The application of intercellular proteins in monitoring tobacco mosaic disease

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Abstract: Tobacco mosaic disease is known as "plant cancer", and it not only drastically decrease the yield but also reduce the appearance quality and intrinsic quality of tobacco product. Therefore, prevention and monitor of tobacco mosaic disease is very vital for tobacco production. The aim of this study was to screen TMV infection-related proteins in intercellular proteins from tobacco leaves, and then use it to monitor and control tobacco mosaic disease. The results show that the early prevention and treatment can decrease effectively the incidence of tobacco mosaic disease. The expression of intercellular proteins in leaves has obvious changes in various treatments after infection, and the 55kD intercellular protein is a potential marker protein for monitoring the occurrence and treatment process of tobacco mosaic disease.

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Keywords: Tobacco mosaic disease; intercellular proteins; monitor

1. Introduction

Viruses are an important class of crop pathogens and are known as "plant cancer" because of their great harmfulness and difficulty controlling their effects (Sun et al, 2001). Tobacco mosaic virus (TMV) is a typical plant virus, and tobacco mosaic is one of the important diseases on tobacco production. After tobacco plants are infected by TMV, the yield will sharply decrease, and the appearance quality and intrinsic quality have also been reduced. Therefore, this disease often causes huge economic losses, and has become one of the important constraint factors to produce high quality tobacco leaves. Since it is difficult to control the harmfulness of tobacco mosaic, prevention and monitor is very important for tobacco production.

Plant intercellular proteins play important roles in the process of growth, disease resistant, degeneration resistant and signal transduction, and have been used in a number of recent studies on plant-fungal interactions. The purpose of the present investigation was to screen infection-related proteins in intercellular proteins from tobacco infected with TMV. If these infection-related proteins in intercellular proteins could be applied to analyze the interactions between tobacco plant and TMV, it might provide an effective means for monitoring tobacco mosaic disease.

2. Material and Methods

2.1 Design of the experiment and plant materials

TMV was multiplied in Nicotiana tabacum cv. K326 and purified using Gooding's method (Gooding and Hebert, 1967). The concentration of TMV was determined with an ultraviolet spectrophotometer. The purified virus was kept at −20 °C and was diluted to 50 μg/ml with 0.01 M phosphate buffered saline before use. Tobacco plants were cultivated and the experiments were conducted when the plants grew to 4-5 leaf stage. Tobacco leaves were infected with TMV solution and sprayed with Ningnanmycin (100mg/L) at different times after infection. The five treatments are as following, (1) No infection, (2) No spraying treatment after infection, (3) Spraying treatment at 1 day after infection, (4) Spraying treatment at 3 days after infection, (5) Spraying treatment at 5 days after infection. The incidence of tobacco mosaic disease was surveyed at 7 days after infection.

2.2 Real-time PCR

Total RNA was extracted from leaves of N. tabacum plants using TRizol solution and treated with RNase free DNase I to remove potential DNA contamination. First-strand cDNA was synthesized using 1 mg of total RNA according to One Step PrimeScript RT-PCR Kit (Perfect Real Time) protocol. Real-time PCR was performed as described (Guo et al, 2011).

2.3 Extraction of intercellular proteins in tobacco

The intercellular proteins were extracted from tobacco leaves according to the reported method (Du et al, 2000; Rathmell and Sequeira, 1974). After washed with water, leaves were cut into strips with the size of 5 cm^2. The strips were immersed in the buffer, and then kept in vacuum for 30 min. The strips were then removed from the buffer, blotted dry, rolled up, and placed in a 50 ml polypropylene centrifuge tube

provided with perforations (2 mm diam) at the bottom. After centrifuged at 4000g for 15 min at 4℃, the collected fluid was mixed with 4 volume of acetone at -20 ℃ overnight for precipitation. The mixture was centrifuged at 12000g for 10 min at 4℃, and then the precipitate was washed with 300 μl isopropanol. After centrifuged at 12000g for 5 min at 4 ℃, the precipitate was dried in the air. The precipitate was dissolved with 100 μl lysis buffer at 26 ℃ for 4 h.

2.4 SDS-PAGE analysis of intercellular proteins in tobacco leaves

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to analyze intercellular proteins in tobacco leaves. Protein samples (30 μg/lane) were loaded to 12% acryl amide gel. Electrophoresis was firstly performed using PowerPac basic (BIO-RAD) at 80 V for 30 min, and then at 110 V for 120 min. At last, the gel was stained with coomassie brilliant blue G-250.

3. Results and discussion

3.1 Incidence of tobacco mosaic disease

As a kind of plant virus disease, tobacco mosaic disease is known as “plant cancer”, Therefore, early prevention and treatment is vital for tobacco production. As shown in table 1 and figure 1, the incidence of tobacco mosaic disease is 100% in no spraying treatment after infection, and those are 10%, 56% and 95% respectively in spraying treatment at 1, 3 and 5 days after infection. These results show that the early prevention and treatment can decrease effectively the incidence of tobacco mosaic disease.

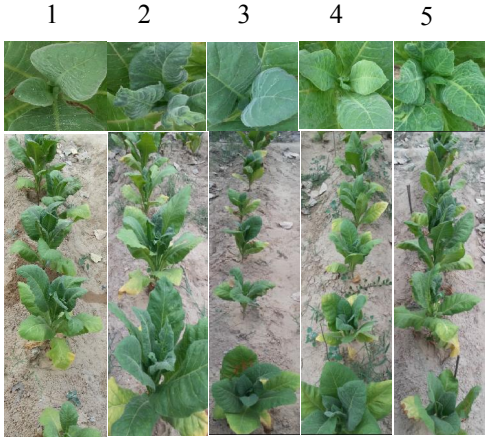

Figure 1. The phenotype of tobacco plant in different treatment. 1,No infection, 2,No spraying treatment after infection, 3, Spraying treatment at 1 day after infection, 4, Spraying treatment at 3 days after infection, 5,Spraying treatment at 5 days after infection.

Table 1. Incidence of tobacco mosaic disease.

Treatment	1	2	3	4	5
Incidence	2%	100%	10%	56%	95%

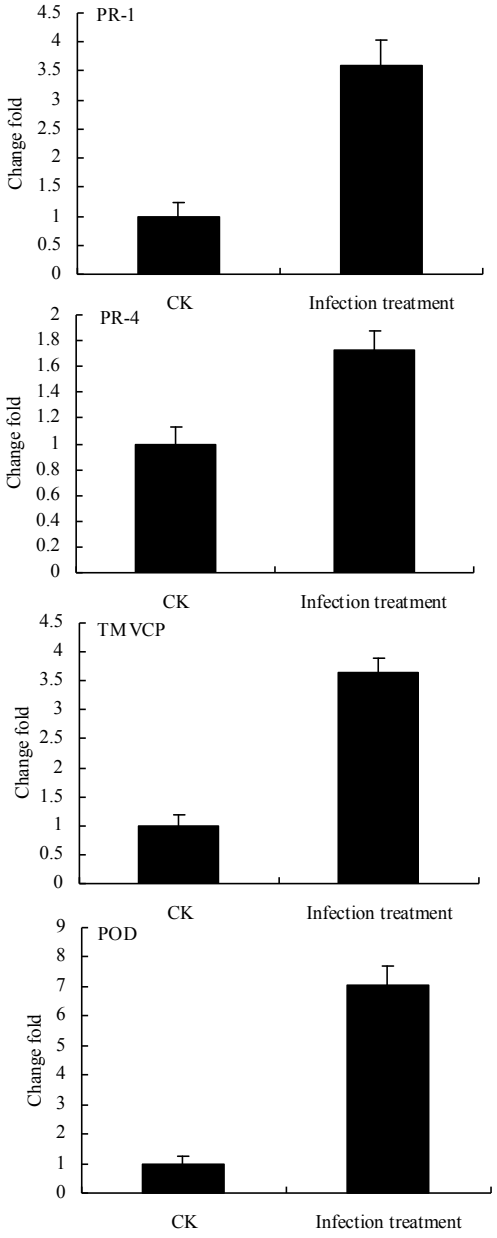

Figure 2. Changes in the expression of several genes related to tobacco mosaic disease.

3.2 Changes in the expression of several genes related to tobacco mosaic disease

TMVCP is the coat protein of TMV, and pathogenesis-related proteins (PR proteins) and

peroxidase (POD) are related to tobacco mosaic disease (Dore, 1991). As shown in figure 2, the expression of TMVCP, PR-1, PR-4, and POD increase obviously at 1 day after infection. Although the symptom of tobacco mosaic disease can not be found at 1 day after infection, these results show that tobacco plants have some physiological response to invasion of the mosaic virus, suggesting that the disease can be monitored at 1 day after infection through screening and detecting the marker of tobacco mosaic disease.

3.3 Intercellular proteins in leaves are related to tobacco mosaic disease

Plants have evolved an effective immune system against pathogen attack, such as induction of PR proteins. PR proteins are some main intercellular proteins accumulating in leaves following inoculation various types of pathogens (Kombrink 1988). Figure 3 show that the expression of intercellular proteins in leaves has obvious changes in various treatments after infection. The expression of 55kD intercellular protein in various spraying treatments is markedly higher than that in no spraying treatment, and it is the highest in spraying treatment at 1 day after infection, implying that early spraying can notably induce the expression of 55kD intercellular protein. Therefore, the 55kD intercellular protein is a potential marker protein for monitoring the occurrence and treatment process of tobacco mosaic disease.

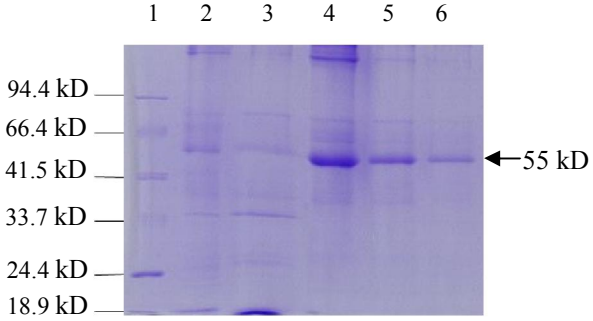

Figure 3. SDS-PAGE of intercellular proteins in leaves.
1, Protein marker, 2,No infection, 3,No spraying treatment after infection, 4, Spraying treatment at 1 day after infection, 5, Spraying treatment at 3 days after infection, 6,Spraying treatment at 5 days after infection.

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