

# Effect of EDTA-assisted Copper Uptake on Photosynthetic Activity and Biomass Production of Sweet Sorghum

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Sweet sorghum (*Sorghum bicolor* L. Moench cv. Róna) is a widely grown sugar crop that is used for bioenergy production. Since sorghum shows increased sensitivity to nutrient deficiency, the objective of this study was to reach an appropriate Cu level in plant tissues using various concentrations of Cu and ethylenediaminetetraacetic acid (EDTA) in order to enhance the photosynthetic activity and biomass production of plants. Copper accumulation increased in the root and stem of plants irrigated for 12 weeks with 0.1  $\mu\text{M}$   $\text{CuCl}_2$  both in the presence and absence of 300  $\mu\text{M}$  EDTA and as a consequence, the plant-available Cu concentration in the soil extracts was lower at harvest. Although the copper content of leaves slightly increased, the transport of Fe and Mn, the microelements participating in light reactions of photosynthesis was negatively affected. In spite of this, 0.1  $\mu\text{M}$   $\text{CuCl}_2$  alone and with 200 or 300  $\mu\text{M}$  EDTA enhanced the maximal  $\text{CO}_2$  assimilation rate ( $A_{\text{max}}$ ) as a function of photon flux density (PPFD) and increased soluble sugar content in all plant parts. The dry mass of plants especially that of stems increased very significantly after 0.1  $\mu\text{M}$   $\text{CuCl}_2$  + 300  $\mu\text{M}$  EDTA treatment. These results show that non-toxic concentration of copper in combination with suitable concentration of EDTA can enhance photosynthesis, biomass production, sugar content and the total copper accumulation in the shoot of sweet sorghum plants.

**Keywords:** biomass production, copper accumulation, microelements, soluble sugars, Sorghum

## Introduction

Renewable energy is an important source of energy that reduces the dependence on fossil fuels and emission of greenhouse gases, thus, the use of biofuel feedstock has a great economic impact. Sweet sorghum (*Sorghum bicolor* L. Moench), a widely grown crop plant accumulates a large quantity of soluble sugars in vegetative tissues, so it is used for ethanol or methane production but it is also applied as animal feed and silage and as raw material in paper industry (Regassa and Wortmann 2014). Sweet sorghum is well-adapted to abiotic stress conditions, such as drought, water logging and salt stress but it shows increased sensitivity to nutrient deficiency (Németh and Izsáki 2007; Tari et al. 2013a).

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Copper deficiency is the most ubiquitous micronutrient deficiency problem in the world and commonly occurs on organic-rich, sandy or calcareous soils. The lower critical concentration of Cu in soils extracted with aqua regia is around 5–10 mg kg<sup>-1</sup> dry soil and about 25–40% of soils are copper deficient in Ireland, Scotland and Germany. Copper deficient soils can also be found in Australia, North America (Alloway 1995; 2005), China (Wu et al. 2010) and in Hungary (Györi 1984). The amount of Cu available to plants varies by soils, the lowest availability is associated with organic or peat soils and it decreases with increasing soil pH. The lower critical concentrations of available copper are 2.2 mg kg<sup>-1</sup> dry soil after diethylenetrinitriolpentaacetic acid (DTPA) extraction and 5–10 mg kg<sup>-1</sup> after ammonium-acetate – acetic acid – EDTA (AAAc-EDTA) extraction (Alloway 2005).

Solubility, availability, uptake and translocation of microelements from root to shoot can be enhanced by the use of synthetic ligands such as ethylenediaminetetraacetic acid (EDTA) (Luo et al. 2006). Among chelating agents used to extract metals from soils, EDTA is regarded as one of the most effective in solubilizing soil-bound Cu<sup>2+</sup> (Hong et al. 1999). It was found that application of EDTA enhanced the accumulation of Cu in *Brassica napus* (Wenzel et al. 2003), *Triticum aestivum* (Luo et al. 2006) and *Sorghum sudanense* (Tari et al. 2013b). The application of excessive EDTA has special physiological and environmental risks. Metal-EDTA complexes are poorly degradable and can cause toxicity in plants and soil organisms (Fuerhacker et al. 2003). Excess of EDTA may remove micronutrients (Fe, Zn, Mn) from the physiologically active macromolecules and may disrupt important biochemical processes in plant cells. The applied concentration of EDTA in induced phytoextraction processes is in the range of 1–10 mmol kg<sup>-1</sup> soil (Wenzel et al. 2003; Luo et al. 2006) and the application of 1.12 kg Cu-EDTA per hectare has been suggested for copper fertilization (Penney et al. 1988). Screening different concentrations of EDTA that can increase the copper uptake and biomass production will not only help to minimize the amount of chelate applied, but also decrease the environmental and physiological risk of mobilized metals.

Copper is an essential microelement for plants (Szira et al. 2014) but it is toxic above 30 mg kg<sup>-1</sup> plant dry mass (DM) (Broadly et al. 2012). It can be found in dissolved forms or in the form of organic Cu<sup>2+</sup> complexes in soil solution. The typical concentration of copper in the soil solution is 10<sup>-9</sup>–10<sup>-6</sup> M and ~10 µg g<sup>-1</sup> DM in the plant tissues (Yruela 2005; Yruela 2009). Plants can uptake both Cu<sup>2+</sup> ions and copper-chelate complexes with specific transporters.

The restricted availability of copper may limit crop yield because Cu<sup>2+</sup> is a constituent of several enzymes and other proteins in plants. Copper deficiency causes strong inhibition of photosynthetic activity and respiration (Droppa and Horváth 1990), it can influence the metabolism of lipids and proteins (Marschner 1995) and plants show deficiency symptoms such as narrow and twisted leaves and depressed growth of internodes due to inhibited cell wall lignification of xylem vessels (Mengel et al. 2001). Much of the yield reduction in Cu-deficient cereals is due to pollen sterility (Marschner 1995). Moreover, copper deficiency has been linked to the incidence of a number of crop diseases. Franzen

et al. (2008) found reduced *Fusarium* disease symptoms in spring and durum wheat cultivars and increased yield after copper fertilization.

Supraoptimal concentration of Cu can also decrease photosynthetic activity, respiration rate, chlorophyll content and thus can cause chlorotic symptoms, inhibition of plant growth and biomass production (Droppa and Horváth 1990), and as a redox-active ion it can destruct cell membranes and proteins (Feigl et al. 2013).

Since the increase in the biomass yield of energy plants even on soils of low or adequate nutrient supply has great agricultural and industrial importance, the purpose of this study is to investigate the EDTA-assisted uptake and accumulation of copper in various plant parts, and the effect of copper fertilization on photosynthetic activity and biomass production of sweet sorghum. The use of low concentration of EDTA, which has low environmental risk but may increase the microelement uptake, was in the focus of this study. The effect of different EDTA and copper combinations on total sugar content of plant organs was also determined.

## Materials and Methods

### *Plant material*

Seeds of sweet sorghum (*Sorghum bicolor* L. Moench cv. Róna) were germinated in the darkness at 26 °C for 2 days then the seedlings were planted into pots (diameter: 24 cm, height: 21 cm). Two healthy plants were grown in 2.5 kg soil (Bioland Tőzefeldolgozó Kft., Biatorbágy, Hungary) containing N (20 to 500 mg L<sup>-1</sup>), P<sub>2</sub>O<sub>5</sub> (200 to 500 mg L<sup>-1</sup>); K<sub>2</sub>O (300 to 600 mg L<sup>-1</sup>), white peat (50%, m/v), black peat (50% m/v), and CaCO<sub>3</sub> (2 kg m<sup>-3</sup>) at pH 7.0. The soil water content was maintained at 60% of the water-holding capacity. The extractable copper content of the soil following full digestion with aqua regia was 28.99 µg g<sup>-1</sup> dry soil at the beginning of the experiment. The plants were grown in a greenhouse under a 12/12 h day/night cycle, at 25/20 °C day/night temperature, at 300 µmol m<sup>-2</sup> s<sup>-1</sup> light intensity and 55 to 60% relative humidity for 12 weeks and were irrigated two times a week with 250 mL of 5×10<sup>-4</sup> M CaSO<sub>4</sub> (pH 5.5) containing 200 or 300 µM EDTA or EDTA in combination with 0.1 mM CuCl<sub>2</sub> (Tari et al. 2013b). The dry mass (DM) of plant parts as well as the biomass produced by 10 plants were recorded. All experiments were repeated three times.

### *Determination of copper content in the soil and plant organs*

Three samples were taken from each treatment to assess the total Cu concentration in the soil (Puskás and Farsang 2009). The mobilizable or plant available element fraction of 5 g soil samples (0.5 M ammonium-acetate + 0.5 M acetic acid + 0.02 M EDTA, AAAc-EDTA extractable fraction) was determined at the end of the experiments after harvesting (Lakanen and Erviö 1971) by atomic absorption spectrometer (Perkin Elmer 3010, Waltham MA, USA).

Cu and other micro elements in plant samples were determined by XSeries II ICP-MS (Thermo Scientific, Bremen, Germany). 100 mg dried plant material was homogenized and placed in test tubes containing 6 mL concentrated nitric acid and 2 mL H<sub>2</sub>O<sub>2</sub> for 20 h. The samples were digested in a microwave destructor (MarsXpress CEM, Matthews NC, USA) at 200 °C for 25 min and were then cooled, diluted with 12 mL double distilled water.

#### *Measurement of photosynthetic activity*

Net photosynthetic rate ( $A$ ,  $\mu\text{mol fixed CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was measured on young, fully expanded leaves using a portable photosynthesis system (LI-6400, LI-COR, Inc.; Lincoln, NE), as described by Poór et al. (2011). Light response curves were recorded under constant conditions (25 °C, 65 ± 10% relative humidity and controlled CO<sub>2</sub> supply of 360  $\mu\text{mol mol}^{-1}$ ) while increasing photosynthetic photon flux density (PPFD) from 0 to 1500  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ . The  $A$  versus PPFD curves were fitted using Sigma plot 11.0 software (Systat Software Inc., Erkrath, Germany) by the equation  $A = A_{\text{max}}(1 - e^{-A_q(\text{PPFD} - L_{cp})})$ , where  $A_{\text{max}}$  is the maximal photosynthetic rate at light saturation,  $A_q$  is the photosynthetic quantum efficiency, the initial slope of the photosynthetic increment at low light levels, and  $L_{cp}$  is the light compensation point (PPFD at  $A=0$ ) (Peek et al. 2002).

#### *Measurement of total soluble sugar content*

Total sugar contents were determined according to Dubois (1956). One g of fresh plant material was homogenized in 10 mL distilled water and was incubated in a 90 °C water bath for 45 min. The samples was centrifuged at 15,000 g for 15 min, at 4 °C, and 40  $\mu\text{L}$  of the supernatant was mixed with 400  $\mu\text{L}$  of 1.8% phenol and 2 mL of concentrated sulfuric acid. The absorbance was measured by a spectrophotometer at 490 nm.

#### *Statistical analysis*

Data are presented as means of at least three independent experiments. Analysis of variance was carried out with Sigma plot 11.0 software (Systat Software Inc., Erkrath, Germany). Statistical analyses were performed using Duncan's multiple range test and differences were considered significant if  $P < 0.05$ .

## **Results**

#### *Plant-available copper content in the soil*

The plant-available copper concentration in the soil extracts decreased significantly to the harvest in case of the treatments with 300 mM EDTA, 0.1 mM CuCl<sub>2</sub> and 0.1 mM CuCl<sub>2</sub> in combination with 200 mM or 300 mM EDTA (Fig. 1).

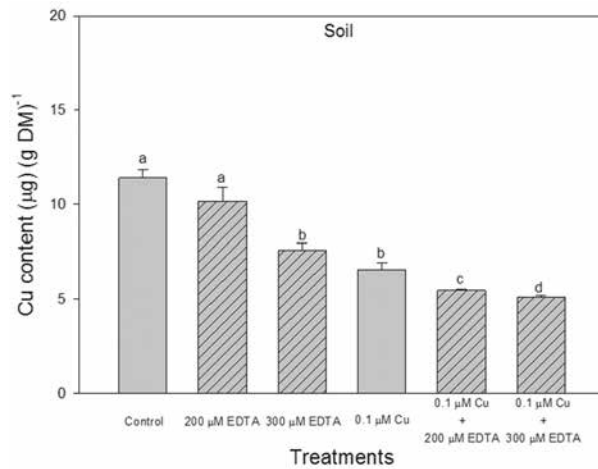


Figure 1. Plant-available copper concentration in soil extracts after harvest of sweet sorghum plants treated with 0.1 µM CuCl<sub>2</sub> alone or in combination with 200 or 300 µM EDTA. Results are means ±SE, n = 9. Means denoted by different letters are significantly different at  $P \leq 0.05$  as determined by Duncan's multiple range test

In parallel, the copper contents increased significantly in the roots of sweet sorghum plants treated with 300 mM EDTA and 0.1 mM CuCl<sub>2</sub> + 300 mM EDTA (Table 1). All of the EDTA, CuCl<sub>2</sub> and combined treatments increased the copper contents of the stems compared to untreated controls but only small part of Cu was transported from stem to leaves (Table 1). However, plants did not show toxicity symptoms during the whole experimental period.

#### *Accumulation of other microelements*

Concentrations of other essential microelements (Fe, Mn, Zn) were determined in plant tissues in order to reveal if they are absorbed more effectively by the root system and transported to the aerial plant parts in the presence of EDTA (Table 1). The first group (Fe, Mn and Zn) includes important microelements participating in the photosynthetic electron transport (Fe, Mn) and detoxification reactions (Zn; Cu-Zn superoxide dismutase) in chloroplast, so they can modify the photosynthetic efficiency in the leaves.

There were no significant changes between treatments in the accumulation of Fe in the root and stem tissues, but both concentrations of EDTA decreased the iron concentration in the leaves in the presence of 0.1 mM CuCl<sub>2</sub>. Similar tendencies could be observed in case of Mn in the leaves and stems of plants treated with 200 or 300 µM EDTA in combination with 0.1 mM CuCl<sub>2</sub>, but EDTA treatments alone increased Mn content in the stem. There were no significant changes in Zn and Co accumulation with the exception of 300 µM EDTA treatment. In these plants Zn and Co transported to the stem to higher extent.

Table 1. Changes in the microelement concentration of root, stem and leaf of sweet sorghum plants treated with 0.1 mM CuCl<sub>2</sub> alone or in combination with 200 or 300 µM EDTA

Plant Organs	Treatments	Microelements (mg g <sup>-1</sup> DM)					
		Cu (18.8)	Zn (16.5)	Co (16.31)	Fe (14.33)	Mn (14.04)	
Root	Control	24.66±0.17 <sup>c</sup>	166.70±6.20 <sup>a</sup>	0.89±0.17 <sup>a</sup>	864.65±91.65 <sup>ns</sup>	23.18±2.97 <sup>ns</sup>	
	200 mM EDTA	26.13±2.63 <sup>c</sup>	179.26±5.44 <sup>a</sup>	0.79±0.05 <sup>a</sup>	1013.15±108.44 <sup>ns</sup>	27.54±1.84 <sup>ns</sup>	
	300 mM EDTA	36.83±4.29 <sup>b</sup>	135.40±2.20 <sup>b</sup>	0.52±0.07 <sup>b</sup>	723.51±157.98 <sup>ns</sup>	20.83±3.80 <sup>ns</sup>	
	0.1 mM CuCl <sub>2</sub>	27.75±3.17 <sup>bc</sup>	148.85±3.36 <sup>a</sup>	0.52±0.14 <sup>a</sup>	740.45±174.47 <sup>ns</sup>	18.73±2.36 <sup>ns</sup>	
	0.1 mM CuCl <sub>2</sub> +200 mM EDTA	32.82±1.88 <sup>bc</sup>	158.40±0.32 <sup>a</sup>	0.60±0.09 <sup>a</sup>	761.91±184.12 <sup>ns</sup>	21.05±1.69 <sup>ns</sup>	
	0.1 mM CuCl <sub>2</sub> +300 mM EDTA	46.86±4.64 <sup>a</sup>	169.23±11.66 <sup>a</sup>	0.62±0.12 <sup>a</sup>	777.71±138.62 <sup>ns</sup>	18.03±1.61 <sup>ns</sup>	
Stem	Control	22.95±1.80 <sup>c</sup>	155.35±20.94 <sup>b</sup>	0.01±0.00 <sup>b</sup>	74.23±20.10 <sup>ns</sup>	8.13±0.37 <sup>c</sup>	
	200 mM EDTA	36.63±1.54 <sup>a</sup>	161.23±16.77 <sup>b</sup>	0.01±0.01 <sup>b</sup>	86.95±37.43 <sup>ns</sup>	10.40±0.23 <sup>b</sup>	
	300 mM EDTA	41.23±1.58 <sup>a</sup>	308.80±63.28 <sup>a</sup>	0.05±0.0 <sup>a</sup>	107.63±22.10 <sup>ns</sup>	15.20±0.04 <sup>a</sup>	
	0.1 mM CuCl <sub>2</sub>	29.22±1.40 <sup>b</sup>	137.63±9.23 <sup>b</sup>	0.02±0.00 <sup>b</sup>	108.43±37.12 <sup>ns</sup>	12.66±1.87 <sup>bc</sup>	
	0.1 mM CuCl <sub>2</sub> +200 mM EDTA	41.43±2.01 <sup>a</sup>	153.11±10.72 <sup>b</sup>	0.01±0.00 <sup>b</sup>	67.81±11.41 <sup>ns</sup>	6.50±0.28 <sup>d</sup>	
	0.1 mM CuCl <sub>2</sub> +300 mM EDTA	40.36±1.03 <sup>a</sup>	170.26±17.90 <sup>b</sup>	0.01±0.00 <sup>b</sup>	74.12±17.59 <sup>ns</sup>	6.20±0.49 <sup>d</sup>	
Leaf	Control	19.81±1.99 <sup>ns</sup>	138.83±12.23 <sup>ns</sup>	0.05±0.04 <sup>ns</sup>	112.11±2.44 <sup>a</sup>	12.38±0.18 <sup>a</sup>	
	200 mM EDTA	24.57±1.57 <sup>ns</sup>	140.70±20.81 <sup>ns</sup>	0.01±0.01 <sup>ns</sup>	101.51±7.19 <sup>a</sup>	11.60±0.53 <sup>a</sup>	
	300 mM EDTA	25.49±1.42 <sup>ns</sup>	147.50±5.80 <sup>ns</sup>	0.02±0.01 <sup>ns</sup>	106.59±6.89 <sup>a</sup>	11.47±0.18 <sup>a</sup>	
	0.1 mM CuCl <sub>2</sub>	23.98±1.54 <sup>ns</sup>	124.30±9.22 <sup>ns</sup>	0.08±0.05 <sup>ns</sup>	105.86±1.79 <sup>a</sup>	11.79±0.21 <sup>a</sup>	
	0.1 mM CuCl <sub>2</sub> +200 mM EDTA	24.73±2.55 <sup>ns</sup>	141.26±11.40 <sup>ns</sup>	0.01±0.01 <sup>ns</sup>	85.61±5.81 <sup>b</sup>	9.67±0.23 <sup>c</sup>	
	0.1 mM CuCl <sub>2</sub> +300 mM EDTA	24.83±1.07 <sup>ns</sup>	141.23±15.01 <sup>ns</sup>	0.01±0.01 <sup>ns</sup>	84.77±1.24 <sup>b</sup>	10.34±0.29 <sup>b</sup>	

EDTA-metal complex stability constants (log K<sub>s</sub>) are in brackets next to the symbol of the metals. Results are means ±SE, n = 3. Means denoted by different letters are significantly different at P ≤ 0.05 as determined by the Duncan's multiple range test (ns: not significant).

Table 2. The parameters of photosynthetic light response ( $A/PPFD$ ) curves in the leaves of sweet sorghum plants treated with 0.1 mM  $\text{CuCl}_2$  alone or in combination with 200 or 300  $\mu\text{M}$  EDTA

Treatments	$A_{\text{max}}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$A_q$ ( $\text{mol CO}_2 \text{ mol}^{-1} \text{ photon}$ )	$R_d$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	$L_{\text{cp}}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )
Control	20.88+0.63 <sup>a</sup>	0.065+0.003 <sup>ns</sup>	1.27+0.16 <sup>ns</sup>	19.28+1.98 <sup>ns</sup>
200 $\mu\text{M}$ EDTA	21.20+0.81 <sup>a</sup>	0.056+0.005 <sup>ns</sup>	0.85+0.30 <sup>ns</sup>	14.17+4.76 <sup>ns</sup>
300 $\mu\text{M}$ EDTA	22.53+0.60 <sup>ab</sup>	0.059+0.006 <sup>ns</sup>	0.99+0.45 <sup>ns</sup>	15.49+5.46 <sup>ns</sup>
0.1 mM $\text{CuCl}_2$	22.89+1.08 <sup>bc</sup>	0.062+0.002 <sup>ns</sup>	1.16+0.33 <sup>ns</sup>	18.80+5.59 <sup>ns</sup>
0.1 mM $\text{CuCl}_2$ + 200 $\mu\text{M}$ EDTA	24.53+1.57 <sup>bc</sup>	0.067+0.002 <sup>ns</sup>	1.63+0.80 <sup>ns</sup>	24.40+1.64 <sup>ns</sup>
0.1 mM $\text{CuCl}_2$ + 300 $\mu\text{M}$ EDTA	24.87+0.94 <sup>c</sup>	0.059+0.001 <sup>ns</sup>	1.24+0.21 <sup>ns</sup>	20.70+3.27 <sup>ns</sup>

$A_{\text{max}}$  = maximal assimilation ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $A_q$  = apparent quantum yield ( $\text{mol CO}_2 \text{ mol}^{-1} \text{ photon}$ );  $R_d$  = dark respiration ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $L_{\text{cp}}$  = light compensation point; ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )

Results are means  $\pm$ SE,  $n = 3$ . Means denoted by different letters are significantly different at  $P \leq 0.05$  as determined by Duncan's multiple range test (ns: not significant).

Although Co is not essential for plants, its deficiency blocks the function of several N-fixing microorganisms (Table 1).

#### Effect of EDTA-, copper- and combined treatments on photosynthesis

Both the excess of copper or copper deficiency can lead to the degradation of photosynthetic pigments and to the decline in photosynthetic activity; thus, it can decrease the accumulation of sugars and biomass production. In these experiments, after treatments with 0.1 mM  $\text{CuCl}_2$  and 0.1 mM  $\text{CuCl}_2$  plus 200 or 300  $\mu\text{M}$  EDTA the maximal  $\text{CO}_2$  assimilation rate ( $A_{\text{max}}$ ) in leaves of sweet sorghum was significantly enhanced, compared to

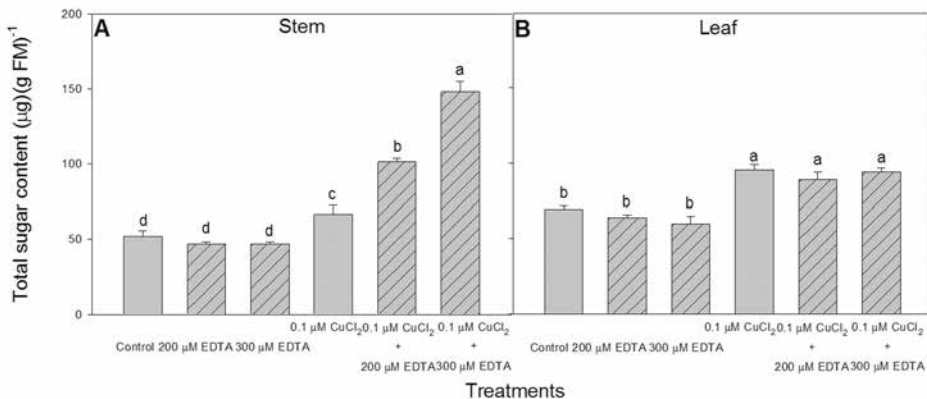


Figure 2. Total sugar content in stem (A) and leaf (B) of sweet sorghum plants treated with 0.1 mM  $\text{CuCl}_2$  alone or in combination with 200 or 300  $\mu\text{M}$  EDTA. Results are means  $\pm$ SE,  $n = 5$ . Means denoted by different letters are significantly different at  $P \leq 0.05$  as determined by Duncan's multiple range test (ns: not significant)

control plants and to EDTA treatments. However,  $A_q$ , the initial slope of the A/PPFD curves, which allows estimates of the apparent quantum yield, the dark respiration ( $R_d$ ) and the light compensation point ( $L_{cp}$ ) were not significantly different from the untreated control (Table 2). It can be concluded that in contrast to net  $CO_2$  assimilation, the dark respiration has not been changed significantly in sorghum plants in the presence of Cu+EDTA.

The photosynthetic pigments, chlorophyll  $a + b$  and carotenoids were not affected significantly by the treatments, suggesting that free radical production remained in the tolerable range in plant tissues (data not shown). However, treatments with 0.1 mM  $CuCl_2$  alone and in combination with 200 or 300 mM EDTA enhanced the accumulation of soluble sugars in all plant parts (stem, leaf) (Fig. 2). Total sugar content increased most significantly in the stem of sweet sorghum plants after treatment with 0.1 mM  $CuCl_2 + 300$  mM EDTA (Fig. 2, A).

#### *Effect of EDTA-, copper- and combined treatments on biomass production*

The application of 0.1 mM  $CuCl_2$  in combination with EDTA contributed to total dry mass accumulation of plants to a very high extent. On whole plant basis, copper nutrition in the presence or absence of EDTA increased the total biomass production very significantly (Fig. 3, A). At the same time, the transport of Cu was greatly enhanced by EDTA to the aboveground plant parts (stems and leaves) both from the control soil or from the soil irrigated with 0.1 mM  $CuCl_2$  (Fig. 3, B). The total Cu content of the shoots reached maximal value in plants treated with 0.1 mM  $CuCl_2 + 300$  mM EDTA.

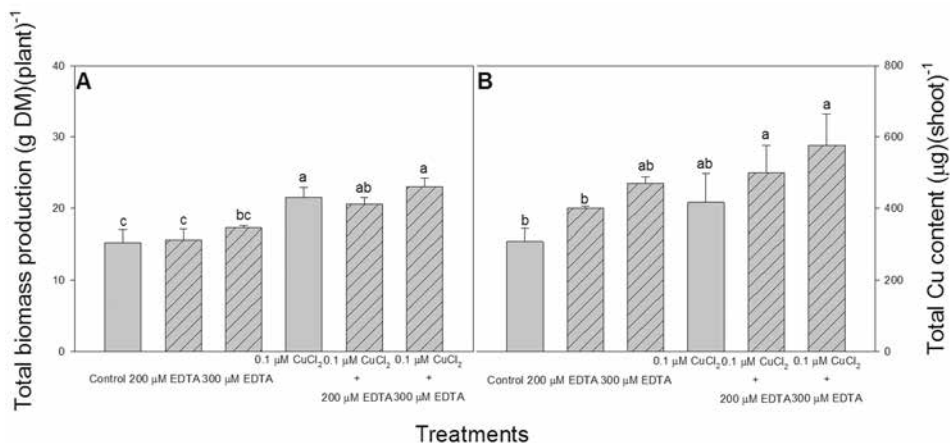


Figure 3. Total biomass production of sorghum plants (A) and total copper content of shoots (B) treated with 0.1 mM  $CuCl_2$  alone or in combination with 200 or 300 µM EDTA. Results are means  $\pm$  SE, n = 10. Means denoted by different letters are significantly different at  $P < 0.05$  as determined by Duncan's multiple range test



## Discussion

Copper deficiency may have major effect both on the quality and yield of crop plants, thus the impact of copper nutrition and its role in photosynthesis, growth and biomass production has great importance in agronomy (Wu et al. 2010; Gonzalez and Alvarez 2013).

The application of a Cu-EDTA complex was suggested for copper fertilization on copper-deficient soils (Penney et al. 1988). However, the effect of EDTA on heavy metal accumulation was contradictory. Some authors reported that EDTA application did not enhance or even decreased heavy metal uptake (Xu et al. 2007), while others found significant metal accumulation in the aerial part of plants after EDTA treatment on heavy metal polluted soils (Luo et al. 2006). It was found that a threshold concentration of EDTA is required to induce the accumulation of metals in plant shoots which was about 1–5 mmol kg<sup>-1</sup> soil in case of Indian mustard grown in soil containing 600 mg kg<sup>-1</sup> Pb (Baylock et al. 1997). The ratio of metal to EDTA concentration is also important. When the ratio of EDTA to Pb was higher than 2, the increase of EDTA was observed in the shoot of Indian mustard with a concomitant inhibition of growth (Xu et al. 2007). Since sweet sorghum was suitable for cleaning heavy metal polluted soils (Angelova et al. 2011), we were interested in whether the biomass and soluble sugar content of this important energy plant could be increased with EDTA treatment on soils of low or adequate available Cu supply. We increased the Cu content of soil with 0.1 mM CuCl<sub>2</sub> applied in the irrigation solution alone or in combination with 200 mM or 300 mM EDTA. These concentrations of EDTA are about one order of magnitude lower (0.48 and 0.72 mmol kg<sup>-1</sup> soil, respectively) than those applied in phytoextraction studies (1–5 mmol kg<sup>-1</sup> soil) (Luo et al. 2006) and they were not toxic to sorghum plants.

The results of the present study indicate that the irrigation with low concentration of copper or copper-EDTA chelate have beneficial effect on copper accumulation and translocation to different plant organs. Copper accumulated mainly in the roots in the presence of 300 mM EDTA even in those plants which were grown on control soil. The highest copper concentrations were observed in the stem treated with 200 or 300 mM EDTA with or without 0.1 mM CuCl<sub>2</sub>. All of the treatments resulted in slight increases in leaf copper contents. In accordance with the results of Geebelen et al. (2002), these treatments did not cause foliar symptoms of copper or EDTA toxicity during the whole experimental period which confirmed that the use of low concentration of EDTA did not induce oxidative stress or any other toxic symptom.

The accumulated copper has positive effects on photosynthesis of plants and thus on the biomass production. Treatments with 0.1 mM CuCl<sub>2</sub> and with 0.1 mM CuCl<sub>2</sub> in combination with 200 or 300 μM EDTA enhanced the maximal net CO<sub>2</sub> assimilation rate ( $A_{\max}$ ) in the leaves at saturating light intensities based on the analysis of fitted photosynthetic light response curves. The dark respiration ( $R_d$ ), the intersection of the A/PPDF curves with y axis, and the light compensation points ( $L_{cps}$ ) did not change significantly compared to untreated control, suggesting that the increases in biomass production was mainly due to the enhanced photosynthetic activity. The uncoordinated ligand is available to bind essential divalent cations in the cells, thus, it was not unexpected that Cu-EDTA

treatments reduced Fe and Mn content but not that of Zn in the leaves. Fe is important metal cofactor of several proteins in the photosynthetic electron transport chain (PSII reaction centre, cytochromes, Fe-S proteins) and Mn ions are cofactors in the active centre of water splitting enzyme (Marschner 1995; Zhao et al. 2014). In spite of their reduced concentrations, the photosynthetic activity was enhanced in the leaves. It is supposed that the formation of a Fe-EDTA complex may ensure a controlled Fe supply in the leaf tissues, preventing the uncontrolled generation of reactive oxygen species.

Since these treatments enhanced the photosynthetic CO<sub>2</sub> assimilation rate, they resulted in the accumulation of photosynthetic products, soluble sugars in all utilizable parts, stems and leaves of sweet sorghum plants. Similar effects of copper treatments on soluble sugar contents were reported by other authors (Jiang et al. 2012) but the effect of copper was very significantly enhanced by EDTA in our system. Higher soluble sugar levels can be detected after 0.1 mM CuCl<sub>2</sub> + 300 mM EDTA treatments in the stem which is the most important plant part in the agricultural and industrial practice. The increased photosynthetic activity may also lead to higher biomass production (Ni et al. 2009; Bharti et al. 2014), thus total dry mass of plants and total sugar content of tissues clearly increased after 0.1 mM CuCl<sub>2</sub> treatment both in the absence and in the presence of EDTA.

These results suggest that irrigation of soil with non toxic concentration of CuCl<sub>2</sub> in combination with appropriate concentration of EDTA can be a useful strategy to enhance the photosynthetic assimilation rate, soluble sugar and biomass production and Cu accumulation in the above-ground plant parts, thus Cu-chelate fertilization may increase the quality of sweet sorghum grown on soils of low Cu concentrations.

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