



Full Length Article

Foliar Application of Proline Improves Salinity Tolerance in Maize by Modulating Growth and Nutrient Dynamics

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Received 01 December 2021; Accepted 05 March 2022; Published 30 April 2022

Abstract

A field experiment was carried out during 2019 and 2020 summer seasons to evaluate the effect of foliar applied proline as osmoprotectant on yield and quality response of maize in calcareous saline soils. Proline was applied to maize as foliar application 100, 200, 300 and 400 mg/L including no spray as control (with only water). These treatments were applied three times in a season with one month interval. The results showed that foliar application of proline increased growth, biochemical parameters, nutrient content, yield and its component including oil percent in maize under saline condition. Foliar application of proline at 400 mg/L gave the highest values of most studied parameters, but there was no significant differences were found for foliar applied 300 mg/L. Yield and its components and oil percent recorded the highest values with treatments 400 mg/L proline foliar application. The foliar applied proline (400 mg/L) showed relative increase in grain yield and oil percent of 157.2 and 79% respectively compared with control. Foliar applied proline at 400 mg/L produced the highest increase in growth, biochemical parameters, and nutrient contents which finally resulted in improved grain yield and oil percent. © 2022 Friends Science Publishers

Keywords: Maize; Salinity stress; Proline; Growth; Yield; Nutrient content

Introduction

Maize (*Zea mays* L.) is one of the main cereal crops, food grains and industrial in many parts of the world for cultivated area and production. For many agricultural goods, maize is a staple human food, a feed for livestock and a raw material. It is an important food crop grown by many resource-poor farmers on a large scale for subsistence. There are many agricultural goods prepared from maize including corn sugar, corn oil, corn flour, starch, syrup, brewer's grit and alcohol (Dutt 2005). Under a broad spectrum of soil and climatic conditions, maize is grown, however, moderately susceptible to abiotic stresses including salinity (Farooq *et al.* 2015).

Salt stress is one of the most significant barriers to crop development in salt-affected areas of the world. Almost 8.5 percent of the world's entire area and about 25 percent of the agricultural land is affected by salinity (Billah *et al.* 2019). One of the main farming problems in semi-arid regions is soil salinity. Plants are vulnerable to extreme

climatic conditions in Egypt, such as high temperatures and drought. Dissolved salts can accumulate in soils due to inadequate ion leaching. An accumulation of salt in the upper layers of the soil can also be due to improper management of irrigation (Mohamed *et al.* 2007).

Proline is an amino acid that accumulates as a result of stress in different tissues of the plant, particularly in the leaves. In the regulation of osmosis in the cell, the accumulation of proline is concentrated in the cytoplasm to counterbalance the osmosis effect. Under stress conditions, proline protects enzymes (Meister 2012) and maintains water-balance in the plant (Tarighaleslami *et al.* 2012). Exogenous proline application has reduced the negative effect of salt stress by controlling cellular osmotic equilibrium (Deivanai *et al.* 2011). Proline as an osmoregulator specifically controls osmotic pressure in the plant to absorb water and play an essential role in many of the plant's critical processes. Proline also protects chloroplast membranes, increases the efficiency of photosynthesis and has the potential to protect cell walls and

membranes, thus, playing an important role in scavenging free radicals, thereby mitigating the adverse impact of stress and improving plant development, productivity and quality (Wu *et al.* 2017). Thus, proline plays an important role in promoting plant growth and seed yield under stress conditions including maize (Abdelhamid *et al.* 2013). As a proteinogenic amino acid, proline plays an important role within plant tissues for different vital metabolic processes (Slama *et al.* 2014; El-Nasharty *et al.* 2017). Proline helps to retain the status of cell water, subcellular structures and protect membranes and proteins from osmotic stress denaturation (Ashraf and Fooland 2007).

Proline plays three major roles during stress, *i.e.*, as a metal chelator, an antioxidative protection molecule, and a signaling molecule (Hayat *et al.* 2012). Furthermore, exogenously applied proline protects enzymes, scavenges free radicals and prevents salinity stress oxidation (Wutipraditkul *et al.* 2015). Wu *et al.* (2017) found that the toxicity of salinity can be decreased by controlling the Na⁺/K⁺ ratio and increasing proline accumulation. This can provide physiological insights into the understanding of the salinity tolerance mechanisms in exogenous proline-treated plants. Perveen and Nazir (2018) and Sary *et al.* (2020) found that proline indicates differential response by regulating different physicochemical parameters not only in different plant species but also under various environmental conditions. Szabados and Savoure (2009) and El-Nwehy *et al.* (2020) explained that multiple proline roles in plants include protein synthesis, osmolyte protection, redox balance maintenance, and mitochondrial function mediated metabolic signaling. Proline improved nutrient acquisition, water uptake and nitrogen fixation are primarily motivated by these positive effects. Exogenous proline also alleviates salt stress by enhancing the activities of antioxidants and reducing the absorption and translocation of Na⁺ and Cl⁻ while improving the assimilation of K⁺ by plants. In addition, L-proline is synthesized by plants in the cytosol and accumulates in chloroplasts. Accumulation in plants is a well-recognized physiological response to salinity-induced osmotic stress (El-Samad *et al.* 2010). The present study therefore investigated the role of foliar applied proline as osmoprotectant in alleviation of salinity stress on growth, yield and quality of maize grown in saline calcareous soil.

Materials and Methods

A field experiment was carried out at the farm of El-Nubaria Agricultural Research Station, Behaira Governorate, Agric. Res. Center (ARC) and Ministry of Agriculture and Land Reclamation (MALR), Egypt during the summer seasons of 2019 and 2020 to evaluate the effect of proline foliar application on maize (*Zea mays* L.) cultivar. The geographical situation features of the farm are 30° 90' N, 29° 96' E, with an altitude of 25 m above sea level. The soil samples (0–30 cm depth) were analyzed according to the

method described by (Page *et al.* 1982). Soil texture was sandy loam and had the following characteristics: pH 8.3, organic matter 0.9%, CaCO₃ 33.6%, EC 4.9 dS/m (3136 mg/kg), K 600, Ca 900, Na 1200, Mg 400, Fe 6.7, Mn 2.9, Zn 1.4 and Cu 2.5 mg/kg.

Experimental design and treatments

The experiment was conducted in a randomized complete blocks design arrangement with three replications. The net plot size was of 10.5 m². The maize crop was planted in each plot with 0.75 m row spacing and plant to plant spacing of 0.20 m.

Treatments were as follows including control, 100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L of foliar application of proline applied three times in a season with one month interval using (L-proline: C₅H₉NO₂, M.W 115.13).

Maize cultivar Giza 310 obtained from Corn Research Section, Agricultural Research Center, Giza, Egypt was used. Maize seed was sown on the 1st of June and harvested on the 3rd of September in both seasons. Nitrogen fertilizer as ammonium sulfate (20.5% N), phosphorus fertilizer as superphosphate (15.5% P₂O₅) and K fertilizer as potassium sulfate (48% K₂O) were added according to the recommendation of the Ministry of Agriculture and Land Reclamation, Egypt. All other farming practices (*i.e.*, fertilizers, irrigation, weeds and diseases control, *etc.*) were done following the recommended practices for the maize crop. Soil samples were taken during each season in June, July and August months from different locations in the experimental site in a randomized way to determine salinity as shown in Table 1.

Growth, yield and yield components determination

At harvest, three plant samples were taken from each plot to determine, plant height (m), fresh and dry weights of plant (kg), ear weight (g), length of ear (cm), the diameter of ear (cm) per plant and the number of row per ear as mean values for two seasons. To determine grain yield (ton/fed), grain was removed and cleaned within 1m² at the center of the plot. Then grain yield is recorded on a dry weight basis. Replicated samples of clean grain (broken grain and foreign material removed) were sampled randomly and 100-grains were counted and weighed.

Biochemical analysis

After the third foliar application of proline, leaves samples were taken to determine:

- (1) The chlorophyll content using Chlorophyll meter Spad502 at 9 am according to Woods *et al.* (1992) and expressed as chlorophyll index.
- (2) Leaf-free proline content was determined according to Bates *et al.* (1973).

Table 1: Mean soil EC values in June, July and August at the different locations in the experiment site for the two experimental seasons

| location | June | July | August |
|----------------|------|------|--------|
| 1 | 3050 | 2850 | 2175.6 |
| 2 | 3216 | 2875 | 2221.5 |
| 3 | 3285 | 2900 | 2128 |
| 4 | 3174 | 2925 | 2240.6 |
| 5 | 3173 | 2500 | 2083.2 |
| 6 | 3233 | 2750 | 2256.8 |
| 7 | 2991 | 2840 | 2486.4 |
| 8 | 3124 | 2880 | 2562 |
| Mean EC (mg/L) | 3156 | 2815 | 2269.3 |
| Mean EC (dS/m) | 4.93 | 4.39 | 3.54 |

(3) Carbohydrate contents in aqueous solutions according to DuBois *et al.* (1956) while nutrient content from grain were determined by method of Cotteneo *et al.* (1982).

Nutrient content

The harvest samples from leaves were also taken for determination of nutrients (N, K, Ca, Na, Mg, Fe, Mn, Zn and Cu) by method as described by Cotteneo *et al.* (1982).

At harvest grains samples were taken to determine:

Seed oil percentage

Seed oil contents was estimated according to AOAC (1990) and expressed as oil content (%) = (weight of the flask + oil - empty flask weight/ weight of sample) x 100.

Statistical analysis

Statistically analysis was performed to compare the means of two seasons (Combined analysis of two successive seasons) data by using the least differences (L.S.D) (Snedecor and Cochran 1990).

Results

Effects on plant growth

Foliar application of proline caused a significant increase in fresh and dry weights of the plant, weights of ear /plant and number of rows per ear compared with control except for foliar applied 100 mg/L. Plant height, length and diameter of ear per plant were not affected. Foliar applied 400 mg/L resulted in an increase of fresh weight of plant (kg) and weight of ear per plant (g) with a relative increase of 106 and 72%, respectively compared with control (Table 2).

Effects on biochemical parameters

The foliar applied proline had a significant improved effect on the biochemical parameters of maize plants except for protein contents. Foliar applied proline with 300 and 400

mg/L gave the highest chlorophyll index value with a relative increase of 35 and 32%, respectively when compared with control without significant differences between the two treatments. Likely, higher proline content was recorded for foliar applied 300 and 400 mg/L with a relative increase of 520 and 544%, respectively compared with control without significant differences between the two treatments. Foliar application increased carbohydrates significantly compared with control but without significant differences between proline treatments (Table 3).

Effects on leaves nutrient content

Foliar application of proline had a significant improved effect on macro and micronutrients in leaves of maize plants. Regarding N% the highest increase was recorded with foliar applied 300 mg/L proline with a relative increase of 83% compared with control. Foliar applied with 300 and 400 mg/L proline gave the highest values of K, K/Na, Mg, Fe and Mn compared with control without significant differences between the two treatments. The similar foliar application showed the lowest Na concentration in leaves of maize plants. While Ca, Zn and Cu had highest increase for 400 mg/L proline, as depicted in Table 4.

Effects on yield and its components

Foliar application of proline significantly enhanced yield and its components including oil percent. Highest yield and its components including oil percent was recorded for foliar applied 300 and 400 mg/L proline without significant differences between the two treatments. The relative increase in grain yield with foliar applied 300 and 400 mg/L were 125.5 and 157.2%, respectively compared with control without significant differences between the two treatments. The relative increase in oil percent (%) with foliar applied 300 and 400 mg/L were 54 and 79%, respectively compared with control without significant differences between the two treatments (Table 3 and Fig. 1).

Effects on grain nutrients content

Foliar application of proline has a significant effect on some macro and micronutrients in grains of maize plants. The results for N concentration in grains were not significant as a result of proline treatments foliar application. K, Ca and Zn increased significantly with foliar applied 200, 300 and 400 mg/L proline without significant differences between these treatments. K/Na, Mg and Mn increased significantly with foliar applied 300 and 400 mg/L proline without significant differences between these treatments. While Na decreased significantly and recorded the lowest value with foliar applied 400 mg/L proline with a relative decrease of 23% compared with control. Fe and Cu recorded the highest value of increase with foliar applied 400 mg/L proline foliar application, as shown in Table 5.

Table 2: Effect of proline application on plant growth of maize grown in calcareous soil under salinity stress

| Foliar application of proline (mg/L) | Plant height (m) | Fresh weight of plant (kg) | Dry weight of plant (kg) | Weight of ear per plant (g) | Length of ear per plant (cm) | Diameter of ear per plant (cm) | No. of rows per ear |
|--------------------------------------|------------------|----------------------------|--------------------------|-----------------------------|------------------------------|--------------------------------|---------------------|
| Control | 2.24 | 0.71 c | 0.17 b | 154.22 b | 16.31 a | 5.07 a | 11.88 b |
| 100 mg/L | 2.34 | 0.92 bc | 0.24 a | 167.33 b | 18.89 a | 5.13 a | 12.67 ab |
| 200 mg/L | 2.45 | 1.06 b | 0.26 a | 238.33 a | 19.89 a | 5.23 a | 12.73 a |
| 300 mg/L | 2.51 | 1.14 b | 0.26 a | 260.78 a | 19.98 a | 5.23 a | 13.10 a |
| 400 mg/L | 2.55 | 1.46 a | 0.23 a | 265.44 a | 19.99 a | 5.33 a | 13.23 a |
| LSD 5% | N.S | 0.2486 | 0.0471 | 52.892 | N.S | N.S | 0.8241 |

Combined analysis of two successive seasons

Table 3: Effect of foliar proline application on biochemical parameters, yield and its components of Maize grown in saline calcareous soil

| Foliar application of proline (mg/L) | Leaves | | Grains | | 100-grains weight (g) | Grain yield (t ha ⁻¹) | Grain oil contents (%) |
|--------------------------------------|-------------------|-------------------------|-----------|-----------------|-----------------------|-----------------------------------|------------------------|
| | Chlorophyll index | Proline $\mu\text{g/g}$ | Protein % | Carbohydrates % | | | |
| Control | 29.67 c | 17.95 d | 5.48 | 86.02 b | 30.67 c | 3.48c | 1.74 b |
| 100 | 33.73 bc | 37.62 c | 5.63 | 87.37 a | 35.33 b | 4.03c | 1.94 b |
| 200 | 34.10 abc | 75.93 b | 5.83 | 87.30 a | 38.67 ab | 6.12b | 2.10 b |
| 300 | 40.17 a | 111.32 a | 5.83 | 87.15 a | 39.67 a | 7.85a | 2.68 a |
| 400 | 39.17 ab | 115.53 a | 5.75 | 87.73 a | 41.33 a | 8.95a | 3.11 a |
| LSD 5% | 6.42 | 17.06 | N.S | 0.89 | 3.51 | 1.45 | 0.52 |

Combined analysis of two successive seasons

Table 4: Effect of foliar proline application on leaves nutrients content of Maize grown in saline calcareous soil

| Foliar application of proline (mg/L) | % | | | | | | mg/L | | | |
|--------------------------------------|--------|---------|---------|---------|---------|---------|-----------|----------|----------|----------|
| | N | K | Ca | Na | K/Na | Mg | Fe | Mn | Zn | Cu |
| Control | 1.37 c | 1.90 c | 0.45 d | 2.83 a | 0.67 c | 0.28 c | 90.67 c | 33.0 b | 18.0 d | 11.0 d |
| 100 | 1.80 b | 2.10 b | 0.46 cd | 2.67 ab | 0.79 b | 0.28 c | 106.67 bc | 35.67 ab | 20.67 cd | 18.0 cd |
| 200 | 2.03 b | 2.17 ab | 0.49 c | 2.60 b | 0.83 ab | 0.29 bc | 108.33 bc | 36.67 ab | 23.33 bc | 26.67 bc |
| 300 | 2.50 a | 2.20 ab | 0.54 b | 2.57 b | 0.86 ab | 0.33 a | 147.33 a | 38.67 a | 27.33 b | 34.0 b |
| 400 | 2.07 b | 2.33 a | 0.70 a | 2.53 b | 0.92 a | 0.31 ab | 128.0 ab | 40.67 a | 36.33 a | 43.33 a |
| LSD 5% | 0.3902 | 0.1758 | 0.0407 | 0.2048 | 0.1058 | 0.0247 | 21.485 | 5.478 | 4.481 | 8.9373 |

Combined analysis of two successive seasons

Table 5: Effect of foliar proline on nutrients content in grains of Maize grown in saline calcareous soil

| Foliar application of proline (mg/L) | % | | | | | | mg/L | | | |
|--------------------------------------|------|---------|---------|---------|---------|----------|---------|---------|----------|----------|
| | N | K | Ca | Na | K/Na | Mg | Fe | Mn | Zn | Cu |
| Control | 0.88 | 0.31 b | 0.16 b | 0.43 a | 0.71 c | 0.055 d | 33.50 d | 2.50 c | 36.0 c | 80.50 c |
| 100 | 0.90 | 0.32 ab | 0.16 ab | 0.41 ab | 0.77 c | 0.057 cd | 36.0 c | 3.50 c | 45.50 b | 84.67 c |
| 200 | 0.93 | 0.34 a | 0.17 a | 0.38 bc | 0.89 b | 0.061 bc | 36.75 c | 5.0 b | 60.0 a | 103.67 b |
| 300 | 0.93 | 0.33 a | 0.17 a | 0.35 cd | 0.94 ab | 0.066 a | 40.50 b | 5.50 ab | 56.0 a | 105.33 b |
| 400 | 0.92 | 0.33 ab | 0.17 ab | 0.33 d | 0.98 a | 0.063 ab | 46.0 a | 6.50 a | 53.50 ab | 153.50 a |
| LSD 5% | N.S | 0.02 | 0.01 | 0.04 | 0.08 | 0.01 | 1.73 | 1.15 | 9.26 | 14.88 |

Combined analysis of two successive seasons

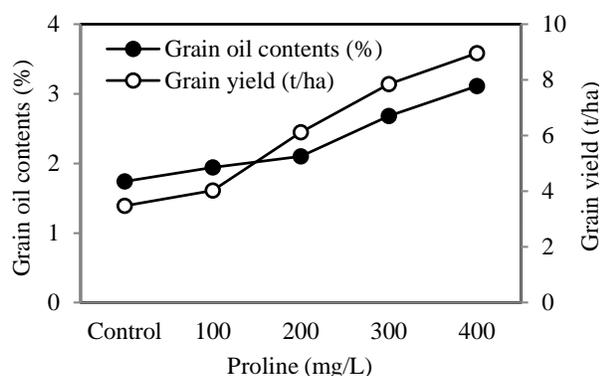


Fig. 1: Effect of foliar proline application on grain yield and oil percent of maize grown in saline calcareous soil

Discussion

The present study showed that the role of proline in stress tolerance as a compatible osmolyte for osmotic adjustment by affecting the uptake and accumulation of inorganic nutrients in maize plants. The proline counteracts the detrimental effects of salinity stress on nutrient uptake since it encouraged K⁺, Ca²⁺ and N uptake in maize as evident from present study. Molazem *et al.* (2010) found an increase in contents of Na⁺ of maize leaves when grown under saline conditions. Increased Na⁺ content in maize thus decreased calcium and potassium content with increased salinity levels, leading to decreased K⁺/Ca²⁺ ratio (Akram *et al.* 2010) also evident from present study. In the rhizosphere, high Na⁺ due to salinity decreases plant uptake of nitrogen,

potassium and calcium, causing serious nutritional imbalances in maize (Farooq *et al.* 2015). The selectivity of Na^+ , K^+ and Ca^{++} in maize was markedly changed by amino acids, especially proline treatments. Proline spraying limited Na^+ uptake and improved the K^+ , K^+/Na^+ ratio and Ca^{2+} selectivity uptake in maize. Zheng *et al.* (2015) noted that in reaction to exogenous proline under salt stress, the increase in water potential of leaves triggered by proline activates K^+ accumulation that helps plants change their cellular osmotic potential and therefore retain higher water content. Cuin and Shabala (2007) showed that solutes such as proline significantly decreased cell K^+ efflux and probably retained cytosolic K^+ homeostasis through improved H^+ -ATPase activity. In turn, this controls voltage-dependent outward-rectifying K^+ channels and created the electrochemical gradient needed for secondary processes of ion transport (Cuin and Shabala 2005). As well as nutrient absorption, exogenous proline is involved in nutrient assimilation under salty conditions. Nitrate reductase is one of the main enzymes involved in the assimilation of nitrogen (Khan *et al.* 2014). Proline is a perfect way to store and recycle nitrogen under conditions of stress (Mansour and Ali 2017). Nitrogen deficiency has explained that proline can be used as a source of nitrogen to develop (Hayat *et al.* 2012). Results of present study are also supported by El-Samad *et al.* (2010) that salinity increased the Na^+ content in maize shoots and roots, while Mg^{2+} accumulation decreased. Proline application had a significantly increased effect on the concentration of Mg^{2+} in shoots and roots under stress conditions (Ali *et al.* 2008). High Na^+ decreases plant absorption of Mg and Fe due to salinity in the rhizosphere and thus induces serious nutritional imbalances in maize (Farooq *et al.* 2015).

Foliar applied proline increase in growth are consistent with Deivanai *et al.* (2011), where major effect on growth traits was also reported for exogenous applied proline. Khan *et al.* (2014) found improved shoots and roots, higher fresh and dry weights of shoots and roots by exogenous applied proline under salt stress showing mitigation effects on plant growth. Under salt-stressed condition, exogenous applied proline significantly increased plant height (Teh *et al.* 2016). The findings of present study obtained are in agreement with Perveen and Nazir (2018) that proline regulating various physiochemical parameters under environmental conditions in increasing development. It seems that foliar application of proline at vegetative stage show differential response in increasing growth by regulating different physico-chemical traits under salinity stress (Perveen and Nazir (2018).

The foliar applied proline had a positive significant effect on the biochemical parameters of maize plants which are also observed by Al-Shaheen and Soh (2016) showing higher chlorophyll content when proline was sprayed on maize leaves. The possible reason can be regulatory function of (proline) in detoxification of free radicals under salinity stress, causing lipid oxidation in the cell membrane

(El-Samad *et al.* 2010; Abuzar *et al.* 2011).

Al-Shaheen and Soh (2016) also reported an increase in endogenous the concentration of leaf proline with exogenous application of proline. Taie *et al.* (2013) found that stressed plants induce a tenfold increase in the proline content of maize leaves which gradually returned to normal level when the stress level decreased.

Lama *et al.* (2016) reported that exogenous application of proline (30 mM), increased relative to the untreated plant subjected to stress. Exogenous proline also decreased salt stress by improving antioxidant activities and reducing the absorption and translocation of Na^+ while improving plant assimilation of K^+ (Bokobana *et al.* 2019; Moukhtari *et al.* 2020). Proline also plays a role in cytoplasmic pH control or constitutes a reserve of nitrogen used by the plant under water deficit (Kishor *et al.* 2005). Likely, foliar applied proline resulted in an increase of carbohydrates and these findings are in agreement with El-Samad *et al.* (2010), where a large accumulation of soluble sugar was found with foliar application of proline. Zheng *et al.* (2015) also observed that exogenous proline under salt stress showed an increase in leaf water content triggered by the aggregation of certain organic compounds such as soluble sugars.

The proline under stress act as a metal chelator, an antioxidative protection molecule, and a signaling molecule (Hayat *et al.* 2012). Proline as an osmoregulator specifically controls osmotic pressure in the plant to absorb water and play an essential and efficient role in many of the plant's critical processes. It also preserves chloroplast membranes and thus increases the efficiency of photosynthesis and has the potential to protect cell walls and membranes and playing an important role in scavenging free radicals, thereby mitigating the adverse impact of stress and improving plant development, productivity, and quality (Wu *et al.* 2017). Moreover, proline showed an important role in promoting plant growth and seed yield under stress conditions, as observed in maize (Abdelhamid *et al.* 2013). Proline helps to retain the status of cell water, subcellular structures and protect membranes and proteins from osmotic stress denaturation (Ashraf and Fooland 2007).

The maize plants, with foliar application of proline showed an increased plant growth with a positive impact on yield characteristics under salt stress, as reported by Alam *et al.* (2016). In different plant species, proline increases salt stress tolerance. and modulate plant growth with increases in photosynthesis and grain yield (Moukhtari *et al.* 2020). Under stress, exogenous applied proline improvement in 100-grain weight and grain yield (Alam *et al.* 2016; Rady *et al.* 2019).

Conclusion

Foliar application of proline especially 400 mg/L can help to improve salt resistance in maize as osmoprotectants or osmoregulator by modulating growth and nutrient dynamics finally by improving grain yield and oil percent.

Acknowledgments

This study was carried out by the National Research Centre (NRC), the Fertilization Technology Department as part of the Egypt-German Project "Micronutrients and Other Plant Nutrition Problems" (Coordinator, Prof. Dr. M. M. El-Fouly) and the Institute for Soil, Water & Climate Research, the Agriculture Research Center.

Author Contributions

All authors significantly contributed to all parts and aspects of the paper.

Conflict of Interest

The authors declared that the present study was performed in absence of any conflict of interest.

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