*Proc. Indian natn. Sci. Acad.* **B54** No. 1 pp. 75–79 (1988)

# Studies on Growth and Activity of Photosynthetic Enzymes in *Sorghum bicolor* (L.) as Influenced by Micronutrients

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(Accepted on 8 February 1988)

Fe, Cu, Zn, B, Mo and Mn were supplied individually to *Sorghum* cultivar CSH-5, as foliar spray. The effect of micronutrients on growth parameters and the activities of photosynthetic enzymes and carbonic anhydrase in the leaves were studied. Application of zinc caused increase in height of the plant; B, Mn, Fe and Cu caused lesser increase in that order. Maximum dry weight accumulation took place in plants treated with Mn followed by Zn, Cu, Mo, Fe and B. The activities of carboxylating enzymes (PEP and RuBP carboxylases), carbonic anhydrase and the rates of photochemical reduction increased with the application of Zn, Mn, Fe, B, Mo and Cu (in the decreased order) as compared to the control.

**Key Words: Micronutrients, Carbonic anhydrase, Carboxylating enzymes, Photochemical reduction**

## Introduction

Effective utilization of mineral nutrients for dry matter production in crop plants is an important problem concerning agricultural production. There are several reports on the increases in chlorophyll content, photosynthesis and respiration of higher plants by the application of micronutrients either to the soil or as foliar sprays. These are reviewed by Nátr (1972). The stimulatory effect of $Mg^{2+}$, $Mn^{2+}$ and $Co^{2+}$ on the activity of PEP carboxylase in corn leaf was observed by Mukerji (1977), and O'Leary et al. (1981). But these observations were made on purified enzymes while determining the effect of divalent cations on the activity of the enzyme.

However, no observations have been made on the effects of micronutrients on the activity of RuBP carboxylase in *Sorghum*. A comprehensive study has been made regarding effect of different heavy metals and B, on the metabolism, growth and yield in CSH-5, a high yielding *Sorghum* cultivar widely cultivated in semi-arid regions. In this paper the effects of metallic ions on the activity of the carboxylating enzymes and Hill reaction and growth of the plant in terms of height and dry matter accumulation alone are presented.

## Material and Methods

The experiments were conducted at All India Coordinated Sorghum Improvement Project, Rajendranagar, Hyderabad in Rabi season (1984). The cultivar selected for the study was CSH-5. The experiment was laid out in randomised complete block design. The micronutrient salts of Fe, Cu, Zn, B, Mo, and Mn were given as foliar sprays (in ppm): Fe EDTA, 2, 5, 10; $CuSO_4.5H_2O$, 0.01, 0.1, 1.0; $ZnSO_4.7H_2O$, 1,2, 5; $H_3BO_3$, 1,2, 5; $H_2MoO_4.4H_2O$, 0.1, 1, 2; and $MnCl_2.4H_2O$, 1,2 and 5.

All the salts used were of BDH or Merck analytical grade. Each micronutrient was supplied to the plants at three growth stages: (i) 15 days after germination, (ii) 28–38 days, and (iii) 60 days of growth.

The initial micronutrient levels in the soil were determined by Atomic absorption spectrophotometric method. The composition of the micronutrients in the soil was found to be as follows (in ppm): Fe - 4000, Cu 28.12, Zn - 69.76, Mn - 354.60.

All the biochemical investigations were confined to the third leaf from the top in all treated plants. The leaf samples were analysed for PEP carboxylase (No. 4.1.1.31) as PEP dependent $H^{14}CO_3^-$ incorporation into

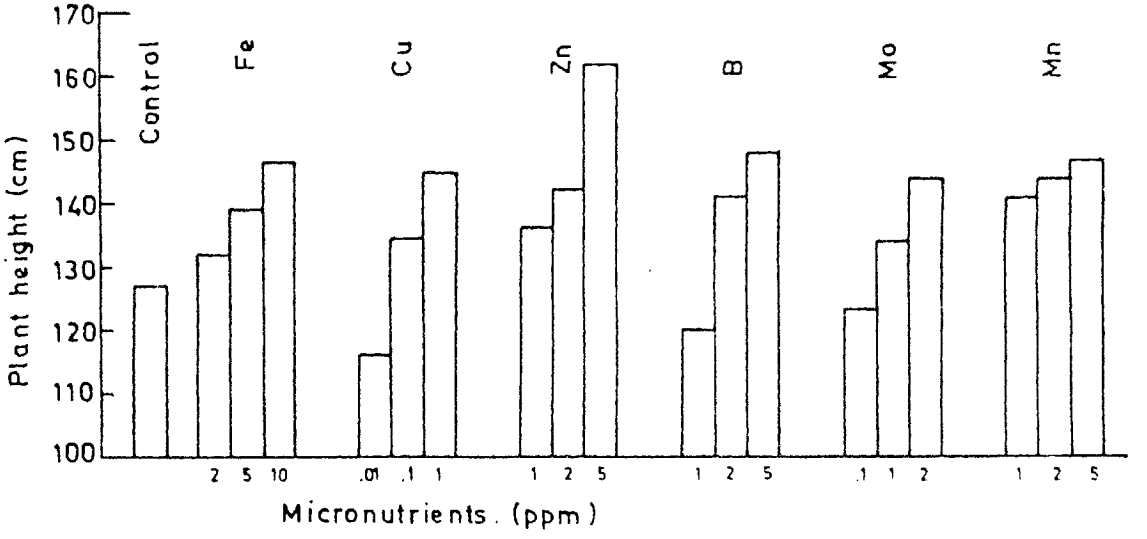


**Figure 1** Effect of micronutrients on the growth (height) of *Sorghum* S.Em ± 3.37; CD at 5% 9.7; F Value 10.62**

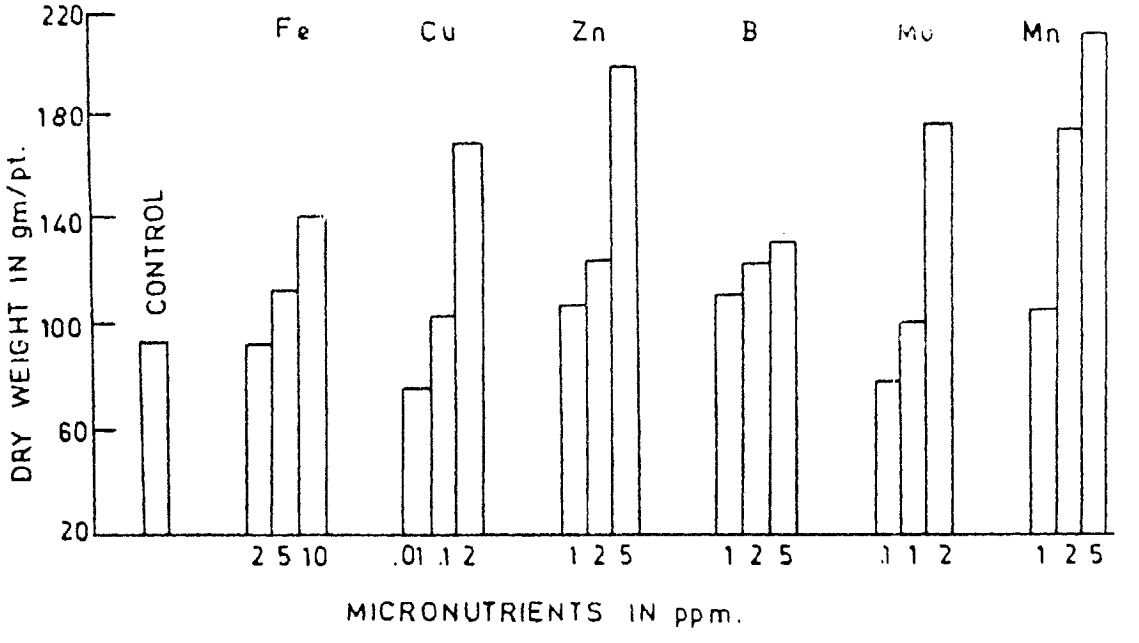


**Figure 2** Effect of micronutrients on the dry matter in *Sorghum* S.Em ± 0.82; CD at 5% 2.37; F Value 2304**

acid soluble products and RuBP carboxylase (No. 4.1.1.39) as PGA dependent $H^{14}CO_3$ incorporation into acid stable products (Raghavendra & Das 1975). Photochemical activities were measured in chloroplasts isolated by the method of Woo et al. (1970). Carbonic anhydrase (No. 4.2.1.1) was extracted and estimated by the method of Everson (1970). Total chlorophyll and total protein soluble determinations were carried out by the methods of Arnon (1949) and Lowry et al. (1951). The plant height and dry matter yield were estimated at the third growth stage.

## Results

The variations in the plant height and dry matter yield as affected by micronutrient treatments at third stage of growth are shown in figures 1 & 2. All micronutrients employed at higher doses caused an increase in the height of the plants over that of control. Amongst the micronutrients used Fe, Zn, Mn increased the height of the plant at all doses, while maximum effect was observed in Zn at 5 ppm. Addition of Cu, B and Mo at lower levels caused small decrease in height of the plant.

The increase in dry matter yield was more pronounced as compared to the effects of micronutrients on the height of the *Sorghum* plant. Application of Mn, and Zn and B increased the dry weight of plants at all doses. The maximum increase in dry weight was caused by Mn and Zn. However, Cu and Mo at the lowest levels caused small decrease in the dry weight of plants.

Table 1 summarizes the effects of micronutrients on carboxylating enzymes, carbonic anhydrase and photochemical activities of *Sorghum* leaves during seedling stage. Carbonic anhydrase activity in treated leaves was enhanced to maximum even at highest doses

**Table 1** *Effects of micronutrients on photosynthetic enzymes, photochemical reductions and carbonic anhydrase activities of* Sorghum leaves

| Treatment No. | Treatment (Conc. in ppm) | Carbonic anhydrase activity in enzyme units | PEP carboxylase activity (Results expressed as ($\mu$moles mg $Chl^{-1}$ h $r^{-1}$) | RuBP carboxylase activity | FeCN reduction Results expressed as ($\mu$moles mg $Chl^{-1}$ h $r^{-1}$) | DCPIP reduction |
|---|---|---|---|---|---|---|
| 1 | Control | 14 | 619 | 217 | 422 | 216 |
| 2 | Fe 2 | 15 | 642 | 213 | 426 | 216 |
| 3 | Fe 5 | 16 | 640 | 232 | 428 | 222 |
| 4 | Fe 10 | 21 | 660 | 254 | 478 | 228 |
| 5 | Cu 0.01 | 13 | 622 | 215 | 414 | 216 |
| 6 | Cu 0.10 | 21 | 640 | 210 | 418 | 220 |
| 7 | Cu 1.0 | 23 | 657 | 248 | 420 | 224 |
| 8 | Zn 1 | 11 | 626 | 235 | 448 | 218 |
| 9 | Zn 2 | 17 | 648 | 270 | 468 | 222 |
| 10 | Zn 5 | 32 | 682 | 345 | 483 | 319 |
| 11 | B 1 | 12 | 609 | 195 | 461 | 220 |
| 12 | B 2 | 18 | 623 | 221 | 467 | 225 |
| 13 | B 5 | 18 | 642 | 242 | 478 | 248 |
| 14 | Mo 0.1 | 15 | 626 | 240 | 465 | 247 |
| 15 | Mo 1.0 | 26 | 645 | 246 | 468 | 268 |
| 16 | Mo 2.0 | 31 | 660 | 248 | 472 | 228 |
| 17 | Mn 1 | 14 | 639 | 217 | 412 | 260 |
| 18 | Mn 2 | 19 | 648 | 232 | 416 | 283 |
| 19 | Mn 5 | 23 | 666 | 294 | 425 | 316 |
| | S Em ± | 0.71 | 1.13 | 2.67 | 1.27 | 0.91 |
| | CD at 5% | 2.05 | 3.25 | 7.69 | 3.65 | 2.61 |
| | F Value | 72.19** | 260.8** | 162.08** | 428.95** | 1308.98** |

of Zn and Mo. The lowest levels of Zn, B and Cu decreased carbonic anhydrase activity marginally. Maximum activities of PEP carboxylase and RuBP carboxylase were recorded in Zn treated plants particularly at the highest dose. In this case, PEP carboxylase activity increased by about 10% and RuBP carboxylase activity by about 59%. Next to Zn, Mn increased the activities of PEP and RuBP carboxylases. Next in order are Fe, Cu, Mo and B which increased the activities of both the carboxylases particularly at higher doses. The rates of ferricyanide, 2, 6-DCPIP reduction were highest in chloroplasts from the leaves treated with higher levels of Fe, Mo, and B (table 1). Copper and manganese at all levels did not significantly alter the reduction of FeCN and 2, 6-DCPIP.

## Discussion

Micronutrients like Fe, Cu, Zn, B, Mo and Mn are biologically very important as they are essential for development, growth and metabolic activity. Several biochemical processess are accelerated by micronutrients. RuBP carboxylase, PEP carboxylase, and carbonic anhydrase are enzymes directly concerned with utilisation of $CO_2$ during photosynthesis. The activity of these enzymes determine net photosynthesis to a large extent. Most of the earlier work on the effects of metallic ions on photosynthetic carboxylating enzymes was confined to the study of the activities of the enzymes under metal deficient conditions.

PEP carboxylase catalyses the irreversible carboxylation of PEP and $HCO^-_3$ to form oxaloacetate and orthophosphate (Maruyama et al. 1966, Utter & Kolenbrander 1972, O'Leary 1982). $Mg^{2+}$ and $Mn^{2+}$ are the most effective activators of this important enzyme (Stiborova & Leblova 1985). Mukerji (1977) also reported that PEP carboxylase activity was stimulated by $Mg^{2+}$, $Mn^{2+}$ and $Co^{2+}$while $Ca^{2+}$ was an inhibitor and zinc had little effect. A significant decrease in the activities of both carboxylating enzymes (PEP carboxylase and RuBP carboxylase) was observed under Zn dificiency in rice and pearl millet (Seethambaram 1983). A reduction in fraction 1-protein and RuBP carboxylase activity was observed in navy beans under Zn deficiency (Jyung et al. 1972, 1973). However, in the present study the activities of both the enzymes were enhanced due to micronutrient spray but the activity was higher in Zn and Mn - treated plants. Zinc application not only enhanced PEP carboxylase but also RuBP carboxylase activity to a maximum extent which enabled *Sorghum* to fix $CO_2$ efficiently.

A reduction in photosynthesis (Botrill et al. 1970) and carbonic anhydrase activity (Edwards and Mohamed 1973) under Zn deficiency reduced net photosynthesis and carbonic anhydrase activity in spinach (Randall & Bouma 1973). A postive correlation for carbonic anhydrase activity with zinc was established by Woo and Sibly (1952). Under zinc deficiency, reduced carbonic anhydrase activity was observed in *Citrullus vulgaris* (Sharma et al. 1981) and increased activity was found with increased levels of zinc application. Veeranjaneyulu and Das (1982) observed that Zn-treated *Ocimum* plants registered an increased carbonic anhydrase activity with increase in concentration of zinc. In the present investigation also carbonic anhydrase activity increased as the supply of zinc to *Sorghum* was raised.

The electron transport process and carbon metabolism are affected either by mineral ion deficiency or by their toxic concentrations (Shrotri et al. 1981, Hampp et al. 1976, Van Assche et al. 1979 and 1986, Tripathy & Mohanty 1980, Baker et al. 1982). In the present invetigation Zn and Mn enhanced the photoreduction of FeCN and 2, 6-DCPIP significantly (table 1). According to Shrotri et al. (1981) zinc deficiency caused a reduction in photosynthetic oxygen evolution and PSII dependent Hill reaction and non-cyclic photophosphorylation of maize plants.

In the present experiments under field conditions, zinc was found to be the most important micronutrient in increasing the growth of *Sorghum*. The height of the plant increased progressively as the level of the zinc increased (figure 1). Foliar application of manganese and zinc treatment enhanced dry matter yields (figure 2).

There seems to be a correlation between increase in growth (in terms of height and dry weight of the plant) and increased activities of the enzymes concerned with photosynthesis at higher levels of zinc and manganese. The effect of any micronutrient need not be uniform on different phases of growth or metabolism. The effects also depend on the levels of micronutrients supplied. Further, the effect of micronutrient at any level is again compounded due to its interaction with other micronutrients and macronutrients present within the system.

## Acknowledgements

The authors are grateful to Professor P Rama Rao (Head of Botany Department), Dr R V Vidyabhusanam, (Project Co-ordinator of AICSIP, Rajendranagar, Hyderabad) for providing laboratory and field facilities and to Dr V Jayamohan Rao in carrying out the statistical analysis. The award of SRF by the Council of Scientific and Industrial Research, New Delhi, to one of the authors (KAK) is gratefully acknowledged.

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