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Physiological Consequences of Structural and Ultra-structural Changes Induced by Zn Stress in *Phaseolus vulgaris*. I. Growth and Photosynthetic Apparatus

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Abstract: Seeds of *Phaseolus vulgaris* L. cv. Limburgs vroege were sown in peat moss supplemented with ZnSO₄ (600 mg kg⁻¹) and irrigated with distilled water for 14 days. Ten growth criteria and 12 anatomical measurements in the shoot (hypocotyl, epicotyl and primary leaves) were reduced by ZnSO₄ but remained unaffected in the root, despite greater accumulation of Zn²⁺ in roots. Shoots and roots of *P. vulgaris* seedlings seemed to show differential sensitivity to Zn stress. Reduction in shoot growth criteria seemed to result from a decrease in parenchyma cell size and diameters of metaxylem vessels in the leaf midrib. Scanning Electron Microscope (SEM) revealed the presence of compacted grana with reduced thylakoids in chloroplasts, which might have contributed to the recorded loss of chl-a, chl-b and carotenoids. In primary leaves, occlusion of intercellular spaces and impregnation of middle lamellae with Zn seemed to hamper substomatal CO₂ diffusion, while increased K⁺-leakage indicated Zn-induced injury to cell membranes. The Zn-induced 6-fold increase in stomatal deformation, reduction in frequency of normal stomata, photosynthetic pigments and substomatal CO₂ concentration in primary leaves, coupled with reductions in cell enlargement, transport capacity of xylem in the shoot, might have resulted collectively in the measured reduction in the photosynthetic rate which would in turn lead to the observed reductions in shoot growth criteria.

Key words: Cell enlargement, *Phaseolus vulgaris*, photosynthetic rate, seedling growth, stomata, ultra-structure, ZnSO₄

INTRODUCTION

Zn is an essential micronutrient for plants but its excessive concentrations in the growth medium are known to induce some morphological and structural changes in plants (Khudsar *et al.*, 2004; Yang *et al.*, 2004; Bertrand and Poirier, 2005). Photosynthesis and transpiration in *Phaseolus vulgaris* were reduced by high concentrations of zinc (Van Assche *et al.*, 1979) and zinc toxicity inhibited chlorophyll formation in young leaves of tomato (Kaya *et al.*, 2000). Other effects of Zn stress on the photosynthetic apparatus include the reduction in net photosynthetic rate, stomatal conductance, substomatal CO₂ concentration and contents of photosynthetic pigments (Khudsar *et al.*, 2004; Van Assche and Clijsters, 1983). Attempts to clarify the relationship between Zn-induced structural and functional alterations in plants are rare (Khudsar *et al.*, 2004).

This study aims to elucidate a possible relationship between the Zn-induced structural and ultra-structural changes in *Phaseolus vulgaris* cv. Limburgs vroege and some of the processes associated with the photosynthetic apparatus, together with their net impact on seedling growth.

MATERIALS AND METHODS

Seeds of *P. vulgaris* cv. Limburgs vroege, supplied by Environmental Biology Centre for Environmental Sciences, Hasselt University, Belgium, were sown in peat moss either with or without a supplement of ZnSO₄ (600 mg kg⁻¹), irrigated with distilled water for 14 days. The experiment was carried out in a growth chamber at 22°C±2 and 62-65% relative humidity and illuminated daily for 16 h with white fluorescent lamps at a photon flux density of 160 μmol m⁻² s⁻¹. The growth criteria recorded in ten replicate plants were: (i) Length of epicotyl, hypocotyl and root, (ii) Fresh and dry weights of stem, primary leaf and root and (iii) Area of primary leaf. Frequencies (number mm⁻²) of normal and anomalous (non-functional) stomata were determined in 20 peels prepared from the central part of the abaxial epidermis of the 20 primary leaves of ten replicate plants.

Cross sections (18-20 μm thick) of homologous parts of blades and petioles of primary leaves, epicotyls, upper and lower parts of hypocotyls and roots of four replicate specimens of each of the control and the Zn-treated seedlings were prepared by embedding in paraffin wax and double staining in safranin and light green. Sections

were cut in the middle parts of leaf midrib, leaf petiole and epicotyl. In the hypocotyl, sections were cut in the upper part at one cm below the cotyledons and in the lower part at two cm above the transition zone between shoot and root. Root sections were cut immediately above the zone of lateral roots. Mean cell areas (in μm^2) of parenchyma in leaf midrib and petiole, pith of epicotyl, cortex and pith of the upper and lower parts of hypocotyl and root cortex were measured by counting the number of cells in accurately specified parts of the cross sections. The number of replicate measurements for each of the eight parenchyma tissues was 16 (four measurements \times four replicate seedlings). To test the accuracy of this method, photos of cross sections were scanned into the computer and mean cell areas were measured directly on the monitor from other randomly selected areas of the eight parenchyma tissues using the Adobe Photoshop v. 7.0 ME program for Windows. Agreement between the results of both methods confirmed their accuracy. Mean diameters (in μm) of the widest five metaxylem vessels in leaf midribs and roots of the four replicate specimens were also measured. Ultra thin sections (60 \AA) were prepared in the same parts of leaf midribs and roots and examined at 80 kvolt by the transmission electron microscope (Jeol 100 CX, Japan) of the Electron Microscopy Unit, Alexandria University.

The pairs of opposite primary leaves of three replicate plants were used for determination of the concentration of photosynthetic pigments (chlorophyll-a, chlorophyll-b and carotenoids) as mg g^{-1} dry weight (Metzner *et al.*, 1965). Net photosynthetic rate, stomatal conductance and substomatal CO_2 concentration were measured using gas-exchange system LI-COR 6200 (Lincoln, USA).

Total Zn in primary leaves, stems and roots of three replicate plants was measured (Cottenie *et al.*, 1982). K^+ leakage was measured to determine membrane permeability (Weckx and Clijsters, 1997) and potassium content of the incubation medium was analyzed using an atomic absorption spectrophotometer (Perkin Elmer 703, Norwalk, CT, USA). Zn concentration, growth conditions and the measured parameters were established on the basis of a preliminary experiment carried out at Environmental Biology Centre for Environmental Sciences, Hasselt University, Belgium.

The results were subjected to statistical analysis using one-way ANOVA to determine the significance of treatment differences using SPSS v. 10 for Windows. Comparison of the main effects was performed using the Least Significant Difference (LSD) from the control.

RESULTS

Figure 1 shows that the length, fresh and dry weights of the stem (epicotyl+hypocotyl) and primary leaves and the area of primary leaves were significantly reduced by ZnSO_4 , while the growth criteria of the root remained unaffected. Significant reductions were recorded in the mean cell area of seven parenchyma tissues of the shoot (leaf midrib, leaf petiole, pith of epicotyl, cortex and pith of the upper and lower parts of the hypocotyl) and in the metaxylem vessel diameter of leaf midrib in response to ZnSO_4 (Fig. 2). In contrast, no significant differences were recorded in the mean cell area of cortical parenchyma and metaxylem vessel diameter between Zn-treated and untreated roots (Fig. 2).

Figure 3 shows cases of stomatal deformation with and without Zn treatment. These included stomata with a single guard cell, with arrested growth, with two unequal guard cells and contiguous stomata. While ZnSO_4 caused a significant reduction in the frequency of fully developed (predominantly paracytic) stomata, it resulted in a 6-fold increase in the frequency of the anomalous types (Fig. 3).

Figure 4 shows accumulation of electron dense Zn deposits in intercellular spaces between all cell types of shoot and root and on the inner surface of parenchyma and xylem vessels while no such deposits were found in tissues of the control seedlings. Zn deposits also extended to impregnate the middle lamellae adjoining these cells. Although no electron-dense deposits were found in chloroplasts, the grana of some internal membranes were clearly compacted and their thylakoids were reduced in primary leaves of the Zn-treated seedlings.

Although Zn was almost equally distributed in the leaves, stems and roots of the control seedlings, Zn content in the Zn-treated primary leaves, stem and root was about 9, 11 and 35 folds that of the control values, respectively (Fig. 5).

ZnSO_4 caused significant reduction in the content of photosynthetic pigments in primary leaves (Fig. 5). The amounts of chlorophyll-a, chlorophyll-b and carotenoids were decreased by about 58.5, 41 and 39% of control values, respectively. Chl-a/chl-b ratio was reduced by about 70% of the control value. Net photosynthetic rate, stomatal conductance and substomatal CO_2 concentration were significantly decreased with Zn treatment by 28, 40 and 12%, respectively relative to the control values (Fig. 5). The release of K^+ through cytoplasmic membranes was also significantly stimulated with Zn treatment in the 14-day-old *P. vulgaris* seedlings (Fig. 5).

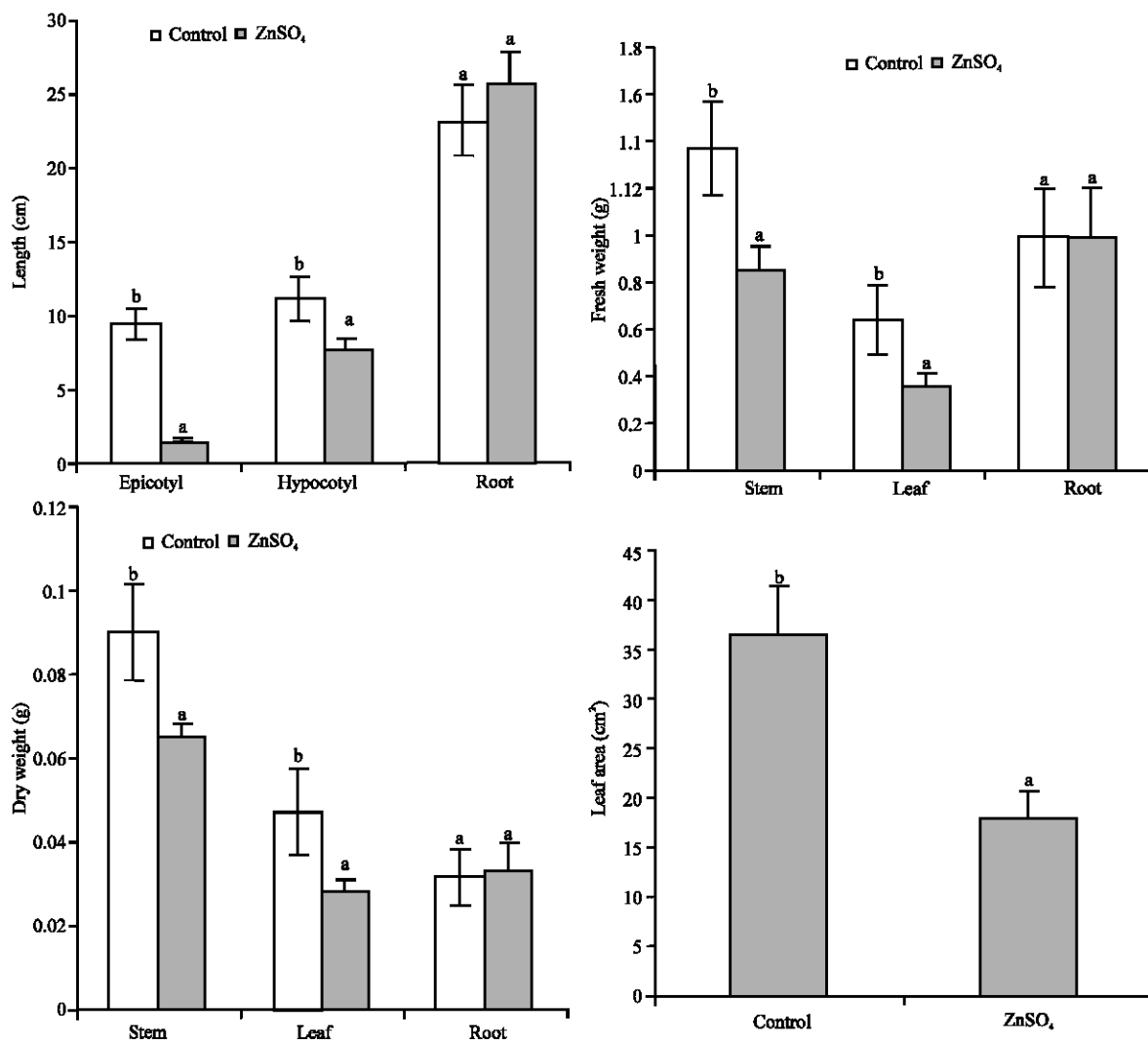


Fig. 1: Effect of ZnSO₄ (600 mg kg⁻¹ peat moss soil) on the length, fresh weight, dry weight of the stem (epicotyl + hypocotyl), primary leaves and root and leaf area of 14-day-old seedlings of *Phaseolus vulgaris*. Value of each bar represents mean±SD; Similar letters on tops of corresponding bars indicate statistically non-significant differences at p≤0.01, based on F-test and LSD, n = 10

DISCUSSION

Roots and shoots of *P. vulgaris* L. cv. Limburgs vroege responded differently to the level of Zn applied in the present experiment. While growth parameters of the root seemed unaffected or slightly enhanced, those of the shoot were significantly reduced (Fig. 1 and Table 1), although Zn accumulation decreased gradually from the basal to the apical parts of seedlings (Fig. 5B and Table 1). Highest reductions were observed in stem length and primary leaf area. Similar trends were reported in the responses of shoots and roots of *Cyperus difformis*, *Chenopodium ambrosoides* and *Digitaria sanguinalis* to stresses caused by Cd, Ni and Pb (Ewais, 1997).

Table 1: The percentage change [(Zn treatment-control/control)×100] in Zn content and growth criteria of 14 day old *Phaseolus vulgaris* seedlings treated with 600 mg ZnSO₄ kg⁻¹ of peat moss soil

Parameters	Percentage change		
	Primary leaf	Stem	Root
Zn content	88.90	90.73	97.10
Length	-	-56.00	10.75
Area	-50.23	-	-
Fresh weight	-44.08	-38.90	-4.97
Dry weight	-40.29	-27.70	3.93
Water content	-44.38	-38.80	-6.49

In the present work, differences in morphological aspects of shoots and roots of *P. vulgaris* were assumed to result mainly from the effect of Zn on cell expansion. While the size of parenchyma cells in seven shoot tissues

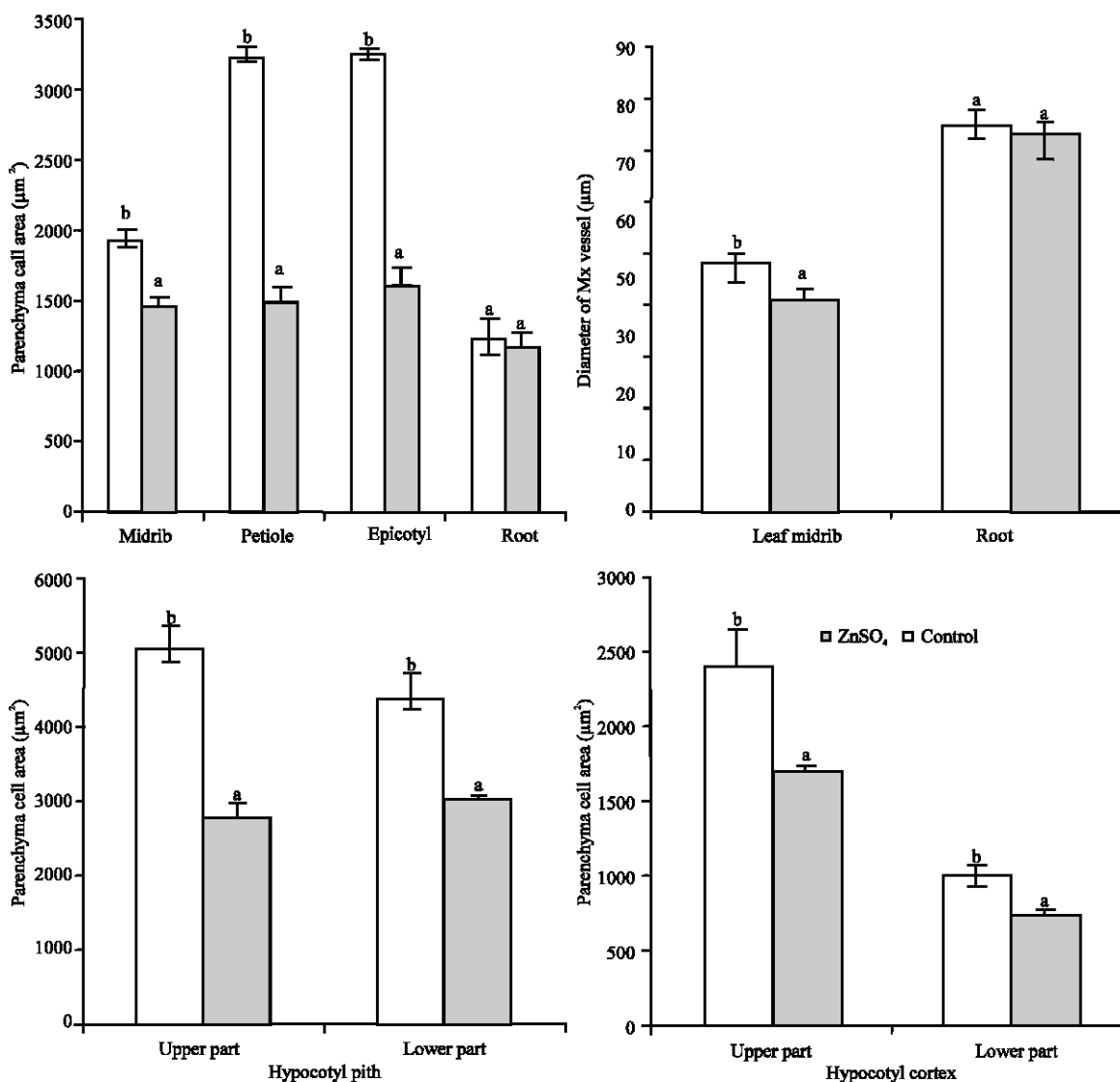


Fig. 2: Effect of ZnSO₄ (600 mg kg⁻¹ peat moss soil) on cell enlargement expressed as parenchyma cell area and diameter of metaxylem (Mx) vessels in 14-day-old seedlings of *Phaseolus vulgaris*. Value of each bar represents mean±SD. Similar letters on tops of corresponding bars indicate statistically non-significant differences at p<0.01, based on F-test and LSD, n = 4

and the mean diameter of metaxylem vessels of leaf midrib were significantly reduced in the Zn-treated plants, the root parenchyma and metaxylem vessels remained almost unaffected (Fig. 2). Shoots and roots of *P. vulgaris* seedlings seem to exhibit differential sensitivity to Zn stress. Similar reductions in cell enlargement were induced by Zn in *Festuca rubra* (Davies *et al.*, 1991) and by Cu and Cd in *Vicia faba* (Kasim, 2005) and in *Sorghum bicolor* (Kasim, 2006).

The diameter of metaxylem vessels is one of the factors affecting their capacity as translocation conduits (Marschner, 1995; Poschenrieder and Barceló, 1999).

Results of the present work demonstrated that Zn treatment led to a significant reduction in the diameter of metaxylem vessels in midribs of primary leaves and this reduction was further exacerbated by the accumulation of electron-dense Zn deposits on their inner surface (Fig. 4). Width of metaxylem vessels in roots was not significantly changed with Zn (Fig. 2). Upward movement of water and minerals from root to the primary leaves might be progressively slowed down. The apoplastic radial hydraulic movement (short distance transport) would also be hampered by the electron-dense Zn deposits completely occluding intercellular spaces and

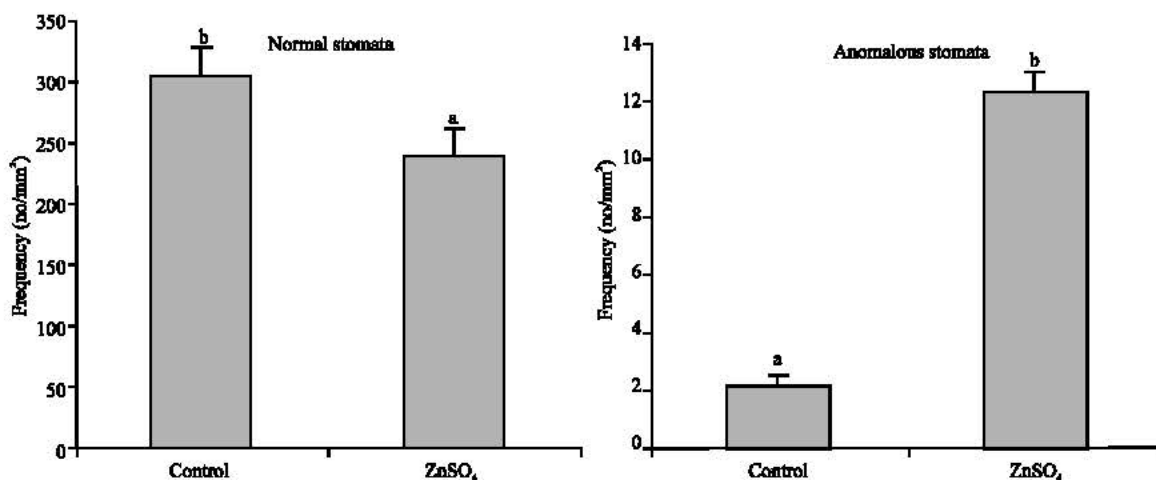


Fig. 3: Effect of ZnSO₄ (600 mg kg⁻¹ of peat moss soil) on the frequencies of normal and anomalous stomata in the primary leaves of 14-day-old seedlings of *Phaseolus vulgaris*. Value of each bar represents mean±SD. Different letters on tops of corresponding bars represent statistically significant differences at p≤0.01, n = 20

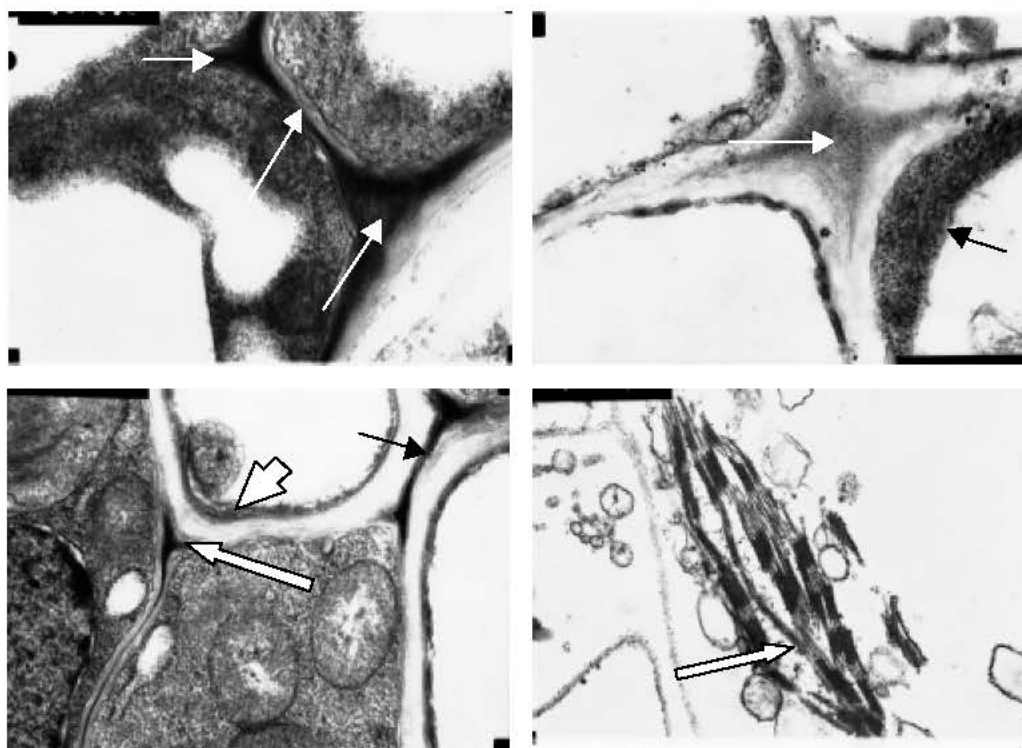


Fig. 4: TEM images of Zn deposits in and between cells of 14-day-old *Phaseolus vulgaris* seedlings treated with 600 mg ZnSO₄ kg⁻¹ of peat moss soil; Upper left: Zn deposits in triangular intercellular spaces and middle lamella between root parenchyma (20,000 x), Upper right: Zn deposits in quadrangular intercellular space (white arrow) between root parenchyma and on the inner surface (black arrow) of cell walls (30,000 x); Lower left: Zn deposits in triangular intercellular spaces (long block arrow), middle lamella (thin black arrow) and on the inner surface of metaxylem vessels (short block arrow) of primary leaves (25,000 x). Lower right: inner membrane (arrowed) of chloroplast with compacted grana (13,000 x)

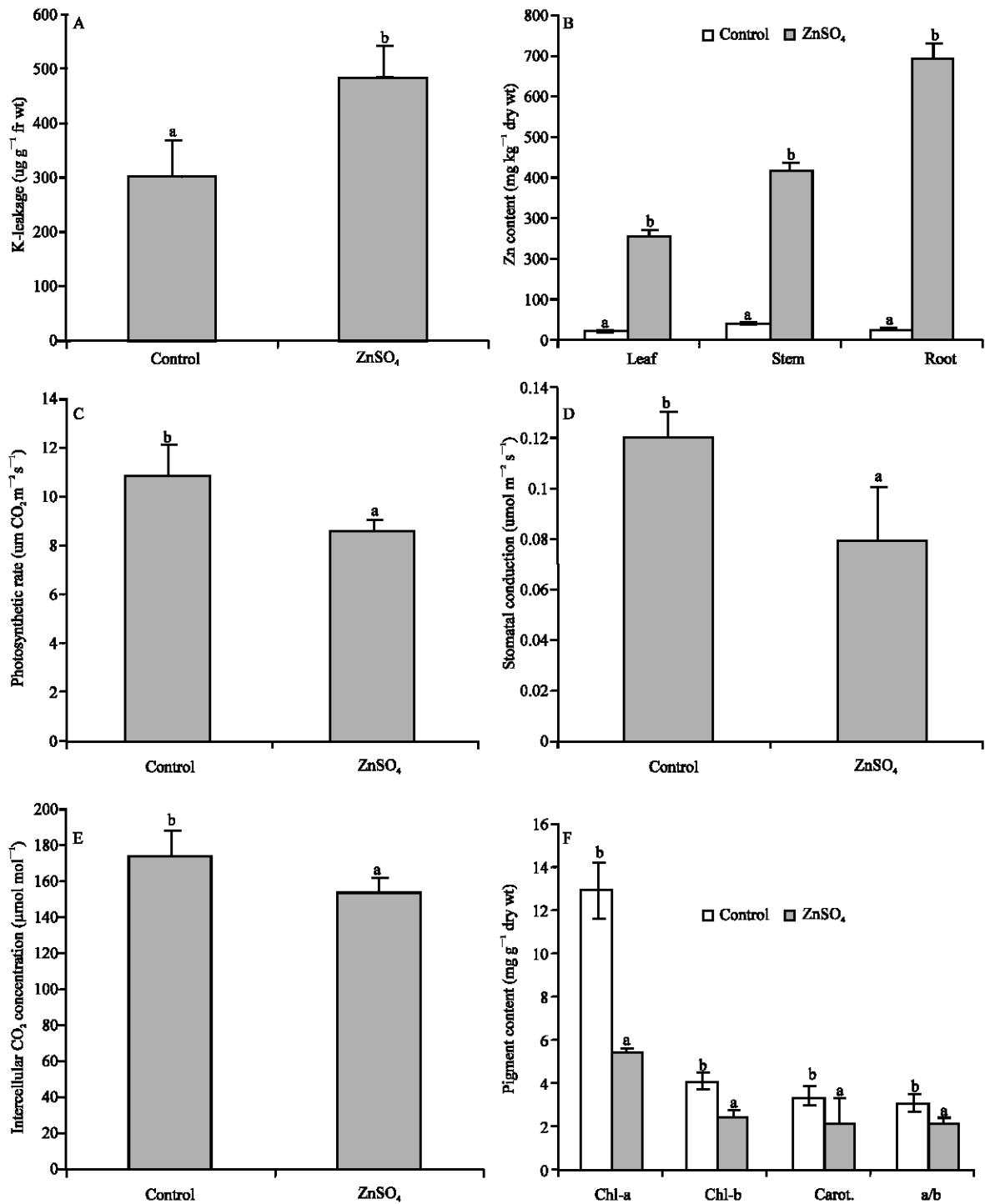


Fig. 5: K-leakage, zinc content, photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and photosynthetic pigments (chl-a, chl-b, carotenoides, chl-a/chl-b ratio) in primary leaves of 14-day-old seedlings of *Phaseolus vulgaris* treated with 600 mg ZnSO₄ kg⁻¹ of peat moss soil. Value of each bar represents mean±SD. Different letters on tops of corresponding bars indicate statistically significant differences at p≤0.01 for (A), (B), (E) and (F) and at p≤0.05 for [C] and [D], based on F-test and LSD, n = 4; for [E] n = 3

impregnating middle lamellae (Fig. 4). Greger and Johansson, (1992) showed that reduction in short distance transport was accompanied with reduction in water loss via the transpiration stream in sugar beet in response to Cd accumulation. In the present study, the required reduction of water loss in *P. vulgaris* seems to be achieved by the Zn-induced decrease in the frequency of normal stomata, the 6-fold increase in the frequency of anomalous (non-functional) stomata and the decrease in surface area of primary leaves which collectively reduced the total number of stomata/leaf. The recorded Zn-induced reduction in leaf area might in turn result in diminished cuticular transpiration which, according to Zyalalov (2004), accounts for 15-25% of total foliar transpiration. Mediavilla *et al.* (2002) and Monteiro and Prado (2006) in their research on oaks and cerrado species, respectively, reported that the increase in deformed (non-functional) stomata might impinge on the rate of photosynthetic electron transport.

The observed Zn-induced increase in K⁺ leakage might be considered as a measure of the injury of cytoplasmic membranes in *P. vulgaris* primary leaves, similar to that caused by Cu stress (Weckx and Clijsters, 1997). Although no Zn deposits were observed in chloroplasts, compaction of grana (leading to narrower thylakoids) was clearly evident (Fig. 4) and seems to have contributed to the recorded decrease in their pigment content (Fig. 5), thus sharing in diminishing the photosynthetic capacity of the Zn-treated seedlings. A similar ultra-structural distortion of chloroplasts was induced by cadmium and resulted in severe reductions in photosynthetic pigments and activity in barley (Vassilev *et al.*, 1995) and *Elodea Canadensis* (Stoyanova and Tchakalova, 1999).

Net photosynthetic rate, stomatal conductance and substomatal CO₂ concentration were reduced significantly with Zn treatment (Fig. 5). Similar results were recorded in *P. vulgaris* (Van Assche *et al.*, 1979, 1980), *Bacopa monniera* exposed to Cd stress (Ali *et al.*, 2000), *Artemisia annua* (Khudsar *et al.* 2004), *Vicia faba* (Kasim, 2005) and *Sorghum bicolor* (Kasim 2001, 2006), where reduction could be attributed to disturbance in chlorophyll biosynthesis and electron transport.

Zn was found to inhibit electron transport (Baker *et al.*, 1982) and possibly to act at the oxidizing site of photosystem II (Miller and Cox, 1983). Baker *et al.* (1982) proposed a site for zinc action between photosystems II and I in the electron transport chain. The photosynthetic electron transport and Rubisco carboxylation (but not oxygenation) activity were inhibited in *P. vulgaris* treated with toxic amounts of

Zn (Van Assche and Clijsters, 1983, 1986) and the inhibition was attributed to the effect of Zn on the oxidizing site of photosystem I and to the displacement of Mg by Zn at the water splitting site in photosystem II, thus producing the photosynthetically inactive Zn-substituted chlorophylls (Küpper *et al.*, 1998).

It might be concluded that accumulation of Zn in *Phaseolus vulgaris* cv. Limburgs vroege seedlings might have limited water supply via retarding both long-distance transport through xylem vessels and short-distance transport between cells. This was underlined by reduction in stomatal frequencies and increased anomalous (non-functional) stomata, injury of cell membranes and metabolic function of the structurally modified chloroplasts. These effects could explain the reduction in the measurable growth criteria of seedlings subjected to Zn treatment.

REFERENCES

- Ali, G., P.S. Srivastava and M. Iqbal, 2000. Influence of cadmium and zinc on growth and photosynthesis of *Bacopa monnieri* cultivated *in vitro*. Biol. Plant, 43: 599-601.
- Baker, N.R., P. Fernyhough and I.T. Meek, 1982. Light dependent inhibition of photosynthetic electron transport by zinc. Physiol. Plant, 56: 217-222.
- Bertrand, M. and I. Poirier, 2005. Photosynthetic organisms and excess of metals. Photosynthetica, 43: 345-353.
- Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlynck, 1982. Chemical Analysis of Plants and Soils. Laboratory of Analytical and Agrochemistry, State University-Ghent, Belgium
- Davies, M.S., D. Francis and I.D. Thomas, 1991. Rapidity of cellular changes induced by zinc in a zinc tolerant and non-tolerant cultivar of *Festuca rubra* L. New Phytol., 117: 103-108.
- Ewais, E.A., 1997. Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. Biol. Plant, 39: 403-410.
- Greger, M. and M. Johansson, 1992. Cadmium effects on leaf transpiration of sugar beet (*Beta vulgaris*). Physiol. Plant, 86: 465-473.
- Kasim, W.A., 2001. Effect of copper and cadmium on some growth criteria and physiological aspects of *Sorghum bicolor*. Egypt. J. Biotechnol., 9: 298-310.
- Kasim, W.A., 2005. The correlation between physiological and structural alterations induced by copper and cadmium in broad beans (*Vicia faba* L.). Egypt. J. Biol., 7: 20-32.

- Kasim, W.A., 2006. Changes induced by copper and cadmium in the anatomy and grain yield of *Sorghum bicolor* (L.) Moench. Int. J. Agric. Biol., 8: 123-128.
- Kaya, C., D. Higgs and A. Burton, 2000. Plant growth, phosphorus nutrition and acid phosphatase enzyme activity in three tomato cultivars grown hydroponically at different zinc concentrations. J. Plant Nutr., 23: 569-579.
- Khudsar, T., Mahmooduzzafar, M. Iqbal and R.K. Sairam, 2004. Zn-induced changes in morpho-physiological and biochemical parameters in *Artemisia annua*. Biol. Plant., 48: 255-260.
- Küpper, H., F. Küpper and M. Spiller, 1998. *In situ* detection of heavy metal substituted chlorophylls in water plants. Photosynth. Res., 58: 123-133.
- Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press London, pp: 83.
- Mediavilla, S., H. Santiago and A. Escudero, 2002. Stomatal and mesophyll limitations to photosynthesis in one evergreen and one deciduous Mediterranean oak species. Photosynthetica, 40: 553-559.
- Metzner, H., H. Rau and H. Senger, 1965. Untersuchungen zur synchronisierbarkeit einzelner pigmentmangelmutanten von *Chlorella*. Planta, 65: 186-194.
- Miller, M. and R.P. Cox, 1983. Effect of Zn²⁺ on photosynthetic oxygen evolution and chloroplast manganese. EFBS Lett., 155: 331-333.
- Monteiro, J.A.F. and C.H.B.A. Prado, 2006. Apparent carboxylation efficiency and relative stomatal and mesophyll limitations to photosynthesis in an evergreen cerrado species during water stress. Photosynthetica, 44: 39-45.
- Poschenrieder, C. and J. Barceló, 1999. Water Relations in Heavy Metal Stressed Plants. In: Heavy Metal Stress in Plants. Prasad, M.N.V. and H. Hagemeyer (Eds.), Springer, Berlin, pp: 207-229.
- Stoyanova, D. and T. Tchakalova, 1999. Cadmium induced ultrastructural changes in shoot apical meristem of *Elodea canadensis* Rich. Photosynthetica, 37: 47-52.
- Van Assche, F., H. Clijsters and R. Marcelle, 1979. Photosynthesis in *Phaseolus vulgaris* L. as Influenced by Supra-Optimal Zinc Nutrition. In: Photosynthesis and Plant Development. Marcelle, R., H. Clijsters and M. Van Pouke (Eds.), Dr. W. Junk (Publisher), The Hague, Boston and London, pp: 175-184.
- Van Assche, F., R. Ceulemans and H. Clijsters, 1980. Zn-mediated effects of leaf CO₂ diffusion conductances and net photosynthesis in *Phaseolus vulgaris* L. Photosynth. Res., 1: 171-180.
- Van Assche, F. and H. Clijsters, 1983. Multiple Effects of Heavy Metal Toxicity on Photosynthesis. In: Effects of Stress on Photosynthesis. Marcelle, R., H. Clijsters and M. Van Pouke (Eds.), Martinus Nijhoff/Dr W. Junk (Publisher). The Hague, Boston and London, pp: 371-382.
- Van Assche, F. and H. Clijsters, 1986. Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentration of zinc: Effect of ribulose-1,5-biphosphate carboxylase/oxygenase. J. Plant Physiol., 125: 355-360.
- Vassilev, A., I. Iordanov, E. Chakalova and V. Derin, 1995. Effect of cadmium stress on growth and photosynthesis of young barley (*Hordeum vulgare*) plants. 2. Structural and functional changes in the photosynthetic apparatus. Bulg. J. Plant Physiol., 21: 12-21.
- Weckx, J.E.J. and H.M.M. Clijsters, 1997. Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiol. Biochem., 35: 405-410.
- Yang, H.M., X.Y. Zhang and G.X. Wang, 2004. Effects of heavy metals on stomatal movements in broad bean leaves. Russ. J. Plant Physiol., 51: 464-468.
- Zyalalov, A.A., 2004. Water flow in higher plants: Physiology, evolution and system analysis. Russ. J. Plant Physiol., 51: 547-555.