

OPEN

Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (*Zea mays* L.)

Cengiz Kaya¹, Mehmet Şenbayram¹, Nudrat Aisha Akram², Muhammed Ashraf³, Mohammed Nasser Alyemini⁴ & Parvaiz Ahmad^{4,5*}

Soil amendments are known to promote several plant growth parameters. In many agro-ecosystems, water scarcity and drought induced phosphorus deficiency limits crop yield significantly. Considering the climate change scenario, drought and related stress factors will be even more severe endangering the global food security. Therefore, two parallel field trials were conducted to examine at what extent soil amendment of leonardite and humic acid would affect drought and phosphorus tolerance of maize. The treatments were: control (C: 100% A pan and 125 kg P ha⁻¹), P deficiency (phosphorus stress (PS): 62.5 kg P ha⁻¹), water deficit stress (water stress (WS): 67% A pan), and PS + WS (67% A pan and 62.5 kg P ha⁻¹). Three organic amendments were (i) no amendment, (ii) 625 kg S + 750 kg leonardite ha⁻¹ and (iii) 1250 kg S + 37.5 kg humic acid ha⁻¹ tested on stress treatments. Drought and P deficiency reduced plant biomass, grain yield, chlorophyll content, F_v/F_m , RWC and antioxidant activity (superoxide dismutase, peroxidase, and catalase), but increased electrolyte leakage and leaf H₂O₂ in maize plants. The combined stress of drought and P deficiency decreased further related plant traits. Humic acid and leonardite enhanced leaf P and yield in maize plants under PS. A significant increase in related parameters was observed with humic acid and leonardite under WS. The largest increase in yield and plant traits in relation to humic acid and leonardite application was observed under combined stress situation. The use of sulfur-enriched amendments can be used effectively to maintain yield of maize crop in water limited calcareous soils.

The global climate change simulations suggest fresh water availability will further deplete in many rainfed and irrigated agricultural areas¹ and thus, threatens food security². Although hydrological, meteorological and agricultural droughts occur simultaneously and are interrelated with each other, agricultural drought is believed to be the most common^{3,4}. Water stress causes a variety of responses from physiological to molecular in plants, allowing them to acclimate to harsh ecological conditions⁵. Drought susceptibility of plants differs according to the plant species, stress level, and growth stages⁶.

Considerable yield gaps have been noticed in agricultural systems^{7,8} and the availability of good quality water and mineral nutrients is critical for overcoming these yield gaps^{9–11}. This is principally reasonable for maize crop, one of the main cereals of the globe, covering 26% and 37% of the total cereal cultivated area and production, respectively¹². Maize is known as one of the highest water-requiring crops. Water deficiency imposed at any stage of its development can reduce grain yield significantly^{13,14}. As it is a fast-growing crop, its requirement for essential nutrients is also high and deficiency of any of the plant nutrients may lead to hamper growth and decrease yield¹⁵. Maize is particularly susceptible to P deficiency, which can suppress growth and grain yield^{16–18}.

¹Harran University, Faculty of Agriculture, Department of Soil Science & Plant Nutrition, Şanlıurfa, Turkey.

²Department of Botany, GC University Faisalabad, Faisalabad, Pakistan. ³University of Agriculture Faisalabad, Faisalabad, Pakistan. ⁴Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. ⁵Department of Botany, S.P. College Srinagar, Srinagar, Jammu and Kashmir, India. *email: parvaizbot@yahoo.com

⁶Department of Botany, S.P. College Srinagar, Srinagar, Jammu and Kashmir, India. *email: parvaizbot@yahoo.com

Phosphorus (P) has been reported to be one of the limited mineral elements in most agro-ecosystems^{19,20}. This element is involved in a number of key energy transfer and photosynthetic oxidation-reduction reactions²¹. Phosphorus is also part of a broad range of biochemical compounds including nucleic acids, structural proteins and enzymes as well as signal transductions^{22,23}. The soil available P for plants is frequently insufficient because of its strong binding in insoluble forms^{24,25}. Plants have developed strategies to alleviate P deficiency²⁶, which include increased efflux of organic acids²⁷, altered root structure²⁸ and enhanced acid phosphatase activity²⁹. All these mechanisms contribute to increased P intake in plants under P-deficient regimes²³.

Organic fertilizer use has gained a great attention as a means to improve crop nutrition and soil fertility. Organic fertilizers have a main function in improving the quantity of organic matter in the root zone³⁰. Leonardite is one of the organic matters with high P content available for this purpose³¹. Leonardite either is an oxidation product of lignite related to subsurface mining^{32,33} or it consists of sediments enriched with humic compounds^{34,35}. It contains a high quantity of humic substances (from 20% to more than 70%). Humic compounds present in the soil affect directly or indirectly plant growth³⁶. Humic acid application leads to an increase in some key plant biochemicals such as nucleic acids, vitamins, amino acids, and nutrients, but it also improves soil chemical properties^{36,37}.

Like phosphorus, sulfur (S) is known to be one of the most crucial major nutrients essential for plant growth^{38–40}. Sulfur plays a role in the building of proteins and chlorophyll^{41–43}. Sulfur deficient regimes suppress cell sap osmotic pressure, which is ascribed to limited accumulation of intracellular solutes⁴⁴. Under S deficit conditions, it is known that SO_4^{2-} is mobilized to sustain plant growth, and that its contribution to osmotic adjustment is compensated by other osmotically active molecules⁴⁵.

Many authors have depicted responses of crops to either phosphorus (P) deficiency^{17,46–48} or water stress^{49–53}, but not much research has been carried out so far to appraise the combined effects of P deficiency and water stress on crops⁵⁴. Furthermore, no sufficient literature exists on the role of sulfur-enriched leonardite and humic acid in maize plants subjected to the combined application of both stresses. In the current study, it was aimed to assess the possible effects of these soil amendments in maize plants subjected to P deficiency and water deficit conditions applied singly or jointly.

Material and Methods

Field conditions. Two parallel field trials were conducted in 2011 at the Agricultural Research Station, University of Harran, Sanliurfa, Turkey, during the appropriate maize growth season (end-June to end-October). Soil samples taken randomly from the top 0–30 cm horizon of the experimental field before planting were analyzed according to Ryan, *et al.*⁵⁵. The texture of the soil used was clay loam with pH of 7.8, CaCO_3 25.2%, organic matter content 1.2%, available P 20 kg ha⁻¹ [0.5 M NaHCO₃ extractable P₂O₅⁵⁶], plant-available S 3.1 μg g⁻¹ [0.01 M CaCl₂ extractable SO₄²⁻-S⁵⁷] and electrical conductivity of saturation extract 1.1 dS m⁻¹. The soil had field capacity 32.6%, permanent wilting point 25.6% and dry bulk density 1.37 g cm⁻³. The exchangeable cation contents of K⁺, Ca²⁺, Mg²⁺ and Na⁺ were 1.35, 25.5, 12.2 and 0.67 cmol kg⁻¹, respectively. Electric conductivity and pH of the water used for irrigation were 0.53 dS m⁻¹ and pH 7.3, respectively.

During the trial, total rainfall and average relative humidity were 3.8 mm and 30.3%, respectively. Maximum, minimum and mean of temperature (°C) during the experiment were 38.7, 18.9 and 29.4, respectively. Weed infestation was controlled manually three times during the season.

Evaporation was appraised using a Class A Evaporation Pan situated close to the field plots for manual measurement of daily evaporation. The volume of water used during each irrigation was calculated following the class A pan evaporation using the below given formula⁵⁸:

$$I_r = E_{pan} - K_{cp}$$

where I_r is the amount of irrigation water used (mm), E_{pan} is the cumulative evaporation at class A pan between two irrigations, and K_{cp} is the plant-pan coefficient.

Experimental design. The experimental design comprised four stress treatments: control (C:100% A pan and 125 kg P/ha), P deficiency (PS: 100% A pan and 62.5 kg P/ha), water deficit stress (WS: 67% A pan and 125 kg P/ha), and PS + WS (67% A pan and 62.5 kg P/ha). In the water stress treatment, plants were irrigated every three days at 67% A pan (E_{pan}) evaporation, whereas the control plants received 100% A pan every day. Phosphorus in the form of superphosphate was incorporated into the soil prior to sowing. According to the findings of a pilot glasshouse trial using a series of concentrations of sulfur (S) and leonardite (LEO) applied individually or in combination, two combinations of soil amendments (SA) (SA1: 625 kg S + 750 kg LEO/ha and SA2: 1250 kg S plus 37.5 kg humic acid/ha) were selected for the present field trial. Leonardite and liquid humic acid were provided by Biotar Company (Ankara, Turkey). Before using these soil amendments, available P was analyzed in order to know whether or not any significant amount of P was released from them into the soil. Leonardite and humic acid contained 350 and 5 mg available P kg⁻¹ which can supply maximum 262.5 and 0.18 g of P from leonardite and humic acid, respectively, based on their application rates per ha. So such amount of P released from LEO and HA could be considered as insignificant compared to the amount of P applied. The trial was designed in a randomized split plot design (stress treatments as main plots, soil amendments as sub-plots). All trial units were replicated thrice. Ninety-six plants of maize (cv. DKC-5789) per treatment were maintained in a planting geometry of 0.25 m plant to plant distances and 0.7 m row to row distance within each experimental unit of 6.0 m × 2.8 m.

All plots were drip-irrigated (4 L h⁻¹ m⁻¹ from 10:00 am to 5:00 pm for two weeks) for a good establishment of seedlings. The drip system operating pressure was fixed at 100 kPa during the entire growth period. For irrigating each row, a single drip tube with 0.5 m emitter spacing was positioned on the surface of soil. For precisely monitoring the schedule of irrigation intervals, soil tensiometers at 30 and 45 cm soil depths centering between

two plants in a row were installed. The tensiometer readings were maintained above the threshold level, i.e., -30 kPa and -20 kPa at 30 and 45 cm depth, respectively, for well-watered plants, and -65 kPa and -50 kPa at 30 and 45 cm depths, respectively, for the water stress treatment.

Nitrogen (urea) and potassium (potassium sulphate) fertilizers at the rate of 200 N and 240 K₂O kg ha⁻¹ were applied to each experimental unit. Potassium fertilizer was broadcast in the soil before planting the crop, while the urea was applied in an equal dose through the drip irrigation three times at two-week intervals.

Plant measurements. Youngest completely extended 3rd leaf from the apex was collected at dawn from each of 10 plants selected randomly from one of 4 rows for the quantity of leaf relative water content (RWC), electrolyte leakage (EL), chlorophyll content, malondialdehyde and hydrogen peroxide contents, acid phosphatase activity, hydrogen peroxide content and maximal quantum yield.

Leaf RWC was assessed using the method of Kaya, *et al.*⁵⁹ adopted from Yamasaki and Dillenburg⁶⁰. Leaf EL was determined using the procedure developed by Dionisio-Sese and Tobita⁶¹. A fresh leaf sample (200 mg) was cut into about 5 mm pieces and kept them in a glass test tube containing 10 mL of distilled water. The sample was retained for 60 min at 32 °C in a water bath, and electrical conductivity (EC₁) was quantified and then the materials were kept in an autoclave at 121 °C for 20 min. The sample solution was cooled down to 25 °C and EC of the solution re-measured (EC₂). The equation followed for determining the membrane permeability was: $MP = EC_1 / EC_2 \times 100$.

Chlorophyll content was determined by using 1.0 g of recently expanded leaf, which was triturated in 90 percent acetone solution. The absorbance of the filtrate was recorded using a UV-visible spectrophotometer (Shimadzu UV-120, Japan) and the chlorophyll pigment was quantified according to Strain and Svec⁶². For enzymes, MDA and hydrogen peroxide assays, the leaf tissues were stored in liquid nitrogen at -80 °C. The activity of acid phosphatase was assessed based on the procedure given by Kaya, *et al.*⁵⁹, adopted from Besford⁶³. For antioxidant enzyme analysis, a fresh leaf tissue (500 mg) was ground in sodium phosphate buffer (50 mM pH 7.0) consisting of polyvinyl pyrrolidone (1%). The material was centrifuged at 10,000 g for ¼ h at 4 °C and the supernatant was used for appraising the activities of catalase⁶⁴, superoxide dismutase⁶⁵ and peroxidase⁶⁶. Malondialdehyde (MDA) content, as a measure of lipid peroxidation, was appraised as described by Weisany, *et al.*⁶⁷. Hydrogen peroxide (H₂O₂) content was assayed as depicted in Loreto and Velikova⁶⁸. For the measurement of maximum potential quantum efficiency of photosystem II (F_v/F_m) previously dark adapted leaves (for 30 min) were subjected to a portable chlorophyll fluorometer (Photosynthesis Yield Analyzer Mini-PAM, Walz, Germany).

At the initiation of grain filling phase, three plants from each of 4 rows were harvested from the ground level to determine above ground shoot fresh weight. The shoots from three plants randomly selected from each experimental unit were dried for 2 days at 70 °C to appraise above ground shoot dry weight.

At day 120 after sowing, 24 plants chosen randomly from the remaining three rows were harvested from each experimental unit. The cobs were removed from the stalks and grain yield assessed at 13% moisture content. Thousand-grain weight (TGW) was also recorded. The dried plant material was processed by ashing at 550 °C for 6 h in a muffle furnace. The concentration of phosphorus was assayed by employing the Vanadate-molybdate method⁶⁹.

Statistical analysis. Statistical analysis of the data gathered from the two parallel experiments was carried out. The two individual experiments did not differ significantly in terms of the analysis of variance of the data, so the data of the two experiments were averaged. Significant differences among the mean values were appraised using the Duncan's test at 5% probability level. Data in all Figures are presented as mean \pm standard error.

Results

Sulfur-enriched soil amendments improve plant growth and grain yield under phosphorus deficiency and water stress. Phosphorus deficiency stress (PS) and water stress (WS) significantly decreased shoot fresh weight (39.2 and 38.6%, respectively), shoot dry weight (35.9 and 36.1%, respectively) and grain yield (12.8 and 18.75%) respectively, but did not affect thousand grain weight (Fig. 1A–D). The combined effect of drought stress and P deficiency caused a significant yield deprivation, in which the decrease in shoot DM, and grain yield were 50.1 and 31.3% relative to those in non-stressed plants, respectively. Application of soil amendments in SA, SA1 and SA2 treatments enhanced the yield parameters significantly in all stress treatments with the exception of thousand grain weight (TGW). The effect of leonardite and humic acid treatment on yield parameters were less significant in the combined stress treatment (PS + WS) compared to that by the single stress treatment. Both additives (humic acid and leonardite) positively affected the yield parameters with no significant difference among them.

Sulfur-enriched soil amendments improve leaf total chlorophyll content and maximum fluorescence yield under phosphorus deficiency and water stress. When P deficiency and water stress applied alone, leaf total chlorophyll contents (16% and 21%, respectively) and maximum fluorescence yield (F_v/F_m) (24% and 21%, respectively) decreased significantly compared to those in the the control treatment (Fig. 2A,B). Furthermore, combination of both stress factors led to a greater decrease in the total chlorophyll content and F_v/F_m by 26 and 41% relative to those in the non-stressed plants, respectively. Application of soil amendments (SA, SA1 and SA2) significantly enhanced total chlorophyll content by 12.1 and 13.7% and F_v/F_m by 19.9 and 14.8%, respectively compared to those in the P deficient treatment (PS). These increases in leaf total chlorophyll content and F_v/F_m were 18.8 and 17.9% in the S1 treatment, and 22.3 and 15.2% in the S2 treatment compared to those in the WS treatments. Application of SAs was not effective in altering these plant traits when plants were subjected to both stresses. Similarly, application of SAs did not affect these parameters in the control plants.

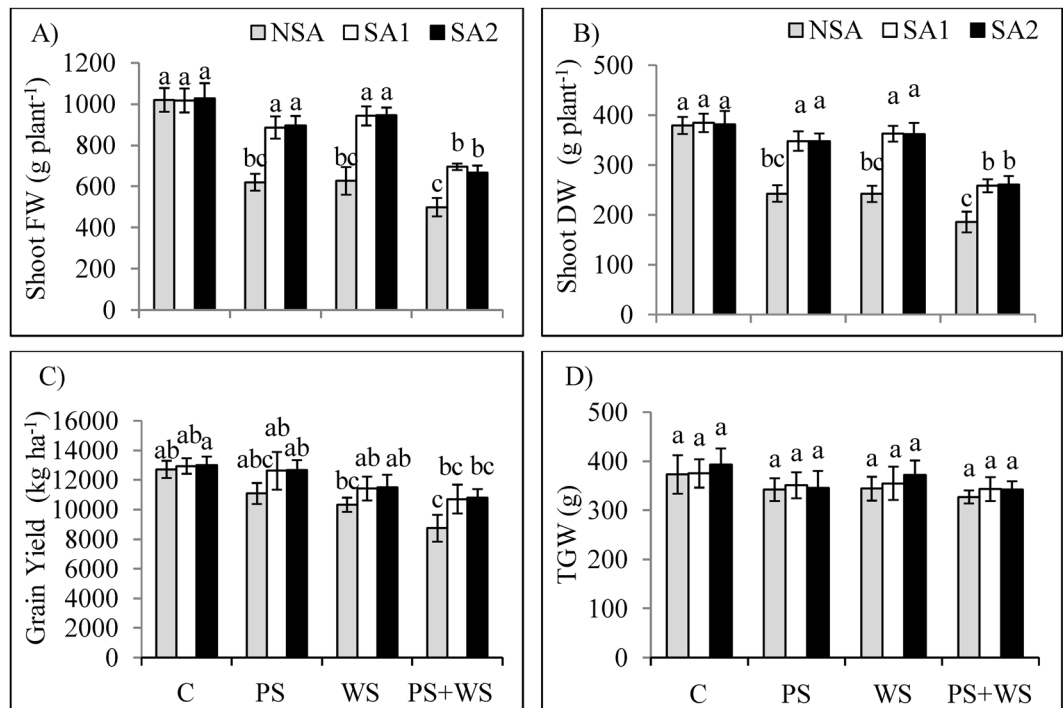


Figure 1. Shoot fresh weight [FW; (A)] and shoot dry weight [DW; (B)], grain yield (C) and thousand grain weight [TGW; (D)] of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments (SA1 and SA2). Mean \pm S.E.; Mean pairs with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. (C) Control treatment (Well-watered; plants were irrigated once every day days at 100% A pan (Epan) evaporation, and adequate P, 125 kg P/ha); PS: phosphorus deficiency stress (62.5 kg P/ha); WS: water stress (Plants were irrigated once every 3 days at 67% A pan (Epan) evaporation); NSA: No soil amendment; SA1: 625 kg sulfur (S) + 750 kg leonardite/ha; SA2: 1250 kg S + 37.5 kg/ha humic acid.

Sulfur-enriched soil amendments improve leaf water potential and leaf relative water content under phosphorus deficiency and water stress. Leaf water potential (Ψ_l) and leaf relative water content (LRWC) decreased by 31.1% and 20.6% in the PS treatment and by 42.8% and 26.7% in the WS treatment, and by 48.5% and 33.1% in the PS + WS treatment (Fig. 3A,B). Application of SAs (in the SA1 and SA2 treatments) improved leaf Ψ_l by 6.2% and 10.4% and LRWC by 14.2% and 14.7% in plants exposed to P deficiency. Similarly, application of SAs also improved both leaf Ψ_l and LRWC in plants treated with water stress. The increase in Ψ_l and LRWC due to the SAs application in the combined stress treatment (PS + WS) was 9.8% and 30.8% in SA1 and 19.5% and 33.2% in SA2 treatments, respectively. In most cases, there were no statistical differences between the two exogenous applications of SAs (SA1 and SA2) with respect to the water relation parameters. Similarly, no significant effects were observed in these parameters by the application of SAs to the control plants.

Sulfur-enriched soil amendments maintain leaf P and acid phosphatase enzyme activity under phosphorus deficiency and water stress. Leaf P content decreased by 80%, but leaf acid phosphatase enzyme activity (APA) increased by 2.4-fold in non-SAs applied P deficient treatment compared to the control plants (Fig. 4A,B). On the other hand, leaf P content and APA remained unaffected under water stress. In the combined stress treatment, however, leaf P content and APA followed the trend as observed in the P deficient treatment. Application of SAs showed positive effects on leaf P content in all treatments. On the other hand, the effect of SAs was negative on APA in all stress treatments.

Sulfur-enriched soil amendments reverse oxidative stress parameters under phosphorus deficiency and water stress. Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents as well as electrolyte leakage (EL) increased significantly under P deficiency and water stress. Here the effect was more pronounced when the stress was applied in combination (Fig. 5A–C). The content of MDA and EL decreased significantly when SAs was applied with the effect being more significant in the SA2 compared to those in the SA1 treatments under water stress alone and in combined stress situation. Similarly, a greater decline in H_2O_2 level was noticed in the SA2 compared to the SA1 treatments in all stress treatments. In the control plants, application of SAs did not have any significant impact on the oxidative stress parameters measured.

Sulfur-enriched soil amendments improve antioxidant defence system under phosphorus deficiency and water stress. Phosphorus deficiency enhanced the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) enzymes by 30.2, 42.9 and 66.6% compared to those in the control

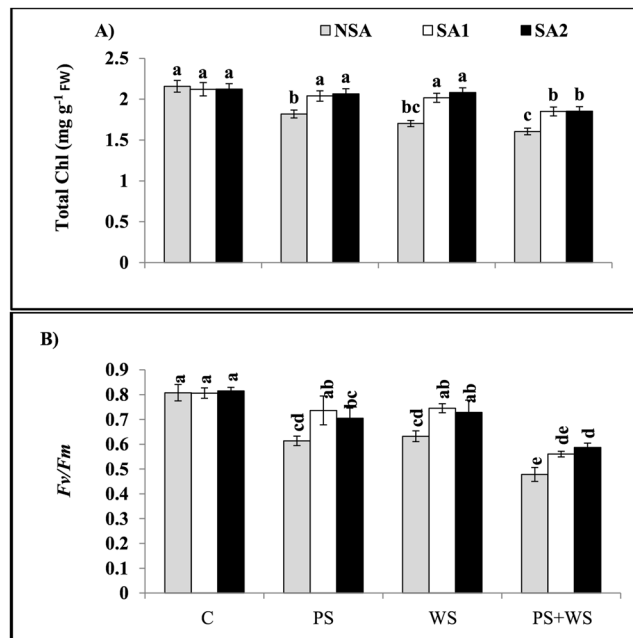


Figure 2. Leaf total chlorophyll contents (A) and maximum fluorescence yield [F_v/F_m ; (B)] of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments (SA1 and SA2). Mean \pm S.E.; Mean pairs with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. (C) Control treatment (Well-watered; plants were irrigated every day at 100% A pan (Epan) evaporation, and adequate P, 125 kg P/ha); PS: phosphorus deficiency stress (62.5 kg P/ha); WS: water stress (Plants were irrigated once every 3 days at 67% A pan (Epan) evaporation); NSA: No soil amendment; SA1: 625 kg sulfur (S) + 750 kg Leonardite/ha; SA2: 1250 kg S + 37.5 kg/ha humic acid.

treatment, respectively. Water deficit conditions suppressed SOD and CAT activities by 22.2 and 47.2%, but increased POD activity by 2.9-fold compared to those in the control plants, respectively. However, when combined stress was applied, CAT activity decreased by 58.4%, but POD increased by 89.5% compared to the controls (Fig. 6A–C). Under P deficiency, application of SAs significantly decreased SOD, CAT and POD activities. However, under water stress, SAs led to an elevation in SOD and CAT activities, but a reduction in POD activity. In the combined stress treatments, POD activity decreased, but the CAT activity increased significantly when SAs was applied.

Discussion

Effect of phosphorus deficiency, water stress and their combination and S-enriched amendments on growth and yield attributes. In our study, single or combined P deficiency and water stress reduced growth and grain yield of maize plants. Nutritional imbalance is one of the major drought-induced disturbances. Among essential nutrients, P is a crucial macronutrient that is mainly responsible for the energy balance of the higher plants. Phosphorus deficiency in soil does not only hamper P uptake and accumulation, but also may limit the uptake of other nutrients, particularly of Mg and K⁷⁰. It is well known that an optimum amount of water as well as essential nutrients including P and S are required to attain optimal yield^{71–73}.

Many investigations have stated alleviating effects of humic acid and sulfur on crop growth. Humic acid (HA) is contemplated as an important bio-stimulator that improves photosynthesis, respiration, permeability of cell membranes, and uptake of phosphate and potassium, as well as contributes to maintain hormonal balance⁷⁴. It also improves the fertility of soil by improving its biological, physical and chemical properties^{75–77}. Nakasha, *et al.*⁷⁸ observed that application of HA before planting of safed musli (*Chlorophytum borivilianum* L.) at the rate of 5, 10, and 15% not only enhanced tuber sprouting, but hastened uniform sprouting pattern, and increased leaf area index, leaf area, number of leaves, and total root length. Similarly, S is an essential plant nutrient needed for maintaining optimal plant growth. It is available usually in the form of anionic sulfate⁷⁹. The positive effect of sulfur on crop yield and growth has also been reported in different studies. For instance, Sanli, *et al.*⁸⁰ reported a marked rise in tuber yield production of potato by exogenously applied Leonardite at 400 kg ha⁻¹. For example, Govahi and Saffari⁸¹ observed in canola (*Brassica napus* L. var. *oleifera*) that dry-matter accumulation increased with the addition of S at a rate of 40 to 120 kg ha⁻¹. However, the optimum dose of sulfur causing a maximal change in growth differs from crop to crop. For example, sulfur application at the rate of 4 mM enhanced vegetative growth, while high concentration (8 mM) delayed the vegetative growth of onion plants⁸². Little information, however, exists until now on the effects of S-enriched humic acid application on crop productivity. Our results indicate that exogenously applied S-enriched Leonardite (SA1 and SA2) is an effective chemical for improving plant growth and yield of maize plants subjected to P deficiency, water stress or under the combination of both. So, application of S-enriched SAs including Leonardite or humic acid might be a quite effective means to surmount the deleterious

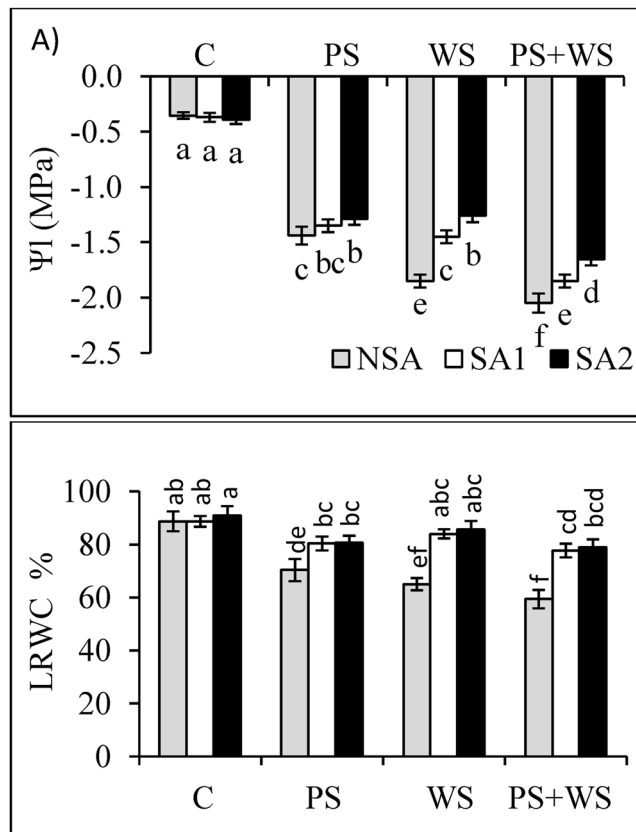


Figure 3. Leaf water potential [Ψ_l ; (A)] and leaf relative water content [LRWC; (B)] of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments (SA1 and SA2). Mean \pm S.E.; Mean pairs with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. (C) Control treatment (Well-watered; plants were irrigated every day at 100% A pan (Epan) evaporation, and adequate P, 125 kg P/ha); PS: phosphorus deficiency stress (62.5 kg P/ha); WS: water stress (Plants were irrigated once every 3 days at 67% A pan (Epan) evaporation); NSA: No soil amendment; SA1: 625 kg sulfur (S) + 750 kg leonardite/ha; SA2: 1250 kg S + 37.5 kg/ha humic acid.

effects of phosphorus deficiency and water stress on plants by improving phosphorus nutrition, water relations and antioxidant defense system, as well as reducing oxidative stress, which have been discussed in detail in the latter sections.

Effect of phosphorus deficiency, water stress and their combination, and S-enriched amendments on chlorophyll contents, and membrane and PSII integrity. Photosynthetic pigments such as chlorophyll are the key components for carrying out light reactions of photosynthesis. These pigments are very fragile, and their ultrastructure and functioning are considerably impaired under stress situations including drought stress^{83,84}. In the current experiment, total chlorophyll content was markedly reduced in the maize crop under water stress and P deficiency, but even more severe effect was observed when both stresses were combined. Previous research reports have shown that the stress-induced decline in chlorophyll content was primarily ascribed to considerable accumulation of H_2O_2 in the plant leaves^{85,86}. So, the decreased total chlorophyll content might be linked to considerable accumulation of H_2O_2 in the maize plant leaves as observed under P deficiency and water stress. Application of SAs specifically in SA2 treatment significantly reduced the H_2O_2 level of the leaf and elevated the total chlorophyll content. Drought-induced decline in chlorophyll pigments has also been reported in different crops in different studies e.g. canola by Akram, *et al.*⁸⁷, quinoa (*Chenopodium quinoa* Willd.) by Aziz, *et al.*⁸⁸, wheat (*Triticum aestivum* L.) by Kosar, *et al.*⁸⁹ and Huseynova, *et al.*⁹⁰, and carrot (*Daucus carota* L. subsp. *sativus*) by Razzaq, *et al.*⁹¹.

One of the main stress-induced cell problems is impaired membrane integrity and permeability. An increase in electrolyte leakage, which reflects a loss of ability of biological membranes to regulate the transport of ions, has been reported under drought stress, e.g. in kochia (*Kochia scoparia* (L.) Schrad.) by Masoumi, *et al.*⁹² and in garden huckleberry (*Solanum scabrum* Mill.) by Assaha, *et al.*⁹³. As phosphorus is directly linked with energy storage and ATP formation in plants⁹⁴, thus its deficiency can impair membrane transport mechanisms and reduce plant growth, particularly under water deficit regimes⁹², and similarly in the current experiment, there was a significant ($P \leq 0.01$) nonlinear correlation ($r = -0.577$) between leaf P content and EL of maize plants (Fig. 7A). Soil amendments with S or leonardite + humic acid significantly enhanced chlorophyll contents and improved membrane and PSII integrity in maize plants subjected to both stresses applied singly or jointly. The latter can be

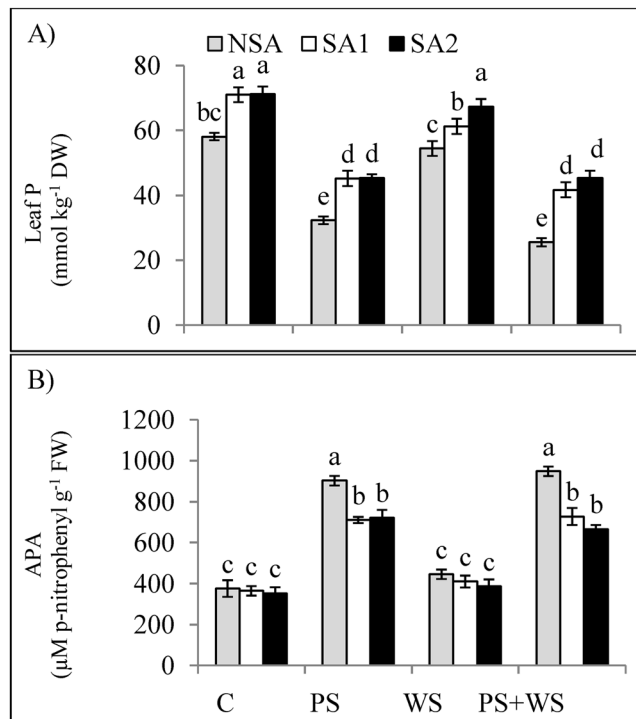


Figure 4. Leaf phosphorus [P; (A)] concentration and leaf acid phosphatase [APA; (B)] of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments (SA1 and SA2). Mean \pm S.E.; Mean pairs with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. (C) Control treatment (Well-watered; plants were irrigated every day at 100% A pan (Epan) evaporation, and adequate P, 125 kg P/ha); PS: phosphorus deficiency stress (62.5 kg P/ha); WS: water stress (Plants were irrigated once every 3 days at 67% A pan (Epan) evaporation); NSA: No soil amendment; SA1: 625 kg sulfur (S) + 750 kg leonardite/ha; SA2: 1250 kg S + 37.5 kg/ha humic acid.

attributed to the reduced P fixation and increased soil P content that is supported by the increased leaf P in the maize plants treated with SAs.

Effect of phosphorus deficiency, water stress and their combination, and S-enriched amendments on water status parameters. Under water and P deficiency stress conditions, RWC decreased significantly. It has been reported that low RWC is generally associated with stomatal closure, thereby leading to reduced CO₂ availability and hence reduced rate of photosynthesis and impaired antioxidant/reactive oxygen species balance^{83,95,96}. In the current experimentation, reduced chlorophyll content and *Fv/Fm* can be associated with low RWC in the maize plants exposed to both P deficiency and water stress. Water stress and P deficiency also significantly affected Ψ_w . Usually, RWC was found to be positively associated with leaf water potential (Ψ_w). For example, water stress reduced leaf water potential and RWC in parallel in soybean⁹⁷. Leaf or tissue water potential (Ψ_w) is frequently used as a prospective selection criterion of plant stress tolerance⁹⁸, because the reduction in Ψ_w caused by enhancement in hydraulic stress leads to reduction in photosynthetic CO₂ assimilation^{99,100}. Although leaf water potential of the maize plants suppressed markedly under both stresses, RWC decreased more prominently when those were applied in combination. Soil amendments with SAs in SA1 and SA2 treatments improved RWC and leaf water potential effectively, particularly when combined stresses were applied. Abuelsoud, *et al.*¹⁰¹ reported that sulfur, in addition to be an important macronutrient, participates in sulfur-containing compounds playing a critical function in osmotic adjustment in plants exposed to water deficit conditions. Therefore, it is still unclear in our study whether the effect of S or other factors in leonardite and humic acid applied soils were responsible for enhanced stress tolerance in the maize plants subjected to SAs amended soils.

Effect of phosphorus deficiency, water stress and their combination and S-enriched amendments on leaf P concentration and acid phosphatase activity. In alkaline clay soils, solubility of P is among the main causes of P deficiency and reduced crop production^{102,103}. One of hypotheses in the present experiment was to test whether S-enriched SAs would affect solubility of P and plant P uptake in alkaline clay soil. Here, application of SAs significantly increased tissue P levels in the P stressed maize plants. As supported by Khan, *et al.*³⁹, application of sulfur fertilizers likely increases the P availability in calcareous soils. This is because, sulfur supplementation to the soil results in the generation of H₂SO₄, which in turn slightly reduces soil pH and increases solubility of P¹⁰⁴. Similarly, an increase in P uptake, plant growth and yield were found due to exogenously applied S-enriched leonardite under clay and loamy sand soils¹⁰⁵. In this study, we did not monitor the soil pH, therefore, it was not clear at what extent change in soil pH was responsible for the observed positive effects

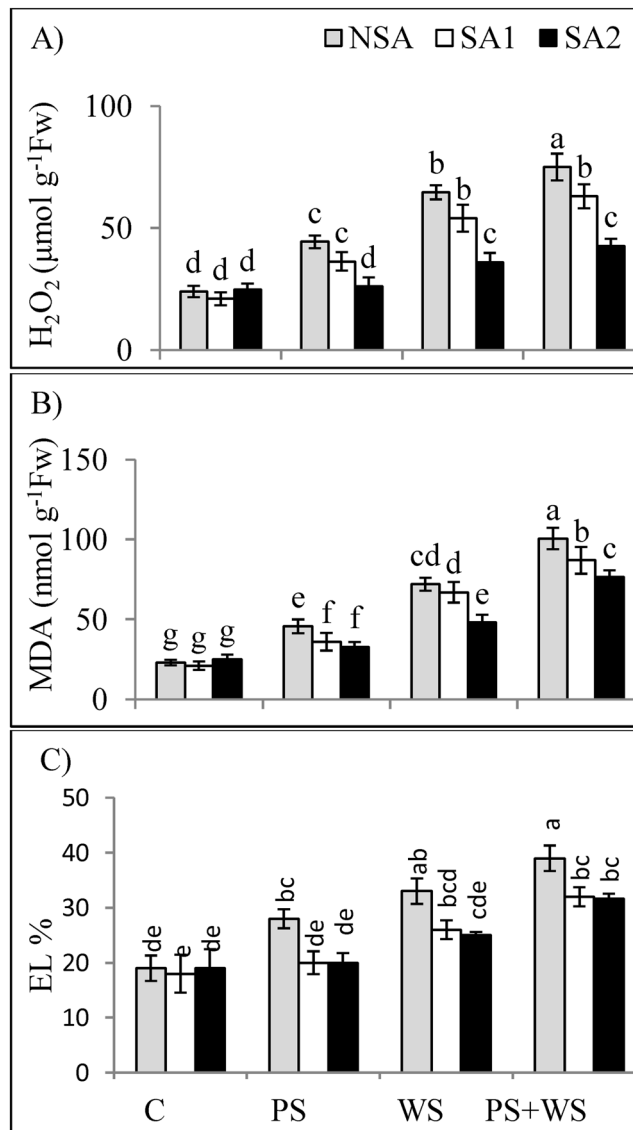


Figure 5. Leaf hydrogen peroxide [H_2O_2 ; (A)], malondialdehyde [MDA; (B)] and electrolyte leakage [EL; (C)] of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments (SA1 and SA2). Mean \pm S.E.; Mean pairs with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. (C) Control treatment (Well-watered; plants were irrigated every day at 100% A pan (Epan) evaporation, and adequate P, 125 kg P/ha); PS: phosphorus deficiency stress (62.5 kg P/ha); WS: water stress (Plants were irrigated once every 3 days at 67% A pan (Epan) evaporation); NSA: No soil amendment; SA1: 625 kg sulfur (S) + 750 kg leonardite/ha; SA2: 1250 kg S + 37.5 kg/ha humic acid.

after application of S-enriched humic acid and leonardite. However, the present study clearly showed that in alkaline clay soil such additives can alleviate P deficiency-induced adverse effects on plants via enhancing maize P uptake.

Acid phosphatase activity (APA) is one of crucial traits because of its substantial role in plant P utilization^{106,107}. The correlation between the APA activity and leaf P was significant ($P \leq 0.01$) ($r = -0.920$) (Fig. 7B), indicating that when plants are subjected to P deficiency, APA activity increases to utilize more P. Wasaki, *et al.*¹⁰⁸ have also shown that APA activity is one of the important plant traits indicating P deficiency.

Effect of phosphorus deficiency, water stress and their combination, and S-enriched amendments on peroxidation.

Free radicals generated by oxidative stress leads to lipid peroxidation thereby causing membrane deterioration in plants⁸⁸. Peroxidation is frequently considered as the most damaging cellular response to stress conditions and is sometimes considered as an indicator of stress severity¹⁰⁹. In the present study, water stress as well as phosphorus deficiency increased the MDA and H_2O_2 contents significantly. Excess accumulation of H_2O_2 can impair the cell redox potential and may lead to increased levels of antioxidants resulting into the alteration of antioxidant system¹¹⁰. In the current experimentation, soil amendments with leonardite and humic acid significantly reduced the P deficiency and water deficit induced oxidative stress. Of both amendments,

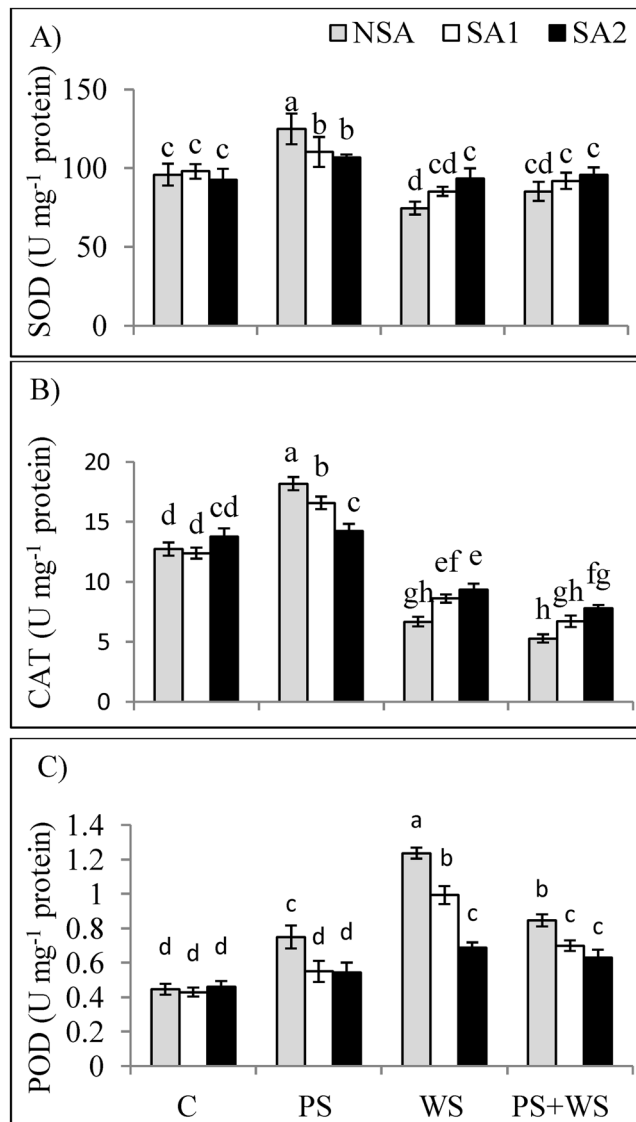


Figure 6. Activities of superoxide dismutase [SOD; (A)], catalase [CAT; (B)] and peroxidase [POD; (C)] in the leaves of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments (SA1 and SA2). Mean \pm S.E.; Mean pairs with different letters are significantly different ($P < 0.05$) by Duncan's multiple range test. (C) Control treatment (Well-watered; plants were irrigated every day at 100% A pan (Epan) evaporation, and adequate P, 125 kg P/ha); PS: phosphorus deficiency stress (62.5 kg P/ha); WS: water stress (Plants were irrigated once every 3 days at 67% A pan (Epan) evaporation); NSA: No soil amendment; SA1: 625 kg sulfur (S) + 750 kg Leonardite/ha; SA2: 1250 kg S + 37.5 kg/ha humic acid.

SA2 was found to be more effective compared to SA1 in reducing both MDA and H_2O_2 contents in the maize plants under all stress conditions. Similarly, in a previous study with maize plants, application of HA was found to be very effective in reducing lipid peroxidation⁴⁸. Moreover, humic acid has been shown to be effective in improving stress-induced lipid peroxidation in maize¹¹¹.

Effect of phosphorus deficiency, water stress and their combination, and S-enriched amendments on antioxidant enzymes. Under water deficit conditions, ROS generally accumulate due to imbalanced ROS/antioxidant activity ratio^{87,88}. Under stress situation, enzymatic antioxidants such as SOD, POD and CAT play a key role in preventing ROS damage^{112–114}. In the current investigation, the activities of these enzymes increased significantly under P deficiency. Application of SAs as SA1 and SA2 treatments effectively increased the activities of enzymatic antioxidants including those of SOD and CAT more dominantly under water deficit conditions. The latter indicates that the putative function of SAs-induced water deficit resistance in the maize plants may be due to their roles in reinforcing the antioxidant defence systems to nullify more H_2O_2 which maintained the leaf chlorophyll content.

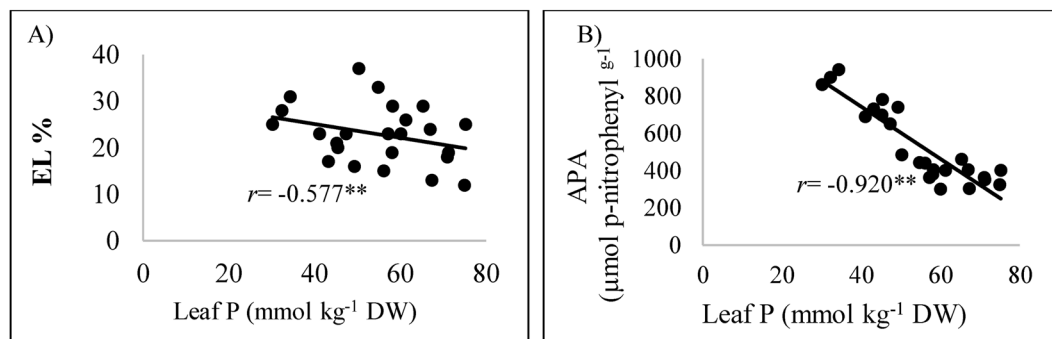


Figure 7. Correlation of leaf phosphorus (P) with electrolyte leakage [EL; (A)] and acid phosphatase [APA; (B)] enzyme activity in the leaves of maize plants grown under phosphorus and water deficiency stresses applied individually or in combination with or without soil amendments. **: correlations are significant at $P \leq 0.01$.

Conclusions

The present study clearly showed that combined stress (water stress and P deficiency) caused a considerable decrease in maize yield and yield related traits such as F_v/F_m , chlorophyll content, and leaf relative water content. However, supply of S-enriched LEO and HA as soil amendments mitigated the negative effects of both stress factors and increased plant growth, and yield. Our data clearly showed that addition of SAs specifically increased the antioxidative defense system and photosynthetic machinery of maize plants under water stress and P deficiency. Therefore, application of S-enriched leonardite and humic acid can be recommended for field application under water limited calcareous soils.

Received: 3 December 2019; Accepted: 10 March 2020;

Published online: 14 April 2020

References

1. FAO. Climate change. (FAO, 2008).
2. Cheeseman, J. In *Halophytes for food security in dry lands* 111–123 (Elsevier, 2016).
3. Kaurin, A. *et al.* Resilience of bacteria, archaea, fungi and N-cycling microbial guilds under plough and conservation tillage, to agricultural drought. *Soil. Biol. Biochem.* **120**, 233–245 (2018).
4. Lal, R. Challenges and opportunities in soil organic matter research. *Eur. J. Soil. Sci.* **60**, 158–169 (2009).
5. Shehab, G. G., AHMED, O. K. & El-Beltagi, H. S. Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). *Not. Bot. Horti Agrobot. Cluj. Napoca* **38**, 139–148 (2010).
6. Lewandowski, W., Erickson, T. E., Dixon, K. W. & Stevens, J. C. Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. *J. Appl. Ecol.* **54**, 997–1007 (2017).
7. Bashir, N., Mahmood, S., Zafar, Z. U. & Rasul, S. Is drought tolerance in maize (*Zea mays* L.) cultivars at the juvenile stage maintained at the reproductive stage? *Pak. J. Bot.* **48**, 1385–1392 (2016).
8. van Ittersum, M. K. *et al.* Yield gap analysis with local to global relevance—A review. *Field Crop. Res.* **143**, 4–17 (2013).
9. Kalra, N., Chakraborty, D., Ramesh Kumar, P., Jolly, M. & Sharma, P. K. An approach to bridging yield gaps, combining response to water and other resource inputs for wheat in northern India, using research trials and farmers' fields data. *Agric. Water Manage.* **93**, 54–64 (2007).
10. Soltani, A., Hajjarpour, A. & Vadez, V. Analysis of chickpea yield gap and water-limited potential yield in Iran. *Field Crop. Res.* **185**, 21–30 (2016).
11. Wang, X. *et al.* Genetic variation in ZmVPP1 contributes to drought tolerance in maize seedlings. *Nat. Genet.* **48**, 1233–1241 (2016).
12. FAO. *FAOSTAT Database – Agricultural Production*. (Food and Agriculture Organization, 2013).
13. Li, Z., Xu, W.-J., Xue, B.-D. & Cao, P. Discuss on evaluating method to drought-resistance of maize in seedling stage. *Journal of Maize Sciences* **2** (2004).
14. Min, H. *et al.* Identification of Drought Tolerant Mechanisms in Maize Seedlings Based on Transcriptome Analysis of Recombination Inbred Lines. *Front. Plant Sci.* **7** (2016).
15. Bender, R. R., Haegerle, J. W., Ruffo, M. L. & Below, F. E. Nutrient Uptake, Partitioning, and Remobilization in Modern, Transgenic Insect-Protected Maize Hybrids. *Agron. J.* **105**, 161 (2013).
16. Plénet, D., Etchebest, S., Mollier, A. & Pellerin, S. Growth analysis of maize field crops under phosphorus deficiency. I. Leaf growth. *Plant. Soil.* **223**, 119–132 (2000).
17. Ramos-Artuso, F., Galatro, A., Buet, A., Santa-María, G. E. & Simontacchi, M. Key acclimation responses to phosphorus deficiency in maize plants are influenced by exogenous nitric oxide. *J. Plant. Physiol.* **222**, 51–58 (2018).
18. Timlin, D. J., Naidu, T. C. M., Fleisher, D. H. & Reddy, V. R. Quantitative Effects of Phosphorus on Maize Canopy Photosynthesis and Biomass. *Crop. Sci.* **57**, 3156 (2017).
19. Lambers, H. & Plaxton, W. C. In *Annual Plant Reviews online* 3–22 (John Wiley & Sons, Ltd, 2018).
20. Obersteiner, M., Peñuelas, J., Ciais, P., van der Velde, M. & Janssens, I. A. The phosphorus trilemma. *Nat. Geosci.* **6**, 897–898 (2013).
21. Singh, S. K., Reddy, V. R., Fleisher, D. H. & Timlin, D. J. Relationship between photosynthetic pigments and chlorophyll fluorescence in soybean under varying phosphorus nutrition at ambient and elevated CO₂. *Photosynthetica* **55**, 421–433 (2017).
22. Duff, S. M. G., Moorhead, G. B. G., Lefebvre, D. D. & Plaxton, W. C. Phosphate Starvation Inducible 'Bypasses' of Adenylate and Phosphate Dependent Glycolytic Enzymes in *Brassica nigra* Suspension Cells. *Plant. Physiol.* **90**, 1275–1278 (1989).
23. Pandey, R. *et al.* Physiological and molecular alterations in plants exposed to high [CO₂] under phosphorus stress. *Biotechnol. Adv.* **33**, 303–316 (2015).
24. Cavagnaro, T. R., Bender, S. E., Asghari, H. R. & Heijden, M. G. A. V. D. The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends Plant. Sci.* **20**, 283–290 (2015).
25. Stutter, M. I. *et al.* Recovering Phosphorus from Soil: A Root Solution? *Environ. Sci. Technol.* **46**, 1977–1978 (2012).

26. Lopez-Arredondo, D. L., Leyva-González, M. A., González-Morales, S. I., López-Bucio, J. & Herrera-Estrella, L. Phosphate nutrition: improving low-phosphate tolerance in crops. *Annu. Rev. Plant. Biol.* **65**, 95–123 (2014).
27. Krishnapriya, V. & Pandey, R. Root exudation index: screening organic acid exudation and phosphorus acquisition efficiency in soybean genotypes. *Crop. Pasture Sci.* **67**, 1096 (2016).
28. Magalhaes, J. V., de Sousa, S. M., Guimaraes, C. T. & Kochian, L. V. In *Plant Macronutrient Use Efficiency* 123–147 (Elsevier, 2017).
29. Qiu, H. *et al.* Identification of QTL for acid phosphatase activity in root and rhizosphere soil of maize under low phosphorus stress. *Euphytica* **197**, 133–143 (2014).
30. Singh, M. *et al.* In *Conservation Agriculture* 113–134 (Springer Singapore, 2016).
31. Fernández-Escobar, R., Benlloch, M., Barranco, D., Dueñas, A. & Gañán, J. A. G. Response of olive trees to foliar application of humic substances extracted from leonardite. *Sci. Hort.* **66**, 191–200 (1996).
32. Terdputtakun, A., Arqueropanyo, O.-A., Janhom, S., Sooksamiti, P. & Naksata, W. Adsorption Characteristics of Leonardite for Removal of Cd(II) and Zn(II) from Aqueous Solutions. *Int. J. Environ. Sci. Dev.* **8**, 393–398 (2017).
33. Tipping, E. In *Cation binding by humic substances* 157–170 (Cambridge University Press, 2004).
34. Kalaitzidis, S., Papazisimou, S., Giannouli, A., Bouzinos, A. & Christanis, K. Preliminary comparative analyses of two Greek leonardites. *Fuel* **82**, 859–861 (2003).
35. Simandl, G., Simandl, J. & Aylen, P. In *Geological Fieldwork 2000* (ed. British Columbia Geological Survey) (British Columbia, 2001).
36. Tan, K. H. (CRC Press, 2014).
37. Sangeetha, M., Singaram, P. & Devi, R. In *Proceedings of 18th World Congress of Soil Science July. 9–15* (2006).
38. De Kok, L. J., Durenkamp, M., Yang, L. & Stulen, I. In *Plant Ecophysiology* 91–106 (Springer Netherlands, 2007).
39. Khan, K. *et al.* Phosphorus Solubility from Rock Phosphate Mixed Compost with Sulphur Application and Its Effect on Yield and Phosphorus Uptake of Wheat Crop. *Open. J. Soil. Sci.* **07**, 401–429 (2017).
40. Nikiforova, V. J. Towards dissecting nutrient metabolism in plants: a systems biology case study on sulphur metabolism. *J. Exp. Bot.* **55**, 1861–1870 (2004).
41. Duke, S. H. & Reisenauer, H. Roles and requirements of sulfur in plant nutrition. *Sulfur in agriculture*, 123–168 (1986).
42. Fox, A., Kwapinski, W., Griffiths, B. S. & Schmalenberger, A. The role of sulfur- and phosphorus-mobilizing bacteria in biochar-induced growth promotion of *Lolium perenne*. *FEMS Microbiol. Ecol.* **90**, 78–91 (2014).
43. Kertesz, M. A., Fellows, E. & Schmalenberger, A. In *Adv. Appl. Microbiol.* 235–268 (Elsevier, 2007).
44. Kusaka, M., Ohta, M. & Fujimura, T. Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. *Physiol. Plant.* **125**, 474–489 (2005).
45. 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).
46. Criado, M. V., Veliz, C. G., Roberts, I. N. & Caputo, C. Phloem transport of amino acids is differentially altered by phosphorus deficiency according to the nitrogen availability in young barley plants. *Plant. Growth Regul.* **82**, 151–160 (2017).
47. Tringovska, I., Naydenov, M., Valcheva, I. & Dintcheva, T. Effect of indigenous bacterial isolates on the development of tomato plants grown under phosphorus deficiency. *Acta Horticulturae*, 149–156 (2016).
48. Zhang, K., Liu, H., Tao, P. & Chen, H. Comparative Proteomic Analyses Provide New Insights into Low Phosphorus Stress Responses in Maize Leaves. *PLoS One* **9**, e98215 (2014).
49. Chen, J. *et al.* Modeling relations of tomato yield and fruit quality with water deficit at different growth stages under greenhouse condition. *Agric. Water Manage.* **146**, 131–148 (2014).
50. Chen, J. *et al.* Quantitative response of greenhouse tomato yield and quality to water deficit at different growth stages. *Agric. Water Manage.* **129**, 152–162 (2013).
51. Cole, J. & Pagay, V. Usefulness of early morning stem water potential as a sensitive indicator of water status of deficit-irrigated grapevines (*Vitis vinifera* L.). *Sci. Hort.* **191**, 10–14 (2015).
52. Ma, F. *et al.* Effect of water deficit in different growth stages on stem sap flux of greenhouse grown pear-jujube tree. *Agric. Water Manage.* **90**, 190–196 (2007).
53. Zhang, D., Jiao, X., Du, Q., Song, X. & Li, J. Reducing the excessive evaporative demand improved photosynthesis capacity at low costs of irrigation via regulating water driving force and moderating plant water stress of two tomato cultivars. *Agric. Water Manage.* **199**, 22–33 (2018).
54. Ghorchiani, M., Etesami, H. & Alikhani, H. A. Improvement of growth and yield of maize under water stress by co-inoculating an arbuscular mycorrhizal fungus and a plant growth promoting rhizobacterium together with phosphate fertilizers. *Agric., Ecosyst. Environ.* **258**, 59–70 (2018).
55. Ryan, J., Estefan, G. & Rashid, A. *Soil and Plant Analysis Laboratory Manual. 2nd edn*, 172 (International Center for Agricultural Research in the Dry Areas (ICARDA), 2001).
56. Olsen, S. R. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* (US Dept. of Agriculture, 1954).
57. Bardsley, C. E. & Lancaster, J. D. Determination of Reserve Sulfur and Soluble Sulfates in Soils. *Soil. Sci. Soc. Am. J.* **24**, 265 (1960).
58. Doorenbos, J. & Pruitt, W. In *Irrigation and Drainage No. 24* 144 (FAO, 1977).
59. Kaya, C., Higgs, D. & Burton, A. Plant growth, phosphorus nutrition, and acid phosphatase enzyme activity in three tomato cultivars grown hydroponically at different zinc concentrations. *J. Plant. Nutr.* **23**, 569–579 (2000).
60. Yamasaki, S. & Dillenburg, L. R. Measurements of leaf relative water content in *Araucaria angustifolia*. *Rev. Brasileira de fisiologia vegetal* **11**, 69–75 (1999).
61. Dionisio-Sese, M. L. & Tobita, S. Antioxidant responses of rice seedlings to salinity stress. *Plant. Sci.* **135**, 1–9 (1998).
62. Strain, H. H. & Svec, W. A. In *The Chlorophylls* 21–66 (Elsevier, 1966).
63. Besford, R. T. Phosphorus nutrition and acid phosphatase activity in the leaves of seven plant species. *J. Sci. Food Agric.* **30**, 281–285 (1979).
64. Kraus, T. E. & Fletcher, R. A. Paclobutrazol protects wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? *Plant. Cell Physiol.* **35**, 45–52 (1994).
65. Beauchamp, C. & Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **44**, 276–287 (1971).
66. Chance, B. & Maehly, A. C. In *Methods Enzymol.* 764–775 (Elsevier, 1955).
67. Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A. & Ghassemi-Golezani, K. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant. Omics* **5**, 60 (2012).
68. Loreto, F. & Velikova, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant. Physiol.* **127**, 1781–1787 (2001).
69. Chapman, H. D. & Pratt, P. F. *Methods of Analysis for Soils, Plants and Waters.* *Soil. Sci.* **93**, 68 (1962).
70. Saleque, M. A., Abedin, M. J., Ahmed, Z. U., Hasan, M. & Panaullah, G. M. Influences of phosphorus deficiency on the uptake of nitrogen, potassium, calcium, magnesium, sulfur, and zinc in lowland rice varieties. *J. Plant. Nutr.* **24**, 1621–1632 (2001).
71. Akter, F., Islam, M. N., Shamsuddoha, A., Bhuiyan, M. & Shilpi, S. Effect of phosphorus and sulphur on growth and yield of soybean (*Glycine max* L.). *Int. J. Bio-resource Stress. Manag.* **4**, 556–561 (2013).

72. Chotchutima, S., Tudsri, S., Kangvansaichol, K. & Sripichitt, P. Effects of sulfur and phosphorus application on the growth, biomass yield and fuel properties of leucaena (*Leucaena leucocephala* (Lam.) de Wit.) as bioenergy crop on sandy infertile soil. *Agriculture Nat. Resour.* **50**, 54–59 (2016).
73. Naheed, G., Shahbaz, M., Akram, N. A. & Ashraf, M. Interactive effect of rooting medium application of phosphorus and NaCl on plant biomass and mineral nutrients of rice (*Oryza sativa* L.). *Pak. J. Bot.* **40**, 1601–1608 (2008).
74. Zhang, L., Sun, X.-Y., Tian, Y. & Gong, X.-Q. Biochar and humic acid amendments improve the quality of composted green waste as a growth medium for the ornamental plant *Calathea insignis*. *Sci. Hort.* **176**, 70–78 (2014).
75. Bakry, M. A., Soliman, Y. R. & Moussa, S. A. Importance of micronutrients, organic manure and biofertilizer for improving maize yield and its components grown in desert sandy soils. *Res. J. Agriculture Biol. Sci.* **5**, 16–23 (2009).
76. Kim, Y.-H. *et al.* Silicon treatment to rice (*Oryza sativa* L. cv. 'Gopumbyeo') plants during different growth periods and its effects on growth and grain yield. *Pak. J. Bot.* **44**, 891–897 (2012).
77. Shah, Z. H. *et al.* Humic Substances: Determining Potential Molecular Regulatory Processes in Plants. *Front. Plant Sci.* **9** (2018).
78. Nakasha, J. J., Sinniah, U. R., Puteh, A. & Hassan, S. A. Potential Regulatory Role of Gibberellic and Humic Acids in Sprouting of *Chlorophytum borivilianum* Tubers. *Sci. World J.* **2014**, 1–9 (2014).
79. Leustek, T. & Saito, K. Sulfate Transport and Assimilation in Plants. *Plant. Physiol.* **120**, 637–644 (1999).
80. Sanli, A., Karadogan, T. & Tonguc, M. Effects of leonardite applications on yield and some quality parameters of potatoes (*Solanum tuberosum* L.). *Turkish J. Field Crop.* **18**, 20–26 (2013).
81. Govahi, M. & Saffari, M. Effect of Potassium and Sulphur Fertilizers on Yield, Yield Components and Seed Quality of Spring Canola (*Brassica napus* L.) Seed. *J. Agron.* **5**, 577–582 (2006).
82. Chandra, N. & Pandey, N. Influence of Sulfur Induced Stress on Oxidative Status and Antioxidative Machinery in Leaves of *Allium cepa* L. *Int. Sch. Res. Not.* **2014**, 1–9 (2014).
83. Ashraf, M. & Harris, P. J. C. Photosynthesis under stressful environments: An overview. *Photosynthetica* **51**, 163–190 (2013).
84. Frosi, G. *et al.* Different physiological responses under drought stress result in different recovery abilities of two tropical woody evergreen species. *Acta Botanica Brasiliica* **31**, 153–160 (2017).
85. Liang, C. *et al.* Melatonin delays leaf senescence and enhances salt stress tolerance in rice. *J. Pineal Res.* **59**, 91–101 (2015).
86. Ni, J. *et al.* Exogenous Melatonin Confers Cadmium Tolerance by Counterbalancing the Hydrogen Peroxide Homeostasis in Wheat Seedlings. *Molecules* **23**, 799 (2018).
87. Akram, N. A. *et al.* Aminolevulinic acid and nitric oxide regulate oxidative defense and secondary metabolisms in canola (*Brassica napus* L.) under drought stress. *Protoplasma* **255**, 163–174 (2018).
88. Aziz, A., Akram, N. A. & Ashraf, M. Influence of natural and synthetic vitamin C (ascorbic acid) on primary and secondary metabolites and associated metabolism in quinoa (*Chenopodium quinoa* Willd.) plants under water deficit regimes. *Plant. Physiol. Biochem.* **123**, 192–203 (2018).
89. Kosar, F., Akram, N. A. & Ashraf, M. Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. *S. Afr. J. Bot.* **96**, 71–77 (2015).
90. Huseynova, I. M., Suleymanov, S. Y., Rustamova, S. M. & Aliyev, J. A. Drought-induced changes in photosynthetic membranes of two wheat (*Triticum aestivum* L.) cultivars. *Biochemistry* **74**, 903–909 (2009).
91. Razzaq, M., Akram, N. A., Ashraf, M., Naz, H. & Al-Qurainy, F. Interactive effect of drought and nitrogen on growth, some key physiological attributes and oxidative defense system in carrot (*Daucus carota* L.) plants. *Sci. Hort.* **225**, 373–379 (2017).
92. Masoumi, A., Kafi, M., Khazaei, H. & Davari, K. Effect of drought stress on water status, electrolyte leakage and enzymatic antioxidants of kochia (*Kochia scoparia*) under saline condition. *Pak. J. Botany* **42**, 3517–3524 (2010).
93. Assaha, D. V., Liu, L., Ueda, A., Nagaoka, T. & Saneoka, H. Effects of drought stress on growth, solute accumulation and membrane stability of leafy vegetable, huckleberry (*Solanum scabrum* Mill.). *J. Environ. Biol.* **37**, 107–114 (2016).
94. Taiz, L. & Zeiger, E. *Plant Physiology*. 4th edn, (Sinauer Associates Inc., 2016).
95. Lawlor, D. W. & Cornic, G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant. Cell Env.* **25**, 275–294 (2002).
96. Williams, T. *et al.* Evaluation of a Baculovirus Bioinsecticide for Small-Scale Maize Growers in Latin America. *Biol. Control.* **14**, 67–75 (1999).
97. Chowdhury, J. A., Karim, M. A., Khaliq, Q. A., Ahmed, A. U. & Mondol, A. M. Effect of drought stress on water relation traits of four soybean genotypes. *SAARC J. Agriculture* **15**, 163–175 (2018).
98. Nogués, S. & Baker, N. R. Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. *J. Exp. Bot.* **51**, 1309–1317 (2000).
99. Kim, J.-S. *et al.* Death mechanisms caused by carotenoid biosynthesis inhibitors in green and in undeveloped plant tissues. *Pestic. Biochem. Physiol.* **78**, 127–139 (2004).
100. Lee, S. C., Lan, W., Buchanan, B. B. & Luan, S. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci.* **106**, 21419–21424 (2009).
101. Abuelsoud, W., Hirschmann, F. & Papenbrock, J. In *Drought Stress Tolerance in Plants, Vol 1* 227–249 (Springer International Publishing, 2016).
102. Memon, K. In *Soil Science* (eds. Bashir & Bantel) 291–316 (National Book Foundation, 1996).
103. Zhou, T. *et al.* Genotypic Differences in Phosphorus Efficiency and the Performance of Physiological Characteristics in Response to Low Phosphorus Stress of Soybean in Southwest of China. *Front. Plant Sci.* **7** (2016).
104. Erdal, I., Kepenek, K. & Kizilgöz, I. Effect of Elemental Sulphur and Sulphur Containing Waste on the Iron Nutrition of Strawberry Plants Grown in a Calcareous Soil. *Biol. Agriculture Horticulture* **23**, 263–272 (2006).
105. Duplessis, G. L. & MacKenzie, A. F. Effects of leonardite applications on phosphorus availability and corn growth. *Can. J. Soil. Sci.* **63**, 749–751 (1983).
106. Bargaz, A. *et al.* A phosphoenol pyruvate phosphatase transcript is induced in the root nodule cortex of *Phaseolus vulgaris* under conditions of phosphorus deficiency. *J. Exp. Bot.* **63**, 4723–4730 (2012).
107. Kouas, S. *et al.* Effect of phosphorus deficiency on acid phosphatase and phytase activities in common bean (*Phaseolus vulgaris* L.) under symbiotic nitrogen fixation. *Symbiosis* **47**, 141–149 (2009).
108. Wasaki, J., Yamamura, T., Shinano, T. & Osaki, M. Secreted acid phosphatase is expressed in cluster roots of lupin in response to phosphorus deficiency. *Plant. Soil.* **248**, 129–136 (2003).
109. Fathi, A. & Tari, D. B. Effect of Drought Stress and its Mechanism in Plants. *Int. J. Life Sci.* **10**, 1–6 (2016).
110. Shahid, M. *et al.* Influence of EDTA and citric acid on lead-induced oxidative stress to *Vicia faba* roots. *J. Soils Sed.* **14**, 835–843 (2013).
111. Moghadam, H. R. T. In *Biological Forum*. 1704 (Research Trend) (2015).
112. Foroozesh, P. *et al.* Effect of Cadmium stress on antioxidant enzymes activity in different bean genotypes. *J. Agric. Biol. Sci.* **7**, 351–356 (2012).
113. Yadav, N. & Sharma, S. Reactive oxygen species, oxidative stress and ROS scavenging system in plants. *J. Chem. Pharm. Res.* **8**, 595–604 (2016).
114. Ahammed, G. J. *et al.* Dopamine alleviates bisphenol A-induced phytotoxicity by enhancing antioxidant and detoxification potential in cucumber. *Environ. Pollut.*, **113957** (2020).

Acknowledgements

This research was partly supported by Harran University, Turkey. The authors would also like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2019/116), King Saud University, Riyadh, Saudi Arabia. Thanks to Professor Dr. Philippe Monneveux for reviewing the paper before submission.

Author contributions

C.K., M.A. and P.A. designed the experimental setup, C.K. performed the experiments. M.S., N.A.A. and M.N.A. carried out data analysis. C.K., M.S., N.A.A. and M.N.A. wrote the initial draft of the manuscript. M.A. and P.A. critically revised the manuscript and edited it to present form. All authors read and approved it for submission.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to P.A.

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