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EFFECT OF COPPER ON GROWTH AND CHLOROPHYLL CONTENT IN TEA PLANTS (*CAMELLIA SINENSIS* (L.) O. KUNTZE)

SARMISHTA DEY[1], P. B. MAZUMDER[2] & S. B. PAUL[3]

[1,2]Plant Biotechnology Laboratory, Department of Biotechnology, Assam University, Silchar, Assam, India

[3]Department of Chemistry, Assam University, Silchar, Assam, India

ABSTRACT

Tea is the oldest, most popular, non alcoholic caffeine containing beverage, in the world. The presence of heavy metal even in trace amount causes adverse effect both in quality and quantitative production. The accumulation of heavy metal causes health issues also. In the present work we have studied the effect of chlorophyll content which is an important photosynthetic parameter with the high concentration of copper stress with respect to the days of the treatment in two different cultivars *viz.* TV-23, TV-17 in hydroponically system. Both shoot and root weight decreased progressively with increasing Cu concentration for *Camellia sinensis*. Negative linear relationships were observed between total Chlorophyll contents and Cu concentration. With the increase in the concentration of Cu even at trace level causes deleterious effect in the metabolism of plant especially photosynthetic activity which caused remarkable breakdown in the photosynthetic parameters. A significant decrease of chlorophyll started for both the cultivars was observed at concentrations above 300µM. From this study, we found the photosynthetic activity of TV-17 is more sensitive to Cu stress than that of TV-23.

KEYWORDS: Tea Plants, Copper Stress, Physiological Characters, Chlorophyll Pigments

INTRODUCTION

In physiological process of plants heavy metal plays a vital role. In trace amounts, several ions required for metabolism, growth and development which is present in soil or as in growth media. But with the increase in the amount, creates problem by leading to cellular damage of plants (Avery, 2001, Schutzendubel and Polle, 2002, Gaetke and Chow, 2003). This leads to the inactivation of biomolecules by blocking essential functional groups or by displacing the essential metal ions (Goyer, 1997). Due to industrialization and globalization the heavy metal contamination became a serious problem (Boominathan and Doran, 2003; Plunkett, 1987; Seiler et al., 1988; Donmez and Aksu, 2002). And heavy metal became a serious threat for the environment due to their extreme toxicity (Kaewsarn & Yu, 2001). Leaves of tea plants are great source of mineral such as Zn, Cu, Mn, F, P etc. Cu is essential for plant growth but increase in the concentration of both the essential and non essential metals will result in inhibition of growth (J.L.Hall, 2002). In the presence of excessive amount of heavy metal shows the toxicity symptoms due to interactions of ions at cellular level. Much of the soluble copper in the apoplast is complexed with organic ligands. Much of Cu^{2+} is tightly bound in the cell wall of roots and can only be replaced by other Cu^{2+} ions (Iwasaki et al., 1990; Walker & Webb, 1981). Carotenoids are the pigments which are more or less produced by all photosynthetic and non photosynthetic organisms. The carotenoids are very important for plants, lack of carotenoids do functioning both in the acquisition of light energy and in protection of photosynthetic apparatus against excessive light damage (Demmimng-Adams et al., 1996). Chlorophylls are essential components for photosynthesis, and occur in chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether. In this study we have investigated the physiological mechanism of Cu induced inhibition of photosynthesis with accumulation of Cu at different concentration.

MATERIALS AND METHODS

Experimental Condition for the Growth of the Plants

Three months old plants of the two cultivars viz. TV-23, TV-17 were collected from the Rosekandy Tea Estate, Assam. Then transferred the plants to the Hoagland solution and allowed them to get stable for 7 days. Then we have treated the plants with $CuSO_4$ at different concentration of 50µM, 200µM, 300µM,400µM, 500µM, 600µM in the nutrient solution. The control plants were left as untreated, comprising only the nutrient solution. The top four leaves were collected for measuring the chlorophyll pigmentations after 2^{nd}, 4^{th} and 7^{th} day of the treatment.

Determination of Chlorophyll

Both the treated leaves and the control leaves at the respective days were harvested and kept in liquid nitrogen for frozen and then lyophilized. The content of chlorophyll of the mature leaf of the plant was determined in 80% acetone extract of 0.1 g leaf and expressed as µg/g fresh weight (Hegedus et al., 2001).

Based on the data obtained from the experiment, the results presented are the mean ± standard deviation (SD) gained from at least three replicate samples. Statistical analysis has done by the least significant difference (LSD) for multiple comparisons, taking $P<0.05$ as significant.

RESULTS

Effect of Copper on Plant Growth

From the above experiment we have observed that at the different concentration of Cu plants are showing different symptoms. With compare to control there is a significant retardation in the growth of the Cu treated plants. At lower concentration leaves are least effected than that of roots and shoots (Figure 1). But with the increase in the concentration of Cu which severely inhibit the root growth with accumulation of Cu in leaves (Figure 2). Black brown spots were observed in the leaves at higher concentration. The roots were suffered a lot by retarding the growth of the length of roots. The number of new leaves slowed down with the less arrival of new stems. As a whole the growth has slowed down. The dry weight of shoots of both the cultivars was retarded with respect to control (Figure 4 & 5). With the increase in the concentration there was a significant decrease in the weight. Through the physiological study we have observed the slow growth rate of the treated plants with respect to control and at higher concentration of 500 µM & 600 µM were almost negligible.

Effect of Copper on Photosynthetic Pigments

There was a significant decrease in the total chlorophyll pigment in both the treated cultivars with respect to control. But the retardation level is high at TV-17. The expanding leaves of TV-23 had higher total chlorophyll than those of TV-17 (Figure 6 & 7).

The contents of pigment in plants showed an almost linear decrease in response to the increase in the concentration of Cu which ranges from 0 µM (control) to 600 µM. At higher concentration the level of decline in the content of chlorophyll has observed more than compare to lower concentration. With the exposure days the chlorophyll content was decreased.

Figure 1: Small Yellow Patches on Leaves at 200µM of Cu in TV-23

Figure 2: Effects of Leaves at 500µM of Cu in Tv-17

Figure 3: Effects of Leaves at 500µM of Cu in Tv-23

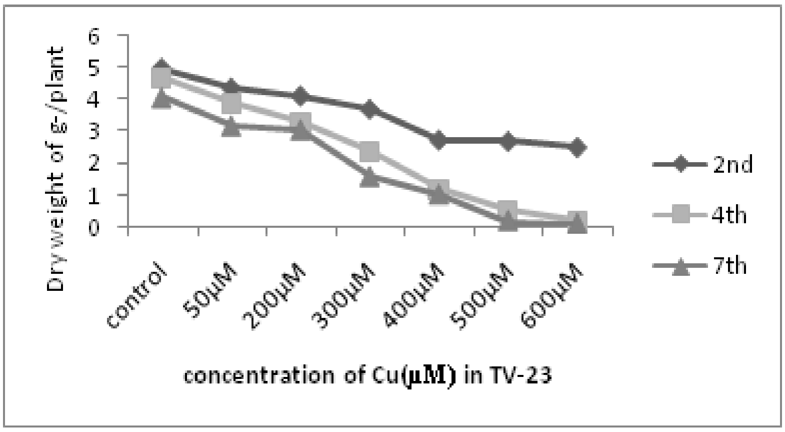

Figure 4: Impacts of Cu on the Dry Weight of Shoot of TV-23. Values are Average of Three Replicates

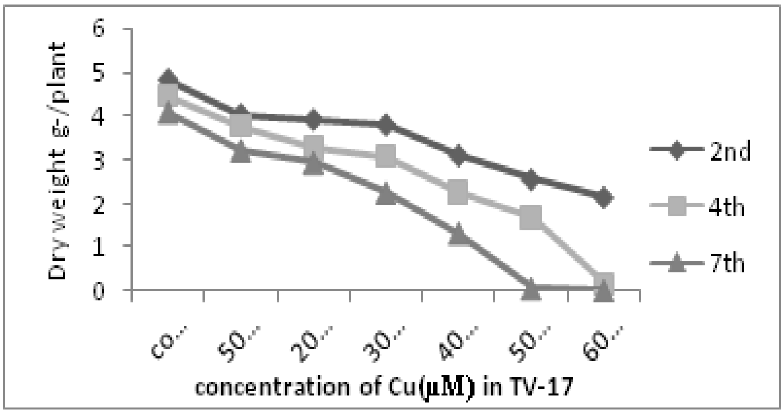

Figure 5: Impacts of Cu on the Dry Weight of Shoot of TV-17. Values are Average of Three Replicates

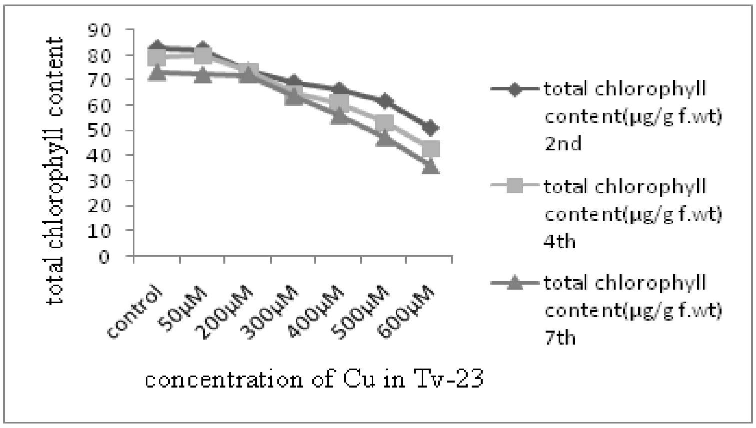

Figure 6: Impacts of Cu on the Total Chlorophyll Content of TV-23. Values are Average of Three Replicates

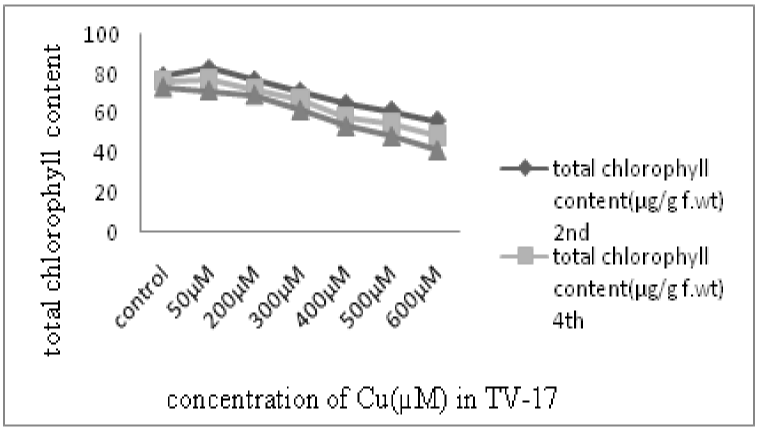

Figure 7: Impacts of Cu on the Total Chlorophyll Content of TV-17. Values are Average of Three Replicates

DISCUSSIONS

Heavy metal inhibits the plant metabolism, but some metals like Cu, Fe, Mn are essential for the photosynthesis. There are number of proteins and enzymes found in heavy metals and so they are essential for the growth and development of plants and also maintain the optimum metabolism. The deficiency of these metals has direct impact on the process of photosynthesis, but with the increase in concentration of these metals become toxic to plant and affects photosynthesis. The growth parameters like biomass have been shown to very sensitive to heavy metals (Arun *et al.*, 2005). Our research has illustrated that Cu inhibited the plant growth by retarding the growth of root, shoot, and decline in the numbers of arrival of new leaves. The dry weight decreased progressively with the increase in the Cu concentration (Figure 4 & 5). At lower concentration the dry weight decrease with less or without showing toxic in leaves (Figure 1). But at higher concentration severely inhibit the root growth with accumulation of Cu in leaves (Figure 2). The chlorophyll contents in the cultivars TV-23, TV-17 has decreased with the increase in the concentration (Figure 6 & Figure 7).

With high concentration of heavy metals, the activities of photosynthetic enzymes degrade which results in the reduction of chlorophyll content (Thapar et al., 2008). In most of the plants Cu is found associated with plastocyanin, an important component of the electron transport chain of PSI in the chloroplast. Copper deficiency reduces PSI electron transport due to decreased formation of plastocyanin (Baszynski et al.; 1988). A decrease in PSII is also observed in Cu deficient plants (Henriques; 1989). The biochemical and photochemical reaction centres of photosynthesis are the important sites of inhibition by the action of heavy metals especially by Cu. Cu ion insert directly to the reaction centre specially PSII which due to the high irradiance causes direct damage to the reaction centre (Kupper et al., 1996, 1998, 2002). Copper interferes with the biosynthesis of the photosynthetic activity by changing the pigment and protein composition of photosynthetic membranes. A lower content of chlorophyll, inactivation of enzymes and proteins linked to photosynthesis process and modification of thylakoid membranes occurs under copper toxicity. Copper-induced chlorosis can result from the inhibition of pigment accumulation and decrease in the chlorophyll integration into photosystems. As a whole it causes damages in the structure and function of the chlorophyll (Kupper et al.; 2003).

Due to the Cu stress many oxidative stress also get generated and this as a whole cause the inhibition in the photosynthetic reactions which has been observed by many authors (Rocchetta and Kupper, 2009). With the increase in the concentration there is remarkable decrease in the chlorophyll pigment with respect to the control. At higher concentration 400µM, 500µM, 600µM with the exposure time the Cu treated plants are showing adverse effect by decline in the growth of shoot, decrease in length of root, growth of new leaves are very low, bleaching of leaves and fall off. All these result in the degradation of chlorophyll pigments. By binding to the various sensitive sites of the photosynthetic apparatus heavy metals can also directly affect the photosynthetic machinery. It gives us an evident that excess Cu has a strong effect on chloroplast fine structure which results in degradation of grana stacks and stroma lamellae (Baszynski et al.; 1988). Many authors have concluded the same in their observation.

Cu stress has decreased the photosynthetic level of *Crassula helmis* (Kupper *et al.*, 2009). Various authors gave evidences that the toxic effect of metal on phototropic organisms strongly appears to be related to the increase in the levels of lipid peroxidation and protein carbonylation as well as the production of antioxidant defense systems (Gallego et al.; 1996, Devi and Prasad; 2005, Tripathi et al.; 2006). In the different season the chlorophyll pigment shows different adverse action. At summer the degradation level is very high with respect to other season like autumn and winter. In high irradiance conditions Cu stressed plants showed different results in leaves of *Triticum aestivum* with the decline in the Fv/Fm (Lanaras *et al.*, 1993). The accumulation of Cd decreased net photosynthesis and transpiration in excised Acer sacchanium L. leaves (Lamoreaux R J et al.; 1978). From the above study it can be concluded that certain concentration of Cu inhibit the growth of plant, cause chlorophyll loss and can affect the photosynthetic activities.

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