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Effect of salinity and zinc on physiological and nutritional responses of rosemary

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A b s t r a c t. This study was conducted to investigate the effect of zinc nutrition on growth, leaf relative water content, membrane permeability, and nutrient uptake of rosemary grown under saline condition. A factorial arranged hydroponics experiment with 3 salinity levels, and 3 levels of zinc was performed. At 0 and 2 μM Zn levels, salinity significantly decreased the leaf relative water content. Salinity treatment increased membrane permeability, Na, Cl and P uptake, accompanied by significant decreases in plant growth, relative water content, and K, Ca and Mg concentration. These changes were greater at the 0 and 2 μM Zn levels compared to the 4 μM Zn treatment. Increases in Zn concentration from 0 μM to 2 and 4 μM counteracted the deleterious effect of salinity on relative water content, membrane permeability and ion uptake. The results showed that Zn nutrition is involved in maintaining of membrane permeability and ion-selective uptake which could improve or accelerate the adaptation of rosemary to salt stress.

K e y w o r d s: rosemary, salt stress, zinc nutrition, oxidative damage, nutrient uptake

INTRODUCTION

Soil salinity affects plant production in many parts of the world, particularly in irrigated lands of arid and semiarid regions (Zorb *et al*., 2004). Salinity reduces plant growth due to adverse water relations, the toxic effects of Na^+ and Cl ions on metabolism, nutrient imbalances and oxidative stress (Tuna *et al*., 2008). Increased production of reactive oxygen species (ROS) under salt stress may lead to plasma membrane peroxidation, thereby affecting cell membrane integrity (Tuna *et al*., 2008). These changes modulate the pattern of ions leakage and uptake (Ashraf and Ali, 2008).

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb that has been accepted as one of the spices with the highest antioxidant activity (Genena *et al*., 2008). Rosemary thrives in a warm and dry climate; however, as in the majority of cultivated plants, growth and yield of rosemary can be affected by salinity (Baatour *et al*., 2009) and Zn deficiency. Zinc deficiency is one of the most important micronutrient disorders limiting crop productivity in calcareous saline soils (Khoshgoftar *et al*., 2004). This element is considered to play a critical physiological role in the structure and function of membrane lipids especially under salt stress (Aktas *et al*., 2006). Loss of membrane stability resulting from Zn deficiency may affect the uptake of Na at toxic levels in salt stressed plants. Therefore, improving the Zn nutritional status of plants could greatly improve their salt stress tolerance. The objective of the present study was to evaluate the effect of Zn on rosemary salt tolerance, mainly as the ability to maintain plant growth and nutrient uptake under salt stress.

MATERIALS AND METHODS

This research was carried out hydroponically in greenhouse conditions between March and August 2008. Plants of rosemary from uniform rooted cuttings were transplanted to 5 l pots containing Hoagland nutrient solution. Pots were covered with black plastic to exclude light from the roots and to prevent evaporation. The experiment was a 3×3 factorial arrangement with three levels – 0, 2, and 4 μM Zn added by dissolving $ZnSO_4$ and three nutrient solution salinity levels (0, 50, and 100 mM NaCl) in 4 replicates.

At harvest, fresh samples of fully young matured leaves were randomly collected from each pot for determination of leaf relative water content (Yamasaki and Dillenburg, 1999) and membrane permeability (Lutts *et al*. 1996). To measure dry mass (DM), shoot and root were separated and oven dried at 70°C for 72 h.

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Total Na and K concentrations were determined by flame emission photometry, Ca, Mg by atomic absorption spectrophotometery, NaCl concentration was assayed by a titrametric procedure, P by a vanadate-molybdate method using a spectrophotometer (Chapman and Pratt, 1982).

The experiment was set up in a factorial design with three levels of salinity and three levels of zinc. Analysis of variance (ANOVA) was performed using SAS program. Differences among treatments means were analyzed by the LSD test at a 5% probability level.

RESULTS AND DISCUSSION

Under Zn sufficient condition (2 and 4 μM), moderate salinity level (50 mM) had no significant effect on the shoot and root dry mass of rosemary grown (Table 1). These results suggest that rosemary could be categorized as a moderately salt tolerant plant (Zaouali *et al*., 2005). The reduction in shoot and root DM due to NaCl treatments was improved in response to Zn application. These findings are in accordance with those obtained by Tavallali *et al*. (2009).

Leaf water content (LRWC) of rosemary was reduced significantly with a rise in NaCl level. Such decrease was greater in nil Zn treatment suggesting that Zn was protective against osmotic changes in rosemary (Fig. 1). The ameliora-tion role of Zn in maintaining of LRWC might be attributed to improvement of vascular tissue (Gadallah, 2000) and more accumulation of Ca and K in the roots. Increasing of Ca and K uptake by rosemary may help the roots to maintain continuous water uptake. Supporting this idea, a fairly good correlation was found between LRWC and root K and Ca concentration (r = 0.62** and r = 0.65**, respectively).

Regardless of zinc treatments, salinity induced significant increase in membrane permeability may be due to ROS (Fig. 2). The enhancement in membrane permeability caused by salinity became more severe at the lowest Zn concentration (0 µM Zn). Plants exposed to 2 and 4 µM Zn had lower membrane permeability (10 and 22%, respectively) in comparison to the plants grown at the Zn-free nutrient solution. These results indicated that Zn may partially ameliorate oxidative damage induced by salt stress (Tavallali *et al*., 2010).

T a b l e 1. The effect of salinity and zinc concentration on shoot and root dry biomasses (g pot^{-1})

NaCl levels (mM)	Zn levels (μM)			
	0	2	4	Mean
		Shoot dry biomass		
0	28.1±0.71 cd	31.0±2.39 ab	31.7±0.45 a	30.3±2.11 A
50	27.2±0.65 d	29.4±0.20 bc	30.3±1.06 ab	29.0±1.51 B
100	18.3±0.75 e	21.0±1.33 e	22.4±0.96 e	20.6±2.00 C
Mean	24.5±4.71 B	27.1±4.88 A	28.2±4.42 A	
		Mean square		
NaCl level		250.4 **		
Zn level		29.5 **		
NaCl×Zn		1.1 n.s.		
		Root dry biomass		
0	5.6±0.14 bc	6.3±0.09 a	6.2±0.48 ab	6.1±0.42 A
50	5.4±0.13 c	5.9±0.04 ab	6.1±0.21 ab	5.8±0.30 B
100	3.7±0.15 e	4.2±0.27 d	5.5±0.19 c	4.4±0.40 C
Mean	4.9±0.94 C	5.5±0.99 B	5.9±0.88 A	
		Mean square		
NaCl level		6.7 **		
Zn level		2.3 **		
NaCl×Zn		0.5 **		

** Significant at P < 0.01. n.s. – not significant at P<0.05. Each value represents the mean SD of four replicates. Values followed by different letters in column are significantly different at the 5% according to LSD test.

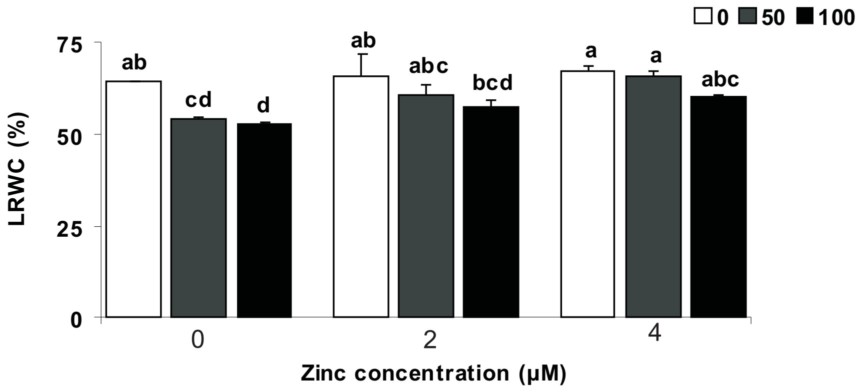


Fig. 1. The effect of salinity (0, 50 and 100 mM NaCl) and zinc concentration (= 0, 2 and 4 μM Zn as $ZnSO_4$) on the LRWC (mean SE) of rosmary. Different letters above bars indicate significant difference at $P<0.05$.

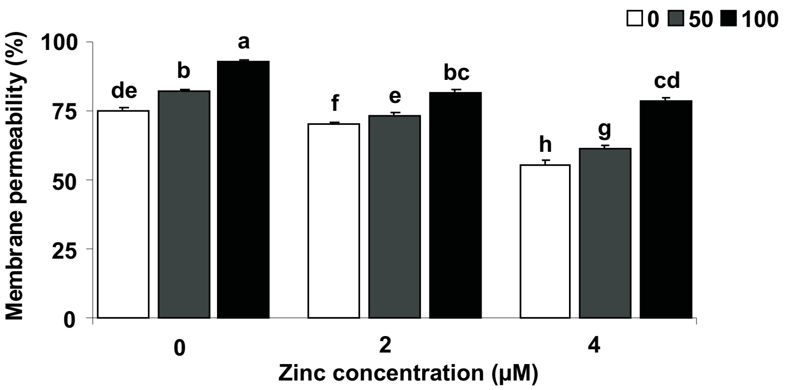


Fig. 2. The effect of salinity (0, 50 and 100 mM NaCl) and zinc concentration (0, 2 and 4 μM Zn as $ZnSO_4$) on membrane permeability (mean SE) of rosmary. Explanations as on Fig. 1.

Shoot concentration of Na and Cl increased with rising salinity levels (Table 2). The cellular membrane dysfunction due to salt stress leads to non-selective uptake of Na and Cl by plants (Kong *et al*., 2005). Shoot Na and Cl concentration was positively correlated to membrane permeability (Fig. 3) indicating that membrane damage resulted in excessive Na and Cl uptake. Plants supplied with adequate Zn, maintained lower concentration of Na and Cl. The results are similar to those reported by Tavallali *et al*. (2009) for pistachio and Aktas *et al*. (2006) for pepper.

At each Zn treatment, increasing salinity level decreased shoot K and Ca concentrations due to Na/K and Na/Ca antagonism (Tuna *et al*., 2008) (Table 2). Increasing Zn level in the nutrient solution had no significant effect on shoot K and Ca concentration of salt-stressed plants.

Salt treatment induced a significant decrease in shoot Mg concentration. Similar results were obtained by Ruiz *et al*. (1997) and Tuna *et al*. (2008) in citrus and maize, respectively. Zinc nutrition at the highest dose (4 μM Zn) resulted in increased shoot Mg concentration.

The shoot P concentration was not affected by salinity. Shoot P accumulation is presumably controlled at the root level and is independent of the salt stress (Kaya *et al*., 2002). Increasing Zn concentration in the nutrient solution had no significant effect on shoot P concentration of rosemary.

CONCLUSIONS

1. The measured decrease in growth induced by salinity suggested that rosemary can be categorized as a relative salt-tolerant species.

2. Zinc nutrition ameliorated adverse effects of salinity stress on the parameters investigated, helped the rosemary to avoid Na and Cl toxicity and improved membrane permeability, leaf relative water content and K, Ca and Mg concentration under saline condition. Greater leaf water content, K, Ca and Mg concentration in plant tissues as much as lower membrane permeability may help in achieving better crop survival under salinity stress.

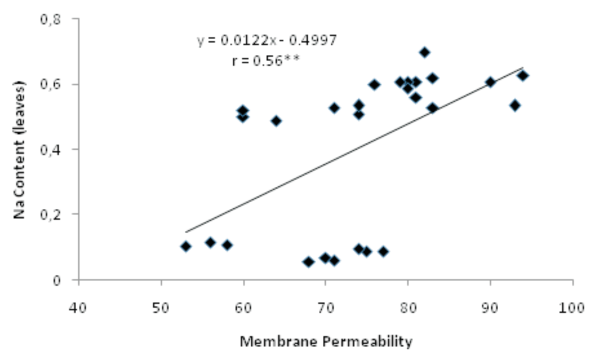
3. Zinc seems to mitigate salt toxicity and thus it increases rosemary tolerance to salinity stress.

T a bl e 2. Concentration of Na, K, Ca, Mg, Cl and P (%) in the leaves of rosemary grown in the nutrient solution with different NaCl and Zn concentration

Zinc levels (μM)	Salinity levels (mM NaCl)	Na	K	Ca	Mg	Cl	P
0	0	0.09± 0.004c	2.65 ± 0.04bc	2.29 ± 0.33c	0.38 ± 0.014b	0.53 ± 0.13d	0.098 ± 0.002bc
	50	0.60± 0.090a	2.41 ± 0.08d	1.36 ± 0.16d	0.34 ± 0.025cd	2.10 ± 0.13bc	0.100 ± 0.002ab
	100	0.60 ± 0.050a	2.41 ± 0.17d	0.87 ± 0.05e	0.30 ± 0.013e	2.39 ± 0.08ab	0.106 ± 0.003ab
2	0	0.06 ± 0.006c	2.83 ± 0.10ab	2.75 ± 0.08b	0.38 ± 0.014b	0.47 ± 0.05d	0.101 ± 0.006ab
	50	0.52 ± 0.013b	2.43 ± 0.05d	1.33 ± 0.14d	0.33 ± 0.007d	1.97 ± 0.10c	0.103 ± 0.004ab
	100	0.61 ± 0.008a	2.45 ± 0.10d	0.97 ± 0.04e	0.34 ± 0.013cd	2.41 ± 0.05a	0.104 ± 0.002ab
4	0	0.11 ± 0.006c	2.91 ± 0.02a	3.17 ± 0.10a	0.40 ± 0.019a	0.47 ± 0.08d	0.87 ± 0.015c
	50	0.50 ± 0.016b	2.55 ± 0.21cd	1.44 ± 0.05d	0.36 ± 0.007bc	1.87 ± 0.06c	0.110 ± 0.013ab
	100	0.60 ± 0.100a	2.52 ± 0.02cd	1.03 ± 0.04e	0.37 ± 0.007b	2.06 ± 0.05c	0.112 ± 0.008a

Explanations as in Table 1.

a



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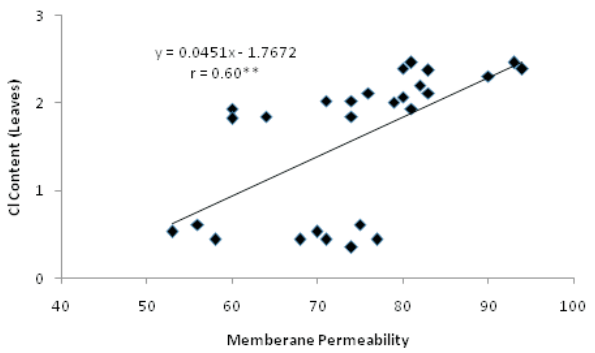

Fig. 3. Relationship between membrane permeability and leaf: a – Na, b – Cl contents of rosemary (n=36).

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