Bangladesh J. Bot. **47**(2): 329-335, 2018 (June)

EFFECTS OF ZINC ON INDOLE CARBOXYLIC ACID AND INDOLE ACETIC ACIDS CONTENTS IN RADISH SHOOT

BALAL HOSSAIN*

Department of Soil Science, National University, Gazipur-1704, Bangladesh

Keywords: Zinc, Indole carbolic acid, Indole acetic acid, Radish shoot

Abstract

Radish (*Raphanus sativus* L.) was grown for several experiments in a glasshouse with and without zinc (Zn) in the nutrient solution. Lack of zinc resulted in stunted growth and reduced leaf of radish shoots. Two-dimensional thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of indole carboxylic acid (ICA) (ester + free) and IAA (ester + free) in Zn-deficient radish shoots. Alkali-labile (1M NaOH) ICA and IAA in Zn deficient radish shoots were estimated with the use of GC. The content of ICA in Zn deficient radish shoots was higher than that of control radish shoots. These results suggest that ICA (ester + free) was accumulated in Zn-deficient radish shoots. But the content of IAA (ester + free) in Zn-deficient radish shoots was almost the same as that of control radish shoots. These results suggest that zinc nutrition did not affect the level of IAA in radish shoots.

Introduction

Tryptophan is considered to be converted to IAA either through indolepyruvic acid or tryptamine and indole acetaldehyde, which appear to be the immediate precursor of IAA (Gibson *et al.* 1972). The conversion of tryptophan to indolealdehyde and ICA was demonstrated, and these compounds were found to derive from the oxidative degradation of IAA using tryptophan-3^{-} ^{14}C (Wightman 1964). Sandberg *et al.* (1984) reported that ICA is a natural constituent of tissues in the higher plants. Accumulation of free ICA in zinc-deficient radish seedlings was also found by Takaki (1980) and Domingo *et al.* (1990).

Takaki and Kushizaki (1976) used tomato and maize plants and detected much higher levels of tryptophan in Zn-deficient plants than in plants with adequate supply of zinc. The highest levels were observed in the most deficient seedlings. This was also true for tryptamine.

It is well established that typical symptom of Zn deficiency in all plants is "stunted growth" and "rosette". Rosette refers to the failure of stem to elongate, causing the leaves of several nodes to lie telescoped together in a plane, rosette fashion (Yamasaki *et al.* 1993, Mengel and Kirkby 1982). Takaki (1980), Takaki *et al.* (1990) and Domingo *et al.* (1992) detected the normal level of IAA in Zn-deficient radish by TLC, GC and GC-MS. However, in spite of the presence of normal level of free IAA detected in Zn-deficient radish seedlings, stunted growth was observed. They speculated that the normal level of free IAA found in Zn-deficient radish seedling may become ineffective or cannot promote plant growth in the absence of Zn.

Apart from the occurrence as a free acid, IAA also exists in conjugated forms (Cohen and Bandurski 1982). Based on the alkali lability of the IAA compounds, Bandurski and Schulze (1977) classified them as free, ester and peptidic forms. Ester IAA and ester ICA were defined as IAA and ICA which were liberated by hydrolysis for 1 hr in 1 M NaOH at room temperature.

This study aimed at clarifying the role of zinc nutrition in plants in relation to the contents of IAA (ester + free) and ICA (ester + free) in radish shoots, using the method of GC and GC-MS.

---

*Author for correspondence: <balalnu78@gmail.com>.

Materials and Methods

Seeds of radish (*Raphanus sativus* L.) were washed thoroughly with deionized water and germinated for 5 days at 30°C. The germinated seeds were transferred to plastic buckets, placed on polyethylene containers filled with a culture solution and grown in a glasshouse under natural light conditions for several weeks in winter season (October to March).

The composition of the culture solution was the same as reported by Takaki 1980. Zinc as $ZnSO_4.7H_2O$ was supplied at two levels, i.e., optimum 0.05 ppm and deficient level of 0 ppm. The culture solution was renewed every 10 days thereafter. Two sets of plant materials were prepared for each nutrient solution. One set was used for the growth measurement and the other for the extraction of IAA and ICA.

After harvest the plants were separated into shoots and roots and the roots were discarded. The fresh weight was measured and the shoots were oven dried for 4 hrs at 100°C. The oven dried weight was measured for the determination of water content. The dry matter was ashed in a muffle furnace at 550°C at 4 hrs. The ash was dissolved in 20 ml of 1 M HCl and was used for the determination of the Zn content by atomic absorption spectrophotometry using an atomic absorption spectrophotometer (Shimadzu Model AA-646).

For extraction of IAA and ICA 50 g of fresh materials from the top growth were homogenized with methanol (MeOH) in a blender. After filtration, MeOH was removed from extract under reduced pressure at 40°C. The aqueous residue was hydrolyzed for 1 hr in 1 M NaOH at room temperature for ICA (ester + free) and IAA (ester + free). The hydrolysate was then placed into an ice bath and adjusted to pH 2.2 with concentrated H_3PO_4 and extracted two times with dichlromethane (CH_2Cl_2). The CH_2Cl_2 was reduced to 100 ml and extraceted 2 times with 250 ml of 1 M $NaHCO_3$ and centrifuged for 5 min at 3000 rpm. The centrifuged sample was adjusted to pH 2.2 with concentrated H_3PO_4 and extracted 3 times with CH_2Cl_2. The CH_2Cl_2 phase was collected into a beaker containing anhydrous Na_2SO_4 to remove water. The CH_2Cl_2 phase was filtered and evaporated to dryness under reduced pressure at 40°C to get a CH_2Cl_2 soluble acidic fraction.

For purification by insoluble polyvinylpyrrolidone (PVP) column, the CH_2Cl_2-soluble acidic fraction was dissolved in a small volume of MeOH and applied into a PVP-column (15 × 2cm) which was equilibrated with a mixture of 0.2 M $Na_2HPO_4.12\ H_2O$ and 0.1 M citric acid solution (pH 8.0) and then eluted with the same mixture. The first 60 ml of the eluted fraction was discarded and the following fractions (suspected IAA, ICA and IBA as internal standard) were collected up to 300 ml. The fraction was acidified to pH 2.2 with 1 : 2 H_3PO_4 and extracted 3 times with CH_2Cl_2. The CH_2Cl_2 extract was collected and combined in a beaker containing anhydrous Na_2SO_4. After filtration the CH_2Cl_2 was evaporated to dryness under reduced pressure at 40°C to get a purified extract.

The gas chromatography-mass spectrometry (GC-MS) analysis of methylated IAA and ICA were performed with a Hitachi GC-MS (Model G-3000 and M-2000 AM). Five micro liters of the methylayed final extract were subjected to GC-MS and compared with the methylated authentic IAA.

Indole carboxylic acid (ICA) and other peaks were also appeared near the peak of IAA, so the collection of IAA was required to obtain its mass spectra. The collection of IAA was done by glass rod from GC at proper time.

For collection of ICA (ester + free) for GC-MS, IAA and other peaks appeared near the peak of ICA, so collection of ICA was required to obtain its mass spectra. The collection of ICA was done by glass rod from the GC at proper time.

For the quantitative estimation of IAA and ICA, 50 µl of 1000 ppm indole butyric acid (IBA) as internal standard and 0.02% butylatedhydroxytoluene (BHT) to protect the oxidative degradation of IAA and ICA were added to a sample weighing 50 g. Extraction, purification and methylation procedures for IAA and ICA were performed. Five microliters of the methylated final extract was applied to a GC (Shimadzu, Model GC-7A). The contents of IAA and ICA were calculated from the peak area of IAA, ICA and compared with that of IBA as internal standard using a chromatopac (Shimadzu, Model C-R1A).

Results and Discussion

In radish seedling lack of Zn resulted the stunted growth of plants, initial symptoms of slight necrosis in the older leaves and reduced leaf area were also observed. There was a marked reduction in the fresh weight of shoots, moisture and Zn contents of plants with the exclusion of Zn in the culture solution.

The critical level of Zn for Zn deficiency in plants is 15 ppm in dry matter (Takaki and Kushizaki 1976). The zinc content of these experiments in Zn-deficient radish seedlings was below 15 ppm. Two dimensional TLC was employed in the identification of putative ICA and IAA in the zinc-deficient radish shoots (Table 1). Van Urk-Salkowsky reactive spots were detected on the chromatogram of the acidic fraction from the zinc-deficient radish shoots which gave the same Rf values and color reactions similar to those of authentic ICA and IAA. The pinkish spot on the chromatogram was considered corresponding to the authentic ICA and the bluish spot on the chromatogram was considered corresponding to the authentic IAA.

Table 1. Effect of Zn on growth of radish shoots.

Expt. No.	DAT	Fresh weight per 5 plants (g)		Moisture content (%)		Zn content (ppm)	
		+Zn	–Zn	+Zn	-Zn	+Zn	–Zn
1	24	12.9	6.90	92.2	91.4	20.1	6.80
2	25	13.0	6.20	91.1	90.7	18.9	6.10
		Fresh wt./10 plants (g)					
3	31	17.7	10.4	89.0	88.5	17.2	5.50

DAT = Days after transplanting.

Putative methylated IAA and ICA in zinc-deficient radish shoots were further identified by GC-MS and the results are presented in Figs 1, 2 and 3. The gas chromatogram presented in Fig. 1 shows the peak of putative IAA, ICA and internal standard IBA from zinc-deficient and control radish shoots.

The peak of putative IAA and ICA from zinc-deficient radish shoots coincided with peak of the authentic IAA and ICA with the same retention time. The mass spectra (Fig. 2) of the putative IAA from zinc-deficient radish shoots showed the high peaks at 189 and 130 m/e. The peak at 189 m/e was the parent of the methylated IAA (ester + free) and the peak at 130 m/z was the base peak of the methylated IAA. These results indicated the presence of IAA in zinc-deficient radish shoots. The mass spectra (Fig. 3) of putative ICA from zinc-deficient radish shoots showed the peak at 175 and 144 m/e. The peak at 175 m/e was parent peak of the methylated ICA and the peak at m/z was the base peak of the methylated ICA. These results indicated the presence of ICA (ester + free) in zinc-deficient radish shoots.

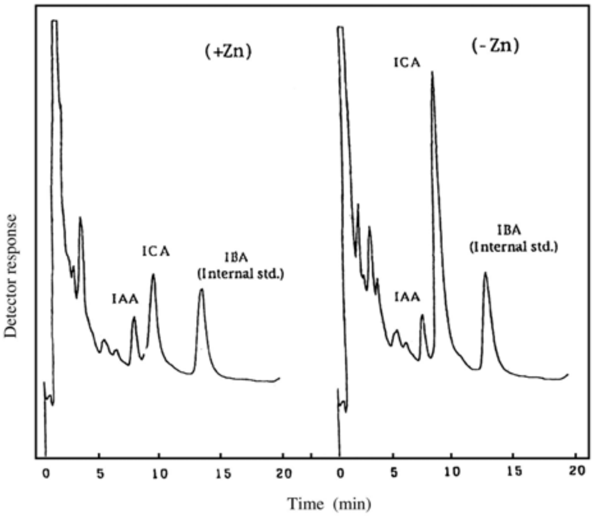

Fig. 1. Gas chromatogram of putative methylated IAA and ICA (ester+free) from the control (+Zn) and the Zn-deficient (–Zn) radish shoots.

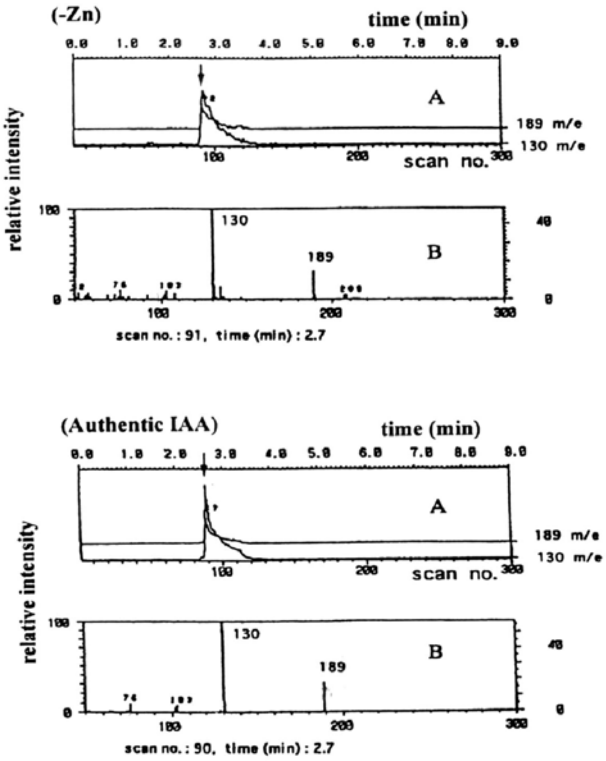

Fig. 2. Electron-impact mass chromatogram and mass spectra of putative methylated IAA (ester + free) from control (+Zn) and Zn-deficient (–Zn) radish shoots and authentic IAA (A: mass chromatogram, B: mass spectra).

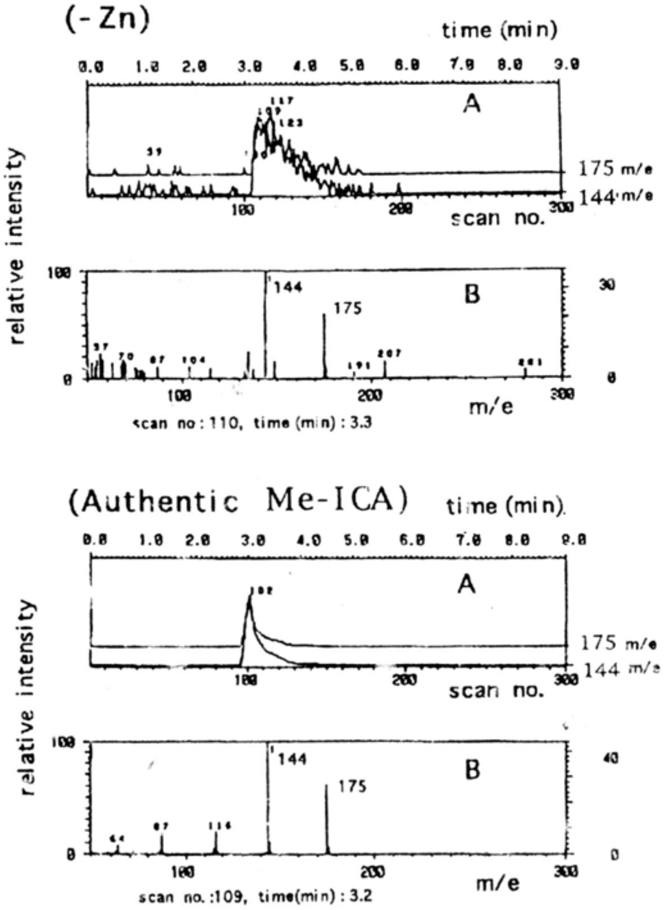

Fig. 3. Electron-impact mass chromatogram and spectra of putative methylated ICA (ester + free) from a purified methylated extract of Zn-deficient (–Zn) radish shoots and methylated authentic ICA. A: Mass chromatogram, B: Mass spectra.

The content of ICA (ester + free) in three different experiments detected in zinc-deficient radish shoots were found to be higher than that of control radish shoots with the value of 61.4, 39.0, 168, and 21.1, 13.0, 72.8 µ g/100 g fresh wt., respectively (Table 2). These results suggested that zinc nutrition affects significantly the content of ICA (ester + free) in radish shoots.

The content of IAA (ester + free) in different three experiments detected in zinc-deficient and control radish shoots were found to be comparable, with values of 10.1, 10.8, 14.9 and 10.3, 11.5, 17.9 µ g/100 g fresh wt., respectively (Table 3). These results suggested that zinc nutrition does not affect the level of IAA in radish shoots.

It was found from the results of the experiments that ICA (ester + free) accumulated in zinc-deficient radish shoots when compared with the values of control shoots.

Although it had been suggested that several indoles may be involved in the ICA metabolism in higher plants, only indole aldehyde (Shindy and Smith 1975), ICA (Jones and Tayler 1957, Sandberg *et al*. 1984) and 3-oxindole acetic acid (Reinecke and Bandurski 1983) had been identified in higher plant extracts on the basis of physiological evidence.

It was found that ICA (free) had no biological activity when compared with IAA (free) but plants contain it in considerable amounts as ICA (ester + free) form. This form of ICA means that plant may use this ICA when it is needed.

Table 2. Sodium hydroxide labile (1N) ICA (ester+ free) contents in control (+Zn) and Zn-deficient (–Zn) radish shoots.

ICA µg/100 g fresh weight			
Expt. No.	DAT	+Zn	–Zn
1	24	21.1	61.4
2	25	13.0	39.0
3	31	72.8	168

DAT = Days after transplanting.

Table 3. Sodium hydroxide labile (1N) IAA (ester + free) contents in control (+Zn) and Zn-deficient (–Zn) radish shoots.

IAA µg/100 g fresh weight			
Expt. No.	DAT	+Zn	-Zn
1	24	10.3	10.1
2	25	11.5	10.8
3	31	17.9	14.9

DAT = Days after transplanting.

The results of the present study showed that in zinc-deficient radish shoots the level of IAA (ester + free) did exist in normal level. Domingo *et al*. (1992) showed that in zinc-deficient radish shoots, the level of IAA (free) did exist in normal level. However, in spite of the normal level of IAA (ester + free) and IAA (free) detected in zinc-deficient radish seedlings, stunted growth, and reduced leaf were observed. So it can be speculated that auxin may not have biological activity without zinc. It has been suggested that IAA conjugates participate in homeostatic control of free IAA levels. This hypothesis implies that IAA conjugation is a reversible process and that IAA can be released by hydrolysis of IAA conjugates (Cohen and Bandurski 1982) and also ICA (free) can be released by hydrolysis of ICA (ester + free). IAA conjugates do not appear as active auxins per/second, but growth responses correlated with their hydrolysis to free IAA had been reported (Bialek*et al*. 1983). In the same way ICA (ester + free) might also be hydrolysed to free ICA.

References

Bandurski RS and Schulze A 1977. Concentration of indole-3-acetic acid and its derivatives in plants. J. Plant Physiol. **60**: 211-213.

Bialek K Meaudt WJ and Cohen JD 1983. Indole-3-acetic acid (IAA) and IAA conjugates applied to bean sections: IAA content and growth response. Plant Physiol. **73**: 130-134.

Cohen JD and Bandurski RS 1982. Chemistry and physiology of the bound auxin. Annu. Rev. Plant Physiol. **33**: 403-430.

Domingo AL, NagatomoY, Tamai M and Takaki H 1990. Indole carboxylic acid in zinc-deficient radish shoots. Soil Sci. Plant Nutr. **36**(4): 555-560.

Domingo AL, NagatomoY,Tamai M and Takaki H 1992. Free tryptophan and indoleacetic acid in zinc-deficient radish shoots. Soil Sci. Plant Nutr. **38**(4): 261-267.

Gibson RA, Schneider AE and Wightman A 1972. Biosynthesis and Metabolism of indole-3yl-acetic acid. II. *In vivo* experiment with 14C-labeled precursor of IAAin tomato and barly shoots. J. Expt. Bot. **23**: 381-399.

Jones ERS and Taylor WC 1957. Some indoleconstituents of cabbage. Nature **179**: 1138.

Mengel K and Kirkby EA 1982. Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland.

Reinecke DM and Bandurski RS 1983. Oxindole-3-acetic acid catabolite in *Zea mays*. Plant Physiol.**71**: 211-213.

Sandberg G Jensen E and Crozier A 1984. Analysis of 3-indole carboxylic acid in *Pinus sylvestris* needles. Phytochemistry **23**: 99-102.

Shindy WW and Smith OE 1975. Identification of plant hormones from cotton ovules. Plant Physiol. **55**: 550-554.

Takaki H and Kushizaki M 1976. Zinc nutrition in higher plants. Bull Nat'l. Inst. Agric. Sci. **28**: 75-118.

Takaki H 1980. Detection of indoleacetic acid and indole carboxylic acid in zinc-deficient radish seedlings by two-dimensional thin layer chromatography. Bull. Fac. Agric. Miyazaki Univ. **27**: 211-215.

Takaki H, Domingo AL, NagamotoY and Tamai M 1990. Effect of zinc on the amount of indoleacetic acid in radish seedlings. Trans. 14th ICSS (Kyoto, Japan) **4**: 497-498.

Wightman F 1964. Pathways of tryptophan metabolism in tomato plants. *In:* Regullateurs Naturels de la Croissance Vegetable. Centre National de la Recherche Scientifique, Paris. **123**: 193-212.

Yamasaki K, Sugiyama T, Takahashi E, Chino M and Asano A 1993. Plant Nutrition and Manure (In Japanese). Asakura-Shoten, Tokyo, Japan.

(Manuscript received on 8 November, 2017; revised on 22 December, 2017)