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Applied Botany

Elixir Appl. Botany 64 (2013) 19199-19201

ISSN: 2229-712X

Growth and physiological attributes of wheat in Zn-contaminated soils

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ARTICLE INFO

Article history:
Received: 26 August 2013;
Received in revised form:
1 November 2013;
Accepted: 6 November 2013;

Keywords
Available zinc,
Biochemical constituents,
Antioxidative defence systems,
Tritium aestivum,
Zinc-contaminated soil.

ABSTRACT

Zn-contaminated soils were rated for various levels of available (DTPA extractable) Zn viz. 0.42, 2.8, 4.5, 9.6 and 26.3 ppm and used to grow wheat (*Tritium aestivum* Linn.) plants. Effect of these soils on growth (length and dry matter production), visible symptoms of toxicity and biochemical constituents (protein, pigments and sugar contents and catalase activity) of wheat were evaluated. Plants grown at high Zn-contaminated soil (26.3 ppm) had visible symptoms of toxicity such as decreased growth, chlorosis and tip burning of young leaves and reduced leaf lamina. Length and dry matter yield of wheat were increased maximum at 4.5 ppm available Zn in soil. Pigment, sugar and protein contents were also stimulated upto 4.5 ppm of available Zn, whereas these values decreased with increase in Zn levels in soil. Antioxidative defense systems with respect to carotenoids and protein contents and catalase activity favoured the dry weight production in wheat which were grown at 4.5 ppm of available Zn in soil. Study revealed the tolerance of wheat found maximum at 4.5 ppm of available Zn in soil, where as tissue concentrations of root and shoot were 30.6 and 35.6 µg Zn g^{-1} of dry weight, respectively. High Zn concentrations (9.6 and 26.3 ppm) were not stimulatory to the wheat growth reduced biochemical constituents in cells and produced visible symptoms of toxicity in wheat.

Introduction

Zinc is an essential nutrient for the plants growth, although elevated concentrations in soil cause growth inhibition. Use of industrial effluent and sewage sludge on agricultural land has become a common practice in India, consequently some heavy metals including Zn are absorbed and concentrated into plant tissues from the soil (Pandey, 2006). These metals have deter - mental effect on plants above a critical concentration through affecting biochemical activities in the cells. Wide spread deficiency of zinc has been recorded in Northern Indian soils of semi-arid regions (Sharma *et al*., 1984). The uptake of Zn in plants is mostly dependent on its concentration and physio-chemical properties of the soil. Zinc is constituent of a multitude of enzymes (Berg and Shi, 1996), functions as a constituent of several regulatory proteins (Sharma, 2006) and play a protective role in cell against oxidative stress (Bettger and ODell, 1981). Despite the beneficial role of Zn, its higher concentrations are toxic and reduced crop yield. Wheat (*Tritium aestivum* Linn.) grown in all parts of the world from the tropics to the subarctic as a most widely cultivated food crop. In India, it is mostly grown in central alluvial plane. Plants can thrive best and tolerate higher levels zinc only through changes in biochemical activities in their cells. Information is scanty and less available regarding tolerant plants with respect to growth and physiological responses of wheat including antioxidative defense by catalase activity subjected to various levels of Zn-contaminated soils. Therefore, study was aimed to investigate tolerance limit of wheat against zinc-stress conditions in alluvial soil of Northern India. The findings of the study may be beneficial to check the suitability of various levels of Zn-contaminated soils to grow wheat crop, also assessment of damage incurred in plants due to Zn-toxicity/deficiency and extent of phytoremedial need.

Materials and Methods

Wheat (*Triticum aestivum* Linn., var) plants were grown in alluvial soil (collected from Badshah baugh area in Lucknow district, U.P. state, India), previously irrigated with 0, 0.25, 1.0, 25 and 100 ppm Zn concentrations to grow *Trigonella foenum-graecum* for 100 days. In post harvested soils, available (DTPA extractable) Zn concentrations were 0.42, 2.8, 4.5, 9.6 and 26.3 ppm respectively. A composite sample of the native soil when analyzed for DTPA extractable available metals (Table 1) by the method of Lindsay and Norvell (1978) revealed its Zn deficiency. Ten seeds were sown in each clay pot (5-6 cm depth) filled with above Zn contaminated soils (0.42 (T_0), 2.8 (T_1), 4.5 (T_2), 9.6 (T_3) and 26.3 (T_4). All experiments were done in triplicate in clay pots, which were placed in a glass top wire house. Twenty five days after emergence of seeds, wheat plants were thinned to four per pot, irrigated with distilled water as per need and observed periodically for growth and visible symptoms appeared on plants. The plants were harvested for dry matter yield on 75th day after sowing (DAS) and analysis of pigments (chlorophyll a, b, total and carotenoids), protein and sugar contents. Catalase activites were carried out on 40th DAS when visible symptoms were started to appear. Pigments were estimated by the method of Lichtenthaler and Wellburn (1983). Protein content was estimated by the method of Lowry *et al*. (1951).The modified method of Bisht (1976) was used for assay of catalase activity. Pigment were determined in 80% acetone and extract absorbance of clear supernatant was measured after centrifugation (10,000 *g*, 20 minutes), at 663, 645 and 652 nm for chlorophyll a, b and total respectively. Results were expressed on fresh weight basis in mg g^{-1}.

Data presented in tables are the mean values of three replicates. All data were tested statistically by ANOVA for L.S.D. (Painse and Sukhatme, 1961).

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Results and discussion

Wheat (*Triticum aestivum* Linn. Var) plants were grown in various levels of Zn-contaminated soils, observed for their growth and some physiological attributes. Growth responses (shoot and dry matter production) were found stimulatory upto 5.6 ppm of available Zn, thereafter gradually decreased, with increase in Zn concentrations in soil (Table 1). Increase in growth could be attributed due to availability of Zn in plants upto their optimum requirement in cell metabolism, since native soil was rated Zn deficient (<0.8 ppm). The critical deficiency level of Zn has been reported less than 0.8 ppm (DTPA extractable) in most of the alluvial soil in northern India (Agarwala ans Sharma, 1979). Zn promotes growth as it is an constituent of carbonic anhydrase enzyme (Sasaki *et al*., 1998), antioxidative superoxide dismutase (Cakmake and Marchner, 1993) and many other enzymes which are involved in many important physiological activities of plants (Pandey and Gautam, 2009). Wheat plants, which were grown in Zn-deficient native soil (T_0), exhibited visible symptoms such as stunted growth, intervenal chlorosis and reduced size of leaf lamina. These symptoms resembled with Zn deficiency, as earlier reported by Sharma (2006) in maize and rice. The Zn-deficiency symptoms did not appeared in plants grown at T_2 and T_3 soils. Some visible symptoms of toxicity such as restricted growth, chlorosis and tip burning of young leaves appeared on plants grown in soil with 26.3 ppm of available Zn (T_4). These symptoms developed due to Zn-toxicity (Pandey and Gautam, 2009) in lentil (*Lens culinaris* Medic). Reduction in growth and appearance of toxicity symptoms on wheat shoot could be attributed due to the inhibition of uptake of some essential metals and ultra structural alterations in plant cells under Zn-toxicity (Sresty *et al*., 1999). In addition, also due to decreased in rate of photosynthesis (Sharma *et al*., 1994).

Pigment contents (chlorophyll a, b, total and carotenoids) were increased maximum up to T_2, whereas decreased at T_3 and T_4 soils (Table 3). Zinc deficiency causes disorganization of chloroplast thylakoids and pigment content and carbonic anhydrase activity which play important role in photosynthesis of plants (Saskaki *et al*., 1998).

Protein and sugar contents also followed the similar trend like the pigment. Maximum protein and sugar contents were observed in wheat exposed with 4.5 ppm of Zn in soil, where declined at higher Zn concentrations in soil. Reduction in protein content at T_3 and T_4 soils might be resulted due to alteration in biochemical activities involved in protein synthesis in cells (Pandey and Shukla, 2009). Zinc forms metalloproteins in stress condition of heavy metals. Reduction in sugar content may be resulted due to decrease in pigment content and corresponding photosynthesis rate (Jyung *et al*., 1975). In our observation sugar content was enhanced by Zn, maximum at T_2 soil showes its beneficial role in photosynthesis and sugar formation. Singhal *et al.* (2007) reported that Zn toxicity retards chlorophyll, carbohydrate and protein content in black gram due to exposure of excess Zn.

Catalase (CAT) activity in wheat leaves was increased up to 4.5 ppm of Zn (T_2), further increased concentration of available Zn in soil showed its declining trend. The increase in CAT activity is an indicative of protection against stresses by plants (Gajewaska and Sklodowaska, 2007). The deficiency of Zn (Yu *et al*., 1998) and its toxicity (Pandey and Gautam, 2009) cause oxidative stress in plants, which produces reactive oxygen species (ROS). ROS in plant cells stimulates production of H_2O_2, which causes lipid peroxidation that damage the cell membrane. Catalase activity protects the plant cells by converting H_2O_2 to H_2O and O_2. Zinc catalyzed rapid detoxification of ROS such as O_2 and OH radicals by producing H_2O_2, which can be process off by the enzymatic action of catalase. Reduction in CAT activity would be due to the failure of antioxidative defence system with Zn toxicity (Sharma, 2006).

Conclusion

In conclusion, growth and biochemical constituents in wheat increased maximum in soil at 4.5 ppm of available Zn (DTPA extractable), whereas started to decline above higher concentrations in soil (9.6 and 26.3 ppm of available Zn). The uptake and translocation of Zn was dose dependent. At higher concentrations (available 9.6 and 26.3 ppm) in soil, Zn declined antioxidative defence with respect to catalase activity and produce visible symptoms of toxicity.

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Table 1: Physico-chemical properties of composite soil sample collected from Badshahbagh area, in Lucknow.

Parameter	Texture	pH	O.M (%)	$CaCO_3$ (%)	E.C (m mhos/cm)	DTPA extractable (ppm)			
						Zn	Cu	Fe	Ni
Average value	Sandy loam	6.7	0.62	0.72	0.48	0.61	0.45	5.15	0.14

OM- organic matter; E.C.- electrical conductance.

Table 2: Growth responses of wheat grown in Zn-contaminated soils 75 DAS.

Treatment	DTPA extractable Zn (g plant^{-1})	Length (cm)	Dry weight in soil (ppm)
T_0	0.42	37.4 (0.0)	0.68 (0.0)
T_1	2.6	40.6 (-8.5)	0.81 (-19.1)
T_2	4.5	38.8 (-3.7)	0.92 (-35.3)
T_3	9.6	33.8 (+9.6)	0.49 (+27.9)
T_4	26.3	28.6 (+23.52)	0.35 (+48.5)
LSD	P= 0.05	5.92	0.28

*-value significant at P<0.05 and **-value significant at P<0.01 levels; Parenthesis indicate percentage increase (+) or decrease (-) over control.

Table 3: Biochemical responses of wheat (*Triticum aestivum* Linn.) grown at Zn-contaminated soils 40 DAS.

Parameters	Available Zn in soils					LSD P=0.05
	0.42 (T_0)	2.8 (T_1)	4.5 (T_2)	9.6 (T_3)	26.3 (T_4)	
Chlorophyll a (mg g^{-1} f.w.)	1.36 (0.00)	1.56 (-14.7)	1.65 (-21.3)	1.39 (-2.2)	1.20 (+11.8)	0.22
Chlorophyll b (mg g^{-1} f.w.)	0.42 (0.00)	0.45 (-7.1)	0.36 (+14.2)	0.33 (+21.4)	0.25 (+40.4)	0.07
Carotenoids (mg g^{-1} f.w.)	1.04 (0.00)	1.15 (-10.5)	1.25 (-20.2)	1.07 (-2.8)	0.90 (+13.4)	0.16
Protein (mg g^{-1} f.w.)	210 (0.00)	271 (-29.0)	285 (-35.7)	190 (+9.5)	175 (+16.6)	61.1
Sugar (µg g^{-1} f.w.)	8.9 (0.00)	10.5 (-17.9)	12.5 (-40.4)	11.8 (-32.5)	7.5 (+15.7)	2.24

*-value significant at P<0.05 and **-value significant at P<0.01 levels; Parenthesis indicate percentage increase (+) or decrease (-) over control.

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