

POTASSIUM AND ZINC CO-FERTILIZATION PROVIDES NEW INSIGHTS TO IMPROVE MAIZE (*ZEA MAYS* L.) PHYSIOLOGY AND PRODUCTIVITY

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Abstract

Potassium (K) and Zinc (Zn) are essential nutrients, and play key role in many physiological processes. The current study aims to identify their interactive impacts (i) in soil and plant body, (ii) on maize physiology and (iii) on production. A field experiment was conducted to assess the potassium co-fertilization with zinc sources and doses. The treatments were (kg ha⁻¹) as: K0 + Zn0, K0 + Zn16, K0 + Zn24, K60 + Zn0, K60 + Zn16, K60 + Zn24, K100 + Zn0, K100 + Zn16 and K100 + Zn24. Murate of potash (MOP) for K, Chelated Zinc (Zn-EDTA; S1) and Zinc Sulphate (ZnSO₄; S2) were the sources of Zn fertilization. Results revealed that co-fertilization have significantly improved maize productivity, and physiological traits. Plant height was significantly increased (27%), cob length (50%), 1000-grain weight (25%), dry weight (203%), membrane stability (191%), relative water content (170%), photosynthesis rate (237%), transpiration rate (353%), stomatal conductance (254%), internal CO₂ (105%) and chlorophyll contents (185%) with MOP + Zn-EDTA fertilization at K60 + Zn16 treatment as compared to control. Moreover, straw K and grain K contents were also improved with combined K and Zn fertilization, while straw and grain Zn was higher with sole Zn application. It is concluded that co-fertilization of MOP + Zn-EDTA at K60 + Zn16 (kg ha⁻¹) is beneficial for productivity, and physiological traits of maize in calcareous soils.

Key words: Maize, Physiology, Potassium, Zinc.

Introduction

Maize (*Zea mays* L.) is one among the important cereal crops grown worldwide, belongs to family Gramineae, and is a C4 plant. After wheat and rice, it is the third most important cereal crop in Pakistan, and contributes about 2.6% to value addition in agriculture and 0.5 percent to the gross domestic product. The area under maize cultivation was increased 5.4% (1.38 million hectares), and productivity was increased (6.9%) about 6.309 million tons compared to the previous year (Pakistan Bureau of Statistics, 2019.). It is a staple food for millions of individuals worldwide, and provide a large number of micro as well as macronutrients. But, the concentration of Zn is not enough for the people, who depend on maize as a staple food. Its cultivation as fodder can reduce the feed shortage issues in the winter season, approximately, 1.6 % of K, more than 6 % Ash contents, 30 % Zn contents, and a reasonable amount of soluble carbohydrates are present in it (Mubeen *et al.*, 2021; Riedell *et al.*, 2009).

Potassium is the third most important primary macronutrient, after Nitrogen (N) and phosphorus (P). It has a key role in many plant physiological processes such as translocation of photo-assimilates, stomatal regulation, and enzymatic activation (Ahmed *et al.*, 2021; Marschner, 2012). It is also involved in protein synthesis, energy transformation, osmoregulation, cation-anion exchange, xylem and phloem transport, root development, and a resistant factor against stresses

(Krasavina *et al.*, 2005). Soils in Pakistan are considered as rich reservoirs of K, but nowadays its deficiency occurs due to excessive removal with tube well water, excessive removal of straw, and clay mineral fixation of applied K (Bukhsh *et al.*, 2012).

Macro as well micronutrients are both important for crop productivity, except that macronutrients are required in large while micronutrients are required in less quantity. Among these micronutrients, Zn is one of the most important nutrients that play a key role in all living organisms for their growth and development (Cakmak, 2008). It is an abundant trace element and acts as co-factor for more than 300 enzymes, also plays a vital role in cell division, protein formation, nucleic acid metabolism, and increases the chlorophyll contents (Cakmak, 2008; Sarwar *et al.*, 2004). Movement of Zn in phloem is relatively higher than its movement from root to shoot, stem and grain or sink, about 50% cereal growing areas around the globe are facing the problem of Zn phyto-availability (Cakmak, 2002). More than 50% of soils in Pakistan are Zn deficient, that is because of less Zn availability due to its fixation with clay particles, high pH range, and soil calcareousness (Bashir *et al.*, 2019).

Increasing K levels can retard the drastic effect of P on Zn significantly. It is also suggested that with an individual application of Zn and K, there is a significant increase in total K uptake by Jute crop (Maitra *et al.*, 2000). Role of K and Zn fertilization on maize growth, productivity and quality are not well documented. It is

possible that higher yield potential of maize in soils with marginal K and Zn deficiency can be achieved by applying K and Zn. This gives rise to the need of the current study to investigate the impacts of K and Zn combined application on physiological and yield traits of maize. The major aims of the current study are to (1) identify the interactive effects of both K and Zn in agricultural ecosystem (2) find the behaviour of maize physiology and production with co-fertilization of K and Zn, and (3) investigate the coordination of different sources and doses of Zn with K on physiological and yield attributes of maize crop.

Material and Methods

Study site: To investigate the above said aims, a field experiment was conducted at the experimental site (30.258° N, 71.515° E, 125.27 m elevation above sea level) of the Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. District Multan is bounded by the district of Khanewal on the North and North East, Vehari district on East and Lodhran district on South. It spreads over an area of 3,721 square Kilometres, including six towns. Several canals flows across the Multan District, providing water from River Chenab for proximate farms. The land of the district is plain and very productive. Multan features a semi-arid to arid climate with very hot summers and mild winters. The maximum recorded temperature is approximately 54°C, and the lowest recorded temperature is approximately 4.5°C. The average rainfall is roughly 186mm. Dust storms are of frequent occurrence within the city (Iqbal *et al.*, 2019).

The trial was conducted during the summer of 2017. Pre-sowing soil samples were collected from the depth of 0-15 cm. Randomly collected triplicate soil samples were air-dried and then passed through a 2 mm sieve. After that, these soil samples were analyzed to study the physico-chemical properties (Table 1). The soil used in the experiment was alkaline calcareous with high pH and less plant-available K (70 mg kg⁻¹) and Zn (AB-DTPA: 0.75 mg kg⁻¹).

Experimental design: The experiment was laid out in split-plot design with the factorial arrangement. The treatments were (1) control with K 0 kg ha⁻¹, and Zn 0 kg ha⁻¹ (Ck), (2) K 0 kg ha⁻¹, and Zn 16 kg ha⁻¹ (K0 + Zn16), (3) K 0 kg ha⁻¹, and Zn 24 kg ha⁻¹ (K0 + Zn24), (4) K 60 kg ha⁻¹, and Zn 0 kg ha⁻¹ (K60 + Zn0), (5) K 60 kg ha⁻¹, and Zn 16 kg ha⁻¹ (K60 + Zn16), (6) K 60 kg ha⁻¹, and Zn 24 kg ha⁻¹ (K60 + Zn24), (7) K 100 kg ha⁻¹, and Zn 0 kg ha⁻¹ (K100 + Zn0), (8) K 100 kg ha⁻¹, and Zn 16 kg ha⁻¹ (K100 + Zn16), and (9) K 100 kg ha⁻¹, and Zn 24 kg ha⁻¹ (K100 + Zn24). The trial plot was divided into three blocks having 51 total plots, net plot size was 4m x 3m and 85 equally sized and healthy maize seeds were grown in all plots. Plant to plant distance was maintained 20cm after thinning and row to

row distance was 75cm. One maize hybrid, Monsanto DK-6142 was used in this experiment. Three K (0, 60, 100Kg ha⁻¹) and Zn (0, 16, 24 Kg ha⁻¹) levels having two different Zn Sources Chelated zinc (Zn-EDTA; S1) and Zinc Sulphate (ZnSO₄; S2) were used. The source of K fertilization was murate of potash (MOP).

The recommended dose of NP was applied in all plots. Nitrogen was applied in two split doses. The first dose of N, P, K, and Zn were applied at the time of sowing. The second dose of N about 60 kg ha⁻¹ was applied after 35 days of germination. To maintain the moisture content during the whole growth period of maize, irrigation was continuously applied, along with all the culture practices (thinning, hoeing, and weeding).

Plant analysis

Agronomic and yield characteristics: The crop was harvested at maturity after 120 days; plant height, cob length, and 1000-grain weight were measured. Five Plants randomly selected from each plot were used to measure the agronomic and yield traits.

Physiological characteristics: Chlorophyll contents (CC) were measured at the blooming/vegetative stage by using chlorophyll meter, and relative water contents (RWC) was measured by taking the difference of fresh weight and turgid weight as suggested by (Wellburn, 1994). Membrane stability index (MSI) was measured by the following formula given by (Premachandra *et al.*, 1990; Sairam, 1994). The gaseous exchange parameters like photosynthetic rate (A), transpiration rate (E), stomatal conductance (Gs), and internal CO₂ concentration (Ci) were measured with the help of infrared gas analyzer (IRGA) Analytical Development Company, Hoddesdon, England.

Nutrients (K and Zn) concentration in straw and grains: The ionic content like K and Zn concentration was measured by the wet digestion method using the di-acid mixture (HNO₃: HClO₄, 2:1 ratio) (Westerman *et al.*, 1990). The potassium contents were measured using a Flame photometer while zinc was measured using Atomic absorption spectrophotometer.

Statistical analysis

Data were presented as the means ± standard deviations in all graphs and tables for the three replicates. General Linear Model (GLM) was used for the analysis of variance of the data by using IBM SPSS® (version 21). The significant differences of all data including agronomic characteristics, grain, straw, and crab yields were determined using the split-plot design analysis of variance (ANOVA) with Duncan multiple comparisons test at p-value 0.05. Microsoft Excel 2016 was used for data processing and graph construction.

Table 1. Soil physio-chemical properties before sowing of maize crop.

Character	Unit	Value	Method
Texture class	-	Loam	USDA classification
Sand	%	46.4	Hydrometer method
Silt	%	35.7	
Clay	%	17.3	
pH _s	-	7.96	pH of saturated soil paste
EC _s	dS m ⁻¹	0.67	Electrical conductivity of saturated soil past extract
CaCO ₃	%	0.57	Acid dissolution
Organic matter	%	0.62	Walkley-Black method
AB-DTPA extractable Zn	mg kg ⁻¹	0.73	Extracted with AB-DTPA
Extractable K	mg kg ⁻¹	70.0	Ammonium acetate extraction

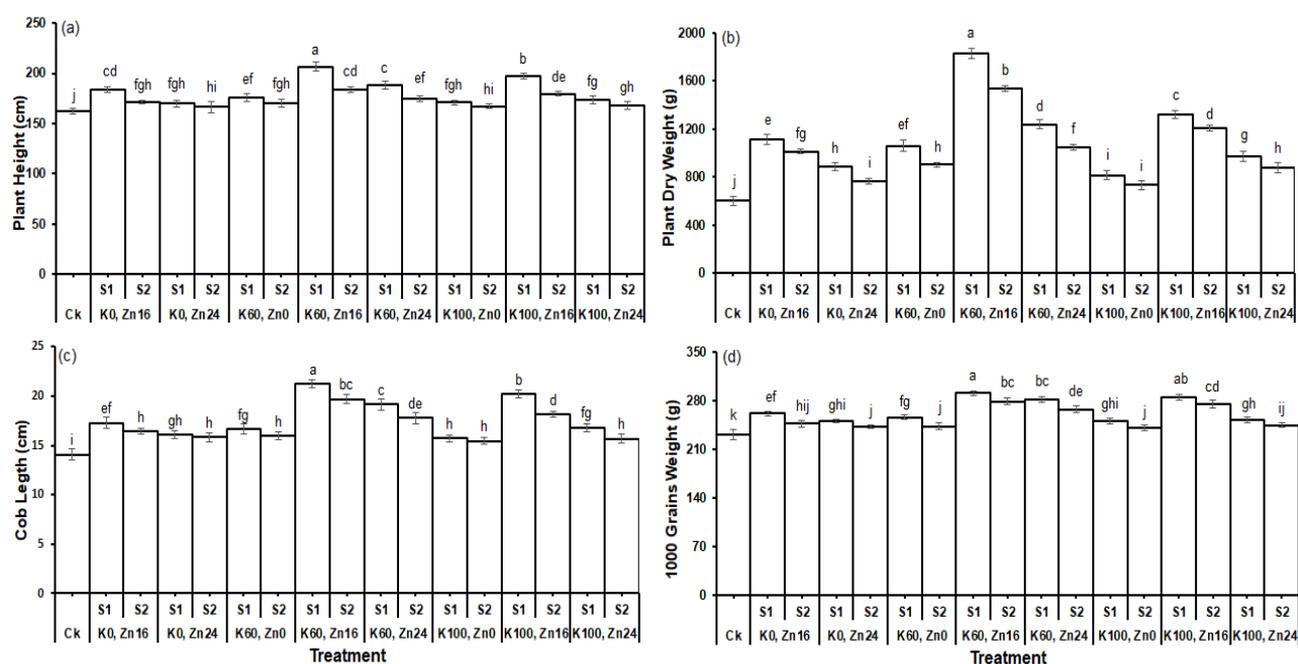


Fig. 1. Effect combined fertilization of K and Zn on (a) plant height and (b) dry weight (c) cob length and (d) 1000-grain weight of maize hybrid with MOP+ Zn-EDTA (S1) and MOP + ZnSO₄ (S2). All the values are given as mean \pm S.D (n=3).

Results

Agronomic and yield characteristics: The combined fertilization of K and Zn significantly increased the plant height, dry weight, cob length, and 1000-grain weight of maize hybrid compared to control conditions (Fig. 1). Maximum plant height (206.30 cm) was observed by the combined application of MOP + Zn-EDTA at K60 + Zn16 treatment, while minimum plant height (162 cm) was observed in the control (Ck) treatment. In the case of dry weight, the application of K and Zn increased the plant dry biomass. Maximum dry weight (1832 g) was observed by the combined application of MOP + Zn-EDTA also at K60 + Zn16 treatment, while minimum dry weight (603 g) in the Ck treatment. The overall range for cob length was observed 14.10-21.20 cm. Maximum cob length (21.20 cm) was observed in the treatments (K60 + Zn16) where MOP + Zn-EDTA were the sources of fertilization, while minimum cob length (14.10 cm) was observed in the Ck treatment. An increment of 50% and 26% was observed in cob length and grain weight respectively.

Physiological attributes of maize: Membrane stability index, RWC, and CC are considered as important parameters for plant growth. A significant increase was observed in MSI, RWC, and CC in the treatments where K and Zn were applied as compared to Ck (Fig. 2). Overall, the MSI was observed in the range of 16.-47.71. The application of MOP + Zn-EDTA has significantly ($p < 0.05$) increased the MSI, maximum MSI (47.71%) was observed by the combined application of MOP + Zn-EDTA at the treatment (K60 + Zn16) while minimum (16.36) MSI in the Ck. The combined application of K and Zn significantly ($p < 0.05$) increased RWC compared to the Ck. Maximum RWC (92.95%) was observed at K60 + Zn16 treatment, while minimum relative water content (34.31%) was observed in the Ck treatment. Regarding the Zn sources combination with MOP, the Zn-EDTA application increased the RWC compared to the ZnSO₄. CC was expressed in the term of Soil Plant Analysis and Development (SPAD value). Maximum CC (55.40 SPAD) was observed in the (K60 + Zn16) treatment with MOP + Zn-EDTA as sources, while minimum CC (19.69 SPAD value) was observed in the Ck.

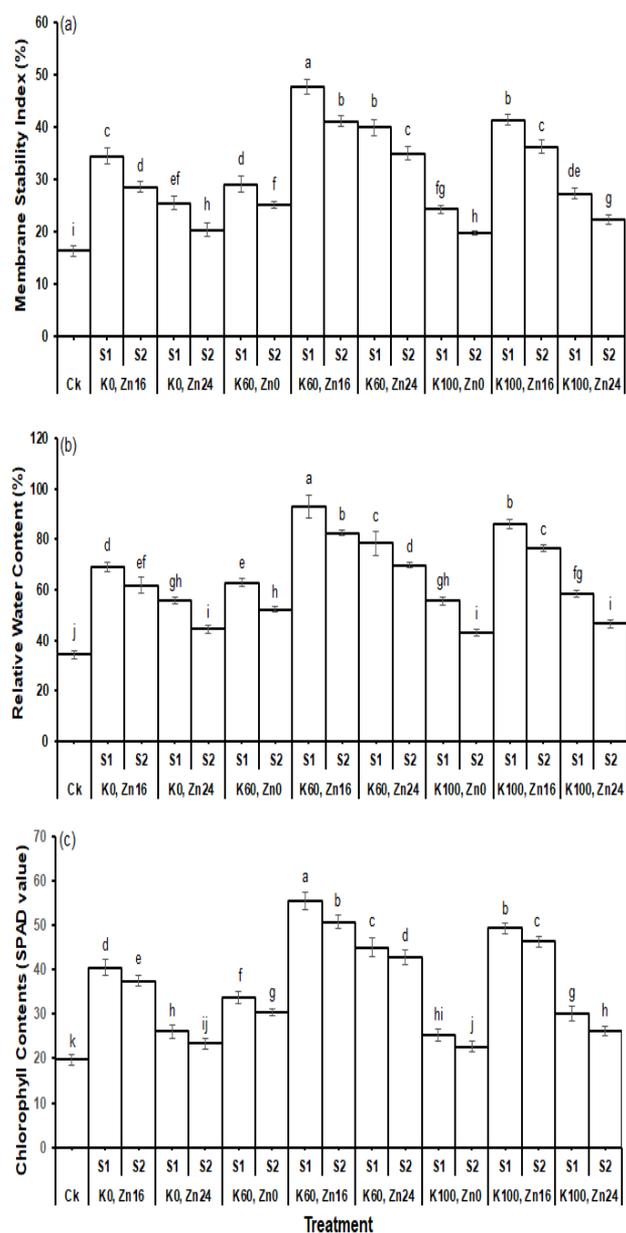


Fig. 2. Effect combined fertilization of K and Zn on (a) membrane stability index, (a) relative water content, and (c) chlorophyll contents of maize hybrid with MOP+ Zn-EDTA (S1) and MOP + ZnSO₄ (S2). All the values are given as mean \pm S.D (n=3).

The gas exchange parameters i-e A, E, Gs, and Ci are the main components for plant growth and development. The application of K and Zn improved the all gas exchange parameters of maize crop (Fig. 3). The application of MOP + Zn-EDTA has resulted in an improvement in Ci, E, and Gs. Maximum values were 393.33 $\mu\text{mol mol}^{-1}$, 10.20 $\text{mmol m}^{-2} \text{sec}^{-1}$ and 0.39 $\text{mmol m}^{-2} \text{sec}^{-1}$ respectively, with combined fertilization while minimum 191.66 $\mu\text{mol mol}^{-1}$, 0.11 $\text{mmol m}^{-2} \text{sec}^{-1}$, and 34.31 $\mu\text{mol m}^{-2} \text{sec}^{-1}$, respectively in Ck treatment.

Nutrients (K and Zn) concentration in grain and straw: The combined fertilization of K and Zn significantly improved the grain straw K concentrations (Fig. 4). Grain K concentration was increased (15%), and straw K concentration was increased (112%) and in K100+Zn16 treatment with Zn source as Zn-EDTA. K concentration was observed in the range of 1.06-1.22% in grain, while in straw K concentration was observed in the range of 1.4-2.98%. Zn concentration was relatively more improved with sole Zn fertilization compared to other treatments. Straw Zn concentration was increased (176%) and grain Zn concentration increased (135%) in K0+Zn24 treatment with Zn source as Zn-EDTA. Additionally, it was also improved with combined fertilization and was high at K100+Zn24 with Zn-EDTA as a Zn source with MOP.

Correlation of physiological parameters with agronomic and productive parameters and nutrients concentration in maize: Pearson correlation indicates strong positive interaction of physiological parameters with agronomic and productive parameters as well as with nutrients concentration of maize (Table 2). Plant height, dry weight, cob length, 1000-grain weight, grain, and straw Zn concentrations were highly positively correlated with all the physiological parameters (CC, RWC, MSI, A, E, Gs, and Ci). Grain K concentration was weakly positively correlated with physiological characters, but straw K concentration was highly positively correlated with MSI, RWC, A, and E; and was weakly positively correlated with Gs, Ci, and CC respectively.

Table 2. Pearson correlation indicating the relationship between physiological, agronomic and productive parameters and nutrients concentrations of maize crop.

	PH	DW	CL	GW	gZn	sZn	gK	sK
MSI	0.906**	0.946**	0.949**	0.955**	0.449**	0.486**	0.315*	0.374**
RWC	0.888**	0.931**	0.946**	0.952**	0.438**	0.478**	0.343*	0.384**
A	0.874**	0.947**	0.943**	0.926**	0.371**	0.424**	0.334*	0.392**
E	0.849**	0.922**	0.920**	0.897**	0.400**	0.442**	0.332*	0.403**
Gs	0.882**	0.947**	0.944**	0.919**	0.412**	0.454**	0.301*	0.356*
Ci	0.893**	0.953**	0.955**	0.931**	0.450**	0.505**	0.296*	0.341*
CC	0.856**	0.932**	0.935**	0.921**	0.404**	0.458**	0.280*	0.347*

Note: Here: PH is plant height, DW is dry weight, CL is cob length, GW is 1000-grain weight, gZn is grain zinc concentration, sZn is straw zinc concentration, gK is grain potassium concentration, and sK is straw potassium concentration, MSI is membrane stability index, RWC is relative water content, A is the photosynthetic rate, E is transpiration rate, Gs is stomatal conductance, Ci is internal CO₂ concentration, and CC is chlorophyll contents respectively

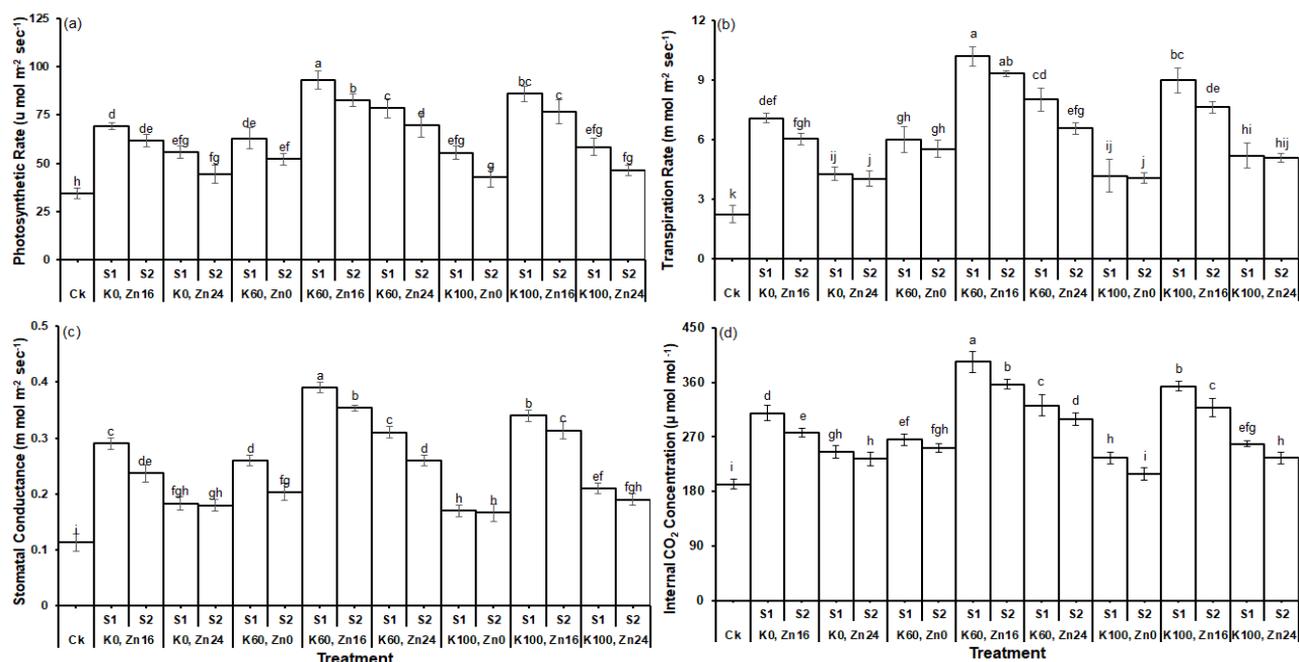


Fig. 3. Effect combined fertilization of K and Zn on (a) photosynthetic rate and (b) transpiration rate (c) stomatal conductance and (d) internal CO₂ concentration of maize hybrid with MOP+ Zn-EDTA (S1) and MOP + ZnSO₄ (S2). All the values are given as mean \pm S.D (n=3).

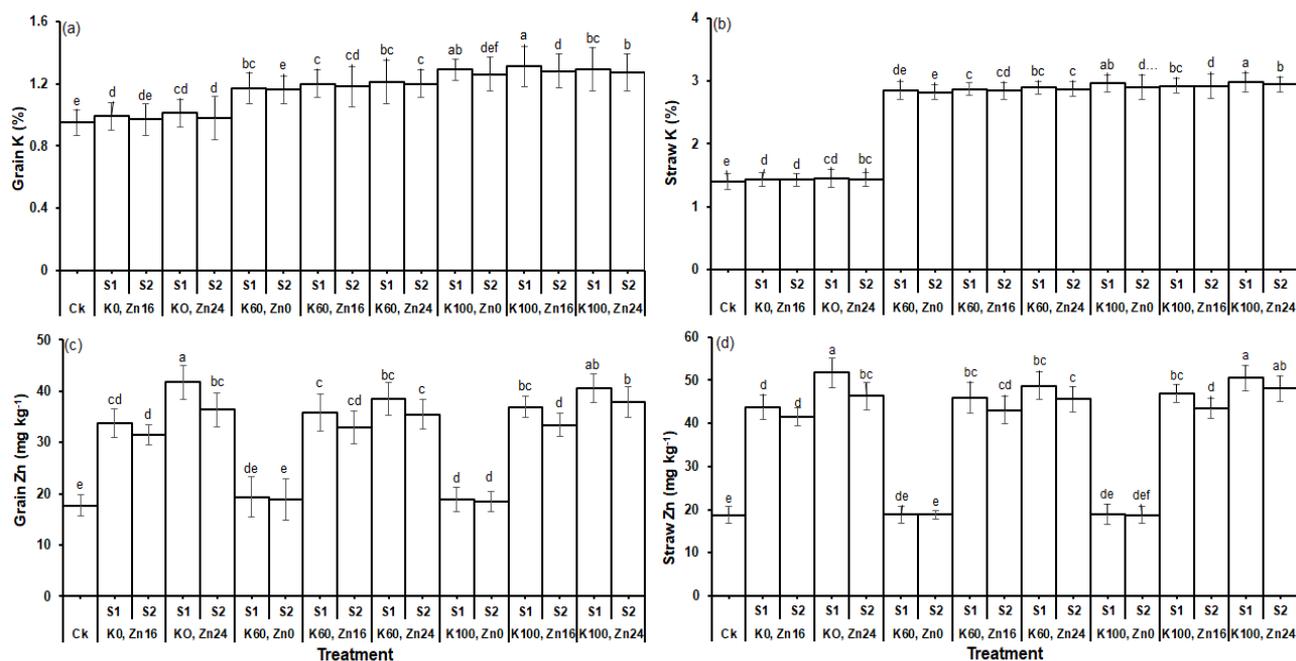


Fig. 4. Effect combined fertilization of K and Zn on (a) potassium concentration in grain, (b) potassium concentration in straw, (c) zinc concentration in grain and (d) zinc concentration in straw of maize hybrid with MOP+ Zn-EDTA (S1) and MOP + ZnSO₄ (S2). All the values are given as mean \pm S.D (n=3).

Discussion

Potassium- zinc application role in maize agronomic and yield attributes: Soils in Pakistan are nutrient deficit due to alkaline calcareous in nature, alkaline pH, arid and semi-arid region, and low availability of canal water. Besides this, frequent use of tube well water is an important reason for K and Zn deficiency (Bashir *et al.*, 2019). The application of K and Zn fertilizers induced a

positive effect on the growth and development of maize. The interactive effect of K and Zn causes a significant increase in agronomic and yield traits of maize crop (Fig. 1). This could be associated with integrated management of nutrient balance that increases their availability and uptake, as observed in certain nutrients like N, P, and K. Potassium fertilization is involved in regulating cell osmosis, photosynthesis, and promoting enzymatic activity, all these helped in increasing plant height.

Similar findings were reported by (Maleki *et al.*, 2014). Zinc improves the parameters by enhancing protein synthesis, the catalytic activity of enzymes, carbohydrates transformation, and chlorophyll synthesis. Chelated Zn is a better Zn source compared to ZnSO₄, can reduce Zn fixation compared to ZnSO₄ due to its slow releasing behavior (Jan *et al.*, 2016).

Cob length was increased by K and Zn fertilization might be due to transportation and remobilization of zinc from leaves and accumulation in grains. Potassium and Zn combined fertilization has increased the 1000-grain weight of maize compared to control, which might be due to more translocation of assimilates to sink (Khan *et al.*, 2001). The role of K is remarkable in water use efficiency, plant growth, cell division, hydrocarbon and protein synthesis, and their quick transfer towards the grain. Zinc fertilization also helps in improving 1000-grain weight significantly (Xue *et al.*, 2012) which, might be due to its remobilization from leaves to the grain.

Potassium- zinc application and physiological attributes of maize: Maize physiology was significantly improved with K+Zn fertilization (Figs. 2, 3). Relative water contents are an important feature for plant growth and development and combined fertilization has increased RWC significantly. An increment in RWC could be associated with K role in the maintenance of water contents of plants, opening and closing of stomata, and in osmotic adjustment. Regarding physiological attributes, potassium had a significant effect on the relative water contents of the leaf, which is also in agreement with the findings of Lawlor & Cornic (2002). This could be due to the increasing root length and more absorption of water by roots and then its maintenance in leaf cells by closing the stomata. The plant absorbs a large amount of potassium from the root zone to maintain normal growth and development of the plant body (Elumalai *et al.*, 2002).

The role of K in agricultural production is directly linked with photosynthesis. It influences the photosynthetic mechanism at different stages including ATP synthesis, translocation of photosynthates, activation of enzymes, and CO₂ uptake (Marschner, 2012). Its abundance can show a boost in the activity of Rubisco for limiting photosynthesis in rice leaves, and deficiency tends to reduce transpiration rate and stomatal closure (Römheld & Kirkby, 2010). Zinc application is also involved in the improvement of gas exchange parameters i.e RWC, MSI, E, A, Gs, and Ci. This role is associated with the activity enhancement of enzymatic systems such as Rubisco and PEPcase, as they have a key role in CO₂ fixation (Hussein & Alva, 2015).

Combined fertilization K and Zn increased the MSI that is mainly associated with K's role as an osmoticum, that maintains the membrane stability and regulates the opening and closing of stomata. Due to the osmoticum role of K in the vacuole, it helps in higher tissue water contents in the plant cell (Gechev *et al.*, 2005). In view of literature, stomatal regulation largely depends on K⁺ distribution in epidermal cells, guard cells, and leaf apoplast (Ahmad *et al.*, 2014). Under mild K deficiency, stomatal limitations are the major factors affecting the

photosynthetic ability of plants, whereas metabolic limitations become a dominant limiting factor (Shabala *et al.*, 2002). Low K availability in soil caused a reduction in leaf K and reduced the photosynthetic ability of plants (Basile *et al.*, 2003).

Potassium- zinc application and ionic concentrations in maize: It was observed that K fertilization has increased the K concentration in grain and straw with combined fertilization (Fig. 4). It has elevated the K nutritional status at the initial stages of crop growth, which is a prerequisite of higher yield (Niu *et al.*, 2011). Combined K and Zn fertilization increased the potassium concentration and uptake by the crop that was associated with their possible positive interaction and help in vigorous root development. Additionally, they can interact to enhance better K₂O fixation, higher photosynthetic activity, and improved growth and development resulted in higher uptake (Jat *et al.*, 2013).

Zinc concentrations showed apparent variation, it was maximum by sole Zn fertilization, which might also be due to its remobilization from leaves to grain at higher dose, but was also improved with combined K and Zn fertilization compared to control. This could be due to higher Zn concentration in the soil solution, which facilitated the uptake through the diffusion process and also a higher share of post-anthesis root Zn uptake in grain Zn concentration. Higher Zn uptake also flourished the synergistic effect of both nutrients. Zinc uptake in combined fertilization might be enhanced due to adsorption of K on clay sites, resulting in the release of fixed Zn at binding sites (Gupta *et al.*, 2018). This phenomenon increased the Zn concentration in soil solution and higher uptake to plant body by mass flow. Somehow, the use of Zn-EDTA as fertilization source also reduced its fixation at binding sites in the form of ZnS, ZnCO₃, and Zn(OH)₂, due to its slow releasing behavior.

The positive interaction of physiological parameters with agronomic and productive parameters, and with nutrients concentration also show that they help to improve each other (Table 2). Further research is required to identify the fertilization method, time of fertilization, and mechanisms improvement due to K and Zn combined fertilization.

Conclusion

The study concludes that combined fertilization of K and Zn is a better approach to improve maize productivity, and its morpho-physiological characteristics. A significant positive influence was observed among all parameters with combined fertilization compared to control. Agronomic, productivity, and physiological characteristics including photosynthesis rate, RWC, MSI, etc. were improved significantly. Zinc sources are also an important factor to classify its role in plant productivity and physiology. Chelated-Zn is recommended as a better source for farming compared to ZnSO₄ under calcareous conditions with high pH.

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