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Effects of zinc and salinity on growth and anatomical structure of *Carthamus tinctorius* L.

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Abstract

Changes in growth and anatomical structure of vascular tissues in stem, root and leaf of safflower plants grown in NaCl and $CaCl_2$ solutions having different osmotic potentials (Ψ_S from 0 to -0.9 MPa) with addition of 0, 10 and 20 mg dm^{-3} zinc were studied. Shoot and root lengths, fresh and dry masses and fresh/dry mass ratio were lower in salt-stressed plants compared to unstressed plants. Salinity induced structural changes in stem, root and leaf tissues; few xylem vessels with smaller size were noticed in stressed plants. The higher concentration of Zn improved growth especially in roots and enhanced xylem formation in comparison to stressed plants grown at the same osmotic potential without Zn. Zn also protected xylem distructure by salinity in leaves.

Additional key words: calcium chloride, safflower, sodium chloride.

Introduction

Soil salinity adversely affects the growth of plants and yield. Also, morphology, anatomy, ultrastructure and metabolism of plants as well as their mineral, water and saccharide contents are deeply affected by saline stress.

High concentrations of Zn inhibit cell division in the roots (Powell *et al.* 1986a,b, Davies *et al.* 1991) and cell elongation (Wainwrigth and Woolhouse 1977, Godbold *et al.* 1983) and causes chlorosis of young leaves (Marschner 1986). In contrast, Zn deficiency decreases auxin concentration and in consequence the growth rate (Mengel and Kirby 1982). The permeability of the plasma membrane is increased in Zn-deficient plants, resulting in increasing the root cell leakage of K, NO_3 and organic compounds (Cakmak and Marschner 1988). The effects of Zn on the anatomical structure of plants are not known. The aim of the present work was to study the response of safflower plants grown under saline conditions to Zn additions. The growth and anatomical characteristics of safflower plants grown in solutions having different osmotic potentials in the presence or absence of Zn were studied.

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Materials and methods

Safflower (*Carthamus tinctorius* L.) plants were grown under natural conditions in plastic pots (0.5 dm^3, 5 plants per pot) filled with aerated nutrient solutions according to Down and Hellmer (1975). Minimum and maximum air temperature and relative humidity were 13 and 28 °C and 38 and 71 %, respectively. After 2 weeks the nutrient solution was replaced by solutions containing NaCl and $CaCl_2$ in concentrations having osmotic potentials 0, -0.3, -0.6 and -0.9 MPa (prepared according to Lagerwerff and Holland 1960 and Lagerwerff and Eagle 1961) and amended with 0, 10, and 20 mg dm^{-3} Zn (as $ZnSO_4$). The plants were kept under treatments for 3 weeks.

For anatomical examination, root and stem segments were taken 1 - 1.5 cm above the root cap and hypocotyl, respectively. Leaf segments were taken from the youngest one. The segments were fixed in 70 % ethanol for 48 h, then dehydrated in a graded ethanol series and cleared in xylol. The segments were embedded in paraffin wax and sectioned at 5 μm on *Leitz 1512* microtome. Prior to light microscope examination, the sections were deparaffinized thrice in xylol for 15 min followed by absolute ethanol for 5 min. The sectioned materials were double stained with safranin and light green (Sass 1951) and were photographed on *Kodak TMAX 100* film. The stem and root diameters, thickness of cortex, vascular tissues and pith, and diameters of vessels were determined by ocular micrometer using a light microscope.

For growth response determination, the plants were removed carefully from the pots and roots were washed thoroughly. Shoot and root lengths and fresh mass were measured, then the plant materials were dried at 70 °C to constant mass.

Results

Growth: In *Carthamus* plants without Zn or in the presence of low concentration of Zn (10 mg dm^{-3}), root fresh and dry masses as well as fresh/dry mass ratio progressively decreased by increasing salinity (-0.3 MPa was an exception). The decrease in fresh/dry mass ratio was more pronounced in roots than in shoots. However, roots of salinity-stressed plants (ψ_S = -0.3 to -0.9 MPa) receiving high Zn doses (20 mg dm^{-3}) exhibited higher growth rate and produced more biomass than the unstressed plants. The same held true for the shoot growth over ψ_S range from -0.6 to -0.9 MPa. Zn addition to unstressed and stressed plants decreased fresh/dry mass ratio in the shoots but not in roots (Table 1).

In the presence or absence of Zn, shoot length was negatively affected by salinity. Salinity effect was concentration dependent. Addition of Zn reduced growth, but simultaneously reduced growth retardation by salinity. In case of root length, reduction by salinity was initiated at higher stress (-0.9 MPa) but lower stress (ψ_S = -0.3 and -0.6 MPa) enhanced root length. Addition of 20 mg dm^{-3} Zn improved root growth of moderately and highly stressed plants (-0.6 and -0.9 MPa) over that of the unstressed plants (Table 1).

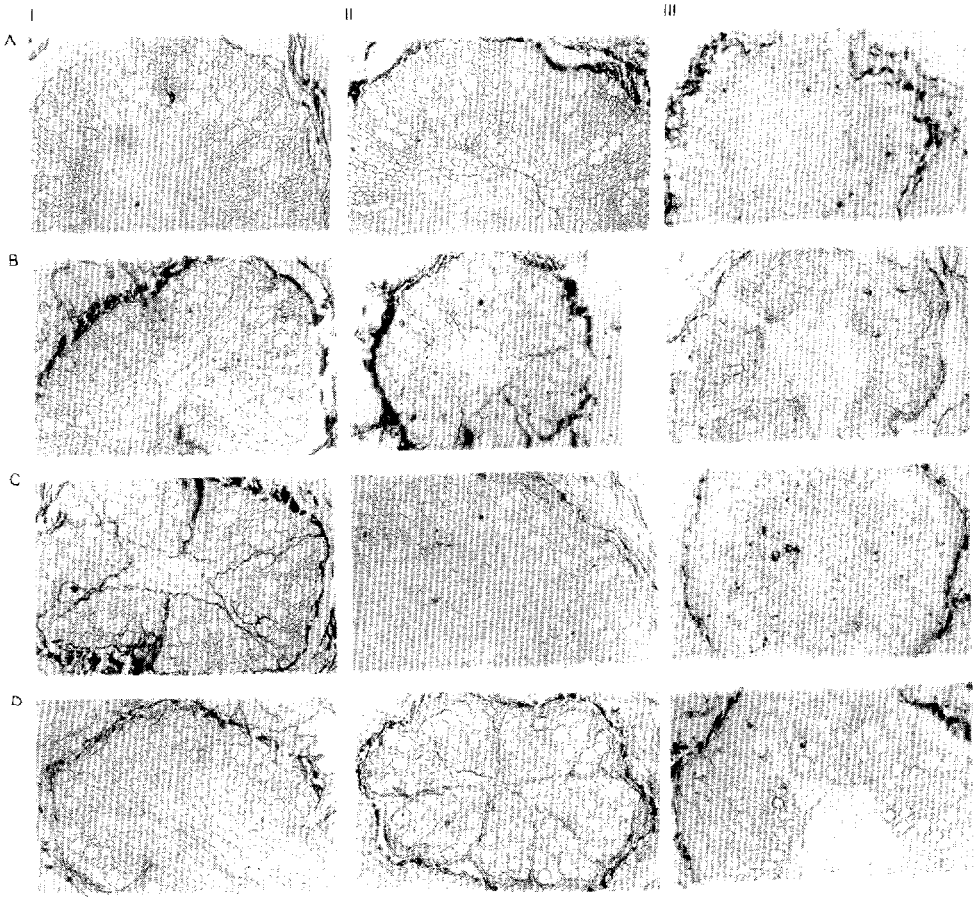

Fig. 1. Cross-sections through stems of safflower plants (showing the vascular cylinder) growing in saline solutions having different osmotic potentials in MPa: 0 (*A*), -0.3 (*B*), -0.6 (*C*), -0.9 (*D*); in the absence (I) and presence of 10 (II) and 20 mg dm^{-3} Zn (III).

Anatomical structure: Salinity and Zn treatments induced structural changes in stem, root and leaf of safflower plants (Fig. 1-3).

In stem the vascular cylinder diameter as well as diameter of vessels in the control plants were much larger than in those treated with Zn and salinity. The effects was concentration dependent (when the two treatments used independently). However, the individual effect of salinity could be modify or reverse in the presence of Zn. For example, while salinity at ψ_S = -0.9 MPa reduced diameter of vessels (Table 2) to

Table 1. Influence of decreased osmotic potential due to salinity stress, zinc and their combination on growth parameters of safflower plants.

Treatment Zn [mg dm^{-3}]	ψ_S [-MPa]	Length [cm] shoot	root	Fresh mass [g] shoot	root	Dry mass [g] shoot	root	f.m./d.m. shoot	root
0	0.0	27.3	31.0	20.00	12.7	1.32	0.46	15.15	27.61
	0.3	23.3	40.3	12.20	13.2	1.10	0.56	11.09	23.57
	0.6	19.3	35.0	8.00	9.5	0.87	0.20	8.99	4.15
	0.9	15.0	28.0	6.80	8.3	0.77	0.20	8.79	4.15
10	0.0	17.0	23.0	6.10	10.6	0.78	0.42	7.82	25.24
	0.3	12.8	35.0	6.80	8.8	0.58	0.40	11.72	22.00
	0.6	15.3	22.0	3.80	9.3	0.64	0.28	5.94	33.21
	0.9	10.3	21.0	2.70	8.3	0.52	0.25	5.19	33.20
20	0.0	11.5	26.0	2.00	8.3	0.50	0.29	4.00	28.62
	0.3	7.0	22.0	2.00	9.1	0.30	0.30	6.67	30.33
	0.6	10.5	31.0	2.38	8.4	0.52	0.42	4.76	20.00
	0.9	7.8	29.0	2.15	10.6	0.68	0.31	3.16	34.19

Table 2. Relative thickness of cortex, vascular tissues (VT) and pith [% of sections diameter] and diameters of vessels (VD) (means [μm] ± SE) in stems and roots of safflower plants as affected by different levels of salinity stress and Zn treatment.

Treatment Zn [mg dm^{-3}]	ψ_S [-MPa]	Stem cortex	VT	pith	VD	Root cortex	VT	VD
0	0.0	31	43	26	58.8±5.03	21	75	51.5±5.69
	0.3	33	38	28	32.6±2.78	41	58	35.3±5.64
	0.6	33	35	29	27.9±3.30	39	57	35.3±2.82
	0.9	30	26	44	24.9±3.26	56	34	37.4±2.82
10	0.0	18	46	35	38.7±2.68	22	75	41.1±3.30
	0.3	38	44	18	28.8±2.78	47	52	29.4±2.65
	0.6	21	48	30	36.7±4.60	41	53	36.8±3.30
	0.9	32	49	18	30.0±2.98	53	46	32.6±3.53
20	0.0	28	37	31	35.3±2.96	45	52	42.6±3.20
	0.3	27	41	31	34.6±3.28	47	36	35.7±2.76
	0.6	35	35	28	38.7±3.21	32	64	33.1±1.22
	0.9	26	48	21	39.6±3.00	29	69	32.4±2.30

42 % in the absence of Zn, the same osmotic potential, reduced diameter of vessels to 77 % of the unstressed plants. On the contrary, combination of salinity at higher osmotic potential (-0.9 MPa) with higher Zn concentration (20 mg dm^{-3}) resulted in larger vascular cylinder and vessels diameters than plants stressed at the same potentials without Zn.

The relative thickness (RT) of vascular tissues (Table 2) progressively decreased with increasing concentration of NaCl and $CaCl_2$ in the solutions. The reverse held true in the case of pith. The RT of cortex was negligibly affected by imposed salt

stress. Addition of Zn was associated with decrease in cortex width (treatment of -0.3 MPa with 10 mg dm^3 Zn was an exception). On the other hand, low Zn concentration improved vascular tissues formation and increased the RT of vascular tissues in unstressed and stressed plants as well. Similar enhancement effects and increase in RT of vascular tissues were noticed with high Zn concentration (20 mg dm^{-3}) at high stress level (-0.9 MPa). The RT of pith were lower in stressed plants treated with Zn than those Zn untreated analogues.

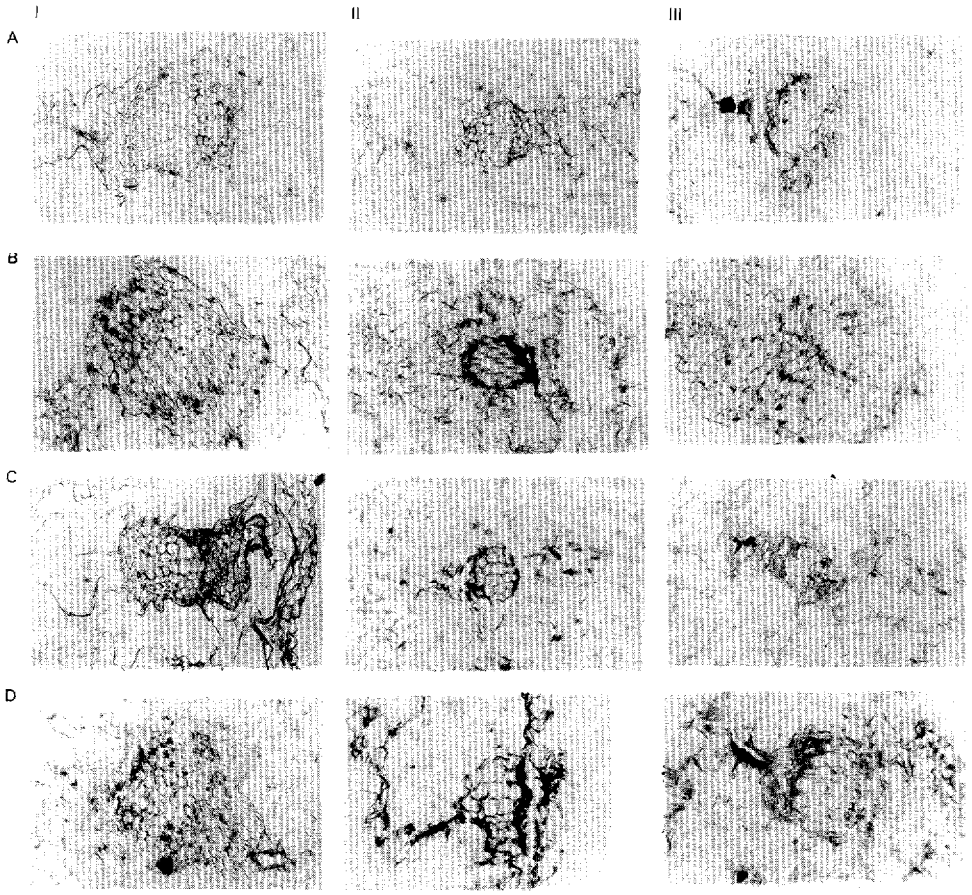

Fig. 2. Cross-sections through leaves of safflower plants (showing the vascular cylinder) growing in saline solutions having different osmotic potentials in MPa: 0 (*A*), -0.3 (*B*), -0.6 (*C*), -0.9 (*D*); in the absence (I) and presence of 10 (II) and 20 mg dm^{-3} Zn (III).

In leaves (Fig. 2) treatment with salinity was associated with disorganization of the vascular cylinder, where small xylem groups with small vessels diameters were

observed in salt stressed plants. Similar response to salinity was reported in the presence of 10 mg dm^{-3} Zn where the distructural effect of salinity on vascular tissues was more pronounced. On the other hand, 20 mg dm^{-3} Zn improved vascular cylinder formation and prevented the distructural effect of salinity. Leaves of plants receiving 20 mg dm^{-3} Zn had large number of xylem vessels with large diameters than those not treated with Zn or received lower concentrations.

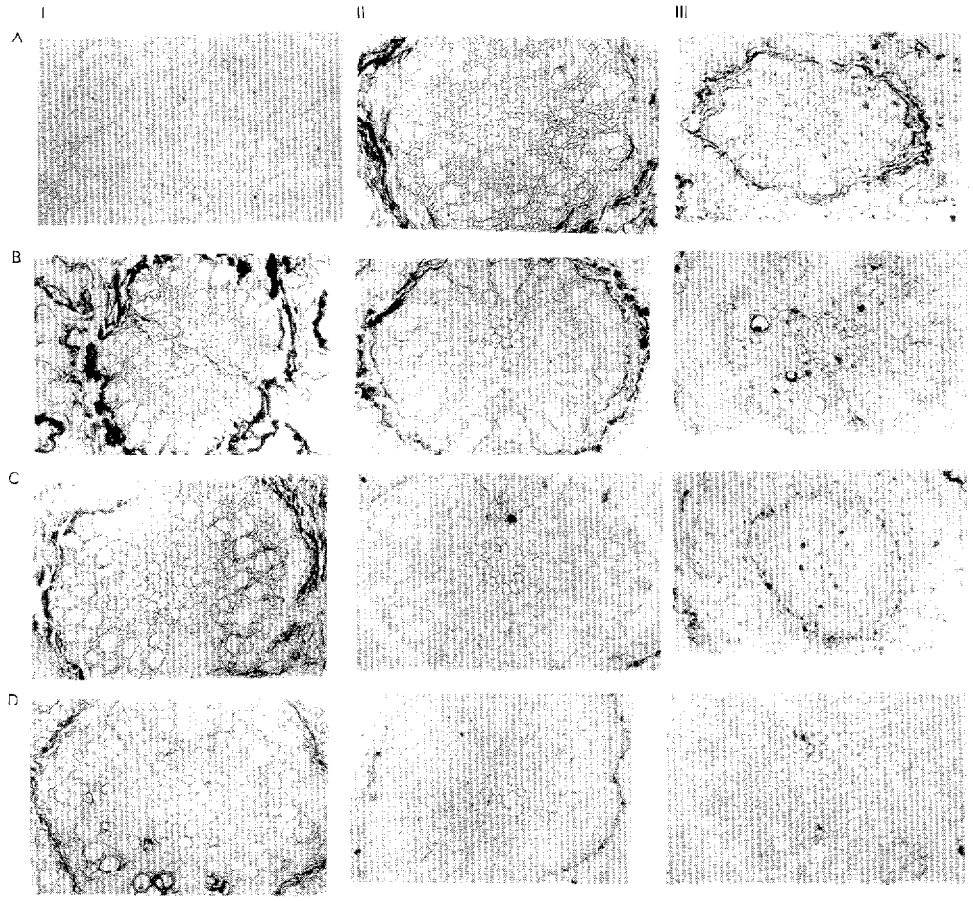

Fig. 3. Cross-sections through roots of safflower plants (showing the vascular cylinder) growing in saline solutions having different osmotic potentials in MPa: 0 (*A*), -0.3 (*B*), -0.6 (*C*), -0.9 (*D*); in the absence (I) and presence of 10 (II) and 20 mg dm^{-3} Zn (III).

Similarly, salinity treatment reduced diameters of root vascular cylinder as well as vessels diameters. The magnitude of salinity effect was modified by Zn addition. For example; in the absence of Zn the diameters of vessels (Table 2) were progressively decreased on salinization (the smallest diameters was noticed at ψ_S = -0.9 MPa). In

the presence of 10 mg dm^{-3} Zn, the smallest diameter in xylem vessels was noticed at ψ_S = -0.3 MPa.

Salinity strongly reduced the relative thickness of vascular tissues in the root (49 % of the unstressed plants at -0.9 MPa). Thickness of cortex was progressively increased by salinization. Lower Zn concentration increased cortex thickness over ψ_S range from 0 to -0.6 MPa. Higher Zn doses enhanced cortex thickness at ψ_S 0 or -0.3 MPa stressed plants and decreased the thickness at ψ_S -0.6 or -0.9 MPa. Zinc addition, especially at higher concentrations, reduced the RT of vascular tissues in unstressed plants and increased the thickness at moderately and highly stressed plants.

Discussion

The results of this work show that in the absence of Zn, salinity had inhibitory effect on the shoot growth (fresh and dry mass, length) of safflower plants. On the contrary, root growth of plants stressed at -0.3 MPa was higher than that of the unstressed plants. Addition of Zn reduced plant growth, however, the salinity effects were alleviated by the presence of high Zn concentration. Thus stressed plants (ψ_S = -0.3 MPa) treated with 20 mg dm^{-3} Zn solution produced more root fresh and dry mass compared to their unstressed analogous. The same held true for shoot growth over -0.6 MPa.

Safflower plants shows different degree of anatomical disturbance in roots, stems and leaves when growing in the presence of increasing concentrations of NaCl and $CaCl_2$. Similar anatomical disturbance have been reported (Hajibagheri *et al.* 1985, Saadeddin and Doddema 1986, Solomon *et al.* 1986, Winter 1988, Huang and Van Steveninck 1990, Serrato Valenti *et al.* 1991, 1992). Generally, plants grown in saline solutions showed smaller vascular tissues and vessels diameter than the unstressed plants. At ψ_S -0.9 MPa disorganization of the vascular cylinder was evident. The relative thickness of vascular tissues was progressively decreased, while that of pith was increased on salinization. This means that salinity reduces vascular tissues while improved the formation of parenchyma cells. However, cortex thickness was not affected by salinity, this means that structural changes occurs only in vascular cylinder. The above findings leads to suggest that the anatomical features in roots, stems and leaves are differentially affected by salinity.

Zinc addition (at lower concentrations) enhanced the anatomical disturbance due to salinity at lower stress levels. On the contrary, higher Zn concentration in combination with saline solution especially at lower water potentials improved xylem formation or protect the xylem reduction against salinity effects. Also, higher Zn concentration had an important role in increasing the absorbing surface through enhancement root elongation.

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