Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

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PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Potential effect of zinc application on chlorophyll stability and metabolic status in *Moringa oleifera* Lam. under salinity stress conditions

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Recently, *Moringa oleifera* has been given attention to cultivation in arid and semi-arid lands not only as a drought tolerant plant, but also for its tremendous nutritional and medical benefits. Many problems such as water scarce, salinity and microelements deficiency faced the cultivation of the economic plants in desert lands. The present work aimed to study the effects of zinc on the chlorophyll content, chlorophyll thermostability and main metabolic compounds, namely, soluble sugars, free amino acids, and total soluble proteins of *Moringa oleifera* under reduced osmotic water potential. Healthy seedlings were incubated in hydroponic solution containing different zinc concentrations and osmotic water potential (ψ_s) levels. The data obtained indicated that the chlorophyll content and its thermostability (CSI) were high under moderate levels of ψ_s and zinc. Differences between the fresh and heated chlorophyll *a/b* ratios were positive in most treatments, with some exceptions due to the destruction of chlorophyll *a*. Statistically, the chlorophyll content and CSI were strongly affected by ψ_s, and the ψ_s × Zn interaction had a subdominant effect, whereas the opposite was true for chlorophyll *b*. Additionally, the same effect of ψ_s and ψ_s × Zn was true for the main metabolic compounds. Particularly, Zn had a subdominant effect on soluble proteins and free amino acids in the shoot and root, respectively. Zn has a crucial role in the function and efficiency of chlorophylls and their thermostability under salinity stress. Commonly, Zn improved the adjustment and increased hydrolytic enzyme activity, leading to increase soluble sugars in water- stressed roots. Also, Zn addition helps in enzyme activities concerning the metabolism of some osmolytes and water binding molecules (proteins) under drastic conditions in arid and semi- arid lands. The correlations between chlorophyll parameters and metabolic compounds were discussed.

Keywords: *Moringa oleifera*, zinc, osmotic water potential, chlorophyll, metabolites

ARTICLE HISTORY
Submitted: August 06, 2024
Accepted: May 29, 2025

CORRESPONDENCE TO
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DOI: 10.21608/ejbo.2025.309944.2957

EDITED BY: W. Amer

INTRODUCTION

Many edaphic problems, such as salinity and micronutrient deficiency during the reclamation and cultivation of hot desert and semidesert lands, are considered. Among these problems, the decreased osmotic water potential of soil causes osmotic stress, ionic stress, ionic imbalance and reactive oxygen species (ROS) in plants, that lead to disruption and damage to cellular structures (Gill and Tuteja, 2010; Budran et al., 2023; Abdel-Magied et al., 2025; Mohammed et al., 2025). The early root length gradually decreased with reduced water potential (Farghali, 2023). Hence, salinity has deleterious effects on different plant stages, and the biosynthesis of photosynthetic pigments results in decreased productivity (Parida and Das, 2005). To overcome these effects of salinity stress, several micronutrients, such as zinc (Zn), are applied to plants to improve various physiological and photochemical processes. Moreover, zinc protects membranes against oxidative and peroxidative damage by stabilizing membrane integrity and permeability and acts as a vital component of various important enzymes (Aravind and Prasad, 2004).

Zinc plays a crucial role in mechanisms and biosynthesis involved in cell membrane stability, chloroplast development, and the production of photosynthetic pigments (Zulfiqar and Ashraf, 2021). Zinc oxide nanoparticles have a constraining effect on the growth and physiological characteristics of Fenugreek plants (El-Shazoly, 2024). Zafar et al. (2022) reported that, ZnO-NPs improved the biosynthesis of chlorophyll *a*, chlorophyll *b*, and carotenoids, when the plants were exposed to salinity. Moreover, salinity obstructs the function of important enzymes responsible for the synthesis of photosynthetic pigments (El-Tayeb, 2005). Zn has a significant effect on alleviating plant abiotic stress. Therefore, increased chlorophyll content contributes to zinc, which acts as a structural and catalytic component of proteins and enzymes and as a co-factor for the normal development of pigment biosynthesis (Samreen, et al., 2017). Furthermore, zinc is involved in the translocation of water-soluble metabolites (e.g. soluble proteins, free amino acids and soluble sugars) to the root, and the subsequent accumulation of more reserve complex materials in plant parts (El-Sharkawi et al., 2016).

Moringa oleifera is a drought-tolerant plant. It is a source of proteins, vitamin C, calcium, and potassium, as well as natural antioxidants (Moyo et al., 2012). Hence, the improvement in the economic value of this species in arid and semiarid lands can be promoted by zinc application. The present work aims to investigate the effect of different concentrations of zinc on moringa plant's biochemical and physiological characteristics such as chlorophyll, soluble sugars, free amino acids and soluble proteins under reduced osmotic water potential.

MATERIALS AND METHODS

Mature seeds of *Moringa oleifera* Lam. (Moringa) were germinated in trays of moist perlite under natural laboratory conditions. After seven days of sowing, uniform healthy seedlings were selected and transferred to one-liter plastic pots, filled with continuously aerated hydroponic cultures.

Adjustments of the osmotic water potential (ψs) and Zn concentration

The hydroponic cultures were treated with a mixture of NaCl and $CaCl_2$ at concentrations that resulted in different osmotic water potentials (SAR=1/8) prepared according to Lagerwerff and Eagle (1961). The used levels of $ψ_s$ were 0 (control), -0.3, -0.7, and -1.0 MPa. Another series of zinc solutions at the same different $ψ_s$ levels ($ψ_s$ +Zn) were prepared by dissolving certain concentrations (0, 5, 10 and 15 ppm) of zinc ($ZnSO_4$) in NaCl +$CaCl_2$ solution. Healthy *M. oleifera* (3-week-old) seedlings were transferred to pots containing treatment solutions (three replicates) at room temperature (day/night temperatures of 28°C/15°C). During the experiments, the illumination was satisfactory when sodium and florescent light were used in combination.

Assessment of chlorophyll (Chl.) content and its thermostability (CSI):

In fresh leaves, chlorophyll *a* and chlorophyll *b* were extracted using 85% acetone and determined according to Lichtenthaler (1987). The chlorophyll thermostability index (CSI) was determined according to Murty and Majumder (1962) as the Chl. The ratio of the content of heated leaves (at 56± 1°C) to that of fresh leaves is expressed as a percentage as follows:

$$CSI\ a = \frac{\text{Content of chlorophyll } a \text{ in heated sample}}{\text{Content of chlorophyll } a \text{ in fresh sample}} X100$$

$$CSI\ b = \frac{\text{Content of chlorophyll } b \text{ in heated sample}}{\text{Content of chlorophyll } b \text{ in fresh sample}} X100$$

Differences between fresh and heated ratios were calculated to evaluate the destruction of Chl. *a* or Chl. *b* according to Farghali (1998b) as the following equation: Specific thermostability of Chl. *a* or *b* = Fresh *a/b*-heated *a/b* = -ve or +ve value

Preparation of plant extract

At the end of the experiment (after 10 days treatment), the seedlings were washed with distilled water and dried thoroughly with filter paper. The excised parts (shoots and roots) were then freshly weighed and immediately blended with 10 ml of ice-cold distilled water. The extracts were subjected to deep freezing until analysis.

Determination of water-soluble metabolites

For the *M. oleifera* shoot or root extracts, the metabolic compound contents, including soluble sugars (Dubois *et al.* 1956), total free amino acid (Lee and Takahonshi 1966) and total soluble protein contents (Lowry et al. 1951) were determined using spectrophotometer device (UV 2000, Ray Wild, and LC.- Gottingen, Germany).

Statistical analysis

The effects of single factors ($ψ_s$ and Zn) and their interaction ($ψ_s$ × Zn) were evaluated via analysis of variance (F values), the sharing coefficient percentage (role of each factor on the tested parameters), and the simple linear correlation coefficient, r (Ostle 1963). These statistical analyses were carried out via the SPSS software (2016). Statistical analysis of Tukey test ($ψ_s$ levels as letters, Zn concentration as numbers), was also assessed.

RESULTS

Chlorophyll contents

The Chl. *a* content in *Moringa oleifera* was strongly affected by the Zn concentration and reached a maximum value (3.11 mg. g^{-1} fresh Wt.) at Zn=10 ppm under moderate $ψ_s$ levels (-0.7 MPa, Figure 1). The same was true in the case of Chl. *b* content (1.84 mg. g^{-1}fresh Wt.). In general, low and moderate Zn concentrations had a positive effect on two chlorophyll fractions (*a* and *b*) with reduced water potential. Additionally, the thermostabilities of both chlorophyll fractions were high (2.82 and 1.97 for Chl. *a* and Chl. *b*, respectively) at -0.7 MPa (Figure 2). Thus, the total content of chlorophyll (*a + b*) increased at $ψ_s$ = -0.3 to -0.7 MPa, especially in Zn concentration ranged between 5-10 ppm. These findings implied that Zn had beneficial effects on the total chlorophyll content under salinity stress. Hence, a high content of total chlorophyll (4.95 mg g^{-1} fresh weight) was observed at Zn = 10 ppm and $ψ_s$ = -0.7 MPa (Figure 3).

The Chl. *a/b* ratios of fresh leaves ranged between 1.31 - 2.19 tended to reach its maximum at high Zn concentrations (15 ppm) and low $ψ_s$ levels (-1.0 MPa). Commonly, the Chl. *a/b* ratios slightly varied under different Zn and $ψ_s$ levels (Figure 4). Additionally, the a*/b* ratios of the chlorophyll stability index (CSI) indicated that the decreased $ψ_s$ stimulated the stability of Chl. *a.* The CSI ratio tended to reach a maximum (3.66) at a5 ppm Zn concentration, which might be due to the destruction of Chl. *b* (Figure 3). In general, the CSI *a/b* ratios ranged from 0.88-3.66.

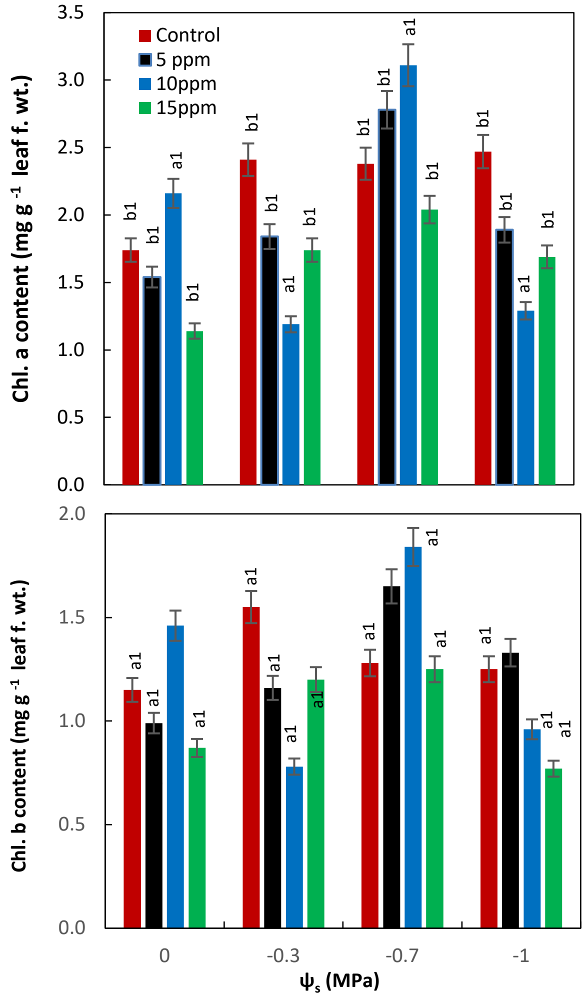

Figure 1. Chlorophyll *a* and *b* contents (mg.g^{-1} fresh weight) at different Zn concentrations and osmotic water potentials, ψ_s levels, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

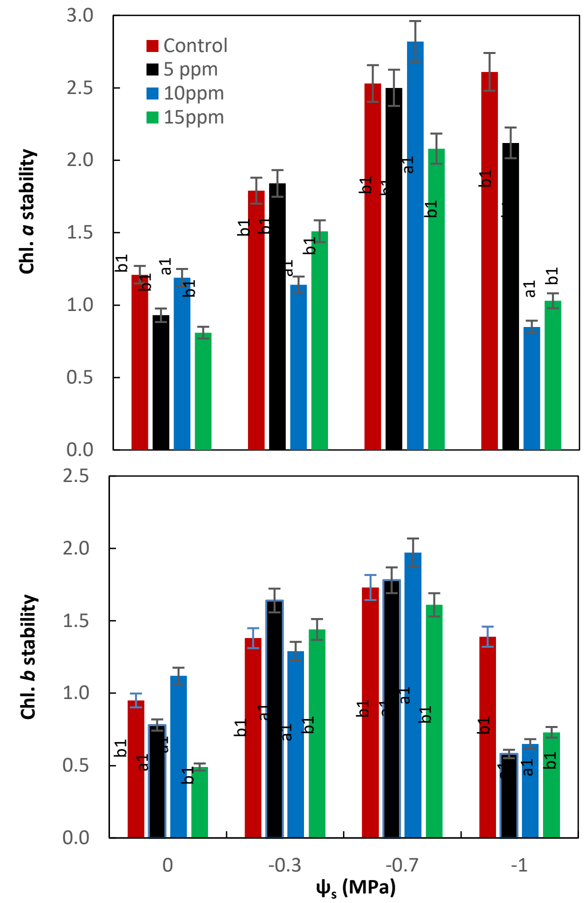

Figure 2. Chlorophyll *a* and *b* contents of heated leaves (CSI) at different Zn concentrations and osmotic water potentials, ψ_s levels, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

Furthermore, the differences between fresh and heated Chl. *a/b* ratios had positive values under different Zn and ψ_s levels due to the destruction of Chl. *a* except under Zn level = 5 ppm and ψ_s = -1.0 MPa due to destruction of Chl. *b* (negative value). The same destruction of Chl. *b* was present at Zn = 15 ppm in unstressed plants (Table 1). All single factors and their interaction (ψ_s × Zn) had significant effects on the Chl. contents and its stability to heat, with some exceptions as illustrated in table (2). Apparently, ψ_s had a predominant role in the chlorophyll *a* content and different chlorophyll stability indices, whereas the interaction (ψ_s × Zn) had a subdominant role. The opposite was found in case of Chl. *b* and total chlorophyll content (*a +b*). This result reflected the crucial role of Zn in the function and efficiency of chlorophylls and their thermostability under salinity stress.

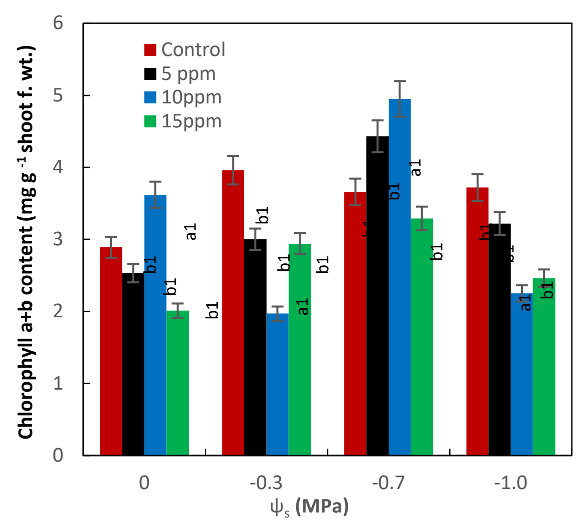

Figure 3. Total chlorophyll content of fresh leaves at different Zn concentrations and osmotic water potentials, ψ_s levels, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

The correlation between the thermostabilities of chlorophyll *a* and chlorophyll *b* was significantly positive under the effect of ψ_s × Zn interaction. The same correlation was found in case of the Chl *a* content with its stability and total CSI under the effects of single factors ψ_s and Zn, respectively (Table 3). This means that Zn application was beneficial for the thermal endurance of both chlorophyll fractions.

Main metabolic compounds

Total soluble proteins (S.P.)

Soluble proteins are helpful in conserving water in plant cells (as water-binding colloids) under harsh conditions. The response of the investigated plant parts of *M. oleifera* to salinity and Zn concentrations essentially differed (Figure 5). In shoots, under relatively high ψ_s levels (0 to -0.3 MPa), the soluble proteins increased at Zn concentrations of 0 and 10 ppm. However, the lowest values of soluble proteins were detected at moderate ψ_s levels under different Zn concentrations. In the roots, the low concentrations stimulated soluble proteins in the presence of Zn concentrations of 5 and 15 ppm. Similarly, Zn = 5 ppm had the same effect on the S.P. in unstressed plant roots. Regardless of ψ_s, Zn = 5 ppm produced the maximum S.P. content (23.55 mg g^{-1} fresh weight). Apparently, moderate ψ_s levels had adverse effects on the S.P. of both the shots and the roots of *M. oleifera*. Statistically, the ψ_s effect was significant and played a major role in the total soluble proteins in the shoot and root parts, and Zn had a secondary role in the S.P. of the shoot (Table 4). However, its interaction with osmotic potential had the same effect on the roots.

Free amino acids (A.A.)

Under the effects of the factors investigated, changes in the amount of A.A. differed according to the investigation. In the shoots, the absence of salinity increased the A.A. content at different Zn concentrations. The highest values of A.A. (5.6-6.5 mg. g^{-1} fresh weight) were detected in response to low and moderate Zn concentrations. While moderate ψs had adverse effects on the A.A. content at different Zn concentrations (Figure 6). The high content of A.A. (3.13 mg. g^{-1} fresh wt.) existed at low ψ_s levels (-1.0 MPa) and Zn = 15 ppm. Therefore, Zn levels of 10 - 15 ppm were accompanied by an increase in the A.A. contents of roots at different ψ_s levels. As in case of soluble proteins, ψ_s had the main effect on the free amino acid contents in both shoot and root parts (Table 4). However, Zn or its interaction with osmotic potential had a secondary role in the case of A.A. in both roots and shoots.

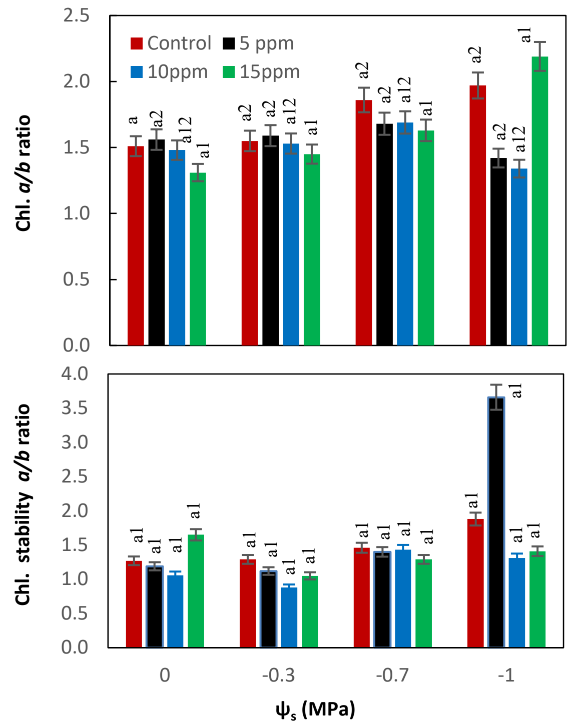

Figure 4. Chlorophyll *a/b* ratio differences between fresh leaves and heated leaves at different Zn concentrations and osmotic water potentials, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

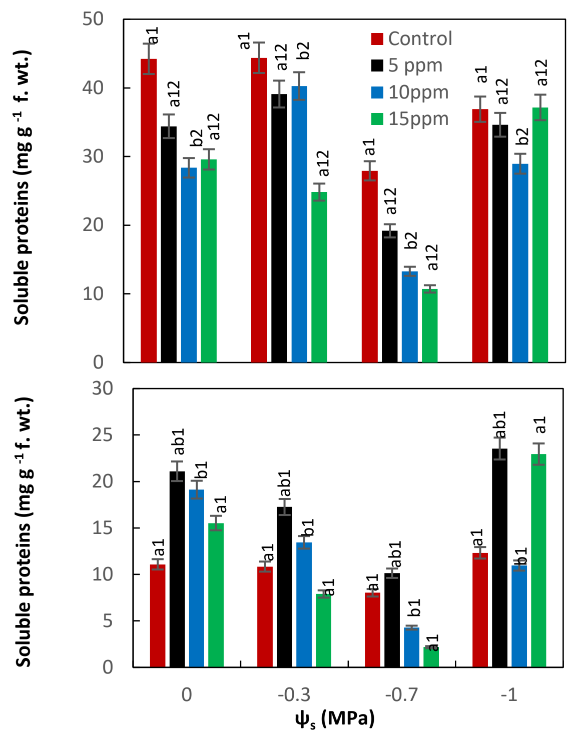

Figure 5. Total soluble protein contents (mg.g^{-1} fresh weight) in shoots and roots at different Zn concentrations and osmotic water potentials, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

Table 1. Differences between fresh and heated chlorophyll *a/b* ratios at different levels of ψ_s and zinc concentrations

Ψ_s Zn Concentration	0	-0.3	-0.7	-1.0	Sum
Control	0.24	0.26	0.4	0.09	0.99
5 ppm	0.37	0.47	0.28	-2.24	-1.12
10ppm	0.42	0.65	0.26	0.03	1.36
15ppm	-0.34	0.4	0.34	0.78	1.18
Sum	0.69	1.78	1.28	-1.34	2.41

Table 2. F values and sharing % of the effect of ψ_s, Zn and their interaction on the chlorophyll content (*a* and *b*) and its thermostability (CSI) of *M. oleifera* leaves.

Contents		Chl. *a*		Chl. *b*		chl. (*a*+*b*)		CSI *a*		CSI *b*		CSI. (*a*+*b*)	
Source of variance	df	F	Shar (%)	F	Share (%)	F	Share (%)	F	Share (%)	F	Share (%)	F	Share (%)
ψ_s	3	8.95**	43.99	3.21*	33.00	6.71**	40.88	19.95**	59.88	29.66**	76.93	40.85**	68.48
Zn	3	3.11*	15.28	1.44	14.81	2.49	15.17	5.40**	16.21	2.09	5.43	6.83**	11.45
ψ_s x Zn	9	2.76*	40.73	1.69	52.18	2.41*	43.94	2.66*	23.91	2.27*	17.64	3.99**	20.07

*Significant at 5% level, ** Significant at 1% level

Table 3. Correlation coefficient (r.) between chlorophyll content (*a* and *b*) and chlorophyll thermo-stability, CSI (fractions and total) of *M. oleifera* under the effect of ψ_s, Zn and their interaction.

Source	Chl. *a* content		Chl. *b* content	
	CSI $_a$	CSI $_{Total}$	CSI $_b$	CSI $_{Total}$
ψ_s	0.962*	0.942	0.884	0.913
Zn	0.935	0.985*	0.869	0.861
ψ_s x Zn	0.868**	0.868**	0.641**	0.748**

*Significant at 5% level, ** Significant at 1% level

Table 4. F values and sharing % of the effect of ψ_s, Zn and their interaction on the metabolic compounds soluble proteins (SP), free amino acids (AA) and soluble sugars (SS) in shoot and root of *M. oleifera*.

Contents		S.P. of shoot		S.P. of root		A.A. of shoot		A.A. of root		S.S. of shoot		S.S. of root	
Source of variance	df	F	Share (%)	F	Share (%)	F	Share (%)	F	Share (%)	F	Share (%)	F	Share (%)
ψ_s	3	5.22**	51.40	4.08*	53.56	5.00**	55.09	3.82*	46.72	4.98**	39.80	19.28**	66.54
Zn	3	3.78*	37.22	1.65	21.71	0.37	4.07	2.55	31.21	1.92	15.33	2.61	9.00
ψ_s x Zn	9	0.39	11.38	0.63	24.72	1.24	40.84	0.6	22.07	1.87	44.87	2.36*	24.46

*Significant at 5% level, ** Significant at 1% level

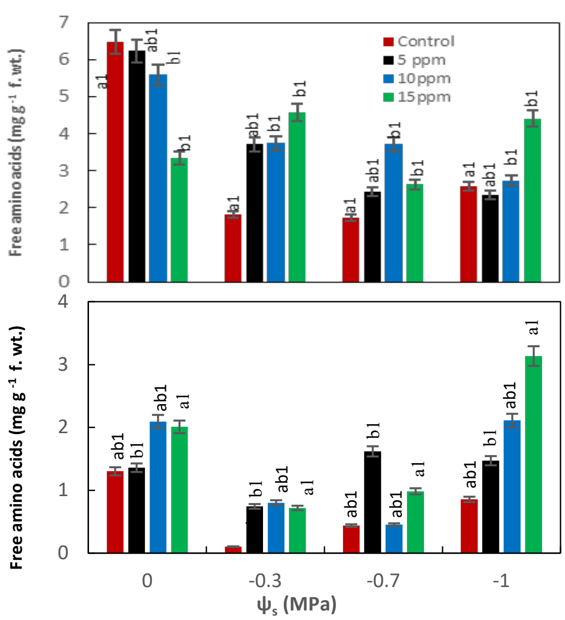

Figure 6. Free amino acid content (mg.g^{-1} fresh weight) in shoots and roots at different Zn concentrations and osmotic water potentials, ψ_s levels, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

Total soluble sugars (S.S.)

Soluble sugar serves as compatible solutes in stressed plants. The data obtained indicated that high S.S. amounts in shoots and roots were present at low ψ_s levels, particularly under low or no Zn (Figure 7). The low ψ_s levels had a positive effect on the S.S. contents at high Zn levels. But S.S. contents gradually decreased with increasing Zn concentration at various ψ_s. In both shoots and roots, a reduced osmotic water potential was more effective at increasing soluble sugar production within a certain range of Zn concentrations. The effects of ψ_s or their interactions with Zn on soluble sugars differed between the two plant parts. Where, the S.S. content in the shot was strongly affected by ψ_s factor. Also, ψ_s had the same effect on the S.S. content of the roots, but interactions (ψ_s × Zn) played a secondary role (Table 3). This may indicate the considerable role of S.S. in roots as a compatible solute against salinity stress.

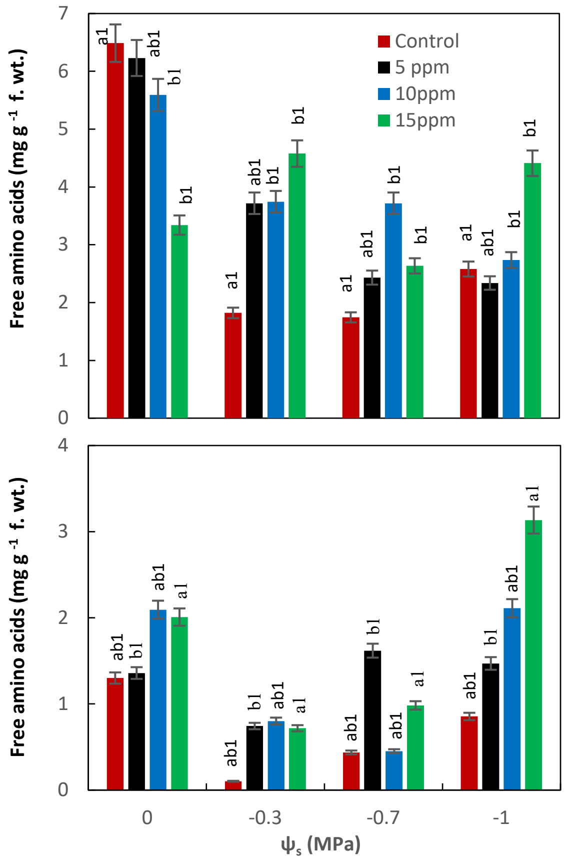

Figure 6. Free amino acid content (mg.g^{-1} fresh weight) in shoots and roots at different Zn concentrations and osmotic water potentials, ψ_s levels, [vales having similar symbols (ψ_s) or numbers (Zn) indicate no significant difference according to Tukey test].

Similarly, the addition of Zn improved the adjustment and increased hydrolytic enzyme activity, leading to increase soluble sugars in *M. oleifera* roots. Apparently, the shoots gained more metabolic compounds than did the roots at different levels of Zn and ψ_s, possibly because of their photosynthetic and enzymatic activities.

Correlation coefficient (r.) between the total chlorophyll content and chlorophyll stability index (CSI) with metabolic compounds:

In the shoots, there was a significant negative correlation between total chlorophyll and soluble proteins under the effect of ψ_s. However, soluble sugars were positively correlated with total chlorophyll in the presence of Zn. The same significant correlation was observed between soluble protein content and total chlorophyll thermostability. Indirectly, a significant negative correlation existed between the CSI and both soluble protein and free amino acid contents of the roots under the effects of ψ_s or its interaction with Zn (Table 5), which it has been implied that soluble protein was translocated from the roots to the shoots in response to Zn and salinity stress. Additionally, the soluble protein content of the shoots was negatively correlated with the free amino acid content of the roots under the effect of Zn factor (Table 6).

DISCUSSION

Zinc, as a micronutrient, plays an important role in enzyme activity, protein production and chlorophyll formation. The internal Zn concentration enhances dry matter accumulation, which is accompanied by an increase in chlorophyll content and photosynthetic activity (Farghali, 1998a). The total chlorophyll content of leaves (Chl *a+b*) and the chlorophyll *a/b* ratio influence the photosynthetic capacity of plants (Croft et al., 2017). In hydroponic solutions, the respiratory CO_2 in the root media (formation of acidic media) facilitates the solubility and uptake of Zn ions. Under salinity stress, the addition of Zn had beneficial effects on the total chlorophyll content.

Therefore, decreasing chlorophyll content during salinity stress can be ameliorated by Zn, which may be attributed to Zn-induced elimination of the sulfhydryl group (Weisany, et al., 2011). The current results revealed that the high contents of the two chlorophyll fractions and total chlorophyll in *Moringa oleifera* were present at moderate levels of Zn and osmotic water stress. The same was true in the case of chlorophyll stability in response to heat. The differences between fresh and heated Chl. The *a/b* ratios had positive values under most levels of Zn and ψ_s due to the destruction of Chl. *a*. This implied that chlorophyll *b* was more stable to heat than was chlorophyll *a*. Statistically, ψ_s played a predominant role in the chlorophyll *a* content and chlorophyll stability index, whereas the interaction ($\psi_s \times$ Zn) had a subdominant role. The opposite was found in the case of Chl. *b* and total chlorophyll content (*a +b*). These findings may reflect the crucial role of Zn in the efficiency of chlorophylls and their thermal stability under salinity stress. Additionally, the positive correlation between both chlorophyll fractions indicated that Zn application was beneficial for the thermal endurance of both chlorophyll fractions, particularly under moderately saline conditions. The interaction between temperature and ψ_s had a main role on the root extension and weight which played a critical role on the plant (*Simmondsia chinensis*) growth under warm desert conditions (Farghali, 2023).

Table 5. Correlation coefficient (r.) between total chlorophyll (*a+ b*) and total thermostability with different investigated metabolites in shoots and roots of *M. oleifera* under the effect of ψ_s, Zn and their interaction.

Parameter	Total Chl. content					
Source of variance	Proteins shoot	Proteins root	Amino acids shoot	Amino acids root	Sugars shoot	Sugars root
ψ_s	-0.961*	-0.931	-0.641	-0.279	0.079	0.235
Zn	0.917	0.279	-0.802	-0.902	0.964*	0.486
ψ_s x Zn	-0.430	-0.427	-0.305	-0.248	0.214	0.247
Parameter	Total Chl. thermo-stability					
Source of variance	Proteins shoot	Proteins root	Amino acids shoot	Amino acids root	Sugars shoot	Sugars root
ψ_s	-0.815	-0.952*	-0.787	-0.513	0.070	0.263
Zn	0.976*	0.014	-0.778	-0.942	0.818	0.420
ψ_s x Zn	-0.450	-0.591*	-0.480	-0.499*	0.244	0.182

*Significant at 5% level, ** Significant at 1% level

Table 6. Correlation coefficient (r.) between soluble proteins with both free amino acids and soluble sugars in shoots and roots of *M. oleifera* under the effect of ψ_s, Zn and their interaction.

Parameter	Soluble proteins of shoot			
Source of variance	Free amino acids		Soluble sugars	
	Shoot	Root	Shoot	root
ψ_s	0.480	0.046	-0.107	-0.218
Zn	-0.895	-0.992**	0.880	0.248
ψ_s x Zn	0.213	-0.147	0.053	-0.177

*Significant at 5% level, ** Significant at 1% level.

The reaction of plants to salinity stress involves a network of interconnected physiological, biochemical, and molecular mechanisms (Badr et al., 2024). Hence, the adaptation of plants to heat stress stimulates the accumulation of metabolic compounds as compatible solutes in the chloroplasts, that are related to an increase in chlorophyll thermostability (Farghali, et al., 2022). The solutes that accumulate in plants during water stress and contribute to the active osmotic adjustment can include soluble carbohydrates, inorganic cations, organic acids or free amino acids (Arabzadeh, 2012). Hence, a high amount of soluble protein is beneficial for maintaining viscous properties and contributes to increasing the osmolality of cytoplasm (Rayan and Farghali, 2007). Under relatively high ψ_s levels, soluble protein (S.P.) content in the shoots of *M. oleifera* was high at Zn = 0 and 10 ppm. The lowest values of soluble proteins were detected at moderate ψ_s levels under different Zn concentrations. This means that the protein content decreases with increasing zinc concentration. Conversely, low ψ_s stimulated soluble protein in the roots at different Zn levels. This implies that the addition of zinc improved the adjustment of roots to water deficiency conditions through increasing the allocation of water-soluble proteins into the roots. (El-Sharkawi et al., 2018). Similarly, a low Zn concentration had the same effect on S.P. content in unstressed plant roots. Regardless of ψ_s, a low Zn concentration produced a maximum S.P. content. However, moderates had adverse effects on S.P. of both shots and roots of *M. oleifera*. The ψ_s effect (judged by sharing %) had a major effect on the total soluble proteins in both shoot and root, and Zn had a secondary effect on S.P. of the shoot.

On the other hand, free amino acids increased under high osmotic water potential, it is possibly because of the hydrolysis of proteins at different Zn levels. The accumulation of amino acids led to soluble protein synthesis, which can be used as the criterion for the evaluation of drought resistance in plants (Rasha et al*.,* 2022). The decreased ψ_s stimulated free amino acids as osmotic compounds, especially in roots, which enhanced water uptake, thus facilitating enzyme activities and hydrolysis of reserve materials (El-Sharkawi et al., 2016). Apparently, ψ_s had the main effect on the free amino acid contents in the shoot and root. Addition of zinc generally increased the translocation of osmotically active metabolites under arid and semi-arid conditions, ultimately resulting in the accumulation of reserve metabolites in the growing parts. This finding was statistically influenced by the secondary role of Zn or its interaction effect on the free amino acid contents of both root and shoot.

The accumulation of soluble sugars (S.S.) in plant tissues plays an osmotic role in maintaining a balance of osmotic force and is one of the main criteria in the stress response (Hajihashemi et al., 2006). An increase in soluble sugar content with low osmotic water potential was reported in the presence of zinc (El-Sharkawi et al., 2016). The present data indicated that high S.S. amounts in shoots and roots were detected at low ψ_s levels, particularly under low or no Zn. Furthermore, the statistical analysis indicated that the S.S. content in both parts was strongly affected by this factor and that the interaction (ψ_s × Zn had a subsidiary effect. The addition of Zn clearly improved the adjustment and increased hydrolytic enzyme activity, leading to increased soluble sugars in water-stressed roots. In general, the amounts of soluble sugars in *M. oleifera* were greater than those in both soluble proteins and free amino acids in the following pattern: Soluble sugars > soluble proteins > free amino acids.

This observation indicated the predominant participation of soluble sugars in plants growing under adverse conditions prevailing in arid and semiarid lands (Farghali and El-Aidarous, 2014). The correlation between chlorophyll parameters and metabolic compounds were variable under the effects of Zn, ψ_s and their interaction. Under the Zn factor, a positive correlation exists between total chlorophyll thermostability and the chlorophyll content with both soluble proteins and soluble sugars, indicating the crucial role of Zn in protection and increased chlorophyll molecule efficiency (Farghali, et al., 2022). Therefore, the presence of some micronutrients, such as zinc, may alleviate the effects of reduced water potential on chlorophyll ability and plant growth (Hera et al., 2018). Additionally, root development and carbohydrate, and chlorophyll formation are dependent on zinc (Cakmak and Kutman, 2018). In the present work, negative correlations were found between the CSI and both the soluble protein and free amino acid contents of roots under the effects of ψ_s or its interaction with Zn, which implies that soluble proteins are translocated from the roots to the shoot in response to Zn and salinity stress.

CONCLUSION

This work revealed the crucial role of Zn in the function and efficiency of chlorophylls and their thermostability under salinity stress. Low and moderate Zn levels improved the thermal endurance of both chlorophyll fractions in *Moringa oleifera*, particularly under moderate salinity conditions. Chlorophyll *b* was more stable under heat than chlorophyll *a.* Additionally, the protein content decreased with increasing zinc concentration. Conversely, low ψ_s levels stimulated soluble proteins in the roots. The amounts of soluble sugars were high compared with those of both soluble proteins and free amino acids. In general, Zn improved the adjustment and increased hydrolytic enzyme activity, leading to increased soluble sugars in water-stressed roots.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY

The data that supported the findings of this study are available within the article.

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