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Effects of selenium and silicon fertilizer on enhancing resistance of flue-cured tobacco

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Abstract: In order to evaluate the role selenium and silicon fertilizer in enhancing resistance of flue-cured tobacco, flue-cured tobacco variety Zhongyan 100 was potted. The effects of selenium and silicon fertilizer were evaluated by analyzing the carbon and nitrogen metabolism, antioxidase and resistance index of flue-cured tobacco. The results showed that selenium and silicon fertilizer could promote the expression of key enzyme genes in carbon and nitrogen metabolism, including Sucrose phosphate synthetase (SPS), sucrose synthetase (SS), soluble starch synthase (SSS), nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH), and increase the activities of resistance-related enzymes Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) and Ascorbate peroxidase (APX). Both selenium and silicon fertilizer can increase the contents of NPT, ASA and GSH, and reduce the content of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in flue-cured tobacco. The effect of selenium fertilizer is more obvious than silicon fertilizer. These results indicate that selenium and silicon fertilizer can promote the formation of healthy tobacco plant through enhancing carbon and nitrogen metabolism and improving the regulation ability of redox balance in flue-cured tobacco.
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1. Introduction

Silicon is the fourth important nutrient element necessary for the growth and development of many crops. The application of silicon fertilizer can not only increase crop yield, but also improve crop resistance to biotic and abiotic stresses. Some studies have shown that silicon fertilizer can increase the germination rate, plant height, number of leaves and tillers, and has important effects on the relative water content of leaves, plasma membrane permeability and proline content of bluegrass, and can enhance the drought resistance of bluegrass [1]. It has also been shown that the rice plants sprayed with silicon fertilizer have greener color, stronger stalk and lodging resistance, and the number of tillers, panicle number, grain number per ear, thousand grain weight and yield are increased [2]. In rice, silicon fertilizer has good disease resistance to blast and brown spot [3]. However, because the molecular mechanism of silicon improving crop resistance is not very clear, the ability of different plants to absorb and utilize silicon is also different, which limits the application of silicon fertilizer to a certain extent. Tobacco is a low-silicon enriched crop. The study on the effect of silicon fertilizer on tobacco resistance can provide the basis for rational application of silicon fertilizer.

Selenium is a very important trace element in ecological environment. There are many researches on the cultivation techniques of selenium-rich rice, selenium-rich rape and selenium-rich wheat, but there are relatively few researches on the application in tobacco. The content of selenium in tobacco leaves was negatively correlated with the content of tar, and selenium could reduce the toxicity of tar in tobacco leaves [4]. With the increasingly prominent problems of smoking and health, the pollution-free production of tobacco leaves has been imminent. To clarify the mechanism and function of selenium in tobacco is conducive to the development and utilization of selenium-rich tobacco and promote the innovation of selenium-rich cigarette brands.

2. Material and Methods

2.1 Experiment materials

The tobacco variety is ZhongYan 100. Nutrient soil contain organic material (>30%), N (500 mg/Kg),

P_2O_5 (300 mg/Kg) and K_2O (550 mg/Kg). Selenium fertilizer is from Sichuan Ailong plant nutrition Technology Co., LTD, including amino acid (100 g/L), Se (10 g/L), Zn (10 g/L) and Mn (4 g/L). Silicon fertilizer contains 0.6g/kg $K_2SiO_3 \cdot 4H_2O$.

2.2 Experiment design

Flue-cured tobacco is potted in the laboratory of Henan Agricultural University. There were three treatments. In CK, conventional fertilization was used. In T1, conventional fertilization + selenium fertilizer were respectively sprayed at 30 days and 50 days after transplanted. In T2, conventional fertilization + silicon fertilizer were sprayed at 30 days and 50 days after transplanted. There were 20 tobacco plants in each treatment.

2.3 Detection of physiological indexes and enzyme activity

After 70 days of transplanting, 5 representative tobacco plants were selected in each treatment. Fresh samples were taken from the upper leaf of each tobacco plant. The fresh samples were mixed and then immediately placed in liquid nitrogen and stored at -80 °C. Each index was repeated 3 times.

QPCR was used to analyze the expression of genes related to carbon and nitrogen metabolism in flue-cured tobacco [5]. The malondialdehyde (MDA) content were determined with the method by haoJianjun et al [6]. The activities of resistance-related enzyme such as superoxide dismutase (SOD), Peroxidase (POD), and CAT were determined with the reported method [7-9]. The activity of APX and ASA content were determined with the kit from Nanjing Jiancheng Bioengineering Research Institute. NPT content was determined with the kit from Hefei Lyle Biological Technology Co., LTD. H_2O_2 and GSH content was analyzed with the kit from Soleibao Biotechnology Co., LTD.

3. Results

3.1 Effects of selenium and silicon fertilizer on chlorophyll content and enzymes related to carbon and nitrogen metabolism in tobacco

Chlorophyll plays a role in the absorption and transfer of light energy in photosynthesis, so the content of chlorophyll is closely related to the photosynthetic rate of plants, and the photosynthetic rate rises with the increase of chlorophyll content in a certain range. As can be seen from Figure 1, the application of selenium fertilizer can significantly increase the chlorophyll content.

Sucrose phosphate synthetase (SPS), soluble starch synthase (SSS) and sucrose synthetase (SS) are the main enzymes related to glycometabolism in plants. SPS activity represents the ability of flag leaves to convert photosynthetic products into sucrose, and can regulate the distribution of photosynthetic products between sucrose and starch. Nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) are key enzymes in plant nitrogen metabolism. Figure 2 shows the effect on the gene expression of key enzymes in carbon and nitrogen metabolism in tobacco, from which it can be seen that the application of selenium and silicon fertilizer can significantly increase the expression of GS, GDH, NR, SPS, SSS and SS in leaves, and the effect of selenium fertilizer is more obvious.

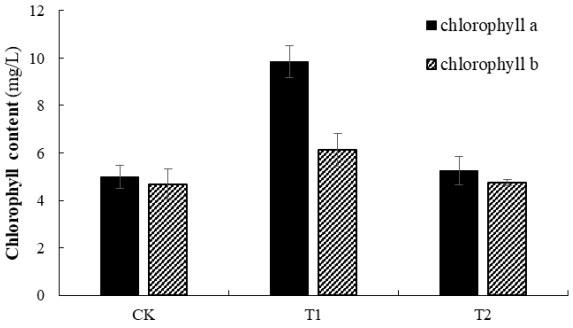

Figure 1. Effects of selenium fertilizer and silicon fertilizer on chlorophyll content of flue-cured tobacco

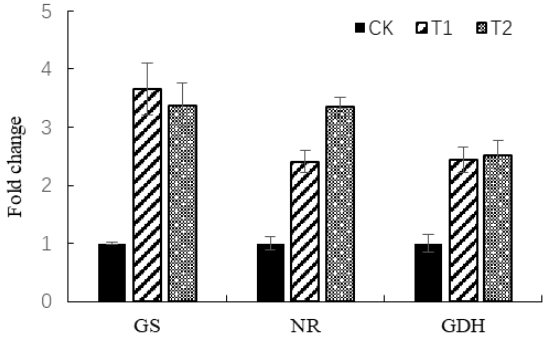

Figure 2. Effects of selenium and silicon fertilizer on nitrogen metabolism of flue-cured tobacco

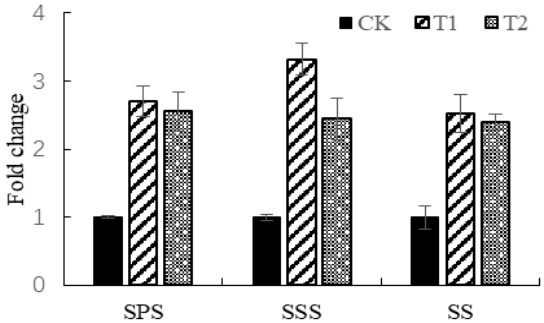

Figure 3. Effects of selenium and silicon fertilizer on carbon metabolism of flue-cured tobacco

3.2 Effects of selenium and silicon fertilizer on antioxidant enzymes in tobacco

Superoxide dismutase (SOD) is a protective enzyme to protect the cell membrane system from the damage of oxygen free radicals. As a superoxide free radical scavenger, its activity has a certain correlation with plant resistance. Under moderate stress induction, its activity was improved to increase plant resilience and adapt to adversity situation. Peroxidase (POD) exists widely in plants and is a kind of enzyme with high activity. It is related to respiration, photosynthesis and auxin oxidation. In the process of plant growth and development, its activity changes constantly. Generally, the activity in aging tissues is higher, and the activity in young tissues is weaker. Studies have shown that POD activity is related to leaf aging, and it is negatively correlated with growth rate. Catalase (CAT) can remove excess hydrogen peroxide in the cell to maintain its normal level in the cell, thereby protecting the structure of the membrane. Ascorbate peroxidase (APX) is one of the important antioxidant enzymes in plant active oxygen metabolism, especially the key enzyme in chloroplast to clear Hydrogen peroxide (H_2O_2). Table 1 shows that selenium fertilizer and silicon fertilizer treatment can increase the activity of antioxidant enzymes (CAT, POD, SOD, APX) in tobacco plants, indicating that selenium fertilizer and silicon fertilizer can regulate the metabolism of nutrient elements and improve the resistance of tobacco plants.

Table 1 Effects of selenium and silicon fertilizer on antioxidant enzymes in tobacco (U/mg protein)

Enzyme	CK	T1	T2
CAT	38.40±1.53	54.54±1.74	43.59±2.36
SOD	203.61±11.17	228.30±1.45	225.87±2.89
POD	212.07±14.24	287.43±21.35	235.67±9.85
APX	1.51±0.13	2.21±0.15	2.13±0.18

3.3 Effects of selenium and silicon fertilizer on redox balance regulation of flue-cured tobacco

Redox balance regulation is an important regulatory mechanism for plant stress resistance. For plants, both biological stress and abiotic stress are accompanied by oxidative stress and imbalance of intracellular redox homeostasis. Studies have shown that various types of stress can affect biological growth and development through intracellular and intercellular redox networks. The glutathione (GSH) metabolic pathway is a very important defensive metabolic pathway, which protects plant cells from oxidative stress. Plant non-protein sulfhydryl (NPT) is essential to maintain many biochemical reactions, and has biological functions such as antioxidant and detoxification. Plant reduced ascorbic acid plays a role in regulating redox balance and scavenging harmful free radicals, and is closely related to plant resistance. Table 2 shows that both selenium and silicon fertilizer can increase the contents of NPT, ASA and GSH in flue-cured tobacco, and the effect of selenium fertilizer is more obvious, indicating that both of them can improve the regulation ability of redox balance in flue-cured tobacco.

Table 2 Effects of selenium and silicon fertilizer on redox balance regulation of flue-cured tobacco

Treatment	NPT (mg/100g FW)	ASA (μg/mg protein)	GSH (mg/100g FW)
CK	538.33±33.31	2.35±0.24	92.84±5.40
T1	3560.08±203.62	2.97±0.09	105.79±21.39
T2	1871.67±152.35	2.55±0.15	104.82±3.37

3.4 Effects of selenium and silicon fertilizer on the contents of hydrogen peroxide and malondialdehyde in tobacco

H_2O_2, one of the most abundant reactive oxygen species (ROS) in cells, is a key signaling molecule for plant growth and development and resistance to stress, and plays an important role in plant response to abiotic stress. The content of malondialdehyde (MDA) reflects the degree of peroxidation of plant cell membrane, and affects the activities of mitochondrial respiratory chain complex and key enzymes in mitochondria in vitro. As can be seen from Table 3, selenium and silicon fertilizer can significantly reduce the content of hydrogen peroxide in tobacco plants, selenium fertilizer can significantly reduce the content of MDA in tobacco plants, indicating that selenium and silicon fertilizer can reduce the damage caused by adversity stress to tobacco plants.

Table 3 Effects of selenium and silicon fertilizer on the contents of hydrogen peroxide and malondialdehyde in tobacco

Treatment	H_2O_2 (mmol/g protein)	MDA (μmol/g)
CK	262.65±38.39	0.0068±0.0009
T1	125.92±23.45	0.0048±0.0004
T2	164.90±18.45	0.0065±0.0009

4. Discussion

Selenium and silicon fertilizer are being used more and more in agriculture to enhance crop resistance and promote crop growth. The application of exogenous silicon may improve the photosynthesis, antioxidation and osmotic adjustment ability of tobacco seedlings under drought stress, relieve drought damage on tobacco seedlings, and promote its growth [10]. Spraying suitable selenium fertilizer can improve the yield and nutritional quality of corn grains, and significantly inhibit the absorption of cadmium and

arsenic in corn [11]. Here, it was found that both selenium and silicon fertilizer can promote the expression of key enzymes in carbon and nitrogen metabolism, selenium fertilizer can significantly increase the chlorophyll content, thus enhancing carbon and nitrogen metabolism in tobacco. It was also proved that both selenium and silicon fertilizer can improve the regulation ability of redox balance in flue-cured tobacco through increasing the activities of resistance-related enzymes SOD, POD, CAT, APX and the contents of NPT, ASA and GSH. Therefore, suitable selenium and silicon fertilizer can enhance resistance and promote growth and development of flue-cured tobacco.

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