# Indole Compounds in Zinc-Deficient Plants

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The first investigation on the relationship between zinc and indole-3-acetic acid (IAA) in plant growth was made by Skoog[1]. He showed that when tomato plants were grown in a zinc-deficient solution, the plants failed to elongate and their IAA content was extremely low as compared with that of the control plants. Addition of zinc to the culture solution caused an increase of IAA in plants within 24 hr with an elongation of stems. The failure in IAA production seemed to be specific to zinc deficiency, since Skoog could find no effect of copper and manganese deficiencies on IAA production before growth reduction.

This relationship between zinc and IAA content was also confirmed by Tsui[2]. IAA formation from tryptophan in higher plants was demonstrated by Wildman et al. However, Tsui found that the conversoin of tryptophan to IAA was not affected by zinc deficiency. On the basis of these findings, Tsui concluded that zinc is required to synthesize tryptophan but not for reactions leading to IAA formation from tryptophan.

Recently, Tsui's conclusion was supported by Salami et al.[3] They grew maize in nutrient solutions to which no zinc was added. The plants developed symptoms characteristic of zinc deficiency and grew poorly. Addition of either zinc or tryptophan to the solutions eliminated these effects—an indirect evidence that zinc is necessary for the synthesis of tryptophan and for maintaining its optimal level.

Tryptophan is thoght to be converted to IAA through either indolepyruvic acid or tryptamine and indoleacetaldehyde, which appear to be the immediate precursors of IAA. Of these two pathways, the oxidative deamination of tryptophan leading to the formation of indolepyruvic acid, as suggested originally by Thimann, has been most favoured.

Recently, Thimann et al.[4] showed that the conversion of tryptamine to IAA occurs in peas and oats under sterile conditions. Muir et al.[5] also showed that the principal pathway of IAA synthesis by the enzyme system from peas was the decarboxylation of tryptophan to tryptamine, followed by oxidation, and that gibberellic acid promoted the conversion of tryptophan and tryptamine to IAA.

The present study aimed to clarify the role of zinc in higher plants in relation to IAA. This report describes the distinct accumulation of free tryptophan and tryptamine observed in zinc deficient plants.

## Chemical components of zinc deficient plants[6]

Contents of soluble nitrogen fraction including amides and amino acids, organic acids, sugars and total phosphate were higher and the content of insoluble nitrogen fraction was lower in zinc deficient maize seedlings.

## Zinc-deficient plants and tryptophan[7]

Identification and determination of tryptophan in plants were made as follows: Cation exchange column chromatography was used for the determination of tryptophan in zinc deficient maize extract. In the 0.35 N Na-citrate buffer system, pH 5.28, an unidentified peak (a) appeared at the elution time of 40 min in front of the lysine peak. Its position was completely coincident with that given by authentic tryptophan. In the 0.38 N Na-citrate buffer system, pH 4.26, an unidentified peak (i) appeared at the elution time of about 125 min and it was the same as that of the authentic tryptophan. Therefore, the peak (a) and (i) were considered to be tryptophan. For the identification of the peak (a), the fraction was collected and treated with cation exchange resin to remove citrate. The resultant slightly yellow substance (a) gave the same Rf value as that of tryptophan on a thin layer chromatogram. This (a) was further identified as tryptophan by its reactions to ninhydrin and Ehrlich reagent.

Changes in free tryptophan content caused by different levels of zinc supply were determined with an amino acid analyzer. Under the condition of adequate zinc nutrition (Zn 50 ppb), free tryptophan was detected in maize leaves but the amounts were very small as compared with those in zinc-deficient plants. The maximum accumulation coincided with the development of severe symptoms of advanced zinc deficiency (Table 1).

Changes in the content of free arginine were quite similar to those of tryptophan.

Under the condition of zinc deficiency, free tryptophan was detected in higher concentrations in tomato, rape and barley seedlings too (Table 2).

On the other hand, total tryptophan contents were not affected by zinc nutrition, except under the condition of extreme zinc deficiency.

## Zinc-deficient plants and tryptamine[7)]

Identification of tryptamine was made as follows: A number of Ehrlich-positive spots were detected on the thin layer chromatogram of the maize extract developed with n-butanol: acetic acid: water (4:1:2) as a solvent. Spot, 1, a dominant spot in zinc deficient maize extract, gave the same Rf value as authentic tryptamine.

The eluate from the spot 1 on the thin layer chromatogram was further chromatographed with a number of solvents. In each case the Rf values coincided with those of the authentic tryptamine, and no difference between the spot 1 and the authentic tryptamine was found in chromogenic reaction such as Ehrlich reaction, ninhydrin reaction, Salkowsky reaction and UV fluorescence.

**Table 1. Effect of zinc nutrition on free tryptophan and free arginine contents in maize leaves**

| Treatment | Zinc concentration in culture soln. (ppb) | Treatment periods (day) | Growth[a)] Top F. W.[b)] g/plant | Free Tryptophan $\mu$ mole/F. W. g | Free Arginine $\mu$ mole/F. W. g |
|---|---|---|---|---|---|
| 1 | 0 | 12 | 7. 0 | 0. 01 | 0. 18 |
| 2 | 0 | 15 | 10. 5 | 0. 03 | 0. 52 |
| 3 | 0 | 17 | 13. 4 | 0. 12 | 0. 86 |
| 4 | 0 | 20 | 12. 8 | 0. 15 | — |
| 5 | 8 | 15 | 18. 8 | 0. 02 | 0. 08 |
| 6 | 50 | 20 | 30. 0 | 0. 01 | 0. 05 |

a) Appearance of plant growth
Treatment 1: Almost normal except that leaves seemed to be dark green
〃 2: Slightly stunted and short internodes
〃 3: Purple leaves, white secretion on the leaf margin
〃 4: Yellowish white buds, old leaves were dead. Typical symptoms of zinc deficiency
〃 5: Slightly stunted and short internodes
〃 6: Normal

b) F. W.=Fresh weight

**Table 2. Effects of zinc nutrition on free tryptophan and free arginine contents in leaves of various plants**

| Plant | Treatment | Growth Top F. W. g/plant | Free Tryptophan $\mu$ mole/F. W. g | Free Arginine $\mu$ mole/F. W. g |
|---|---|---|---|---|
| Tomato | Control | 36.9 | 0.01 | 0.03 |
| | -Zn | 16.4 | 0.07 | 0.30 |
| Rape | Control | 45.4 | 0.01 | 1.76 |
| | -Zn | 8.3 | 0.04 | 34.20 |
| Barley | Control | 36.1 | 0.03 | 0.08 |
| | -Zn | 18.8 | 0.16 | 1.03 |

Treatment periods: Tomato var. Fukuju No. 2 (Oct. 7–Nov. 8)
Rape (Dec. 14–Feb. 4)
Barley var. New golden (Jan. 6–Mar. 13)

**Table 3. Effect of zinc nutrition on tryptamine content in maize leaves**

| Treatment | Zinc concentration in culture soln. (ppb) | Treatment periods (day) | growth[a] (Top F. W. g/plant) | Tryptamine ($\mu$ mole/F. W. 100 g) |
|---|---|---|---|---|
| 1 | 0 | 18 | 11.4 | 0.13 |
| 2 | 0 | 24 | 16.5 | 0.20 |
| 3 | 0 | 27 | 15.0 | 0.25 |
| 4 | 50 | 24 | 35.0 | 0.02 |

a) Appearance of plant growth:
Treatment 1: Slightly stunted
〃 2: Purple leaves
〃 3: Typical symptoms of zinc deficiency
〃 4: Normal

The UV absorption spectrum of the spot 1 was compared with that of tryptamine. In this experiment, the spot 1 was further purified by rechromatography with acetic acid : methanol : $CHCl_3$ (4 : 1 : 15) followed by a second chromatography (paper chromatography) with 8% NaCl. A section of the chromatogram containing a purified spot 1 was cut out and extracted with 80% ethanol. The UV absorption spectrum of the spot 1 in 80% ethanol coincided completely with that of authentic tryptamine. Both the spot 1 and tryptamine gave the same absorption spectra having a maximum at 280 m$\mu$ with a characteristic shoulder at about 285 m$\mu$ and a minimum at 245–250 m$\mu$.

Changes in tryptamine content caused by different zinc levels were determined by means of paper chromatography and thin layer chromatography. The content of tryptamine was highest in maize leaves which developed symptoms of zinc deficiency (Table 3). With an adequate zinc nutrition (Zn 50 ppb), tryptamine was also found in maize leaves, although the amounts were very small in contrast to those in zinc dificient plants. Tryptamine in maize seedlings accumulated at an early stage of zinc deficiency, and its concentration increased considerably when the external symptoms of zinc deficiency became severe.

Considerable accumulations of tryptamine were not observed in plants deficient of iron, copper, manganese and boron (Table 4).

Tryptamine was detected in high concentrations in zinc-deficient tomato and barley plants too (Table 5).

Tryptamine accumulated in the zinc-deficient maize seedlings was decreased in its concentration and amount by the addition of zinc to the culture solution (Zn 0.1 ppm), and the profound elongation of stem internodes

**Table 4. Effect of micro nutrients on tryptamine contents in maize leaves**

| Treatment | Growth Top F.W. g/pot | Tryptamine g/F.W. 100 g Roots | Leaves | Appearance of plant growth |
|---|---|---|---|---|
| -B | 11.0 | 2 | 4 | Damaged meristematic regions |
| -Cu | 30.0 | 2 | 6 | Chlorotic leaves with margins rolled up |
| -Mn | 40.0 | 1 | 4 | Interveinal chlorosis |
| -Fe | 29.0 | 1 | 4 | Chlorosis of young leaves |
| -Zn | 23.0 | 10 | 30 | Failure of internode to elongate |
| Control | 35.5 | 1 | 4 | Normal |

Treatment periods
-B : Aug. 26-Sep. 7
-Cu : Aug. 26-Sep. 11
-Mn : Aug. 26-Sep. 11
-Fe : Sep. 17-Oct. 8
-Zn : Sep. 19-Oct. 16
Control: Sep. 19-Oct. 16

**Table 5. Effect of zinc nutrition on tryptamine contents in young plants of several species**

| Plant species | Zinc concentration in culture soln. (ppb) | Growth Top F.W. g/pot | Tryptamine g/F.W. 100 g Roots | Leaves |
|---|---|---|---|---|
| Tomato | 0 | 19.5 | 1 | 6 |
| | 5 | 34.0 | Trace | 4 |
| | 50 | 36.0 | Trace | 4 |
| Rape | 0 | 9.3 | Trace | Trace |
| | 5 | 26.0 | Trace | Trace |
| | 50 | 46.0 | Not detected | Not detected |
| Barley | 0 | 15.5 | 2 | 800 |
| | 5 | 20.0 | 2 | 200 |
| | 50 | 17.5 | 2 | 80 |
| Maize | 0 | 12.8 | 20 | 50 |
| | 8 | 20.5 | 10 | 20 |
| | 50 | 30.0 | 2 | 4 |

took place soon after (Plate 1).

## Zinc-deficient plants and IAA[6]

An aliquot of the acid ether fraction, equivalent to 100 g of fresh weight (F.W.) of shoots, was spotted on a plate and the plate was developed by using benzen:acetic acid:water (2:2:1) as a solvent. The thin layer chromatogram was sprayed with Ehrlich reagent and the absorbance of the IAA-Ehrlich reaction product was determined at 560 m$\mu$ using a Shimadzu Model CS-900 Dual Wavelength TLC Scanner. The IAA level in normal tomato shoots was about 0.01 $\mu$g per 1 g F.W. Free IAA was also found in zinc-deficient tomato shoots, although the amounts were very small in comparison with normal plants.

## Role of zinc in IAA metabolism

As indicated by the above results, contents of free tryptophan and tryptamine were much higher in zinc-deficient plants than in plants

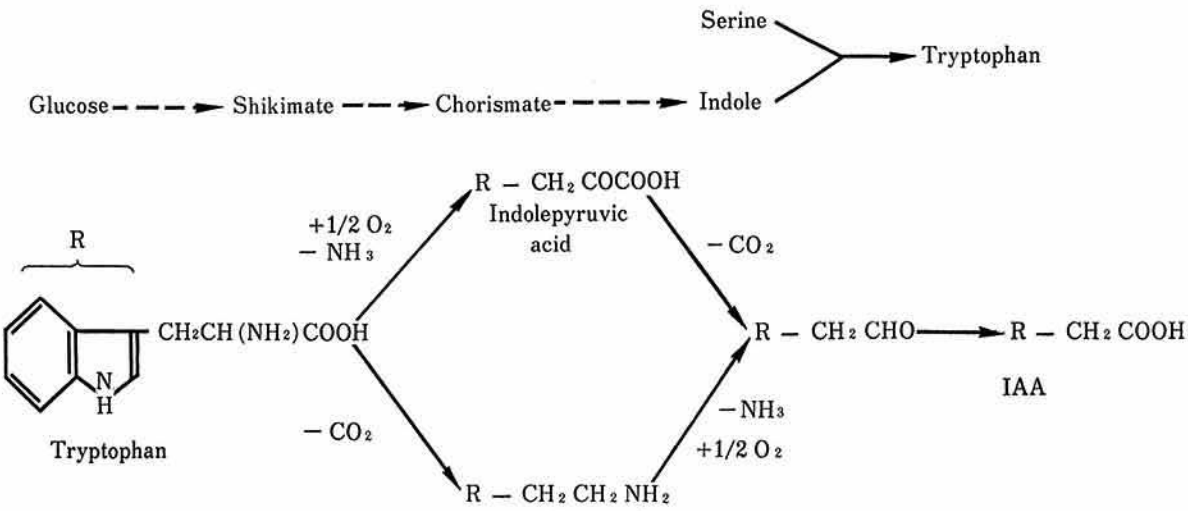


Fig. 1. IAA biogenesis from tryptophan

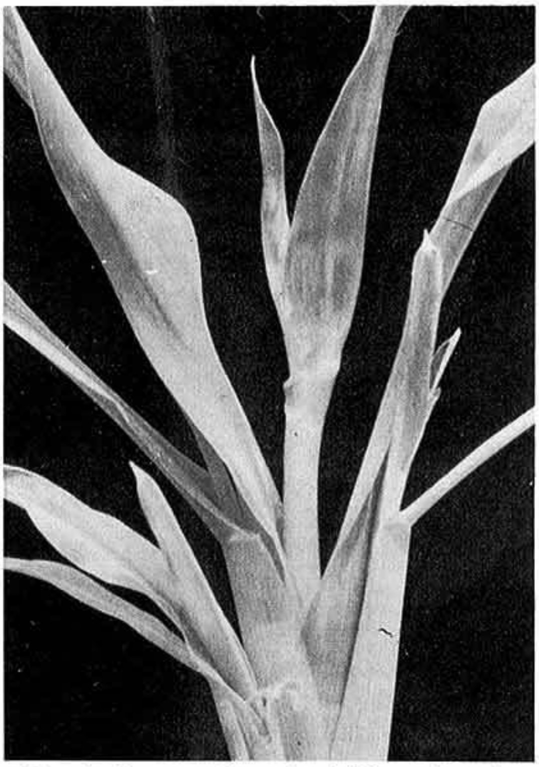

Plate 1. Recovery from zinc deficiency in maize seedling.
Concentration of tryptamine accumulated in zinc deficient plant was decreased by the addition of zinc to the culture solution (0.1 ppm), and the elongation of stem internode took place drastically soon after.

with sufficient zinc, and the content of IAA in the zinc-dificient tomato shoots was low.

To demonstrate the role of zinc in metabolism of these indole compounds, several kinds of tracer experiments were caried out. Results obtained are as follows:[6]

1. Radioactivity from labelled tryptamine was incorporated into IAA, indicating that this compound can serve as a precursor of IAA in tomato seedlings.

2. The inactivation of IAA caused by the zinc-deficient stem sections was almost the same as that caused by zinc-sufficient sections of tomato seedlings. This result suggests that zinc is required for IAA synthesis, but not for maintaining it in the active state.

Whether both of the indolepyruvic acid and tryptamine pathway are actualy working under natural condition, or either pathway is predominant in different plant species or under different physiological conditions is a matter of argument.

Judging from the above data, it can be concluded that zinc plays a role in the metabolic pathway from tryptophan to IAA, especially from tryptamine to IAA.

## References

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