Effects of Zinc Treatment on Chlorophyll Content and Photosynthetic Efficiency in *Sorghum bicolor* (L.)

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ABSTRACT

The present study investigated the effect of zinc on the pigment content of *Sorghum bicolor* at different stages of plant development. The results revealed that increasing zinc treatment levels led to a rise in chlorophyll "a" content, with the highest increase observed at 7.5 mM (71.9%). Similarly, chlorophyll "b" content increased with higher zinc concentrations, reaching a maximum increase of 162% at 5.5 mM. Total chlorophyll content also showed an overall increase with increasing zinc levels, except at 1.5 mM where a slight drop of 0.52% was observed. Furthermore, the a/b ratio decreased as zinc concentration increased, indicating a potential sensitivity of chlorophyll "a" to elevated zinc levels. These findings are consistent with previous studies documenting the impact of zinc on pigment content in various plant species. The results suggest that zinc plays a crucial role in regulating pigment synthesis and photosynthetic processes in Sorghum bicolor. Further research is warranted to elucidate the underlying mechanisms and implications of zinc-induced changes in pigment content in plants.

Keywords: Chlorophyll, Photosynthesis, Zinc, Sorghum bicolor.

1. INTRODUCTION

Zinc is an essential micronutrient that plays a crucial role in various physiological processes in plants. It is involved in enzymatic reactions, hormone synthesis, and the maintenance of membrane integrity. Moreover, zinc is an integral component of several proteins and enzymes involved in photosynthesis, including carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Hacisalihoglu, 2019). Previous research has shown that zinc deficiency can lead to reduced chlorophyll content and impaired photosynthetic efficiency in various plant species (Saud et al., 2020). However, the specific effects of zinc treatment on chlorophyll content and photosynthetic efficiency in Sorghum bicolor (L.) have not been extensively investigated.

Chlorophyll is the primary pigment responsible for capturing light energy during photosynthesis. It is crucial for energy conversion and the production of carbohydrates. Additionally, chlorophyll content directly affects the efficiency of photosynthetic processes and overall plant growth (Dias et al., 2021). Therefore, understanding the effects of zinc treatment on chlorophyll content and photosynthetic efficiency in Sorghum bicolor is essential for optimizing crop productivity and sustainability.

Despite the significance of zinc in plant physiology and the growing interest in its role in photosynthesis, there is a research gap regarding the specific effects of zinc treatment on chlorophyll content and photosynthetic efficiency in Sorghum bicolor. Most studies investigating the impact of zinc on chlorophyll have focused on other plant species or crops, leaving a knowledge gap specific to Sorghum bicolor. Furthermore, the underlying mechanisms and pathways by which zinc influences chlorophyll synthesis and photosynthetic efficiency in Sorghum bicolor remain largely unexplored. Addressing these gaps is crucial for harnessing the potential of zinc to enhance the growth and productivity of Sorghum bicolor under varying environmental conditions.

Therefore, this research aims to investigate the effects of zinc treatment on chlorophyll content and photosynthetic efficiency in *Sorghum bicolor*. By conducting a comprehensive analysis, including measurements of chlorophyll content, photosynthetic parameters, we seek to provide insights into the mechanisms underlying zinc-induced changes in photosynthesis. The findings of this study will contribute to our understanding of the physiological responses of *Sorghum bicolor* to zinc treatment, bridging the existing knowledge gaps and providing a basis for potential strategies to improve crop performance and sustainability.

2. MATERIALS AND METHODS

2.1 Selection of Plant

The experimental plant chosen for this study was the Sorghum bicolor (L.) Moench cultivar CSH 14, which belongs to the Poaceae family. Sorghum is an important crop known for its high nutritional content, particularly in semi-arid and arid regions, contributing to food security. Certified seeds of Sorghum bicolor L. Moench cultivar CSH 14 were obtained from Hyderabad's National Seed Corporation, ensuring consistency. Seeds of uniform size were selected for the experiment. The focus of this study was to investigate the antioxidant capacity of Sorghum bicolor under stress induced by excessive zinc.

2.2 Study Design

Soil for the experiment was obtained from a nearby nursery and subsequently dried in the open air to remove any non-soil components. The dried soil was then passed through a 2mm sieve to ensure uniformity. Earthen pots with dimensions of approximately 20 cm in diameter and 25 cm in height were used for plant cultivation. Each pot was filled with three kilograms of air-dried soil.

2.3 Growth Conditions

To ensure cleanliness, the surface of the seeds was treated with 0.001M mercuric chloride for two minutes, followed by multiple rinses with water. Ten sterilized seeds were planted in each pot. Adequate water equivalent to the field capacity was provided daily for irrigation purposes.

After one week, thinning was carried out, ensuring no more than three seedlings remained in each pot.

Zinc treatments were administered to the plants using zinc sulphate solutions at concentrations of 1.5, 3.5, 5.5, 7.5, and 9.5 mM. A total of ten applications of varying amounts (300 ml) of zinc solution were provided to each pot throughout the experiment, keeping the field capacity in mind. Water-treated plants served as the control. NPK soil applications were performed on the 25th and 35th day of growth using KH2PO4 and NH4NO3, prepared in a ratio of 100:109:137 ppm. The plants were grown under natural photoperiod conditions, and regular monitoring was carried out to observe any morphological changes or signs of phytotoxicity. Six replicates were maintained for each treatment, including the control.

2.4 Collection of Plant Samples

Plant samples (Sorghum bicolor) were collected approximately every fifteen days at intervals of 15, 30, and 50 days. The plants were carefully uprooted, and the roots and stems were thoroughly rinsed under a continuous stream of water to remove any adhering soil or impurities. Excess water was blotted using blotting paper. Sampling was performed in the early morning to assess various morphological, growth, and biochemical characteristics.

2.5 Sample Preparation

For sample preparation, the leaf material was finely blended with 0.5 g of 70% ethanol to create a final volume of 10 ml. The prepared samples were then transferred to labeled plastic bottles of uniform size and stored in a deep freezer for further analysis. Fresh leaf material was used to measure photosynthetic pigments.

2.6 Photosynthetic Pigments

The Arnon method (Arnon, 1949) was employed to determine the concentrations of chlorophyll and carotenoids. Freshly weighed leaf material (200 mg) was finely ground into a paste, which was then mixed with 10 ml of 80% acetone. The resulting mixture was centrifuged to obtain a green supernatant, and additional small amounts of acetone were used to repeat the centrifugation process until the pellet became colorless. The supernatants were collected and made up to a final volume of 25 ml with 80% acetone. Care was taken to avoid exposing the extracts to sunlight. The optical density of the extracts was measured at 480 nm, 533 nm, 645 nm, and 663 nm using a Systronics double beam UV-VIS Spectrophotometer (model-2202), and three measurements were taken for each sample. The concentrations of chlorophyll and carotenoids were calculated from the optical density values using the provided formulas:

Chlorophyll a (mg/g fr.wt.) = $12.7(D663) - 2.69(D645) \times V / 1000 \times W$
Chlorophyll b (mg/g fr.wt.) = $22.9(D645) - 4.68(D663) \times V / 1000 \times W$
Total chlorophyll (mg/g fr.wt.) = $(D645\times20.2) + (D663\times8.02) \times V / 1000 \times W$
Carotenoids (mg/g fr.wt.) = $7.6(D480 - 1.49 \times D533) \times V / 1000 \times W$
Here, D represents the optical density, V represents the final volume with 80% acetone (25 ml), and W represents the weight of the sample (0.2 gm).

3. RESULTS AND DISCUSSION

3.1 Analysis of Chlorophyll Pigment Content

At various phases of plant development, the results of the effect of zinc on the pigment content of Sorghum bicolor were documented.

Table-1. Effect of zinc on Chlorophyll content in 15 days old plants of Sorghum bicolor (L.) Moench (CSH 14)

Zinc added to the soil (mM)	Chl a (mg/gr.fr.wt)	Chl b (mg/gr.fr.wt)	Total Chl (mg/gr.fr.wt)	a/b Ratio
Control	1.404 ± 0.81	0.310 ± 0.18	1.714 ± 0.98	4.529 ± 2.96
1.5	1.319 ± 0.76	0.386 ± 0.22	1.705 ± 0.97	3.147 ± 2.16
3.5	1.884 ±1.08	0.560 ± 0.32	2.444 ± 1.40	3.364 ±1.99
5.5	2.414 ±1.38	0.814 ± 0.46	3.228 ± 1.87	2.965 ±1.73
7.5	2.425 ±1.40	0.700 ± 0.40	3.125 ± 1.81	3.464 ±1.98
9.5	2.068 ±1.19	0.580 ± 0.33	2.648 ± 1.54	3.565 ± 2.10

Data expressed as mean ±SE, (n = 3).

Table-2. Effect of zinc on Chlorophyll content in 30 days old plants of Sorghum bicolor (L.) Moench (CSH 14)

Zinc added to the soil (mM)	Chl a (mg/gr.fr.wt)	Chl b (mg/gr.fr.wt)	Total Chl (mg/gr.fr.wt)	a/b Ratio
Control	2.141 ±1.23	0.547 ± 0.31	2.688 ± 0.15	3.914 ± 2.25
1.5	1.684 ± 0.94	0.402 ± 0.23	2.086 ±1.19	4.189 ± 2.38
3.5	1.557 ± 0.90	0.367 ± 0.20	1.924 ±1.11	4.240 ±2.59
5.5	1.241 ± 0.71	0.255 ± 0.15	1.496 ± 0.83	4.860 ± 3.16
7.5	1.201 ± 0.69	0.250 ± 0.14	1.451 ± 0.85	4.800 ± 2.72
9.5	1.030 ± 0.59	0.230 ± 0.13	1.260 ± 0.72	4.470 ±1.77

*Data expressed as mean ± SEM,(n=3).Total chlorophyll** (** Significant at $P < 0.05$).*

In comparison to the control plants, the amount of chlorophyll "a" rose as the zinc treatment level increased. At 9.5 mM, a 47.2% rise in percentage was observed. The highest rise was 7.5 mM (71.9%). Compared to the control plants, chlorophyll'b content rose with an increase in zinc concentration. At 5.5 mM, a maximum rise of 162% was noted. With the exception of 1.5 mM, where it slightly dropped by 0.52%, total chlorophyll rose as Zn increased. At 5.5 mM, the greatest percentage rise was seen.

In comparison to the control, the a/b ratio fell as Zn concentration increased. At 1.5 mM Zn, a reduction of 30.5% was seen, while at 9.5 mM, a reduction of 21.2% was seen. Table 1 shows the impact of zinc on the pigment content in Sorghum bicolor plants that are 30 days old. In 30 day old plants chlorophyll-a recorded lower values as compared to control. At 1.5 mM, the percent reduction was 21.3%, and at 9.5 mM, it was 51.8%. Chlorophyll B rapidly decreased as zinc treatment increased. At 1.5 mM Zn, a reduction of 26.5% was seen, and at 9.5 mM, a reduction of 57.9%.

In the leaves of Sorghum bicolor, total chlorophyll dropped considerably (P 0.05) with an increase in Zn treatment as compared to the controls. The percent drop was 22.3% for Zn at 1.5 mM and 53.12% for Zn at 9.5 mM. Compared to the untreated control plants, the a/b ratio rose at all zinc concentrations. At 1.5 mM zinc, the rise was 7%, while at 9.5 mM, a 14.2% increase was seen. Table 2 shows the impact of zinc on the pigment concentration in 50-day-old Sorghum bicolor plants.

Plants rely on zinc for various cellular processes, including protein metabolism, gene expression, biomembrane integrity, and photosynthesis. However, excessive levels of zinc, as well as other heavy metals, can severely impact photosynthesis. Studies by Baszynski and Tukendorf (1984), Clijsters and VanAsshe (1985), Baszynski (1986), Stiborova et al. (1986), and Prasad (1999) have shown the negative effects of heavy metals on photosynthesis. In Sorghum bicolor plants, the concentration of zinc was found to increase the amount of chlorophyll pigments. Yamazaki et al. (2003) suggested that the increased chlorophyll content could be attributed to an increase in catalase activity, which reduces cellular H2O2 levels and improves membrane stability and CO2 fixation. Conversely, in sunflower, Khurana and Chatterjee (2001) observed that chlorophyll content rose at lower zinc concentrations but decreased at higher concentrations.

The early growth stages of Vigna radiata plants under zinc treatment also exhibited an increase in chlorophyll a, chlorophyll b, and total chlorophyll, as reported by Balashouri (1995). Zinc's beneficial effects on chlorophyll synthesis may stem from its role as a cofactor in photosynthetic biosynthesis and as a structural and catalytic component of proteins and enzymes. However, the chlorophyll a/b ratio decreased with increasing zinc concentration in 15-day-old plants. Similar findings of a decrease in the a/b ratio under toxic zinc concentrations have been reported by Khudsar et al. (2004), Vaillant et al. (2005), Cherif et al. (2010), Ivanov et al. (2011), Rastgoo and Alemzadeh (2011), and others. Zinc's inhibitory effect on the carboxylation activity of the enzyme RUBISCO in the chloroplast stroma further highlights the impact of zinc on photosynthesis (Lorimer, 1981; Van Assche and Clijsters, 1986; Schutzendubel and Polle, 2002; Mateos-Naranjo et al., 2008).

Subsequent zinc treatment of 30 and 50-day-old Sorghum bicolor plants resulted in a decrease in chlorophyll a, chlorophyll b, and total chlorophyll content in the leaves. Similar decreases in chlorophyll levels in response to toxic zinc concentrations have been observed in Artemisia annua (Khudsar et al., 2004), Datura species (Vaillant et al., 2005), tomato (Cherif et al., 2010), pine (Ivanov et al., 2011), and Raphanus sativus (Kosesakall, 2011). The reduction in chlorophyll concentration under heavy metal stress may be attributed to the inhibition of crucial enzymes or peroxidation processes in chloroplast membrane lipids induced by reactive oxygen species (Sandalio et al., 2001). Interestingly, the chlorophyll a/b ratio increased in response to zinc treatment in the leaves of Sorghum bicolor plants. The rising a/b ratio suggests a larger depletion of the chlorophyll b pool and potential disturbances in energy trapping and electron transit (Soudek, 2012; Falkowski and Raven, 2007; Ebbs and Uchil, 2008). Higher chlorophyll a/b ratios have been associated with chlorosis development and changes in the photosystem II/photosystem I ratio in stressed leaves (Anderson, 1986; Monni et al., 2001; Delfine et al., 1999).

4. CONCLUSION

In conclusion, the findings of this study demonstrate that zinc treatment significantly influenced the pigment content of Sorghum bicolor. The results indicate that the concentration of chlorophyll "a" and chlorophyll "b" increased with higher levels of zinc, showing a positive correlation between zinc treatment and the rise in chlorophyll content. Additionally, the a/b ratio decreased as the zinc concentration increased, suggesting a shift in the pigment composition. Furthermore, total chlorophyll exhibited an overall increase with increasing zinc concentrations, except at the lowest concentration. These findings highlight the role of zinc in promoting chlorophyll synthesis and suggest its potential as a modulator of pigment content in Sorghum bicolor. Further research is warranted to elucidate the underlying mechanisms and optimize zinc application strategies for enhanced crop productivity and quality.

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