Journal of Phytology 2011, 3(6): 58-62
ISSN: 2075-6240
www.scholarjournals.org

JOURNAL OF PHYTOLOGY

www.journal-phytology.com

Effects of Excess Zn on Growth and Photosynthetic Performance of Young Bean Plants

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Article Info

Article History

Received : 22-03-2011
Revised : 05-06-2011
Accepted : 07-06-2011

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Summary

Phaseolus vulgaris L. cv. Lodi plants were grown in a medium containing 200-500 µM of Zn to surpass the threshold of toxicity and assess the inhibitory action on growth, following anatomical changes, the photosynthetic pattern, and Mg, Fe and Mn accumulation. It was found that with increasing Zn accumulation in root and shoot tissues the decreased fresh mass and leaf area correlated with the inhibition of the net photosynthetic rate, transpiration, stomatal conductance, rate of apparent photosynthetic electron transport and isoprenoids accumulation. Additionally, the ratio of variable and maximum fluorescence also remained slightly inhibited with increasing Zn accumulation in the leaves. These alterations further accomplished with a decreasing stomatal index and a thickness of leaf lamina, whereas in the roots the bark, the radius of the central cylinder and diameter of the largest tracheae also diminished. Additionally, the contents of Mg, Fe and Mn in root and shoots tissues also became affected at different levels. It is concluded that excess Zn triggers disturbances in the waters relations, which affect photosynthesis, namely stomatal conductance and therefore plants growth. It is also pointed that as the accumulation of Mg and Fe is affected chlorophylls and carotenoids synthesis become inhibited.

Key Words: Excess zinc; Iron accumulation; Fluorescence; Magnesium accumulation; Manganese accumulation; *Phaseolus vulgaris* L.; Photosynthesis

Abbreviation list: A = Net photosynthetic rate; Chl = Chlorophyll; E = Transpiration rate; ETR = Rate of apparent photosynthetic electron transport; FA = Formaldehyde alcohol; FAA = Formaldehyde acetic acid alcohol; F_0 = Initial or basal fluorescence; F_m = Maximum fluorescence; F_v = Variable fluorescence; gs = Stomatal conductivity; OEC = Oxygen Evolving Complex; PPFD = Photosyntetic Photon Flux Density; PS2 = Photosystem 2; Rubisco = Ribulose bisphosphate carboxylase

Introduction

The average concentrations of zinc required for optimal plant growth varies between 25 and 150 mg kg^{-1}, whereas symptoms of zinc toxicity, namely inhibition of growth, develop when the threshold of toxicity (400-500 mg kg^{-1}) is surpassed [1-7].

Although there isn't consensus regarding the hierarchy of limiting factors, it has been recognized that excess zinc inhibits CO_2 assimilation mostly due to structural and functional disturbances in the photosynthetic process [8-11]. The transpiration rates and the stomata functioning might also be inhibited [12, 6], mostly due to disturbances in the vascular system mediated by xylem elements blockage, as it implicates low water content in the leaves [4]. It also seems that zinc can induce iron and magnesium deficit, concomitantly inducing a decreased accumulation of photosynthetic pigments [5, 6, 10], since magnesium directly participates in chl synthesis and iron interacts as a co-factor [13-15]. According to Van Assche and Clijsters [16] the possible replacement of manganese for zinc in the OEC of PS2 can also inhibit the photosynthetic electron transport and the non-cyclic photophosphorilation. Vangronsveld and Clijsters [17] further considered that Rubisco activity becomes inhibited in this process. The global dynamics of the unbalanced photosynthesis finally might prompt the oxidative burst [18, 19] and therefore further disturbances in chloroplasts ultrastructure [3].

Considering the implications of the environmental pollution with zinc, which is mostly due to industrial and agricultural activities, such as smelter and incinerator emissions, dispersal from mine wastes, excessive applications of Zn-containing fertilizers and pesticides, use of zinc contaminated sewage sludges, manures or industrial wastes as fertilizers and release from galvanized surfaces [20, 21], using *Phaseolus vulgaris* L. cv. Lodi as a test system, this study aims to characterize the outcome of increased zinc concentrations on growth and photosynthetic performance of young bean plants.

Materials and Methods

Plant Material and Growth Conditions

Seeds of *Phaseolus vulgaris* L. cv. Lodi were sterilized for 10 minutes with 10% H_2O_2, washed with distilled water and germinated on vermiculite moistened with distilled water.

Three days following germination the plants were placed in containers, submitted to a PPFD of 250 μmol m^{-2} s^{-1} (14 /10 hours, light/dark), at 22±1°C, RH of 55–60 % and grown in modified ½ strength Hoagland nutrient solution, that was changed every five days. After the primary leaves had fully developed, $ZnSO_4$ was added to the aerated solutions, to obtain final Zn concentrations of 200, 300, 400 and 500 μM. The control plants were grown under a background amount of 1 μM Zn in the nutrient solution. All treatments were carried out using three replicates (i.e., 3 containers), having 4 plants each. At the end of the 10th day the plants were used for biometric, physiological, microscopic, and mineral analyses.

Growth and mineral analysis

Bean plants were chosen at random, after all physically damaged or deformed plants were discarded. The fresh mass and the leaf area were determined with a digital area meter NEO-2 (TU, Bulgaria).

After the removal from the solution, the entire roots and shoots were put to a constant flow of deionized water (10 L min^{-1}, for 5 min) to remove exogenous contaminants. The content of Zn, Fe, Mg and Mn in plant organs was determined by atomic emission spectrophotometry (Perkin Elmer, Analyst 200, Deutschland) after samples drying at 100ºC (30 min) and thereafter at 65ºC for 5 days, followed by mineralization, at 600 ºC for 7 hours.

Photosynthetic analysis

The parameters of leaf gas exchange A), E and gs were determined by a portable photosynthetic system LCpro+ (ADC, England) in the middle section of the first true leaf of the plants. The analysis was conducted in the morning period (10 – 12 AM). Four measurements were taken in each separate treatment.

The photosynthetic pigments were extracted from the middle part of the first true leaves with cooled 100% acetone, measured spectrophotometrically using a Pharo 300 (Merck, Darmstadt, Germany) and calculated according to Lichtenthaler [22].

F_v/F_m and ETR were determined in the middle section of the first leaves by a pulse modulated fluorimeter MINI-PAM (H. Walz, Effeltrich, Germany). F_v/F_m was determined after a 30-minute adaptation in the dark, during which F_0 and F_m were measured, where the intensity of the measuring red light was 0.15 μmol m^{-2} s^{-1}, the frequency was 0.6 kHz, and the saturating impulse for F_m lasted 0.8 s with an intensity of over 5500 μmol m^{-2} s^{-1}. The ETR was calculated using the formula ETR = Y*0.5*PPFD*0.84, where Y is the quantum yield. The quantum yield was determined at steady-state photosynthesis after 30 minutes adaptation of plants at 250 μmol m^{-2} s^{-1} using identical saturating light impulse.

Anatomical analysis

Samples from roots and leaves were collected at the end of the test period, and fixed in FAA for 24 hours and transferred for storage in FA. Semi-lasting glycerine samples were prepared, and the different variants had 50 measurements each. The observations, measurements and photographs of samples were made with a digital light microscope Motic DMBA210 (Motic Incorporation Ltd., China), using the image analysis software Motic Images Plus version 2.0., with a general magnification of x100. The following parameters were followed: stomatal index, thickness of leaf lamina, thickness of the bark of the primary root and the diameter of the central cylinder of the root, the diameter of the widest conductive elements [23].

Statistics

Statistical analysis was performed using a one-way ANOVA (for $P \leq 0.05$). Based on the ANOVA results, a Tukey test for mean comparison was performed, for a 95% confidential level, to test for significant differences among treatments. In the tables, different letters (a, b, c, d, e) express significant differences.

Results

Zinc stressed bean plants developed a more compact root system with reduced linear dimensions. The inhibition of the roots was accompanied by browning of some areas, probably associated with enhanced processes of lignification [24]. The plants had a shorter height and more tightly arranged leaves. The leaf lamina of the lower leaves was slightly curled, whereas upper ones had a reduced size and were brighter with signs of chlorosis.

As the concentration of Zn in the root medium increases, the fresh mass of the bean plants was reliably reduced (Table 1). The inhibition of the fresh mass in the treatment having 200 μM Zn was 21%, whereas in the treatment with the highest concentration of 500 μM Zn reached 43%. Identical results were also detected with the formation of the leaf area. Additionally, with Zn augmentation the leaf area decreased from 23% to 46%.

The amount of Zn in the leaves and roots of the control bean plants reached 138 mg Zn/kg and 143 mg/kg, respectively. These values are within the range of typical concentrations of Zn in the plants [7]. As the concentration of Zn increased to 200 μM, the metal content in the leaves doubled, and in the roots increased several times till 1982 mg/kg. As expected, the increased external concentration of Zn triggered a progressive augmentation of this metal accumulation and therefore the difference between the content in roots and leaves became diminished. Excess Zn also became strongly inhibitory to leaf gas exchanges (Table 2). At the 200 μM Zn treatment A and E decreased by 39% and 31%, respectively. Stomatal conductivity decreased more significantly (about and above 50%). At the 500 μM Zn treatment A and E became inhibited to 8% and 16%, respectively.

The ratio F_v/F_m, which indicates the maximum quantum efficiency of PS2, did not change significantly when the concentration of Zn in the root medium increased, remaining within 0.75 - 0.83, which is typical for the functionally of healthy leaves [25]. Moreover, the rate of photosynthetic electron transport (ETR) gradually decreased, but did not exceed 22% at the 500 μM Zn treatment.

The sharp decrease of E in heavy metal-treated plants is an expression of adaptation to water deficit [24, 12, 6]. This perspective was supported by the established anatomical changes in the leaves of the Zn-treated bean plants, which generally was characterized by a denser mesophyll and reduced intercellular spaces (Figure 1; Table 3).

At the 500 μM Zn treatment the thickness of the leaf lamina, was reduced by 18%. The control plants revealed larger intercellular spaces in the spongy parenchyma and in the palisade parenchyma, while mesophyll of the treated plants remained more compact, hence the reduced thickness of the leaf lamina. The number of stomata cells on the lower surface of the plants treated with Zn was higher, but their size decreased. Nevertheless, excess of Zn did not induce significant changes in the anatomical structure of roots. The radius of the central cylinder of the plants treated with 500 μM Zn was also reduced by 11%. Although there was some reduction in the width of the primary cortex and in the diameter of the widest conductive elements of xylem, the differences were small and statistically unproven. The results of the effects of Zn on the anatomical characteristics of leaves and roots of bean plants also corresponded to the research of other authors [3, 26, 4].

Excess Zn strongly decreased the photosynthetic pigments content in the bean plants (Table 4). The amount of chl *a* showed a higher reduction, followed by chl *b* and carotenoids. The strongest inhibition was found in the treatment of 500 μM Zn that reached ca. 52%, 41% and 31%, respectively.

The translocation of Fe to the leaves of Zn treated plants decreased sharply as the external Zn concentration increased above 300 μM (Table 5). Excess Zn reduced the absorption and translocation of Mg and to a lesser extend of Fe. In addition, high Zn concentrations further decreased the content of Mn in roots and leaves about 2 to 3 fold relatively to the control bean plants, which clearly indicated that excess Zn inhibited the absorption and translocation of those elements, which play an important role in the photosynthetic process.

Table 1. Effect of excess Zn on some biometric parameters and Zn accumulation of *Phaseolus vulgaris* L. cv. Lodi. Parentheses contain the percentage values of each parameter relatively to the control. Different letters (a, b, c, d, and e) indicate statistically differences (P ≤ 0.05).

Treatments (μM Zn)	Fresh mass (g)	Leaf area (cm^2)	Zn content (mg / kg)	
			Leaves	roots
1	10.80 (100)^a	247.0 (100)^a	138^a	143^a
200	8.58 (79)^b	190.1 (77)^b	242^b	1982^b
300	7.11 (66)^b	167.7 (68)^b	865^c	2392^c
400	6.69 (62)^b	133.0 (54)^b	1366^d	2598^c
500	6.19 (57)^c	141.1 (57)^c	2065^e	2705^d

Table 2. Effect of excess Zn on photosynthetic performance of *Phaseolus vulgaris* (cv. Lodi) plants. Parentheses contain the percentage values of each parameter relatively to the control. Different letters (a, b, c, d, e) indicate statistically differences (P ≤ 0.05).

Treatments (μM Zn)	A (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	F_v/F_m	ETR (μmol m^{-2} s^{-1})
1	7.78 (100)^a	0.90 (100)^a	0.02^a	0.79	89 (100)
200	4.78 (61)^b	0.58 (64)^b	0.01^b	0.78	83 (93)
300	2.45 (31)^c	0.44 (49)^c	0.01^b	0.76	80 (90)
400	1.41 (18)^d	0.34 (38)^c	0.00^b	0.76	85 (96)
500	0.61 (8)^e	0.16 (18)^d	0.00^b	0.75	69 (78)

Table 3. The effect of excess Zn on the anatomical structure of leaves and roots in *Phaseolus vulgaris* L. cv. Lodi. Parentheses contain the percentage values of each parameter relatively to the control. Different letters (a, b, c, d) indicate statistically differences (P ≤ 0.05).

Organ	Parameters	Treatments (μM Zn)	
		1	500
Leaf	Stomatal index (%)	29.00^a (100)	27.70^a (95)
	Thickness of leaf lamina (μm)	144.66^a (100)	118.2^b (82)
Root	Bark (μm)	683.7^a (100)	662.9 (97)
	Radius of the central cylinder (μm)	683.6^a (100)	609.9^b (89)
	Diameter of the largest tracheae (μm)	49.34^a (100)	46.29 (94)

Table 4. Effect of excess Zn on photosynthetic pigments of *Phaseolus vulgaris* L. cv. Lodi. Parentheses contain the percentage values of each parameter relatively to the control. Different letters (a, b, c, d) indicate statistically differences (P ≤ 0.05).

Treatments (μM Zn)	Chls				Carotenoids (mg/g_{FW})	(Chl *a*+*b*) / Carotenoids
	a (mg/g_{FW})	*b* (mg/g_{FW})	*a*+*b* (mg/g_{FW})	a / b		
1	1.41 (100)^a	0.51 (100)^a	1.91	2.79	0.59 (100)^a	3.25
200	1.14 (81)^b	0.44 (86)^b	1.57	2.61	0.48 (81)^b	3.26
300	1.03 (73)^c	0.41 (80)^b	1.44	2.54	0.43 (73)^b	3.35
400	0.87 (62)^c	0.35 (69)^c	1.23	2.46	0.44 (75)^b	2.77
500	0.68 (48)^d	0.30 (59)^c	0.98	2.27	0.41 (69)^c	2.42

Table 5. Effect of excess Zn on Mg, Fe and Mn accumulation of *Phaseolus vulgaris* L. cv. Lodi. Parentheses contain the percentage values of each parameter relatively to the control. Different letters (a, b, c, d) indicate statistically differences (P ≤ 0.05).

Treatments (μM Zn)	Mg	Fe	Mn
	Leaves (mg / kg)		
1	2276 [a]	126[a]	40[a]
200	2564[b]	132[a]	38[a]
300	2354[a]	107[a]	23[b]
400	2068 [a]	60[b]	10[c]
500	1922[a]	65[b]	15[c]
	Roots (mg / kg)		
1	1952[a]	245[a]	9.2[a]
200	2020[a]	297[a]	6.1[b]
300	1830[a]	341[b]	2.9[c]
400	1728[b]	348[b]	2.8[c]
500	1710[b]	312[a]	3.0[c]

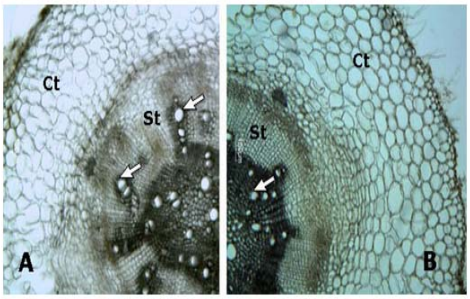

Figure 1. Transverse section of *Phaseolus vulgaris* L. cv. Lodi roots, magnification x100. A – untreated plants; B – plants treated with 500 μM Zn. Ct – cortex; St – stele. Conductive elements of the xylem are marked with arrows.

Discussion

Zinc stressed bean plants developed a more compact root system as previously reported by other authors [27]. Generally, excess Zn inhibits plant growth, causing browning of roots, as well as chlorosis and necrosis in the leaves [1, 2]. In our study on bean plants grown at high Zn concentrations, visual symptoms of toxicity were developed, but there were no necrotic areas in the leaves, as was previously found with wheat plants [4]. Even in the case of the 200 μM Zn treatment, the accumulation of this nutrient in the roots exceeded the threshold toxicity (400-500 mg/kg) [7]. The high concentration of Zn in roots reflected not only the assimilation and absorption capabilities, but also the slightly adsorbed Zn ions in root cells surface. Despite this it was in the toxic range and corresponded to the observed toxic effects on growth.

The drastic reduction of the net photosynthesis rate and the almost identical reduction of the transpiration rate (Table 2) supported that the main factor limiting photosynthesis in Zn-treated bean plants implicates stomatal limitions. The decrease of the thickness of the leaf lamina, which was mainly due to the reduction of the intercellular spaces of the leaf mesophyll, additionally reduced the flow of CO_2 within the leaf. All these changes hindered CO_2 diffusion to the chloroplasts leading to lower carboxylase activity of Rubisco [8, 17].

The net photosynthetic rate of Zn-treated plants can be inhibited also, as a result of other disturbances in the integral photosynthetic process [10, 8]. In addition to being the result of increased stomatal limitation, our research indicated that the decrease of A can be linked to severely reduced content of photosynthetic pigments as well. The reduced content of pigments can be an integral result of many possible causes – disturbed biosynthesis and / or enhanced degradation, as well as reduced content of thylakoids, and others [6]. In this particular case, we assume that the reduced content of pigments in Zn-treated bean plants is partly due to the inhibited biosynthesis because of limited absorption and translocation of Fe and Mg. The stronger inhibition of chl *a* relatively to chl *b* was probably due to its higher sensitivity to stress factors, whereas the smaller effect of Zn on carotenoids possibly was linked to their protective role against the chlorophylls photoxidation.

In conclusion, as excess Zn in the root medium increased, considerable anatomical changes and functional disorders in young bean plants where observed. The most significant effect was detected on the rate of CO_2 assimilation, which at 500 μM Zn became inhibited over 90%. The negative effect of Zn on the photochemical processes was less pronounced and did not exceed 22%. In the particular case of our study, the photosynthetic rate in Zn-treated bean plants was mostly limited by the reduced access of CO_2, due to disturbances in the water relations. Xeromorphic changes in leaf anatomy were observed, which became an expression of the adaptation of plants treated with Zn to disturbances in water regime.

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