Journal of Animal & Plant Sciences, 33(6): 2023, Page: 1292-1303
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.6.0669

PHOSPHORUS AND ZINC NUTRITION IN MAIZE (*ZEA MAYS* L.) UNDER DROUGHT STRESS

T. Afzal[1], A. Wakeel[1], T. Shahzad[2], S. Hussain[2] and M. Sanaullah[1]*

[1]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad.
[2]Department of Environmental Sciences & Engineering, Government College University Faisalabad.
*Corresponding author's email: sanasial@gmail.com; msanaullah@uaf.edu.pk

ABSTRACT

Drought stress causes negative effects on soil nutrients dynamics as well as their mutual interactions. Negative interaction between phosphorus (P) and zinc (Zn) vis-à-vis plant uptake exists under adequate moisture availability but this phenomenon is still unclear under drought condition. A pot study was conducted to investigate the effect of this interaction on maize plants (var. FH-1046) at three moisture levels, viz., optimum (80% of water holding capacity-WHC), moderate drought (50% of WHC) and severe drought (30% of WHC). The P and Zn were added alone (only-P and only-Zn) or in combination (P + Zn) at the rate of 57 and 6 mg kg^{-1} soil at three moisture levels with complete randomization. Soil moisture levels were introduced after two weeks of plant growth and maintained after every three days by weighing. After 60 days of plant growth, plants were harvested and analyzed for their growth and nutrients contents, viz., P, Zn, nitrogen (N), and potassium (K). The findings showed that combined P and Zn caused a significant reduction in P and Zn contents at the root and shoot levels at higher moisture (80% and 50% of WHC) relative to alone P and Zn additions, but the extent of this reduction reduced with increasing drought intensity, showing the negative effect of drought on plant nutrients uptake. The effect of combined nutrients addition was positive on plant growth and the absence of P (only Zn addition) reduced plant height by 14% and 28% at 80% and 30% of WHC levels. Shoot and root dry weights increased significantly by 41% and 43% with combined P and Zn applications. The plant growth also declined with the increasing drought levels. Under severe drought (30% of WHC), the extent of the negative interaction of P and Zn diminished considerably but there was an overall negative effect on plant growth and nutrient contents at 30% of WHC. The findings suggest that increasing drought intensity can reduce the negative effect of P and Zn on each other's uptake and accumulation in plants but with concurrent reductions in absolute nutrients contents and plant growth.

Keywords: Phosphorus; Zinc; Drought; Maize; Water holding capacity

INTRODUCTION

Drought is the most predominant abiotic stress across the globe (Silva *et al*., 2010). The severity and frequency of drought events is predicted to exacerbate in the future by the global climate models (Barnett *et al*., 2005). The quantitative assessment demonstrates that arid and semiarid regions may be highly vulnerable to drought events compared to humid regions in the future (Mohammad and Scholz, 2017). The soil moisture is prerequisite to the nutrients mobility and regulation in soil and plant systems (Hu and Schmidhalter, 2005). Drought stress affects plants in two ways; either by directly influencing plant metabolic processes or indirectly by causing a reduction in nutrients uptake from soil, which might be linked with drought-induced inhibition on root activity, uploading into xylem vessels, and transpiration flow (Page and Feller, 2015). Reduced capacity of roots to take up nutrients from soil generally causes a decline in their concentrations in upper plant parts (Etienne *et al*., 2018). The moisture deficiency in soil pores under drought typically causes notable changes in soil physicochemical and biological characteristics, which can affect nutrients dynamics in soil (Faye *et al*., 2006; Amtmann and Blatt, 2009). However, the effect of drought on nutrients availability may be variable in accordance with the intensity and duration of drought events. The severe drought has been reported to pose a more negative effect on the uptake, transport, and allocation of nutrients within plant tissues (Fischer *et al*., 2019). However, moderate drought stress was found to induce a positive effect on the N allocation and the protein contents of plant tissues (Barron *et al*., 2003; Etienne *et al*., 2018).

The mobility of minerals in soil and within plant tissues shows great variations; and macronutrients may be more mobile than micronutrients (Etienne *et al*., 2018). Thus, P may be more mobile within plants than Zn under drought condition. However, in soil, P acts as immobile mineral which may be attributed to its less solubility and very reactive nature (Paz-Ares *et al.* 2022). The main pathway governing the movement of P and Zn is diffusion which is dependent on the soil moisture availability. Under moist-deficit conditions, the dimensionality of the soil pores reduces, and tortuosity increases, which causes to reduce the nutrients mobility in soil especially of P and Zn (Faye *et al*., 2006). Thus, drought condition can constrain the mobility of these minerals (P and Zn) by limiting the diffusion pathway in soil, consequently reducing the plant root uptake of minerals, loading into xylem vessels, and translocation to other plant parts (Ahanger *et al*., 2018).

The negative interaction between P and Zn is widely recognized phenomenon in plant nutrition (Marschner, 1995; Zhang *et al.* 2012; Zhang *et al.* 2016), which may cause reduction in plant growth and productivity (Soltangheisi *et al*., 2013; Sacristán *et al*., 2019). Marschner (2011) reported that P and Zn may bind with root fabric under their co-existence that may reduce their upward loading to xylem vessels and subsequent translocation to other plant parts. Under higher or even adequate P supply, a boom in plant growth and development may cause a dilution in plant Zn content (Sacristán *et al*., 2019). Another factor that indirectly poses this negative interaction might be associated with suppressive effect of high P fertilization on the mycorrhizal associations (Ryan *et al*., 2008; Ova *et al*., 2015) that may be important in the uptake of micronutrients such as Zn. The supply of Zn can also affect P dynamics within plant tissues probably due to the formation of Zn-phytate within root cells, thereby obstructing P transport from root to shoot (Hopkins *et al*., 1998; Rupa *et al*., 2003). To the best of our knowledge, past research seems to be focused on this antagonistic interaction under adequate moisture availability and the possible effect of soil moisture in this regard has not been fully explored yet. However, the escalation in drought events under ongoing global warming threat necessitates to explore this nutrient interaction under moist-scarce conditions. A pot experiment was designed to investigate the effects of drought on this mineral interaction by applying P and Zn alone (only-P and only-Zn) as well as in combination (P + Zn) at three moisture levels, 80% of WHC (optimum moisture), 50% of WHC (moderate drought) and 30% of WHC (severe drought). It was hypothesized that drought stress would intensify the P and Zn interaction by affecting their uptake from soil and nutrient contents in plants.

MATERIALS AND METHODS

This pot study was carried out at wire house located in the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad Pakistan. After removing the field trash, the soil was sampled from the upper 20 cm using soil auger. The sampling site was situated on experimental farm of the Institute under wheat and maize rotation for the last three years. A composite sample was prepared from the collected soil and analyzed for various physicochemical characteristics after air-drying, grinding with a pestle and mortar, and sifting via 2-mm sieve.

The physicochemical analyses of soil included soil texture (Bouyoucos, 1962), pH of saturated paste, electrical conductivity of extract (ECe), organic matter by Walkley and Black method (Nelson and Sommers, 1996), soil total N (Jackson, 1962), Olsen-P (Olsen and Sommers, 1982), NH_4-acetate exchangeable K by flame photometer (Jenway PFP-7), and DTPA-extractable Zn using an atomic absorption spectrometer (AAS) (Hitachi, Solaar S-100, CISA). The soil texture was loamy with sand (40%), silt (38%) and clay (23%) and slightly alkaline (pH 7.50) with non-saline conditions (ECe 1.48 dS m^{-1}). The soil had field capacity (17%), determined by thermo-gravimetric method, and low organic matter content (< 0.70 %). Soil total N, Olsen-P, NH_4-acetate exchangeable K, and DTPA-extractable Zn contents were 200, 7.30, 161, and 0.05 mg kg^{-1} soil, respectively.

Treatment plan and experimentation: After having imbibed in 0.01 M $CaSO_4$ solution for 4 hours, the seeds of hybrid maize (FH-1046) were germinated on moist filter paper under dark conditions for 2 days. Thirty-six pots were filled with 5 kg of soil. The recommended doses of N and K (136 and 31 mg kg^{-1} soil, respectively) were uniformly applied to all pots, equivalent to 272 and 62 kg ha^{-1}, respectively. The split application of N was done after 2 and 4 weeks of transplantation. The experiment included three treatments as follows: i) only P (57 mg kg^{-1}), ii) only Zn (6.3 mg kg^{-1}) and iii) their combined addition (P and Zn, 57 and 6.3 mg kg^{-1}). The amounts of P and Zn were equivalent to 114 and 12.6 kg ha^{-1}, respectively, on a field scale. Six germinated seedlings (two-days old) were transplanted to each pot and moisture content was maintained at 80% of WHC for 14 days in all pots. The pots were then split into 80%, 50%, and 30% of WHC levels and the respective moisture levels in the pots were maintained by weighing. The treatments (only P, only Zn, and combined P + Zn) at three moisture levels were replicated 4 times (n = 4) with complete randomization. To avoid the competition of weeds for nutrients, these were manually eliminated from the pots regularly.

Harvesting and plant analysis: The plants were harvested using scissors on completing 60 days of growth. Before harvest, plant height was measured with a meter rod. The roots were manually removed from the soil and their length was measured. Both shoot and root samples were washed with tap and distilled water. Oven-dried shoot and root samples (at 65°C) till constant weight were ground using a stainless-steel grinder for nutrient analysis.

Shoot and root samples (250 mg) were wet-digested using a di-acid mixture (HNO_3 and $HClO_4$) at 380°C till the colorless appearance. The P concentration of samples was detected at λ = 410 nm on a UV-VIS Spectrophotometer (Shimadzu UV-1201) using ammonium vanado-molybdate as the color developing reagent (CDR) (Chapman and Pratt, 1962). A calibration curve from the anatomized standards was formed and used to evaluate the P concentration in the samples. The DTPA-extractable Zn concentration of the samples was determined on AAS (Hitachi, Solaar S-100, CISA). The N and K concentrations of the samples were determined using Kjeldhals' distillation apparatus (UDK, D126) and flame photometer (Jenway PFP-7), respectively. The translocation of P and Zn from root to shoot was determined by dividing the shoot uptake of P and Zn with root uptake of P and Zn, respectively. The shoot uptake and root uptake of P and Zn were determined by multiplying their concentrations (g kg^{-1}) in shoot and root with their shoot and root dry weights, respectively and then dividing the final value with 1000.

Statistical analysis: All parameters were subjected to the two-way analysis of variance (ANOVA) with moisture and P and Zn levels as fixed factors and the difference among the treatment means at three moisture levels were compared by least significant difference (LSD) test at 5% level of probability using Statistix 8.1 software.

RESULTS

Plant growth variables: The combined P and Zn and only-P treatments had a positive effect on the shoot length at 80% and 30% of WHC levels compared to only Zn, which was not recorded in case of 50% of WHC (Fig. 1). The maximum shoot length (83.5 cm) was recorded under combined addition, which was 12% higher than only Zn addition (Fig. 1a). The root length also showed a similar trend, which was found to be greatest (64.8 cm) under combined addition at 80% of WHC level, while only Zn at 30% of WHC caused a significant reduction ($p < 0.05$) in root length (27.9 cm), as shown in Fig. 1b.

The combined P and Zn enhanced the shoot dry weight at 30% of WHC by 19% and 29% compared to alone additions of P and Zn, respectively. No significant change in shoot dry weight was found for combined (P and Zn) and only P at 50% and 80% of WHC levels. At 80% of WHC, only Zn reduced shoot dry weight significantly by 26% compared to only P. Shoot dry weight was the highest (6.8 g) at 80% of WHC with combined P and Zn, and the lowest (2.8 g) at 30% of WHC in the absence of P (only Zn addition) (Fig. 2a). While no significant effect of P and Zn levels was found on root dry weight at 30% of WHC level. Under combined P and Zn, root dry weight increased by 30% and 9% relative to alone additions of P and Zn at 50% of WHC. The root dry weight was recorded highest (2.4 g) by combined P and Zn at 80% of WHC, which was about 27% and 9% higher than alone Zn and P additions, respectively (Fig. 2b).

Phosphorus and zinc uptake and allocation: The interaction of P and Zn had a significant negative effect on their content at both shoot and root levels. Shoot P content was significantly higher by 24% and 27% by only P treatment than combined (P + Zn) at 80% and 30% of WHC levels, respectively (Fig. 3a). However, at 30% of WHC, shoot P content was found to be same with combined (P and Zn) and only P additions. The shoot P content was recorded lowest with only Zn than combined addition and only P additions (Fig. 3a).

The P content of roots was also consistent with shoot where interaction of P and Zn negatively affected the P content at all moistures. At 80% of WHC, the P content of root was the highest (6.60 g kg^{-1} dry matter) with only P, which was about 33% higher than combined addition. With increasing drought intensity (50% and 30% of WHC levels), combined addition also resulted in 28% and 25% reductions in root content of P than only P, respectively (Fig. 3b).

Root to shoot translocation of P was not affected by alone and combined P and Zn additions at 80% and 50% of WHC levels. However, P translocation was considerably reduced at 30% of WHC level with combined P and Zn (Fig. 3c). Higher P translocation was recorded with only P addition at 30% of WHC, but the magnitude was not significant compared to combined P and Zn at all moisture levels (Fig. 3c).

Like the P content in plants, the interactive effect was inhibitory on shoot Zn content. Shoot Zn content was significantly higher by 13% and 10% with only Zn than combined P and Zn at 80% and 50% of WHC, respectively (Fig. 4a). At 30% of WHC, combined P and Zn also decreased the shoot Zn content than only Zn addition, although non-significant, being similar with shoot P concentration at 30% of WHC. There was also a consistent trend for root Zn content. Combined addition also reduced the root Zn content by 23%, 18%, and 13% relative to only Zn at 80%, 50%, and 30% of WHC levels, respectively (Fig. 4b).

Root to shoot translocation of Zn was largely affected by soil moisture contents. The Zn translocation was not affected by alone and combined P and Zn at 80% of WHC. While an increase in Zn translocation was found at 50% and 30% of WHC levels in the absence of Zn (only P addition), as shown in Fig. 4c.

Plant nitrogen and potassium uptake: The N and K contents in shoot and root were found to be positively related with only P and combined (P and Zn) additions. At 80% of WHC, shoot N contents increased by 10% and

15% under combined P and Zn compared to alone P and Zn additions, respectively. This increase in shoot N content with combined P and Zn was consistent with increasing drought intensity (50% and 30% of WHC levels) with reduction in absolute nutrient contents due to moisture deficiency (Fig. 5a). The presence of P was found to induce positive effect on root N content at 50% and 80% of WHC levels. With only Zn, the root N content reduced by 18% and 10% at higher moisture (80% and 50% of WHC levels). The effect of P and Zn additions (combined or alone) on root N content was decreased with increasing drought intensity (30% of WHC level) (Fig. 5b). The K content of shoot and root were not affected (reduced) by the absence of Zn (with only P addition) at all moisture levels (Fig. 6).

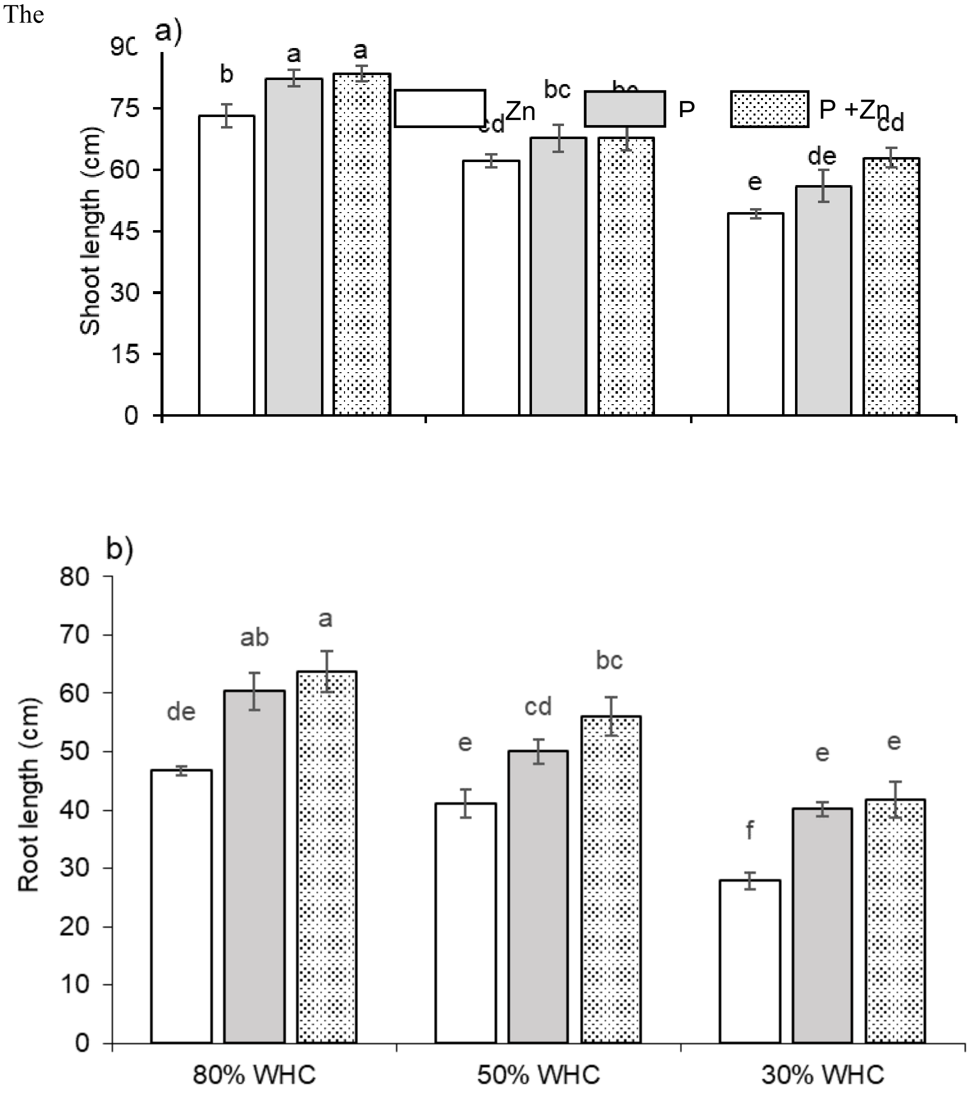

Figure 1: The length of shoot (a) and root (b) with separate as well as combined P and Zn applications under three moisture levels. Data represented as mean ± standard error (SE; n = 4). The bars with different letters are significant statistically.

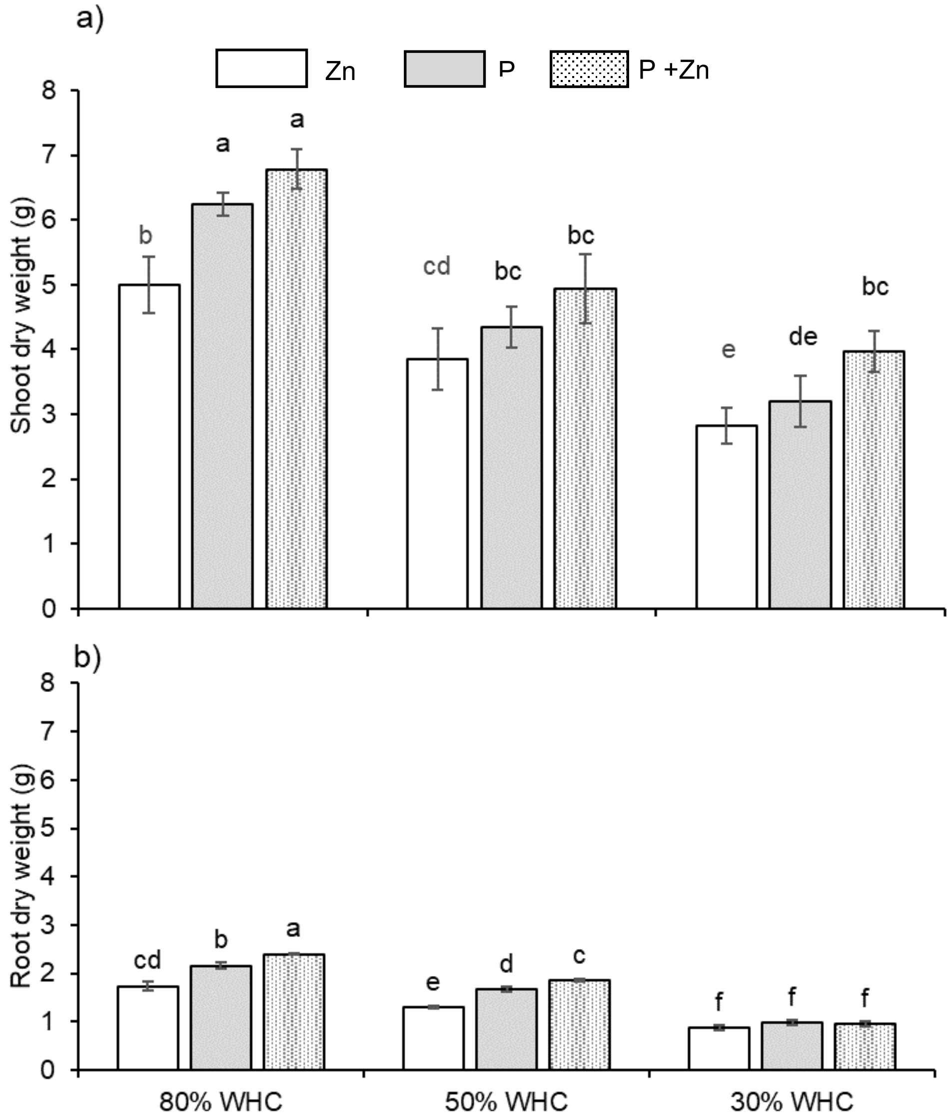

Figure 2: The dry weight of shoot (a) and root (b) with separate and combined P and Zn applications at three moisture levels. Data represented as mean ± SE (n = 4). The bars with different letters are significant statistically.

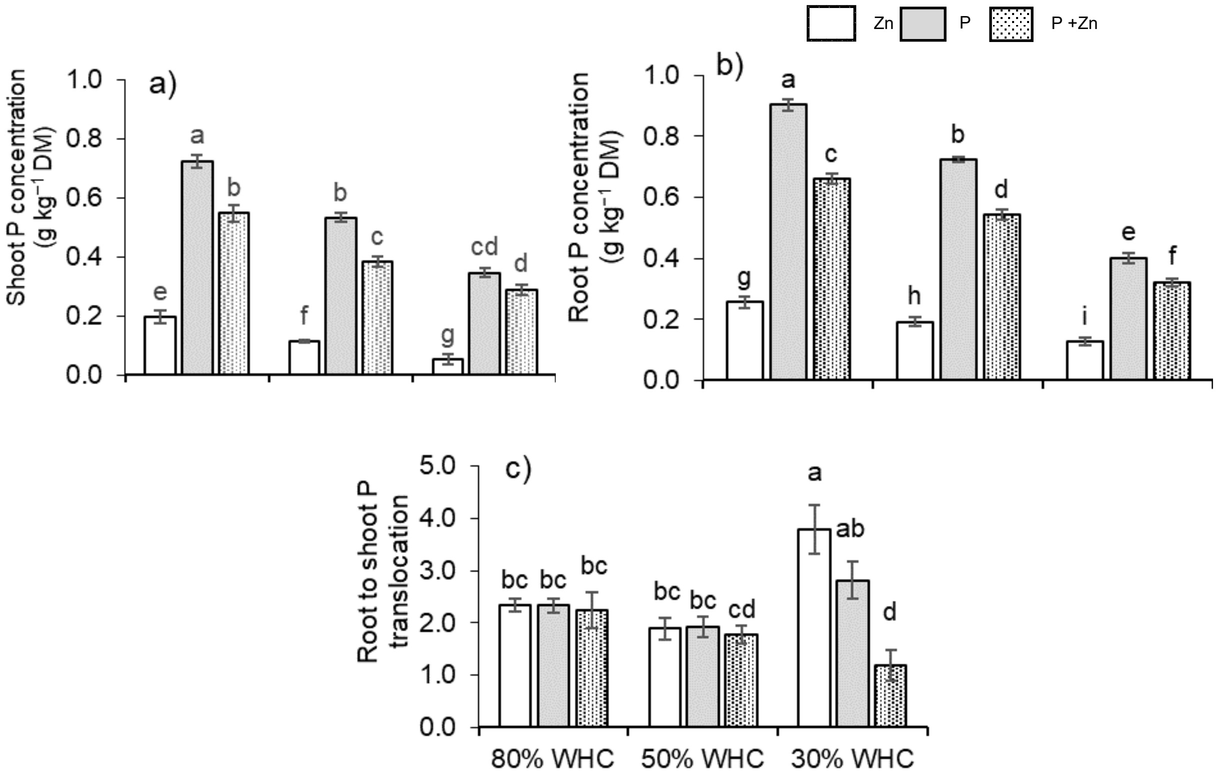

Figure 3: The mean P concentration (g kg^{-1} dry matter) of shoot (a) and root (b) as well as P translocation from root to shoot (c) with separate and combined P and Zn applications at three moisture levels. The bars with different letters are significant statistically. The data represented as mean ± SE (n = 4).

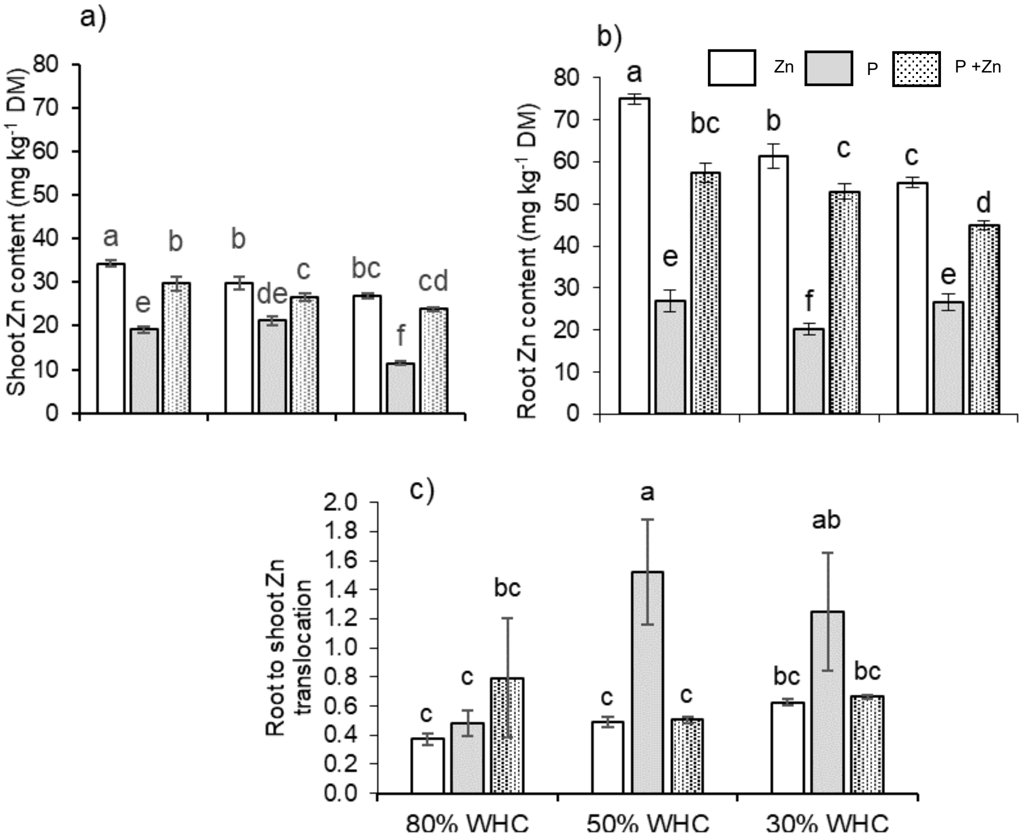

Figure 4: The mean Zn concentration (mg kg^{-1} dry matter) of shoot (a) and root (b) as well as root to shoot Zn translocation (c) with separate and combined P and Zn applications at three moisture levels. The bars with different letters are significant statistically. The data represented as mean ± SE (n = 4).

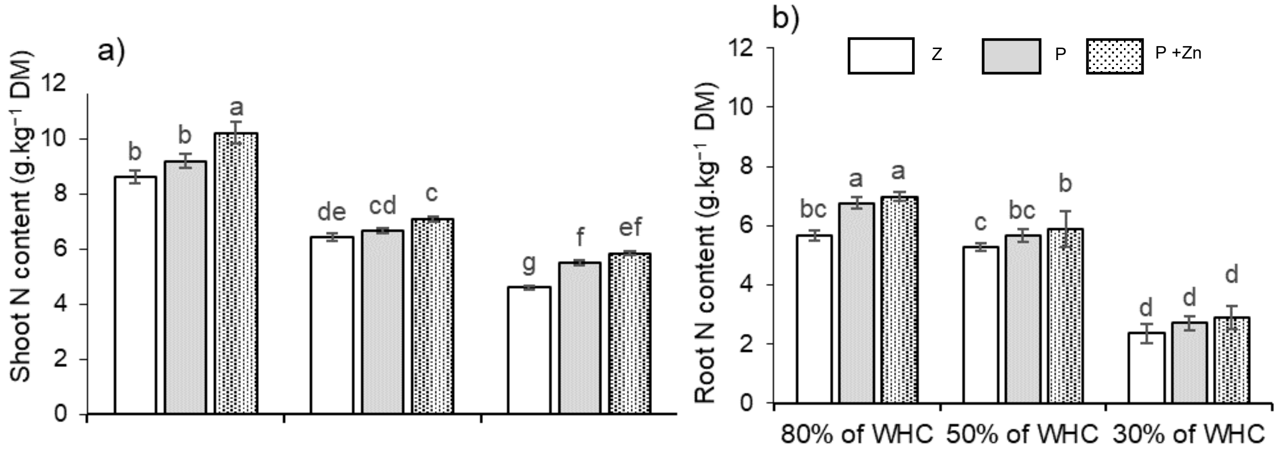

Figure 5: The mean N concentration (g kg^{-1} dry matter) of shoot (a) and root (b) with separate and combined P and Zn applications at three moisture levels. The bars with different letters are significant statistically. The data represented as mean ± SE (n = 4).

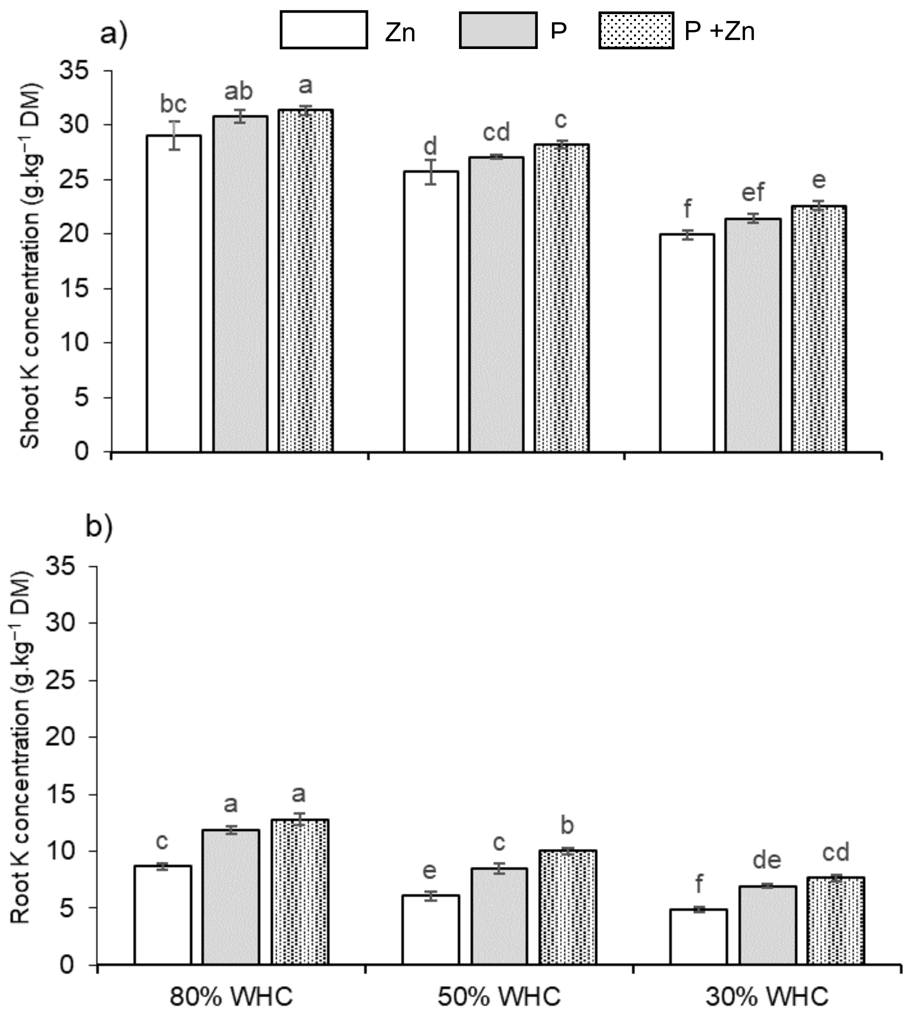

Figure 6: The mean K concentration (g kg^{-1} dry matter) of shoot (a) and root (b) with separate and combined P and Zn applications at three moisture levels. The bars with different letters are significant statistically. The data represented as mean ± SE (n = 4).

DISCUSSION

The notable increase in plant growth (plant height and root length; Fig. 1a-b), and plant dry weights (Fig. 2 a-b) with the addition of P or combined P and Zn at all moisture levels demonstrates the vitality of P in plant developmental processes, which was corroborated by a number of studies in the past (Singh and Sale, 2000; Plénet *et al*., 2000; Mumtaz *et al*., 2014; Servani *et al*., 2014; Kim and Li, 2016). The involvement of P is central to the plant development since it is involved in the biosynthesis of key biomolecules (ATP; adenosine triphosphate and nucleic acids), intactness of cellular membranes, cell division, utilization of carbohydrates, and enzyme activities (Marschner, 1995). The plant growth was found to be highly sensitive to the lack of P compared to Zn.

Under combined P and Zn application, the supply of Zn may also be important in improving plant growth attributes particularly under stressed condition. The Zn is critical to the functioning of a large number of enzymes (Etienne *et al*., 2018), protection of several cellular structures (membrane phospholipids and nucleic acids), and also a structural part of a key enzyme (Zn-SOD; superoxide dismutase) (Cakmak and Marschner, 1988; Cakmak, 2000; Marschner, 2011). This enzyme typically provides aid to plants to cope with the deleterious effects of drought stress, such as the detoxification of ROS (reactive oxygen species). Under combined P and Zn application, the significant increases in root dry weight at all moisture levels and shoot dry weight at severe drought (Fig. 2) substantiated the importance of Zn in plant metabolism. The P also plays a crucial part in improving plants' resiliency under stressful conditions (Motalebifard *et al*., 2013) since it is central to the synthesis of ATP and structure of cellular components, as discussed above. Thus, combined application of P and Zn was found to be highly efficacious in maintaining the plant development under moisture deficit conditions.

A severe reduction in plant growth attributes under drought condition (Fig. 1-2) demonstrated its deleterious effects on plant metabolism. The moisture deficiency poses a multitude of negative effects on plant physiological and metabolic processes, such as root activity (nutrient uptake per unit of root size), transpirational stream (plant water relations), reduced stomatal conductance, and cell division (Cakmak *et al*., 1996; Alam, 1999). A cascade of these changes in plants disturbs the assimilatory processes (photo-assimilation or C assimilation), consequently posing negative impacts on plant developmental processes (Farooq *et al*., 2009; Taiz and Zeiger, 2006). Under moist-deficit conditions, an improvement in plant development with the combined addition of P and Zn rather than alone additions demonstrates the significance of proper nutrient management to enhance the plants' resiliency under stressful conditions.

Despite the positive effects of combined P and Zn addition on plant development, both nutrients were found to reduce each other's contents at root and shoot levels. The significant decrease in P content of root and shoot under combined P and Zn addition (Fig. 3) demonstrates the negative effect of Zn on its root uptake and upward translocation. This negative effect of Zn was found to be reduced with the intensification of drought levels even non-significant on shoot P content at severe drought compared to combined P and Zn addition (Fig. 3a). This highlights that drought conditions can reduce the extent of P and Zn interactions by posing negative effects on nutrients mobility in soil and root uptake with concurrent reductions of absolute contents of P and Zn at root and shoot levels (Marschner, 2011). The mobility of both P and Zn is highly sensitive to moisture deficiency since both of these moves in soil via diffusion pathway. At higher moisture levels, under combined P and Zn addition, the mutual interactions of P and Zn and binding of P with plant phytate contents (Yousaf *et al*., 2019) at the root level can limit its loading into xylem and subsequently disturbing its translocation and remobilization (Cakmak and Marschner, 1987; Rupa *et al*., 2003; Yousaf *et al*., 2019).

The Zn contents of shoot and root in response to P and Zn additions were affected in the same way as of P, demonstrating a negative impact of P on the uptake and upward translocation of Zn. The extent of the negative effect of P on plant Zn content was decreased with increasing drought intensity like the negative effect of Zn on plant P contents, as discussed above. The reduction in plant Zn content under adequate or excessive P addition may be caused by the vitality of P as being structural part in several cellular compartments and thereby it can result in the dilution of plant Zn contents by promoting plant development (P-induced dilution in Zn content) (Cakmak and Marschner, 1987). The decrease in the magnitude of P and Zn interactions with concurrent reductions in absolute nutrient concentrations with increasing drought intensity may be caused by reduced mineral solubility, limited root nutrient uptake activity, and impaired mineral dynamics in plant due to poor water relations of plants under drought stress condition (Drissi *et al*., 2015). Under combined P and Zn addition, the extent of P translocating from root to shoot was found to decrease with increasing drought intensity, but vice versa was the case with alone applications (Fig. 3c). Despite the low solubility and phyto-availability in soil under moist-deficit conditions, the requirement of P acquisition by plants increases under moisture scarcity to cope with the deleterious effects of drought (Jin *et al*. 2006; Rose *et al*., 2013). The P translocation to shoot under moist-deficit conditions with combined P and Zn application may have been reduced due to the negative interaction of P and Zn.

The dramatic increase in Zn translocation to shoot with only P addition (in the absence of Zn) under drought levels (50% and 30% of WHC levels) (Fig. 4c) might be due to early sensing of Zn deficiency at the shoot level which may stimulate its accumulation in shoot by promoting root activity (higher Zn uptake) (Assunção *et al*., 2013; Bista *et al*., 2018). This might also explain the higher upward translocation of Zn in the absence of Zn (with only P) under moist-scarce conditions (50% and 30% of WHC levels) (Fig. 4a-b). Under excessive P supply, lettuce roots were found to be deficient in Zn content (Bouain *et al*., 2014). This also shows that a negative effect of P on plant Zn content largely prevails at the root level and the Zn accumulation in upper plant parts is reduced under enhanced P nutrition. Upward translocation of both P and Zn with their combined addition was found to be significantly lower with increased drought intensity.

The reduction in root content of P and Zn under their combined addition may also be caused by their mutual reaction and the precipitation of Zn phosphates in soil (Drissi *et al*., 2015). The significant decreases in root Zn content with increasing drought levels under combined addition may be due to an increase in the extent of their mutual reaction under moist-deficit conditions. This reaction between P and Zn may be increased in P-rich clayey soil and poses a negative effect on the mobility and phyto-availability of Zn (Imran *et al*., 2016; Moreno-Lora and Delgado, 2020).

In the absence of P (only Zn addition), the significant decrease in N and K contents of root and shoot demonstrates the positive effect of P addition on their uptake and upward translocation. No change in plant N and K contents with combined P and Zn shows that their negative interaction has no effect on the other plant nutrients. The reduced plant development with increased drought intensity may explain the reduction in plant mineral contents (N and K) in moisture scarcity. The physiological disturbances in plants such as reduced photo-assimilation and limited root activity caused by drought stress may reduce plant development (Grossman and Takahashi, 2001; Bista *et al*. 2018). The physicochemical changes in soil caused by drought might also affect the mobility and uptake of nutrients (Amtmann and Blatt, 2009), thereby causing a reduction in nutrient concentrations in plants.

Conclusions: The study findings demonstrate the negative effect of P and Zn on each other's contents at the root and shoot level. The extent of this interaction was reduced with intensification of drought levels with concurrent decreases in absolute nutrient contents. This negative interaction was exclusive for P and Zn, and it had no effect on plant growth and plant contents of N and P at the root and shoot levels. With increasing drought intensity, the increases in plant growth with combined P and Zn shows the significance of adequate nutrient management to improve the plants' resiliency to withstand stressful conditions. The reduction in nutrient contents and the intensity of P and Zn interaction under drought stress depicts the reduction in nutrient uptake and translocation due to reduced nutrients mobility in soil and disturbance of plant physiological and metabolic processes. This study suggests that the intensity of the negative interaction of P and Zn reduces with the intensification of drought with reduction of absolute nutrient contents and plant development due to moisture deficiency.

Acknowledgments: We acknowledge the Environmental Biogeochemistry Lab, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad Pakistan for provision of necessary materials and technical support for analytical work and the anonymous reviewers for their time in reviewing this manuscript which helped a lot to improve its quality.

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