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Physiological Response of Different Cucumber Hybrid Combinations to Inoculation of Powdery Mildew

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Abstract In order to study the physiological changes of different cucumber combinations in South China to powdery mildew, on the 0, 1, 3, 6 and 9 days after inoculation of powdery mildew, the changes of disease index, chlorophyll content, defense enzyme activity and osmoregulation substance content of 5 South China hybrid combinations were studied, and relationship between the changes of physiological indexes and the resistance of cucumber powdery mildew was analyzed. The results showed that, according to the disease index, the resistance of 5 cucumber combinations was graded, 18C-3 was disease resistance, 18C-2 and 18C-4 were medium resistance, 18C-1 and 18C-5 were susceptible; after inoculation of 5 cucumber combinations with powdery mildew, the content of chlorophyll decreased, and the content of chlorophyll in 18C-3 was higher than that in other combinations; The activity of superoxide dismutase (SOD), peroxidation(POD) and phenylalanine ammonia lyase (PAL) of most combinations decreased first, and then increased. the activity of polyphenol oxidase (PPO) was up – down – up again. The activity of defense enzyme of resistant combination 18C-3 was significantly higher than that of other combinations, and maintained high proline (PRO) content and low malondialdehyde (MDA) content all the time, indicating that the resistant combination had stronger defense ability and resistance to membrane lipid peroxidation than the susceptible combinations. By analyzing the changes of physiological indexes and the relationship with resistance to powdery mildew of cucumber, the resistant combination of South China type was selected, which provided more theoretical basis for resistance identification and genetic breeding of cucumber powdery mildew.

Keywords Cucumber; Hybrid combination; Powdery mildew; Physiological response

Powdery mildew (PM) is one of the main leaf diseases of cucumber (*Cucumis sativus* L.) with the characteristics of short incubation period, fast transmission speed, and annual occurrence (Liu et al., 2009; Luo, 2010). It occurs in greenhouses, shed and open field cultivation in the north and south of China. The untimely prevention and control will easily lead to premature senescence of cucumber plants, affecting quality and yield (Qu and Qin, 2007). With the development of cucumber protected cultivation and the gradual expansion of the area, the harm of powdery mildew has become increasingly serious. At present, the main means of prevention and control in production are chemical pesticides, which have a great impact on the green production of cucumber, but also have a certain harm to the environment and people's health (Xi et al., 2009). Therefore, breeding resistant varieties is an effective way to prevent and control cucumber powdery mildew.

A series of complex physiological and biochemical changes will occur in the host plant after the invasion of pathogenic fungi. These changes are closely related to the plant resistance, and many enzymes, such as superoxide dismutase (SOD), peroxidation (POD), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), play an important role in the plant disease resistance mechanism (Liu et al., 2018). SOD is an important antioxidant enzyme. POD can strengthen cell wall and increase disease resistance by participating in the lignification process of cell wall. PAL is a key enzyme in plant phytoalexin synthesis. PPO can oxidize phenols into quinones, which can inhibit and poison bacteria (Chai et al., 2018). Many studies have shown that the activities of defense enzymes in plants will increase after being attacked by pathogens, and the higher the activity, the stronger the resistance of

plants (Tian et al., 2015; Liu et al., 2018). Many pathogens, especially those harmful to plant leaves, often interact with chloroplasts after infecting plants, leading to the degradation of chloroplasts, chlorosis, etiolation and other symptoms of leaves, and thus affecting the photosynthetic capacity of leaves (Liu et al., 2018). South China type cucumbers are more susceptible to powdery mildew than European type cucumbers. At present, there are few varieties resistant to powdery mildew in South China type cucumbers. Therefore, five South China type combinations were selected for identification of powdery mildew resistance in this study, and the physiological and biochemical changes after inoculation of powdery mildew were studied to screen out South China type combinations resistant to powdery mildew, which provided more theoretical basis for resistance identification and genetic breeding of cucumber powdery mildew.

1 Results and Analysis

1.1 Resistance to powdery mildew in different cucumber hybrid combination

The disease index of powdery mildew of 18C-3 was the lowest (19.29) and that of 18C-1 was the highest (62.71) among the 5 cucumber combinations of South China type (Table 1). According to the disease index, the resistance of 5 cucumber combinations was graded, 18C-3 was disease resistance, 18C-2 and 18C-4 were medium resistance, 18C-1 and 18C-5 were susceptible.

Table 1 Disease index and resistance classification of cucumber hybrid combinations

Combination	Disease index	Resistance classification
18C-1	62.71 a	S (Susceptible)
18C-2	51.43 b	MR (Middle resistance)
18C-3	19.29 d	R (Resistance)
18C-4	42.86 c	MR (Middle resistance)
18C-5	58.43 a	S (Susceptible)

Note: The different normal letters within same column indicate significant difference among materials at 0.05 level (p<0.05)

1.2 Changes of chlorophyll content in leaves of different cucumber hybrid combinations inoculated with pathogens

After the cucumber seedlings of the 5 combinations were inoculated with the pathogen, the contents of chlorophyll a, chlorophyll b and relative chlorophyll showed a gradual downward trend (Figure 1). Chlorophyll a content of all combinations had little difference within 3 days after inoculation compared with that before inoculation. From the 6th day, except for 18C-3, other combinations decreased significantly. On the 9th day, the content of chlorophyll a in all combination decreased to the lowest, and the content of chlorophyll a in 18C-3 was 20.56 mg/g, which was significantly higher than that in other combinations. Followed by 18C-4, which was 19.37 mg/g. And the content of chlorophyll a in 18C-5 was the lowest on the 9th day, which was 15.34 mg/g. After inoculation, the chlorophyll b content and relative chlorophyll content of 18C-3 were always higher than those of other combinations, while the chlorophyll b content of 18C-1 was significantly lower than that of other combinations. The changes of carotenoid content in the 5 combinations were different. In general, 18C-2, 18C-3 and 18C-4 showed a slow upward trend. 18C-2 rose to the highest level of 4.52 mg/g on the 6th day. 18C-3 decreased slightly on the 6th day, and rose rapidly to the highest level (5.11 mg/g) on the 9th day.

1.3 Changes of SOD and POD activities in leaves of different cucumber hybrid combinations inoculated with pathogens

Except for 18C-4, the SOD activity of other combinations of cucumber seedlings decreased first and then increased after inoculation (Figure 2A). The SOD activity of 18C-1, 18C-2 and 18C-5 decreased rapidly after inoculation, and 18C-5 decreased to the lowest level on the first day after inoculation, and then increased slowly. 18C-1 and 18C-2 decreased to the lowest on the 6th day after inoculation. The activity of 18C-3 decreased to the lowest on the 3rd day, and then began to rise, and the activity on the 6th and 9th days was 510.19 U/g and 737.71 U/g, respectively, which were significantly higher than other combinations. 18C-4 increased slowly and decreased slightly on the 6th day. On the 9th day, the activity was 553.44 U/g, which was significantly higher than that of 18C-1 and 18C-5.

POD activity of all combinations decreased first and then increased (Figure 2B). Except for 18C-1, other combinations reached the peak on the 6th day after inoculation. The peak value of 18C-3 was 19 340 U/g, which was significantly higher than that of other combinations. On the 9th day, it decreased slightly to 18 453 U/g, which was still higher than that of other combinations. 18C-4 still maintained high activity on the 6th day after inoculation.

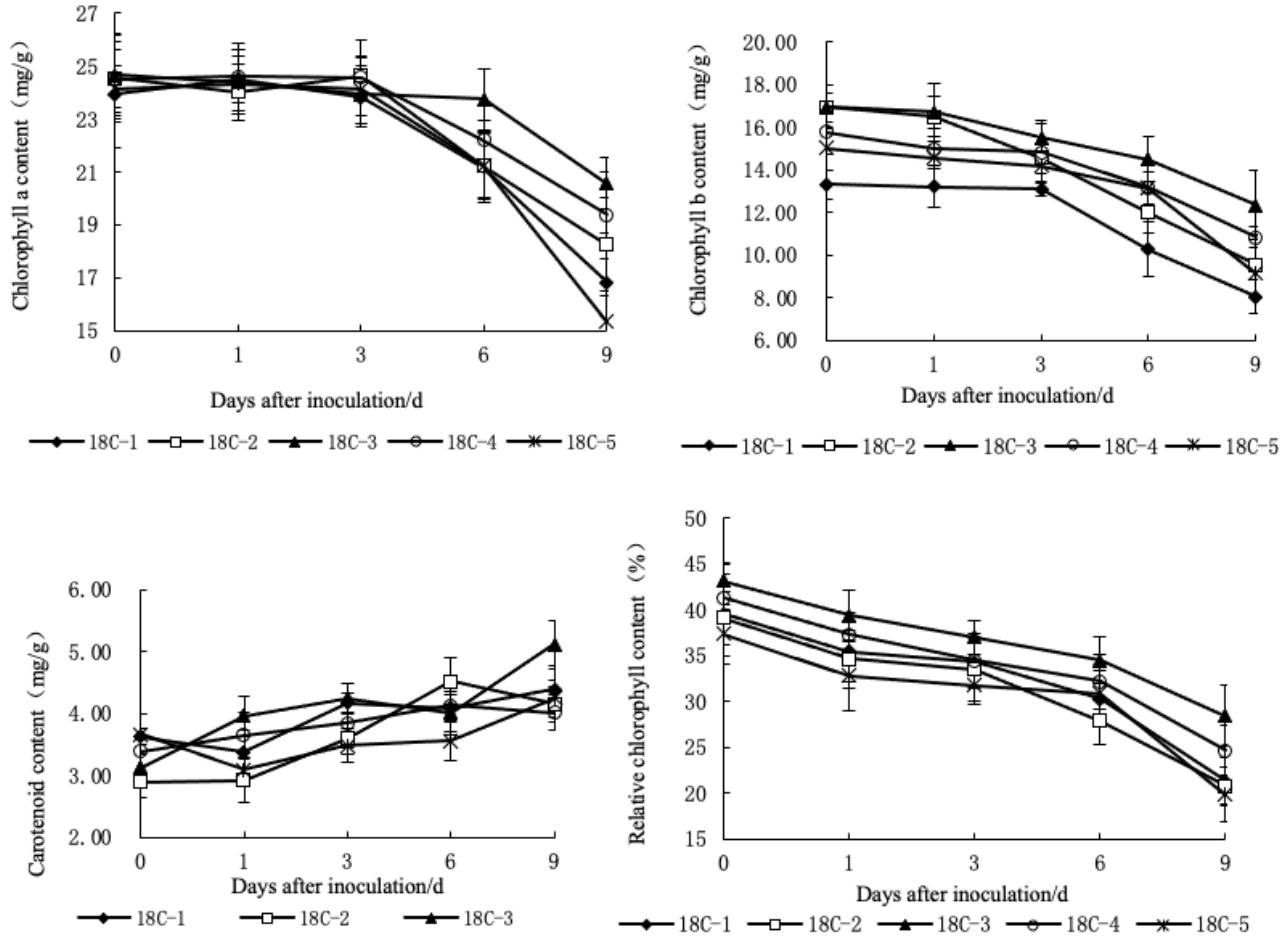

Figure 1 Changes of chlorophyll content in leaves of cucumber seedlings inoculated with powdery mildew

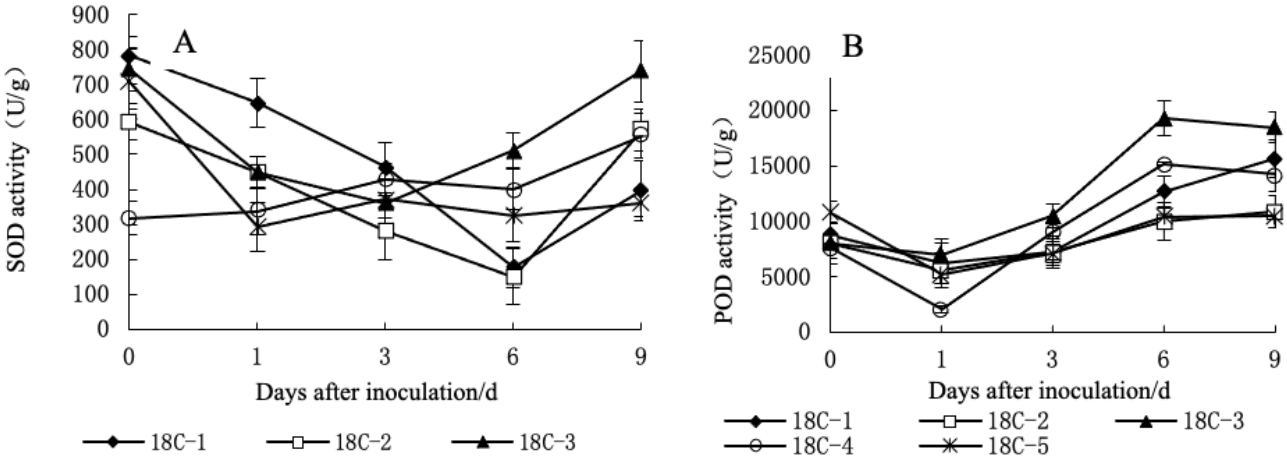

Figure 2 Changes of SOD and POD activities in leaves of cucumber seedlings inoculated with pathogen

1.4 Changes of PAL and PPO activities in leaves of different cucumber hybrid combinations inoculated with pathogens

After inoculation with powdery mildew, PAL activity of seedling leaves of each combination decreased first and then increased (Figure 3A). PAL activity of 18C-1, 18C-3, 18C-4 and 18C-5 decreased to the lowest on the first day after inoculation, and then began to increase. 18C-2 decreased to the lowest of 61.98 U/g on the 3rd day after treatment, which was significantly lower than other combinations. The PAL activity of 18C-3 reached the peak value of 119.17 U/g on the 9th day after inoculation, which was significantly higher than that of other combinations.

The activity of PPO in the leaves of seedlings of each combination showed a trend of up–down–up again inoculation (Figure 3B) and reached a trough on the 3rd day after inoculation. The PPO activity of 18C-1 and 18C-2 was always low, and 18C-1 was lower than other combinations on the first and ninth days after treatment, which were 136 U/g and 296.4 U/g, respectively. The POD activity of 18C-3 on the 6th and 9th day after inoculation was higher than that of other combinations, and the POD activity on the 9th day was 427.2 U/g, followed by 18C-4.

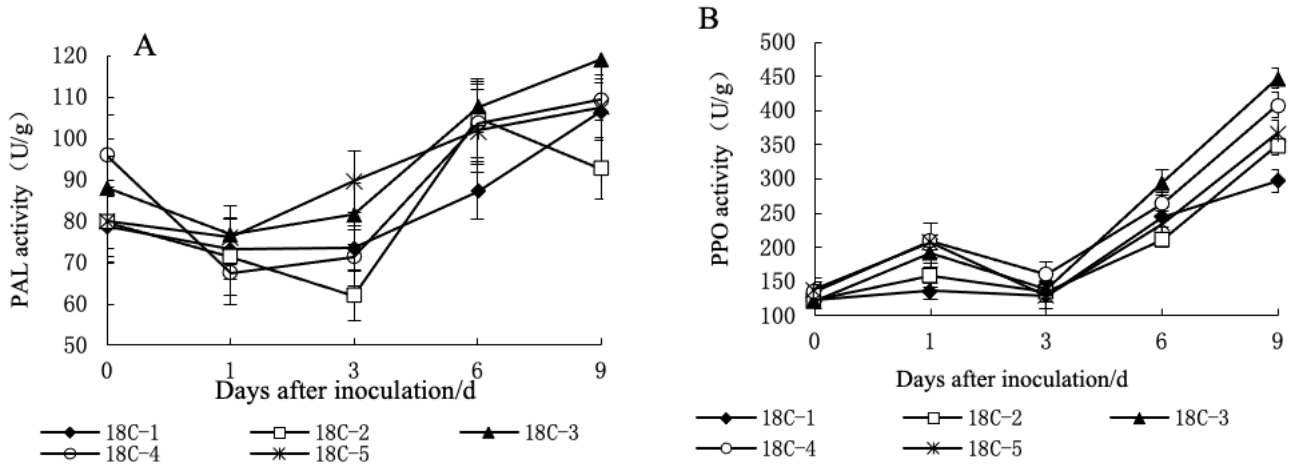

Figure 3 Changes of PAL and PPO activity in leaves of cucumber seedlings inoculated with pathogen

1.5 Changes of PRO and MDA activities in leaves of different cucumber hybrid combinations inoculated with pathogens

After inoculation of powdery mildew, the proline (PRO) content in the leaves of five cucumber combinations showed a trend of up-down-up-down again with the extension of time (Figure 4A) and reached a trough on the 3rd day after inoculation. The PRO content of 18C-3 was significantly higher than that of other combinations from the 3rd day after inoculation.

Except that the MDA content of 18C-3 in the five combinations decreased on the 2nd day, the MDA content of other combinations increased gradually with the extension of inoculation time (Figure 4B). 18C-3 always maintained a low MDA content, and the MDA content on the 9th day after inoculation was 60.89 nmol/g, which was significantly lower than other combinations, followed by 18C-4. The content of MDA in 18C-5 was the highest (92.54 nmol/g).

2 Discussion

Chlorophyll is an important pigment in plant photosynthesis and an important indicator of plant photosynthetic capacity. Shen et al. (2003) found that after cucumber plants were infected with pathogens, the content of chlorophyll in leaves decreased significantly, and chlorophyll b was more sensitive than chlorophyll a. Han et al. (2017) studied the changes of chlorophyll in the leaves of wheat with different resistance after inoculation of powdery mildew, and found that the relative chlorophyll content decreased, and the decline of susceptible varieties was greater. In this study, after inoculation with powdery mildew, chlorophyll a, chlorophyll b and relative

chlorophyll content of different South China cucumber combinations showed a downward trend, chlorophyll a did not change much before 4 days after inoculation, and chlorophyll b was more sensitive to disease infection. The chlorophyll content of 18C-3 was significantly higher than that of other combinations and showed stronger resistance to the disease.

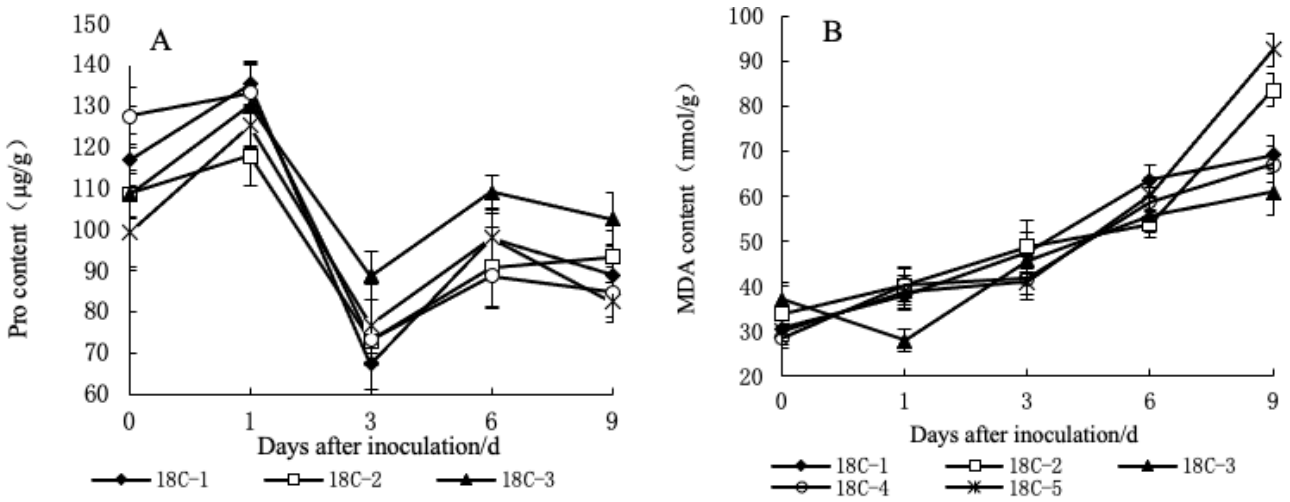

Figure 4 Changes of proline and MDA content in leaves of cucumber seedlings inoculated with pathogen

As important antioxidant enzymes in plants, SOD and POD can eliminate free radicals and maintain the metabolic balance of active oxygen in plants (Zhu et al., 2015). PPO and PAL are important defense enzymes related to plant disease resistance, which play an important role in strengthening and repairing cell wall structure, synthesis of disease resistance related substances, etc. (Chai et al., 2018), and can enhance the resistance of plants to pathogens. At present, the research conclusions about the relationship between defense enzyme activity and plant disease resistance are not consistent because of different plants, diseases and determination methods. Zhou et al. (2013) believed that the activities of POD, PPO and PAL were significantly positively correlated with disease resistance. Li and Si (2007) found that after inoculation of *Fusarium oxysporum* at seedling stage in different cucumber varieties, the three enzyme activities of resistant and susceptible varieties increased, and the increase of enzyme activities of resistant varieties was higher than that of susceptible varieties. And Liu et al. (2009) found that the POD and PPO activities of susceptible varieties increased rapidly and greatly in their research on cucumber inoculation with *Fusarium oxysporum*. Han et al. (2017) found that after inoculation of wheat powdery mildew, the activities of SOD, POD and CAT in different resistant wheat showed a trend of little change at first, then began to increase, and finally decreased. Zhang et al. (2016) found that the activities of PAL, PPO and POD in leaves of melon varieties with different resistance increased first and then decreased after inoculated with *Didymella bryoniae*. In this experiment, after cucumber seedlings were inoculated with powdery mildew, except for the change trend of SOD activity of 18C-4, which showed a trend of up-down-up again, the activities of SOD, POD, PAL in other combinations of leaves showed a trend of down-up, and the activity of PPO showed a trend of up-down-up again. The enzyme activity of the disease resistant combination 18C-3 reached the peak at the first rising stage, and the increasing range was significantly higher than that of other combinations. Various defense enzyme systems in plants will cooperate with each other and react differently after being infected by pathogen. This is because the disease resistance mechanism of plants is relatively complex, and the dominant factors of host resistance to different diseases are also different (Dang et al., 2011).

Host plants will produce a large amount of active oxygen after being infected by pathogen, which will directly destroy the unsaturated fatty acids in the membrane system, causing serious lipid peroxidation. Mycotoxin can also cause ion exosmosis and aggravate membrane lipid peroxidation (Li et al., 2012). MDA content can reflect the severity of membrane lipid peroxidation and is negatively correlated with plant resistance. Li et al. (2012) showed that after cucumber was inoculated with *Pseudoperonospora cubensis*, the MDA content of resistant varieties was lower than that of susceptible varieties. Proline accumulation is an adaptive response of plants to

stress. Zhang et al. (2017) believed that bottle gourd varieties with strong resistance to powdery mildew can accumulate higher proline content, which can reduce the damage caused by disease. In this experiment, after inoculated with powdery mildew, the MDA content in the leaves of different South China cucumber combinations showed an upward trend except that 18C-3 decreased on the 1st day. The PRO content of different combinations reached the peak on the 1st day after inoculation and dropped to the trough on the 3rd day. The disease resistant combination 18C-3 maintained low MDA content and high PRO content.

The interaction between pathogens and plants is very complex, and different plants or varieties have different disease resistance mechanisms to different pathogens. In this study, among the 5 cucumber combinations of different South China types, the disease resistant combination 18C-3 could resist the infection of powdery mildew by maintaining high chlorophyll content, defense enzyme activity and osmoregulation substance content.

3 Materials and Methods

3.1 Experimental materials

The tested 5 cucumber (*Cucumis sativus* L.) combinations of South China type were independently selected by the Cucumber Research Group of the Horticulture Research Institute of Shanghai Academy of Agricultural Sciences, numbered 18C-1, 18C-2, 18C-3, 18C-4 and 18C-5, respectively, and the basic characteristics were obtained (Table 2).

Table 2 Basic characteristics of 5 cucumber combinations in South China

Combination	Female	Fruit length (cm)	Fruit color	Thorn color	Tumor size
18C-1	All female	27.62	Dark green	White	Small
18C-2	All female	25.00	Dark green	White	Small
18C-3	All female	25.50	Dark green	White	Small
18C-4	All female	19.37	Yellow Green	White	Middle
18C-5	All female	25.37	Green	Black	Middle

Cucumber powdery mildew (*Sphaerotheca fuliginea*) was collected from naturally infected cucumber plants in the greenhouse of Zhuangxing Comprehensive Test Base of Shanghai Academy of Agricultural Sciences. Collected diseased cucumber leaves, washed off the debris on the leaves and the original sporangia, cultivated them in a light incubator at 20℃~24℃ for 24 h, and then brushed the newly grown powdery mildew spores on cucumber leaves with a brush dipped in pure water to prepare the suspension of 10~15 spores per field of view under 10× microscope for inoculation.

3.2 Experimental methods

The experiment was conducted in the Key Laboratory of Horticulture Research Institute of Shanghai Academy of Agricultural Sciences from April to July 2018. Soaked the seeds of 5 cucumber combinations for germination, and when the bud length was 0.5 cm, the plug seedling was started. When the seedlings with two leaves and one heart, they were inoculated with powdery mildew by spray method. Each variety was inoculated with 30 (including 3 replicates, 10 per replicate), and the other 30 (including 3 replicates, 10 per replicate) were used as the control (spraying water). The first real leaf was taken on the 0, 1st, 3rd, 6th and 9th d after inoculation, and stored at -80℃ for the determination of the relevant physiological indexes.

3.3 Investigation of powdery mildew index

The incidence was investigated 10 d after inoculation. The disease was investigated according to the grading standard of Zhang et al. (2005), and the disease index (DI) was calculated. The resistance classification standard is as follows: High resistance (HR): 0≤DI≤15; Resistance (R): 15＜DI≤35; Middle resistance (MR): 35＜DI≤55; Susceptible (S): DI＞55.

3.4 Physiological index measurement

Referred to the method of Li (2000, Higher Education Press, pp.134-137) to determine the chlorophyll content. Determined the activity of SOD, POD, PAL and PPO according to the instructions of the kit of Suzhou Comin

Biotechnology Co., Ltd. The contents of PRO and MDA were determined according to the method of Hao et al. (2014).

SPSS20.0 and Excel were used to analyze the data.

Authors' Contributions

ZHM and YJZ jointly participated in the experimental design. ZHM conducted data analysis and paper writing. JHJ, BLJ, DXT, HLZ, CJW, and HJJ jointly participated in the experimental design and results analysis. YJZ made the final revision of the paper. All authors read and approved the final manuscript.

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