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Effects of combined application of biochar and microorganism on carbon and nitrogen metabolism and disease resistance of flue-cured tobacco

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Abstract: In order to solve the problems of soil degradation and yield reduction in tobacco production, the experiment of biochar application with microbial bacteria was carried out in Zhumadian tobacco field of Henan Province with Zhongyan 100 as material. The effects of biochar application with microbial bacteria were evaluated by analyzing the carbon and nitrogen metabolism and disease resistance of flue-cured tobacco. The results showed that the combination of biochar and microorganism could promote the expression of key enzyme genes in carbon and nitrogen metabolism and increase their activities in tobacco leaves. The soluble sugar content and the activities of resistance-related enzymes PAL, SOD, POD and CAT were increased, the disease resistance of tobacco leaf was enhanced, the occurrence of black shank disease, root rot disease and bacterial wilt disease was significantly reduced, and the average price, yield and output value of tobacco leaf were increased.

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Keywords: Flue-cured tobacco; Biochar； Microbial bacteria; Carbon and nitrogen metabolism; Disease resistance

1. Introduction

Tobacco is a kind of no-continuous cropping cash crop. Because of cultivated land area, economic benefit, cultivation conditions and other factors, continuous cropping of tobacco has been a common phenomenon. Short-term continuous cropping of tobacco will lead to tobacco quality and yield decline, tobacco plant growth and development retarded. Long-term continuous cropping will change the soil ecological environment and physical and chemical properties, and make soil fertility decline, soil microecological imbalance, disease and insect pest aggravation. Continuous cropping obstacle has become a key restricting factor for improving tobacco yield and quality. The causes of continuous cropping obstacles have soil nutrient imbalance, the accumulation of roots toxic secretions, rhizosphere micro ecosystem unbalance and so on, so further study of soil improvement mechanism, and exploring comprehensive management measures of soil, improving soil physical and chemical properties, increasing the productivity of the soil are the important way to reduce and overcome the influence of continuous cropping obstacle.

Biochar can not only reduce soil bulk density, improve soil pH value and water holding capacity, but also delay the release of fertilizer in soil and reduce nutrient leaching loss as a natural nitrogen fertilizer sustained-release agent. A large number of studies have shown that as a kind of high carbon and low nitrogen fertilizer, high carbon base soil remediation fertilizer can significantly promote crop growth and yield. Based on the analysis of carbon and nitrogen metabolism, resistance index, disease occurrence and economic characters of tobacco plant, the effect of applying high carbon base fertilizer and microbial agent on improving soil was evaluated in this paper, so as to form the application scheme of high carbon base fertilizer suitable for tobacco-growing area, and provide scientific basis for sustainable development and rational fertilization technology of tobacco field.

2. Material and Methods

2.1 Experiment materials

The tobacco variety is ZhongYan 100, and the high-carbon biochar-based fertilizer is from Henan Huinong Soil Conservation and Development Co., LTD., including the total nutrient ($N+P_2O_5+K_2O$) ≥ 5%, organic matter (dry base) ≥ 45%, biochar ≥ 20%, crude fat ≥ 1%, water content ≤ 20%. The tobacco specific fertilizer contains N 10%, P_2O_5 12% and K_2O 18%. The microbial agents such as Bacillus subtilis, Bacillus licheniformis, Bacillus laterosporus and Trichoderma harzianum are from Qiming Biological Engineering Co., LTD.

2.2 Experiment design

In zhumadian city, low- and medium-yield fields (pH 4.98, organic matter 11.9 g/Kg, hydrolytic nitrogen 77.4 mg/Kg, available phosphorus 11.6 mg/Kg, and rapidly available potassium 175 mg/Kg) where tobacco has been continuously planted for more than 3 years were selected. There were two treatments. In CK, conventional fertilization (including tobacco-special fertilizer 750 Kg/hm^2, cake fertilizer 600 Kg/hm^2) was used. In T1, conventional fertilization (the reduction of 149.92 kg of tobacco-specific fertilizer/hm^2) + high-carbon based soil remediation fertilizer 750 kg/hm^2 + microbial agents were applied. Each treatment was repeated three times.

2.3 Investigation of tobacco plant disease

According to GB/T 232222--2008 "Classification and Investigation Methods of Tobacco Diseases and Insect Pests", 50 plants were randomly sampled from each plot at mature stage. The incidence of black shank disease, bacterial wilt, Mosaic disease and root rot disease in each treatment was investigated and the incidence was calculated.

2.4 Measuration of physiological indexes and enzyme activity

After 60 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 for the determination of physiological indicators and enzyme activities. Each index was repeated 3 times.

The soluble sugar content and malondialdehyde (MDA) content were determined with the method by hao Jianjun et al [1]. The activities of resistance-related enzyme such as superoxide dismutase (SOD), Peroxidase (POD), phenylalaninase (PAL) and CAT were determined with the reported method [2-4]. The activities of key enzymes in carbon and nitrogen metabolism, such as sucrose phosphate synthase (SPS), invertase (INV), sucrose synthase (SS), amylase (AMS), nitrate reductase (NR), glutamine synthase (GS), glutamate dehydrogenase (GDH), and glutamate synthase (GOGAT), were determined with the reported method [5-11].

2.4 Gene expression detection

QPCR was used to analyze the expression of genes related to carbon and nitrogen metabolism in flue-cured tobacco. Total root RNA was extracted by TRIzol method and cDNA was synthesized by TaKaRa One Step PrimeScript RT-PCR Kit (Perfect Real Time). TaKaRa SYBR_Premix Ex TaqTM II (Perfect Real Time) kit was used for qPCR detection. Each reaction system contained 2 μL diluted cDNA, 0.5 μL positive and negative primers, 12.5 μL 2×SYBR_Premix Ex TaqTM II and 9.5 μL water (nuclease-free). The reaction procedure was as follows: after 95 ℃ for 30 s, 40 cycles (95 ℃ for 5 s, 57 ℃ for 30 s, 72 ℃ for 30 s) were run, and each sample was repeated 3 times. *NtActin* was used as an internal reference gene, primer sequences were shown in Table 1, and expression levels were calculated by $2^{-\triangle\triangle CT}$ method.

3. Results

3.1 Effects of combined application of biochar and microbial agent on enzymes related to carbon and nitrogen metabolism in tobacco.

The balance and coordination of carbon and nitrogen metabolism are closely related to the formation of tobacco quality during leaf growth and maturation. SPS, INV, SS and AMS are enzymes related to carbon metabolism in plants. As can be seen from Figure 1, the combined application of biochar and microbial bacteria can increase the expression of SS by 3.5 times, while the expression of INV, SPS and AMS increases by more than 2 times, indicating that the combined application of biochar and microbial bacteria can promote the carbon metabolism of tobacco leaves. NR, GS, GDH and GOGAT are key enzymes in plant nitrogen metabolism. As can be seen from Figure 1, the combined application of biochar and microbial bacteria can increase the expression of NR in tobacco leaves by more than 3 times, and the expression of GS, GDH, and GOGAT by more than 2 times. The results showed that the combined application of biochar and microorganism could promote the nitrogen metabolism of tobacco leaves. Table 2 showed that the combined application of biochar and microbial bacteria could significantly improve the activities of enzymes related.

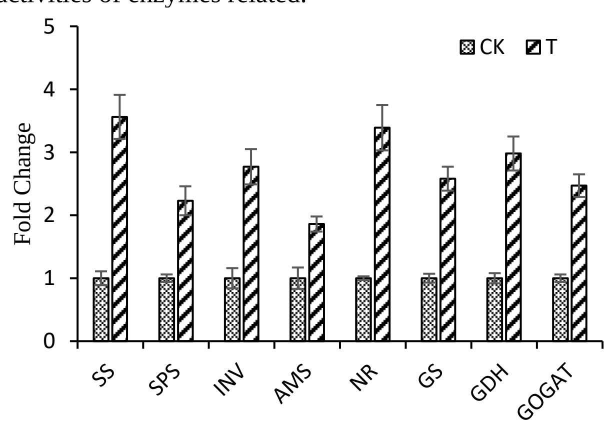

Figure 1. Effects of combined application of biochar and microbial bacteria on gene expression of enzymes related to carbon and nitrogen metabolism in tobacco.

to carbon and nitrogen metabolism, indicating that carbon and nitrogen metabolism of tobacco was significantly enhanced.

Table 2. Effects of combined application of biochar and microbial bacteria on activities of enzymes related to carbon and nitrogen metabolism in tobacco

Enzyme activity	CK	T
NR（ug/(g•h)）	24.33±1.27	27.68±1.38
GS（U/(g•h)）	71.71±3.51	87.22±4.63
INV（mg/(g•h)）	18.24±1.04	19.82±0.88
SS（U/(g•h)）	0.39±0.03	0.49±0.05
GDH（ng/mg protein）	54.22±4.93	62.47±4.87
GOGAT（ng/mg protein）	48.64±3.26	54.51±4.33
AMS（U/mg protein）	0.13±0.04	0.22±0.05
SPS（mg/(g•min)）	2.99±0.16	3.27±0.19

3.2 Effects of combined application of biochar and microbe on resistance of flue-cured tobacco

MDA is an indicator of membrane lipid peroxidation and plant senescence, which can reflect the strength of the response to adverse conditions. The accumulation of soluble sugar can protect the cell membrane from degradation and destruction, which is beneficial to the improvement of plant resistance. As shown in table 3, the combined application of biochar and microbial agents can significantly increase soluble sugar content and reduce MDA content, indicating that the ability of tobacco plant to resist adversity has been improved. PAL is a key enzyme in the phenylpropanoid metabolic pathway, which plays an important role in the synthesis of antibacterial substances such as phytoprotectin, lignin and phenolic compounds and can reflect plant disease resistance. SOD, CAT and POD are important enzymes in plant antioxidant system, maintaining the normal level of intracellular reactive oxygen species. Table 3 showed that the combined application of biochar and microbial agent could significantly increase the activities of PAL, SOD, CAT and POD, indicating that the disease resistance and antioxidant capacity of tobacco plant were improved.

Black shank, root rot and bacterial wilt are common soil-borne diseases of flue-cured tobacco, which have great harm to tobacco. As can be seen in table 4, their incidence disease decreased from 2.05% to 0.66%, 5.85% to 1.67%, 6.21% to 2.05% after the combination of biochar and microbial agents. The incidence of tobacco common Mosaic disease decreased from 8.33% to 2.52%, indicating that the combined application of biochar and microbial bacteria could effectively reduce the occurrence of main tobacco diseases, and improve the quality of tobacco leaves.

Table 3. Effects of combined application of biochar and microbe on flue-cured tobacco resistance

Resistance index	CK	T
Soluble sugar	1.35±0.38	1.96±0.21
MDA (nmol /g•FW)	40.26±1.13	31.34±2.35
SOD (U/g•FW)	115.64±2.06	156.39±2.97
POD (U/g•FW)	74.27±2.65	90.18±3.34
PAL (U/min•mg)	5.64±0.37	7.98±0.42
CAT (U/min•mg)	0.59±0.03	0.86±0.05

Table 4. Effects of biochar and microorganism on flue-cured tobacco diseases

Diseases	CK	T
Black shank /(g•FW)/(g•FW))	2.05±0.13	0.66±0.04
Root rot	5.85±0.41	1.67±0.09
Bacterial wilt	6.21±0.38	2.05±0.14
Common Mosaic disease	8.33±0.23	2.52±0.15

3.3 Effects of combined application of biochar and microorganism on economic characters of cured tobacco leaves

As shown in table 5, the combined application of biochar and microbial agents could significantly increase the average price, yield and output value of tobacco leaves by 15.83%, 13.86% and 31.89%, respectively.

Table 5. Effects of combined application of biochar and microorganism on economic characters of cured tobacco leaves

Economic characters	CK	T
Average price (yuan/Kg)	24.38+0.13	24.38+0.13
Yield (kg/hm^2)	1619.37	1843.86
Output value (yuan/hm^2)	39480.24	52070.61

4. Discussion

Carbon and nitrogen metabolism is a basic metabolic process of plants, which is not only closely related to the yield and quality of tobacco leaves, but also affects the formation of healthy tobacco plants. Among the enzymes related to carbon metabolism, SPS is the key enzyme in sucrose synthesis and can reflect the ability of photosynthate to sucrose. INV catalyzes the conversion of sucrose to monosaccharide, reduces the accumulation of starch in chloroplast and strengthens the carbon fixation process of photosynthesis, which can represent the intensity of carbon metabolism. SS decomposes sucrose to produce monosaccharides, which are further converted into carbohydrates such as starch. AMS is an enzyme that degrades starch. During nitrogen metabolism, nitrate in the body is synonymized into organic nitrogen compounds under the action of NR, GS, GDH and GOGAT, and these enzyme activities reflect the nutritional status and nitrogen metabolism level of the plant. Soil nutrition is an important factor affecting the carbon and nitrogen metabolism of tobacco. The combination of biochar and microbial agent can increase the expression and activity of these key enzymes in carbon and nitrogen metabolism, suggesting that the interaction among soil, biochar and microbial agent promotes the transformation of soil nutrients, and thus enhances the carbon and nitrogen metabolism of tobacco plant.

Biochar can not only promote the expression of genes related to carbon and nitrogen metabolism in plant leaves, but also improve the expression of resistance genes in tomato [12-13]. In this study, it was found that high carbon-based soil remediation fertilizer could significantly increase the activities of resistance-related enzymes and reduce the incidence of tobacco plant diseases, indicating that high carbon-based soil remediation fertilizer could improve tobacco plant resistance and promote the formation of healthy tobacco plants.

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