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# EFFECT OF ZINC NUTRITION ON SUCROSE BIOSYNTHESIS IN MAIZE

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**Key Word Index**—*Zea mays*; Gramineae; maize; Zn nutrition; sucrose synthetase.

**Abstract**—Zinc deficiency caused an accumulation of $^{14}C$ into malic acid, sugar phosphates, sugar nucleotides, glucose, fructose, phosphoenolpyruvate, glycine and alanine, whereas the $^{14}C$ labelling in sucrose decreased. The activity of sucrose synthetase (EC 2.4.1.13) was unaffected up to the 15th day and thereafter it declined. Severe Zn deficiency reduced the biosynthesis of total protein and sucrose synthetase by 50 and 20%, respectively.

## INTRODUCTION

Zinc is well known as an essential micronutrient for the normal growth of plants and seems to be involved in sucrose metabolism. Zn deficiency caused a decline in the level of sucrose in sugarbeet [1] and maize shoots [2]. The present investigation was undertaken to determine the level of sucrose synthetase in Zn-deficient maize plants.

## RESULTS AND DISCUSSION

Data on the distribution of $^{14}C$ in various products of photosynthesis are presented in Table 1. Zinc affected the intermediates of the $C_3$- and $C_4$-pathway for $CO_2$ fixation. Greater accumulation of $^{14}C$ was located in sugar phosphates and nucleotides, glucose, fructose, alanine, glycine, phosphoenolpyruvate and malic acid in Zn-deficient plants (0 ppm Zn). At the same time Zn-deficient plants had a smaller accumulation of $^{14}C$ in sucrose. These results are similar to those of our previous report on maize [2].

Data on enzyme activity at various growth stages are depicted in Table 2. Initially, after 5 days there was no significant effect of Zn deficiency on the activity of sucrose synthetase. However, on the 20th day, the enzyme activity was significantly reduced due to severe Zn deficiency (0 ppm Zn). After this stage, the Zn-deficient plants were unable to survive.

The incorporation of phenylalanine-[$^{14}C$] into sucrose synthetase and homogenate protein was reduced by 20 and 50%, respectively, in Zn-deficient leaves (Table 3). The effect of 0.05 ppm Zn application on the incorporation of phenylalanine-[$^{14}C$] into enzyme and homogenate protein was found to be intermediate. Inhibition in protein synthesis under Zn-stressed conditions has been reported earlier [3]. Zn deficiency in *Euglena gracilis* resulted in the disappearance of cytoplasmic ribosomes [4] which were the sites of protein biosynthesis. Moreover, the accumulation of free amino acids (alanine and glycine, Table 1)

Table 1. Effect of Zn deficiency on % distribution of $^{14}C$ in various photosynthates. Each value is the mean of 2 replicates

| Photosynthates | % of total $^{14}C$ 0.0 ppm-Zn | 0.1 ppm-Zn |
|---|---|---|
| 1. Sugar phosphates + Sugar nucleotides | 37.0 ± 2.1 | 26.0 ± 1.4 |
| 2. Glucose | 9.5 ± 1.2 | 4.5 ± 0.3 |
| 3. Fructose | 8.2 ± 0.8 | 5.3 ± 1.3 |
| 4. Alanine | 12.1 ± 1.7 | 6.0 ± 0.4 |
| 5. Glycine | 8.0 ± 0.4 | 3.5 ± 0.7 |
| 6. Malic acid | 13.0 ± 0.8 | 8.5 ± 0.9 |
| 7. PEP | 8.0 ± 0.6 | 4.5 ± 0.8 |
| 8. Sucrose | 4.2 ± 1.6 | 41.7 ± 3.6 |

Table 2. Effect of Zn nutrition on sucrose synthetase activity. Each value is the mean of 3 replicates

| Plant age (days) | Sucrose synthetase activity (EU/mg protein) 0.0 ppm-Zn | 0.05 ppm-Zn | 0.1 ppm-Zn |
|---|---|---|---|
| 5 | 0.07 | 0.06 | 0.07 |
| 10 | 0.08 | 0.07 | 0.09 |
| 15 | 0.06 | 0.09 | 0.09 |
| 20 | 0.04 | 0.08 | 0.11* |

* Significant at 5% level. CV = 7.2%.

Table 3. Effect of Zn nutrition on the incorporation of phenylalanine-[$^{14}C$] into the homogenate protein and purified enzyme protein (sucrose synthetase, EC 2.4.1.13). Each value is the mean of 3 replicates

| Zn treatment (ppm) | Purified enzyme protein (dpm/mg protein) | Homogenate protein (dpm/mg protein) |
|---|---|---|
| 0.0 | 248 ± 7.2 | 3150 ± 31.3 |
| 0.05 | 260 ± 4.6 | 4180 ± 23.7 |
| 0.10 | 305* ± 6.3 | 6200* ± 43.4 |

* Significant at 5%.

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under Zn deficiency also suggested that the incorporation of amino acids into proteins was restricted. The accumulation of sugar nucleotides (ADPG or UDPG) under Zn deficiency showed decreased utilization in the biosynthesis of sucrose via sucrose synthetase.

## EXPERIMENTAL

*Plant culture.* Maize (*Zea mays* L. cv Ganga-5) seeds were surface-sterilized with 0.01% $HgCl_2$ soln for 5 min and washed repeatedly with $H_2O$. The seeds were then germinated in Petri dishes lined with moist filter paper at 28 ± 2° in the dark. After 4 days, the seeds were removed and the seedlings transferred to 250 ml conical flasks containing aerated, purified [5, 6] nutrient soln [7]. Three Zn levels, viz. 0, 0.05 and 0.1 ppm, were supplied in the form of $ZnSO_4$. The plants were grown under green-house conditions.

*Exposure to* $^{14}CO_2$. Plants (10 days old) were exposed at normal atmospheric $CO_2$ concn to $^{14}CO_2$ (50 $\mu$Ci) in an assimilation chamber (20 × 30 × 25 cm) in bright sunlight for 10 min. The plant shoots were then killed and extracted in 80% EtOH. The EtOH-soluble fraction was collected after centrifugation. The products were separated and $^{14}C$ was counted by the method described earlier [2].

*Extraction and assay of sucrose synthetase.* Leaf samples were ground with 5 vol. of Tris–HCl 0.1 M (pH 7.8), $MgCl_2$ (10 mM), EDTA (2 mM), mercaptoethanol (10 mM) and PVP (5%). The homogenate was filtered through nylon mesh. The filtrate was centrifuged at 10 000 g for 20 min at 0°. The method of ref. [8] was used for the enzyme assay of the supernataut obtained.

*Incorporation of phenylalanine-*[$^{14}C$] *into enzyme protein.* Leaf discs (0.4 cm) were taken from the young leaves of Zn-deficient plants. Leaf discs (100 per dish) were floated on 5 ml of a soln containing 0.04 M Tris–HCl (pH 7.3), 0.005% Triton X-100, 0.1 $\mu$Ci phenylalanine-[$^{14}C$] and a designated amount of $ZnSO_4$. The discs were shaken in the light (1.2 mW/cm$^2$ for 20 hr).

*Enzyme purification.* Leaf discs, treated in the same way, were then pooled for the extraction and purification of sucrose synthetase. A known amount of leaf discs was ground in Tris–HCl (pH 7.8). Na bisulphite (5 mmol) followed by 20 mmol cysteine was added and the pH of the homogenate was adjusted to 8 with NaOH. The homogenate was then centrifuged at 9000 g for 30 min. Solid $(NH_4)_2SO_4$ was added to the supernatant to 30% satn. The ppt. formed was removed by centrifugation. The concn of $(NH_4)_2SO_4$ was increased to 35% satn and the ppt. formed was again removed by centrifugation and collected. The ppt. was dissolved in 0.1 M Pi buffer (pH 8) followed by dialysis for 18 hr at 4° against this buffer. The dialysed fraction was then applied to a 6 × 40 cm of DEAE-Sephadex A-50 column pre-equilibrated with 0.1 M Pi buffer containing 0.1 M NaCl and 0.1 M cysteine (pH 8). Fractions showing maximum sucrose synthetase activities were collected and precipitated by $(NH_4)_2SO_4$ at 50% satn. The ppt. was dissolved in 10 ml of 0.1 M Pi buffer (pH 8) containing 0.1 M NaCl and 0.1 M cysteine. This enzyme soln was dialysed once again, and the dialysed enzyme obtained was used for enzymatic assay. The radioactivity of phenylalanine-[$^{14}C$] in the aliquots of initial homogenate and purified enzyme prepn was determined by liquid scintillation counting. Protein was estimated by the method of ref. [9] with crystalline BSA as standard.

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