	1.41^{d}	0.35^{c}	0.23^{c}	525.21^{l}	50.60^{a}	104.68^{c}	33.12^{c}	38.32^{c}	12.63^{b}	5.12^{j}
	50	3.59^{a}	0.37^{b}	2.74^{b}	1.55^{b}	0.38^{b}	0.26^{b}	557.68^{l}	46.07^{b}	114.13^{b}	37.08^{a}	36.37^{cd}	13.49^{b}	5.40^{j}
	75	3.19^{b}	0.34^{c}	2.57^{c}	1.48^{c}	0.39^{b}	0.24^{c}	524.60^{l}	46.21^{b}	104.22^{c}	34.60^{bc}	41.07^{b}	12.78^{b}	4.21^{j}
	100	3.34^{b}	0.50^{a}	3.57^{a}	2.07^{a}	0.50^{a}	0.34^{a}	744.14^{l}	46.75^{b}	152.46^{a}	36.46^{ab}	54.48^{a}	18.51^{a}	4.09^{j}
50	0	1.68^{i}	0.19^{kl}	1.28^{j}	0.84^{k}	0.16^{k}	0.10^{hi}	10,600.78^{e}	24.57^{d}	51.56^{f}	16.67^{gh}	25.35^{ef}	4.56^{gh}	23.69^{g}
	25	2.36^{de}	0.24^{f}	1.86^{d}	1.08^{f}	0.23^{def}	0.14^{de}	8,581.26^{f}	24.06^{de}	70.56^{d}	25.56^{d}	26.25^{e}	6.72^{cd}	19.74^{gh}
	50	2.38^{d}	0.24^{f}	1.56^{fg}	0.89^{ijk}	0.24^{de}	0.11$^{f–i}$	3,128.59^{j}	25.16^{d}	58.45^{e}	20.58^{ef}	23.40^{fg}	5.66^{def}	17.10^{ghi}
	75	2.10^{gh}	0.20^{jk}	1.49^{gh}	1.00^{gh}	0.25^{d}	0.12^{fg}	2,746.13^{jk}	24.45^{d}	60.89^{e}	20.35^{ef}	23.48^{fg}	7.34^{c}	13.39^{hi}
	100	2.35^{def}	0.28^{e}	1.90^{d}	1.18^{e}	0.23^{def}	0.15^{d}	2,011.59^{k}	22.41^{def}	50.21^{f}	24.83^{d}	25.35^{ef}	7.40^{c}	12.60^{i}
75	0	1.51^{j}	0.16^{m}	1.11^{kl}	0.74^{l}	0.13^{l}	0.08^{j}	14,382.00^{d}	16.83^{ij}	41.86^{gh}	15.49^{hi}	22.44^{g}	4.11^{h}	106.43^{c}
	25	2.20^{fg}	0.22^{hi}	1.72^{e}	1.04^{fg}	0.21$^{f–i}$	0.13^{ef}	10,892.77^{e}	19.62^{gh}	66.86^{d}	21.87^{e}	22.12^{g}	6.06^{de}	93.82^{d}
	50	2.11^{gh}	0.22^{hi}	1.45^{hi}	0.87^{jk}	0.20$^{g–j}$	0.11$^{f–i}$	7,204.05^{g}	21.44^{efg}	58.65^{e}	18.84^{fg}	18.29^{ij}	5.31^{efg}	71.37^{e}
	75	1.80^{i}	0.18^{l}	1.33^{j}	0.89^{ijk}	0.20$^{g–j}$	0.11$^{f–i}$	6,205.27^{h}	17.61^{hi}	55.41^{ef}	16.88^{gh}	15.88^{jk}	5.96^{de}	56.67^{f}
	100	2.21^{efg}	0.24^{f}	1.60^{f}	1.07^{f}	0.22$^{e–h}$	0.13^{ef}	5,267.44^{i}	17.86^{hi}	39.24^{gh}	20.58^{ef}	19.21^{hi}	5.94^{de}	50.85^{f}
100	0	1.22^{k}	0.13^{n}	0.93^{m}	0.58^{m}	0.10^{m}	0.07^{j}	22,855.97^{a}	10.30^{l}	32.57^{i}	11.92^{j}	19.36^{hi}	2.79^{i}	274.98^{a}
	25	1.80^{i}	0.21^{ij}	1.38^{ij}	0.94^{hij}	0.17^{k}	0.12^{fg}	17,718.48^{b}	14.88^{jk}	57.66^{e}	17.58^{gh}	21.54^{gh}	4.79^{fgh}	166.83^{b}
	50	1.79^{i}	0.20^{jk}	1.35^{j}	0.87^{jk}	0.18^{ijk}	0.13^{ef}	16,416.52^{c}	20.87^{fg}	51.11^{f}	18.80^{fg}	10.41^{l}	5.09$^{e–h}$	111.30^{c}
	75	1.37^{j}	0.15^{m}	1.06^{l}	0.69^{l}	0.18^{ijk}	0.10^{hi}	10,205.08^{e}	12.72^{kl}	43.92^{g}	13.71^{ij}	13.98^{k}	4.07^{h}	93.38^{d}
	100	1.96^{h}	0.23^{g}	1.18^{k}	0.95^{hi}	0.18^{ijk}	0.11$^{f–i}$	7,581.26^{g}	14.05^{k}	37.89^{h}	20.07^{ef}	14.58^{k}	4.72^{fgh}	67.86^{e}

eggplant (Ekinci *et al.* 2021). Lai *et al.* (2014) pointed out that these positive impacts could be obtained by the maintenance of integrity in the membrane in plants.

Exogenous H_2S lowered the degradation of Chl under salinity, thus enhancing the photosynthetic capacity of the bean. We found that the most effective dose was 25 µM H_2S. Zhang *et al.* (2009) demonstrated that sodium sulfide (NaHS) attenuated the osmotic-induced reduction in Chl concentrations in sweet potatoes. In our study, we observed that H_2S applications increased photosynthetic properties in beans under non-salt stress conditions (Table 2). In addition, we also found that the decrease in photosynthetic properties in saline conditions was lower with H_2S applications. Similar to our findings, Ding *et al.* (2019) stated that H_2S alleviates the impact of stress on plants by increasing photosynthetic activity under salt stress. The effects of H_2S applications on the photosynthetic characteristics of bean was different under different salt concentrations. For example, 50 µM H_2S applications in 50 and 100 mM NaCl and 25 µM H_2S applications in 75 mM NaCl were more effective in terms of improving P_N and C_i as compared to other application doses of H_2S under the same salt stress levels. Similarly, results of 25 µM H_2S application dose were more favorable in 50 and 70 mM NaCl in terms of improving g_s. It is thought that improved photosynthetic activity with H_2S application might be thanks to the reduction of ROS accumulation in particular. It has been known that chloroplast biogenesis and photosynthetic enzyme expression increase with H_2S applications in spinach, and an increase in photosynthetic activity occurs (Chen *et al.* 2011). The enhanced photosynthetic activity with exogenous H_2S under salt stress conditions was also determined in crops such as eggplant (Ekinci *et al.* 2021), rice (Wei *et al.* 2021), and cucumber (Sun *et al.* 2021). It was also reported that H_2S can elevate photosynthetic electron transfer and chlorophyll biosynthesis in cucumber and *Kandelia obovata* leaves under salt stress (Jiang *et al*. 2020). Consequently, the negative impact of salinity on these properties could be alleviated by external H_2S applications (Table 2, Fig. 2).

H_2S applications increased the survival rate of beans under examined salt concentrations by increasing various antioxidant enzyme activities. The results of our study indicated that exogenous H_2S treatments decreased the antioxidant enzyme activities, proline, and sugar content of bean seedlings under saline conditions (Table 3). It has been determined that the accumulation of various osmolytes (sucrose, proline, and soluble total sugars) can be effective in increasing stress tolerance in plants (Shi *et al.* 2013). This may be due to the reduction of ROS accumulation thanks to H_2S applications. Ding *et al.* (2019) also found out that H_2S changes antioxidant enzyme activity to increase plant stress tolerance, and coordinates signal transmission pathways in wheat. Exogenous H_2S applications significantly reduced MDA and H_2O_2 accumulation in bean seedlings. The reducing effect of H_2S applications on oxidative stress markers such as H_2O_2 and MDA was explained by its outcome of providing a protective shield against oxidative damage. The mitigative effect of H_2S is explained earlier by its favorable effect on defense mechanisms through antioxidant activities, and ROS detoxification (Mostofa *et al*. 2015).

Zhang *et al.* (2010b) also mentioned that NaHS (H_2S donor) applications in plants exposed to osmotic stress resulted in a low MDA content in seedlings and limited lipid peroxidation. In addition, the augmentation in MDA accumulation in maize raised by salt stress was significantly lowered by H_2S (Shan *et al.* 2014), while the H_2O_2 and MDA content increased by salinity stress in wheat decreased by foliar H_2S application (Ding *et al.* 2019). Papanatsiou *et al*. (2015) stated earlier that H_2S traverses the intracellular and intercellular domains, and regulates the homeostasis in plant cells. It is well known now that when ROS accumulation started to cause oxidative stress to plants, the increase in H_2S concentration helps to decrease the ROS concentration with different enzymatic and nonenzymatic signal pathways. We found in our study that exogenous H_2S application lowered ROS content. In our experiment, the activities of POD, CAT, and SOD enzymes in bean leaves significantly increased under saline conditions with H_2S applications. The results from previous studies showed that apart from increasing some enzyme activities, such as L/D-cysteine desulfhydrase and O-acetylserine (thiol) lyase, exogenous H_2S application also boosts the amount of endogenous H_2S and cysteine (Khan *et al*. 2018, Li *et al*. 2019).

Foliar H_2S applications reduced ABA content but increased IAA and GA content (Fig. 4). Under stress conditions, H_2S interacts closely with plant hormones to increase tolerance to environmental stress factors. H_2S interacts with phytohormones, such as ethylene, ABA, melatonin, jasmonic acid, and polyamines, to maintain plant responses to abiotic stresses (Huang *et al.* 2021). Specifically, H_2S, a new plant gasotransmitter, is the ABA signalosome for cross-adaptation. By interacting with ABA, it adjusts stomatal closure during different abiotic stresses. H_2S can play role in ABA-induced stomatal closure of stressed plants (Liu and Xue 2021). In addition, H_2S positively modulates ABA signaling in guard cells through persulfidation proteins encoded by the *OST1* and *SnRK2.6* genes (Zhang *et al.* 2021). Moreover, Mei *et al.* (2017) suggested that H_2S is crucial for the progress of lateral roots by interacting with IAA. Fang *et al.* (2014) reported that H_2S-treated tomato seedlings showed induced upregulation of an auxin-dependent gene, *i.e.*, the cyclin-dependent kinase (CDK) gene (*SlCDKA1*). Moreover, in another study, they determined that the production of IAA significantly increased with NaHS application, and as an IAA transport inhibitor, NPA (N-1-naphthylphthalamic acid) lessened the H_2S effect on the root development in sweet potato, soybean, and willow (Zhang *et al.* 2009).

H_2S treatments increased the mineral content of beans (except for Na and Cl) slightly under salt stress (Table 4). H_2S has a significant effect on nutrient balance in plants and ensures their survival under environmental stress factors (Raza *et al.* 2022). It is stated that H_2S acts together with Ca and contributes to the regulation of antioxidant defense and ion homeostasis during K-deficient NaCl stress. In addition, Ca and H_2S act synergistically and increase H^+-ATPase activity and Na^+/H^+-antiport system in plants under NaCl stress (Zhao *et al*. 2018, Khan *et al.* 2021). This process induces the expression of several genes which encode the isoforms of the plasma membrane proton pump in a plant (Zhao *et al*. 2018). It is also known that the accumulation of intracellular Na^+ and the Na^+/K^+ ratio can be reduced by H_2S application and the exosmosis of intracellular K^+ can be inhibited (Li *et al*. 2022). Similarly, Mostofa *et al.* (2015) reported that H_2S pretreatment decreased the Na content in salt-stressed rice. The role of H_2S also has been found important by Deng *et al.* (2016) in mitigating growth inhibition of wheat under salinity, decreasing Na concentration, and increasing the selective carrying capacity from K^+ to Na^+ under salt stress. Previous studies relate decreased root growth under salt stress with a decrease in K^+ content and the K^+/Na^+ ratio in the cytoplasm (Zhao *et al* 2018). H_2S application was reported to restrict the NaCl-induced K^+ content in both salt-tolerant and salt-sensitive grape roots. Additionally, it is stated that H_2S elevates Na^+ efflux and the influx of H^+ to support the plasma membrane polarity (Zhao *et al*. 2018). Similar to our findings, the K^+/Na^+ ratio in alfalfa roots increased with H_2S applications (Wang *et al.* 2012).

Conclusion: The effects of salt stress, which is one of the important problems in agricultural production, cause losses in yield and quality. One of the important types of vegetables affected by salinity is beans. In our study, we determined that salt stress caused significant damage to bean seedlings, and the damage was observed starting from the concentration of 50 mM NaCl. However, the application of H_2S had important effects on beans under salt stress. As a result, it was determined in the study that the damage caused by salt stress in beans can be reduced by exogenous H_2S applications.

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