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Impact of Essential Micronutrient, Zn, on Growth and Chlorophyll Biosynthesis in Young *Zea mays* Seedlings

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Abstract

The present study analyses growth and chlorophyll biosynthesis in young maize seedlings in response to Zn supply over a wide range of concentrations. Supply of 0 - 5 mM $ZnCl_2$ to 3 days old light grown maize seedlings led to gradually increased accumulation of Zn in the shoot tissue, while in the root tissue substantial increase was observed at/and above 0.1 mM $ZnCl_2$. Zn supply significantly reduced the overall growth of maize seedlings mostly at 1 - 5 mM $ZnCl_2$ exerting strong correlation and the observed effect was more substantial for root tissue. Amongst the biochemical parameters, increase in protein and proline content was more prominent in root tissue than the shoot, while RNA content was reduced in shoot tissue. Zn treatment to light grown seedlings significantly increased the chlorophyll, carotenoid content, while in dark grown seedlings it had marginal/no effect. Delta amino levulinic acid (ALA) content in both the regimes was increased at higher Zn concentrations. Also ALA synthesis was increased in both the regimes, but non significantly. Zn enhanced ALA dehydratase (ALAD) activity of light as well as dark grown seedlings being significant in former. The results demonstrate that the Zn accumulation and growth effect at higher Zn concentrations in maize depend upon the tissue with root as the target site and shoot growth are mainly influenced by ALA and subsequently ALAD in maize seedlings.

Keywords

Amino Levulinic Acid, Amino Levulinic Acid Dehydratase, Chlorophyll Biosynthesis, Growth Effects, Maize, Zn Effects

1. Introduction

Zinc (Zn) being an essential micronutrient for plants, is required for optimum

growth and development. Its deficiency as well as toxic symptoms has been found in different plants. Zn deficiency reduces various physiological processes, such as, net photosynthesis [1], tissue water content and P and Mg concentrations [2] in plants. Toxic effects of Zn in plants influencing different functions have also been reported, such as, high concentrations of Zn decrease net photosynthesis and respiration rate in *Beta vulgaris* [3], generate reactive oxygen species, and decrease the chlorophyll content and shoot yield [4]. Zn effects on plant growth varies in different species, thus decrease has been observed in ryegrass [5], clusterbean [6], in sugarbeet [7], and in Sorghum [8] while in *Eruca sativa* seedlings increase is reported [9].

Chlorophylls are the vital pigments being analyzed to assess the impact of deficiency as well as the toxicity of various causative factors. Chlorophylls are one of the essential tetrapyrroles responsible for their light trapping and energy transduction activities. Chlorophyll biosynthesis is a vital physiological process of the green plants, which is regulated at several steps [10]. Its biosynthesis involves a complex pathway having a large number of intermediates with amino levulinic acid (ALA) as the common precursor for all tetrapyrroles. ALA is synthesized by the two pathways, the Shemin pathway involving glycine and succinyl Co-A and the Beale pathway, involving the C-5 compounds, like glutamate and 2-oxoglutarate by an ALA synthesizing system in plants [10]. ALA dehydratase (ALAD, E.C. 4.2.1.24) catalyses the next step *i.e.* conversion of ALA to porphobilinogen, the basic unit of tetrapyrrole. ALADs from different sources are metalloenzymes that utilize a variety of divalent and monovalent cations [11]. Plant ALAD requires Mg^{2+} and Zn^{2+} for its activity. Decrease in chlorophyll content due to Zn supply has been reported in several plant species [6] [7] and [8] and also the photosynthetic activity [5]. However, Zn effects on enzymes of chlorophyll biosynthesis including earlier steps are rare. Early stages of seedling growth are a crucial part of any plant life. Thus, to study impact of Zn three days old seedlings were used involving hydroponic technique with the objective to evaluate the Zn effects in relation to growth and chlorophyll biosynthesis in young maize seedlings.

2. Materials and Methodology

2.1. Plant Material and Treatment

Seeds of *Zea mays* L. cv. Ganga safed-2, were surface sterilized with $HgCl_2$ (0.1%) for 1 - 2 min followed by thorough washing and then soaked in distilled water for half an hour. About 20 seeds were placed in petri plates lined with moistened whatman filter paper and the seedlings were grown for three days in continuous light of 30 Wm^{-2} at 26˚C ± 2˚C. For some experiments, the seedlings were raised in continuous dark for three days and then used for treatment, which reflected the analysis of light induced chlorophyll biosynthesis. For Zn treatment, 4 uniformly grown seedlings were transferred to 40 ml of 0 - 5 mM $ZnCl_2$ solution contained in small petri plates for 24 h in continuous light as

above and the treated seedlings were used for various analyses.

2.2. Analytical Procedures

Determination of growth and Zn content—Treated seedlings were used to analyze Zn content, fresh weight, dry weight, number of roots, weight and length of root and shoot. For Zn content analysis, root and shoot tissue of the seedlings were dried in oven at 80˚C for 72 hours. The dried material (400 mg) was wet digested with 5 ml of conc. HNO_3 and diluted 5× to 50× times *i.e.* (5× (control), 10× (0.001, 0.01, 0.1 mM), 20× (1 and 2 mM) and 50× (5 mM) with distil water. Zn content was determined by using atomic absorption spectrophotometer, make "GBC, Scientific Equipment Ltd—AVANTA".

Biochemical parameters—Total protein and RNA content of root and shoot tissue were measured by the method of [12] and [13], respectively. For protein content analysis, treated tissues were first boiled with and then extracted using 5.0 ml of 80% ethanol for each step. The extract was centrifuged at 5000 rpm for 10 min and the pellet was suspended in 5.0 ml of 10% TCA for 30 min and again centrifuged similarly. The resulting pellet was dissolved in 5.0 ml of 0.1 N NaOH and kept for at least 1hr. After centrifugation, the supernatant was used for protein estimation with Folin Ciocaulteau's phenol reagent. For RNA extraction similar 80% ethanol treatment was used and then the pellet was suspended in 2.0 ml of 1% PCA mixed thoroughly and centrifuged. The pellet was treated with 5.0 ml of ethanol: diethyl ether: chloroform (2:2:1 v/v) mixture and then centrifuged. Three ml of 0.3 N KOH was added to the residue and was incubated at 37˚C for 18 hr. Next the pH was adjusted to 2.0 using 1 N PCA, centrifuged and appropriately diluted supernatent was used to estimate total RNA by orcinol reagent. For proline content determination, method of [14] was used.

Chlorophyll extraction and estimation—Pigments were extracted from the treated shoot tissue using 80% acetone and estimated spectrophotometrically by measuring the absorbance at 470 nm, 646 nm, and 663 nm. The pigment content was calculated using the following equations according to the method of [15].

$$\text{Chl a}\left(\mu\text{g ml}^{-1}\right) = 12.21\left(A_{663}\right) - 2.81\left(A_{646}\right)$$

$$\text{Chl b}\left(\mu\text{g ml}^{-1}\right) = 20.13\left(A_{646}\right) - 5.03\ \left(A_{663}\right)$$

$$\text{Total Chlorophyll} = \text{Chl a} + \text{Chl b}$$

$$\text{Caratenoids}\left(\mu\text{g ml}^{-1}\right) = 1000\left(A_{470}\right) - 3.27\ \left(\text{Chl a}\right) - 104\ \left(\text{Chl b}\right)/229.$$

ALA content determination—Estimation was performed according to the method of [16]. For extraction of ALA, shoot tissue was extracted with 1 M sodium acetate buffer. Homogenate was further centrifuged at 10,000 rpm for 10 min at 4˚C. ALA was reacted with ethyl acetoacetate followed by modified Ehlrich reagent for estimation. Color formation was measured at absorbance 555 nm on Shimadzu UV-1800 spectrophotometer.

ALA Formation—method of [16] was used to estimate the activity. Light and dark grown seedlings were used which were further incubated with levulinic acid

which is responsible for inhibition of ALA synthesis. Incubation was performed in light and dark conditions. Modified Ehlrich reagent was used to estimate amount of ALA as above.

ALAD assay—ALAD activity was assayed spectrophometrically by estimating the amount of PBG (porphobilinogen) formed using modified Ehlrich reagent [17] according to the method of [18]. Treated shoot was extracted in 50 mM Tris HCl buffer (pH 8.2) containing 10 mM DTT. Extract was centrifuged at 15,000 rpm for 30 min at 4˚C and supernatant was used as enzyme preparation. Reaction mixture of ALA, enzyme and $MgCl_2$ was used to which TCA was added to stop the reaction. One unit of enzyme activity is defined as 1 nmol of PBG formed per hour.

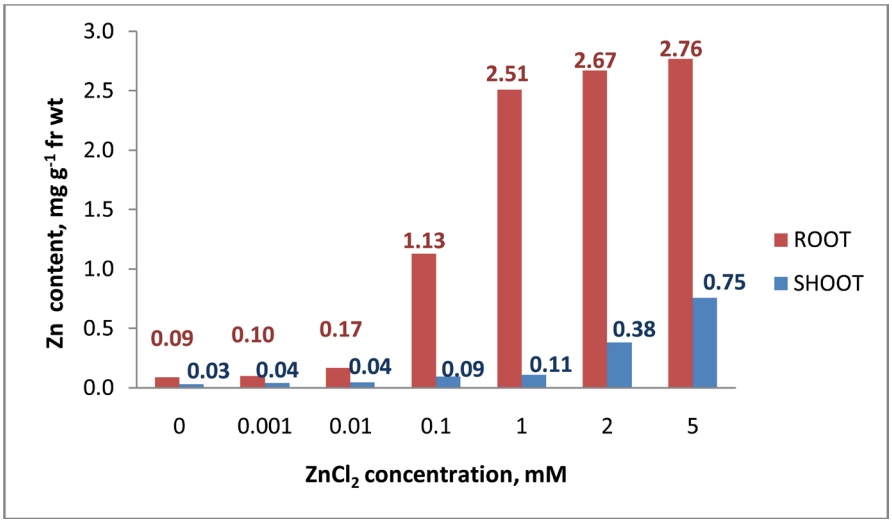
2.3. Data Analysis

Data presented in the paper are average of at least four independent experiments with ± S.E. Significance of difference obtained for various treatments was tested by the Student's t-test. Compound correlation was calculated by Graphpad Prism 7.04.

3. Results

3.1. Effect on Zn Accumulation in Root and Shoot of Maize Seedlings

Supply of 0 - 5 mM $ZnCl_2$ to three days old maize seedlings for 24 h resulted in concentration dependent increase in accumulation of Zn in root as well as shoot tissue with the effect being more substantial in the former (Figure 1). Sharp increase in Zn content in root tissue was observed at/above 0.1 mM, while in shoot



Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C and Zn content of root and shoot tissue was measured.

Figure 1. Effect of supply of $ZnCl_2$ on accumulation of Zn in root and shoot tissue of the seedlings.

tissue the increase was gradual; hence, perfect correlation resulted for later with R^2 values being 0.591 and 0.976, respectively (Figure 1).

3.2. Effect of $ZnCl_2$ on Growth and Biochemical Parameters of Maize Seedlings

Zinc treatment of maize seedlings reduced significantly the fresh wt of the seedlings at higher concentrations of 1 - 5 mM, while the dry wt was reduced slightly (Table 1). Root parameters, like number and length were decreased more prominently and significantly in the concentration range of 0.1 to 5 mM Zn, but effect on shoot length was less severe and less significant (Table 1). Further, root wt was decreased more prominently and significantly than shoot weight. Moreover, all the growth parameters were correlated with Zn treatment with R^2 values being in the range of 0.542 (For root length) to 0.744 (For shoot wt).

Incubation of maize seedlings with $ZnCl_2$ increased the protein content in both the root as well as shoot tissue with the effect being more prominent and significant in former (Table 2). Proline content was also increased in both the tissue with more significant change in roots (Table 2). The RNA content in shoot tissue was decreased, while it was increased in root issue but the effect was not significant except at 1 mM Zn (Table 2).

Compound correlation analyses yielded correlation between each pair of the parameters. Thus, in root tissue a perfect correlation of Zn content with root wt and protein and also between root wt and protein was observed having the values −0.989, 0.947 and −0.969, respectively (Table 3(a)). For Zn content, root wt and protein with RNA and proline, strong correlation with values being in the range of 0.654 to 0.740 resulted, however between RNA and proline there was no correlation (Table 3(a)). In shoot tissue perfect correlation was observed for Zn content and shoots wt with proline having the values 0.939 and −0.949, respectively (Table 3(b)). Very strong correlation of shoot wt with Zn content

Table 1. Effect of supply of $ZnCl_2$ on fresh wt, dry wt, root no., root and shoot length, root and shoot wt of the seedlings. Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C and various growth parameters were measured.

$ZnCl_2$ conc, mM	Fresh wt, mg	Dry wt, mg	Root No	Root length, cm	Shoot length, cm	Root wt, mg	Shoot wt, mg
0.0	612 ± 9 (100)	271 ± 11 (100)	5 ± 0.2 (100)	5.7 ± 0.3 (100)	2.9 ± 0.4 (100)	114 ± 10 (100)	122 ± 10 (100)
0.001	612 ± 10 (100)	263 ± 10 (97)	5 ± 0.2 (100)	4.8 ± 0.5 (84)	2.9 ± 0.4 (100)	109 ± 13 (96)	108 ± 6 (89)
0.01	612 ± 15 (100)	259 ± 8 (96)	5 ± 0.4 (100)	4.7 ± 0.4 (82)	2.3 ± 0.1 (79)	101 ± 13 (89)	106 ± 2 (87)
0.1	604 ± 19 (99)	254 ± 19 (94)	3 ± 0.2** (60)	3.3 ± 0.1** (58)	2.3 ± 0.2 (79)	82 ± 9 (72)	95 ± 8 (78)
1	544 ± 21* (89)	251 ± 9 (93)	3 ± 0.2** (60)	3.0 ± 0.3** (53)	2.1 ± 0.1 (72)	56 ± 5** (49)	82 ± 6* (67)
2	535 ± 29 (87)	247 ± 12 (91)	3 ± 0.1** (60)	2.8 ± 0.1** (49)	1.6 ± 0.1* (55)	46 ± 4** (40)	77 ± 7* (63)
5	528 ± 12** (86)	241 ± 15 (89)	2 ± 0.2*** (40)	2.3 ± 0.2***(40)	1.5 ± 0.1* (52)	39 ± 3** (34)	62 ± 8** (51)
R^2 value	**0.681**	**0.659**	**0.597**	**0.542**	**0.659**	**0.659**	**0.744**

Values relative to control are given in parentheses. The level of significance tested was denoted as p values * < 0.05, ** < 0.01, *** < 0.001.

Table 2. Effect of supply of $ZnCl_2$ on total protein, RNA and proline content of root and shoot tissue of the seedlings. Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C and various biochemical parameters were measured.

$ZnCl_2$]conc, mM	Root			Shoot		
	Total protein, mg g^{-1} fr wt	Total RNA, mg g^{-1} fr wt	Proline, µg g^{-1} fr wt	Total protein, mg g^{-1} fr wt	Total RNA, mg g^{-1} fr wt	Proline, µg g^{-1} fr wt
0.0	6.0 ± 0.4 (100)	18.1 ± 1.6 (100)	259 ± 14 (100)	6.8 ± 0.4 (100)	12.3 ± 1.3 (100)	171 ± 17 (100)
0.001	5.8 ± 0.4 (97)	16.0 ± 1.9 (88)	358 ± 43 (138)	6.9 ± 0.5 (102)	10.6 ± 0.7 (86)	183 ± 24 (107)
0.01	6.4 ± 0.5 (106)	15.5 ± 2.7 (86)	399 ± 14*** (154)	6.5 ± 0.4 (96)	9.9 ± 0.8 (81)	185 ± 14 (108)
0.1	8.6 ± 1.1 (143)	24.0 ± 3.2 (133)	342 ± 40 (132)	6.5 ± 0.1 (96)	9.6 ± 0.8 (78)	199 ± 22 (116)
1	9.6 ± 0.7** (160)	22.4 ± 0.4* (124)	381 ± 82 (147)	8.7 ± 1.2 (128)	9.3 ± 1.8 (76)	236 ± 23 (138)
2	12.9 ± 1.5** (215)	21.4 ± 1.4 (118)	405 ± 41** (156)	9.2 ± 0.45* (135)	9.7 ± 1.7 (79)	237 ± 33 (139)
5	13.3 ± 1.2***(222)	21.3 ± 1.1 (118)	456 ± 42** (176)	8.4 ± 0.60 (124)	9.2 ± 1.5 (75)	310 ± 37** (181)

Values relative to control are given in parentheses. The level of significance tested was denoted as p values * < 0.05, ** < 0.01, *** < 0.001.

Table 3. (a) Compound Correlation data of Zn Content, Root wt, Protein, RNA, Proline content in root tissues. (b) Compound Correlation data of Zn Content, Shoot wt, Protein, RNA, Proline content in shoot tissues.

(a)

	Zn content	Root wt	Protein	RNA	Proline
Zn content		−0.989	0.947	0.729	0.654
Root wt			−0.969	−0.695	−0.740
Protein				0.656	0.700
RNA					0.169
Proline					

(b)

	Zn content	Shoot wt	Protein	RNA	Proline
Zn content		−0.854	0.632	−0.494	0.939
Shoot wt			−0.787	0.826	−0.949
Protein				−0.481	0.727
RNA					−0.670
Proline					

(−0.854) and RNA (0.826), strong correlation of protein with Zn content (0.632), shoot wt (−0.787) and proline (0.727) and between RNA and proline −0.670) also resulted (Table 3(b)). While for RNA with Zn content and protein lesser correlation was observed.

3.3. Effect of $ZnCl_2$ on Chlorophyll Biosynthesis in Shoot Tissue of Light Grown Maize Seedlings

Treatment of light grown maize seedlings with $ZnCl_2$ at 1 - 5 mM increased the

chlorophyll, carotenoid and ALA content with significant effect for carotenoids at 5 mM Zn and for ALA at 2 and 5 mM both (Table 4(a)). To analyse ALA formation activity $ZnCl_2$ treated seedlings were incubated with 60 mM levulinic acid for 4 h either in light or dark corresponding to chloroplastic and mitochondrial activities respectively, the ALA accumulation in both was slightly enhanced and exerted no correlation (Table 4(b)), However, $ZnCl_2$ significantly increased ALAD activity at all the concentrations (Table 4(b)).

The compound correlation analysed for chlorophyll and carotenoid contents with Zn content exhibited very strong +ve correlation (0.862) but had shown very strong −ve correlation (−0.836 and −0.807) with shoot wt (Table 5(a)). For ALA with Zn content the correlation was strong being 0.703 and with shoot wt it was

Table 4. (a) Effect of supply of $ZnCl_2$ on total chlorophylls, carotenoids and ALA content in shoot tissue of the light grown seedlings. Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C. (b) Effect of $ZnCl_2$ on ALAS and ALAD activity in shoot tissue of light grown seedlings. Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C.

(a)

$ZnCl_2$ conc, mM	Total chlorophylls, μg g^{-1} fr wt	Carotenoids, μg g^{-1} fr wt	ALA content, nmoles g^{-1} fr wt
0.0	207 ± 8 (100)	23.3 ± 2.4 (100)	234 ± 13 (100)
0.001	206 ± 13 (100)	21.4 ± 2.7 (93)	235 ± 11 (100)
0.01	211 ± 13 (102)	24.3 ± 1.8 (104)	260 ± 18 (111)
0.1	196 ± 12.2 (95)	20.5 ± 1.8 (93)	259 ± 18 (111)
1	240 ± 12 (116)	29.0 ± 1.7 (126)	352 ± 70 (150)
2	229 ± 16 (111)	26.8 ± 3.8 (117)	312 ± 13* (133)
5	270 ± 19 (131)	35.9 ± 1.7** (157)	351 ± 16** (150)

Values relative to control are given in parentheses. The level of significance tested was denoted as p values * < 0.05, ** < 0.01, *** < 0.001.

(b)

$ZnCl_2$ conc, mM	ALAS activity, nmoles ALA formed h^{-1} g^{-1} fr wt In Light	ALAS activity, nmoles ALA formed h^{-1} g^{-1} fr wt In Dark	ALAD activity, nmoles PBG formed h^{-1} g^{-1} fr wt
0	88 ± 6 (100)	83 ± 5 (100)	200 ± 7 (100)
0.001	88 ± 3 (100)	93 ± 8 (112)	249 ± 4** (125)
0.1	99 ± 8 (113)	93 ± 7 (112)	263 ± 4** (132)
1	92 ± 5 (105)	91 ± 7 (110)	279 ± 5*** (140)
5	97 ± 5 (111)	96 ± 2 (116)	345 ± 11*** (173)

Values relative to control are given in parentheses. The level of significance tested was denoted as p values * < 0.05, ** < 0.01, *** < 0.001.

Table 5. (a) Compound Correlation data of Zn content, shoot wt, total chlorophyll, carotenoids, ALA content in shoot tissues of light grown maize seedlings; (b) Compound Correlation data of Zn content, shoot wt, ALAS light and dark treated and ALAD in shoot tissues of light grown maize seedlings.

(a)

	Zn content	Shoot wt	Chlorophyll	Carotenoid	ALA
Zn content		−0.854	0.862	0.862	0.703
Shoot wt			−0.836	−0.807	−0.908
Chlorophyll				0.992	0.891
Carotenoid					0.870
ALA					

(b)

	Zn content	Shoot wt	ALAS in light	ALAS in dark	ALAD
Zn content		−0.830	0.527	0.590	0.875
Shoot wt			−0.681	−0.776	−0.979
ALAS in light				0.624	0.658
ALAS in dark					0.852
ALAD					

perfect being −0.908 (Table 5(a)). Further, observed correlation of chlorophyll with carotenoid and ALA was perfect being 0.992 and 0.891, respectively and between carotenoid and ALA was 0.870 (Table 5(a)). Positive correlation for Zn content with ALA formation in light and dark was lesser (0.527 and 0.590), but for ALAD activity was very strong (0.875). Observed correlation of shoot wt with these parameters was negative and stronger being perfect with ALAD (−0.979) (Table 5(b)). Further, ALAD activity exerted strong correlation with ALA formation in light and dark both being 0.658 and 0.852, respectively and stronger with later (Table 5(b)).

3.4. Effect of $ZnCl_2$ on Chlorophyll Metabolism in Shoot Tissue of Dark Grown Maize Seedlings

Treatment of dark grown maize seedlings with $ZnCl_2$ decreased the chlorophyll content slightly and the carotenoid content remained unaffected, while the ALA content was significantly increased at 2 and 5 mM $ZnCl_2$ (Table 6(a)). The observed correlation with Zn treatment was found to be very strong for ALA content with R^2 value of 0.818 (Table 6(a)). When maize seedlings were treated with $ZnCl_2$, ALA formation activity both in light and dark and ALAD activity were enhanced at 1 and 5 mM showing significant increase for ALA formation in light at 1 mM treatment only (Table 6(b)). Zn treatment and these parameters also exerted strong correlation with R^2 value of 0.960, 0.728 and 0.691 respectively (Table 6(b)).

Table 6. (a) Effect of supply of $ZnCl_2$ on total chlorophylls, carotenoid and ALA content in shoot tissue of the dark grown seedlings. Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C. (b) Effect of $ZnCl_2$ on ALAS and ALAD activity in shoot tissue of dark grown seedlings. Three days old maize seedlings were treated with varying concentrations of $ZnCl_2$ for 24 h at continuous light intensity of 30 Wm^{-2} and temperature 26˚C ± 2˚C.

(a)

$ZnCl_2$ conc., mM	Total chlorophylls, µg g^{-1} fr wt.	Carotenoids, µg g^{-1} fr wt.	ALA, nmoles g^{-1} fr wt
0.0	115 ± 5 (100)	16.0 ± 1.3 (100)	333 ± 8 (100)
0.001	94 ± 7 (82)	15.9 ± 1.0 (99)	346 ± 9 (104)
0.01	108 ± 9 (94)	15.5 ± 1.1 (97)	355 ± 13 (107)
0.1	101 ± 9 (88)	15.4 ± 1.1 (96)	375 ± 14 (113)
1	121 ± 7 (105)	15.6 ± 1.4 (98)	410 ± 15 (123)
2	109 ± 12 (95)	16.0 ± 1.9 (100)	449 ± 5** (135)
5	106 ± 9 (92)	15.9 ± 0.6 (99)	475 ± 9** (143)
R^2 value	**0.007**	**0.152**	**0.818**

Values relative to control are given in parentheses. The level of significance tested was denoted as p values * < 0.05, ** < 0.01 *** < 0.001.

(b)

$ZnCl_2$ conc, mM	ALAS activity, nmoles ALA formed h^{-1} g^{-1} fr wt In Light	ALAS activity,moles ALA formed h^{-1} g^{-1} fr wt In Dark	ALAD activity, nmoles PBG formed h^{-1} g^{-1} fr wt
0	64 ± 4 (100)	77 ± 5 (100)	140 ± 25 (100)
0.001	69 ± 7 (108)	71 ± 5 (92)	149 ± 10 (107)
0.1	74 ± 7 (116)	68 ± 8 (88)	159 ± 10 (114)
1	80 ± 3* (125)	88 ± 7 (114)	168 ± 6 (121)
5	108 ± 19 (169)	96 ± 10 (125)	180 ± 3 (129)
R^2 value	**0.960**	**0.728**	**0.691**

Values relative to control are given in parentheses. The level of significance tested was denoted as p values * < 0.05, ** < 0.01, *** < 0.001.

4. Discussion

4.1. Growth Effects

Zn is an essential micronutrient for plants, but is toxic at high concentrations, hence its effect on growth depends upon the concentration. Thus decrease in growth due to Zn supply has been reported in ryegrass [5], sugar beet [7] cluster bean [6] and Sorgham [8], however, in mungbean [19] and *Eruca sativa* [9] increased growth resulted. In the present study, with the supply of $ZnCl_2$ to 3 days old maize seedlings, the effect on Zn accumulation and morphological and biochemical growth parameters have been analysed. The Zn treatment led to gradually increased accumulation of Zn in the shoot tissue, while in the root tissue

substantial increase was observed at/and above 0.1 mM $ZnCl_2$ (**Figure 1**). Also the root parameters were significantly reduced at/and above 0.1 mM $ZnCl_2$ (**Table 1**). Thus Zn supply significantly reduced the overall growth of maize seedlings mostly at 1 - 5 mM $ZnCl_2$ exerting strong correlation and the observed effect was more substantial for root tissue. Amongst biochemical parameters, increase in protein and proline content was more prominent in root tissue than the shoot, while RNA content was reduced in shoot tissue (**Table 2**). Reduction in RNA may be due to faster degradation and the increased protein due to translation of pre synthesized RNA. It seems that enhanced protein and proline synthesis is associated with Zn effects in root, as these contents are significantly increased. Increased levels of protein may be required for the synthesis of stress induced proteins [20] and of proline, for osmotic adjustments in response to Zn. Accumulation of proline is well documented against various stresses in plants, such as, *Spinacia oleracea* [21] and *Cucurbeta pepo* [22]. Further, the observed correlation for Zn accumulation in root with as well as between root wt and protein was perfect (**Table 3(a)**). Also perfect correlation for Zn accumulation in shoot and shoot wt with proline resulted (**Table 3(b)**). Thus the Zn accumulation and growth effect in maize depends upon the tissue with root as the target site.

4.2. Chlorophyll Biosynthesis Effects

Chlorophylls, the vital photosynthetic pigments and carotenoids, the light harvesting pigments in green tissues affect the overall photosynthetic activity and hence the growth and development of the plant. Chlorosis due to both Zn deficiency [23] and toxicity [2] have been observed in plants. Reports of decrease in chlorophyll content due to Zn supply in sugar beet [7], cluster bean [6] and Sorgham [8] but increase in mungbean [19] has been documented. In the present study, Zn treatment to light grown seedlings prominently increased the chlorophyll and carotenoid content, while in dark grown seedlings it had marginal/no effect (**Table 4(a)** and **Table 6(a)**). This indicated that the steady state level of the pigments is getting affected by Zn rather than inducible level. Content of ALA, a regulatory metabolite and universal precursor for all the tetrapyrroles, was increased at higher Zn concentrations in both the regimes (**Table 4(a)** and **Table 6(a)**). Thus increased synthesis of chlorophylls as well as other heme containing proteins is likely to result by Zn supply. Increased ALA content may involve enhanced synthesis and the ALA synthesis was also found to be increased in both the regimes in light as well as dark that corresponds to chloroplastic and mitochondrial activities respectively, however, the effect was non significant (**Table 4(b)** and **Table 6(b)**). Thus it seems that Zn exerts lesser effect to enhance ALA synthesis. Further, Zn supply increased ALAD activity of light as well as dark grown seedlings being significant in former (**Table 4(b)** and **Table 6(b)**). Hence, the next step of chlorophyll biosynthetic pathway catalysed by ALAD involving formation of porphobilinogen from ALA seems to get affected

by Zn. Further, ALADs from different sources are metalloenzymes that utilize a variety of divalent and monovalent cations [11] ALAD requires Mg^{2+} and Zn^{2+} for its activity [10] hence its activity is enhanced by Zn treatment. Moreover, in light grown seedlings the observed correlation for pigment content with Zn accumulation and shoot wt was very strong for both, +ve (0.862 and 0.862) and –ve (0.836 and 0.807), respectively (**Table 5(a)**). Also for ALA content and ALAD with shoot wt perfect –ve correlation resulted (**Table 5(a)** and **Table 5(b)**). Moreover, ALAD has been reported to be a substrate-modulated enzyme [24]. Hence, it is likely that during Zn treatment shoot growth and chlorophyll formation is mainly influenced by ALA and subsequently by ALAD in maize seedlings.

5. Conclusion

$ZnCl_2$ treatment accumulated Zn in root as well as shoot tissue with more substantial increase in former. Zn supply reduced the overall growth of the maize seedlings at higher concentrations with more prominent effect in roots. Increase in protein and proline content by Zn was more prominent in root tissue than the shoot. Zn increased the chlorophyll, carotenoid and ALA content in the shoot tissue of light grown seedlings and the ALA content in dark grown seedlings also. Increase in ALA formation and ALAD activity also resulted by Zn being most significant in light grown seedlings. The results suggest that Zn accumulation and growth effect at higher Zn concentrations in maize depend upon the tissue with root as the target site and shoot growth are mainly influenced by ALA and subsequently ALAD in maize seedlings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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