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Effect of seed priming with zinc oxide nanoparticles on growth rate and antioxidant system of maize seedling (Triple-hybrid 321) under salinity stress

Naglaa Loutfy*, Mona F. Abou Alhamd, Rhab A. Galal

Department of Botany and Microbiology, Faculty of Science, South Valley University, Qena, Egypt.

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Abstract: It has been shown that application of inorganic nanomaterials on cereal plants during their growth cycle improves the rate of plant productivity by supplying a micro-nutrient source. This study aimed to evaluate the impact of seed priming with zinc oxide nanoparticles (ZnONPs) (0.0 and 100 ppm) on maize Triple-hybrid 321 seedlings grown in soil containing different levels of salinity (0.0, 50, 100 and 150 mM NaCl) for three weeks. Parameters were determined for growth (fresh and dry mass (FM, DM), osmotic concentration (OC) of organic/inorganic solutes, photosynthesis pigment content (PPC), and selective antioxidant system (AOS) enzymes that might be involved in the stress remediation. The toxic effects of salinity on *Zea mays* L plants were assessed by determining growth parameters, antioxidant enzymes activity [catalase (CAT), peroxidase (POX) and ascrobate peroxidase (APOX)], osmotic concentration (OC) of organic/inorganic solutes, organic solutes (soluble sugars, soluble proteins, free amino acids and proline) and ions content (Na^+, K^+, Ca^2 and Mg^{2+}) in the presence or absence of ZnONPs. The obtained results revealed that salinity stress resulted in a significant increase in antioxidant enzymes activity, organic solutes and ions content which are associated with significant reduction in growth parameters compared with control plants. Seed priming with ZnONPs significantly alleviated the harmful effect of salinity on *Zea mays* L seedlings and increased the activity of antioxidant enzymes, organic solutes and ions content (Na^+, K^+, Ca^{2+} and Mg^{2+}), which could be an induced defensive mechanism against salinity stress.

Key words: zinc oxide nanoparticles; antioxidant enzymes; organic and inorganic solutes; salinity stress; maize; seed priming

Introduction

Maize (*Zea mays* L.), which is widely cultivated throughout the world and has the highest production among all the cereals, is one of the important grown cereal crops in Egypt, plays an essential role and is used in both human and animal feeding a draw material for many industries (Loutfy *et al.* 2020). Abiotic environmental conditions, such as salinity, are critical elements towards restricting the crop efficiency having adverse effect on yield capability of plant. The effect of salinization in lowering crop yields has been the major problem especially in arid and semiarid areas (Al-Naggar *et al.* 2015). In the arid regions of countries including Egypt, many crops are cultivated on low quality soils deficient with major nutrients, under extreme severe conditions of high temperature, salt stress (salinity) and sometimes combination of both. These conditions occurred due to the unnecessary use of chemical fertilizers which result in accumulation of extra salts in the soils due to evaporation especially after unsuitable irrigation (Mariani & Ferrante 2017; Negrao *et al.* 2017). Salty soils result in disturbance of osmotic regulation and ion imbalance in roots which leads to impair metabolism, growth and yield of the cultivated plant (Loutfy *et al.* 2020). Micronutrients are required in small amounts, and they affect directly or indirectly vital processes in plant such as photosynthesis, respiration, protein synthesis, reproduction phase (Marschner 1998; Alpaslan *et al.* 1999). Zn plays a very important role in plant metabolism by influencing the activities of oxidoreductases, transferases, hydrolases, ligases, and isomerases (Auld 2001). Plant

* Corresponding author E-mail: naglaaloutfy@yahoo.com

enzymes activated by Zn are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation (Marschner 2002). Zn can interfere with reactive oxygen species (ROS) produced by the membrane bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and thus represents an excellent protective antioxidant against the oxidation of several vital cell components such as chlorophyll, membrane lipids and proteins (Cakmak 2000). Nanotechnology, a new emerging and fascinating field of science, permits advanced research in many areas, and nanotechnological discoveries could open up novel applications in the field of biotechnology and agriculture. In agriculture, using nanoparticles is expected to improve the crop productivity by enhancing plant nutrition, precision farming, water use efficiency, crop protection against predators and diseases, innovative tools for pathogen detection, molecular biology, and environmental protection (Panwar *et al*. 2012). ZnONPs is one of the metal-based NPs (MNPs) that are commonly investigated with regards to human and ecosystem health as well as nanotoxicological effect on plants (Chenetal 2015; VanAken 2015; Zhang *et al*. 2015). The increased popularity of using Zn in fertilizers and pesticides is also commissioned due to its natural demand as a micronutrient in the body (Prasad *et al*. 2012).

The aim of this study is to evaluate the possible roles of ZnONPs in mitigating the adverse impacts of salt stress on some growth parameters, photosynthetic pigments content, organic solutes of maize plants under saline condition.

Materials and methods

Plant material and growth conditions

Seeds of maize (Triple-hybrid 321) were obtained from the Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Seeds sterilized with sodium hypochlorite solution (5%) for five minutes, washed thoroughly with distilled water and classified into two groups. The first group was soaked in 100 ppm freshly prepared solution of Zinc oxide nanoparticles (ZnONPs) for 8 h and the other group was soaked in distilled water for the same time and used as control. Ten seeds were planted in plastic pots containing 2 Kg of washed sandy soil for three weeks.

Determination of growth parameters

The harvested seedlings were divided into roots and shoots and the fresh mass (FM) of each sample was determined. The harvested plant's organs were quickly frozen and stored at –30 °C for biochemical analyses. Some parts of the samples were rapidly dried in an oven at 70 °C to estimate constant weight for determination of dry mass (DM).

Photosynthetic pigments

According to Lichtenthaler (1987), chlorophyll a (Chl. a), chlorophyll b (Chl. b) and carotenoids (car.) in leaves were determined spectrophotometry. About 100 mg of fully expanded young leaves was used for pigment extraction in 80 % acetone. The extract of pigments was assayed against a blank of pure 80 % acetone at wavelengths of 663, 644 and 452.5 nm for chl. a, chl. b and carotenoid contents, respectively.

Determination of organic solutes

Soluble sugars contents were estimated according to Badour (1959) by anthrone sulphuric acid method. According to the method of Bradford (1976), soluble proteins content was determined. Proline was determined according to the procedures described by Bates *et al*. (1973). Total free amino acids were extracted from plant tissues and determined according to (Moore & Stein 1948).

Estimation of Na, K, Mg and Ca:

Sodium and potassium were determined by the flame emission technique (Williams & Twine 1960). Calcium and magnesium were determined volumetrically by versene titration method as described by Schwarzenbach & Biedermann (1948).

ROS scavenging enzyme activities

About 500 mg of plant tissue was homogenized in 1 ml K-phosphate buffer pH.7, containing 0.1 mM Na_2EDTA and 1 % of PVP, and then centrifuged at 10,000 rpm at 4 ^{0}C for 20 minutes. The supernatant was used for enzyme assay. Catalase (CAT) activity was determined following Aebi (1984). Peroxidase (POX) activity was determined according to MacAdam *et al.* (1992). Ascorbate peroxidase (APOX) was determined using the technique mentioned by Nakano and Asada (1981).

Statistical analysis

All data were subjected to the analyses of variance (ANOVA) to test the significant difference between the mean (n = 3) of measured variables. Tukey's multiple comparison tests were performed to compare means at $P < 0.05$, different letters (a, b, c, d) indicate significant differences at $P < 0.05$ based on Tukey's test.

Results

Growth parameters and Photosynthetic pigments

Several growth parameters including shoot and root lengths, FM and DM of root or shoot were determined in maize seedlings (Triple-hybrid 321) to evaluate the effect of priming with ZnONPs on seedling growth under salinity stress (Tables 1 and 2). The studied

parameters were decreased by increasing salinity in root medium compared to control. At the level of 150 mM NaCl (the highest salinity level), the reduction in shoot length, root length, shoot fresh mass and root fresh mass was about 60%, 55%, 80% and 65% respectively. While the lessening in DM of shoot and root was 80% and 50% respectively. The decrease in photosynthetic pigments, Chl. a, Chl. b and car. was about 46%, 41% and 44% respectively. On the other side, zinc nanoparticles resulted in a stimulatory effect on length, fresh weight, dry weight and photosynthetic pigments in the tested organs as compared with those of the corresponding unstressed and stressed plants (Tables 1 and 2).

Organic solutes

Exposure of Triple-hybrid 321 to salt stress resulted in a noticeable accumulation in the contents of soluble sugars, soluble proteins, proline and total free amino content in both shoot and root. The concentration 150 mM NaCl recorded the highest accumulation in soluble sugars (about 43% and 217% in shoot and root respectively), soluble proteins (about 20% and 36% in shoot and root respectively), proline (about 155% and 274% in shoot and root respectively) and total free amino acids (about 85% and 65% in shoot and root respectively) as compared to the corresponding values of the untreated control.

The accumulation of soluble sugars, soluble proteins and proline in roots was higher than shoot. Seed priming with ZnONPs alone or in combination with salt significantly elevated the content of the above parameters over those of either untreated control or NaCl- stressed seedlings (Tables 3, 4, 5 and 6).

Mineral ions

The data in Table 7 demonstrate that, the Na^+ content was positively affected by increasing NaCl stress. Reversibly, the contents of K^+, $Ca^{2+,}$ and Mg^{2+} were negatively affected by increasing NaCl stress and attained their lowest values at the highest salt level (150 mM NaCl). On the other hand, application of ZnONPs lowered the levels of Na^+, and increased the content of K^+, Ca^{2+}, Mg^2 compared with the corresponding ZnONPs untreated seedlings (Table 7).

Antioxidant enzymes activity

Table 8 indicates that CAT, POX and APOX activities were significantly increased with the rise in salt concentration and the maximum increase of 62%, 122% and 67% respectively was recorded at 150 mM NaCl over the untreated control. Treatment with ZnONPs further improved CAT, POX and APOX activities in stressed plants relative to non-stressed and stressed plants alone.

Discussion

Salt stress is one of the most significant abiotic factors restricting growth of plant (Qayyum *et al*. 2016). Usage of nano-fertilizers is one of the appropriate techniques for plant production and lessening environmental contamination (Taha *et al*. 2016). In this work, ZnO nanoparticales were used to alleviate the salinity stress effect. The results found in the present work revealed that maize cultivar [Triple-hybrid 321] demonstrate salt stress produced a marked decrease in shoot & root length and fresh & dry mass with increasing of NaCl level. This inhibitory impact of salt stress could be recognized to the osmotic effect of NaCl salt (salter *et al*. 2007).

Table 1: Effect of ZnO-NPs and different concentrations of NaCl (0.0, 50, 100 and 150mM) on growth parameters of maize cultivar Triple-hybrid 321(TH 321) Seeds were soaked in water or 100 ppm ZnO- NPs.

Triple-hybrid 321							
Treatments		**Shoot**			**Root**		
Soaking in	**NaCl (mM)**	**Length (cm)**	**Fresh mass (g)**	**Dry mass (g)**	**Length (cm)**	**Fresh mass (g)**	**Dry mass (g)**
Water	0	20.10 ±1.07a	4.22±0.39a	0.39±0.03a	16.22±0.39a	2,19±0.03a	0.15±0.03a
	50 mM	16.40±0.39b	2.89±0.19b	0.21±0.02b	12.89±0.19b	1.65±0.11b	0.13±0.05a
	100 mM	12.30±0.34c	1.5. ±0.67c	0.13±0.02c	9.00±0.67c	1.02±0.01a	0.09±0.04b
	150 mM	7.60±0.88d	0.87±0.51d	0.08±0.12dc	7.11±0.51d	0.75±0.02ca	0.07±0.05cb
Zn NPs	0	27.44±0.20a	5.01±0.19a	0.59±0.01a	17.11±0.19a	2.34±0.12a	0.20±0.02a
	50 mM	22.44±0.20b	3.56±0.20b	0.29±0.07b	13.56±0.20b	1.99±0.11b	0.16±0.02b
	100 mM	14.22±0.39c	2.67±0.34c	0.21±0.04b	11.67±0.34b	1.83±0.05b	0.13±0.03c
	150 mM	11.44±0.20d	1.5±0.69d	0.12±0.01c	9.45±0.69c	1.67±0.04c	0.09±0.01d

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$.

Table 2: Effect of ZnO-NPs and different concentrations of NaCl (0.0, 50, 100 and 150 mM) on the content of pigments of maize cultivars Triple-hybrid 321(TH 321). Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		**Triple-hybrid 321**		
Soaking in	**NaCl(mM)**	**Chl. a (mg/g FM)**	**Chl. b(mg/g FM)**	**Carotenoid (mg/g FM)**
Water	**0**	0.847±.051^{a}	0.253±.012^{a}	0.145±.011^{a}
	50mM	0.612±.008^{b}	0.230±.011^{b}	0.118±.003^{b}
	100 mM	0.525±.008^{c}	0.178±.007^{c}	0.100±.003^{bc}
	150 mM	0.456±.012^{d}	0.148±.002^{d}	0.081±.005^{d}
ZnO- NPs	**0**	1.132±.012^{a}	0.423±.041^{a}	0.240±.012^{a}
	50mM	0.934±.011^{b}	0.300±.008^{b}	0.232±.021^{a}
	100 mM	0.800±.010^{bc}	0.230±.012^{c}	0.133±.015^{c}
	150 mM	0.654±.009^{d}	0.201±.003^{c}	0.111±.031^{cd}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$.

Table 3: Effect of ZnO-NPs and different concentrations of NaCl (0.0, 50, 100 and 150mM) on the content of soluble carbohydrates of maize cultivars (Triple-hybrid 321(TH 321) . Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		**Triple-hybrid 321**	
Soaking	**NaCl (mM)**	**Shoot**	**Root**
Water	**0**	34.27±0.38^{a}	7.47±0.16^{a}
	50 mM	39.62±0.88^{b}	11.38±0.47^{b}
	100 mM	42.96±0.07^{cb}	15.05±0.19^{c}
	150 mM	49.19±0.52^{d}	23.71±0.26^{a}
ZnO- NPs	**0**	40.44±0.16 ^{a}	12.10±0.22^{a}
	50 mM	43.54±2.29 ^{ab}	18.17±0.05^{b}
	100 mM	52.09±0.35 ^{c}	26.80±0.22^{c}
	150 mM	64.84±6.29 ^{d}	35.52±0.19^{d}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$. The values are expressed as mg g^{-1}dry mass

Table 4: Effect of ZnO-NPs and different concentrations of NaCl (0.0, 50, 100 and 150 mM) on the content of soluble protein of maize cultivar (Triple-hybrid 321(TH 321) .Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		**Triple-hybrid 321**	
Soaking	**NaCl (mM)**	**Shoot**	**Root**
Water	**0**	91.47±2.46^{a}	32.04±0.82^{a}
	50 mM	111.12±2.45^{b}	39.99±0.77^{b}
	100 mM	115.83±0.99^{cb}	41.26±0.58^{c}
	150 mM	109.20±1.67^{db}	43.58±0.55^{cd}
ZnO-NPs	**0**	110.25±1.47^{a}	40.92±0.99a
	50 mM	120.60±0.55^{b}	52.16±1.17^{b}
	100 mM	125.04±0.14^{bc}	54.94±0.90^{c}
	150 mM	128.94±1.04^{cd}	59.54±0.55^{d}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$. The values are expressed as mg g^{-1}dry mass

Table 5: Effect of ZnO- NPs and different concentrations of NaCl (0.0, 50, 100 and 150mM) on the content of free total amino acids of maize cultivar Triple-hybrid 321(TH 321) . Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		Triple-hybrid 321	
Soaking in	**NaCl(mM)**	**Shoot**	**Root**
Water	**0**	6.68±0.08^{a}	6.26±0.14^{a}
	50 mM	9.33±0.21^{b}	8.06±0.04^{b}
	100 mM	10.69±0.20^{c}	8.92±0.21^{c}
	150 mM	12.33±0.12^{d}	10.24±0.22^{d}
ZnO- NPs	**0**	7.16±0.10^{a}	10.10±0.16^{a}
	50 mM	12.87±0.16^{b}	11.32±0.20^{b}
	100 mM	13.36±0.10^{c}	12.31±0.10^{b}
	150 mM	20.62±0.14^{d}	14.22±0.33^{c}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$. The values are expressed as mg g^{-1}dry mass

Table 6: Effect of ZnO-NPs and different concentrations of NaCl (0.0, 50, 100 and 150 mM) on the content of proline of maize cultivars Triple-hybrid 321(TH 321) Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		Triple-hybrid 321	
Soaking in	**NaCl(mM)**	**Shoot**	**Root**
Water	**0**	3.01±0.07^{a}	2.73±0.08^{a}
	50 mM	5.50±0.13^{b}	6.47±0.13^{b}
	100 mM	6.27±0.09^{c}	7.97±0.13^{c}
	150 mM	7.69±0.10^{d}	10.22±0.31^{d}
ZnO- NPs	**0**	5.36±0.04^{a}	5.35±0.04^{a}
	50 mM	6.40±0.10^{ab}	11.74±0.20^{b}
	100 mM	8.28±0.42^{c}	13.46±0.09^{c}
	150 mM	9.73±0.12^{d}	14.94±0.10^{d}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$.The values are expressed as mg g^{-1}dry mass

Table 7: Effect of ZnO-NPs and different concentrations of NaCl (0.0, 50, 100 and 150 mM) on mineral contents (mg g^{-1} dry weight) of maize cultivar Triple-hybrid 321(TH 321). Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		Triple-hybrid 321							
		Shoot				Root			
Soaking in	**NaCl (mM)**	**Na**	**K**	**Ca**	**Mg**	**Na**	**K**	**Ca**	**Mg**
Water	**0**	15.16±.1.32^{a}	26.05±.0.13^{a}	14.12±.0.62^{a}	10.23±0.42^{a}	11.34±021^{a}	20.05±.0.10^{a}	16.12±.0.42^{a}	12.23±0.65^{a}
	50mM	31.45±0.18^{b}	21.87±.081^{b}	12.87±.0.91^{b}	8.56±0.35^{b}	26.45±093^{b}	16.77±.091^{b}	13.97±.0.84^{b}	10.56±0.95^{b}
	100 mM	37.12±0.25^{c}	18.23±.0.27^{c}	9.87±.0.97^{c}	6.98±0.17^{c}	31.25±013^{c}	11.23±.0.77^{c}	11.57±.0.97^{c}	8.98±0.57^{c}
	150 mM	43.72±0.22^{d}	14.97±.012^{d}	7.65±.0.42^{d}	4.23±.052^{d}	36.12±095^{d}	8.67±.042^{d}	9.35±.1.42^{d}	6.23±.042^{d}
ZnO-NPs	**0**	10.34±.042^{a}	27.89±0.31^{a}	18.78±.2.00^{a}	15.78±0.41^{a}	9.24±.008^{a}	22.99±0.51^{a}	20.33±.1.00^{a}	16.08±1.41^{a}
	50mM	22.11±.0.11^{b}	25.67±0.18^{b}	14,85± 0.58^{b}	13.12±0.58^{b}	22.32±.021^{a}	20.67±0.38^{b}	17.82± 0.88^{b}	13.12±0.68^{b}
	100 mM	32.67±0.18^{c}	21.56±0.22^{c}	11.78±0.92^{c}	9.34±0.72^{c}	27.23±.055^{c}	14.76±0.32^{c}	14.77±0.92^{c}	11.84±0.62^{c}
	150 mM	36.12±.0.9^{cd}	18.34±0.43^{d}	9.65±.003^{c}	7.56±0.25^{c}	32.78±.071^{d}	11.94±0.73^{d}	11.55±0.13^{d}	9.16±0.15^{d}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$.The values are expressed as mg g^{-1}dry mass.

Table 8: Effect of ZnO- NPs and different concentrations of NaCl (0.0, 50, 100 and 150mM) on enzyme activity of maize cultivar (Triple-hybrid 321(TH 321) and Single-hybrid 6 (SH 6). Seeds were soaked in water or 100 ppm ZnO-NPs.

Treatments		Triple-hybrid 321		
Soaking	**NaCl(mM)**	**Catalase**	**Peroxidase**	**Ascorbate Peroxidase**
Water	0	5.04±0.04^{a}	11.08±0.16^{a}	6.96±0.11^{a}
	50 mM	5.51±0.05^{a}	13.49±0.20^{b}	7.71±0.06^{b}
	100 mM	6.39±0.21^{b}	15.68±0.24^{c}	8.88±.12^{c}
	150 mM	8.17±0.08^{c}	24.55±0.26^{e}	11.65±0.02^{e}
ZnO-NPs	0	6.93±0.18^{a}	13.25±0.10^{a}	8.00±0.11^{a}
	50 mM	7.52±0.04^{b}	14.91±0.31^{b}	9.25±0.14^{b}
	100 mM	8.33±0.26^{c}	16.77±0.31^{c}	10.28±0.08^{bc}
	150 mM	9.65±0.45^{d}	27.71±0.58^{d}	12.41±0.05^{d}

Values are means of three replicates ± standard deviation (SD).Statistical significance of differences compared to control: Means not followed by the same letter are significantly different at $P < 0.05$.

Likewise, salt stress caused nutritional and metabolic imbalances due to ion toxicity which led to decrease in plant growth (Khan *et al.* 2020). Also, it induced the reduction in new cell production (Boscaiu *et al.* 2005). The ZnONPs stimulated an increase in growth criteria (root & shoot lengths and fresh & dry matter yields of both root and shoot of tested plant and consequently lightened the effect of NaCl stress. These results are agreed with Abdel Latef *et al.* 2017 and Hussein & Abou-Baker 2018. Zn is an important factor for both growth and development of plants (Pathak *et al.* 2012). since, Zn plays a signficant role in various purposes such as natural auxin building and therefore activating cell division and enlargement (Ali & Mahmoud 2013), maintenance of the structural integrity of biomembranes (Weisany *et al.*, 2012), enhancement of protein synthesis (Ebrahimian & Bybordi 2011), accumulation of phospholipids, scavenging active oxgen species, translocation of nutrients from the old cells to new cells), and reducing the uptake of excess of Na^{+} and Cl^{-} (Jiang *et al.* 2014 and Pooja *et al.* 2020). Tawfik *et al.* (2017) indicated that ZnONPs improved growth parameters of *Atriplex halimus*. Maize Plants growing under saltinity concentrations showed altered synthesis of photosynthetic pigments. Decrease in pigments synthesis is a cumulative effect of various factors such as osmotic stress-induced decrease in water content, altered mineral uptake and down-regulation in the activities of key photosynthetic enzymes (Padmaja *et al.* 1990; Abd-Allah *et al.* 2015). In the present study, seed priming with ZnONPs improved the chlorophyll contents under normal and NaCl circumstances. Carotenoids were markedly improved due to ZnONPs application which might be assumed to contribute to protection of macromolecules including proteins, DNA and RNA from the poisonous effects of free adicals (Ahmad *et al.* 2015; Abdel-Latef & Tran 2016).

Soluble carbohydrates considerably increased in both shoot and root organs of maize cultivar (TH 321). The accumulation of soluble carbohydrates in tested plants play a significant role in cell homeostasis (Ahmad *et al.* 2016). Enhanced accumulation of total soluble carbohydrates in response to salinity was revealed by Abdel-Latef *et al.* 2017 and El-Katony *et al.* 2019. In the present work, ZnO nanoparticles caused a noticeable increase in soluble carbohydrates in the tested organs of unstressed and stressed maize cultivar when compared with the corresponding levels. Zn acts a key role in sugar formation (Soliman *et al.* 2015).

The distinct accumulation of organic solutes due to soaking with ZnONPs might raise salinity tolerance of cell through osmotic adjustment, thus enhancing the growth of plants (Abdel-Latef *et al.* 2017). The data of soluble proteins content showed that increase in NaCl concentration markedly increased the soluble proteins in two root and shoot of the tested plant, our results were compatible with the finding of (Azooz *et al.* 2004 and Abdel-Latef *et al.* 2017). The high soluble proteins might be play a vital role in osmoregulation under salinity and can provide a storage form of nitrogen (Ahmad *et al.* 2016). Also, soluble protein accumulation under stress might be the effect of enhanced synthesis of specific stress-related proteins (Abdel-Latef *et al.* 2017). In this study, ZnONPs induced a considerable increase in soluble proteins content in unstressed and stressed plants in maize cultivar. This increase of soluble proteins might improve salt tolerance of plants through osmoregulation (Abdel-Latef *et al.* 2017). Proline content obviously accumulated in the two organs (root & shoot) of salinized maize cultivar. These results are in close with the results found by Kukreja *et al.* 2005, Haripriya *et al.* 2018 and Abdel-Latef *et al.* 2020. Lutts *et al.* (1996) stated that speedy increase of

proline in plant organs and tissues is one of the most obvious metabolic effects of salt stress. Proline increase might be used as a marker for tolerating NaCl stress through the contribution in osmoregulation (Abou Alhamd 2005). Furthermore, Proline acts as a sink for energy to adjust redox potential, such as a hydroxy radical scavenger, a solute that keeps macromolecules against denaturation and a way of lowering the acitity in the cell (Tammam 2003). The data of proline in our work showed that there is a negative correlation between growth criteria and proline accumulation in both organs of Triple-hybrid 321. These results agree with Perez-Alfocea *et al*., 1994 and Omarn, 2000. Treatment with ZnO nanoparticles results in a pronounced increase in proline accumulation in the two organs of tested cultivar as compared with the corresponding plants. Abdel-Latef *et al.* 2017 found that seed-priming with ZnONPs stimulated a marked accumulation in proline compared to control and salt stressed plants and this accumulation may be used this accumulation in osmotic adjustment to tolerate salinity stress. Total free amino acids increased in the two organs of the salinized maize cultivar. These results agree with the results achieved by Shah *et al.* (2001), Kumar *et al.* (2003) and Azooz *et al.* (2004). Total free amino acids has an important role in cell osmoregulation under salt stress (Azooz *et al.* 2004). Gilbert *et al.* (1998) reported that some functions of total amino acids for instance serving as an accessible energy source, nitrogen supply during restricted growth and phytosynthesis, detoxification of excess ammonia under stress and stablilisation of enzymes and/or membranes might be attained. Concerning the interactive effect between ZnONPs and salinity stress, it can be noticed that the to ZnONPs increasd the total free amino acids in tested maize cultivar. The marked increase of total in plants treated with ZnO NPs might improve salt tolerance of plants through osmotic adjustment (Abdel-Latef *et al.* 2017).

There is a variation in mineral contents in the two organs of tested maize cultivar (TH 321) under salinity stress. The content of sodium in the root & shoot of maize cultivar (TH 321) noticeably increased with increasing NaCl in the medium. In this matter, many authors stated that salinity caused an increase in sodium absorption (Saied *et al.* 2005; Qayyum *et al.* 2016; Khan *et al.*, 2020). Higher concentrations of Na^+ ions are harmful to plants and cause disorder in the mobility of potassium and calcium within the plant (Iqbal *et al.*, 2015). Several studies have witnessed the antagonistic relationship of Na^+ with several other important ions like K^+ (Ahmad *et al.* 2014; Abd Allah *et al.* 2015; Ahanger *et al.* 2015). The present study also revealed an improved uptake of Na^+ posing a concomitant negative effect on the uptake of other ions like K^+, Ca^{2+} and Mg^{2+}. Our results of reduced uptake of essential mineral ions due to salt stress are in concurrence with the findings of Azooz *et al.* (2015) for okra and Iqbal *et al.* (2015) for *Brassica juncea*. Application of ZnONPs not only allayed the harmful effect of excess sodium by constraining its uptake but also produced a marked increase in the uptake of essential mineral elements (K^+ , Ca^{2+} and Mg^{2+}). Enhanced K^+ uptake has positive control overgrowth performances through its active contribution in various metabolically significant processes like enzyme activation, osmoregulation, and the selective accumulation of sodium (Ahmad *et al.* 2014; Ahanger *et al.* 2015). Improvement in the Mg^{2+} uptake in ZnONPs -treated plants may contribute to enhanced chlorophyll pigment synthesis. Soliman *et al.* 2015 demonstrated that The application of nanoparticles increased aacumulation of K^+, Ca^{++} and Mg^{++}in salt stressed *Moringa* plants.

It has been illustrated in several studies that exposure to salinity stress could generate reactive oxyen species (ROS) producing enhanced activity of antioxidant enzymes as a protection system (Weisany *et al.* 2012; Soliman *et al.* 2015). This is in agreement with the stated results which indicated that tested maize cultivar in salinity conditions led to an increase in the antioxidant enzymes (CAT, POX and APOX) which can be considered as good proof of ROS production. It indicated that the increase in the activity of antioxidant enzymes may be recognized to the adaptive protection system of tested maize cultivar against the damaging impact enforced by NaCl salt. Soaked in ZnONPs increased the activity of antioxidant enzymes (CAT, POX and APOX) in the tested maize cultivar compared with the control and salinized plants.These results showed that the enhancement in antioxidant enzyme activity in response to ZnONPs treatment was due to oxidative stress produced by NaCl and the defense against salinity stress by antioxidant enzymes induced by ZnONPs. Perhaps Zn is able to help the enzymes antioxidant biosynthesis (Weisany *et al.* 2012; Rezaie & Abbasi 2014). Siddiqui (2018) and Zafar *et al*., 2021 reported that usage of ZnONPs produced an increase in antioxidant enzymes (CAT, POX and APOX). Moreover, Haripriya *et al.* 2018 reported that ZnONPs increased POX, CAT and AOPX activities in finger millet leaves under salinity stress.

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