内容提要

铜(Cu)是植物生长发育中不可缺少的微量元素，但是其含量过高也会影响植物生长和人类健康。近年来，由于农业生产中含 Cu 农药、粪肥的不合理使用，垃圾焚烧、污水灌溉、铜矿开采冶炼等人为行为导致土壤中重金属 Cu 含量严重超标，已成为影响植物生长和人类健康的主要因素。菠菜(*Spinacia oleracea* L.)是石竹目的一种草本植物，该植物具有叶面积大、相对生长率高、重金属吸收率较高等特点。

本研究以菠菜幼苗作为试验材料，采用盆栽试验，研究了不同 Cu 浓度处理下菠菜幼苗生长量、抗氧化物酶、气体交换参数、叶绿素荧光参数、矿质元素吸收和运输，以及细胞超微结构等生理特性的变化，旨在全面地揭示菠菜的 Cu 胁迫响应机制，随后，向 Cu 胁迫幼苗叶部喷施三种不同的植物激素(GA_3、IAA、NAA)，探寻喷施植物激素的最适浓度，进一步揭示植物激素对菠菜耐 Cu 机理的调控效应。通过研究，得出以下主要结果：

(1) 不同浓度 Cu 处理对菠菜幼苗生长生理指标的影响。本试验设置 11 组 Cu 浓度，观测不同浓度 Cu 处理下菠菜幼苗生长生理指标的变化。结果显示，100 $mg \cdot L^{-1}$ Cu 浓度时，菠菜幼苗的生物量明显增加，而抗氧化物酶活性(SOD、POD、CAT、APX)变化不大，光合参数(P_n、g_s、T_r、Y (II)、qP 值)有所增加，叶部的 N、K、Mg、Ca、Fe、Zn 含量增加，根部的 Ca、Fe、Zn 含量均为增加趋势，细胞器结构完整、清晰，表明 100 $mg \cdot L^{-1}$ Cu 浓度能促进植物生长、提高光合速率、促进矿质元素吸收；而在 800 - 1000

$mg·L^{-1}$ Cu 环境中，菠菜幼苗生物量下降，MDA 含量显著升高，抗氧化物酶活性增至最高后有不同程度的下降，叶绿素 a、b 含量降低，P_n、g_s、T_r 值减小，Fv/Fm、qP、NPQ、Y (II)值下降，除细胞核、线粒体结构模糊外，其他细胞器均完整，表明较高 Cu 浓度处理影响了植物的生命活动，但是植物通过调节体内抗氧化系统，使生理活动维持较好状态，表现出植物具有较强的耐 Cu 性。

(2) 高浓度 Cu 胁迫对菠菜幼苗生长生理参数的影响。当添加 700 $mg·kg^{-1}$ 高浓度 Cu 浓度处理时，菠菜幼苗生长量下降，MDA、O_2^-、H_2O_2 含量增加，脯氨酸、可溶性蛋白以及抗氧化物酶活性也呈增加趋势，叶绿素 a、叶绿素 b、类胡萝卜素含量、光合参数(P_n、g_s、T_r 值)，以及叶绿素荧光参数(Fv/Fm、qP、NPQ、Y (II))均减少。叶部和根部 Cu 含量显著升高，且叶部 Cu 含量明显高于根部。叶部和根部的 Mg、Ca 含量减少，N、P、K、Fe、Zn 含量增加。叶部叶绿体肿胀，细胞核模糊，但是其余细胞器均清晰可见，表明高浓度 Cu 胁迫下，菠菜幼苗叶部和根部吸收了大量的 Cu^{2+}，并主要聚集在叶部，导致植物体内氧化应激反应加剧，叶绿素合成、气体交换、部分矿质元素吸收等生理活动受阻，部分细胞器结构发生了变化，即使这样，植物仍然能够主动提高渗透调节物质含量，增加抗氧化物酶活性，抵御高浓度 Cu 对植物的伤害，由此可见，菠菜幼苗具有很强的耐 Cu 性。

(3) 添加不同浓度 GA_3 溶液对高浓度 Cu 胁迫菠菜幼苗的调控效应。本试验将 7 组不同浓度的 GA_3 溶液喷施到 Cu 胁迫幼苗的叶部，7 天后取样测定各项参数。试验结果显示，当喷施 3 - 5 $mg·L^{-1}$ 的 GA_3 溶液时，菠菜幼苗

叶部 Cu 含量显著下降，O_2^-、H_2O_2 含量减少，氧化伤害明显降低，可溶性蛋白含量增加，气体交换参数、叶绿素荧光参数增加，抗氧化酶活性升高，矿质元素吸收得到了促进，植物生物量增加。而当 GA_3 浓度高于 40 mg·L^{-1} 时，植物体内表现出氧化损伤增强，抗氧化酶活性降低、光合参数、叶绿素荧光参数都有所下降，生物量也呈下降趋势，表明高浓度 GA_3 反而加剧了 Cu 胁迫损伤，严重影响了菠菜幼苗的生理功能。因此，从本试验结果推断 3 - 5 mg·L^{-1} GA_3 的浓度对 Cu 胁迫下菠菜幼苗毒害作用的缓解效果最好。

(4) 喷施不同浓度的 IAA 溶液对高浓度 Cu 胁迫菠菜幼苗的调控效应。本试验将 6 组不同浓度的 IAA 溶液喷施到 Cu 胁迫幼苗叶部，处理 7 天后取样测定生长生理参数，结果显示，低浓度的 IAA (10 - 40 mg·L^{-1})能提高 Cu 胁迫下幼苗根部的 Cu 含量，降低叶部的 Cu 含量。此时，MDA O_2^-、H_2O_2 含量降低，抗氧化酶(SOD、CAT)活性增加，叶绿素 a、叶绿素 b、类胡萝卜素、P_n 和 g_s 值、以及 Fv/Fm、NPQ、qP、ETR 值、矿质元素吸收量均为增加趋势，叶部细胞器的受损现象也得到了缓解，表明外源添加此浓度的 IAA 可以诱导抗氧化酶的合成，提升光合作用，促进矿质元素吸收，减缓高浓度对菠菜幼苗的胁迫伤害，而这种缓解作用在 IAA 浓度达到 60 mg·L^{-1} 时最明显，此时叶部、根部的 Cu 含量降至最低，幼苗体内的氧化应激反应最低，植物生长量、光合作用、矿质元素吸收均增至最高，由此可见，施用 60 mg·L^{-1} IAA 较其他 IAA 浓度相比，对 Cu 胁迫伤害缓解的作用最明显。

(5) 外源喷施不同浓度的 NAA 溶液对高浓度 Cu 胁迫菠菜幼苗的调控效应。Cu 胁迫下，向幼苗叶部喷施 5 种不同浓度的 NAA 溶液时，10 μmol·L^{-1} NAA 溶液的调控效应表现最为明显。此时，菠菜幼苗叶部 Cu 含量显著降

低，根部 Cu 含量明显增加，其生长量也显著升高，MDA、O_2^-、H_2O_2 含量降至最低，抗氧化物酶活性(SOD、POD、CAT、APX)以及脯氨酸、可溶性蛋白含量均为增加趋势，幼苗叶部叶绿素含量、P_n、g_s、C_i 值增至最大，qP 和 ETR 值也增至最大值，根部矿质元素含量呈增加趋势，叶绿体数量增多，基粒片层排列整齐，细胞壁、细胞膜、细胞核、线粒体、液泡清晰可见，细胞核与核液清晰分开，表明添加 10 μmol·L^{-1} NAA 溶液时，能提高植物抗氧化能力，增加渗透调节物质含量，减少细胞内部的膜质过氧化伤害，降低叶绿素合成的干扰，促进光合作用，缓解 Cu 对细胞器的伤害，从而，减轻了高浓度对菠菜幼苗的胁迫作用。

Summary

Copper (Cu) is an indispensable trace element in plant growth and development, but its excessive content affected plant growth and human health. In recent years, due to the unreasonable use of Cu-containing pesticides and manure in agricultural production, garbage incineration, sewage irrigation, copper mining and smelting and other human behaviors, the content of heavy metal Cu in the soil has seriously exceeded the standard. Spinach (*Spinacia oleracea* L.), a herb belonging to the Caryophyllales order, contains large amounts of vitamins, carotenoids, organic acids and alkaline minerals, as well as antioxidantsis, and is characterized by large leaf area, high relative growth rate, and high rate of heavy metal absorption.

In this study, spinach seedlings were used as pot experimental materials. The changes of growth, antioxidant enzymes, gas exchange parameters, chlorophyll fluorescence parameters, absorption and transport of mineral elements, and cell ultrastructure of spinach seedlings were first studied under different treatments of Cu concentrations. Then, three different plant hormones (GA_3, IAA and NAA) were sprayed into the leaves of Cu-stressed seedlings, and the optimal concentration of plant hormones was explored to further reveal the regulatory effect of plant hormones on the mechanism of Cu-tolerance in spinach seedling. The main results were as follow:

(1) Effects of different concentrations Cu treatment on growth physiologi-

cal indexes of spinach seedlings. In this experiment, 11 groups of Cu concentrations were set, and aimed at observation the changes of growth physiological indexes of spinach seedlings. The results showed that at the 100 $mg \cdot L^{-1}$ Cu concentration, the biomass increased significantly, and antioxidant enzymes (SOD, POD, CAT and APX) were no significant change, photosynthetic parameters (P_n, g_s, T_r, Y (II) and qP) increased, N, K, Mg, Ca, Fe and Zn content in leaf increased, the root of Ca, Fe and Zn contents increased, the organelles structure were complete and clearly visible. It shows that the 100 $mg \cdot L^{-1}$ Cu concentration can promote plant growth, increase the photosynthetic rate, and promote the mineral elements absorption. However, at the Cu concentration (800 - 1000 $mg \cdot L^{-1}$ Cu concentration), the biomass decreased, MDA content significantly increased, the antioxidant enzymes activities increased to the highest after different degrees of decline, the contents of chlorophyll a and b reduced, the P_n, g_s and T_r decreased, and Fv/Fm, qP, NPQ and Y (II) reduced, except for the ambiguous structure of nucleus and mitochondria, all other organelles were completely visible. It showed that higher Cu concentration affected the life activities of plants, but plants maintained a better state of physiological activities by regulating the antioxidant system, which showed that plants had a strong Cu tolerance.

(2) Changes of growth physiological parameters of spinach seedlings under high concentration Cu stress. When adding 700 $mg \cdot kg^{-1}$ Cu concentration, spinach seedling growth decreased, MDA, O_2^- and H_2O_2 contents increases, pro-

line content, soluble protein content and antioxidant enzyme activity also increased, chlorophyll a and chlorophyll b, carotenoid content, photosynthetic parameters (P_n, g_s and T_r), and chlorophyll fluorescence parameters (Fv/Fm, qP, NPQ, Y (II)) reduced. The Cu content in leaves and roots increased significantly, and the Cu content in leaves was significantly higher than that in roots. In the leaf and root, Mg and Ca contents decreases, and N, P, K, Fe and Zn contents increased. The chloroplast in leaf cells swelled, the nucleolus blurred, but the rest of the organelles were clearly visible, indicate that under high concentration of Cu stress, the leaves and roots absorbed a large amount of Cu^{2+} and the Cu^{2+} mainly accumulated in the leaf, leading to the intensification of oxidative stress response in plants, chlorophyll synthesis, gas exchange, some mineral element absorption and other physiological activities were affected, and some organelle structure changed. Even so, the plants can still actively increase the content of osmotic regulating substances, increase the activity of antioxidant enzymes, and resist the damage of high Cu concentration to plants. It can be seen that spinach seedlings have strong Cu tolerance.

(3) Regulation effect of adding different concentrations of GA_3 on spinach seedlings under high concentration of Cu stress. In this experiment, different concentrations of GA_3 solutions were applied to the leaves of 700 $mg \cdot kg^{-1}$ Cu stressed seedlings. After 7 days, plant samples were collected for measurement of indicators. The results show that the application of 3 - 5 $mg \cdot L^{-1}$ GA_3 significantly decreased the Cu content in the leaf, O_2^- and H_2O_2 content decreased,

oxidative damage significantly reduced, the content of soluble protein increased, the parameters of gas exchange and chlorophyll fluorescence increased, the activities of antioxidant enzymes increased, the absorption of mineral elements promoted, and the plant biomass increased. However, when the concentration of GA_3 was higher than 40 $mg \cdot L^{-1}$, the oxidative damage in plants enhanced, the activity of antioxidant enzymes, photosynthetic parameters and chlorophyll fluorescence parameters decreased, and the biomass also decreased, indicating that the high concentration of GA_3 intensified the damage of Cu stress and seriously affected the physiological function of spinach seedlings. Therefore, it can be concluded that the concentration of 3 - 5 $mg \cdot L^{-1}$ GA_3 has the best alleviating effect on the toxicity of spinach seedlings under Cu stress.

(4) Regulation effect of spraying IAA solution with different concentrations on spinach seedlings under high concentration of Cu stress. In this experiment, 6 groups of IAA solutions with different concentrations were sprayed into the leaves of seedlings under Cu stress. After 7 days of treatment, the physiological parameters were measured. The results showed that low concentration of IAA (10 - 40 $mg \cdot L^{-1}$) could increase the Cu content in the roots and decrease the Cu content in the leaves of seedlings under Cu stress. At this point, the MDA, O_2^- and H_2O_2 content reduced, antioxidant enzyme (SOD, CAT) activity increased, chlorophyll a and chlorophyll b, carotenoids, P_n and g_s, and Fv/Fm, NPQ, qP, ETR, mineral element absorption increased, the damaged to leaf organelles also alleviated, showed that exogenous add this concentration of IAA

can induce the synthesis of antioxidant enzymes and enhance photosynthesis, promote the mineral elements absorption, alleviate the stress injury of high concentration to the spinach seedlings, and the alleviating effect was most obvious when the concentration of IAA reached 60 $mg \cdot L^{-1}$. At this time, Cu content in leaves and roots decreased to the lowest level, oxidative stress reaction in seedlings was the lowest, plant growth, photosynthesis and mineral element absorption all increased to the highest level. Therefore, compared with other IAA concentrations, 60 $mg \cdot L^{-1}$ IAA had the most obvious effect on Cu stress injury relief.

(5) Regulation effect of exogenous spraying of different concentrations of NAA solution on spinach seedlings under high concentration of Cu stress. Under Cu stress, 10 $\mu mol \cdot L^{-1}$ NAA solution showed the most obvious regulation effect when spraying 5 different concentrations of NAA solution to spinach seedling leaves. At this time, the seedling leaves of Cu content decreased significantly, the roots of Cu content significantly increased, the growth also significantly increased, MDA, O_2^- and H_2O_2 content decreased to a minimum, antioxidant enzymes (SOD, POD, CAT and APX) and proline, soluble protein contents increased, the chlorophyll content and P_n, g_s and C_i increased to the largest, qP and ETR also increased to the maximum, the root of the mineral elements contents enhanced. The chloroplast number increase, grana lamella neatly, cell wall, cell membrane, nucleus, mitochondria and vacuoles were clearly visible, the nucleus was clearly separated from the nuclear fluid. It

showed that add 10 μmol·L^{-1} NAA solution can improve plant antioxidant capacity, increase the contents of osmotic regulation substances, reduce intracellular membranous peroxide damage, reduce the interference of chlorophyll synthesis, promote the photosynthesis, alleviate the Cu damage to the organelles, thus, the stress effect of high concentration on spinach seedlings was alleviated.