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Unveiling the mechanisms of silicon-induced salinity stress tolerance in *Panicum turgidum*: Insights from antioxidant defense system and comprehensive metabolic and nutritional profiling

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ABSTRACT

Salinity is a global challenge to sustainable agriculture, impacting plant growth at cellular and functional levels. Nevertheless, silicon (Si), a multifunctional micro-element, plays a vital role in restoring and maintaining growth and development during unfavourable abiotic conditions such as high salinity exposure. Therefore, in the current research, two salinity levels [S1; 1 M (1000 mM) NaCl and S2; 2 M (2000 mM) NaCl] were used to assess the effects of exogenous Si (Si-1; 150 mg/L and Si-2; 250 mg/L) on key biological characteristics and especially the metabolite profiles of *Panicum turgidum* plants. Our findings revealed that the salt stress negatively affected the plants through high salt content (Na^+ and Cl^-) that further antagonized the essential nutrient balance in tissues; increased NH_4^+, but lowered NO_3^- and K^+ in both roots and leaves. The excessive production of NH_4^+ led to over-accumulation of methylglyoxal (MG), resulting in the hyper-accumulation of sugars and altering the concentrations of amino acids, thereby inducing diabetes-like symptoms in *P. turgidum* plants. Interestingly, Si application restored the growth of *P. turgidum* plants by reducing oxidative damage thereby modifying the nutritional status, metabolic and biochemical characteristics of the plants. Specifically, the application of Si-2 showed improvement of key biological indictors in leaves and roots under both salinity levels. The current study also demonstrated that Si substantially reduced the NH_4^+-mediated MG-induced stress by lowering the concentration of MG, up-regulating the antioxidant capacity of various enzymes glyoxalase I (Gly-I), glyoxalase II (Gly-II), glutathione (GSH), glutamine: 2-oxoglutarate aminotransferase (GOGAT), nitrate reductase (NR), glutamine synthetase (GS), glutamate dehydrogenase (GDH); with concomitant changes in the levels of sugar/carbohydrates in roots and leaves of *P. turgidum*.

1. Introduction

Soil salinity, a frequently encountered abiotic stress globally, severely impairs crop productivity by impeding growth through ionic, osmotic, and nutritional imbalances, along with salient biological disruptions within the whole plant (Isayenkov and Maathuis 2019; He et al., 2023). Moreover, salt induces oxidative stress by producing extremely reactive molecules, including methylglyoxal (MG), as a byproduct of glycolysis (Isayenkov and Maathuis, 2019). The overproduction of ionic salts, such as sodium (Na^+), magnesium (Mg^{2+}), chloride (Cl^-), potassium (K^+), carbonate (CO_3^{-2}), and calcium (Ca^{2+}), leads to nutritional deficiencies within plant tissues such as ammonium (NH_4^+) and nitrate ions (NO_3^-) and this eventually impede the growth. The imbalance between CO_2 and O_2 levels led to subtle alterations in glycolytic flux, resulting in the excessive accumulation of metabolites by-products including methylglyoxal (MG). This ultimately triggered symptoms reminiscent of diabetes in wheat and spinach leaves (Saito et al., 2011; Takagi et al., 2014). Whereas

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the nutritional imbalance caused by NH_4^+ stress in *Arabidopsis thaliana* resulted in an upsurge in sugar metabolism, resulted in a higher accumulation of MG, a highly toxic dicarbonyl metabolite that denatures the phospholipids, proteins, the amino acids, and nucleic acids like lysine, cysteine, and arginine and converts them into advanced glycation end products (AGEs) and damages many biological functions in targeted cells in plants under abiotic stresses (Borysiuk et al., 2018; Li, 2016). In stressed plants, MG can exhibit both detrimental and signaling effects, which are contingent on the concentration of MG, the plant species involved, and the type and severity of the stress conditions (Li et al., 2019). Additionally, plants experience oxidative stress due to MG's involvement in the reduction of O_2 (Li, 2016). Contrastingly, plants possess a clearly defined two-step catabolic process for methylglyoxal (MG), involving two metalloenzymes: the Glyoxalase I (Gly-I) and the Glyoxalase II (Gly-II). This process utilizes GSH as a cofactor to convert the toxic MG into non-toxic d-lactate (Bhatt et al., 2020; Hasanuzzaman et al., 2018). Detoxifying methylglyoxal (MG) is a vital element in achieving salinity stress tolerance, with glutathione (GSH) playing a pivotal role in this process. Despite its importance, MG remains a relatively novel concept in plant physiology, necessitating further scientific studies. Gaining a comprehensive understanding of NH_4^+-induced MG stress across different plant is imperative essential, exploring its repercussions on both primary and secondary metabolism. Additionally, such studies should investigate the morphological alterations resulting from MG stress, ultimately contributing to insights into yield and quality losses under salinity stress. Although, NH_4^+ is a non-toxic compound and plays a critical role in the metabolic activities in the plants. It requires glutamine synthetase (GS), glutamate dehydrogenase (GDH), glutamine: 2-oxoglutarate aminotransferase (GOGAT), nitrate reductase (NiR) and nitrate reductase (NR) into amino acids (Shilpha et al., 2023; Song et al., 2022).

Silicon (Si), an effective element, improves physiological attributes, biochemical and metabolic processes in plants by enhancing nutritional intake, decreasing osmotic and the ionic stress, and increasing plants' resistance to various abiotic stresses i.e. drought, salinity, heavy metals and metalloids (Campos et al., 2020; Silva Júnior et al., 2019; Song et al., 2022; Viciedo et al., 2019). Briefly, Si creates a double-silicate layer in plants under a various environmental stresses, which increases stomatal conductance and internal CO_2 concentration in plants to improve photosynthetic activity (Patel et al., 2021). Additionally, it is also posited that Si not only plays a crucial role in regulating metabolites such as sugar amino acids, by maintaining carbon and nitrogen levels in plants, but also aids in protecting plants from severe NH_4^+ accumulation under abiotic stress (Ahmad et al., 2019; Hasanuzzaman et al., 2018; Li et al., 2020; Yan et al., 2020; Jalil et al., 2023). While a wealth of knowledge exists regarding the role of Si application in mitigating abiotic stresses in plant, however, no research on the impact of exogenous Si application in alleviating MG-induced diabetes-like symptoms in *Panicum turgidum* plants under salt stress have been identified to our knowledge. Grasses such as *P. turgidum* have a significant importance due to higher carbohydrates, fibers, fats, and the protein, and are used both as animal fodder and land stability and regeneration (Bhatt et al., 2020). Though considered as moderately salt-tolerant however evidences showed that at severe salinity stress *P. tugidum* faced alterations in physiological and metabolic profile. Halophytes or the use of salt-tolerant plant species as phytoremediation under salt-affected soils has received greater attention in recent years. Phytoremediation method is considered one of the most efficient, cost-effective, and eco-friendly method available to date. For this reason, a wide range of halophytes plant species, such as Brassicaceae, Cyperaceae, and Poaceae, are well-known for their high salt tolerance up to 1 M (Adam, 2015; Hasanuzzaman et al., 2014) Given the dominant salt conditions, it becomes imperative to formulate a strategy of salt-tolerant for *P. turgidum* (Ahmadi et al., 2022; Camacho-Sanchez et al., 2020; Wang et al., 2018). Considering the importance of *P. turgidum* and its performance in the saline environments, along with the potential mitigating effects of silicon (Si), the experiment was planned with the following objectives: i) To assess the capability of *P. turgidum* under saline stress conditions. ii) to study the nutritional, morpho- physiological, biochemical, and metabolic aspects of *P. turgidum*, and to evaluate how Si application could potentially improve the adversative impacts of salinity. iii) To examine the degree of damage caused by nutritional imbalance due to the elevated accumulation of NH_4^+under salinity stress in the *P. divisum* tissues (roots and leaves). iv) To discover the stress induced by MG leading to diabetes-like symptoms and the detoxifying pathway, with a focus on the protective role of Si as a stress mitigator.

2. Materials and methods

2.1. Experimental description

A pot experiment was executed at the research site of Imam Abdulrahman bin Faisal University in Dammam, Saudi Arabia. The nursery stock of *Panicum turgidum* was purchased from a local nursery when it reached the 2–4 true leaf stage. The planting medium consisted of a 1:1 ratio of sand to garden soil. Plastic containers of 50 cm high and 20 cm wide (larger sized containers were chosen to avoid root restriction in grass species) were filled with 5 kg of properly sieved soil. The soil physio-chemical properties were evaluated and the findings were reported earlier (Yong et al., 2010; Wu et al., 2023). As per the greenhouse-management methods, soil filled pots were fertilized with 12 mM of a complete nutrient solution of Hewitt's nitrate nutrient solution (Hewitt and Smith, 1974). The seedlings of *P. turgidum* were then transplanted into these plastic bins at the 2–4 true leaf stage.

2.2. Treatments

The experiment comprised seven treatments, involving two salinity levels: 1 M (1000 mM) and 2 M (2000 mM). Additionally, two levels of silicon (Si) were applied, namely 150 mg/L (Si-1) and 250 mg/L (Si-2), administered in form of potassium silicate ($K_2(SiO_3)$). The treatments were as follows; T0: 0 M salinity or No salinity was taken as control and represented as CK, T1: 1 M Salinity+ Si-0; T2: 1 M Salinity+ Si-1; T3: 1 M Salinity+Si-2; T4: 2 M Salinity+Si-0; T5: 2 M Salinity+Si-1 T6: 2 M Salinity+Si-2. Saline conditions were introduced to the soil through irrigation waters containing the desired levels of sodium chloride (NaCl) ten days after transplanting. Subsequently, during the fourth week post-transplanting, Si was applied to the seedlings through foliar application was done twice a week for the four successive weeks. The plants were harvested in the tenth week, and various parameters were studied based on established protocols.

2.3. Sampling and measurements

2.3.1. Plant growth traits

The growth attributes, such as leaf quantity per plant (LN/plant), were manually computed, and leaf expanse (LA) was ascertained with a leaf expanse gauge. Following the harvest, the botanical segments (roots and foliage) were meticulously separated. Shoot and root dimensions (SL and RL, respectively) were logged in "cm" with a measuring gauge, while shoot and root novel masses (SFW and SDW) were assessed in "mg" with a digital equilibrium. For the scrutiny of desiccated biomass, specimens were deposited in receptacles, then subjected to 70 °C for 48 h within an oven. Following the stipulated interval, variables such as shoot desiccated mass (SDW) and the root desiccated mass (RDW) were gauged in "mg," and averages were documented.

2.3.2. Nutrients uptake

The constituents of sodium (Na^+), potassium (K^+), chloride (Cl^-), ammonium (NH_4^+), and nitrate (NO_3^-) levels in the desiccated roots and foliage were gauged in accordance with a formerly documented technique (Rahman et al., 2016; Parvin et al., 2019). An acidic blend (HNO_3: $HClO_4$; 5:1) was employed to dissolve 0.1 g of pulverized, uniform desiccated botanical specimens. Measurements of Na^+, Cl^-, K^+, NH_4^+, and NO_3^- levels in the digested solution were performed using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan).

2.3.3. Methylglyoxal (MG) quantification method

The MG quantification was done according to the previous method (Mustafiz et al., 2010). The 250 mg of fresh roots and leaves were homogenized using liquid nitrogen in a mortar and pestle. The mixture was mixed using 2.5 mL of $HClO_4$ (0.5 M), transported to a tube, and allowed to incubate for 20 min on ice. After centrifuging extract for 10 min at 4 °C at 11,000 g, it was transferred to a fresh tube. The pH was then measured after a progressive addition of saturated potassium carbonate (K_2CO_3). After thoroughly mixing the supernatant and allowing CO_2 bubbles to emerge, each K_2CO_3 addition was made. The supernatant was centrifuged for 15 min at 11,000 g after being stored at room temperature for 20 min. Then, the supernatant was utilized for MG estimation. To measure the MG, the supernatant consisted of the 250 mL of 1,2-diaminobenzene at 7.2 mM, and the 5 M of the 100 mL $HClO_4$, and 650 mL of neutralized supernatant. Following at room temperature a 30 min incubation period, mixture's absorbance at 336 nm was measured. The MG (Sigma) standard curve was used to record and represent the total amount of MG as nmol/g FW.

2.3.4. Enzymes extractions and assays

The enzymatic activities such as Gly-I and Gly-II were computed by molar absorption coefficients of $3.37 \times 10^3\ M^{-1}\ cm^{-1}$ for (S-d-lactoylglutathione), and $1.36 \times 10^4\ M^{-1}\ cm^{-1}$ (2-nitro-5-thiobenzoic acid) respectively, and described as nmol/g FW (Li et al., 2019).

The activity of the reduced glutathione (GSH) was measured using previous method (Griffith, 1980). 2 mL of 5 % sulfosalicylic acid were used to homogenize the 0.5 g of fresh leaves and roots at a low temperature. Following a 15 min centrifugation at 15,000 rpm, 50μL of 5′,5′-dithiobis-2-nitrobenzoic acid (DTNB) and 0.6 mL of K-phosphate buffer (100 mM, pH 7.3) were added to 0.5 mL of supernatant. Using a spectrophotometer (AA-7000, Shimadzu, Japan), the absorbance was measured at 412 nm after two min.

A previously published technique was used to measure GDH activity (Turano et al., 1998). The NADH-GDH activity was measured in 0.03 M Tris–HCl with a pH of 8.4 and 7 mM mercaptoethanol. 1.5 mM reduced NADH—Na_2 salt, 1.5 mM $CaCl_2$, 350 mM of $(NH_4)_2SO_4$, and 3 mM 2-oxoglutarate were the components of the reaction mixture. Using a spectrophotometer (AA-7000, Shimadzu, Japan), absorbance drop was recorded at 340 nm.

The activity of GOGAT and GS was assayed by the previous protocols (O'Neal and Joy, 1973; Rachim and Nicholas, 1985). Briefly, an enzyme extract was added to a solution containing 35μmol $MgSO_4$, 100μmol α-ketoglutarate, 8μmol hydroxylamine, 10μmol ATP, and 0.5 mol/l $FeCl_3$. The mixture was allowed to incubate for 50 min at 15 °C before the enzymatic reaction was stopped by adding 0.5 mol/l $FeCl_3$, 0.8 mol/l TCA, and 0.5 mol/l HCl. A spectrophotometer (AA-7000, Shimadzu, Japan) was utilized to record the absorbances at 620 nm.

The samples of frozen leaves and roots were suspended in the two liters of buffer containing 70 mM HEPES-KOH (pH 8.2), 40 mM $MgCl_2$, 15 mM FAD, and 2 mM DTT. Following the thawing, suspension was cleared by centrifugation at 10,000 rpm at 6 °C for 15 min. The resulting mixture was then run over a Sephadex G-25 column. The mixture was used for measuring NR activity (Hageman and Hucklesby, 1971). The 600 μl of supernatant was injected into 1000μl of the extraction buffer consisting of 7 mM KNO_3 and 0.5 mM NADH. 130 μl of Zn $(CH_3CO_2)_2$ was added to stop the reaction. After subtracting unreacted NADH, a spectrophotometer (AA-7000, Shimadzu, Japan) was used to quantify NR at 580 nm.

The NiR was assayed using a previously described method (Losada and Paneque, 1971). 800 mg of crushed samples (leaves and roots) were kept in flask containing the mixture of 15 ml of infiltration media, 80 mM Tris–HCl (pH 8.5), 0.8 mM $[(C_6H_7N)_2]$ (methyl viologen), 4 mM KNO_2 (potassium nitrite) and the mixture was evacuated for 60 s and the dithionate was oxidized by vigorous shaking. In addition, 1.0 ml of sulphanilamide ($C_6H_8N_2O_2S$) and 0.50 ml of *n*(1-napthyl) ethylene diamine dihydrochloride ($C_{12}H_{16}C_{l2}N_2$) of 0.02 % were added, and mixture was incubated for 50 min in the dark. The UV–vis spectrophotometer (AA-7000, Shimadzu, Japan) was utilized to measure absorbance at 580 nm in comparison to a blank.

2.3.5. Metabolites extraction

All the metabolites including sugars, and the sugar alcohols (glucose, sucrose fructose, fructose-6-P, ribose, mannose, xylose, galactose, maltose, and inositol), amino acids (proline, valine, serine, cysteine, glcine, leucine, propanamine, butlyamine, lysine, asparagine, isoleucine, glutamine, ornithine, GABA, 4-hdroxyproline), and organic acids (citrate, isocitrate, cis-aconitate, 2-oxoglutarate, oxaloacetate, fumarate, succinate, aspartic acid, aspartate, glutamate, glyceric acid-3-P, glyceric acid, 2-momo-isobutyrine, phosphophenylpyruvic acid, phenylalanine, propaonic acid, pyruvate, and lactate) in the leaves and roots of *P. turgidum* were analyzed by the earlier reported protocol (Müller et al., 2015). The leaves and root samples were processed for GC–MS analysis using AS2000 (Thermo Electron, Dreieich, Germany). Helium was employed as the carrier gas with a flow rate of 1 mL/min. The temperature of interface was at 250 °C, and ionic source temperature was set to 220 °C. The oven maintained a temperature of 80 °C for three minutes after every examination. Mass spectra were acquired at a rate of 1 scan/s across a 50 to 750 *m/z* scanning range. Metabolites were recognized using standards from Sigma-Aldrich, and additional identification was facilitated using the Golm Metabolome Database, which is freely available for use (Kopka et al., 2005). The identification of compounds was confirmed by matching mass spectrum data and chromatographic retention time with reference standards. The specific metabolites relative concentrations were automatically measured by the peak areas integrating of corresponding ions utilizing the specific processing configuration provided by Thermo Electron's Xcalibur 1.4 software (Dreieich, Germany) (Fiehn et al., 2000). The ratio of relative response was computed by dividing the obtained result using dry weights of sample and then each peak area comparing to peak area of internal standard ribitol. Measurements for each of three replications of both the control and Si-treated plants were conducted in technical duplicates.

2.4. Statistical analysis

The pot experiment, comprising a total of 7 treatments with 3 replications each, was conducted. All data underwent statistical analysis by the analysis of variance (ANOVA) within a CRD (complete randomized design). The means of treatments were further scrutinized by the LSD (least significant difference) test through SPSS software at a level of 5 % probability. Heatmaps illustrating the data were generated using the MultiExperiment Viewer (MeV) software.

Table 1
Influence of Silicon applications on Panicum turgidum growth traits. LN/plant (leaf count per plant), SL (shoot length), LA (leaf expanse), RL (root length), SFW (shoot novel mass), RFW (root novel mass), SDW (shoot desiccated mass), RDW (root desiccated mass), CK (control regimen), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). Distinct lowercase letters indicate statistically significant differences at $p \leq 0.05$ levels (ANOVA).

Salt treatment		LN/plant	SL (cm)	LA (cm^2)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
	CK	17.33a	63.27a	29.98a	32.81a	51.86a	22.41a	26.77ab	11.1ab
1 M salinity	Si-0	10.67b	51.98b	19.53cd	22.04bc	37.98c	13.75c	19.39d	6.94cd
	Si-1	11.33b	55.05b	23.72bc	24.92b	45.55b	18.54ab	23.48bc	8.49abc
	Si-2	14ab	53.82b	26.71ab	26.39b	46.73b	21.38a	26.92ab	11.74a
2 M Salinity	Si-0	5.33c	51.06b	10.69e	16.75d	32.47d	8.28d	13.05e	4.71d
	Si-1	10.33bc	53.78b	16.43d	17.60cd	39.99c	15.59bc	20.79cd	7.96bcd
	Si-2	10.33bc	53.96b	20.07cd	23.95b	47.11b	19.25ab	27.77a	8.58abc

3. Results

3.1. Growth parameters

The current results showed that the salinity stress negatively affected the morphological characteristics of *Penicum turgidum.* All growth indicators including the LN/plant, SL, LA, RL, SFW, RFW, SDW, and RDW reduced by 38, 18, 35, 33, 27, 39, 28, and 37 % under moderate salt stress (1 M salinity), while 70, 19, 64, 49, 37, 63, 51, and 58 % inhibition was noticed under severe salinity stress (2 M), respectively. However, the various applications of Si-2 significantly altered the growth indices and decreased salinity stress in *P. turgidum* plants by improving the studied parameter up to 1.31-, 1.04-, 1.37-, 1.19-, 1.23-, 155-, 1.38-, and 1.69-fold under moderate stress conditions, whereas, 1.93-, 1.06-, 1.88-, 1.43-, 1.45-, 2.32-, 2.13-, and 1.82-fold under severe/high salt stress, respectively (Table 1).

3.2. Salinity-induced ionic stress and its alleviation by silicon application

Furthermore, salinity induced ionic toxicity and caused nutritional imbalance in *P. turgidum's* root and leaves. The salinity stress resulted in accumulation of Na^+, Cl^-, and NH_4^+up to 1.58-, 1.37-, and 1.11-fold in roots, whereas 1.35-, 1.19-, and 1.16-fold in *P. turgidum* leaves under 1 M salt stress, respectively. Similarly, the toxic ion concentration further increased up to 2.12-, 1.52-, and 1.27-fold in roots, while 1.69-, 1.45-, and 1.34-fold in *P. turgidum* leaves under severe saline stress (2 M), respectively (Fig. 1). However, Si application considerably alleviated salt stress in *P. turgidum* roots and leaves. Specifically, Si-2 application inhibited Na^+, Cl^-, and $NH_4^+$by 38, 18, and 17 % under moderate stress, whereas 14, 19, and 11 % under severe salt stress in *P. turgidum* roots, respectively. Similarly in leaves, Si-2 reduced the toxic ion uptake by 25, 13, and 12 % under moderate stress and 20, 13, and 7 % under severe salt stress, respectively (Fig. 1).

Moreover, the NO_3^- and K^+ substantially reduced by 34 and 36 % in roots and 13 and 41 % in leaves of *P. turgidum* under moderate salinity stress. Furthermore, 61 and 64 % in roots and 34 and 69 % in leaves under severe salt stress, respectively. However, Si-2 application improved the NO_3^- and K^+concentration up to 1.43- and 1.36-fold in roots while 1.21- and 1.62-fold in leaves under moderate stress, whereas 1.87-and 1.67-fold in roots, and 1.31- and 2.34-fold in leaves under severe salinity (2 M) stress severe saline stress, respectively (Fig. 1).

3.3. Ammonia assimilating enzymes GS/GOGAT pathway

Ionic toxicity and nutritional imbalance caused NH_4^+-induced stress in pants under excessive saline conditions. In response to NH_4^+-induced stress, the activity of antioxidants including GOGAT, GS, GDH, NR, and NiR considerably reduced by 35, 30, 12, 9, and 30 % in roots and 16, 28, 19, 16, and 23 % in leaves under moderate salt stress, while 44, 50, 24, 29, and 61 % in roots and 24, 64, 28, 27, and 45 % in leaves under severe salt stress, respectively (Fig. 2). Nevertheless, Si-2 application considerably increased the GS/GOGAT enzymes by 1.27-, 1.18-, 1.11-, 1.11-, and 1.39-fold in roots and 1.08-, 1.37-, 1.19-, 1.14-, and 1.19-fold in leaves under moderate salt stress while 1.53-, 1.33-, 1.17-, 1.27-, and 1.86-fold in roots and 1.13-, 1.70-, 1.16-, 1.16-, and 1.41-fold in the leaves under critical salinity stress respectively (Fig. 2).

3.4. MG stress and its detoxification mechanism

Excessive saline conditions exacerbated the oxidative stress induced by MGin *P. turgidum* plants. In *P. turgidum* roots, there was an up-regulation of 1.31- and 1.71-fold, while the leaves showed an increment of 1.36- and 2-fold under moderate and severe salinity stress, respectively. However, Si-2 application considerably reduced the MG content by 50 and 30 % in roots whereas 38 and 39 % in leaves under moderate and severe salinity stress respectively (Fig. 3).

Plants activated GSH, Gly-I, and Gly-II as part of their defensive mechanism in response to MG toxicity. Specifically, Gly-I and Gly-II showed down-regulation of 40 and 37 % in roots and 20 and 23 % in leaves under moderate stress, whereas 58 and 78 % in roots and 74 and 47 % in leaves under severe salt stress, respectively. Nonetheless, Si-2 application significantly improved the Gly-I and GlyII levels up to 1.75- and 1.21-fold in roots and 1.12- and 1.75-fold in leaves under moderate salinity, whereas 2.86- and 5.47-fold in roots and 2.91- and 2.68-fold in the leaves under critical salinity stress, respectively (Fig. 3). Conversely, GSH levels considerably enhanced up to 1.3- and 2.04-fold in roots while 1.15- and 1.68-fold in *P. turgidum*'s leaves exposed to moderate and severe salt stress, respectively and the application of Si-2 further enhanced the GSH level irrespective of the salinity stress (Fig. 3).

3.5. Metabolic profile

The metabolic profile of roots and leaves of *P. turgidum* faced alterations under over-salted conditions. The separated cluster of the untreated seedlings indicates the significant effect of salt and silicon treatments. Briefly, among sugars and sugar alcohols, glucose, fructose, sucrose, fructose-6-P, ribose, and mannose showed an up-regulation in both leaves and roots of *P. turgium* under both salinity levels, respectively. Nevertheless, the sugar content in roots and leaves was significantly decreased by application of Si-2. Whereas xylose, galactose, maltose, and inositol are negatively impacted by saline stress. In contrast, Si-2 application alleviated the salinity stress and improved xylose, galactose, maltose, and inositol levels in *P. turgidum* roots and leaves, respectively (Fig. 4a-b).

Moreover, the amino acids including proline, glutamine, cysteine, and serine increased, whereas glycine, propanamine, leucine, lysine, asparagine, butylamine, isoleucine, threonine, valine, ornithine, GABA, and 4-hdroxyproline were considerably reduced under both the salinity stress levels in *P. turgidum* leaves, respectively. However, Si-2 application negatively impacted glutamine, serine, and cysteine content, while it had positive influence on proline, glycine, leucine, propanamine, butylamine, lysine, asparagine, threonine, isoleucine

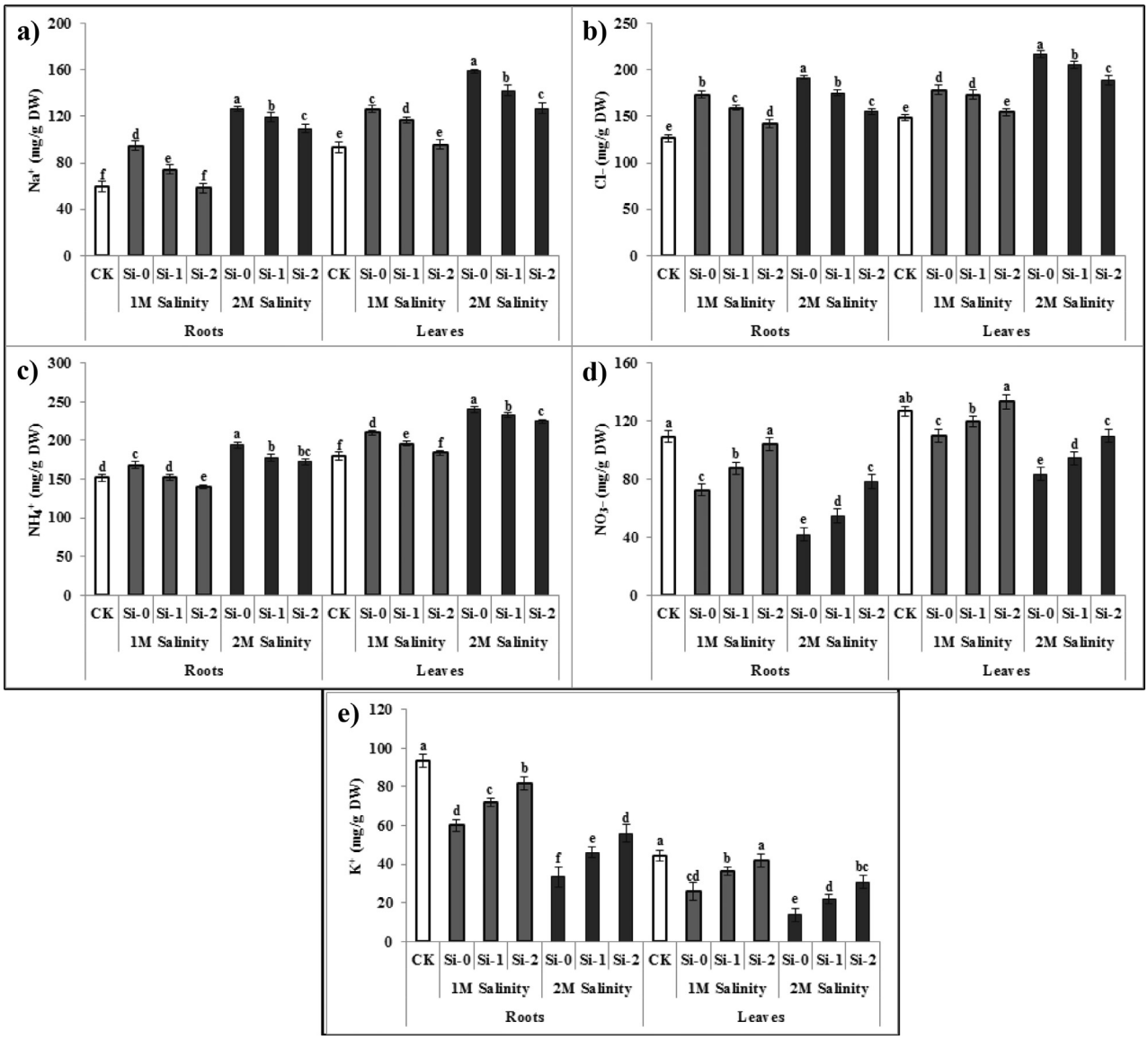

Fig. 1. The effects of exogenous applications of Silicon on ionic toxicity and nutrients uptake of *P. turgidum* under the salinity stress. (a); Na^+ (sodium), (b); Cl^- (chloride), (c); NH_4^+ (ammonium), (d); NO_3^- (nitrate), (e); K^+ (potassium), CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). The different small letters are significantly different with each other at $p \leq 0.05$ levels (ANOVA).

valine, ornithine, GABA, and 4-hdroxyproline content in *P. turgidum* leaves (Fig. 5a).

Similar to the leaves, amino acids such as serine, cysteine, lysine and glutamine were up-regulated in *P. turgidum* roots. Conversely, proline, valine, glycine, leucine, propanamine, butylamine, asparagine, threonine, isoleucine, ornithine, GABA, and 4-hydroxyproline were down-regulated under both the salinity levels, respectively. Conversely, Si-2 application down-regulated serine, cysteine, lysine and glutamine content, whereas, the enhancement was noticed in proline, valine, glycine, leucine, propanamine, butylamine, asparagine, threonine, isoleucine, ornithine, GABA, and 4-hydroxyproline concentration in *P. turgidum* roots (Fig. 5b).

In addition to others, organic acids including citrate, isocitrate, cis-aconitate, 2-oxoglutarate, succinate, fumarate, oxaloacetate, aspartate, aspartic acid, glyceric acid-3-P, glyceric acid, 2-momo-isobutyrine, phosphophenylpyruvic acid, phenylalanine, propanoic acid, pyruvate, and lactate experienced a significant reduction in roots under both the salinity levels. Though, Si-2 application alleviated the salinity stress and considerably improved the organic acid content in *P. turgidum* roots. Conversely, glutamate content slightly enhanced under both the salinity levels, whereas Si-2 reduced its concentration in roots (Fig. 6b). Moreover, leaves displayed same pattern of results under both the salinity levels and application of Si-2 significantly improved the organic acids content in *P. turgidum* (Fig. 6a).

3.6. Principle component analysis

The principal component analysis (PCA) was conducted to elucidate the effects of Si application on the nutritional, morpho- physiological, biochemical, and metabolic aspects in leaves and roots of *P. turgidum* under salinity stress. The first two principal components (PCs) described 86 % in leaves and 83 % in roots of the morphological, enzymatic, and nutritional variations in leaves and roots under different Si applications. The analysis of leaves and roots revealed that morphological and enzymatic activities displayed a significant positive relation, while in leaves the *GlyII* showed a negative correlation between the nutritional and morphological parameters (Fig. 7a&b).

Furthermore, the metabolites of leaves and roots displayed 95 % and 97 % of the first two principles respectively. The result of PCA depicted that Si application (Si-2) had a significant impact on the leaves metabolites including (propanam, isoleucine, valine, pyruvate, prop acid, gly acid, GABA) (Fig. 7c). In roots, the PCA results exhibited

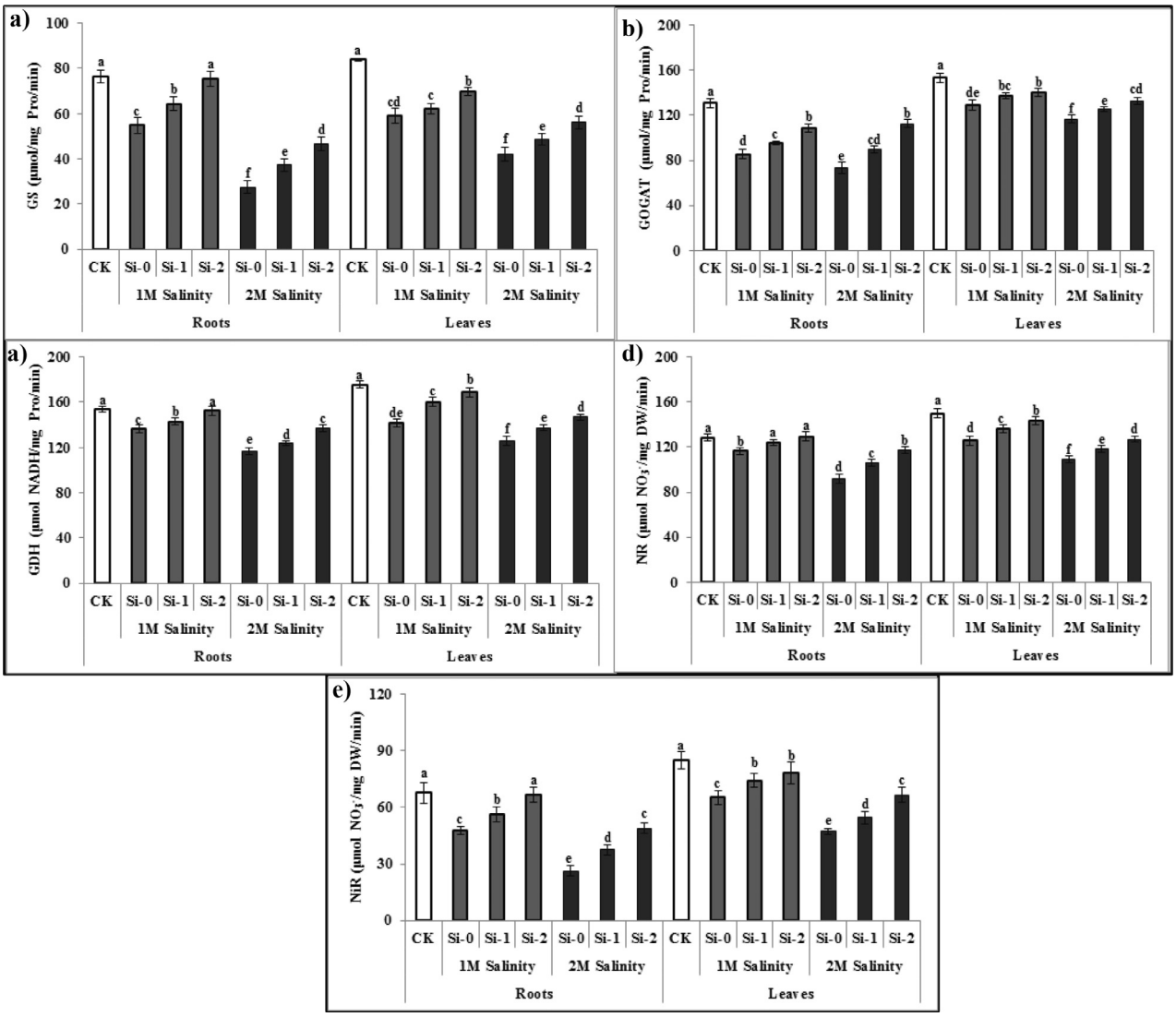

Fig. 2. The effects of exogenous applications of silicon on ammonia assimilating enzymatic activity of *P. turgidum* under salinity stress. (a); GS (glutamine synthetase), (b); GOGAT (glutamate synthetase), (c); GDH (glutamate dehydrogenase), (d); NR (nitrate reductase), (e); NiR (nitrite reductase), CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). The different small letters are significantly different with each other at $p \leq 0.05$ levels (ANOVA).

that Si application including (Si-1 and Si-2) significantly improved the metabolites content indicating the protective role of Si as a stress mitigator under salinity stress (Fig. 7d).

4. Discussion

Excessive soil salinity represents a paramount challenge among the various abiotic factors, imposing negative effects on plant growth at multiple levels, encompassing physio-biochemical and molecular levels. This single abiotic factor is a significant risk to sustainable agriculture and, by extension, our planet. Salt stress destructively influences crop quality and yield through various mechanisms, including the osmotic and ionic imbalances, alterations in the metabolic pathways, nutritional deficiencies, impairment of physiological functions, and disruptions in biochemical processes (Isayenkov and Maathuis, 2019; He et al., 2023). Furthermore, overabundance of toxic salt ions Na^+ and Cl^- generates ionic imbalance, and causes necrosis in leaves, resulting in decreased leaf area, reduced photosynthetic activity, and the overall crop production under severe saline conditions (He et al., 2023; Wu et al., 2023). The current results were consistent with earlier findings (Etesami et al., 2021; Joshi et al., 2022). The current investigation revealed that, *P. turgidum* leaves exhibit far greater accumulation of salt ions than in roots, indicating that *P. turgidum* could be considered to be a salt-excreting halophyte in the family Poaceae (Fogliatto et al., 2019; Wang et al., 2020; Zeeshan et al., 2020). Previous study revealed that higher salt ion levels increased the growth under moderate salt conditions; however under severe salinity, *P. turgidum* (Koyro et al., 2013), and *Panicum antidotale* (Ahmad et al., 2010) plants experienced a significant reduction in biological activities resulted in the downregulation of growth and nutrients status in aerial plant parts (Atia et al., 2019; Moinuddin et al., 2014). Moreover, higher salt accumulation resulted in the rise of osmolytes such as proline, glycine, sugars and other amino acids in leaves of *P. turgidum* plants indicating that osmotic adjustments were achieved using a combination of organic (sugars, amino acids) and inorganic solutes (Na^+ and K^+) (Abideen et al., 2014; Hasegawa, 2013; Hussain et al., 2015; Karan and Subudhi, 2012). However, the exogenous Si application considerably reduced the hazardous impacts of salinity by improving the morphological, physiological, biochemical and metabolic activity in *P. turgidum* plants under salinity stress, a recent study confirmed the current results where Si and proline enhanced the salinity tolerance in *Zea mays* (Gou et al., 2023), and salt and cadmium (Cd) tolerance in *Phaseolus vulgaris* (Rady et al., 2019). Similar to exogenous Si application, Silicon nanoparticles

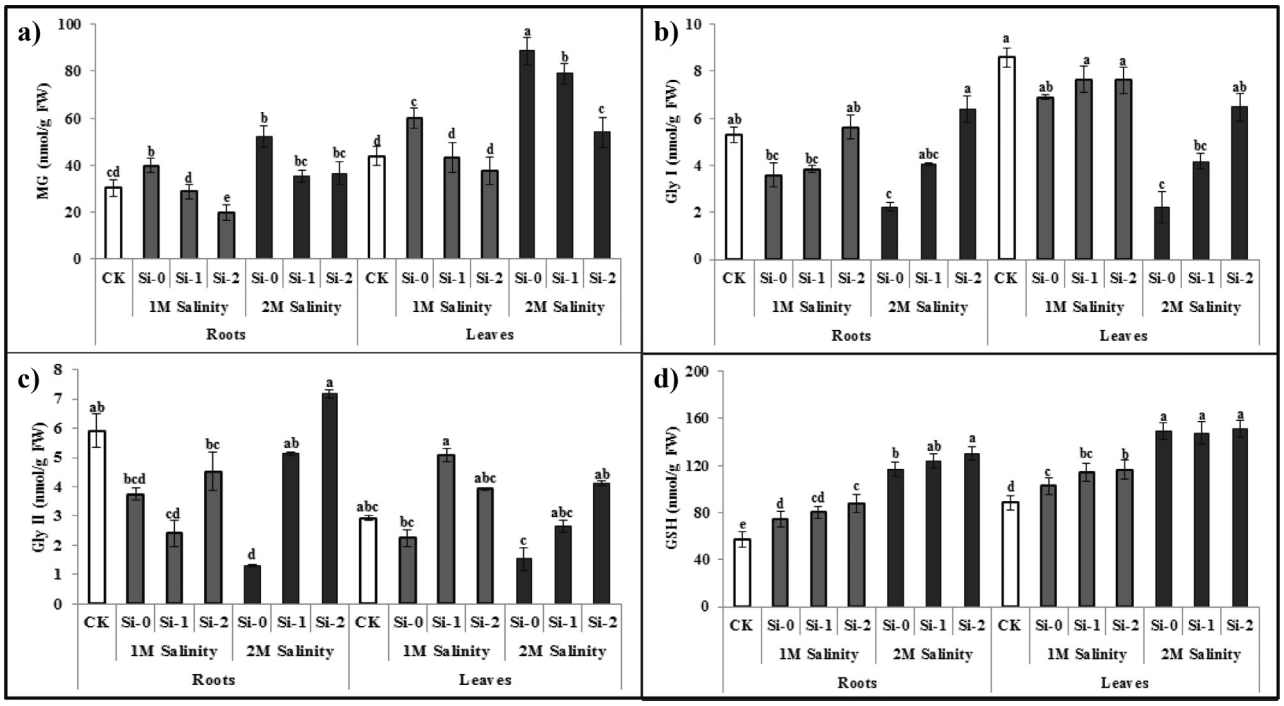

Fig. 3. The effects of exogenous applications of Silicon on MG and its scavenging enzymes of *P. turgidum* under the salinity stress. (a); MG (methylglyoxal), (b); Gly-I (glyoxalase I), (c); Gly-II (glyoxalase II), (d); GSH (glutathione), CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). The different small letters are significantly different from each other at $p \leq 0.05$ levels (ANOVA).

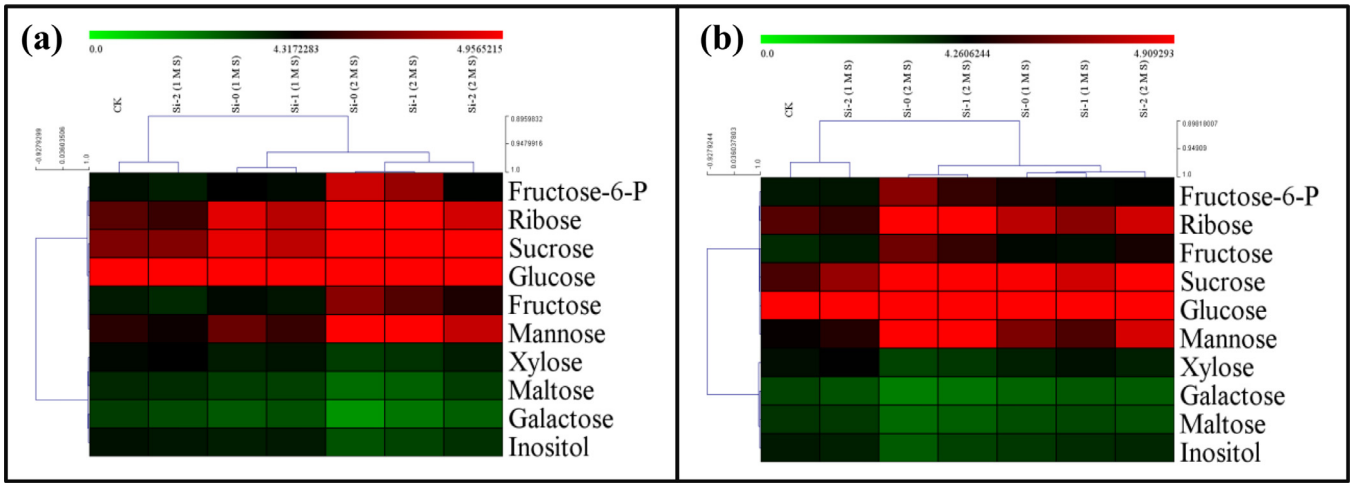

Fig. 4. The effects of exogenous applications of Silicon on the metabolite profiles of *P. turgidum* under the salinity stress. (a); leaves sugars and sugar alcohols, (b); roots sugars and sugar alcohols, CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). The heatmap created with the help of Multiviewer Experiment software using the log-2 values. The coloured cell on the map refers to the normalized log value of studied metabolic levels, with metabolites in rows and samples in columns. Red colour refers the increase, while green represents the decrease in metabolic activity in *P. turgidum* plants.

(SiNPs) application produced the same results. Briefly, integrative application of bacterial carbonates and SiNPs at the rate of 1.0–1.5 mM enhanced the abiotic stress resistance in *Triticum aestivum* by improving the physiological and biochemical attributes, reduced the ROS activity, enhanced the osmolytes and decreased the osmotic stress in wheat plants grown under sami-arid conditions, these results were found indirect in line with our current results (Desoky et al., 2022). The current findings described that, ionic salt (Na^+ and Cl^-) accumulation lead to not only decreased morphological development but also caused nutritional deficiency such as NH_4^+, NO_3^{-} and K^+ imbalance in *P. turgidum* the leaves and roots, earlier researches were found parallel to our present results (Gao et al., 2016; Zhang et al., 2023; 2018). Furthermore, an additional mechanism contributing to ion toxicity under salinity stress involves the excessive influx of sodium ions (Na^+) replacing potassium ions (K^+), thereby influencing numerous biochemical, physiological, and the metabolic characteristics in the stressed plants. These findings are inconsistent with those reported in a previous study (Shahid et al., 2020). Studies also demonstrated that increased accumulation of the ionic salts significantly affected the K^+ and N-nutrients including NH_4^+ and NO_3^-. The current results suggested that Na^+ and K^+ imbalance led to higher accumulation of NH_4^+ ions which ultimately reduced the NO_3^- concentration caused NH_4^+-toxicity in *P. turgidum* roots and leaves. The higher accumulation of NH_4^+ led to the impairment of glycolysis and over-production of carbohydrates and sugars, resulting in excessive generation of MG in both roots and leaves of *P. tugidum.* This over-expression of toxic MG is linked to dicarbonyl stress or diabetes-like symptoms in pants. The current

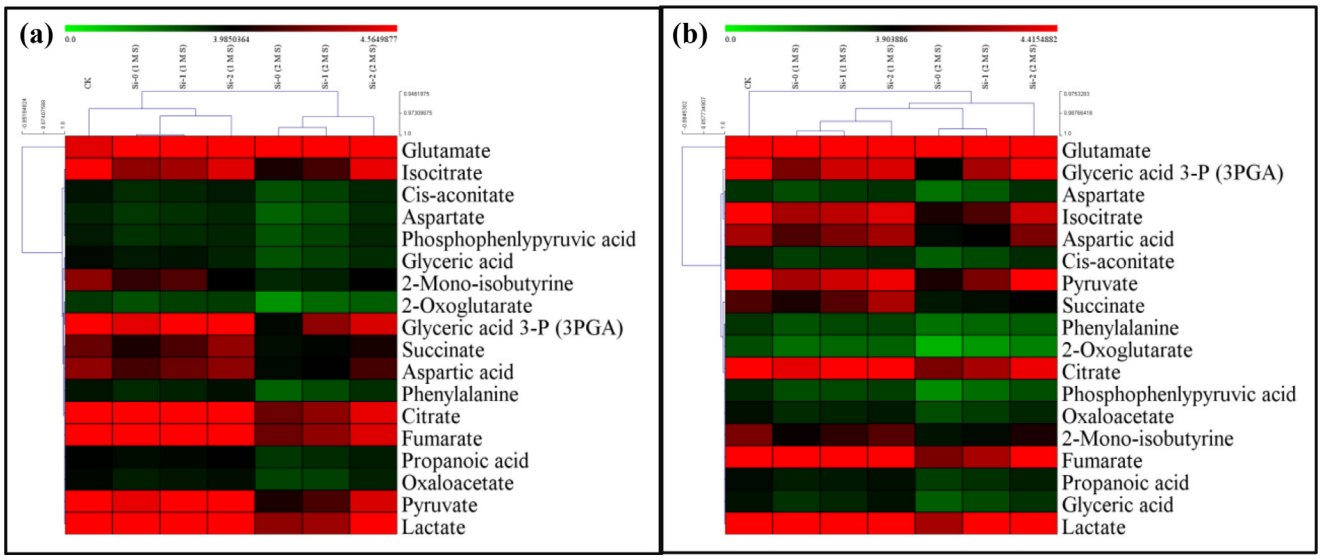

Fig. 5. The effects of exogenous applications of Silicon on the metabolite profiles of *P. turgidum* under the salinity stress. (a); leaves amino acids, (b); roots amino acids, CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). The heatmap was created with the help of Multiviewer Experiment software using the log-2 values. The coloured cell on map refers to a normalized log value of the studied metabolic levels, with metabolites in rows and samples in columns. Red colour refers the increase, while green represents the decrease in metabolic activity in *P. turgidum* plants.

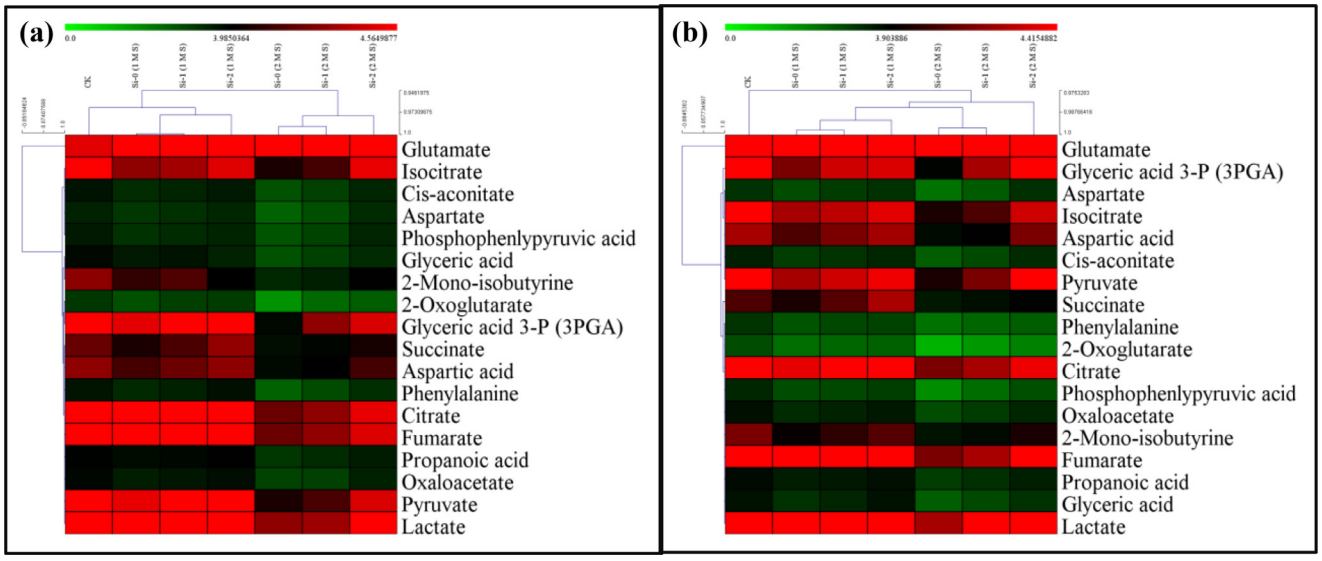

Fig. 6. The effects of exogenous applications of Silicon on the metabolite profiles of *P. turgidum* under the salinity stress. (a); leaves organic acids and others, (b); roots organic acids and others, CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L). The heatmap was created with the help of Multiviewer Experiment software using the log-2 values. The coloured cell on map refers to a normalized log response value of studied metabolic levels, with metabolites in rows and samples in columns. Red colour refers the increase, while green represents the decrease in metabolic activity in *P. turgidum* plants.

results were found reliable with earlier studies (Saito et al., 2011; Takagi et al., 2014). Some previous studies revealed MG-induced oxidative stress in different grass species under salinity (Mohsin et al., 2020; Siddiqui et al., 2020a; Talaat et al., 2023), drought (Saleem et al., 2023b) and heavy metal stress (Saleem et al., 2023a), these results were consistent with the present findings. Nonetheless, exogenous Si significantly alleviated the NH_4^+ stress, leading to downregulation of the MG content and restoring the metabolic profile of *P. tugidum* (Fig. 7). Although, ample of evidences are present describing the silicon role alleviating toxic ammonia effects in different plants (Campos et al., 2020; Kochanová et al., 2014; Song et al., 2022; Viciedo et al., 2019). However, to the best of our knowledge no study was found describing the role of Si alleviating toxic MG-induced diabetes like indicators in the plants. To fill this knowledge gap more research focused on silicon mitigating NH_4^+- and-MG-induced toxicity by altering the metabolic response in the plants is needed in future. Moreover, the present findings described that, under severe salinity (2 M) stress and higher NH_4^+ production showed severe chlorosis in *P. turgidum* leaves. These results suggested that combined stress of salinity and NH_4^+ exerted drastic effects on the growth and development of *P. turgidum.* Nevertheless, introduction of low or moderate salinity stress can be an effective approach alleviating the negative impacts of NH_4^+ on growth (Camalle et al., 2020); interestingly, these earlier results were in line with our current observations.

Furthermore, besides affecting sugar-related metabolism, salt stress-induced NH_4^+ elevation in plants leads to alteration in nitrogen metabolism, potentially causing alterations and/or reductions in amino acids and organic acid contents in *P. tugidum* roots and leaves (Fig. 8). The current results revealed that amino acids including glutamine, proline, cysteine, serine and lysine showed an up-regulation while glutamate among organic acids whereas the other metabolites showed a significant decline under high NH_4^+ and salinity stress

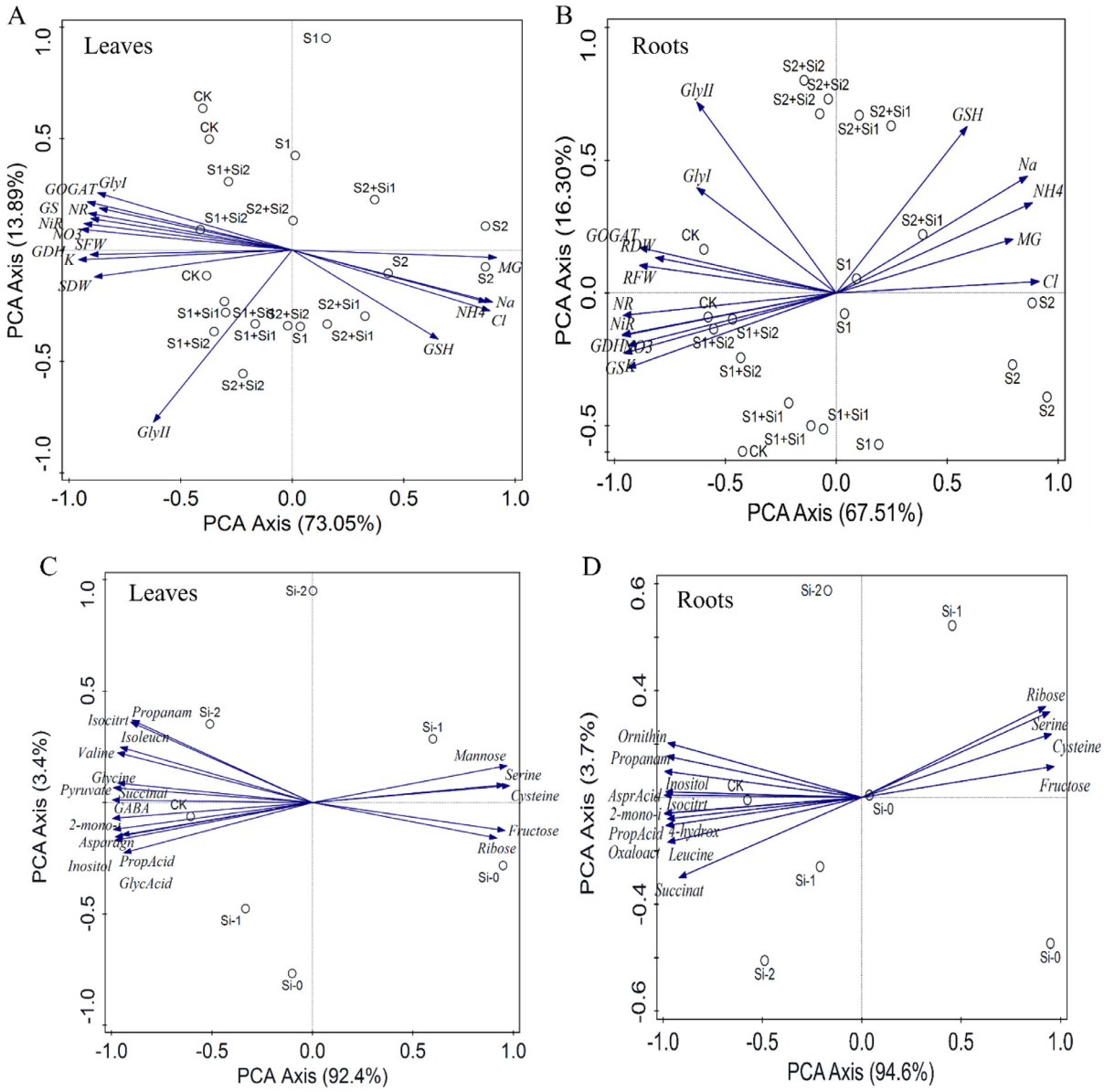

Fig. 7. Scores of principal component analysis (PCA) highlighting the Silicon effects on metabolite profile of *P. turgidum* under the salinity stress. (A) Physiological parameters of leaves, (B) Physiological and biochemical parameters of roots (C), Metabolites of leaves (D). Metabolites of leaves CK (control treatment), Si-0 (0 mg/L), Si-1 (150 mg/L), Si-2 (250 mg/L).

might be due to the combined effect of NH_4^+ and salinity. Previous studies on *Brassica oleracea* (Barreto et al., 2016), *Zea mays* (Zanin et al., 2015), *Passiflora edulis* (Silva Júnior et al., 2019), and *Solanum lycopersicum* (Borgognone et al., 2013) had shown that ammonium toxicity depend on the NH_4^+ion concentration, plant species, pH, and the severity of salinity. Furthermore, Si application decreased the NH_4^+ toxicity and enhanced the metabolite profile of *P. turgidum* plants under all the salinity levels, suggested that exogenous Si-2 (250 mg/L) application could effectively mitigate the salinity mediated NH_4^+-induced toxic effects on *P. turgidum*'s metabolic profile including amino acids, sugars, and organic acids. Some previous studies on *Acacia gerrardii* (Al-Huqail et al., 2019), *Beat vulgaris* (Viciedo et al., 2019), *Passiflora edulis* (Silva Júnior et al., 2019), *Halogeton glomeratus* (Wang et al., 2021) *Arachis hypogaea* (Patel et al., 2021), apple (Karagiannis et al., 2021), and *Zea mays* (Kochanová et al., 2014) under the abiotic stresses involving the NH_4^+, drought stress and salinity respectively.

Plants are able to counter MG-induced oxidative stress by activating their inherent defense mechanism, which relies on two metalloenzymes, Gly-I and Gly-II, and utilizes glutathione (GSH) as a cofactor. Although, the genetic study limitations hindered the exploration of the activation process of Gly-I and Gly-II, and the present findings align with previous results that elucidated MG-detoxification mechanisms under various abiotic stressors in several plant species. (Dorion et al., 2021; Borysiuk et al., 2022; Siddiqui et al., 2020b; Li et al., 2019; Saito et al., 2011; Hossain et al., 2021). According to the current findings, glutathione (GSH) levels increased in both roots and leaves during salt stress, while there was a significant reduction in the activity of Gly-I and Gly-II enzymes. However, Si treatment alleviated MG stress by enhancing the levels of GSH, Gly-I, and Gly-II enzymes in both the leaves and roots of P. turgidum. These findings aligned with previous studies that shown exogenous Si successfully reduced MG stress and elevated Gly-I and Gly-II activity in plants displayed to a diversity of the abiotic stressors, such as heavy metals, salinity, and

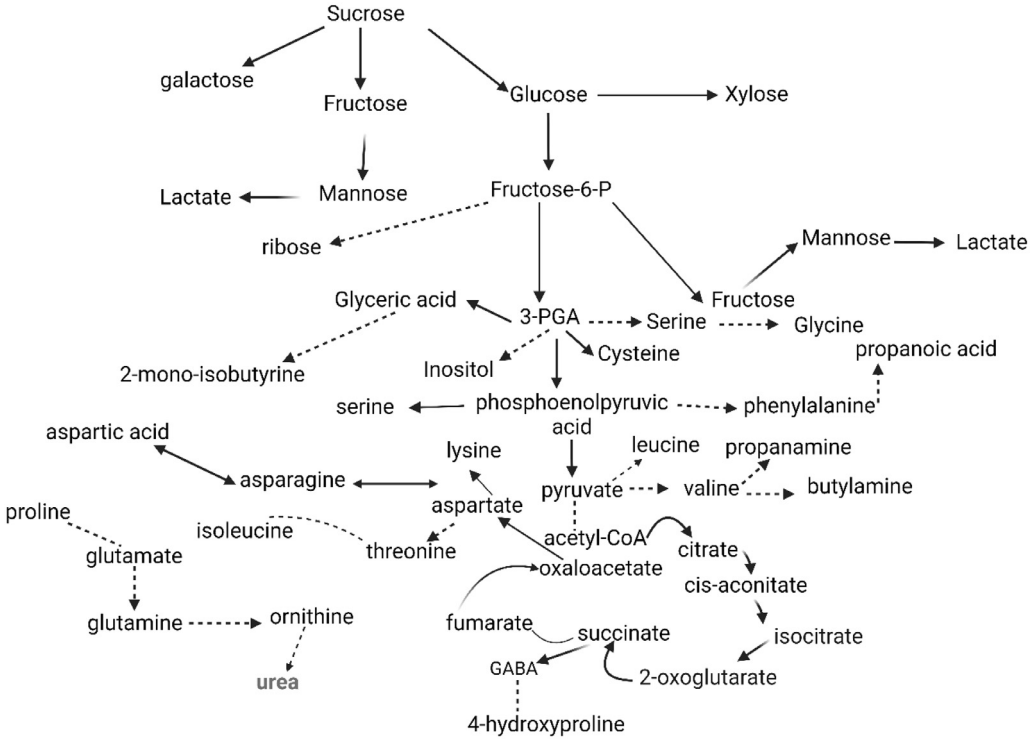

Fig. 8. Schematic diagram of common metabolites of *P. turgidum* involved in sugar, amino acids metabolism.

drought (Ahmad et al., 2019; Hasanuzzaman et al., 2019; 2018; Li et al., 2020; Singh and Roychoudhury, 2020). On the contrary, the activities of ammonium assimilating enzymes involved in the GS/GOGAT cycle, such as GOGAT, GDH, GS, NR, and NiR, were inhibited under both moderate and severe salinity stress, indicating some degree of metabolic dysfunctioning in roots and leaves of *P. turgidum* plants.. Previous studies on *Oryza sativa* (Sathee et al., 2021), *Sophora japonica* (Tian et al., 2021), *Hordeum vulgare* (Ben Azaiez et al., 2020), *Solanum lycopersicum* (Khan and AlZuaibr, 2022), and *Zostera marina* (Wang et al., 2021) were found in accordance with the present results. Whereas, the Si application greatly improved the GS/GOGAT enzymes by reducing the toxic salt ions and NH_4^+ concentration in both the roots and leaves of *P. turgidum* plants. Some previous studies found parallel to our present results, where Si application improved the GS/GOGAT cycle enzymes in plants under salinity (Song et al., 2022), drought (Cui et al., 2021), autotoxicity (Lyu et al., 2022), and NH_4^+ stress (Campos et al., 2020).

5. Conclusion

Elevated salinity stress negatively affected the overall growth and development of *P. turgidum* plants. Specifically, the leaves and roots experienced ionic stress from ionic salts (Na^+ and Cl^-), leading to over-accumulation of NH_4^+ ions and suppression of plant growth. Furthermore, the high levels of salt ions and NH_4^+ altered the concentrations of NO_3^- and K^+, thereby significantly impacting sugar/carbohydrate metabolism in plants. These changes led to an overabundance of methylglyoxal (MG) in both roots and leaves. Consequently, the MG-detoxification pathway was activated, involving Gly-I and Gly-II catalysts, along with supplementary antioxidants such as GSH. Gly-I and II interacted to transform MG into lactate, with GSH serving as a coadjutant, subsequently metamorphosing it into an innocuous pyruvate. Although the NH_4^+ assimilating enzymes associated with the GS/GOGAT cycle were inhibited under both salinity levels, *P. turgidum* plants exhibited more severe toxicity symptoms under high salinity stress (2 M), indicating that *P. turgidum* is a temperately salt-tolerant grass species. However, the application of exogenous Si significantly enhanced the nutritional status, morphological development, and metabolic profile by mitigating NH_4^+ induced methylglyoxal (MG) toxicity in P. turgidum plants. Nevertheless, a comprehensive understanding of the biological pathways involved in NH_4^+-induced MG toxicity when using Si as a stress-protectant is still incomplete. To obtain a more comprehensive understanding of NH4+-induced plant diabetes and/or MG production, future research should prioritize electrophysiological studies focused on $NH_4^+$and K^+transport in both roots and leaves.

Conflict of interests

The authors declare that they have no conflict of interests.

CRediT authorship contribution statement

Nadiyah M. Alabdallah: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – review & editing. **Aisha Saud Al-Shammari:** Formal analysis, Funding acquisition. **Khansa Saleem:** Supervision, Writing – review & editing. **Saleha S. AlZahrani:** Formal analysis, Funding acquisition. **Ali Raza:** Supervision, Writing – review & editing. **Muhammad Ahsan Asghar:** Supervision, Writing – original draft, Writing – review & editing. **Abd Ullah:** Supervision, Writing – review & editing. **Muhammad Iftikhar Hussain:** Supervision, Writing – review & editing. **Jean Wan Hong Yong:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft.

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