

Protective Effect of Attapulgite Clay in Substrate on *Angelica sinensis* Seedlings under Copper Stress (Postprint)

Authors: Zhang Mudan, Sai Nao Wang Qing, Ran Ruilan, Da Mengting, Jia Lingyun, Sun Kun, Feng Hanqing

Date: 2018-07-18T00:00:00+00:00

Abstract

Angelica sinensis seedlings were cultivated in mixed substrates containing different proportions of attapulgite clay (attapulgite clay:substrate volume ratios of 0/1, 1/100, 1/70, 1/50, 1/20) to investigate the physiological effects of Cu stress on *Angelica sinensis* seedlings and the protective effect of attapulgite clay on *Angelica sinensis* seedlings under Cu stress. The results showed: (1) When no attapulgite clay was present in the substrate, with increasing CuCl₂ treatment concentrations, the actual photochemical efficiency [Y(II)], PSII electron transport rate (ETR), photochemical quenching (qP), and chlorophyll content in *Angelica sinensis* seedling leaves gradually decreased; whereas leaf non-photochemical quenching (qN), soluble protein content, antioxidant enzyme activities (SOD, POD, CAT, APX), hydrogen peroxide content, and Cu content in roots and leaves all increased significantly, indicating that Cu stress reduced the photochemical efficiency and chlorophyll synthesis of *Angelica sinensis* seedlings while increasing tissue Cu content and oxidative stress; (2) Under CuCl₂ stress, when different proportions of attapulgite clay were present in the substrate, the Cu stress-induced reductions in leaf Y(II), ETR, qP, and chlorophyll content, as well as the increases in leaf qN, soluble protein content, antioxidant enzyme activities, hydrogen peroxide content, and Cu content in roots and leaves, were all alleviated. This occurred because attapulgite clay adsorbed or immobilized free and exchangeable Cu ions in the substrate, reducing the absorption of available Cu ions by *Angelica sinensis* seedlings and thereby mitigating the physiological pressure caused by Cu ions on the plants. The alleviating effect followed the order: 1/70 > 1/50 > 1/20 > 1/100 > 0/1, which was attributed to the fact that when excessive attapulgite clay was present in the substrate, while absorbing Cu ions from the substrate, it also affected substrate aeration, water permeability, and pH, among other properties. These results demonstrate that appropriate

addition of attapulgite clay to the substrate can alleviate the physiological pressure imposed by Cu stress on *Angelica sinensis* seedlings. These experimental findings provide certain reference and guidance for reducing heavy metal contamination in soilless cultivation of *Angelica sinensis*.

Full Text

Preamble

Protective Effect of Attapulgite Clay on *Angelica sinensis* Seedlings Under Cu Stress

ZHANG Mudan, SAI NAO Wangqing, RAN Ruilan, DA Mengting, JIA Lingyun, SUN Kun, FENG Hanqing*

College of Life Sciences, Northwest Normal University, Lanzhou 730070, China

Abstract

Angelica sinensis seeds were cultivated in a substrate containing different proportions of attapulgite clay (volume ratios of attapulgite clay to substrate: 0/1, 1/100, 1/70, 1/50, 1/20) to investigate the physiological effects of Cu stress on *A. sinensis* seedlings and the protective role of attapulgite clay under CuCl stress. The results showed: (1) In the absence of attapulgite clay, increasing CuCl concentrations led to gradual decreases in the actual photochemical efficiency [Y(II)], PSII electron transport rate (ETR), photochemical quenching (qP), and chlorophyll content of seedling leaves, while non-photochemical quenching (qN), soluble protein content, antioxidant enzyme activities (SOD, POD, CAT, APX), hydrogen peroxide content, and Cu accumulation in roots and leaves all increased significantly. These findings indicate that Cu stress reduced photochemical efficiency and chlorophyll synthesis while increasing tissue Cu content and oxidative stress. (2) Under CuCl stress, the presence of attapulgite clay in the substrate alleviated the Cu-induced reductions in leaf Y(II), ETR, qP, and chlorophyll content, as well as the Cu-induced increases in qN, soluble protein content, antioxidant enzyme activities, hydrogen peroxide content, and Cu accumulation in roots and leaves. This protective effect occurred because attapulgite clay adsorbed or immobilized free and exchangeable Cu ions in the substrate, thereby reducing the absorption of bioavailable Cu ions by *A. sinensis* seedlings and mitigating the physiological pressure caused by Cu ions. The mitigation effect followed the order: 1/70 > 1/50 > 1/20 > 1/100 > 0/1, likely because excessive attapulgite clay, while absorbing Cu ions, also affected substrate aeration, water permeability, and pH. These results demonstrate that appropriate incorporation of attapulgite clay into the substrate can alleviate the physiological stress imposed by Cu toxicity on *A. sinensis* seedlings. This study provides valuable insights and references for reducing heavy metal contamination in the soilless cultivation of *Angelica sinensis*.

Keywords: *Angelica sinensis* seedlings, copper stress, attapulgite clay, oxidative stress, alleviating effect

Funding: National Natural Science Foundation of China (3150070, 31260059); Fundamental Research Funds for the Gansu Universities of Gansu Provincial Department of Finance; Basic Scientific Research Business expenses of Universities in Gansu Province; Youth Teacher Scientific Research Ability Promotion Plan Innovation Team Program of Northwest Normal University; University Scientific Research Program of Gansu Province (2015A-007)

Corresponding Author: FENG Hanqing, Professor, Master's Supervisor, Research interests: Plant Physiology, Email: fenghanq@nwnu.edu.cn

Introduction

Angelica sinensis (Oliv.) Diels, a member of the Apiaceae family, is a traditional Chinese medicinal herb whose roots are used to enrich blood, moisten the intestines, treat menstrual disorders, cold-deficiency abdominal pain, rheumatic arthralgia, traumatic injuries, and as a chronic laxative for constipation in elderly patients with physical weakness (Wei et al, 2009; Fang et al, 2012). Currently, *A. sinensis* is cultivated in Gansu, Sichuan, Yunnan, Hubei, and other provinces, with the largest cultivation area in Gansu Province, primarily distributed in the foothill regions on both sides of the eastern branch of the Minshan Mountains (Qiu et al, 2010).

Copper (Cu) is an essential micronutrient for plants, playing crucial roles in protein structure, photosynthetic electron transport, and cell wall metabolism (Guerrero, 2005). However, excessive Cu ions adversely affect plant growth and development. Maksymiec and Krupa (2006) found that during early stages of heavy metal Cu stress, H₂O₂ content and antioxidant enzyme activities increased in plant tissues, indicating that excess Cu induces oxidative stress in plant cells. Furthermore, studies have shown that Cu stress can inhibit photosynthesis and affect chlorophyll synthesis (Xie et al, 2018). These physiological changes ultimately lead to impaired plant growth and development, and can even cause plant death (Kopittke et al, 2006).

In recent years, Cu ion pollution has become increasingly severe (Sharma & Agrawal, 2005; Xie et al, 2018), particularly soil and water contamination from copper mining (Li, 2008; Zhao, 2009) and the extensive application of copper-containing pesticides such as Bordeaux mixture (Cu ion concentration of 1.5 g · L⁻¹) (Druart et al, 2012), which subject plants to serious Cu ion stress. Therefore, studying the physiological effects of Cu stress on plants—especially medicinal plants with strict heavy metal standards—is of significant importance. However, most research on Cu stress physiology has focused on model plants like *Arabidopsis* or important crops, with few reports on medicinal plants such as *A. sinensis*.

Meanwhile, effective strategies to reduce Cu ion toxicity represent a current research hotspot. Attapulgite clay, a non-metallic mineral resource with both clay and stone characteristics (Hu et al, 2005), can reduce free heavy metal ions in the environment through adsorption and ion exchange at low cost (Wei & Cui, 2005; Zhu & Zhou, 2004). Previous studies have used attapulgite clay to remediate ecosystems contaminated with Pb, Cu, Cd, and other heavy metals (Wang, 2016; Yang & Hu, 2004; Tan, 2011), demonstrating its ability to adsorb and immobilize free heavy metals in substrates, thereby protecting the environment. Based on these properties, incorporating attapulgite clay into cultivation substrates may alleviate the adverse effects of Cu stress on medicinal plants like *A. sinensis*. However, no such studies have been reported domestically or internationally.

For these reasons, this study used *A. sinensis* seedlings to investigate their physiological changes under short-term heavy metal Cu stress and the alleviating effects of attapulgite clay, aiming to expand the application of attapulgite clay in agricultural research and provide references for reducing heavy metal toxicity in plants.

1.1.1 Plant Material Cultivation

The experimental material was *Angelica sinensis* cv. Mingui. Seeds were rinsed four times with distilled water and sterilized with 1% NaClO for 10 minutes, then washed eight times with distilled water to remove residual NaClO. The seeds were placed in petri dishes lined with double-layer gauze and germinated in an incubator at 25°C. After 7 days, uniformly germinated seeds were planted in mixed substrates with different attapulgite clay ratios (attapulgite clay:substrate volume ratios of 0/1, 1/100, 1/70, 1/50, and 1/20, with equal total substrate volume for all treatments), and irrigated with 1/2-strength Hoagland nutrient solution until the substrate was completely moist. Seedlings were grown at 25°C under $200 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ light intensity, and nutrient solution was applied every 3 days after cotyledon expansion.

1.1.2 Material Treatment

Thirty-day-old, uniformly growing *A. sinensis* seedlings were selected for two experiments: (1) 60 mL of CuCl₂ solution at concentrations of 5, 15, 25, and 35 mmol · L⁻¹ (Cu ion concentrations based on reported Cu pollution levels) (Zhao, 2009) was applied to substrates without attapulgite clay; control groups received equal volumes of distilled water. Measurements were taken after 24 hours (four independent samples per treatment, three replicates per measurement). (2) 60 mL of 25 mmol · L⁻¹ CuCl₂ solution was applied to each mixed substrate (attapulgite clay:substrate volume ratios of 0/1, 1/100, 1/70, 1/50, and 1/20); control groups received equal volumes of distilled water, followed by 24 hours of cultivation before physiological parameter measurements.

1.2.1 Determination of Chlorophyll Fluorescence Parameters

Following methods described by Genty et al (1989), Flexas et al (1999), and Hazrati et al (2016), a modulated chlorophyll fluorescence imaging system (IMAGING-PAM) was used to measure *A. sinensis* seedlings. After instrument connection and normal operation, initial fluorescence (F_o) and maximum fluorescence (F_m) were measured in dark-adapted state. When real-time fluorescence (F_s) under actinic light reached steady state (20 s), a saturation pulse was applied to measure maximum fluorescence (F_m'), followed by far-red light to measure minimum fluorescence (F_o'). The following formulas were used: $Y(II) = (F_m' - F_s)/F_m'$; $ETR = [(F_m' - F_s)/F_m'] \times PAR \times 0.5 \times 0.84$; $qP = (F_m' - F_s)/(F_m' - F_o')$; $qN = (F_m' - F_o')/(F_m - F_o)$.

1.2.2 Determination of Chlorophyll Content

Chlorophyll content in *A. sinensis* seedling leaves was determined according to Arnon (1949). Fresh leaves (0.02 g) were chopped and placed in a 5 mL centrifuge tube with 4 mL of 80% acetone extraction solution, then extracted in darkness at 4°C until leaves turned white. After homogenization, 3 mL of extract was transferred to a cuvette and absorbance was measured at 645 nm and 663 nm to calculate chlorophyll content.

1.2.3 Determination of Soluble Protein Content

Soluble protein content in *A. sinensis* seedling leaves was measured according to Zhao (2016). Fresh leaves (0.01 g) were ground in a mortar with 4 mL distilled water, homogenized, and left for 30 minutes before centrifugation at $4,000 \text{ r} \cdot \text{min}^{-1}$ for 20 minutes. The supernatant was transferred to another tube and diluted to volume with distilled water. One milliliter of extract was mixed with 5 mL of Coomassie Brilliant Blue G-250 protein solution, incubated for 2 minutes, and absorbance was measured at 595 nm. Protein content was calculated using a standard curve ($\text{mg} \cdot \text{g}^{-1}$).

1.2.4 Determination of Antioxidant Enzyme Activities and Hydrogen Peroxide Content

Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium (NBT) reduction method of Nishikimi et al (1972). Peroxidase (POD) activity was determined using the guaiacol method of Fernández (2013) and Martins (2011). Catalase (CAT) activity was measured using the method of Chance and Maehly (1995). Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981). Hydrogen peroxide content was measured using the method of Ferguson (1983).

1.2.5 Determination of Copper Content in Plant Tissues

To determine Cu content, plant materials (roots and leaves of *A. sinensis* seedlings) were digested with 70% HNO₃ solution under microwave conditions and analyzed by inductively coupled plasma atomic emission spectrometry according to Chen et al (2004).

1.3 Statistical Analysis

Raw data were processed and graphed using Excel and Origin software, with values expressed as mean \pm standard deviation (SD). Data were analyzed using two-tailed t-tests, with $P < 0.05$ indicating statistically significant differences.

2.1 Effects of Different CuCl Concentrations on Copper Content in Angelica Seedling Tissues

The results showed that Cu content in both roots and leaves of *A. sinensis* seedlings increased with rising CuCl concentrations. At 5 mmol \cdot L⁻¹ CuCl, leaf Cu content showed no significant difference compared to the control, while root Cu content was significantly higher. When CuCl concentration reached 15 mmol \cdot L⁻¹, leaf Cu content increased significantly compared to the control. These findings indicate that CuCl stress caused substantial Cu accumulation in plant roots and leaves, with increasing CuCl concentrations leading to progressive Cu accumulation in *A. sinensis* seedling tissues.

2.2 Effects of Different CuCl Concentrations on Chlorophyll Fluorescence Parameters, Chlorophyll Content, and Soluble Protein Content in Angelica Seedling Leaves

The previous results demonstrated that Cu content in *A. sinensis* seedling roots and leaves increased significantly with rising CuCl concentrations. As shown in , treatment with 5 mmol \cdot L⁻¹ CuCl caused no significant changes in Y(II) and ETR compared to the control. However, when CuCl concentration increased to 15 mmol \cdot L⁻¹, both Y(II) and ETR decreased significantly and continued to decline with further concentration increases. Compared to Y(II) and ETR, qP and qN were more sensitive to CuCl stress: qP decreased significantly while qN increased significantly with rising CuCl concentrations. These results indicate that increasing exogenous CuCl concentrations not only elevated Cu content in root cells but also caused structural changes in QB-binding proteins and electron transport-related proteins on the oxidizing or reducing side of PSII, leading to disordered PSII electron transport (Wei, 2016; Baker et al, 1998; Amina et al, 2013). This ultimately resulted in Cu accumulation in leaves and decreased PSII reaction center openness, excitation energy capture efficiency, and operational efficiency, thereby altering chlorophyll fluorescence parameters by affecting electron transport component functions in PSII. Furthermore, the

study found that chlorophyll content decreased concurrently with changes in leaf Cu content and chlorophyll fluorescence parameters, presumably because Cu replaced Fe^{2+} , Zn^{2+} , Mg^{2+} , and other ions in chlorophyll, altering central ion composition and inactivating chlorophyll molecules (Rousos & Harrison, 1987; Liu et al, 2008; Peng & Song, 2011; Xue et al, 2016). Since chlorophyll content also affects PSII light utilization efficiency (Yang et al, 2016; Feng et al, 2016), the changes in chlorophyll fluorescence parameters caused by CuCl likely resulted from combined effects of altered electron transport component functions and chlorophyll structural damage.

Soluble protein content serves as an important osmotic regulator in plant stress resistance, and its changes can reflect environmental impacts on plants (Bao et al, 2001; Chen et al, 2010). The study found that soluble protein content in *A. sinensis* seedling leaves increased significantly with rising CuCl concentrations compared to the control, indicating that CuCl stress affected protein metabolism in seedling leaves.

2.3 Effects of Different CuCl Concentrations on Antioxidant Enzyme Activities and Hydrogen Peroxide Content in Angelica Seedling Leaves

SOD, POD, CAT, and APX are key enzymes in the reactive oxygen species scavenging system that protect cells from oxidative damage, and their activity changes are important indicators of plant antioxidant capacity (Gong et al, 2008; Sun et al, 2005). For example, SOD can effectively prevent reactive oxygen species damage to cell membranes (Cakmak & Horst, 2006; Korniyev et al, 2001); POD, as a highly active adaptive enzyme, can reflect plant metabolic status and environmental adaptability (Zhou et al, 2016); APX and CAT belong to different H₂O₂ scavenging enzyme categories (Mittler, 2002). Increased H₂O₂ content indicates environmental stress on plant cells (Qrozco-Cárdenas et al, 2001; Foyer & Noctor, 2003; Knight, 2004). This study found that compared to the control, antioxidant enzyme activities (SOD, POD, CAT, APX) in *A. sinensis* seedling leaves increased significantly with rising CuCl concentrations, with SOD increasing by 3.2-, 5.7-, and 6.7-fold, and APX increasing by 2.0-, 3.0-, 3.1-, and 4.1-fold. Concurrently, H₂O₂ content in seedling leaves also increased progressively with CuCl concentration. These results demonstrate that excess Cu in the cultivation substrate caused physiological stress on *A. sinensis* seedlings.

2.4 Effects of Attapulgitic Clay in Substrate on Copper Content in Angelica Seedling Tissues Under CuCl Stress

The above results showed that Cu stress adversely affected both roots and leaves of *A. sinensis* seedlings. Under 25 mmol · L⁻¹ CuCl stress, physiological parameters changed significantly, and this Cu concentration approximated that in Bordeaux mixture. Therefore, we further investigated whether adding attapulgitic

clay to the substrate could alleviate the physiological pressure caused by CuCl by comparing physiological parameters of seedlings grown in mixed substrates (attapulgite clay:substrate volume ratios of 0/1, 1/100, 1/70, 1/50, and 1/20) under 25 mmol · L⁻¹ CuCl stress.

We first measured Cu content in roots and leaves of *A. sinensis* seedlings after CuCl treatment. Under CuCl stress, when the attapulgite clay:substrate ratio was 1/100, Cu content in roots and leaves showed no significant difference compared to seedlings grown in substrate without attapulgite clay. However, as attapulgite clay content in the substrate increased, Cu content in both roots and leaves decreased significantly compared to the control without attapulgite clay. This reduction likely occurred because attapulgite clay decreased free Cu ions in the substrate through adsorption and ion exchange.

2.5 Effects of Attapulgite Clay in Substrate on Chlorophyll Fluorescence and Chlorophyll Content in Angelica Seedling Leaves Under CuCl Stress

Measurements revealed that under CuCl stress, the presence of attapulgite clay in the substrate increased Y(II), ETR, and qP while decreasing qN in *A. sinensis* seedling leaves compared to substrate without attapulgite clay [Figure 1: see original paper]. This indicates that attapulgite clay adsorbed excess Cu ions, preventing structural changes in QB-binding proteins and electron transport-related proteins on the oxidizing or reducing side of PSII, thereby reducing PSII electron transport disorder and alleviating the adverse effects of CuCl stress on PSII. Further observation showed that as the attapulgite clay ratio increased from 1/100 to 1/70, its alleviating effect on CuCl-induced PSII damage was enhanced. However, with further increases in attapulgite clay ratio, this effect persisted but was weakened compared to the 1/70 treatment, with the mitigation order being 1/70 > 1/50 > 1/20 > 1/100 > 0/1. This may be because excessive attapulgite clay, while absorbing Cu ions, also affected substrate physical properties such as aeration, water permeability, and pH, though the specific mechanisms require further investigation.

As shown in [Figure 2: see original paper], under CuCl stress, the presence of 1/70 and 1/50 attapulgite clay in the substrate significantly increased chlorophyll content compared to substrate without attapulgite clay, with the 1/70 ratio showing the most pronounced effect. This suggests that attapulgite clay reduced the replacement of Fe²⁺, Zn²⁺, and Mg²⁺ in chlorophyll by Cu²⁺, thereby decreasing changes or inactivation of central ion composition and alleviating the inhibitory effect of CuCl stress on chlorophyll synthesis.

2.6 Effects of Attapulgite Clay in Substrate on Soluble Protein Content, Antioxidant Enzyme Activities, and Hydrogen Peroxide Content in Angelica Seedling Leaves Under CuCl Stress

This study investigated the effects of different attapulgite clay proportions in the substrate on soluble protein content, antioxidant enzyme activities, and hydrogen peroxide content in *A. sinensis* leaves under CuCl stress [FIGURE:3, 4, 5]. When CuCl stress increased leaf soluble protein content, CAT activity, and hydrogen peroxide content, attapulgite clay in the substrate alleviated these increases, with the mitigation effect generally following the order: $1/70 > 1/50 > 1/20 > 1/100 > 0/1$.

When CuCl stress elevated SOD and POD activities [Figure 4: see original paper], attapulgite clay significantly slowed these increases. For POD, the alleviating effect strengthened as the attapulgite clay ratio increased from 1/100 to 1/50, but weakened with further ratio increases. For APX activity, the 1/70 attapulgite clay ratio significantly reduced the CuCl-induced increase [Figure 4D: see original paper].

These results indicate that an attapulgite clay:substrate volume ratio of 1/70 provides the strongest adsorption capacity, effectively alleviating CuCl-induced increases in leaf soluble protein content, antioxidant enzyme activities, and hydrogen peroxide content.

3 Discussion and Conclusion

In this study, CuCl stress caused decreases in $Y(II)$, ETR, qP, and chlorophyll content in *A. sinensis* seedlings, indicating reduced PSII capacity and operational efficiency under light, as well as decreased electron acceptance and transfer abilities. Correspondingly, qN, which represents energy dissipation, increased significantly under CuCl stress, suggesting that more light energy was dissipated as heat rather than being used for photochemical reactions (Xie et al, 2018). Meanwhile, as Cu accumulated in plant tissues, soluble protein content, antioxidant enzyme activities, and hydrogen peroxide content in *A. sinensis* leaves increased significantly [TABLE:1, TABLE:3, TABLE:4], demonstrating severe physiological pressure from Cu stress.

As mentioned above, attapulgite clay exhibits excellent heavy metal adsorption properties (Hu et al, 2005; Wei & Cui, 2005) and can immobilize heavy metals in cultivation substrates through adsorption or ion exchange, thereby reducing plant heavy metal absorption (Tan et al, 2011). Therefore, we investigated whether attapulgite clay addition could effectively alleviate the physiological pressure caused by Cu stress. In further experiments, we compared physiological parameters of seedlings grown in mixed substrates (attapulgite clay:substrate volume ratios of 0/1, 1/100, 1/70, 1/50, and 1/20) under $25 \text{ mmol} \cdot \text{L}^{-1}$ CuCl

stress (a concentration approximating that in Bordeaux mixture). The results showed that under CuCl stress, substrates containing attapulgite clay significantly reduced Cu content, soluble protein content, antioxidant enzyme activities (SOD, POD, CAT, APX), and hydrogen peroxide content while increasing photochemical efficiency and chlorophyll content compared to substrates without attapulgite clay. This indicates that attapulgite clay addition can alleviate physiological pressure from Cu ions on *A. sinensis* seedlings, likely by adsorbing or immobilizing free and exchangeable Cu ions in the substrate, thereby reducing absorption of bioavailable Cu ions. Notably, although increasing attapulgite clay ratios continuously reduced Cu accumulation in plant tissues, the alleviation of other physiological pressures did not show an absolute parallel relationship. Overall, when the attapulgite clay ratio was below 1/70, its alleviating effect on CuCl-induced physiological pressure strengthened with increasing ratio. However, when the ratio exceeded 1/70, the alleviating effect weakened to varying degrees, with the mitigation order being $1/70 > 1/50 > 1/20 > 1/100 > 0/1$. This may be because excessive attapulgite clay, while absorbing Cu ions, also affected substrate aeration, water permeability, and pH (Tan et al, 2011), though specific mechanisms require further investigation.

In conclusion, appropriate amounts of attapulgite clay can alleviate physiological pressure caused by CuCl stress. In field cultivation of *A. sinensis*, attapulgite clay addition can mitigate heavy metal toxicity, with an attapulgite clay:substrate volume ratio of 1/70 providing optimal alleviation.

References

- AMINA B, REDA DM, RACHID R, et al, 2013. Physiological and biochemical changes observed in alternative cellular model: Paramecium tetraurelia treated with Paracetamol[J]. Int J Biosci, 9(3): 132-141.
- ARNON DI, 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris[J]. Plant Physiol, 24(1): 1.
- BAKER J, DENNSTEELE CV, III LD, 1988. Sequence and characterization of 6 Lea proteins and their genes from cotton[J]. Plant Mol Biol, 11(3): 277-291.
- BAO SW, TAN F, LIAO ZH, 2001. Effects of soil drought on the osmoregulating ability of Vicia faba leaves[J]. J Southwest Agric Univ, 23(4): 353-359. [鲍思伟, 谈锋, 廖志华, 2001. 土壤干旱对蚕豆叶片渗透调节能力的影响 [J]. 西南农业大学学报, 23(4): 353-359.]
- BOULASSEL A, DJEBAR M R, ROUABHI R, et al, 2013. Physiological and biochemical changes observed in alternative cellular model: Paramecium tetraurelia treated with paracetamol[J]. Int J Biol. 3(9): 132-141.
- CAKRNMAK I, HORST WJ, 2006. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean

(Glycine max)[J]. *Physiol Plant*, 83(3): 463-468.

CHANCE B, MAEHLY AC, 1995. Assay of catalase and peroxidase[J]. *Meth Enzymol*, 2: 764-775.

CHEN CT, CHEN TH, LO KF, et al, 2004. Effects of proline on copper transport in rice seedlings under excess copper stress. *Plant Sci*. 166: 103-111.

CHEN MT, ZHAO Z, QUAN JE, 2010. Variation of soluble protein components and contents in seedling root tips of four trees under drought stress[J]. *Acta Bot boreal-Occident Sin*, 30(6): 1157-1165. [陈明涛, 赵忠, 权金娥, 2010. 干旱对 4 种苗木根尖可溶性蛋白组分和含量的影响 [J]. *西北植物学报*, 30(6): 1157-1165.]

DRUART C, SCHEIFLER R, MILLET M, et al, 2012. Landsnail eggs bioassays: A new tool to assess embryotoxicity of contaminants in the solid, liquid or gaseous phase of soil[J]. *Appl Soil Ecol*, 53(1): 56-64.

FANG L, XIAO XF, LIU CX, et al, 2012. Recent advance in studies on *Angelica sinensis*[J]. *Chinese Herbal Medicines*, 4(1): 12-25.

FENG P, SUN L, SHEN XH, et al, 2016. Response and enrichment ability of perennial ryegrass under lead and cadmium stress[J]. *Acta Pratac Sin*, 25(1): 153-162. [冯鹏, 孙力, 申晓慧, 等. 多年生黑麦草对 Pb、Cd 胁迫的响应及富集能力研究 [J]. *草业学报*, 2016, 25(1): 153-162.]

FERGUSON IB, WATKINS CB, HARMAN JE, 1983. Inhibition by calcium of senescence of detached cucumber cotyledons: effect on ethylene and hydroperoxide production[J]. *Plant Physiol*, 71(1): 182-186.

FERNANDEZ R, BERTRAND A, REIS R, et al, 2013. Growth and physiological responses to cadmium stress of two populations of *Dittrichia viscosa*, (L.) Greuter[J]. *J Hzar Mater*, 244-245(2): 555-562.

FLEXAS J, ESCALONA JM, MEDRANO H, 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines[J]. *Plant Cell Environ*, 22(1): 39-48.

FOYER CH, NOCTOR G, 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria[J]. *Physiol Plant*, 119(3): 355-364.

GENTY B, BRIANTAIS JM, BAKER NR, 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence[J]. *Biochim Biophys Acta*, 990(1): 87-92.

GONG SJ, MA TW, JIANG YF, CHEN J, LIU YD, LI J, 2008. Antioxidant enzyme activities induced by Cadmium stress in three species of Mosses[J]. *Acta Bot boreal-Occident Sin*, 28(9): 1765-1771. [龚双姣, 马陶武, 姜业芳, 等, 2008. 镉胁迫下 3 种藓类植物抗氧化酶活性变化的比较研究 [J]. *西北植物学报*, 28(9): 1765-1771.]

GUERRERO IY, 2005. Copper in plants[J]. *Braz J Pl Physio*, 17(1).

- HAZRATI S, TAHMASEBI-SARVESTANI Z, MODARRES-SANAVY SA, et al, 2016. Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of Aloe vera L.[J]. *Plant Physiol Biochem*, 106: 141-148.
- HU T, QIAN YH, JIN YL, et al, 2005. Study on the application of Attapulgite clay[J]. *China Min Maga*, 14(10): 73-76. [胡涛, 钱运华, 金叶玲, 等, 2005. 凹凸棒土的应用研究 [J]. *中国矿业*, 14(10): 73-76.]
- KNIGHT MR, 2004. Oxidative Stress-Induced Calcium Signaling in Arabidopsis[J]. *Plant Physiol*, 135(3): 1471-1479.
- KOPITTKE PM, DART PJ, MENZIES NW, 2006. Effect of Cu on the growth and nodulation of Cowpea (*Vigna unguiculata*)[C]// World Congress of Soil Science - Frontiers of Soil Science[J]. *Int Union Soil Sci*, 1-1.
- KORNYEYEV D, LOGAN BA, PAYTON P, et al, 2001. Enhanced photochemical light utilization and decreased chilling-induced photoinhibition of photosystem II in cotton overexpressing genes encoding chloroplast-targeted antioxidant enzymes[J]. *Physiol Plant*, 113(3): 323-331.
- LI H, GUO A, WANG H, 2008. Mechanisms of oxidative browning of wine[J]. *Food Chem*, 108(1): 1-13.
- LIU SH, LIU XH, HOU J, et al, 2008. Study on the spectral response of Brassica *Campestris* L. leaf to the copper pollution[J]. *Sci China*, 51(2): 202-208.
- MAKSYMIEC W, KRUPA Z, 2006. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in Arabidopsis thaliana[J]. *Environ & Ex Botany*, 57(1-2): 187-194.
- MARTINS LL, MOURATO MP, CARDOSO AI, et al, 2011. Oxidative stress induced by cadmium in *Nicotiana tabacum*, L.: effects on growth parameters, oxidative damage and antioxidant responses in different plant parts[J]. *Acta Physiol Plant*, 33(4): 1375-1383.
- MITTLER R, 2002. Oxidative stress, antioxidants and stress tolerance[J]. *Trends Plant Sci*, 7(9): 405.
- NAKANO Y, ASADA K, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in Spinach chloroplasts[J]. *Plant Cell Physiol*. 22: 867-880.
- NISHIKIMI M, RAO AN, YAGI K, 1972. The occurrence of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen[J]. *Biochem Biophys Res Comm*. 46: 849-854.
- PENG XY, SONG M, 2011. Effects of exogenous cysteine on growth, copper accumulation and antioxidative systems in Wheat seedlings under Cu stress[J]. *Acta Ecol Sin*, 31(12): 3504-3511. [彭向永, 宋敏, 2011. 外源半胱氨酸对铜胁迫下小麦幼苗生长、铜积累量及抗氧化系统的影响 [J]. *生态学报*, 31(12): 3504-3511.]

QIU DY, LIN HM, CHEN Y, et al, 2010. Effect of altitude, longitude and altitude on Angelica growth and early bolting in Medicine formation period[J]. Acta Agr Sin, 18(6): 838-843. [邱黛玉, 蔺海明, 陈垣, 等, 2010. 经纬度和海拔对当归成药期植株长势和早期抽薹的影响 [J]. 草地学报, 18(6): 838-843.]

QROZCO-Cárdenas ML, RYAN CA. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate[J]. Plant Cell. 13(1): 179-91.

ROUSOS PA, HARRISON HC, 1987. Identification of differential responses of cabbage cultivars to copper toxicity in solution culture[J]. J Amer Soc Hortic Sci, 112(6): 928-931.

SHARMA RK, AGRAWAL M, 2005. Biological effects of heavy metals: an overview[J]. Journal of Environ Biol, 26(2 Suppl): 301.

SUN WH, WANG WQ, MENG QW, 2005. Functional mechanism and enzymatic and molecular characteristic of ascorbate peroxidase in plants[J]. Acta Phytophysiol Sin, 41(2): 143-147. [孙卫红, 王伟青, 孟庆伟, 2005. 植物抗坏血酸过氧化物酶的作用机制、酶学及分子特性 [J]. 植物生理学报, 41(2): 143-147.]

TAN KY, LIU XD, LIU JC, TANG QF, HUANG YY, LUO SG. Remediation experiments of Attapulgitic clay to heavy metal contaminated soil[J]. Rock and Min Anal, 2011, 30(4): 451-456. [谭科艳, 刘晓端, 刘久臣, 等, 2011. 凹凸棒石用于修复铜锌镉重金属污染土壤的研究 [J]. 岩矿测试, 30(4): 451-456.]

WAI DD, 2016. The Protection mechanism of glycinebetaine on photosynthesis under chilling Stress in Tomato leaves[D]. Shang dong: J Shangdong Agric Univ. [卫丹丹, 2016. 低温胁迫下甜菜碱对番茄叶片光合作用的保护机制 [D]. 山东: 山东农业大学]

WANG C, CAO JS, XIE K, SHANG KH, 2016. Research progress of the modification of attapulgitic and its application in water treatment[J]. Appl Chem Ind, 45(8): 1575-1578. [王成, 操家顺, 谢坤, 等, 2016. 凹凸棒土改性及其在水处理中的应用研究进展 [J]. 应用化工, 45(8): 1575-1578.]

WEI RD, CUI Q, 2005. The exploitation and application of the Attapulgitic clay deposit in Linze, Gansu Province[J]. Gansu Sci Sin, 17(3): 43-45. [魏荣道, 崔娇, 2005. 甘肃临泽凹凸棒石粘土矿开发应用研究 [J]. 甘肃科学学报, 17(3): 43-45.]

WEI W, GONG SX, ZHANG TJ, 2009. Research progress on the compositions of angelica polysaccharide and their pharmacological action[J]. Drug Eval Res, 32(2): 130-134.

XIE JJ, LU LN, SHI DL, et al., 2018. Protective effects of alternative respiratory pathway on Photosystem II in Phaseolus vulgaris under CuCl₂ stress[J]. Bull Bot Res, (1): 75-80. [谢佳佳, 芦丽娜, 石岱龙, 等, 2018. 交替呼吸途径对 CuCl₂ 胁迫下菜豆叶片光系统的保护作用 [J]. 植物研究, (1): 75-80.]

XUE YW, WANG YF, ZHAO CJ, et al., 2016. Effects of copper stress on germination and antioxidant system in wheat seedlings[J]. Acta Agric Univ Jiangxiensis (Nat Sci Ed), 38(1): 54-59. [薛盈文, 王玉凤, 赵长江, 等, 2016. 铜胁迫对小麦种

子萌发及幼苗抗氧化系统的影响 [J]. 江西农业大学学报, 38(1): 54-59.]

YANG XM, HU GJ, 2004. Study on remediation of cadmium contaminated soil by attitugite[J]. J Heilongjiang Sci-Tech Univ, 14(2): 80-82. [杨秀敏, 胡桂娟, 2004. 凹凸棒石修复镉污染的土壤 [J]. 黑龙江科技大学学报, 14(2): 80-82.]

YANG Y, WANG XY, WANG J, et al., 2016. Effects of species diversity on plant growth and remediation of Cd contamination in soil[J]. Acta Sci Circumst, 36(6): 2013-2113. [杨扬, 王晓燕, 王江, 等, 2016. 物种多样性对植物生长与土壤镉污染修复的影响 [J]. 环境科学学报, 36(6): 2103-2113.]

ZHAO J, 2009. Elsholtzia splendens remediation of copper contaminated soil assisted by trichoderma[D]. Wuhan: Huazhong Agricultural University. [赵静, 2009. 铜污染土壤的木霉强化海州香薷修复研究 [D]. 武汉: 华中农业大学]

ZHAO YY, DAI Y, CUI XM, et al, 2016. Determination of protein contents of radix aconiti kusnezoffii using Coomassie Brilliant Blue G-250 dye binding[J]. J Yunnan Nat Univ (Nat Sci Ed), 15(3): 235-237. [赵英永, 戴云, 崔秀明, 等, 2016. 考马斯亮蓝 G-250 染色法测定草乌中可溶性蛋白质含量 [J]. 云南民族大学学报 (自然科学版), 15(3): 235-237.]

ZHOU CF, WU GX, LI T, et al, 2016. Effect of exogenous hydrogen sulfide on photosynthesis and antioxidant system of cucumber leaves under low temperature in solar-greenhouse[J]. Acta Horti Sin, 43(3): 462-472. [周超凡, 吴帽秀, 李婷, 等, 2016. 外源 H₂S 对低温下日光温室黄瓜光合作用及抗氧化系统的影响 [J]. 园艺学报, 43(3): 462-472.]

ZHU HQ, ZHOU J, 2004. The present exploitation situation and development trend of Attapulgitic clay[J]. Conserv Utiliz Min Res, 4: 14-17. [朱海清, 周杰, 2004. 凹凸棒石粘土的开发利用现状及发展趋势 [J]. 矿产保护与利用, 4: 14-17.]

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.