

OPEN

Sulfate-mediated Drought Tolerance in Maize Involves Regulation at Physiological and Biochemical Levels

Muhammad Munir Usmani¹, Fahim Nawaz^{1*}, Sadia Majeed², Muhammad Asif Shehzad¹, Khawaja Shafique Ahmad³, Gulzar Akhtar⁴, Muhammad Aqib¹ & Rana Nauman Shabbir⁵

Restriction in nutrient acquisition is one of the primary causes for reduced growth and yield in water deficient soils. Sulfur (S) is an important secondary macronutrient that interacts with several stress metabolites to improve performance of food crops under various environmental stresses including drought. Increased S supply influences uptake and distribution of essential nutrients to confer nutritional homeostasis in plants exposed to limited water conditions. The regulation of S metabolism in plants, resulting in synthesis of numerous S-containing compounds, is crucial to the acclimation response to drought stress. Two different experiments were laid out in semi-controlled conditions to investigate the effects of different S sources on physiological and biochemical mechanisms of maize (*Zea mays* L. cv. P1574). Initially, the rate of S application in maize was optimized in terms of improved biomass and nutrient uptake. The maize seedlings were grown in sandy loam soil fertigated with various doses (0, 15, 30 and 45 kg ha⁻¹) of different S fertilizers viz. K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄. The optimized S dose of each fertilizer was later tested in second experiment to determine its role in improving drought tolerance of maize plants. A marked effect of S fertilization was observed on biomass accumulation and nutrients uptake in maize. In addition, the optimized doses significantly increased the gas exchange characteristics and activity of antioxidant enzymes to improve yield of maize. Among various S sources, application of K₂SO₄ resulted in maximum photosynthetic rate (43%), stomatal conductance (98%), transpiration rate (61%) and sub-stomatal conductance (127%) compared to no S supply. Moreover, it also increased catalase, guaiacol peroxidase and superoxide dismutase activities by 55, 87 and 65%, respectively that ultimately improved maize yield by 33% with respect to control under water deficit conditions. These results highlight the importance of S fertilizers that would likely be helpful for farmers to get better yield in water deficient soils.

Maize (*Zea mays* L.) is one of the major cereal crops that provide food for humans and feed for livestock. The demand for maize seed has increased significantly during past few decades due to its consumption in poultry feed and wet milling industry. The importance of maize as a food crop is well recognized and is used as a staple food in many parts of the world¹. Maize seed is an abundant source of energy as 100 g seed provides 365 kilocalories of energy². However, it is an extensive nutrient crop and excessive use of fertilizers to obtain high yield has resulted in the depletion of nutrients particularly sulfur (S) in soils³. Moreover, water shortage due to climate change may induce further losses in maize production in future.

Adaptation of maize to limited water conditions has received great interest from farmers, researchers and policy makers considering its importance in nutritional food security. Since maize requires large quantities of water to complete its life cycle, water deficiency at critical growth stages significantly reduces maize yield⁴. Exposure to drought stress induces marked changes in water status, chlorophyll content and photosynthetic apparatus of

¹Department of Agronomy, MNS University of Agriculture, Multan, Pakistan. ²Department of Agronomy, University College of Agriculture and Environmental Sciences, Bahawalpur, Pakistan. ³Department of Botany, University of Poonch, Rawalakot, 12350, Pakistan. ⁴Department of Horticulture, MNS University of Agriculture, Multan, Pakistan. ⁵Department of Agronomy, Faculty of Agriculture, Bahauddin Zakariya University, Multan, Pakistan. *email: fahim5382@gmail.com

Physical Characteristics		Chemical Characteristics					
Texture	Saturation percentage	pH	Organic matter (%)	Nitrogen (mg kg ⁻¹)	Phosphorus (mg kg ⁻¹)	Potassium (mg kg ⁻¹)	Sulfur (mg kg ⁻¹)
Sandy loam	24	8.1	0.79	102	8.50	240	2.1

Table 1. Physicochemical characteristics of soil used for the pot experiments.

plants⁵. It influences water use efficiency and biomass accumulation in plants⁶. Drought induced reduction in nutrients absorption, redistribution and transport adversely affects the plant production^{7,8}. Due to utilization of large amount of nutrients, maize is considered sensitive to nutrient deficiency³, which may be further aggravated by limited water conditions. Increased deficiency of nutrients in agricultural soils is considered one of the major factors for reduced maize yield⁹.

Sulfur (S) is recognized as the fourth major nutrient after nitrogen (N), phosphorus (P) and potassium (K). It not only improves crop yield but also influences the quality due to its key role in protein synthesis. It is main constituent of proteins, thioredoxin (TRx), methionine (Met), cysteine (Cys), vitamins (Vit), sulfo-lipids (SL) and iron-sulfur (Fe-S) clusters system that play important role in regulation of physiological metabolism of plants¹⁰. Plants uptake S in metabolically inactive form known as sulfate (SO₄²⁻) from soil surface. It is reduced into sulfide (S⁻²) and assimilated into cysteine (Cys) by the activity of ATP-sulfuryase¹¹. A variety of S compounds such as glutathione (Glu), Met, phytochelatin (PCs) are synthesized from Cys-residues which play an important role in alleviating the drastic effects of environmental stresses like drought¹². Interestingly, S is the only macronutrient that accumulates in the xylem sap of water stressed maize plants¹³. Recent research suggests a coordinated action of several drought-responsive stress metabolites with S assimilation in plants exposed to drought stress¹⁴. For example, abscisic acid (ABA) induced closure of stomata is directly linked with S metabolism in maize¹³. The increased S demand in drought-stressed plants reflects the regulatory importance of S in ABA signalling and detoxification of reactive oxygen species (ROS)¹⁵. The highly reductive glutathione or GSH scavenges ROS such as OH[•], O^{•-2} and H₂O₂ through activation of enzymatic antioxidants viz. catalase (CAT), guaiacol peroxidase (GPX) and superoxide dismutase (SOD). Both CAT and GPX eliminate excess H₂O₂ by generating H₂O and O₂, whereas SOD prevents OH[•] formation by removing O^{•-2}^{16,17}. Additionally, S metabolism is linked to polyamine and ethylene through salvage pathway involved in maize response to drought stress^{3,11}.

Studies involving the use of S fertilizers to improve crop produce and productivity are well documented^{18–22}. However, the questions pertaining to comparative effects of S fertilizers on uptake and metabolism of other nutrients have largely remained unanswered. It is momentous to unravel the effects of S on mineral elements particularly nitrogen (N), potassium (K) and phosphorus (P). Evidence suggests that decrease in SO₄²⁻ availability during drought may restrict nitrate (NO₃⁻) uptake due to limited CO₂ fixation and decreased flux of SO₄²⁻ into cysteine in maize¹¹. Therefore, a balanced N:S ratio is essential to obtain high yield and quality in cereal crops²³. Remarkably, rhizospheric S availability regulates K content in shoot indicating that K⁺ acts as counter-ion to compensate for SO₄²⁻ deficiency in soil²⁴. Likewise, remobilization of vacuolar SO₄²⁻ during S deprivation was compensated osmotically by accumulation of NO₃⁻ and phosphate (PO₄³⁻) in vacuole to sustain plant growth²⁵.

A constant decline of water resources, due to climate change, is one of the major threats to the future food security of ever increasing world population. The challenge of water scarcity is more urgent than ever: acute water shortage pose serious threats to productivity of major food crops; decreasing water flows put us in seriously water-scarce countries. The frequent shortage of water and deterioration of eco-environment due to progressive global climate change have greatly influenced agricultural production in arid and semi-arid regions of the world. Adoption of drought mitigation approaches such as selection for physiologically efficient S fertilizers may have value in management programs aimed at improving drought stress tolerance to increase grain yield of food crops like maize. In this study, we hypothesized that S induced improvement in drought tolerance may be attributed to increased photosynthetic activity and activation of antioxidant machinery. To test this hypothesis, we firstly optimized doses for S fertilization in maize seedlings that were later used in second experiment to evaluate the effects of S on physiological and biochemical processes of maize under drought stress.

Materials and Methods

Experimental material and conditions. Two pot experiments were conducted in wire house of MNS-University of Agriculture, Multan (MNS-UAM), Pakistan using completely randomized design with factorial arrangement and three replications. Seeds of indigenous maize hybrid viz. P1574 characterized as drought sensitive by Majeed *et al.*²⁶ were obtained from local seed dealer of Pioneer Pakistan Pvt. Ltd. It is a highly digestible spring maize hybrid also used for silage purposes by local farmers. The seeds were initially treated with recommended doses of Topsin-M-70-WP (fungicide) and Imidacloprid (insecticide) for disinfection. The pots were filled with sandy loam soil collected from research field area of MNS-UAM. Before filling the pots, soil samples were randomly taken from collected soil to determine the physicochemical characteristics following the procedure reported by Jackson and Barak²⁷ (Table 1).

Pot experiment-I. First pot experiment was carried out to optimize sulfur (S) dose for selected maize hybrid using different S sources. Randomly selected healthy, uniform ten seeds were sown in plastic pots of 10 kg capacity of soil (25 cm diameter × 45 cm length). The seedlings were thinned later and only five seedlings were maintained after emergence in each pot. Nutrient solutions containing N, P and K were applied as fertilizers at the start of the experiment using urea (0.6 kg per pot), diammonium phosphate (0.3 kg per pot) and potassium oxide (0.2 kg per

pot). Sulfur (S) fertilization was done one week after seedling emergence (V4, four leaf stage) through fertigation using various sources of S viz. K_2SO_4 , $CuSO_4$, $FeSO_4$ and Na_2SO_4 applied at different rates of 15, 30 and 45 kg ha⁻¹ (75, 150 and 225 mg per pot). All pots were weighed daily to estimate water lost through evapotranspiration and supplied with required amount of water. After five weeks of seedling emergence (V10), the seedlings were harvested for estimation of biomass attributes and later dried in an oven at 65 °C for at least 72 h to record dry weight and NPK analysis.

Pot experiment-II. The second pot experiment was conducted to evaluate the effects of optimized S doses on physiological and biochemical processes of maize under drought stress. Ten seeds of same maize hybrid were sown in earthen pots (diameter 45 cm × length 60 cm) filled with 24 kg soil. Only three seedlings per pot were maintained after emergence, which were later reduced to only one healthy seedling in each pot. Recommended doses of P and K (80 kg ha⁻¹ each) and 1/8th N (120 kg ha⁻¹) were fertigated at the time of sowing using diammonium phosphate, potassium oxide (0.96 g per pot) and urea (0.18 g per pot), whereas remaining N was applied at in three equal split doses of 0.42 g per pot as described by Naeem *et al.*²⁸.

Drought stress and sulfur fertilization. Drought stress was imposed one week after seedling emergence by keeping one set of plants (normal plants) at 100% water holding capacity (WHC), whereas water stressed plants were kept at 30% WHC based on gravimetric method as described by Nachabe²⁹. The soil moisture content was measured daily using soil moisture meter ML-3 Theta Probe (Delta-T Devices, United Kingdom) and maintained by adding the amount of water lost through evapotranspiration.

Sulfur fertilization was done through fertigation, before initiation of drought stress, using optimized doses of K_2SO_4 (30 kg ha⁻¹), Na_2SO_4 (30 kg ha⁻¹), $CuSO_4$ (45 kg ha⁻¹) and $FeSO_4$ (45 kg ha⁻¹). The youngest mature leaves from each experimental unit were selected for the estimation of water status, SPAD value and activity of antioxidative enzymes at tasseling (VT) stage. The plants were harvested at physiological maturity and data regarding yield attributes was recorded from harvested plant material following standard procedures.

Determination of NPK content. The above ground plant material including leaves (0.5 g) was dried in an oven and later grounded using Willey mill. The dried material was used for the determination of N, P and K content following the method described by Wolf³⁰. Briefly, the powdered plant tissue was acid-digested with 5 ml of H_2SO_4 using BD50 digestion block (SEAL Analytical, Malaysia). Then 2 ml of H_2O_2 was added in tubes and heated at 350 °C for three and half hours until fumes were produced. Volume of extract was maintained by adding distilled water up to 50 ml in volumetric flask. The extract was filtered with Whatman-40 filter paper and N was determined using Kjeldhal method. The vanadium molybdate yellow colorimetric method was used for P determination, whereas K content was assayed using flame photometer (Sherwood M410, UK).

Estimation of leaf relative turgidity and SPAD value. The detached youngest, fully expanded leaf was weighed immediately to record fresh weight (FW) and then dipped in distilled water for 24 h at 4 °C. Later, the leaves were taken out from distilled water, wiped with tissue paper and weighed to determine turgid weight (TW). Then same leaves were placed in an oven for 72 h at 65 °C to record dry weight (DW). Leaf relative turgidity (RT) was measured using following formula reported by Barrs³¹:

$$RT = [(FW - DW)/(TW - DW)] \times 100$$

The fully expanded young leaves were used to estimate leaf chlorophyll content expressed as SPAD value. The observations were made early in the morning between 9.00 and 11.00 a.m. using chlorophyll meter (SPAD-502, Minolta Corp.).

Gas exchange measurements. The gas exchange characteristics were measured with a CIRAS-3 portable open-flow gas exchange system (PP Systems, Amesbury, USA). The system was used to record net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and sub-stomatal conductance (C_i) of uppermost fully expanded leaf of each seedling. The chamber was adjusted at 100 mL min⁻¹ mL airflow rate, 1200 μ mol·m⁻²·s⁻¹ density of photosynthetic photon flux, 390 ± 5 μ mol·mol⁻¹ CO₂ concentration rate, 99.9 kPa atmospheric pressure.

Assay of antioxidative enzymes. Leaf samples were homogenized (1:5) in pestle and mortar using 50 mM Na_2HPO_4 , pH 7.0 containing 1 M Sodium chloride, 1 mM EDTA and 1% polyvinylpyrrolidone. Enzyme activities were determined using supernatant of enzyme extract (EE) produced from sample solution after centrifugation (20,000 × g, 15 min) at 4 °C.

Catalase activity (CAT) activity was determined by monitoring the degradation of hydrogen peroxide (H_2O_2) according to Chance and Maehly³². Enzyme extract (200 μ L) was added in reaction mixture (1.8 mL), which contained H_2O_2 (30 mM) and K-P-buffer (50 mM) of 7.0 pH. The decline in H_2O_2 was estimated as a reduction in optical density at 240 nm.

The procedure reported by Urbanek *et al.*³³ was followed to estimate guaiacol peroxidase (GPX) activity. The reaction mixture (2 mL) was prepared by mixing 50 mM K-P buffer (pH 6.8) with H_2O_2 (20 mM) and guaiacol (20 Mm). The mixture was incubated at room temperature for 10 min. The reaction was stopped by adding 0.5 mM H_2SO_4 (5%) and absorbance was measured at 480 nm.

The enzymatic activity of superoxide dismutase (SOD) was recorded following the method of Van Rossun *et al.*³⁴. The enzyme extract (50 μ L) containing 50 mM K-P-buffer (pH 7.8). was added to 2 μ M riboflavin, 75 μ M nitroblue tetrazolium chloride (NBT), 100 μ M EDTA and 13 mM L-methionine. The reaction was initiated in a chamber under illumination of a 30 W-fluorescent lamp for 10 min. The blue color formazane, produced as a

Observations	K ₂ SO ₄ (kg ha ⁻¹)				FeSO ₄ (kg ha ⁻¹)				CuSO ₄ (kg ha ⁻¹)				Na ₂ SO ₄ (kg ha ⁻¹)			
	0	15	30	45	0	15	30	45	0	15	30	45	0	15	30	45
SL	26.0 ± 1.76 ^g	39.7 ± 4.17 ^{c-f}	57.0 ± 2.12 ^a	54.3 ± 3.9 ^{ab}	28.3 ± 1.8 ^{fg}	38.3 ± 2.65 ^{c-f}	40 ± 1.8 ^{c-f}	46.7 ± 2.37 ^{a-c}	31.0 ± 1.17 ^{e-g}	31.0 ± 1.17 ^{e-g}	39.0 ± 1.17 ^{c-f}	43.0 ± 2.7 ^{b-e}	30.0 ± 1.15 ^{fg}	38.6 ± 1.5 ^{c-f}	44.7 ± 4.5 ^{b-d}	32.7 ± 2.06 ^{d-g}
RL	27.7 ± 2.44 ^c	36.0 ± 3.57 ^{a-c}	47.3 ± 6.12 ^a	45.0 ± 4.07 ^{ab}	25.66 ± 1.48 ^e	36.3 ± 1.8 ^{a-c}	34.3 ± 2.96 ^{a-c}	35.3 ± 2.37 ^{a-c}	29.0 ± 0.58 ^c	28.0 ± 1.17 ^c	34.0 ± 1.76 ^{a-c}	34.0 ± 2.56 ^{a-c}	28.7 ± 0.89 ^c	33.7 ± 1.48 ^{a-c}	39.0 ± 4.11 ^{a-c}	31.3 ± 1.79 ^{b-c}
SFW	2.60 ± 0.38 ^{bc}	3.40 ± 0.35 ^{bc}	5.82 ± 0.38 ^a	5.82 ± 0.45 ^a	2.64 ± 0.32 ^{bc}	3.40 ± 0.53 ^{bc}	3.66 ± 0.17 ^{bc}	4.30 ± 0.54 ^{ab}	2.34 ± 0.06 ^c	3.61 ± 0.33 ^{bc}	3.61 ± 0.26 ^{bc}	4.30 ± 0.36 ^{bc}	2.69 ± 0.23 ^{bc}	3.61 ± 0.27 ^{bc}	3.91 ± 0.32 ^{bc}	3.51 ± 0.27 ^{bc}
RFW	2.07 ± 0.08 ^{fg}	2.29 ± 0.08 ^{e-g}	3.49 ± 0.07 ^{ab}	3.27 ± 0.06 ^{bc}	2.11 ± 0.09 ^{e-g}	2.40 ± 0.12 ^{e-g}	2.88 ± 0.10 ^{cd}	3.75 ± 0.12 ^a	2.15 ± 0.09 ^{e-g}	2.28 ± 0.07 ^{e-g}	2.57 ± 0.08 ^{de}	3.21 ± 0.11 ^{bc}	2.05 ± 0.10 ^g	2.53 ± 0.08 ^{d-f}	3.10 ± 0.10 ^{bc}	2.96 ± 0.09 ^{cd}
SDW	0.47 ± 0.04 ^c	0.54 ± 0.02 ^c	1.11 ± 0.22 ^a	0.97 ± 0.10 ^{ab}	0.50 ± 0.06 ^c	0.55 ± 0.03 ^{bc}	0.58 ± 0.05 ^{bc}	0.67 ± 0.02 ^{bc}	0.54 ± 0.04 ^c	0.58 ± 0.05 ^{bc}	0.59 ± 0.06 ^{bc}	0.71 ± 0.03 ^{a-c}	0.44 ± 0.05 ^c	0.71 ± 0.06 ^{a-c}	0.83 ± 0.03 ^{a-c}	0.60 ± 0.05 ^{bc}
RDW	1.04 ± 0.03 ^{de}	1.19 ± 0.04 ^{c-e}	1.77 ± 0.09 ^a	1.72 ± 0.09 ^a	0.96 ± 0.11 ^c	1.08 ± 0.06 ^{de}	1.35 ± 0.07 ^{b-d}	1.75 ± 0.08 ^a	1.04 ± 0.04 ^{de}	1.14 ± 0.07 ^{de}	1.32 ± 0.05 ^{b-e}	1.56 ± 0.06 ^{ab}	1.02 ± 0.08 ^{de}	1.15 ± 0.08 ^{de}	1.55 ± 0.06 ^{a-c}	1.57 ± 0.06 ^{ab}

Table 2. Biomass attributes of maize seedlings applied with various sources of sulfur fertilizers viz. K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄ at 0, 15, 30 and 45 kg ha⁻¹. Shoot length = SL, Root length = RL, Shoot fresh weight = SFW, Root fresh weight = RFW, Shoot dry weight = SDW and Root dry weight = RDW. Different alphabets represent significant difference between mean values ± standard error.

result of photoreduction caused by nitroblue tetrazolium (NBT), was noted as increase in absorbance at 560 nm. No enzyme extract was added in reaction mixture used as control and kept in the dark. One SOD unit was defined as the quantity of enzyme required to inhibit 50% photoreduction of the NBT.

Economic analysis. A benefit-cost analysis was carried out to conclude the economic feasibility of various sulfate fertilizers to alleviate the drastic influence of drought stress in maize. The rate of each S fertilizer i.e. K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄ used in this experiment was 30, 45, 45 and 30 kg ha⁻¹ respectively. The cost of K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄ was 176, 1200, 1200 and 65 kg⁻¹ in PKR (Pakistani rupees) respectively. Land preparation, sowing seed, irrigation, fertilizing, plant protection measures (insecticide, herbicide), harvesting and threshing was included in fixed cost. The gross income was estimated by using prevailing average marketing price in Pakistan, PKR 900 per 40 kg.

Statistical analysis. All collected data were analyzed statistically using Fisher's Analysis of Variance (ANOVA) technique on computer programme Statistix (version 9.1). The treatment means were compared using Tukey's *post-hoc* test at 0.05 probability level.

Results

Biomass accumulation. Application of S fertilizers significantly ($P < 0.01$) influenced biomass attributes i.e. shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) of maize seedlings (Suppl. Table 1). Maize seedlings fertilized with K₂SO₄ at 30 kg ha⁻¹ exhibited the highest increase in SL (119.24%) RL (71.08%), SFW (124.46%), RFW (68.59%), SDW (136.17%) and RDW (62.43%) compared to control (no S supply). Higher concentration of K₂SO₄ and Na₂SO₄ significantly ($P < 0.01$) reduced biomass accumulation, whereas application of CuSO₄ and FeSO₄ at 45 kg ha⁻¹ gave maximum values for these attributes with respect to no S supply (Table 2).

NPK content. Fertigation with various S sources markedly ($P < 0.01$) affected NPK accumulation in shoots of maize seedlings (Suppl. Table 1). Excess S supply significantly ($P < 0.001$) improved N content and resulted in mean maximum N accumulation in seedlings treated with K₂SO₄ (53.67 mg kg⁻¹), FeSO₄ (53.0 mg kg⁻¹) and CuSO₄ (51.50 mg kg⁻¹) at 45 kg ha⁻¹. However, high dose of Na₂SO₄ i.e. 45 kg ha⁻¹ reduced N content by 23% compared to 30 kg ha⁻¹ that exhibited maximum N accumulation (52.0 mg kg⁻¹) in shoot (Fig. 1a). Similar trend was noted for shoot P and K content as application of K₂SO₄, FeSO₄ and CuSO₄ at high S dose of 45 kg ha⁻¹ significantly ($P < 0.05$) improved P accumulation by 53, 51 and 48% in comparison with no S supply, whereas mean maximum P content (15.25 mg kg⁻¹) using Na₂SO₄ was recorded at 30 kg ha⁻¹ and it declined significantly by 22% at higher dose of 45 kg ha⁻¹ (Fig. 1b). Similarly, relative to no S treatment, maize seedlings fertigated with K₂SO₄, FeSO₄ and CuSO₄ at 45 kg ha⁻¹ exhibited 56, 54 and 49% higher K content in shoot. In maize seedlings treated with Na₂SO₄, the highest K content (102.50 mg kg⁻¹) was recorded at 30 kg ha⁻¹ in comparison with 45 kg ha⁻¹ that considerably reduced shoot K content by 17% (Fig. 1c).

Leaf relative turgidity and SPAD value. The main effects of drought stress (D) and sulfur sources (S) were found significant ($P < 0.01$) for leaf relative turgidity (RT) and chlorophyll content (Chl) (Suppl. Table 2). Drought stress caused a substantial decline ($P < 0.01$) in RT and Chl of maize seedlings by 13 and 10%, respectively. Application of S fertilizers significantly ($P < 0.01$) ameliorated the drastic effects of drought stress. Maize plants treated with FeSO₄ and CuSO₄ maintained the highest RT i.e. 86.78 and 85.68%, respectively under water deficit conditions. Similarly, K₂SO₄ and Na₂SO₄ application increased RT by 12 and 10% compared to no S supply in maize under drought stress (Fig. 2a). The highest Chl was recorded in maize plants fertilized with K₂SO₄ under normal (42.67 SPAD value) as well as drought stress (40.73 SPAD value) conditions. Interestingly, CuSO₄

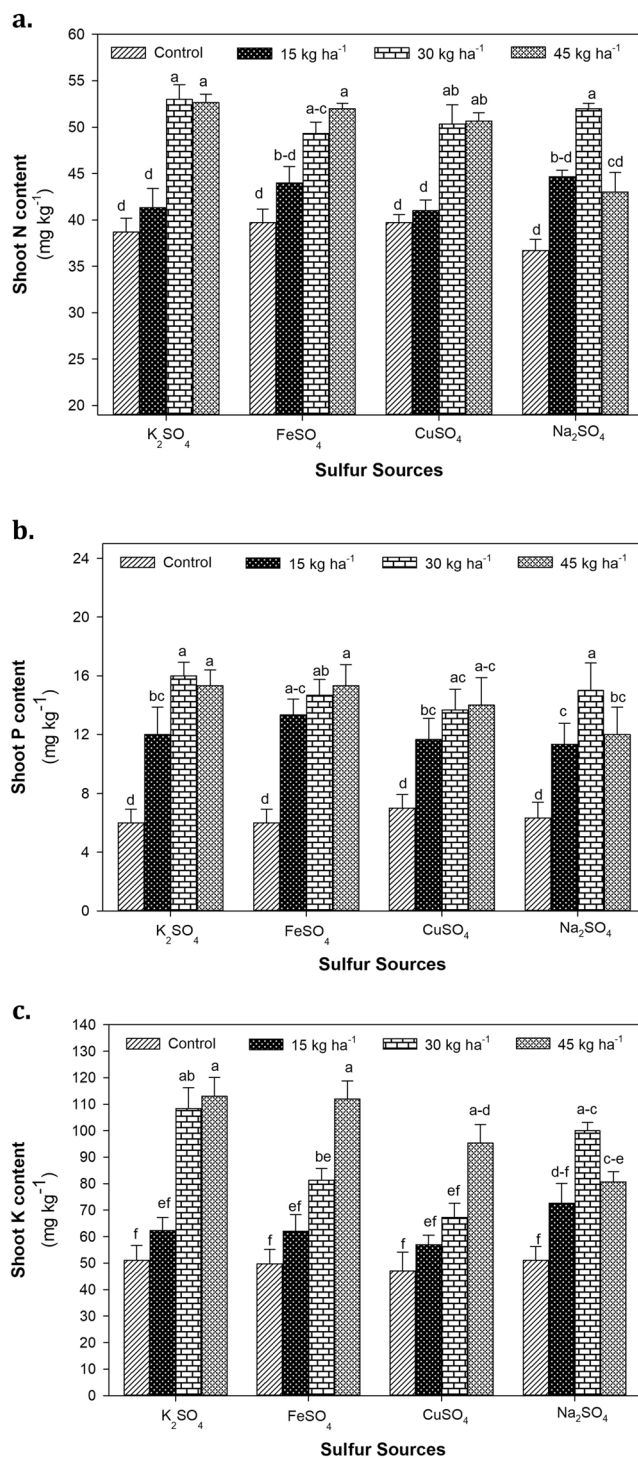


Figure 1. (a) The nitrogen (N), (b) phosphorous (P) and (c) potassium (K) content of maize seedlings affected by the application of various doses (0, 15, 30 and 45 kg ha⁻¹) of sulfate fertilizers (K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄). The mean values with different letters indicate significant difference ($P \leq 0.05$), according to *post hoc* Tukey's test.

application negatively influenced Chl and resulted in the lowest values along with control in both normal (37.17 SPAD value) and water stressed (31.78 SPAD value) maize plants (Fig. 2b).

Gas exchange characteristics. Exposure to drought stress considerably ($P < 0.001$) reduced A (37.75%), E (52.47%), g_s (31.24%) and C_i (65.49%) of maize plants compared to normal conditions, irrespective of S application (Suppl. Table 2). Exogenous S fertilization with K₂SO₄ resulted in the highest increase in A (43%) and E

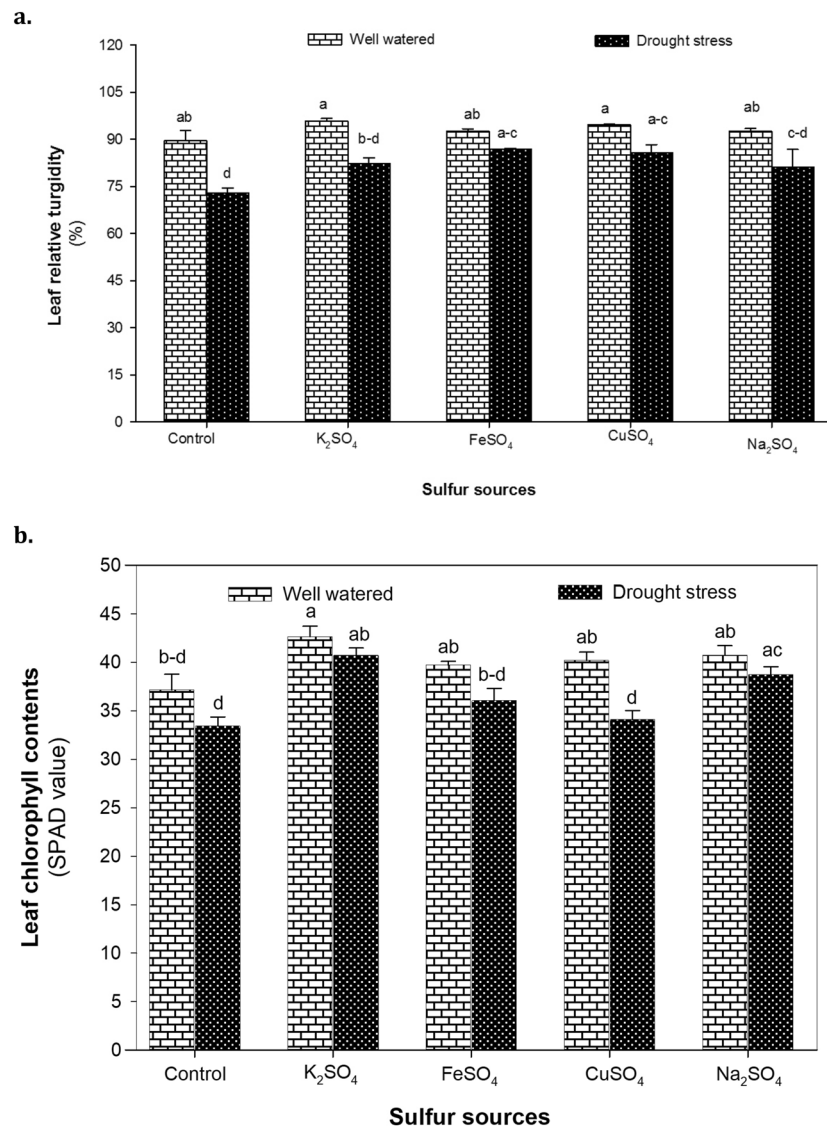


Figure 2. (a) The leaf relative turgidity (RT) and (b) the chlorophyll content (Chl) of maize plants affected by the application of optimized doses of sulfate fertilizers (K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄) under normal (100% WHC) and drought stress (30% WHC) conditions. The mean values with different letters indicate significant difference ($P \leq 0.05$), according to *post hoc* Tukey's test.

(61%) of maize plants subjected to drought stress (Fig. 3a,b). A marked increase of 43 and 23% in A and E, respectively was also observed by FeSO₄ application under water deficit conditions (Fig. 3a,b).

The regulation of stomatal apparatus was also significantly ($P < 0.001$) influenced by the application of various S fertilizers (Suppl. Table 2). Application of K₂SO₄ effectively improved g_s and C_i of maize plants by 98 and 127%, respectively under drought stress. Fertilization with FeSO₄ and Na₂SO₄ also increased g_s by 53% (Fig. 3c), whereas application of Na₂SO₄ enhanced C_i by 105% under water deficit conditions (Fig. 3d).

Antioxidant enzyme activities. Drought stress markedly ($P < 0.001$) markedly increased the activities CAT (131%), GPX (79%) and SOD (137%) compared to well-watered conditions (Suppl. Table 2). The highest CAT activity (253.33 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$) was noted in leaves of water stressed maize plants supplemented with K₂SO₄ and did not differ significantly from FeSO₄ (237.0 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$) and Na₂SO₄ (215.0 $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$) (Fig. 4a). Similarly, the maize plants treated with K₂SO₄, FeSO₄ and Na₂SO₄ exhibited 87, 34 and 30% higher GPX activity, respectively in comparison to plants with no S application (control) (Fig. 4b). Application of S fertilizers effectively enhanced SOD activity ($P < 0.001$) and gave the maximum increase (65%) in plants supplemented with K₂SO₄ under water deficit conditions. Likewise, Na₂SO₄, FeSO₄ and CuSO₄ upregulated SOD activity by 43, 31 and 29% compared to control under drought stress (Fig. 4c).

Yield and yield components. The main effects of D and S were significant ($P < 0.01$) for all maize yield attributes viz. kernels per cob (KC), 1000-grain weight (GW), grain yield (GY) and biological yield (BY), however,

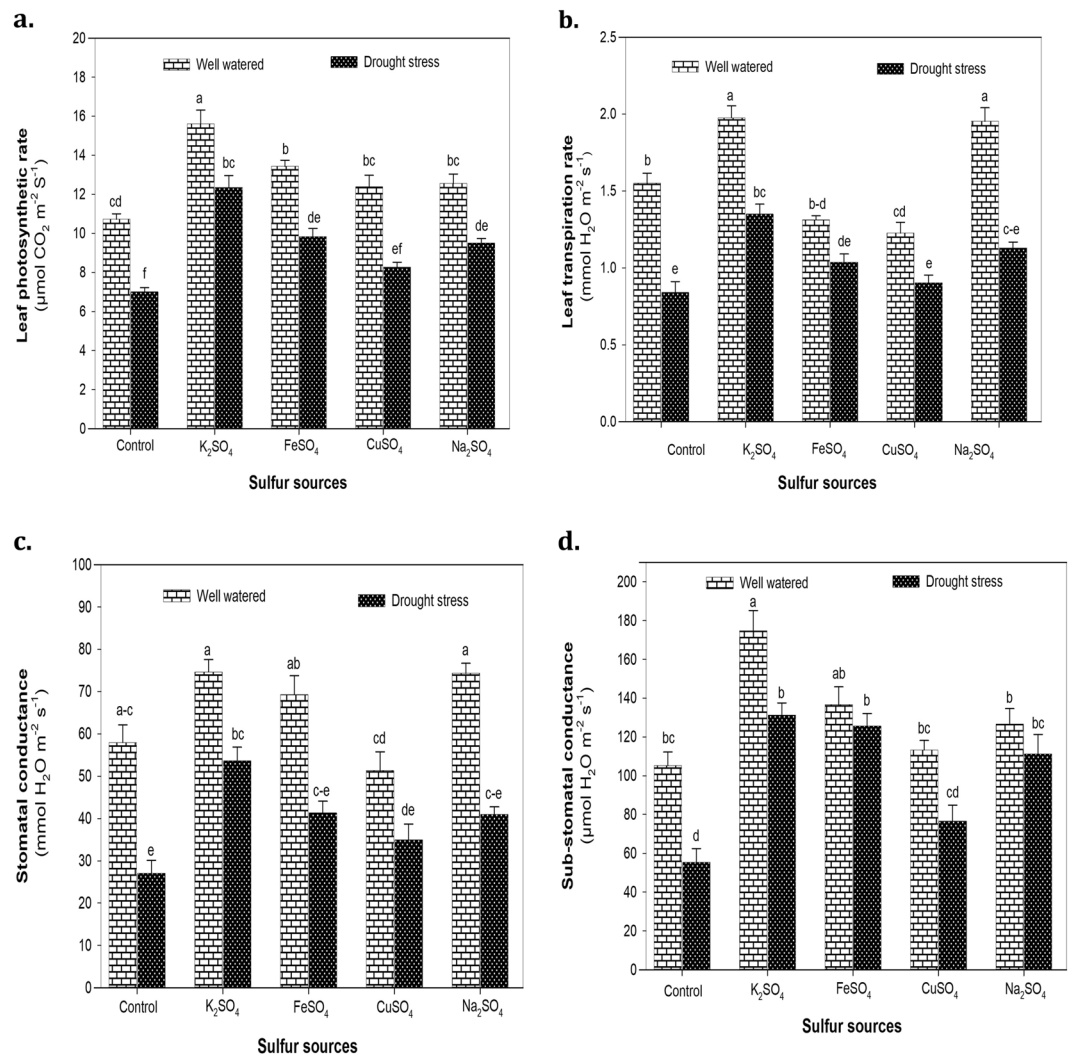


Figure 3. (a) The leaf photosynthetic rate (A), (b) transpiration rate (E), (c) stomatal conductance (g_s) and (d) sub-stomatal conductance (C_i) of maize plants affected by the application of optimized doses of sulfate fertilizers (K_2SO_4 , $FeSO_4$, $CuSO_4$ and Na_2SO_4) under normal (100% WHC) and drought stress (30% WHC) conditions. The mean values with different letters indicate significant difference ($P \leq 0.05$), according to *post hoc* Tukey's test.

significant ($P < 0.05$) two way interaction ($D \times S$) was only observed for KC, GW and GY (Suppl. Table 3). Exposure to drought stress considerably reduced KC, GW, GY and BY by 24, 19, 23 and 41%, respectively with respect to normal conditions. The highest increase in KC (41%) and GW (27%) was recorded by K_2SO_4 application compared to control (no S supply) under drought stress conditions (Fig. 5a,b). Interestingly, $CuSO_4$ application reduced GW by 11% in water stressed maize plants compared to normal ones (Fig. 5b). Similar trend was observed for GY and BY as K_2SO_4 supply significantly increased GY by 17 and 33% compared to control under normal and water deficit conditions, respectively (Fig. 5c). Likewise, it improved BY by 15 and 21% in normal and water stressed maize plants (Fig. 5d). A marked increase in GY and BY was also observed by $FeSO_4$ (26 and 15%) and Na_2SO_4 (13 and 7%) application under drought stress conditions (Fig. 5c,d).

Application of various sulfate fertilizers improved the net benefit cost ratio, however, high market cost of $FeSO_4$ resulted in negative net income. Similarly, $CuSO_4$ and Na_2SO_4 induced toxicity also negatively influenced the net benefit ratio, whereas K_2SO_4 application was found to be most economical for improving maize yield under water deficit conditions (Table 3).

Discussion

This study presents data to interpret firstly the effects of various S fertilizers and their doses on biomass accumulation and NPK uptake in maize seedlings. Secondly, the physiological and biochemical significance of S fertilizers in drought stress tolerance, with a particular focus on regulation of gas exchange characteristics and enzymatic antioxidants to improve maize yield will be discussed. Exogenous S application significantly ($P < 0.01$) enhanced SL, RL, SFW, RFW, SDW and RDW of maize seedlings (Suppl. Table 1). As biomass attributes were increased by

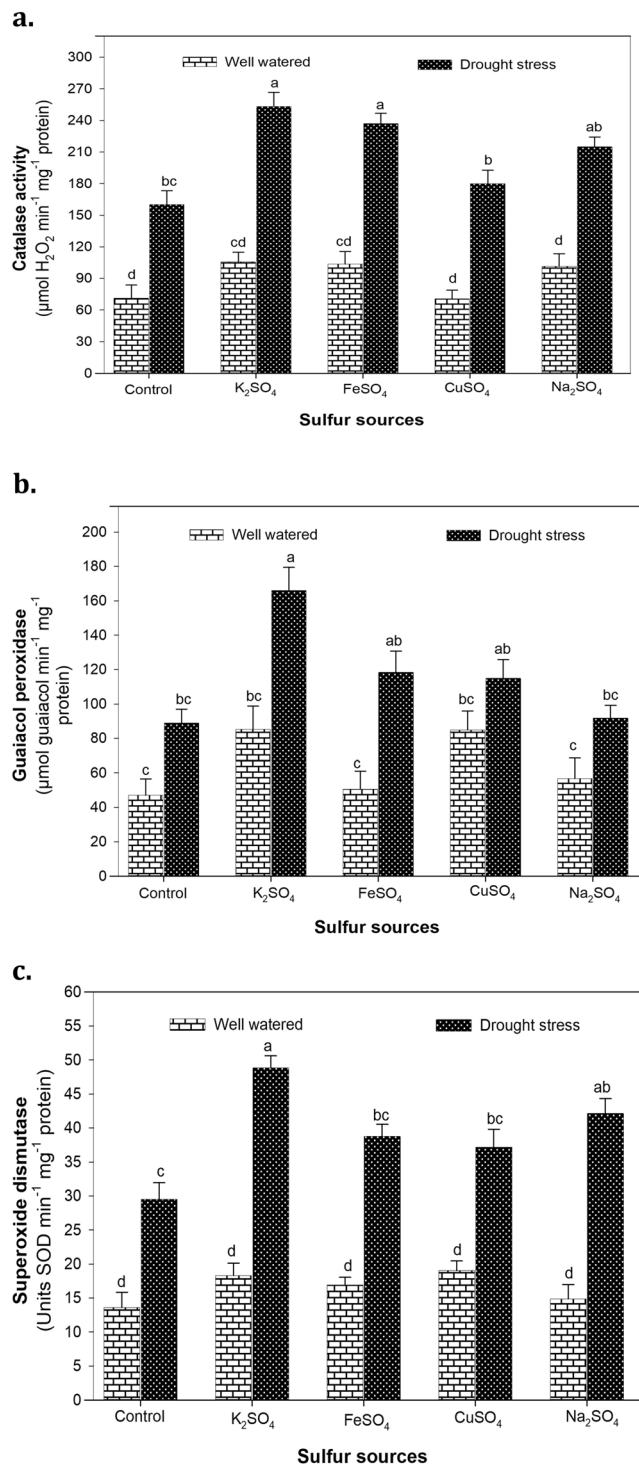


Figure 4. (a) The catalase (CAT), (b) guaiacol peroxidase (GPX) and (c) superoxide dismutase (SOD) activity of maize plants affected by the application of optimized doses of sulfate fertilizers (K₂SO₄, FeSO₄, CuSO₄ and Na₂SO₄) under normal (100% WHC) and drought stress (30% WHC) conditions. The mean values with different letters indicate significant difference ($P \leq 0.05$), according to *post hoc* Tukey's test.

S fertilization, it may be concluded that S availability enhances photosynthetic rate and stimulates translocation of photosynthates towards sink²². However, this stimulating effect of S fertilizers was found to be dose dependent and considerable variation was observed among various doses (Table 2). High doses of K₂SO₄ and Na₂SO₄ (45 kg ha⁻¹) showed adverse effects on biomass accumulation as reported earlier in studies involving maize³⁵ and *Brassica rapa*³⁶. The possible general explanation for this reduction may be the altered toxicity of sulfate anion (SO₄²⁻) by the presence of Na⁺³⁷.

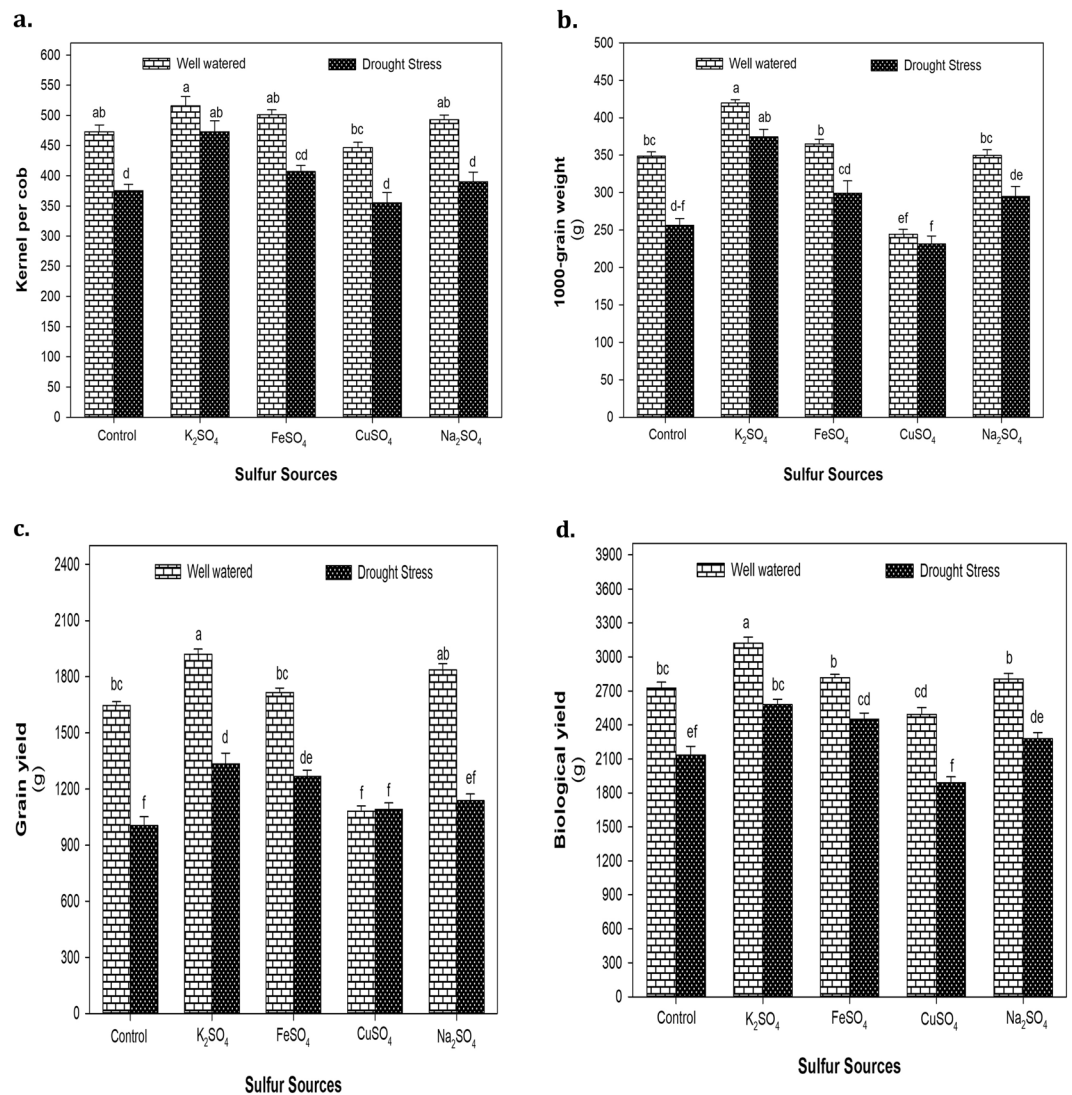


Figure 5. (a) The number of kernels per cob (KC), (b) 1000-grain weight (GW), (c) grain yield (GY) and (d) biological yield (BY) of maize plants affected by the application of optimized doses of sulfate fertilizers (K_2SO_4 , $FeSO_4$, $CuSO_4$ and Na_2SO_4) under normal (100% WHC) and drought stress (30% WHC) conditions. The mean values with different letters indicate significant difference ($P \leq 0.05$), according to *post hoc* Tukey's test.

Treatment	Total expenditure (*PKR ha ⁻¹)		Gross income (PKR ha ⁻¹)		Net Income (PKR ha ⁻¹)		Benefit: cost ratio	
	Normal	Drought	Normal	Drought	Normal	Drought	Normal	Drought
Control	134394	132754	174020	97900	39626	-34854	1.29	0.74
K_2SO_4	139794	138154	229526	166100	89732	27946	1.64	1.20
$FeSO_4$	188394	186754	208560	143726	20166	-43028	1.11	0.77
$CuSO_4$	188394	186754	155540	95700	32854	-91054	0.83	0.51
Na_2SO_4	137394	135754	185174	121000	47780	-14754	1.35	0.89

Table 3. Effect of various sulfate fertilizers application on net income and benefit-to cost ratio of maize under normal and drought conditions. *1.00 USD = 159.7 PKR.

Addition of sufficient amount of nutrients is first agricultural measure to increase crop performance³⁸, however, the uptake of mineral nutrients is considerably influenced by the interactions between metal ions at different physiological levels²⁴. This is particularly true for S as drought stress results in low SO_4^{2-} absorption and its subsequent translocation to leaves that also limits NO_3^- translocation, consequently reducing nitrogen use efficiency³⁹. Similarly, S-deprivation influences K accumulation in shoots providing evidence that K^+ acts as counter-cation in the absence of SO_4^{2-} in leaf tissue^{24,40}. The results in present study showed that low water availability considerably

($P < 0.01$) restricts NPK accumulation in shoots of maize seedlings (Fig. 1a–c). Drought induced restriction in nutrient accumulation might be associated to reduced E , resulting in limited root absorbing power to uptake N, P and K⁴¹. Drought stress drastically influences maize at early growth stages by changing root maintenance system and architecture⁴². Recent studies by Studer *et al.*⁴³ confirmed that the seedling growth of maize is most sensitive to nutrient deficiency, particularly under drought stress conditions. In agreement with the findings of Raza *et al.*²² in sesame, our results showed that S fertilization significantly ($P < 0.05$) increased NPK content in shoots of maize seedlings (Suppl. Table 1), this may be associated with improved nutrient acquisition and utilization of these macronutrients with S application. Shoot N content were considerably ($P < 0.001$) increased by S application (Fig. 1a) providing further evidence that these nutrients are highly inter-related³² and significantly influence protein metabolism, thereby quality of crop plants^{44,45}. Lošák *et al.*⁴⁶ found positive correlation between N and S to increase camelina seed yield in S deficient soils. They suggested that combined S and N application could be utilized as an effective approach to improve oil and protein yield. High concentration of these nutrients obtained by K₂SO₄ might also be attributed to presence of K⁺ considering the importance of this cation to improve drought tolerance in crop plants^{47,48}. Previous studies on *Eruca sativa*⁴⁹ sesame⁵⁰ and maize⁵¹ also reported an increased P and K content by S application. It may be inferred that S fertilization after biochemical oxidation produces H₂SO₄, which improves nutrient availability to plants^{52,53}. Recent report of Reich *et al.*³¹ showed that S deficiency reduced K⁺ accumulation in shoots of Chinese cabbage highlighting the role of S in xylem loading and translocation of K⁺ to the shoot. Positive effect of FeSO₄ on nutrient accumulation in maize seedlings (Fig. 1a–c) suggests the cooperative role of S and Fe in plant metabolism, for example Fe-S clusters in the electron transport chain⁵⁴. Cross talk between S and Fe uptake and metabolism in plants is particularly important because S deficiency not only limits the synthesis of Fe-S cluster proteins but also reduces the translation in general⁵⁵.

Maintenance of leaf RT and Chl indicate plant ability to tolerate water stress conditions^{28,56}. In the present study, drought induced reduction ($P < 0.001$) in leaf RT (Suppl. Table 2) might be the result of loss in turgor or impaired photosynthetic rate or combination of these factors^{57,58}. Leaf RT is often used as a criterion to determine the degree of drought stress as loss in RT leads to protoplasm dehydration⁵⁹ and decrease in cell enlargement and expansion⁶⁰. Application of S fertilizers significantly ($P < 0.01$) enhanced leaf RT (Fig. 2a) indicating that S availability restricts the movement of ions and solutes in the cells associated with reduced osmotic potential under water deficit conditions⁴⁹. High RT by S application might be associated with increased water uptake by roots as drought stress reduces translocation of newly absorbed SO₄²⁻ to shoot also reported in *B. napus*⁶¹.

Biosynthesis and maintenance of photosynthetic pigments is considered to be a potential indicator of drought tolerance in crop plants⁴. Measurement of total Chl using chlorophyll meters such as SPAD-502 is an effective, non-destructive and inexpensive method that provides absolute values of Chl per unit leaf area⁶². A marked decline in SPAD value ($P < 0.001$) was noted in S deficient maize plants (control) under drought stress (Fig. 2b), which may be due to decrease in S compounds such as Cys and Met that serve as integral component of chloroplast targeted proteins. Our findings are in line with reports of Houhou *et al.*⁶³ in *E. sativa* and Kassem *et al.*⁶⁴ in *Lycopersicon esculentum* providing further evidence that S starvation influences the coordination between light reaction and Calvin cycle in chloroplast ultimately reducing Chl in leaves. In rice, Lunde *et al.*⁶⁵ found a significant decrease in Chl of S deficient plants followed by a notable reduction in photochemical performance and decreased photosynthetic activity. Maize seedlings supplemented with CuSO₄ exhibited a significant ($P < 0.001$) decrease in Chl (Fig. 2b) that might be attributed to Cu ion toxicity leading to ultra-structural alterations and photochemical oxidation in chloroplast⁶⁶. Excess Cu inhibits carboxylase activity and causes swelling of thylakoids as reported by Ibrahim *et al.*⁶⁷ in *Gynura procumbens*.

The reduction in gas exchange attributes viz. A , E , g_s and C_i is generally considered to be the first effects of drought stress due to chlorophyll degradation and restriction in available CO₂^{68,69}. Reduced gas exchange under S deficient conditions has been previously reported in several plant species such as barley⁷⁰, mustard⁷¹ and rape⁷². In this study, application of S fertilizers considerably ($P < 0.001$) increased A (Fig. 3a) suggesting that S availability improves CO₂ assimilation and protein abundance to alleviate drought induced inhibition of photosynthetic capacity in maize plants. Adequate S-supplementation favors formation of S containing amino acids (Cys and Met) and reduces oxidative stress (high CAT, GPX and SOD activity) to stabilize Rubisco and thylakoid membrane proteins under drought stress conditions¹⁴. Compared to no S supply, application of K₂SO₄ and FeSO₄ markedly ($P < 0.001$) enhanced g_s and C_i (Fig. 3c,d) accompanied by high E (Fig. 3b) suggesting that these fertilizers influence stomatal regulation and further stabilize Fe-S clusters to improve the functioning of vital cellular processes such as photosynthesis and respiration under water deficit conditions^{73,74}. In a study involving contrasting *B. napus* genotypes (Mosa and Saturnin), Lee *et al.*³⁹ reported higher photosynthetic activity in genotype (Saturnin) with high sulfur use efficiency that ultimately contributed to better drought tolerance.

Drought stress as well as S deprivation causes metabolic imbalance that leads to oxidative burst in plant cell⁷⁵. S deficiency stimulates peroxidation of biomolecules due to excessive accumulation of reactive oxygen species (ROS) and reduced synthesis of S-containing compounds⁷⁶. In contrast to no S supply, application of S fertilizers considerably ($P < 0.001$) increased the activities of CAT, GPX and SOD (Suppl. Table 2), which was consistent with the maintenance of photosynthetic capacity following S supplementation under drought stress as reported by Ma *et al.*⁷⁷ in wheat. Adequate S supply helps to counteract the drastic effects of ROS on nucleic acids and proteins through upregulation of antioxidant enzymes such as CAT, GPX and SOD⁷⁸. These antioxidative enzymes serve as scavengers of O₂ and H₂O₂ and help to prevent the production of toxic HO⁷⁹. It is evident from the results that S mediated high antioxidant activity (Fig. 4a–c) corresponds to drought tolerance in maize. The combined action of CAT and SOD converts highly toxic O₂⁻ and H₂O₂ into molecular oxygen and water, respectively to prevent ROS induced cellular damage in plants^{41,80}. The availability of S promotes photosynthetic assimilation of SO₄²⁻ to produce Cys that may be used to synthesize Met or converted into glutathione to regulate protein and cell function⁸¹ under environmental stresses like drought¹¹. Compared to other S sources, higher antioxidant activity by K₂SO₄ application is in agreement with the recent reports of Zareei *et al.*⁸² in black grapes and Marques *et al.*⁸³ in

eggplant suggesting the coordinated action of K^+ and SO_4^{2-} to regulate photosynthesis and translocation of photosynthates from roots to shoots, thereby alleviating drought induced oxidative damage in maize plants.

The overall impact of drought stress on yield and yield attributes of maize plants was highly significant ($P < 0.001$) with no S supply (Suppl. Table 3) that might be associated with reduced translocation of sugars to developing kernels under water deficit conditions⁸⁴. This poor supply of sugars starves embryo and induces abortion of ovary ultimately affecting grain formation and yield of maize⁸⁵. Positive effects of S fertilization on yield (Fig. 5a-d) suggest that SO_4^{2-} availability stimulates movement of assimilates from phloem into the apoplast due to higher photosynthetic activity and enhanced stomatal regulation (also observed in present study) under drought stress conditions. In line with our findings, early reports of Dewal and Pareek⁸⁶ and Shahzad *et al.*⁴⁷ showed ameliorative effects of K_2SO_4 application to improve GY in water stressed wheat and maize, respectively. Our results also showed a significant effect of $FeSO_4$ application on maize yield attributes (Fig. 5a-d) as reported by Farokhi *et al.*⁸⁷ in sunflower and Heidari *et al.*⁵⁰ in sesame. The possible explanation for this increased yield by S fertilization could be the involvement of S-containing compounds in vital physiological and biochemical processes to modulate stress response under water deficit conditions⁸⁸. The reduction in GY by $CuSO_4$ application might be due to toxic effects of Cu^{2+} on photosynthetic electron transport chain resulting in protein denaturation and deactivation of antioxidant enzymes in plant cell⁸⁹.

Conclusion

The present study concludes that S starvation has a diverse impact on physiological and biochemical processes with important implications for maize yield under drought stress conditions. In contrast, S availability positively influenced the leaf water status, gas exchange characteristics and antioxidative machinery in water stressed maize plants. Among various S sources, K_2SO_4 application resulted in the maximum increase in yield providing further evidence that K^+ and SO_4^{2-} are strongly correlated to improve yield in crop plants. A marked increase in growth and yield was also noted by $FeSO_4$ fertigation indicating that application of this fertilizer influences vital cellular processes including synthesis of Fe-S cluster proteins to improve drought tolerance in maize. However, high market cost of this fertilizer resulted in negative cost:benefit ratio for this fertilizer. Similarly, Na_2SO_4 supply improved maize yield but high dose of this fertilizer induces toxicity, which may be due to accumulation of Na^+ in rhizosphere. Similarly, $CuSO_4$ application causes toxicity and significantly reduced yield compared to other S fertilizers. Moreover, these fertilizers were not found to be economical for improving yield under drought stress conditions.

Received: 16 August 2019; Accepted: 13 January 2020;

Published online: 24 January 2020

References

- Boomsma, C. R. & Vyn, T. J. Maize drought tolerance: potential improvements through arbuscular mycorrhizal symbiosis. *Field Crops Res.* **108**, 14–31 (2008).
- Nuss, E. T. & Tanumihardjo, S. A. Maize a paramount staple crop in the context of global nutrition. *Compr. Rev. Food Sci. Food Safety.* **9**, 417–436 (2010).
- Sutar, R. K., Pujar, A. M., Kumar, B. A. & Hebsur, N. S. Sulphur Nutrition in Maize-A Critical Review. *Int. J. Pure Applied Biosci.* **5**, 1582–1596 (2017).
- Naeem, M., Naeem, M. S., Ahmad, R. & Ahmad, R. Foliar-applied calcium induces drought stress tolerance in maize by manipulating osmolyte accumulation and antioxidative responses. *Pak. J. Bot.* **49**, 427–434 (2017).
- Yang, X. *et al.* Drought induced root aerenchyma formation restricts water uptake in rice seedling supplied with nitrate. *Plant Cell Physiol.* **53**, 495–504 (2012).
- Farooq, W. A. *et al.* Elemental analysis of fertilizer using laser induced breakdown spectroscopy. *Optics Spec.* **112**, 874–880 (2012).
- Rouphael, Y., Cardarelli, M., Schwarz, D., Franken P. & Colla, G. Effects of drought on nutrient uptake and assimilation in vegetable crops. In: *Plant responses to drought stress* (Ed. Aroca, R). 171–195 (Springer Nature, 2012).
- Osakabe, Y., Osakabe, K., Shinozaki, K. & Tran, L. S. P. Response of plants to water stress. *Front. Plant Sci.* **5**, 86 (2014).
- Khan, M. J., Khan, M. H., Khattak, R. A. & Jan, M. T. Response of maize to different levels of sulfur. *Commun. Soil Sci. Plant Anal.* **37**, 41–51 (2006).
- Khan, N. A. *et al.* Salinity tolerance in plants: revisiting the role of sulfur metabolites. *J. Plant Biochem. Physiol.* **2**, 120 (2014).
- Ahmad, N., Malagoli, M., Wirtz, M. & Hell, R. Drought stress in maize causes differential acclimation responses of glutathione and sulfur metabolism in leaves and roots. *BMC Plant Biol.* **16**, 247 (2016).
- Anjum, N. A. *et al.* ATP-sulfurylase, sulfur-compounds, and plant stress tolerance. *Front. Plant Sci.* **6**, 210 (2015).
- Ernst, L. *et al.* Sulphate as a xylem-borne chemical signal precedes the expression of ABA biosynthetic genes in maize roots. *J. Exp. Bot.* **61**, 3395–3405 (2010).
- Chan, K. X., Wirtz, M., Phua, S. Y., Estavillo, G. M. & Pogson, B. J. Balancing metabolites in drought: the sulfur assimilation conundrum. *Trends Plant Sci.* **18**, 18–29 (2013).
- Nawaz, F. *et al.* Reactive Sulfur Species-Key Regulators of Abiotic Stress Tolerance in Plants. In: *Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms* (Ed. Hasanuzzaman *et al.*) pp. 685–713 (2019).
- Das, K. & Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2**, 53 (2014).
- Sarker, U. & Oba, S. Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. *Sci. Rep.* **8**, 16496 (2018).
- Vazin, F. Effect of zinc sulfate on quantitative and qualitative characteristics of corn (*Zea mays*) in drought stress. *Cer. Agron. Mold.* **45**, 15–24 (2012).
- Bahadur, L. & Tiwari, D. D. Nutrient management in mung bean (*Vigna radiata* L.) through sulphur and biofertilizers. *Legume Res.* **37**, 180–187 (2014).
- Kausar, A. *et al.* Alleviation of salt stress by K_2SO_4 in two wheat (*Triticum aestivum* L.) cultivars. *Appl. Ecol. Environ. Res.* **14**, 137–147 (2016).
- Khan, R. *et al.* Effect of foliar application of zinc and manganese on growth and some biochemical constituents of *Brassica juncea* grown under water stress. *Americ-Eura. J. Agric. Environ. Sci.* **16**, 984–997 (2016).
- Raza, M. *et al.* Effect of sulphur application on photosynthesis and biomass accumulation of sesame varieties under rainfed conditions. *Agron.* **8**, 149 (2018).

23. Klikocka, H. & Marx, M. Sulphur and nitrogen fertilization as a potential means of agronomic biofortification to improve the content and uptake of microelements in spring wheat grain DM. *J. Chem.* **2018**, 1–12 (2018).
24. Reich, M. *et al.* Interactions of sulfate with other nutrients as revealed by H₂S fumigation of Chinese cabbage. *Front. Plant Sci.* **7**, 541 (2016).
25. Sorin, E. *et al.* Effect of sulphur deprivation on osmotic potential components and nitrogen metabolism in oilseed rape leaves: identification of a new early indicator. *J. Exp. Bot.* **66**, 6175–6189 (2015).
26. Majeed, S., Nawaz, F., Naeem, M. & Ashraf, M. Y. Effect of exogenous nitric oxide on sulfur and nitrate assimilation pathway enzymes in maize (*Zea mays* L.) under drought stress. *Acta Physiol. Plantar.* **40**, 206 (2018).
27. Jackson, M. L. & Barak, P. Soil chemical analysis: advanced course. Madison (WI): UW-Madison Libraries Parallel Press (2005).
28. Naem, M. *et al.* Improving drought tolerance in maize by foliar application of boron: water status, antioxidative defense and photosynthetic capacity. *Arch. Agron. Soil Sci.* **64**, 626–639 (2018).
29. Nachabe, M. H. Refining the definition of field capacity in the literature. *J. Irrig. Drain. Engin.* **124**, 230–232 (1998).
30. Wolf, B. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Commun. Soil Sci. Plant Anal.* **13**, 1035–1059 (1982).
31. Barrs, H. D. Determination of water deficits in plant tissues. In: Water Deficits and Plant Growth (Ed. T.T. Kozlowski), Vol. I, 235 (Academic Press London, 1968).
32. Chance, B. & Maehly, C. Assay of catalase and peroxidases. *Methods Enzymol.* **2**, 764–775 (1955).
33. Urbanek, H., Kuzniak-Gebarowska, E. & Herka, K. Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta Physiol. Plantar.* **13**, 43–50 (1991).
34. Van Rossum, M. W. P. C., Alberda, M. & van der Plas, L. H. W. Role of oxidative damage in tulip bulb scale micro propagation. *Plant Sci.* **130**, 207–216 (1997).
35. Tariq, M. U., Saeed, A., Nisar, M. U., Mian, I. A. & Afzal, M. Effect of potassium rates and sources on the growth performance and on chloride accumulation of maize in two different textured soils of Haripur, Hazara division. *Sarhad J. Agric.* **27**, 415–22 (2011).
36. Reich, M. *et al.* Chloride and sulfate salinity differently affect biomass, mineral nutrient composition and expression of sulfate transport and assimilation genes in *Brassica rapa*. *Plant Soil.* **411**, 319–332 (2017).
37. Jamal, A., Moon, Y. S. & Abdin, M. Z. Enzyme activity assessment of peanut (*Arachis hypogea*) under slow-release sulphur fertilization. *Aust. J. Crop Sci.* **4**, 169–174 (2010).
38. Bloem, E., Haneklaus, S. & Schnug, E. Milestones in plant sulfur research on sulfur-induced-resistance (SIR) in Europe. *Front. Plant Sci.* **5**, 779 (2015).
39. Lee, B. R., Zaman, R., Avic, J. C., Ourry, A. & Kim, T. H. Sulfur use efficiency is a significant determinant of drought stress tolerance in relation to photosynthetic activity in *Brassica napus* cultivars. *Front. Plant Sci.* **7**, 459 (2015).
40. Hasanuzzaman, M. *et al.* Interaction of sulfur with phytohormones and signaling molecules in conferring abiotic stress tolerance to plants. *Plant Signal. Behav.* **13**, 1477905 (2018).
41. Shabbir, R. N. *et al.* Supplemental exogenous NPK application alters biochemical processes to improve yield and drought tolerance in wheat (*Triticum aestivum* L.). *Environ. Sci. Pollut. Res.* **23**, 2651–2662 (2016).
42. Hu, Y. C., Burucs, Z., von Tucher, S. & Schmidhalter, U. Short-term effects of drought and salinity on mineral nutrient distribution along growing leaves of maize seedlings. *Environ. Exp. Bot.* **60**, 268–275 (2007).
43. Studer, C., Hu, Y. & Schmidhalter, U. Interactive effects of N-, P- and K-nutrition and drought stress on the development of maize seedlings. *Agriculture*. **7**, 90 (2017).
44. Fazili, I. S. *et al.* Interactive effect of sulfur and nitrogen on growth and yield attributes of oilseed crops (*Brassica campestris* L. and *Eruca sativa* Mill.) Differing in yield potential. *J. Plant Nutri.* **33**, 1216–1228 (2010b).
45. Fazili, I. S. *et al.* Oil Biosynthesis and its Related Variables in Developing Seeds of Mustard (*Brassica juncea* L.) as Influenced by Sulphur Fertilization. *J. Crop Sci. Biotech.* **13**, 39–46 (2010a).
46. Lošák, T. *et al.* Effect of combined nitrogen and sulphur fertilization on yield and qualitative parameters of *Camelina sativa* [L.] Crtz. (false flax). *Acta Agric. Scand. Section B-Soil Plant Sci.* **61**, 313–321 (2011).
47. Shahzad, A. N. *et al.* Foliar application of potassium sulfate partially alleviates pre-anthesis drought-induced kernel abortion in maize. *Int. J. Agric. Biol.* **19**, 495 (2017).
48. Adhikari, B., Dhungana, S. K., Kim, I. D. & Shin, D. H. Effect of foliar application of potassium fertilizers on soybean plants under salinity stress. *J. Saudi Soc. Agric. Sci.* In press (2019).
49. Singh, S. & Pareek, B. L. Effect of different levels of nitrogen and sulphur on content and uptake of nutrient and quality of taramira. *Annu. Agric. Res. New Series.* **24**, 200–202 (2003).
50. Heidari, M., Galavi, M. & Hassani, M. Effect of sulfur and iron fertilizers on yield, yield components and nutrient uptake in sesame (*Sesamum indicum* L.) under water stress. *African J. Biotech.* **10**, 8816–8822 (2011).
51. Rahman, M. M., Soaug, A. A., Darwish, F. H. A., Golam, F. & Sofian-Azirun, M. Growth and nutrient uptake of maize plants as affected by elemental sulfur and nitrogen fertilizer in sandy calcareous soil. *African J. Biotech.* **10**, 12882–12889 (2011).
52. El-Tarabily, K. A., Abdou, A. S., Maher, E. S. & Satoshi, M. Isolation and characterization of sulfur-oxidizing bacteria, including strains of *Rhizobium* from calcareous sandy soils and their effects on nutrient uptake and growth of maize. *Aust. J. Agril. Res.* **57**, 10–111 (2006).
53. Abdou, A. S. Effect of applied elemental sulfur and sulfur-oxidizing bacteria (*Parococcus versutus*) into calcareous sandy soils on the availability of native and applied phosphorus and some micronutrients. In: 18th World Congress of Soil Science, Philadelphia, Pennsylvania, USA. July 9–15. Aebi, H., 1984. Catalase *in vitro*. *Meth. Enzymol.* **105**, 121–126 (2006).
54. Liu, J., Zhang, H., Yin, Y. & Chen, H. Effects of exogenous hydrogen sulfide on antioxidant metabolism of rice seed germinated under drought stress. *J. South Agric.* **48**, 31–37 (2017).
55. Forieri, I., Wirtz, M. & Hell, R. Toward new perspectives on the interaction of iron and sulfur metabolism in plants. *Front. Plant Sci.* **4**, 357 (2013).
56. Nawaz, F. *et al.* Supplemental selenium improves wheat grain yield and quality through alterations in biochemical processes under normal and water deficit conditions. *Food Chem.* **175**, 350–357 (2015).
57. Hajiboland, R. & Farhanghi, F. Effect of low boron supply in turnip plants under drought stress. *Biol Plant.* **55**, 775 (2011).
58. Hussain, R. A., Ahmad, R., Nawaz, F., Ashraf, M. Y. & Warraich, E. A. Foliar NK application mitigates drought effects in sunflower (*Helianthus annuus* L.). *Acta Physiol. Plantar.* **38**, 83 (2016).
59. Yagmur, M. & Kaydan, D. Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. *Afr. J. Biotechnol.* **7**, 2156–2162 (2008).
60. Živčák, M., Repková, J., Olšovská, K. & Brestič, M. Osmotic adjustment in winter wheat varieties and its importance as a mechanism of drought tolerance. *Cereal Res. Commun.* **37**, 569–572 (2009).
61. Lee, B. R. *et al.* Genotypic variation in N uptake and assimilation estimated by ¹⁵N tracing water deficit-stressed *Brassica napus*. *Environ. Exp. Bot.* **109**, 73–79 (2015).
62. Shah, S., Houborg, R. & McCabe, M. Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agron.* **7**, 61 (2017).
63. Houhou, M., Joutei, K. A. & Louhalia, S. Biomass production, chlorophyll content and morphological parameters are affected by sulfur deficiency in *Eruca sativa* L. *Int. J. Ecol. Environ. Sci.* **44**, 67–75 (2018).

64. Kassem, A. S., Mohammed, H. F. A. & EL-Sayed, S. A. A. Influence of sulfur deprivation on biomass allocation, mineral composition and fruit quality of tomato plants. *Middle East J. Agric. Res.* **4**, 42–48 (2015).
65. Lunde, C. *et al.* Sulfur starvation in rice: the effect on photosynthesis, carbohydrate metabolism, and oxidative stress protective pathways. *Physiol. Plantar.* **134**, 508–521 (2008).
66. Jain, P., Kachhwaha, S. & Kothari, S. L. Chloroplast ultra structure, photosynthesis and enzyme activities in regenerated plants of *Stevia rebaudiana* (Bert.) Bertoni as influenced by copper sulphate in the medium. *Ind. J. Exp. Bio.* **52**, 898–904 (2014).
67. Ibrahim, M., Chee Kong, Y. & Mohd Zain, N. Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr. *Molecules* **22**, 1623 (2017).
68. Pietrini, F., Iannelli, M. A., Pasqualini, S. & Massacci, A. Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. exsteudel. *Plant Physiol.* **133**, 829–837 (2003).
69. Lee, B. R. *et al.* Increased proline loading to phloem and its effect on nitrogen uptake and assimilation in water stressed white clover (*Trifolium repens*). *New Phytol.* **182**, 654–663 (2009).
70. Astolfi, S. *et al.* Response of barley plants to Fe deficiency and Cd contamination as affected by S starvation. *J. Exp. Bot.* **63**, 1241–1250 (2012).
71. Fatma, M., Asgher, M., Masood, A. & Khan, N. A. Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione. *Environ. Exp. Bot.* **107**, 55–63 (2014).
72. Muneer, S. *et al.* Involvement of sulphur nutrition in modulating iron deficiency responses in photosynthetic organelles of oilseed rape (*Brassica napus* L.). *Photosynth. Res.* **119**, 319–329 (2014).
73. Balk, J. & Pilon, M. Ancient and essential, the assembly of iron–sulfur clusters in plants. *Trends Plant Sci.* **16**, 218–226 (2011).
74. Duan, J. *et al.* Response of gas-exchange characteristics and chlorophyll fluorescence to acute sulfur dioxide exposure in landscape plants. *Ecotox. Environ. Saf.* **171**, 122–129 (2019).
75. Chandra, N. & Pandey, N. Influence of Sulfur Induced Stress on Oxidative Status and Antioxidative Machinery in Leaves of *Allium cepa* L. *Inter. Schol. Res. Not.* Article ID 568081 (2014).
76. Bashir, H., Ahmad, J., Bagheri, R., Nauman, M. & Qureshi, M. I. Limited sulfur resource forces *Arabidopsis thaliana* to shift towards non-sulfur tolerance under cadmium stress. *Environ. Exp. Bot.* **94**, 19–32 (2013).
77. Ma, D. *et al.* Alleviation of drought stress by hydrogen sulfide is partially related to the abscisic acid signaling pathway in wheat. *PLoS one* **11**, 0163082 (2016).
78. Hasan, M. K. *et al.* Melatonin alleviates low-sulfur stress by promoting sulfur homeostasis in tomato plants. *Sci. Rep.* **8**, 10182 (2018).
79. Mittler, R., Vanderauwera, S., Gollery, M. & Van Breusegem, F. Reactive oxygen gene network of plants. *Trends Plant Sci.* **9**, 490–498 (2004).
80. Tahri, H., Wahbi, S., El Modafar, C., Aganchich, A. & Serraj, R. Changes in antioxidant activities and phenol content in tomato plants subjected to partial root drying and regulated deficit irrigation. *Plant Biosys.* **142**, 550–562 (2008).
81. Gotor, C. *et al.* Signaling in the plant cytosol: cysteine or sulfide? *Amino Acids* **47**, 2155–2164 (2015).
82. Zareei, E., Javadi, T. & Aryal, R. Biochemical composition and antioxidant activity affected by spraying potassium sulfate in black grape (*Vitis vinifera* L. cv. Rasha). *J. Sci. Food Agric.* **98**, 5632–5638 (2018).
83. Marques, D. J. *et al.* Effect of potassium sources on the antioxidant activity of eggplant. *Rev. Brasil. Ciên. Solo* **38**, 1836–1842 (2014).
84. Hütsch, B. W., Jung, S. & Schubert, S. Comparison of Salt and Drought-Stress Effects on Maize Growth and Yield Formation with Regard to Acid Invertase Activity in the Kernels. *J. Agron. Crop Sci.* **201**, 353–367 (2015).
85. Setter, T. L., Flannigan, B. A. & Melkonian, J. Loss of kernel set due to water deficit and shade in maize. *Crop Sci.* **41**, 1530–1540 (2001).
86. Dewal, G. S. & Pareek, R. G. Effect of phosphorus, sulphur and zinc on growth, yield and nutrient uptake of wheat (*Triticum aestivum*). *Indian J. Agron.* **49**, 160–162 (2004).
87. Farokhi, H., Shirzadi, M. H., Afsharmanesh, G. & Ahmadzadeh, M. Response of azargol sunflower cultivar to different micronutrients in Jiroft region, southeast of Iran. *South West. J. Hortic. Biol. Environ.* **6**, 53–64 (2015).
88. Hasanuzzaman, *et al.* Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: *Crop stress and its management: Perspectives and strategies*. pp. 261–315 (Springer, Dordrecht, 2012).
89. Barbosa, R. H. *et al.* Foliar copper uptake by maize plants: effects on growth and yield. *Ciência Rural* **43**, 1561–1568 (2013).

Author contributions

F.N. designed and supervised the experiments and wrote the manuscript. M.M.U. performed the experiments and analytical work. S.M. and K.S.A. provided the reagents for analytical work and assisted in preparing the final draft of manuscript. R.N.S. and M.A. provided inputs for experiments and guided in preparing figures. M.A.S. and G.A. co-supervised the experiments carried out as a part of MSc. (Hons.) thesis work of M. M.U.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41598-020-58169-2>.

Correspondence and requests for materials should be addressed to F.N.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020