

Hydrogen sulfide and potassium synergistically induce osmotic stress tolerance through regulation of H⁺-ATPase activity and sugar metabolism in tomato seedlings

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Abstract

Potassium (K) is an essential macronutrient which is known to regulate key metabolic processes, modulate enzyme activity and plays a decisive role in osmotic adjustment in plants. Present work evaluates the role of K in the regulation of endogenous hydrogen sulfide (H_2S) signaling in modulating the tolerance of tomato (*Solanum lycopersicum* L. Mill.) seedlings to osmotic stress. The findings reveal that exposure of seedlings to 15% (w/v) polyethylene glycol 8000 (PEG) led to a substantial decrease in leaf K content which was associated with reduced H^+ -ATPase activity. Exogenous application of K to the stressed seedlings significantly improved endogenous K content. Treatment with sodium orthovanadate (SOV, PM H^+ -ATPase inhibitor) and tetraethylammonium chloride (TEA, K channel blocker) suggests that exogenous K stimulated H^+ -ATPase activity that further regulated endogenous K content in tomato seedlings subjected to osmotic stress. Moreover, reduction in H^+ -ATPase activity by hypotaurine (H_2S scavenger) substantiates the role of endogenous H_2S in the regulation of H^+ -ATPase activity. Elevation in endogenous K content enhanced the biosynthesis of hydrogen sulfide (H_2S) through enhancing the synthesis of cysteine, the H_2S precursor. Synergistic action of H_2S and K effectively neutralized osmotic stress by regulating sugar metabolism that resulted in osmotic adjustment, as witnessed by reduced water loss and improved hydration level of the stressed seedlings. Cross talk of H_2S and K also assisted the seedlings in the activation of antioxidant enzymes that controlled the generation of reactive oxygen species and led to the protection against oxidative stress. The integrative role of H_2S and K signaling was validated using hypotaurine (H_2S scavenger) and TEA (K channel blocker) which weakened the protection against osmotic stress induced impairments. In conclusion, exogenous K and endogenous H_2S regulate H^+ -ATPase activity which plays a decisive role in the maintenance of endogenous K homeostasis. Thus, present work reveals that K and H_2S crosstalk is essential for modulation of osmotic stress tolerance in tomato seedlings.

Key Messages

Potassium (K) and hydrogen sulfide (H_2S) crosstalk, during osmotic stress tolerance, operates through regulating H^+ -ATPase activity and sugar metabolism in tomato seedlings.

1. Introduction

Modulation of plant growth under water scarcity is important for crop sustainability and optimum yield. Owing to sedentary nature of plants, availability of optimum concentration of water in the vicinity becomes more imperative for endurance of plants. Water not only acts as medium for numerous cellular activities but assures plants access to vital nutrients required for proper growth and blossoming harvests. However, availability of water to the plants is often challenged by environmental stresses such as drought, salinity, high and low temperature, heavy metal etc. Manifestation of osmotic stress is a common attribute of these abiotic stresses that restricts water availability to the plants. Osmotic stress

results from imbalance between extracellular and intracellular solute concentration that reduces soil osmotic potential and subsequently imposes osmotic stress to the plants. Higher solute concentration may result from excess accumulation of salt and evaporation of water from the soil that leaves behind excess of ions in the soil. Owing to accumulation of salts, osmotic pressure in soil exceeds the osmotic pressure of plant cells that restricts water and minerals availability to the plants. Osmotic stress adversely affects the cell wall integrity, apoplast pH, cytosolic fluidity, photosynthetic activity, and structure of chloroplast and mitochondria followed by changes in gene expression, regulation of transcription factors and enzyme activities (Liu et al. 2010; García-Morales et al. 2018; Darko et al. 2019). These alterations caused by osmotic stress severely affect plants at morphological, physiological, biochemical, and molecular level (Khan et al. 2017; García-Morales et al. 2018). Under osmotic stress, depleted hydration level in plants induces leakage of potassium (K) that results in hydropassive stomatal closure (Kim et al. 2010). Stomatal closure delimits CO₂ availability in the mesophyll cells which results in altered primary metabolism accompanied by over production of reactive oxygen species (ROS) (Mehler 1951). Although at lower concentration ROS signaling appears to be beneficial, their higher concentration creates oxidative stress and damages macromolecules and causes deformity and dysfunction of cell membranes through peroxidation of lipids (Hasanuzzaman et al. 2020; Khan et al. 2020a, b).

Therefore, alleviation of osmotic stress appears to be an important integrative strategy against various abiotic stresses. To counter osmotic stress-induced constraints, plants should maintain osmotic, ionic, and oxidative equilibrium. In this regard, accumulation of soluble carbohydrates is one of the key strategies which plants adopt to counter osmotic stress. Carbohydrates act as osmolytes that stabilize the membranes, and as signaling molecules that trigger essential metabolic events during plant adaptive responses to various abiotic stresses (Ahmad et al. 2020a). Sucrose, the predominant soluble carbohydrate, fulfils high energy demand under stressful conditions and plays essential role in the maintenance of cellular activities, cell wall biosynthesis, respiration, and serves as a major storage carbohydrate in the form of starch for later use (Sami et al. 2016). Besides sugars, K, the essential macronutrient, has been reported to act as a substantial inorganic osmoticum and affects stomatal conductance, axial growth, and tropism in plants (Marschner 1995). Significant evidence shows the role of K in osmotic adjustment, nitrogen metabolism, regulation of enzyme activities, and biosynthesis of chlorophyll (Chl) (Anschütz et al. 2014; Zahoor et al. 2017a, b). However, abiotic stresses are known to induce cellular K efflux and reduction in K influx that results in K loss (Shabala and Cuin 2008) thus leading to weak protection against the stress. Therefore, maintenance of intracellular K homeostasis is a key factor to mediate plant adaptive responses to abiotic stresses (Anschütz et al. 2014; Khan et al. 2020a, b).

Plants counter oxidative stress through the inherent antioxidative defense system which is operated through a complex network of non-enzymes and enzymes. The enzymatic antioxidant superoxide dismutase (SOD) provides first line of defense that converts superoxide (O₂^{•-}) to hydrogen peroxide (H₂O₂). At lower concentration H₂O₂ participates in signaling cascade and contributes to the defense mechanisms (Hasanuzzaman et al. 2020), whereas H₂O₂ becomes phytotoxic when its increasing level

crosses a threshold (Khan et al. 2020a, b; Siddiqui et al. 2020). However, to control H_2O_2 level, plants possess a set of enzymes such as peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). These enzymes convert H_2O_2 into water and molecular oxygen. In spite of an array of defense strategies, survival of plants depends on the activation of defense system prior to the commencement of damage. In this regard, plants possess a dedicated signaling network that assures well-timed activation of the defense system under any stressful condition.

In the last decade of investigations in plants, hydrogen sulfide (H_2S) has emerged as an endogenous gaseous transmitter and one of the key players of the signaling network. In plants, H_2S is synthesized in a cysteine (Cys) degradation reaction catalyzed by L-cysteine desulfhydrase (LCD, EC 4.4.1.1) and D-cysteine desulfhydrase (DCD; EC 4.4.1.15). Hydrogen sulfide regulates key processes of plants including seed germination, organogenesis, stomatal movement, and photosynthesis (Arif et al. 2020; Khan et al. 2017, 2018, 2020). In addition, H_2S mediates responses to various abiotic stresses and regulates oxidative and ionic homeostasis and enzyme activities during abiotic stress tolerance in plants (Arif et al. 2020; Khan et al. 2017, 2018, 2020a; Mukherjee and Bhatla 2020). Although interaction of H_2S with other signaling molecules, phytohormones, and second messengers has been well studied (Khan et al. 2020a, b; Mukherjee and Bhatla 2020) but no substantial information is available on the role of H_2S and K crosstalk in plant adaptive responses to abiotic stresses. Therefore, present investigation aims to comprehend the significance of K in the regulation of endogenous H_2S homeostasis during tolerance of plants to osmotic stress. Role of endogenous H_2S in K homeostasis and their crosstalk in the regulation of carbohydrate metabolism and antioxidative defense system was also elucidated.

2. Materials And Methods

2.1. Seed germination and treatments

Role of K and H_2S under osmotic stress conditions was tested in tomato (*Solanum lycopersicum* L. Mill.) seedlings. Healthy and uniform seeds of tomato were surface sterilized with 10% sodium hypochlorite solution for 10 min. followed by three washings with double deionized water (DDW). The sterilized seeds were germinated in filter paper-lined Petri dishes containing Raoult's nutrient solution for 10 days prior to the treatment. The Petri dishes were kept in the dark at average day/night temperature of $26/15 \pm 3$ °C for 8 days. After 8 days, germinated seeds were transferred to the new filter paper-lined Petri dishes containing: (1) DDW (Control), (2) 5 mM K_2CO_3 (K), (3) 15% (w/v) polyethylene glycol 8000 (PEG), (4) K+PEG, (5) K+PEG+1 mM hypotaurine (K+PEG+HT), (6) K+PEG+20 mM tetraethylammonium chloride (K+PEG+TEA), and (7) K+PEG+TEA+HT. The seedlings were treated for 24 h and each treatment was replicated three times. After 24 h of treatments, the seedling leaves were used for the estimation of various characteristics. Osmotic stress was induced by PEG, whereas TEA was used as K-channel blocker and HT as H_2S scavenger.

To evaluate the K transport mechanism, 8-days old seedlings, raised with nutrient solution, were treated with 20 mM TEA or 500 μ M sodium orthovanadate (SOV, PM H⁺-ATPase inhibitor) for 30 min (Zhao et al., 2018). After 30 min. the seedlings were subjected to 15% (w/v) PEG for 24 h, in the presence or absence of 5 mM K. After 24 h, concentration of K was estimated in the leaves of the seedlings (Fig. 2 A and B).

2.2. Crude enzyme extract preparation

A crude extract was prepared from the leaves (0.5 g) of treated tomato seedling. The extract was used for the assay of enzymes activity. Protein quantification was carried out according to Bradford (1976) using Bovine Serum Albumin (BSA) as a standard.

2.3. Estimation of K content and plasma membrane H⁺-ATPase activity

Concentration of K in the leaves was estimated using flame photometer. The leaves were dried for 72 h at 75 °C followed by grinding to fine powder. 1 g of the powder was placed in a furnace at 500 °C to get ash which was dissolved in 5 mL HCl (20%). The solution was diluted to 50 mL using DDW and was used for the estimation of K content.

Activity of plasma membrane H⁺-ATPase (PM H⁺-ATPase) was measured by the method of Hejl and Koster (2004) with slight modification (Majumdar and Kar 2018). The enzyme activity of PM H⁺-ATPase was determined by quantifying the production of inorganic phosphate at the wavelength of 700 nm.

2.4 Estimation of starch, sucrose, and total soluble carbohydrates

Starch was quantified spectrophotometrically at 620 nm by adopting the method of Kuai et al. (2014). Soluble sugar and sucrose content were also determined spectrophotometrically at 620 nm and 480 nm, respectively by adopting the modified method of Xu et al. (2015).

2.5 Estimation of the activity of carbohydrate metabolizing enzymes

Activity of α -amylase (EC 3.2.1.1), β -amylase (EC 3.2.1.2) was measured by the method of Li et al. (2013). Assay of sucrose phosphate synthase (SPS; EC 2.4.1.14), sucrose synthase (SuSy; EC 2.4.1.13), and soluble acid invertase (SAI) was performed by adopting the method of Kalwade and Devarumath (2013).

2.6. Estimation of Cys, H₂S content and activity of their biosynthesizing enzymes

Quantitative analysis of Cys was carried out by the method of Gaitonde (1967). Activity Cys-biosynthesizing enzymes, O-acetylserine (thiol) lyase (OAS-TL; EC 2.5.1.47) and serine acetyltransferase (SAT; EC 2.2.1.30) was measured according to Riemenschneider et al. (2005a) and Nakamura et al. (1987), respectively. Quantification of H₂S was performed by the method of Li (2015). The activity of L-cysteine desulphydrase (LCD; EC 4.4.1.1) and D-cysteine desulphydrase (DCD; EC 4.4.1.15) was estimated by the method of Bloem et al. (2004) and Riemenschneider et al. (2005b), respectively.

2.7. Determination of oxidative stress markers

Effect of drought and other treatments on oxidative stress was evaluated by estimating the level of $O_2^{\cdot-}$ and H_2O_2 according to the method of Elstner and Heupel (1976) and Velikova et al. (2000), respectively.

Effect of treatments on oxidative stress-induced damage was assessed by estimating electrolyte leakage (ELKG) according to Lutts et al. (1995) and lipid peroxidation by measuring TBARS (Cakmak and Horst 1991). The detailed method was adopted from Khan et al. (2020b). Relative water content (RWC) was also measured according to Yamasaki and Dillenburg (1999) using fresh weight, dry weight, and turgid weight of the roots (Khan et al., 2020b). Moreover, rate of water loss was also determined periodically from 1 h to 6 h as per the method explained by Khan et al. (2018).

2.8. Antioxidant enzymes assay

Enzyme activity of SOD (EC 1.15.1.1), POX (EC 1.11.1.7), and CAT (EC 1.11.1.6) was estimated according to Upadhyaya et al. (1985), Beauchamp and Fridovich (1971), and Cakmak and Marschner (1992), respectively. Activity of APX (EC 1.11.1.11) was measured by the method of Nakano and Asada (1981). Method of Foyer and Halliwell (1976) was used for the estimation of GR (EC 1.6.4.2) activity.

2.9. Estimation of chlorophyll (Chl) content, activity of Chl metabolizing enzymes, and carbonic anhydrase

Concentration of Chl content ($mg\ g^{-1}$ leaf DW) in the leaves of treated seedlings was estimated by the method of Lichtenthaler and Buschmann (2001). The activity of δ -aminolevulinic acid dehydratase (δ -ALAD) was determined according to Jain and Gadre (2004). Chlorophyllase (Chlase) activity was determined by the method of McFeeters et al. (1971) and Fang et al. (1998).

2.10. Data analysis

One-way analysis of variance (ANOVA) was applied for statistical analysis of the data. The values were expressed as means \pm standard error of three independent replicates. Differences between treatment means were compared statistically using Duncan's multiple range test (DMRT) at $p < 0.05$ by SPSS Ver. 20 statistical software (SPSS Inc., Chicago, IL, USA)

3. Results And Discussion

3.1. Endogenous K homeostasis and H^+ -ATPase activity in tomato leaves is regulated by endogenous H_2S signaling during PEG-induced osmotic stress

Tomato seedlings (8d old) were subjected to 15% (w/v) PEG treatment in the presence and absence of exogenous 5 mM K_2CO_3 (K). Osmotic stress-induced changes in endogenous K content coincides with the modulation of H^+ -ATPase activity. In the present work PEG-induced osmotic stress significantly reduces K

content (45.8%) which corresponds to a concomitant reduction in the activity of H^+ -ATPase (19.1%) in comparison with their respective controls (Fig. 1 A and B). In order to investigate the role of endogenous H_2S on the modulation of K content, tomato seedlings were also treated with 20 mM TEA (K channel blocker) and 1 mM HT (H_2S scavenger). Although exogenous K treatment partially recovered PEG-induced decrease in endogenous K content. However, HT and TEA application (separate or in combination) reduced the K content (Fig. 1 A). A similar trend of reduction in the H^+ -ATPase activity was observed in the presence of exogenous HT and TEA. Furthermore, treatment with sodium orthovanadate (SOV, PM H^+ -ATPase inhibitor) resulted in decreased K content in leaves of tomato seedlings treated with PEG and K (Fig. 2 A). Treatment with TEA exhibits different trends in the levels of K content in the presence of PEG and PEG+K. TEA treatment increased K content in the presence of PEG application (PEG) which, however, decreased in the presence of PEG+K treatment (Fig. 2 B). It indicates that TEA substantially inhibited K efflux in the stressed seedlings that did not receive exogenous K. However, TEA did not significantly inhibited K efflux in the stressed seedlings subjected to K treatment (Fig. 1 A, 2 B). This observation indicates that under K supplementation, H^+ -ATPase activity regulates endogenous K content in tomato seedlings leaves subjected to osmotic stress (Fig. 1 B, 2 A).

Drought stress significantly affects ion uptake mechanisms in plants including K (Asif et al. 2017; Ahmad et al. 2018). Reduction in the leaf water potential is usually associated with an alteration in K content during drought stress (Gunes et al. 2007). Among the various effects of drought stress, reduced water availability in roots affects membrane permeability and reduces active transport of nutrients which are attributed to reduced levels of K in plant organs (Ahmad et al. 2018). Drought stress is known to promote the expression of high affinity K transporter (OsHAK1) in rice thus improving K acquisition in the plant (Chen et al. 2017). K homeostasis in mesophyll cells of drought stressed tea plants have been reported to be mediated by the activity of H^+ -ATPase (Zhang et al. 2018). In comparison with PEG treatment, endogenous K content exhibits 69.1% increase in the presence of PEG+K treatment which also corresponds to a 41.6% increase in the H^+ -ATPase activity (Fig. 1 A, B). Thus, exogenous K supplementation during PEG-induced osmotic stress is likely to normalize H^+ -ATPase activity (Palmgren 2001) thus increasing K content in leaves of drought stressed tomato seedlings. H^+ -ATPase activity in the plasma membrane is crucial for maintaining K uptake which is mediated by secondary transport mechanisms (K transporters or H^+/K^+ symporters) (Shabala 2017; Khan et al. 2020b). In the present work, reduction in the H^+ -ATPase activity during PEG treatment likely results in net H^+ -influx and subsequent membrane depolarization. Thus, reduction in K content during PEG treatment might be resulting from depolarization of membrane (Wang et al. 2015) that activated K-efflux systems across the membranes. Exogenous K supplementation alleviated the PEG-induced K loss by activating H^+ -ATPase activity (Palmgren 2001) in the leaves (Fig. 1 A, B). Furthermore, H^+ -ATPase dependent K accumulation was reversed upon treatment with SOV which resulted in a reduction in K content (Fig. 2 A). Exogenous K-induced increment in endogenous K content was also evident to be mediated by H^+ -ATPase activity, as shown by subsequent reduction in K content in the presence of SOV treatment (Fig. 2A).

It is note-worthy that treatment with HT (endogenous H₂S scavenger) resulted in a reduction in the endogenous K content (19.5%) and H⁺-ATPase activity (42.1%) in comparison with the stressed seedlings treated with K only (PEG+K) (Fig. 1 A, B). Thus, H⁺-ATPase dependent modulation of K content during osmotic stress is evident to be operative through endogenous H₂S signaling. Reduction in endogenous H₂S content brought about by HT treatment down regulates H⁺-ATPase activity and reduces K content as a downstream signaling event. It is evident that H₂S treatment exerts promotive effects manifested by the upregulation of gene expression and phosphorylation of plasma membrane associated H⁺-ATPase activity in Arabidopsis (Li et al. 2014). The increase in K content due to H₂S signaling is attributed to the upregulation of Na/K antiport system being energized by the activity of H⁺-ATPase (Khan et al., 2020b). H₂S is a positive regulator of K retention and mediates ion homeostasis during salinity stress (Mostofa et al. 2015; Deng et al. 2016). *The present evidence reveal that endogenous H₂S modulates H⁺-ATPase dependent-K content in leaves of stressed tomato seedlings. Furthermore, exogenous K-mediated alleviation of induced osmotic stress and partial recovery in endogenous K content is also mediated by the endogenous H₂S (Fig. 10).*

3.2. K and endogenous H₂S exert additive effects in modulating sugar metabolism during osmotic stress

Exogenous K treatment led to an increase in starch (13.3%) and sucrose (66.4%) content in tomato seedling leaves in comparison with their control (Fig. 3 A, B). However, osmotic stress being induced by PEG treatment resulted in decreased starch content accompanied by increase in sucrose level (Fig. 3 A, B). Exogenous K supplementation to PEG treatment compensates PEG-induced reduction in starch content also followed by a reduction in sucrose content. However, HT and TEA treatment reduce both starch and sucrose content in comparison with their controls. Total soluble sugars (TSS) exhibit marginal increase in the presence of K which, however, decreases during osmotic stress induced by PEG treatment (46.8%). Treatments with HT and TEA reversed the effect of K-mediated increase in TSS content during osmotic stress. Changes in starch and sucrose content correlate with variations in the activity of enzymes associated with starch hydrolysis (α -amylase, β -amylase), sucrose biosynthesis (SPS), and sucrose hydrolysis (SuSy and SAI) (Fig. 4 A-E). Apart from its role as a storage molecule, starch level is known to exhibit dynamic changes during abiotic stress thus indicating its involvement in metabolic fitness of plants (Thalmann and Santelia 2017). Starch degradation during abiotic stress is associated with improved osmotic tolerance attained by higher sugar accumulation. Drought resistant variety of broad bean (*Phaseolus vulgaris*) has been reported to undergo higher starch breakdown in comparison with the sensitive strains (González-Cruz and Pastenes 2012). Starch hydrolysing enzymes (α -amylase, β -amylase) undergo upregulation in the presence of K+PEG treatment which also get reduced upon HT and TEA treatment. Similar trend was observed for the enzymes associated with sucrose metabolism. Thus, K and endogenous H₂S appear to positively upregulate carbohydrate metabolism in drought stressed tomato leaves (Fig. 10).

Source tissues like leaves undergo a precise control of starch-sugar inter-conversion during abiotic stress signals (Thitisaksakul et al. 2017; Thalmann and Santelia 2017). PEG-induced osmotic stress in tomato

leaves results in decreased starch content (18.3%) in comparison with the control (Fig. 3A). However, in the presence of K and K+ PEG treatment a recovery in the starch content has been observed (Fig. 3 A). Interestingly, higher endogenous K content (Fig. 1A) correlates with higher retention of starch during PEG-induced osmotic stress. K concentrations are likely to improve the osmotic potential of cells thus compensating the need of higher osmolyte accumulation (Fig. 10). Increased degradation of starch in osmotic-stressed tomato leaves coincides with higher sucrose accumulation (Fig. 3B). A surge in starch catabolism is observed during PEG-induced osmotic stress and exogenous K application which is evident by the increased activity of α -amylase and β -amylase in tomato leaves (Fig. 4 A, B). K appears to be a positive regulator of α -amylase and β -amylase enzymes (Hasanuzzaman et al. 2018) thus upregulating their activity in the presence of PEG-induced osmotic stress (Fig. 10). Water stress in cucumber cotyledons is known to induce starch degradation which is associated with increased β -amylase activity (Todaka et al. 2000). In the present work, increased activity of α -amylase and β -amylase enzymes corroborate the fact that PEG-induced sucrose accumulation possibly results from increased starch degradation. Leaves are the primary source organs which accumulate sucrose and starch as the major forms of photosynthates. However, K application during osmotic stress (PEG) exert differential effects to starch and sucrose content (Fig. 3A and B). However, total soluble sugar (TSS) content exhibits a reduction in the presence of PEG (40.3%) which, however, is increased upon K supplementation (Fig. 3 C). Interestingly, reduced TSS content in tomato leaves (Fig. 3 C) is likely attributed to increased long distance transport of solutes from leaves to other plant organs during osmotic stress (Cramer et al. 2007). Source-sink relations involve precise modulation in the mobilization of sugar assimilates during drought stress (Lemoine et al. 2013). Increased K content (exogenous) partially compensates the effect of osmotic stress that might result in a higher retention of TSS in leaves (Fig. 3 C).

Sucrose metabolism during osmotic stress and K supplementation has been analyzed by investigations on the activity of sucrose phosphate synthase (SPS), sucrose synthase (SuSy) and soluble acid invertase (SAI) activity (Fig. 4 C, D, E). Interestingly, SPS and SuSy/SAI activity exhibit opposite trends in the presence of K and PEG treatments. K and PEG treatment resulted in 11.6 % and 35.0 % increment in SPS activity respectively which all the more increased 65.0% in the presence of PEG+K treatment as compared with control (Fig. 4 C). This signifies the role of K and PEG in upregulating sucrose biosynthesis catalysed by the activity of SPS. SuSy activity exhibits 43.9% increase in the presence of K treatment, which, however, decreases by 26.5% in the presence of PEG as compared with the control (Fig. 4 D). However, K supplementation of stressed seedlings (K+PEG) improved SuSy activity by 63.0% than the stressed seedlings raised without K supply (PEG). Increased endogenous K content promotes sucrose hydrolysis in tomato leaves which is evident from increased SuSy and SAI activity. Higher accumulation of sucrose in PEG-treated tomato leaves corresponds with reduced sucrose hydrolysis (SuSy/SAI activity) and increased sucrose biosynthesis (SPS activity) (Fig. 3B, Fig. 4 C, D, E). Although various sucrose cleaving enzymes act differently during abiotic stress, SuSY and SAI activity during drought stress pertains to higher accumulation of hexose sugars (Pinheiro et al. 2001; Praxedes et al. 2006). Drought stress is known to enhance sugar metabolism by increasing the activity of SPS and SuSy in roots of soybean

seedlings (Du et al. 2020). K application has been known to upregulate sugar metabolism by enhancing the activity of SPS, SuSy and SAI in water stressed cotton plants (Zahoor et al. 2017b).

Interestingly, in the present work, HT and TEA treatment reduced starch, sucrose and TSS content (Fig. 3 A-C) followed by down-regulation in the activity of starch and sucrose metabolizing enzymes (Fig. 4 A-E). Fewer reports are present to elucidate the regulatory role of H₂S in modulation of carbohydrate metabolism during drought stress. Proteomic analysis by Jiang et al. (2020) deciphers the role of H₂S in regulating various enzymes associated with carbohydrate metabolism in salt-stressed cucumber leaves. *Present findings reveal that K and endogenous H₂S appear to exert additive effects in the positive regulation of carbohydrate metabolism in leaves of osmotic-stressed tomato seedlings (Fig. 10).*

3.3. K regulates endogenous H₂S homeostasis during osmotic stress in tomato leaves

Cysteine functions as the major precursor for H₂S biosynthesis in plant cells where the reaction is catalyzed by the activity of cysteine desulfhydrases (LCD or DCD). In order to investigate the possible role of K in regulating endogenous Cys and H₂S biosynthesis, drought stressed tomato leaves were subjected to enzymatic assay for SAT, OASTL, LCD and DCD. PEG-induced osmotic stress upregulated SAT and OASTL activity which all the more increased in the presence of K supplementation (K+PEG) (Fig. 5 A-B). A similar trend was observed in the activity of LCD and DCD under PEG induced stress (Fig. 6 A) The increase in activity of the enzymes correlates with increased Cys and H₂S content in the presence of K and PEG treatment (Fig. 5 C, 6 B). It is noteworthy that PEG-induced osmotic stress upregulated H₂S biosynthesis in leaves of tomato seedlings. L-cysteine desulhydrase (LCD) activity was observed to be more abundant in comparison with D-cysteine desulhydrase activity (DCD) in leaves of tomato seedlings (Fig. 6 A, B). However, both the enzymes showed upregulation in the presence of K and PEG treatment. Interestingly, HT and TEA treatment downregulated both Cys and H₂S biosynthesis during PEG-induced osmotic stress in tomato leaves (Fig. 5A-C; Fig. 6 A and B). It is noteworthy that PEG-induced osmotic stress upregulated H₂S biosynthesis in leaves of tomato seedlings. L-cysteine desulhydrase (LCD) activity was observed to be more abundant in comparison with D-cysteine desulhydrase activity (DCD) in leaves of tomato seedlings (Fig. 6 A, B). However, both the enzymes showed upregulation in the presence of K and PEG treatment.

Exogenous H₂S is known to upregulate both Cys and H₂S biosynthesis during water deficit in *Eruca sativa* (Khan et al. 2018). The activity of OASTL, LCD, and DCD were reported to be upregulated in the presence of exogenous NaHS (H₂S donor) where a surge in Cys and H₂S accumulation was observed (Khan et al. 2018). OASTL and LCD function as crucial enzymes for Cys homeostasis in Arabidopsis (Álvarez et al. 2010). Recent investigations reveal the role of LCD and H₂S homeostasis to be associated with salinity tolerance and regulation of electrolytic leakage in *Zea mays* and *Solanum lycopersicum* (Kaya et al. 2019; Mukherjee and Bhatla 2020). Among the various roles of LCD and H₂S during abiotic stress, they are known to be associated with the evocation of glutathione-mediated antioxidative defense, hormonal signaling and silicon-induced signaling (Kaya et al. 2020a, b, c; Mukherjee and Corpas 2020). In

the present work in tomato leaves H_2S content has been observed to steadily increase as a function of both LCD and DCD activity (Fig. 6B). Tissue levels of H_2S as a signaling molecule has been reported to be increased up to 2.5-fold in response to various biotic and abiotic stress (Fang et al. 2014; Khan et al. 2020a). Upregulation of LCD/DCD activity and associated H_2S levels are suggestive of their possible role in ROS homeostasis and drought stress tolerance (Kaya et al. 2020a, b). Recent evidence has provided significant insights to the antioxidative role of H_2S as a priming and anti-senescence molecule for alleviation of drought and heavy metal stress in plants (Jin et al. 2018; Khan et al. 2020a; Zhou et al. 2020). Leaves of tomato seedlings subjected to drought stress exhibit higher accumulation of H_2S (Fig. 6 B) which is possibly attributed to its downstream functional regulation of redox homeostasis and senescence control during drought stress. *Present findings therefore reveal that H_2S homeostasis is crucially regulated by K, endogenous Cys metabolism, and H_2S levels during PEG-induced osmotic stress in tomato leaves (Fig. 10).*

3.4. K and H_2S modulate ROS homeostasis and enzymatic antioxidative defense in osmotic stressed-tomato leaves

Drought stress (PEG) in tomato seedling leaves resulted in increased $O_2^{\bullet-}$ and H_2O_2 content which was, however, observed to be depleted during PEG+K treatment (Fig. 7 A and B). Furthermore, exogenous K-mediated decrease in ROS levels undergo surge in the presence of HT and TEA treatments. The increased ROS levels in the presence of PEG, HT, and TEA correlate to modulation in the activity of antioxidative enzymes (SOD, POX, CAT, APX and GR) (Fig. 9 A-D). Osmotic stress induced by PEG treatment leads to a significant increase in H_2O_2 and $O_2^{\bullet-}$ content in comparison with their control (Fig. 7A and B) (Khan et al. 2017; Liu et al. 2019). However, exogenous K compensates the impact of drought stress, thus reducing the levels of free radical generation. Although the treatments with HT and TEA (separately) increased the levels of ROS, their treatment in combination (K+PEG+HT+TEA) led to a note-worthy increase in $O_2^{\bullet-}$ and H_2O_2 levels in tomato leaves. Osmotic stress in tomato leaves resulted in increased lipid peroxidation (TBARS) which all the more increases in the presence of TEA and HT in combination (Fig. 7 C). Thus, the extent of lipid peroxidation during osmotic stress correlates with the increased levels of $O_2^{\bullet-}$ and H_2O_2 generated during PEG-induced osmotic stress. Furthermore, interesting correlations are obtained among endogenous K (Fig. 1A), H_2S level (Fig. 6 B) and ROS generation during PEG-induced osmotic stress. High levels of ROS generated during TEA and HT treatment signify the beneficial role of K and H_2S in regulating ROS levels during osmotic stress. Various investigations reveal the beneficial role of H_2S signaling in regulating the antioxidant state during drought and other abiotic stress (Ahmad et al. 2020b; Khan et al. 2017, 2020a, b). Present work reveals K and endogenous H_2S -mediated regulation of antioxidative enzymes during osmotic stress in tomato leaves (Fig. 9 A-D). Osmotic stress (PEG) upregulates the activity of SOD, POX, CAT, APX and GR, which, however, gets reduced in the presence of HT and TEA treatment. Thus, endogenous K and H_2S positively upregulate the activity of the antioxidative enzymes. SOD catalyzes the dismutation of superoxide ions to form H_2O_2 . Potassium supplementation during drought stress reduces superoxide levels (Fig. 7 B) which correspond to the

higher activity of SOD (Fig. 9 A). Furthermore, PEG, K and H₂S-mediated changes in H₂O₂ levels exhibit inverse correlation with POX, CAT, and APX activity (Fig. 9 A and B). Interestingly, all the antioxidative enzymes analyzed in the present work (SOD, POX, CAT, APX and GR) exhibit highest activity in the presence of PEG+K treatment, which, however, gets reduced in the presence of HT and TEA (Fig. 9 A-D). Thus, K-mediated alleviation of osmotic stress accompanies a surge in the H₂S biosynthesis and mediates a H₂S-dependent induction of antioxidative defense. H₂S is known to modulate the activity of SOD, CAT, POD, APX and GR in plants subjected to abiotic stress (Ahmad et al. 2020b; Khan et al. 2017; Khan et al. 2020a, b), wherein H₂S also functions as an important regulator of glutathione-ascorbate cycle (Kaya et al. 2020a; Khan et al. 2018, 2020a, b). Glutathione reductase catalyzes the conversion of GSSG into GSH which is crucial in maintaining tolerance to oxidative stress. Present work reveals an elevation in GR activity (PEG+K) (Fig. 9D) thus signifying higher accumulation of GSH in leaves of drought stressed tomato seedlings. Enhanced levels of GSH along with H₂S has been reported to be effective in imparting tolerance to oxidative stress (Kaya et al. 2020a; Khan et al. 2018, 2020a, b). *Our findings are indicative of the fact that endogenous K functions as a positive regulator of H₂S-mediated antioxidative defense against osmotic stress (Fig. 10).*

3.5. K mediated-H₂S signaling reduces osmotic stress and leaf water loss

Analysis of relative water content (RWC) and electrolytic leakage (ELKG) in osmotic-stressed tomato leaves reveal variations associated with PEG, HT, and TEA treatment. Osmotic stress (PEG) increases ELKG followed by reduced RWC (Fig. 8 A) in tomato leaves. Electrolytic leakage from the osmotic-stressed leaves is further enhanced in the presence of HT and TEA treatment (Fig. 8A). Increased ELKG correlates with reduced RWC in the presence of PEG and HT/TEA treatment in tomato leave (Fig. 8A). Exogenous K appears beneficial in alleviating the effect of osmotic stress by causing increase in RWC and subsequent reduction in ELKG. Furthermore, HT treatment reveals the role of endogenous H₂S in regulation of ELKG. Leaf water loss exhibits increase in the presence of PEG, HT, and TEA treatment with maximum increase at 6 h after osmotic stress (Fig. 8 B).

Membrane permeability and ion homeostasis are crucial for imparting osmotic tolerance to plants. Drought stress in general reduces RWC in plant tissues (Khan et al. 2017, 2018; Chen et al. 2016). H₂S exerts beneficial role in increasing RWC accompanied by increase in osmoprotectants in *Spinacia* seedlings (Chen et al., 2016). In the present work, K and H₂S upregulate osmotic tolerance (RWC, sucrose content) in a synergistic manner (Fig. 3B, Fig. 8 A). Furthermore, analysis of leaf water loss in the presence of K and H₂S treatment during osmotic stress reveals its temporal increase from 1-6h in the presence of PEG, HT and TEA treatment (Fig. 8 B). K and H₂S are likely to impart beneficial role in regulating stomatal movement during drought stress which is attributed to the reduction in water loss in K or K+PEG treated seedling leaves. H₂S-mediated stomatal closure during water stress is attributed to the evocation of ABA biosynthesis and its signaling in guard cells (Chen et al. 2020; Zhou et al. 2020). *Thus, it is advocated that K and H₂S signaling orchestrate osmotic tolerance, sucrose accumulation, stomatal conductance and hydroactive stomatal closure in drought stressed leaves (Fig. 10).*

3.6. K and H₂S regulate chlorophyll metabolism and carbonic anhydrase activity in osmotic-stressed tomato leaves

Chlorophyll metabolism (in the presence of K, PEG, HT, and TEA treatments) exhibits precise regulation by endogenous K and H₂S in drought stressed tomato leaves. PEG treatment reduces chlorophyll content (49.6%) of tomato leaves accompanied by reduction (32.9%) in the activity of δ -ALAD (chlorophyll precursor biosynthesizing enzyme) (Table 1) which is, however, reversed by HT and TEA treatments. Chlorophyllase activity shows upregulation in the presence of PEG which even more increases in the presence of HT and TEA (Table 1). CA activity is downregulated during osmotic stress which, however, gets recovered in the presence of PEG+K (Table 1).

Drought stress is known to reduce Chl biosynthesis in plants (Chen et al. 2015; Meher et al. 2018; Siddiqui et al. 2015). PEG-induced osmotic stress instigates ROS levels, increases lipid peroxidation (Fig. 7 A-C) which is accompanied by a significant decrease in Chl content and reduced activity of Chl-metabolizing enzymes (δ -ALAD) (Table 1). Potassium supplementation during PEG-induced osmotic stress partially recovers chl content and δ -ALAD activity. Thus, delayed senescence due to K supplementation in osmotic-stressed leaves are likely to maintain optimum photosynthesis and carbon assimilation in cells. δ -ALAD activity is responsible for synthesis of Chl precursor in leaves. Furthermore, chl catabolizing enzyme Chlas gets increased in the presence of PEG treatment and all the more in the presence of HT and TEA (Table 1). Evidence by Jin et al. (2018) reveal H₂S-mediated regulation of senescence associated gene expression and delay in senescence in drought stressed-Arabidopsis. Endogenous K and H₂S levels are positive regulators of Chl metabolism during osmotic stress in tomato leaves (Table 1).

Carbonic anhydrase (CA) activity is crucial for maintaining dissolved CO₂ at the carboxylation site of RUBISCO present in mesophyll cells (Gruber and Feiz 2018). Moreover, CA activity serves as a marker for stress which responds to external environmental stimulus in plants (Khan et al. 2018). CA activity is essential to maintain calvin cycle, stomatal conductance thus enabling CO₂ diffusion across guard cells. Water stress negatively affects CA activity in mesophyll cells thus causing subsequent reduction in photosynthetic assimilation (Król and Weidner 2017). Present work reveals PEG-induced reduction in the activity of CA which is, however, recovered by exogenous K supplementation (Table 1). Moreover, K appears to function as a significant positive regulator of CA activity in the presence of PEG treatment where CA activity is significantly enhanced (70.5%) in comparison with the stressed seedlings (PEG). Endogenous K and H₂S positively upregulate the activity of CA, where treatment with TEA+HT causes 68.0% reduction in CA activity than K+PEG (Table 1). CA activity is essential to protect the function of RUBISCO and regulate stomatal closure during drought stress (Gruber and Feiz 2018). Endogenous K and sucrose levels (present work) are likely to regulate osmotic balance in mesophyll cells of stressed tomato leaves. Thus, alleviation of osmotic stress is associated with increased CA activity which is expected to improve stomatal conductance and CO₂ diffusion thus maintaining optimum carbon assimilation. In this context it is noteworthy that endogenous H₂S is crucial for maintaining optimum CA

activity during osmotic stress. *Thus, K and H₂S-mediated drought tolerance and regulation of photosynthetic assimilation is accompanied by modulation of CA activity in tomato leaves.*

3.7. K and H₂S signaling exhibit positive correlations in orchestration of long-distance osmotic stress tolerance in tomato leaves

A significant positive relationship is observed among endogenous H₂S content, K and H⁺-ATPase activity in the presence of PEG-induced drought stress in tomato seedling leaves (Fig. 1 A and B, Fig. 6B). Interestingly, increased H⁺-ATPase activity positively upregulates secondary transport of K in leaves which is further accompanied by increased sucrose accumulation, higher RWC and elevated starch degradation (Fig. 10). In this context exogenous supplementation of K appears to positively upregulates Cys and H₂S biosynthesis (SAT, OASTL, LCD, DCD) in osmotic-stressed tomato leaves. Thus, improved accumulation of sucrose is indicative of restored photosynthetic assimilation which correlates with retention of chlorophyll, δ -ALAD activity and reduced Chlase activity under osmotic stress. Reduced TSS under osmotic-stressed leaves are indicative of reallocation of organic nutrients from source to sink organs. However, K and H₂S tend to retain TSS in the leaves of stressed tomato seedlings. A reduction in the ROS levels is observed in the presence of K supplementation during osmotic stress which correlates with altered activity of antioxidative enzymes. Thus, K transport and endogenous H₂S homeostasis associate with H⁺-ATPase activity to improve carbohydrate metabolism and promote tolerance to osmotic stress. *To sum up, present evidence reveals the synergistic role of K and H₂S in regulating carbohydrate metabolism and osmotic stress tolerance (Fig. 10).*

4. Conclusions

Results of the study show that osmotic stress-induced reduction in leaf K content was associated with the reduction in H⁺-ATPase activity. Also, osmotic stress adversely affected carbohydrate and chlorophyll metabolism in tomato seedlings. Whereas antioxidative defense system and H₂S metabolism was activated by osmotic stress. Contrastingly, generation of reactive oxygen species was enhanced with an associated increase in lipid peroxidation, rate of water loss and decreased RWC. It indicates that activated defense system of stressed plants failed to counter osmotic stress. However, exogenous application of K enhanced endogenous K content which further elevated the accumulation of soluble carbohydrates and causes activation of antioxidative defense system to a level required to counter osmotic and oxidative stress. Cumulative effect of these processes led to the osmotic adjustment and reduction in ROS generation coupled with reduced water loss, improved RWC and reduced peroxidation of lipids. Use of SOV (H⁺-ATPase inhibitor) and TEA (K channel blocker) indicates that increase in endogenous K content in K supplemented seedlings was carried out by H⁺-ATPase. Reduction in H⁺-ATPase activity by H₂S scavenger HT suggests the involvement of endogenous H₂S in the activation of H⁺-ATPase activity that improved K influx mechanism as shown by increased K content in osmotic-

stressed tomato seedling leaves. *To sum up, present evidence provides insights into the role of K and H₂S signaling in modulation of osmotic stress tolerance in tomato seedlings*

Declarations

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Author contribution statement:

M.H.S. S.M. and S.A. designed the experiments, and wrote the manuscript. , A.A.A, Q.D.A., B.M.A.M, and H.M.A participated in the experiments. Y.H., S.A. and M.H.S analyzed the data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest:

The authors declare that they have no conflict of interest.

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Table

Table 1. Effect of various treatments on chlorophyll metabolism and carbonic anhydrase activity in the leaves of tomato. Chlorophyll (Chl) content (mg g⁻¹ DW), δ-aminolevulinic acid dehydratase (δ-ALAD) activity (Units g⁻¹ DW), chlorophyllase (Chlase) activity (n mol chl^{ide} g⁻¹ DW), and carbonic anhydrase (CA) activity (μ CO₂ kg⁻¹ DW s⁻¹).

Treatments	Parameters			
	Chl content	δ-ALAD activity	Chlase activity	CA activity
Control	9.27±0.19 ^{ab}	110.46±5.39 ^b	8.51±0.56 ^{fg}	1468.37±44.67 ^{bc}
K	9.85±0.28 ^a	128.51±3.61 ^a	6.42±0.39 ^h	1563.79±51.32 ^b
PEG	4.67±0.35 ^d	74.08±7.82 ^g	11.58±1.20 ^{de}	1165.52±88.69 ^d
K+PEG	7.58±0.10 ^c	108.44±2.81 ^{bc}	9.36±0.29 ^f	1987.65±66.00 ^a
K+PEG+TEA	3.86±0.26 ^e	96.07±6.49 ^d	11.74±0.69 ^d	935.65±44.87 ^e
K+PEG+HT	3.09±0.35 ^f	88.72±2.88 ^{de}	13.61±0.35 ^{bc}	817.05±47.32 ^f
K+PEG+TEA+HT	2.64±0.11 ^g	84.31±4.77 ^{ef}	14.88±0.47 ^a	634.82±71.98 ^g

Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT). Double deionized water (control), 5 mM K_2CO_3 (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K^+ channel blocker), and 1 mM hypotaurine (HT: H_2S scavenger).

Figures

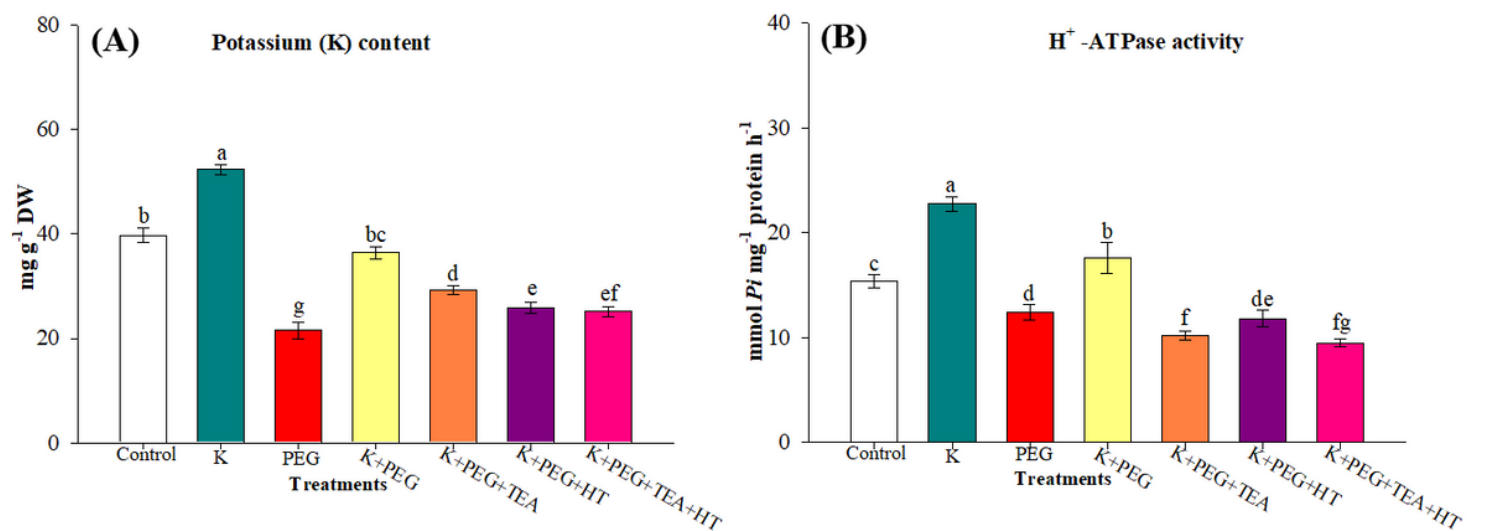


Figure 1

Effect of various treatments on (A) Leaf potassium (K) content and (B) and H^+ -ATPase activity in tomato seedling leaves treated with double deionized water (control), 5 mM K_2CO_3 (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K^+ channel blocker), and 1 mM hypotaurine (HT: H_2S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

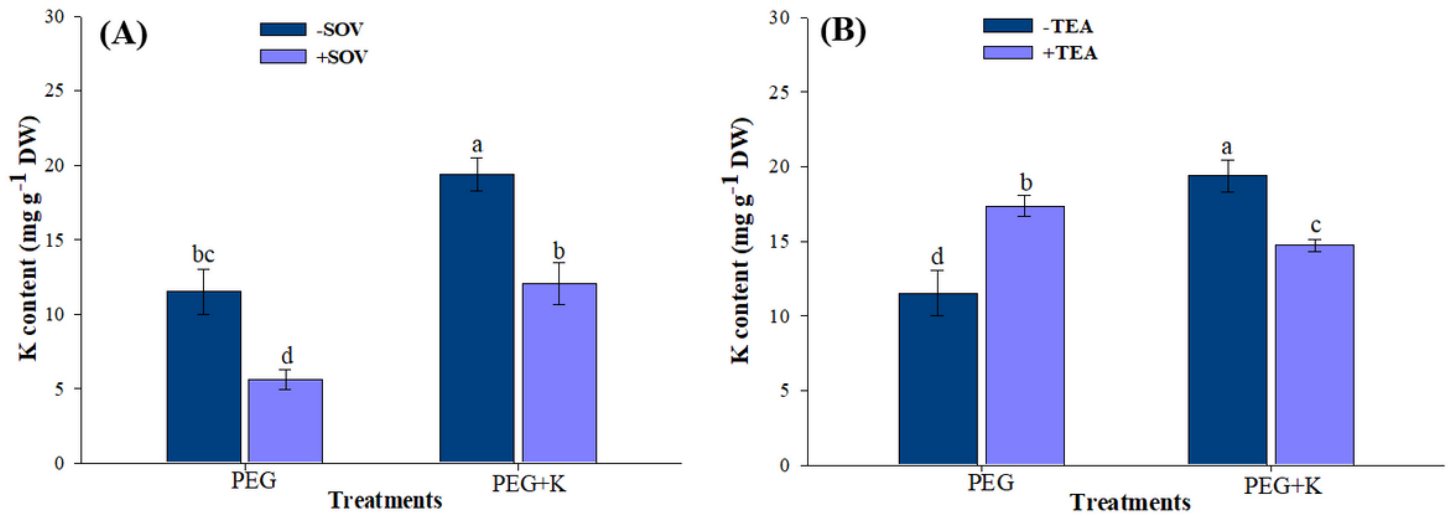


Figure 2

Effect of plasma membrane inhibitors on potassium (K) content in tomato seedling leaves. Prior to K estimation, seedlings were treated with 500 μ M sodium orthovanadate (SOV: PM H⁺-ATPase inhibitor) or 20 mM tetraethylammonium chloride (TEA: K-channel blocker) for 30 min followed by exposure to 15% (w/v) polyethylene glycol 8000 (PEG) for 24 h, in presence or absence of K. Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

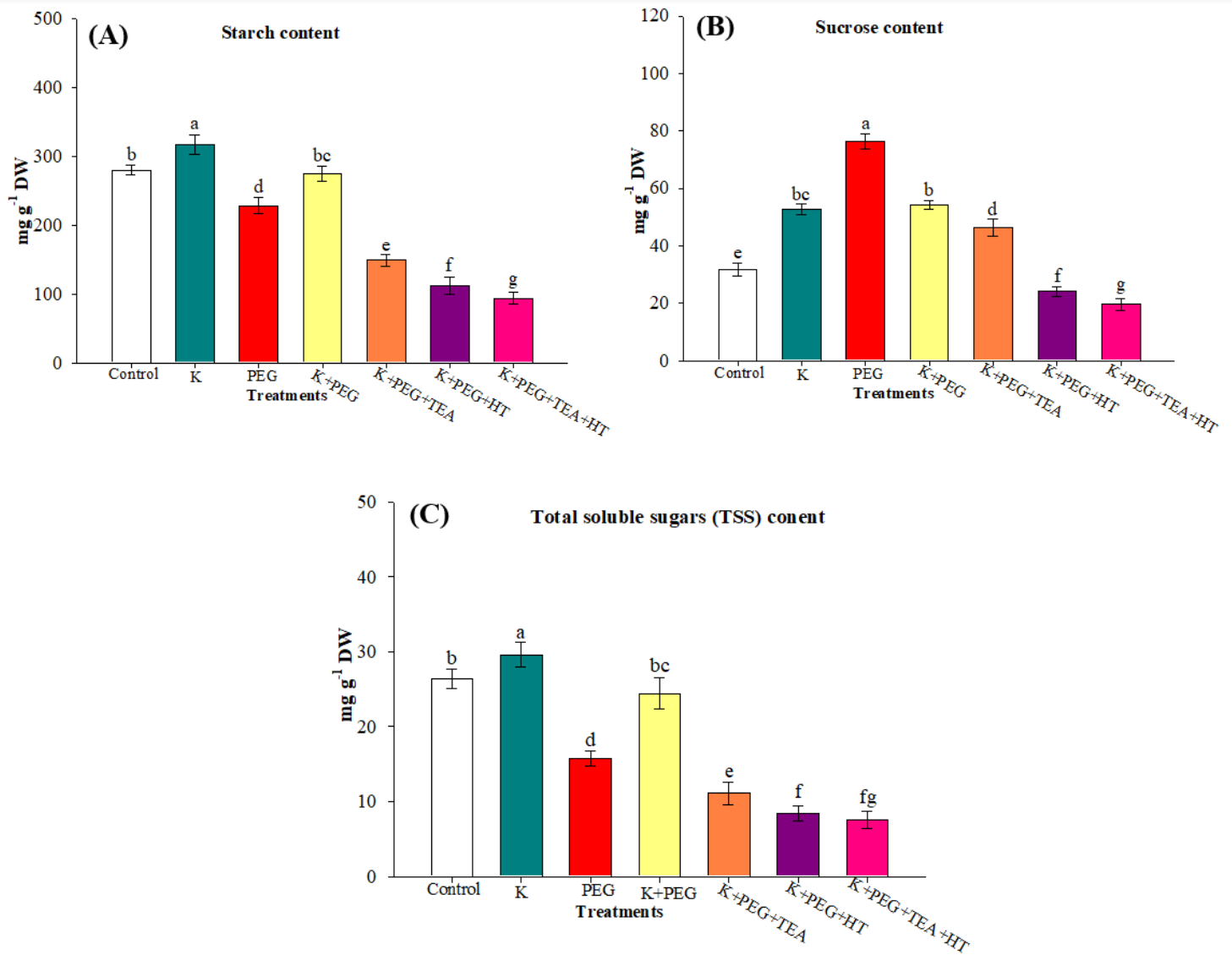


Figure 3

Effect of various treatments on the concentration of carbohydrates in tomato leaves. (A) Starch content, (B) Sucrose content, and (C) Total soluble carbohydrates (TSC) content. Double deionized water (control), 5 mM K₂CO₃ (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K⁺ channel blocker), and 1 mM hypotaurine (HT: H₂S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

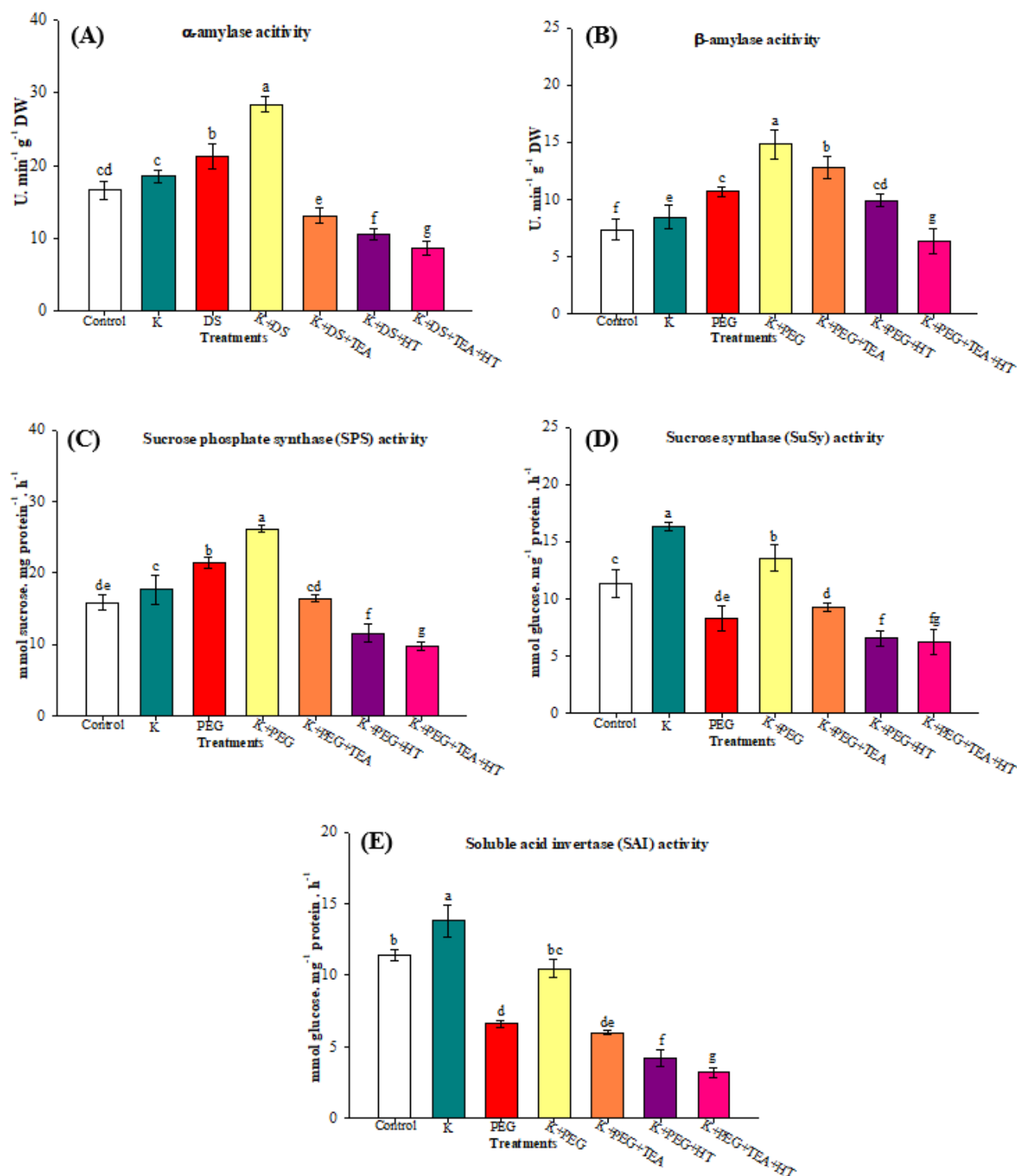


Figure 4

Activity of the enzymes associated with carbohydrate metabolism. (A) α -amylase activity, (B) β -amylase activity, (C) Sucrose phosphate synthase (SPS) activity, (D) Sucrose synthase (SuSy) activity, and (E) Soluble acid invertase (SAI) activity in tomato leaves. Double deionized water (control), 5 mM K₂CO₃ (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K⁺ channel blocker), and 1 mM hypotaurine (HT: H₂S scavenger). Data is mean of three independent replicates, with

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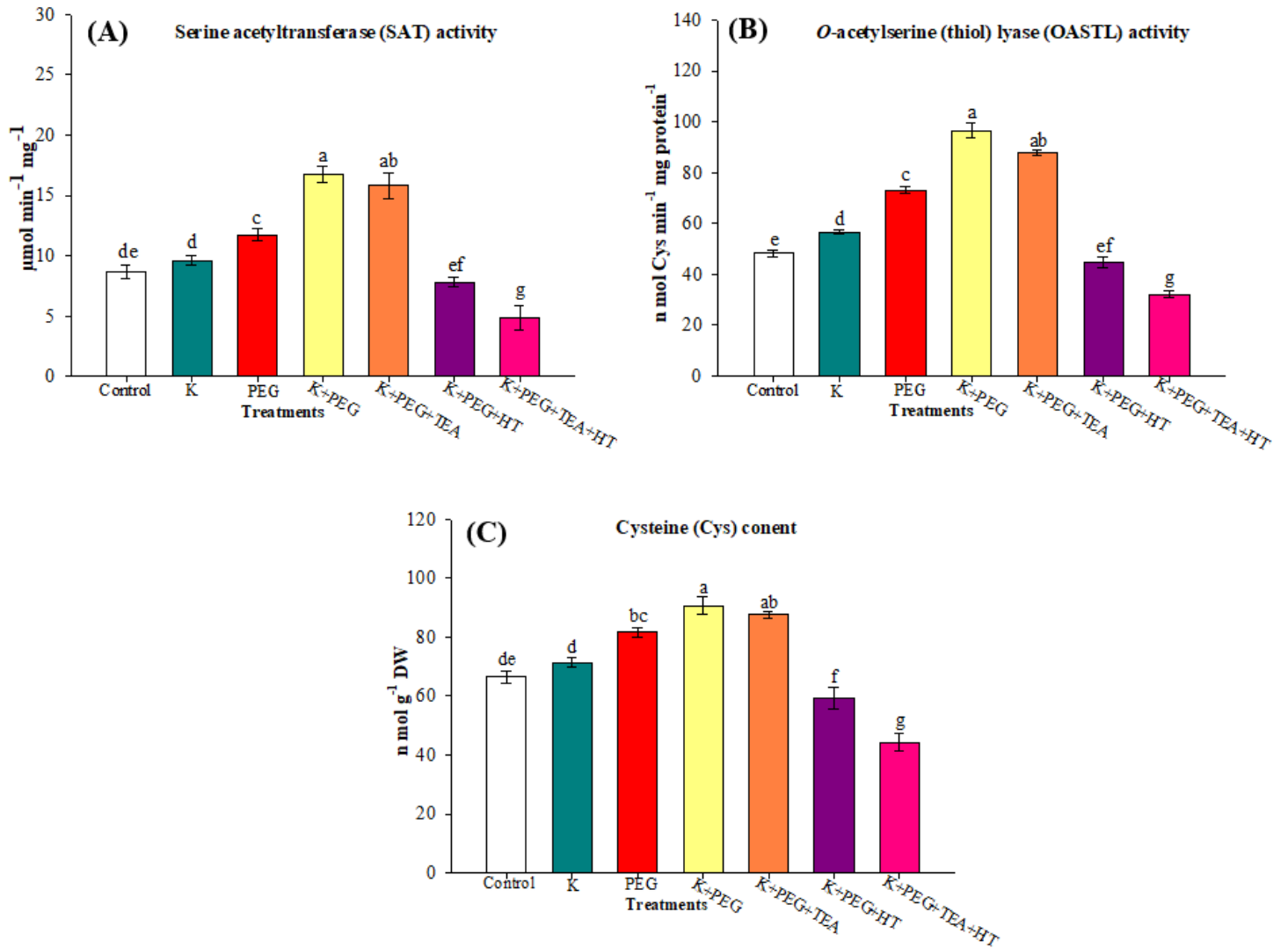


Figure 5

Cysteine (Cys) content and activity of its biosynthesizing enzymes in the leaves of tomato. (A) Serine acetyltransferase (SAT) activity, (B) O-acetylserine (thiol) lyase (OASTL) activity, and (D) Cys content. Double deionized water (control), 5 mM K_2CO_3 (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K⁺ channel blocker), and 1 mM hypotaurine (HT: H_2S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

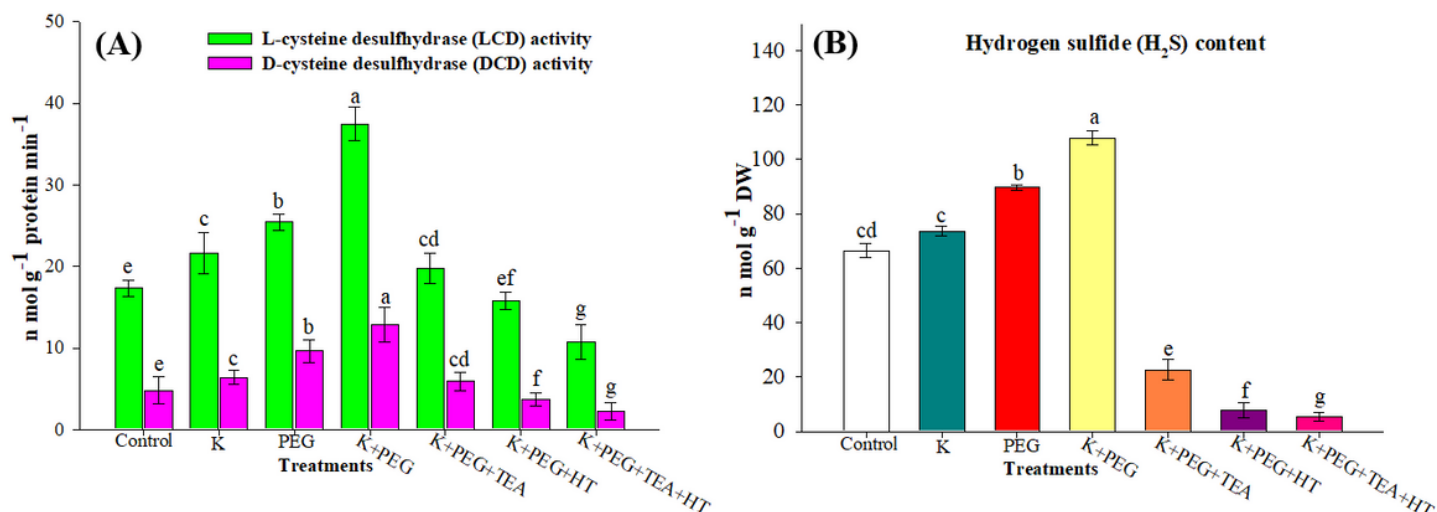


Figure 6

Effect of various treatments on the activity of biosynthesizing enzymes of hydrogen sulfide (H₂S) and its concentration in tomato leaves. (A) Activity of L-cysteine desulfhydrase (LCD) and D-cysteine desulfhydrase (DCD) and (B) Hydrogen sulfide (H₂S) content. Double deionized water (control), 5 mM K₂CO₃ (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K⁺ channel blocker), and 1 mM hypotaurine (HT: H₂S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT). .

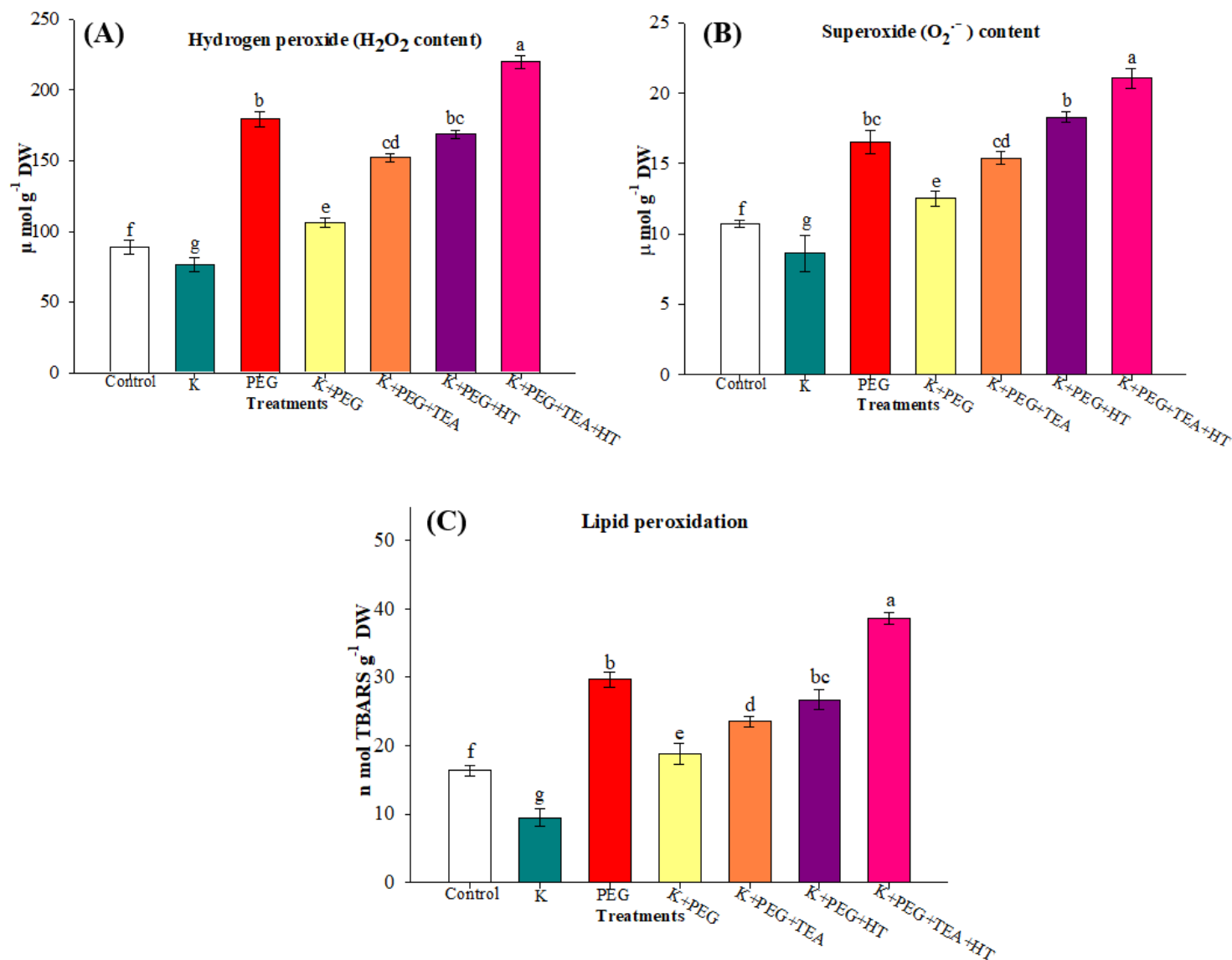


Figure 7

Reactive oxygen species production and associated damage in the leaves of tomato. (A) Hydrogen peroxide (H_2O_2) content, (B) Superoxide ($O_2^{\bullet-}$) content, and (C) Lipid peroxidation. Double deionized water (control), 5 mM K_2CO_3 (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K^+ channel blocker), and 1 mM hypotaurine (HT: H_2S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

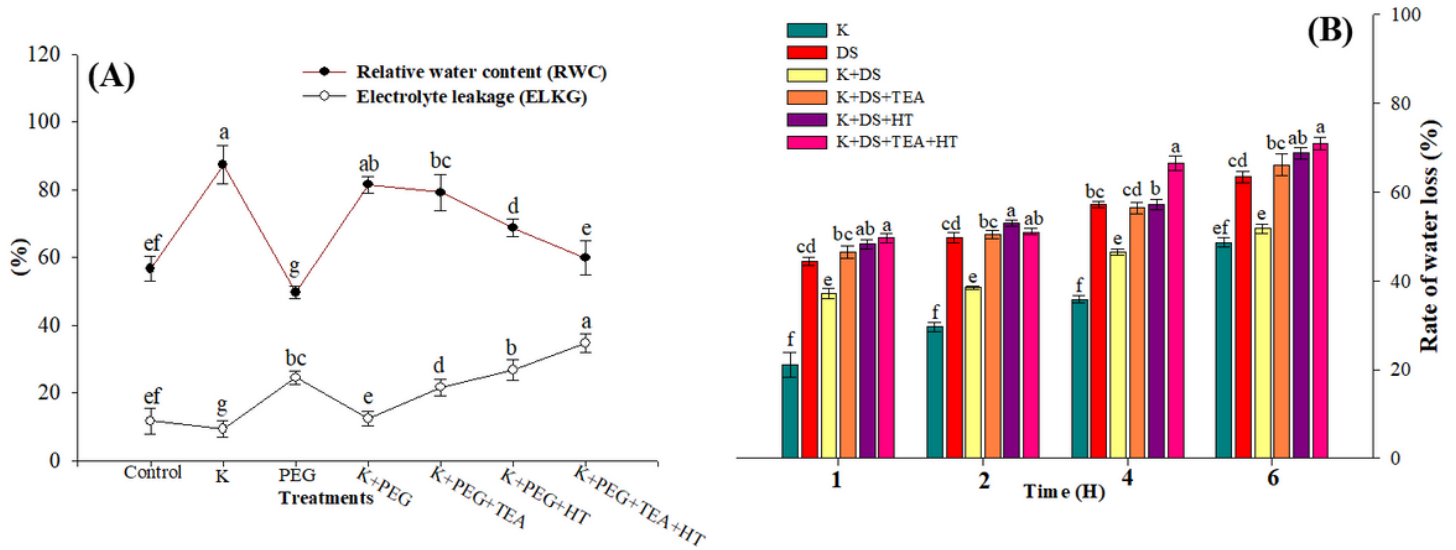


Figure 8

Effect of treatments on the stress markers in tomato seedlings. (A) Relative water content and electrolyte leakage and (B) Rate of water loss at different intervals. Double deionized water (control), 5 mM K₂CO₃ (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K⁺ channel blocker), and 1 mM hypotaurine (HT: H₂S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

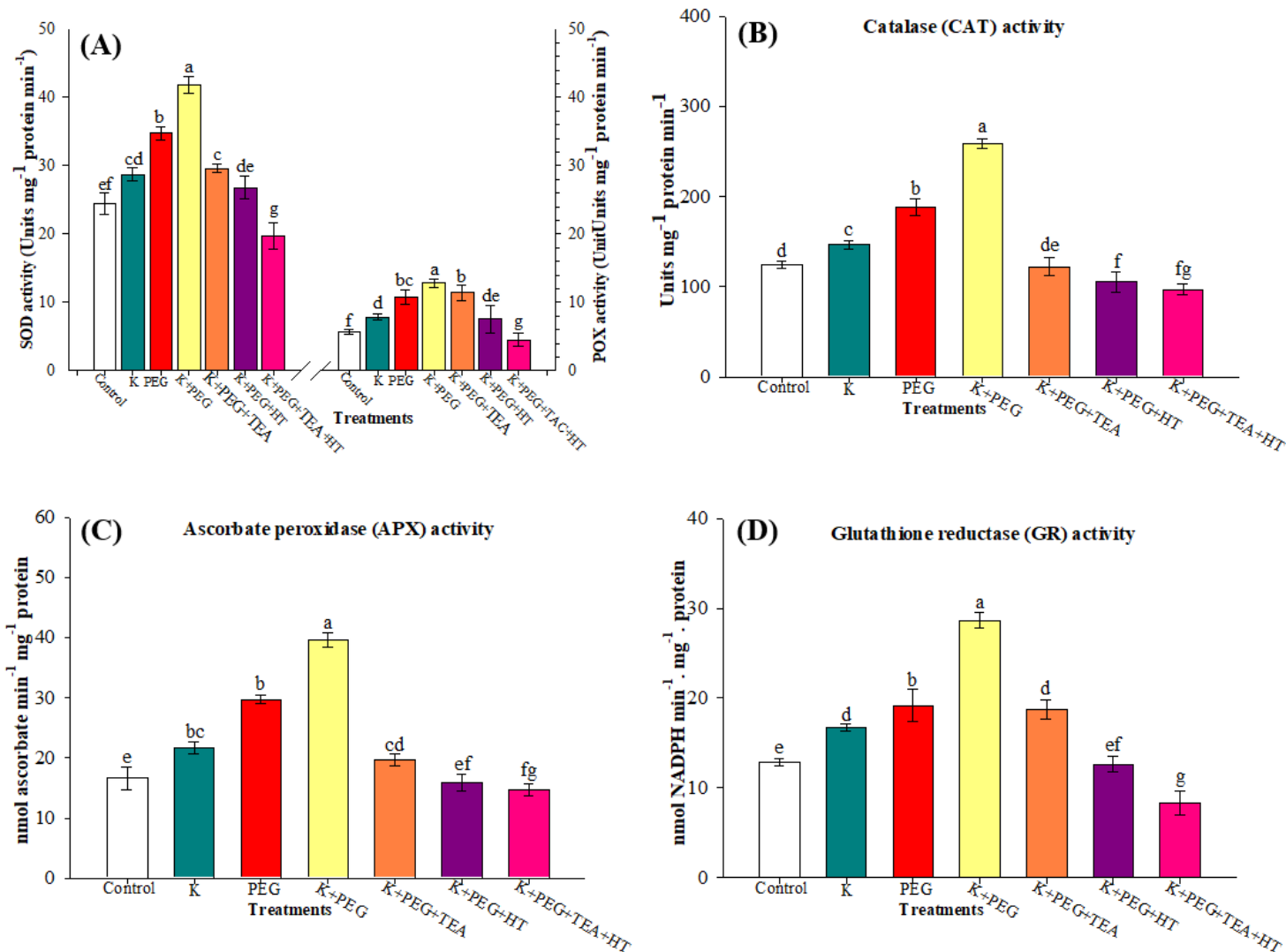


Figure 9

Activity of antioxidant enzymes in tomato seedlings. (A) Superoxide dismutase (SOD) and peroxidase (POX) activity, (B) Catalase (CAT) activity, (C) Ascorbate peroxidase (APX) activity and (D) Glutathione reductase (GR) activity. Double deionized water (control), 5 mM K₂CO₃ (K), 15% (w/v) polyethylene glycol 8000 (PEG), 20 mM tetraethylammonium chloride (TEA, K⁺ channel blocker), and 1 mM hypotaurine (HT: H₂S scavenger). Data is mean of three independent replicates, with bars indicating SE. Bars with different letters indicate that differences were statistically significant at $p < 0.05$ (DMRT).

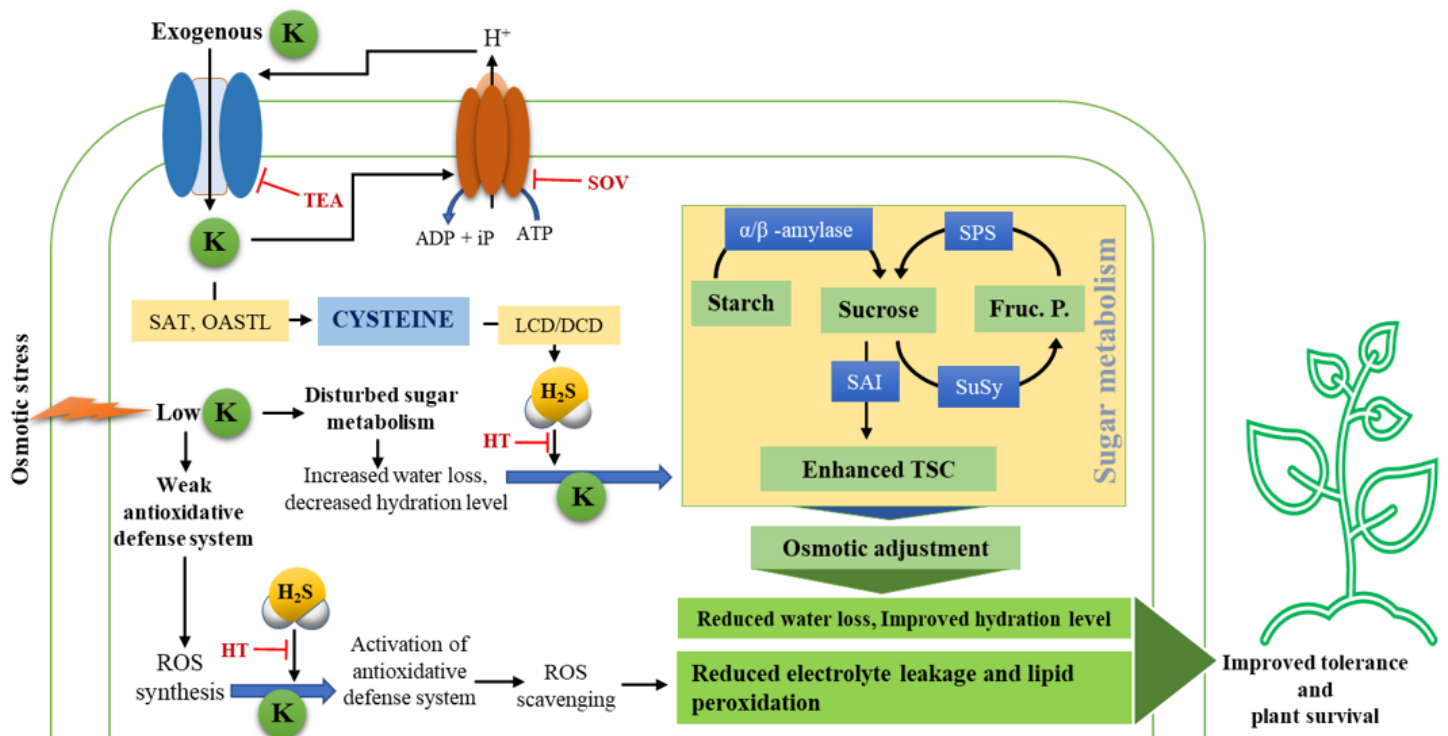


Figure 10

Diagrammatic representation of the role of potassium (K) and hydrogen sulfide (H₂S) in the regulation of sugar metabolism and antioxidative defense system under osmotic stress. DCD: D-cysteine desulfhydrase, Fruc. P.: Fructose-6-phosphate, HT: hypotaurine, LCD: L-cysteine desulfhydrase, OASTL: O-acetylserine (thiol) lyase, ROS: Reactive oxygen species, SAI: Soluble acid invertase, SAT: Serine acetyltransferase, SOV: Sodium ortho vanadate (H⁺-ATPase inhibitor), SPS: Sucrose phosphate synthase, SuSy: Sucrose synthase, TEA: Tetraethylammonium chloride (K Channel blocker), TSC: Total soluble carbohydrates