Physiological and Biochemical Properties of Cotton in Response to Copper Stress

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Research article

Keywords: copper, cotton, tolerance, physiological and biochemical characteristics

Posted Date: August 27th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51780/v1

Abstract

Background

copper (Cu) is an essential micronutrient, required for plant growth and development. However, high concentrations of Cu can be extremely toxic to plant. This study investigate the tolerance mechanism of cotton under copper stress and its potential for soil pollution improvement.

Results

The hybrid cotton variety (Zhongmian 63) and its two parent lines were selected as materials. Cotton seedling were treated with different Cu concentrations (0, 0.2, 50, 100, 200 μM) for 10 days in hydroponic condition. The results showed that the stem height, root length, and leaf area of cotton seedlings appear to have a down trend with the increase of Cu concentration. Increasing Cu concentration promoted Cu accumulation in roots, stems, and leaves of all the three cotton genotypes, however, the roots region was the main Cu storage organ, followed by leaves and stems regions. Compared with the parent lines, the roots of Zhongmian 63 are more capable of enriching Cu and have the least amount of Cu transported to the shoots. Therefore, the toxicity of Cu to cotton seedling is effectively alleviated. Cu-caused oxidative stress to cotton leaves was evident by over accumulation of H_2O_2 and MDA. POD activity and soluble sugar content increased firstly and then decreased compared with the control group. GSH content increased and photosynthetic pigment content decreased with increasing copper concentration in nutrient solution.

Conclusion

Our results suggest that the hybrid cotton variety Zhongmian 63 performed well under Cu stress. This lays the theoretical foundation for further analysis on molecular mechanism of cotton resistance to copper and promoting the large-scale planting Zhongmian 63 in the copper-contaminated area.

Background

Heavy metal contamination is one of the vital environmental issues to the present world because of its increasing levels caused by both nature and anthropogenic activities [1]. Among the heavy metal, copper (Cu) is an essential micronutrient, required for plant growth and development. Visible symptoms of Cu deficiency include stunted growth, chlorosis of young leaves, loss in biomass and fruit yield and ultimately death of the plant [2,3]. However, high concentrations of Cu can be extremely toxic to plant. For example, it interferes with vital physiological processes and metabolic functions of essential elements, causing cellular redox imbalance, and oxidative stress [4].

Plants have evolved certain homeostatic mechanisms to avoid heavy metal toxicity such as metal exclusion, retention in the roots, immobilization in the cell wall, metal compartmentation and binding of heavy metal by strong ligands like glutathione, Metal-lothioneins (MTs) and thiol-rich peptides phytochelatins (PCs) [5,6]. Moreover, plants employ antioxidant system and non-enzymatic antioxidants to combat the oxidative injury induced by the heavy metal[7]. The antioxidative enzymes include superoxide dismutase (SOD), catalases (CAT), peroxidase (POD), etc. Non-enzymatic antioxidative defense systems include small molecule antioxidants like glutathione (GSH), ascorbate (AsA) and others. GSH plays a multiple role against metal toxicity by reducing metal uptake [8], chelating metal ions in cell or as a precursor of PCs [9,10]. These induced cellular antioxidants scavenged reactive oxygen species (ROS) thereby prevented the damage caused by the overproduction of ROS. Soluble sugars pose a critical role in the cellular redox balance as they do have a close relationship with photosynthesis and respiration [11]. Sugars on the other hand, function as ROS eliminator or cell signal in response to stresses in plants [12].

Cotton is known to be one of the major economic crops in the word. Nonetheless it is a heavily fertilized crop during growth and production, and it is subjected to constant threat of Cu toxicity [13]. But it is very resistant to heavy metals and other abiotic stresses, because of its non-edible characteristics of cotton fiber, and also its ability to restore soil quality when grown on heavy metal polluted areas [14]. Our main aims are to study the Cu toxicity syndrome among two parental lines and their hybrid variety, and to explore the mechanisms of Cu tolerance in cotton.

Results

Plant growth conditions under Cu stress

Cotton seedlings were cultured in nutrient solution containing different Cu concentrations for 10 days. The results found that low Cu concentrations could promote cotton seedlings growth, but high Cu concentrations inhibited the seedlings growth (Fig. 1). When the content of Cu was 200 μM, the growth of seedlings was badly restricted (Fig. 1). Although the toxicity of Cu significantly inhibited the growth of cotton seedlings, the effects of different Cu concentrations on the growth of three cotton varieties seedlings were apparently different. The average data based on plant growth also showed that the parental line sGK9708 and the hybrid variety Zhongmian 63 were more tolerant to Cu than the maternal line 9053. However, the stem height and leaf area of the three cotton varieties showed strong tolerance when the Cu concentration was 50 μM (Fig. 2A, 2B), but the root length was inhibited (Fig. 2C). It indicates that the root is more sensitive to Cu stress than the shoot. Moreover, the stem height, leaf area and root length of three cotton varieties were inhibited when the contents of Cu were increased (Fig. 2).

The content of photosynthetic pigments

The toxicity of Cu severely reduced the chlorophyll content of chlorophyll a and chlorophyll b in cotton seedling leaves (Table 1). In the absence of Cu, chlorophyll content in three cotton seedlings decreased comparing with the control group. When Cu was excessive, the chlorophyll content of the three cotton seedlings decreased with the increasing of Cu concentration comparing with the control group. But the chlorophyll contents of sGK9708 and Zhongmian 63 increased temporarily when the Cu concentration was 50 μM and increased significantly in Zhongmia 63, but subsequently decreased with further increasing of Cu concentration. The ratio of chlorophyll a to chlorophyll b tended to reduce with the increasing of Cu concentrations in the nutrient solution. This indicates that the rate of chlorophyll a content declination is higher than that of chlorophyll b.

Determination of cu content

Under Cu stress, different cotton varieties exhibited differences in the Cu uptake and transport of Cu (Table 2). Although the absorption of Cu was different, the trend of Cu accumulation in different tissues of all three varieties was similar. Cu concentration was the highest in root, following in leaves, and the lowest in stems under different Cu treatment. However, Cu accumulation was the highest in Zhongmian 63, followed in sGK9708 and 9053. Comparing to the parent line, Zhongmian 63 transportation of Cu from the roots to the shoots was lower than that of the parental lines. This indicates that Zhongmian 63 is more capable of enriching Cu in root and effectively reduce the toxicity of Cu to cotton seedlings.

Determination of H_2O_2 and MDA content

The leaf H_2O_2 and MDA (malondialdehyde) content of the three varieties increased with the increasing of Cu concentration (Fig.3). At the Cu concentration of 200 μM, the content of H_2O_2 and MDA in the three cotton varieties reached the highest. However, compared with the parent lines, the H_2O_2 and MDA content in the Zhongmian 63 leaves increased slowly with increasing Cu concentration. This indicates that Zhongmian 63 has a stronger ability to scavenge ROS.

GSH content and POD activity

The leaf GSH content of the three varieties increased with increasing Cu concentration (Fig.4A). When Cu was excessive, the GSH content increased with the increase of Cu concentration in the three cotton varieties. At the Cu concentration of 200 μM, the GSH content in the leaves of the three cotton varieties also reached the highest. However, under the conditions of Cu concentration treatment, the GSH content in the Zhongmian 63 leaves was significantly higher than that of the parent lines.

Under Cu stress conditions, the activities of POD in the leaves of three cotton seedlings increased first and then decreased (Fig.4B). At Cu concentration of 50 μM, the POD activity in the leaves of 9053 seedlings reached the highest, and then the activity significantly reduced. At the Cu concentration of 100 μM, sGK9708 and Zhongmian 63 reached the highest POD activity, but POD activity of Zhongmian 63 was higher than that of sGK9708, and when Cu concentration was 200 μM, the POD activity decreased significantly.

Determination of soluble sugar content

Effect of different concentrations of Cu on soluble sugar content of all three cotton varieties was assessed and results are presented in Fig.5. Among the three cotton varieties, the soluble sugar content in leaves reached the maximum at 50 μM Cu concentration, and then decreased with further increase of Cu levels. However, at Cu concentration of 100 μM and 200 μM, the soluble sugar content of Zhongmian 63 was significantly higher than that of the parent lines.

Discussion

In recent decades, due to the increase in human activities, such as the exploitation of ore, the frequent application of heavy metal-containing pesticides and fertilizers in agriculture, and the discharge of wastewater from industrial wasters resulted in a gradual increase in the concentration of heavy metals in environment. Heavy metal poisoning is also becoming one of the major environment problems facing the world today. A large number of studies have shown that heavy metal pollution in soil, such as copper pollution, can cause drastic changes in physiological and biochemical characteristics of plants. However, in the face of heavy metal stress, plants have evolved a defense mechanism to resist stress, in order to maintain their normal growth and development. In this study, we investigated the effect of Cu on the morphology of three cotton varieties by setting a medium containing different Cu concentrations (Fig.1), including stem height (Fig.2A), leaf area (Fig.2B) and root length (Fig.2C). In the present study, when the Cu concentration is 50 μM in the culture solution, the stem height and leaf area of the three cotton varieties showed an increasing trend. Thounaojam et al. [15] stated that as the concentration of Cu increased, the stem height of rice seedling gradually decreased compared with the control group, and the Zhongmian 63 are more tolerant to Cu than the parent lines. Similar to our finding, Lei et al. [13] found the hybrid lines cotton seedlings performed better under Cu stress than the parent lines. Growth retardation might be due to its interference with various cellular processes such as photosynthesis and respiration [2]. This statement can correspond to a significant reduction in the photosynthetic pigments.

Among the three cotton varieties, the Cu content accumulated in the roots of cotton seedlings was higher than that in stems and leaf tissues (Table 2). In general, the accumulation ability of Zhongmian 63 Cu is much higher than that of its parental lines. There could be some additive genetic effects that might result in an increased in its Cu absorption properties. To explore the Cu-resistant mechanism of the hybrid line, transcriptomics techniques can be used to explore the differential genes for further explanation. Our results confirmed the previous findings, showing a positive correlation between Cu tolerance and plant potential to sequester Cu into root tissues with a limited translocation to shoot[13].

The production of ROS is a common phenomenon of stresses, which can attack polyunsaturated fatty acids and lead to lipid peroxidation[16]. H_2O_2 is the main ROS and increases with increasing Cu concentration. In this study, increasing Cu concentration in culture medium led to the increase in H_2O_2 and MDA in the leaves of cotton seedling（Fig.3A）. The results were in harmony with findings of previous studies in maize leaves (Liu *et al.,* 2018) and in Medicago sativa seedling shoot[17] In this current study, Cu induced H_2O_2 incited directly lipid membrane damage in cotton leaves. High concentration of ROS caused enzyme inactivation, lipid peroxidation, and oxidation of nucleic acids[18].

At the cellular level, plants protect cells from oxidative toxicity through a range of biochemical mechanisms (e.g. enzymatic and non-enzymatic antioxidant) [19]. POD is a major part of H_2O_2 scavenging enzymes that removes H_2O_2 from chloroplasts and cytosol of the plant cell [20]. In our research study, exposure of the cotton seedlings to higher Cu concentration resulted an increased and then decreased with further increase of Cu levels (Fig. 4B). Muzammual et al. [21] have reported that POD activity in ramie (Boehmeria nivea L.) leaves increased first and then decreased with increasing Cu concentration, while some studies reported inhibition of POD activity under Cu stress in the leaves of Rhizophagus clarus [22] and also reported the enhancement of POD activity under Cu stress in the leaves of Medicago sativa [17]. These results indicate that changes in heavy metal-induced antioxidant enzyme activities are related to treatment concentrations and plant species. GSH acts as an antioxidant, detoxifies H_2O_2 via the ascorbate-glutathione cycle. In this study, results showed that GSH content enhanced with increasing Cu concentration (Fig. 4A). Cu-induced elevation in GSH content has also been observed in rice [15]. GSH also acts as a precursor of phytochelatins (PCs). PCs may participate in the detoxification and tolerance by chelating with metals [6].

Soluble sugar could be part of a series of defenses and signals useful to plants, not only to sense and control photosynthetic activity, but to sense and control the ROS balance [11]. Previous literature reported that soluble sugar was decreased in *Capsicum annuum* L. under Cu stress [23]. In the present work, the content of soluble sugar in the leaves of cotton seedling increased first then decreased with the increase of Cu concentration (Fig.5). The possible reason is that lower Cu concentration accelerates the decomposition of high-density carbohydrates in plants and inhibits their synthesis, so the photosynthetic products directly forms low molecular mass substances, such as sucrose, resulting in soluble sugar content increase. At high Cu concentrations, the anabolism and growth of plants are inhibited, and the photosynthetic capacity of plants is reduced, resulting in a decrease in soluble sugar content.

Plant converts inorganic matter into organic matter to satisfy their growth and development through photosynthesis. Chlorophyll is a very important biomolecule that plays a vital role in photosynthesis and enable plants to absorb light energy. Therefore, chlorophyll was considered to be important biomarkers against a variety of abiotic stress that include heavy metals [24]. During our experiment, it was found that the chlorophyll content in the leaves of cotton seedling decrease with the increasing of Cu concentration (Table 1). However, the rate of declination of the parental line was more pronounced. These results are supported by previous results that showed higher Cu concentration reduced chlorophyll content in rice and in tomato [25]. It has been proposed that excess Cu substitutes Mg in chlorophyll molecule [26]. However, the carotenoid content increased under Cu stress conditions compared to the control group (Table 1).

Conclusion

In summary, this study shows that an excessive Cu concentration severely restricts root, shoot, leaf growth and photosynthetic parameters of cotton seedling. However, cotton seedling showed strong tolerance in a range of Cu concentrations. The higher levels of Cu-induced oxidative stress increase H_2O_2 production in cotton seedlings. On the other hand, when compared with the parent line, the hybrid lines effectively increased their tolerance to Cu by increase GSH content, POD activity and soluble sugar content and reduce the transfer of Cu to the ground.

Abbreviations

Cu: copper

MTs: Metal-lothioneins

PCs: phytochelatins

SOD: superoxide dismutase

CAT: catalases

POD: peroxidase

GSH: glutathione

AsA: ascorbate

ROS: reactive oxygen species

MDA: malondialdehyde

Methods

Plant materials, growth condition, and treatments

The glasshouse experiment was conducted using cotton parental lines, namely, the female 9053 (general upland cotton), the male parent sGK9708 (insect resistant), and their hybrids variety Zhongmian 63. Their seeds were provided by Dr. Daigang Yang (Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang, China). Uniformly sized seeds were surface sterilized with 1%(v/v) sodium hypochlorite solution for 20 minutes, washed with distilled water for 3 times and allowed for water imbibition for 24 h before growing in the sand culture medium. Seed germination was allowed for 5 days at 28°C in dark. On the 6th day, uniform seedlings were transferred to hydroponic media and allowed to acclimatize for 10 days. The nutrient solution composition is 20 μM H_3BO_3, 1 μM $ZnSO_4·7H_2O$, 0.2 μM $CuSO_4·5H_20$, 1 μM $MnSO_4·H_2O$, 0.005 μM $(NH_4)_6MO_7O_{24}·4H_2O$, 2498.41 μM $Ca(NO_3)_2·4H_2O$, 499.7 μM KH_2PO_4, 1000.12 μM $MgSO_4·7H_2O$, 2504.70 μM KNO_3、100 μM Na Fe·EDTA, at 22°C/27°C during 8/16 h dark/light periods respectively, with a light intensity of 180 $μmol·m^{-2}·s^{-1}$ and 60% relative humidity. Fifteen days of equally sized cotton seedlings were transferred into the nutrient solution with different Cu concentrations. The treatments concentrations were composed of 0 (without addition of Cu), 0.2 (control group), 50, 100, 200 μM, and Cu is supplied as $CuSO_4·5H_2O$. The nutrient solutions were continuously renewed every 4 days, and the pH value was adjusted to 5.6-5.7 with 0.1M HCl or 0.1M NaOH. Plants were harvested after 10 days with Cu treatments for future analysis.

Determination of growth parameters

Growth parameters of the cotton seedlings were evaluated by determining leaf area, root, and shoot lengths. The leaf area of seedlings was determined by area-weighing method; the lengths of roots and shoots were recorded using the meter ruler.

Measurement of photosynthetic pigments contents

Chlorophyll and carotenoid concentrations were determined according to Lichtenthaler and Wellburn [27]. Briefly, the top-most expanded leaves were randomly cut and soaked in 80% acetone in the ratio of 1:10 w/v until the pigments were completely extracted when leaf became colorless. The process was performed in darkness. The extracts were centrifuged for 15 min at 4000 g to remove any adhering residues. The supernatant was measured at 663, 646 and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. 80% acetone was used as a blank control.

Determination of copper content

For analysis Cu content, the root and leaf samples were harvested separately, and were rinsed with tap water, and immersed in 20 mM Na_2-EDTA for 15 min to remove any trace elements adhering to the tissue. The root and leaf samples were oven dried at 75°C for 48 h. Dried samples (0.1 g) were ground and acid digested with HNO_3 mixture for 24 h at 80°C, followed by Cu estimation using an atomic absorption spectrophotometer.

Detection of H_2O_2 and MDA level

The accumulation of H_2O_2 in leaves was measured by monitoring the A415 of the titanium-peroxide complex following the method described by Liu et al. [28]. Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2. Recovery was checked by adding various amounts of H_2O_2 to the leaf extracts as an internal standard. The level of lipid peroxidation was determined according to Thounaojam et al. [15]. Fresh leaves (0.2 g) were homogenized with 5 ml 0.25% TBA. The homogenate was boiled for 30 min at 95 °C and centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was recorded at 532 nm and 600 nm using an extinction coefficient of 155 $mM^{-1} \cdot cm^{-1}$.

Measurement of POD activity and GSH content

Frozen leaf segments were homogenized, centrifuged, and then supernatant was immediately used for the antioxidant enzyme assays. The POD activity was measured by guaiacol oxidation according to the method of Li et al. [29]. To determine the GSH content, samples (0.5 g) were crashed with 5 ml of 10% (w/v) TCA, and the homogenate was centrifuged at 15,000×g for 15 min at 4°C. The GSH content was determined according to the method described previously [30].

Determination of soluble sugar content

Soluble sugar content was determined according to the procedure reported by Muhammad et al [31]. Fresh leaves (0.5g) were homogenized in 80% ethanol, and then incubated at 75°C for 10 minutes. Forty (40) microliters of the supernatant were mixed with 80% of carbolic acid and 4 mL of concentrated sulfuric acid and then the absorbance was recorded at 490nm. The concentration of soluble sugar was determined by a calibration curve prepared from a sucrose solution and was expressed as $mg \cdot g^{-1}$ FW.

Statistical analysis

All statistical analysis was performed by SPSS 21.0 computer software package. Data were expressed as mean values ± S.D. with three replicates for each treatment. Differences among the groups were examined by one-way ANOVA followed by LSD. $P<0.05$ was considered as statistically significant.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are available from the corresponding author upon reasonable request. The plant materials were provided by Dr. Daigang Yang (Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang, China).

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by the National Natural Science Foundation of China (No.31672497) and National Key Research and Development Projects of China (No. 2018YFD0100403). The funding bodies played no role in the design of the experiment and data collection, analysis, or preparation of the manuscript.

Contributions

GJS and MXF designed the experiments. ZH, ZKH, ZG and WPP performed most of experiments and analyzed the data. YDG assisted in experiments. ZH and ZKH wrote the manuscript. GJS and MXF revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank the Master student Anane.G.Owusu for providing the help of English writting. We thank Prof. Yan Meng (College of Life Sciences, Anhui Agricultural University) and Prof. De-Xiang Wu (College of Agronomy, Anhui Agricultural University) for critical comments to the manuscript.

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Tables

Table 1

Effect of Cu treatments on the contents of chlorophyll and carotenoid in cotton seedlings

Cotton variety	Cu concentration (μM)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll (a+b) (mg/g FW)	Chlorophyll a/b	Carotenoid (mg/g FW)
9053	0	0.28±0.01bcd	0.08±0.01b	0.36±0.02bcd	3.36±0.14c	0.051±0.003bc
	0.2	0.33±0.05cd	0.09±0.02bc	0.43±0.06cd	3.52±0.09c	0.048±0.006ab
	50	0.30±0.04cd	0.09±0.02bc	0.39±0.06cd	3.12±0.09b	0.045±0.004ab
	100	0.27±0.03bcd	0.08±0.01b	0.35±0.04bcd	3.26±0.06bc	0.049±0.007abc
	200	0.11±0.01a	0.04±0.01a	0.16±0.01a	2.64±0.21a	0.049±0.004abc
Zhong mian63	0	0.24±0.02bcd	0.08±0.01b	0.31±0.02bcd	3.06±0.20c	0.044±0.003ab
	0.2	0.25±0.04bc	0.08±0.02b	0.32±0.05bc	3.06±0.11b	0.038±0.004a
	50	0.35±0.06d	0.11±0.02c	0.46±0.08d	3.15±0.03b	0.050±0.005bc
	100	0.28±0.10cd	0.09±0.03bc	0.37±0.13bcd	3.11±0.10b	0.048±0.016abc
	200	0.19±0.02ab	0.07±0.01b	0.26±0.03ab	2.67±0.07a	0.055±0.006bc
sGK9708	0	0.30±0.03cd	0.10±0.02bc	0.40±0.04cd	3.09±0.17bc	0.050±0.003bc
	0.2	0.32±0.08cd	0.09±0.03bc	0.42±0.11cd	3.37±0.03bc	0.044±0.005ab
	50	0.34±0.06cd	0.10±0.02bc	0.43±0.08cd	3.39±0.13bc	0.068±0.004abc
	100	0.29±0.03cd	0.09±0.01bc	0.38±0.04bcd	3.13±0.09b	0.049±0.003abc
	200	0.17±0.08a	0.07±0.02b	0.24±0.1ab	2.34±0.4a	0.058±0.007c

Data are means ± S.D.(n=3). Those with different superscript letters (a, b, c and d) in the same column are significantly different (*p*<0.05, Tukey multiple comparison).

Table 2
The Cu contents in the leaf, stem and root of cotton under different Cu concentrations treatment

Cotton variety	Cu concentration (μM)	Leaf (μg/g DW)	Stem (μg/g DW)	Root (μg/g DW)
9053	0	8.67±0.85a	4.43±0.70a	20.27±1.46a
	0.2	10.40±1.20a	5.33±0.35a	22.20±2.00a
	50	28.95±3.13b	8.70±1.45bc	121.67±5.8b
	100	46.27±4.76c	15.87±2.06e	262.87±23.1de
	200	75.54±11.40e	25.37±2.80g	386.30±31.96f
Zhongmian 63	0	7.60±0.36a	4.47±0.70a	20.47±1.74a
	0.2	8.83±0.81a	5.43±0.59a	24.77±3.32a
	50	23.79±2.36b	9.03±0.83c	158.63±10.92bc
	100	39.43±5.80c	13.60±1.55de	286.20±17.76de
	200	65.07±4.73d	20.57±2.41f	489.20±32.03g
sGK9708	0	8.50±0.70a	5.63±0.45ab	18.57±1.12a
	0.2	9.47±1.20a	6.30±0.50abc	20.07±2.14a
	50	30.27±1.99b	12.40±2.05d	139.83±10.63bc
	100	43.53±3.56c	20.27±1.96f	253.00±10.50d
	200	76.97±8.06e	33.90±3.60h	475.73±24.83g

Data are means ± S.D.(n=3). Those with different superscript letters (a, b, c and d) in the same column are significantly different (p<0.05, Tukey multiple comparison).

Figures

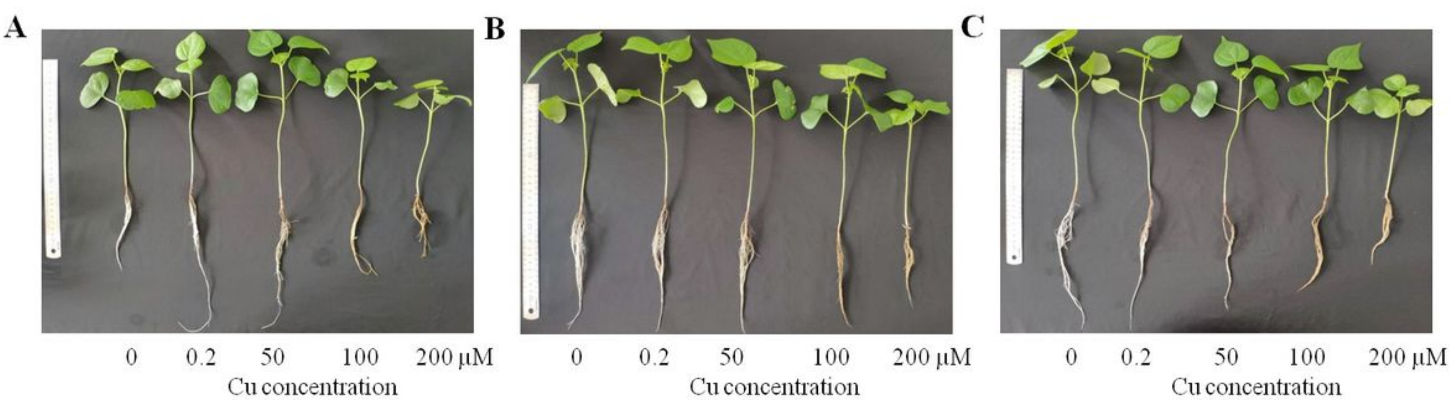

Figure 1

Effect of different Cu concentrations on morphology of plant seedlings. A: 9053; B: sGK9708; C: Zhongmian 63

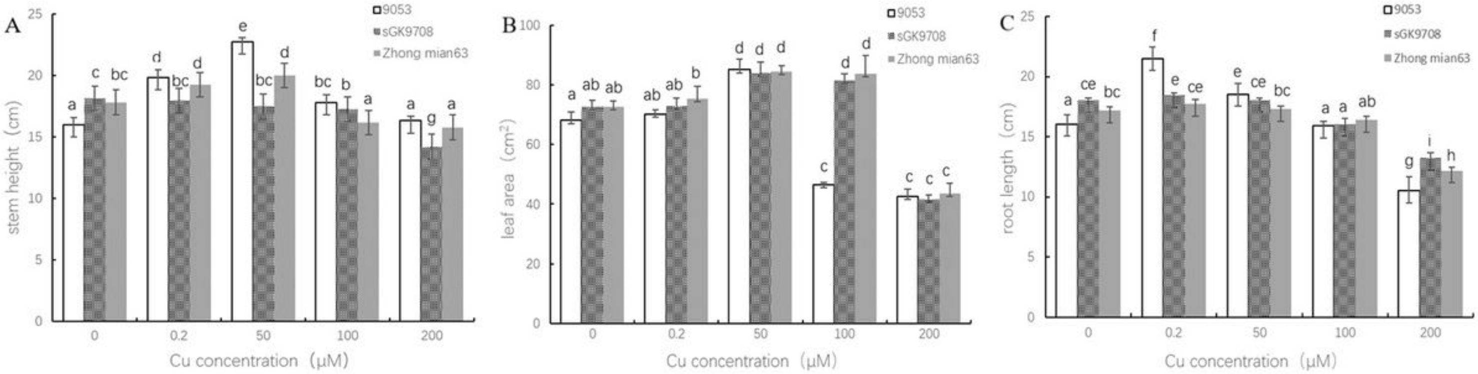

Figure 2

Effect of different Cu concentrations on plant growth of three cotton cultivars after 10th day of treatment. A: stem height; B: leaf area; C: root length The data are mean of three separate experiments. ±S.D. indicates significant mean difference from control at P=0.05 according to LSD test.

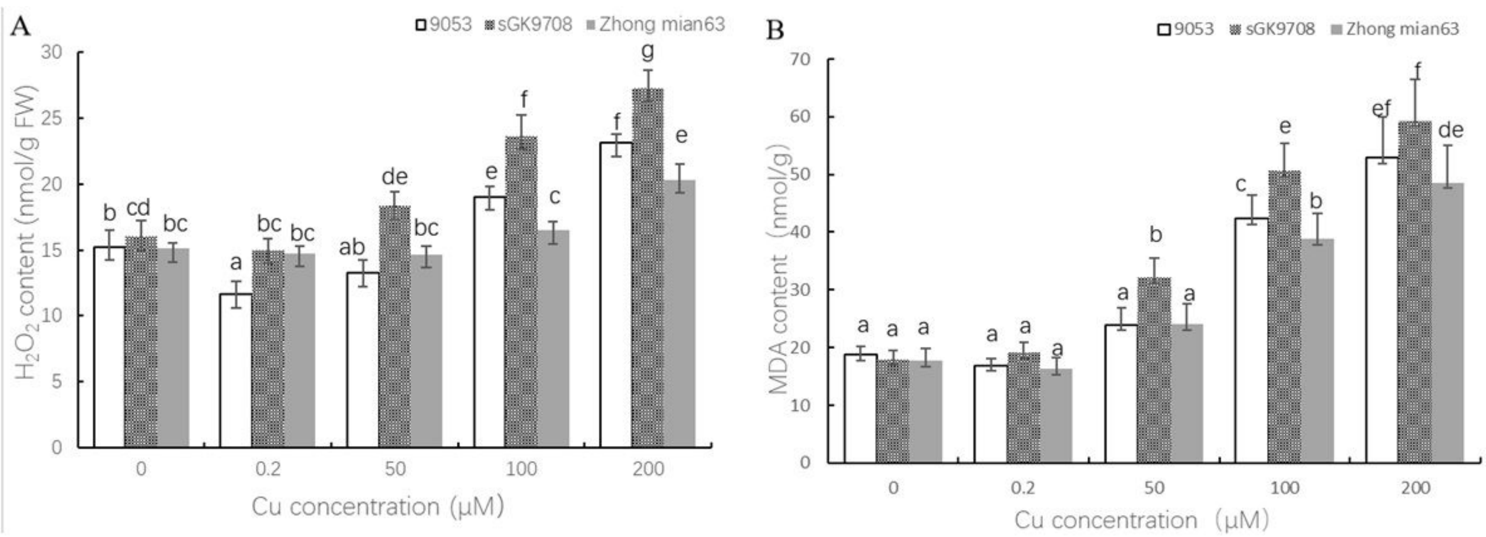

Figure 3

Effect of different Cu concentrations on the contents of H2O2 and MDA in leaves of three cotton cultivars. The data are mean± S.D. (n=3). Significant mean difference from the control at P=0.05 in multiple comparison by LSD test.

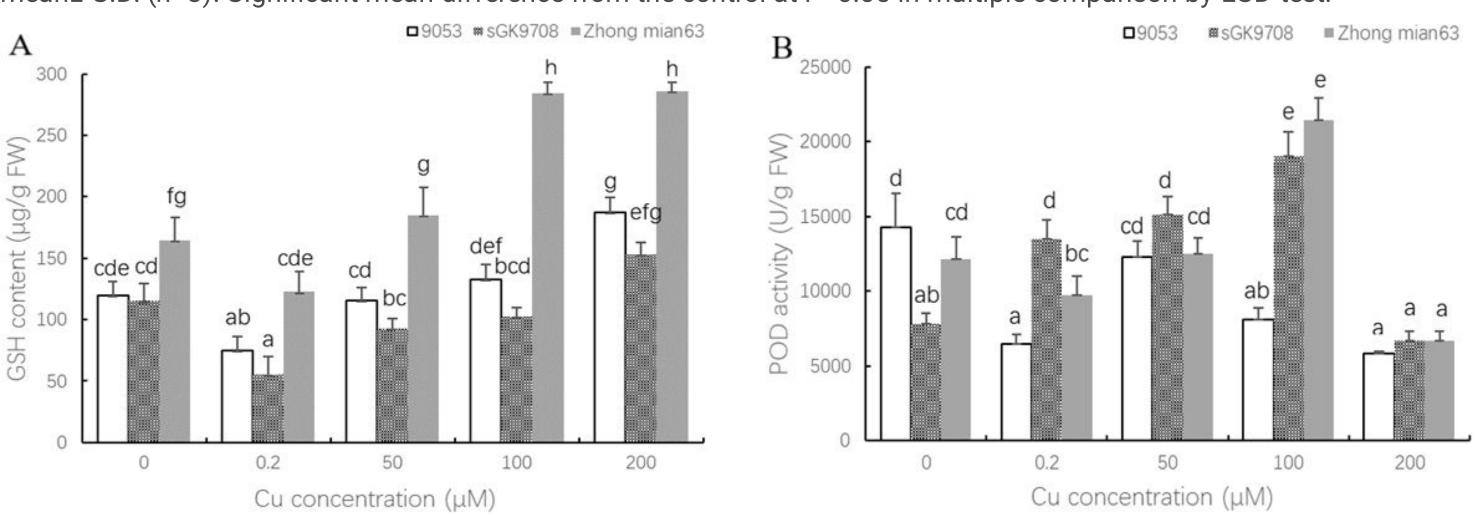

Figure 4

Effect of different Cu concentrations on GSH content and POD activity in leaves of three cotton cultivars. The data are mean± S.D. (n=3). Significant mean difference from the control at P=0.05 in multiple comparison by LSD test.

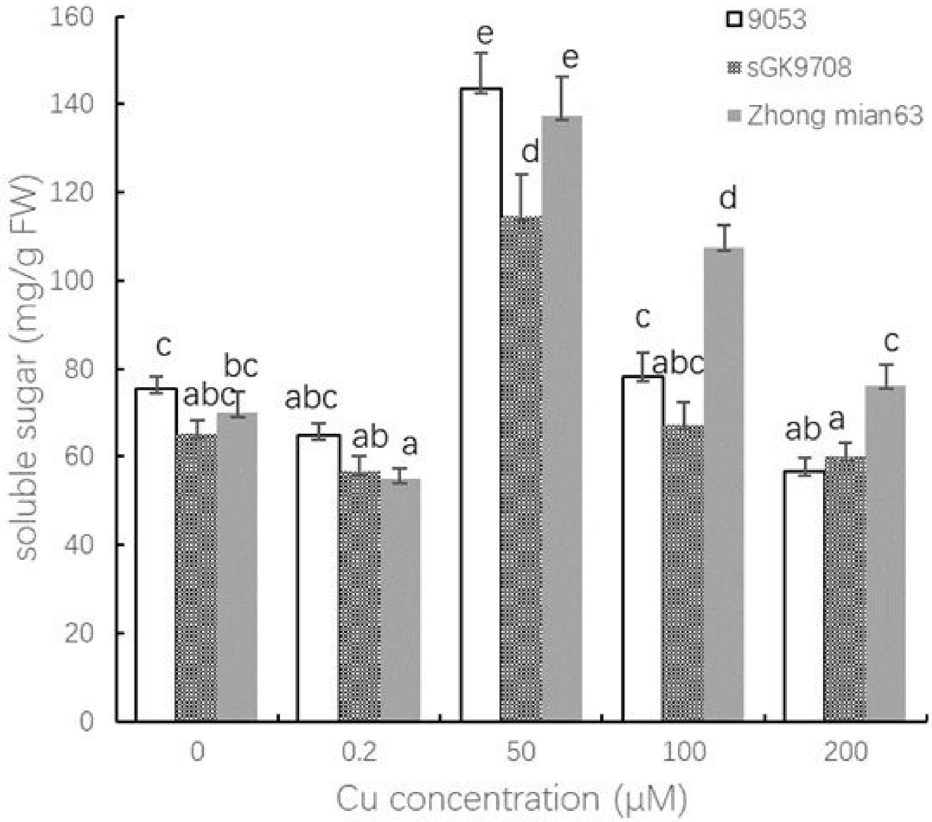

Figure 5

Effect of different Cu concentrations on the content of soluble sugar in leaves of three cotton cultivars. The data are mean± S.D. (n=3). Significant mean difference from the control at P=0.05 in multiple comparison by LSD test.