Short Research Article

The Effect of Jingdusha on Cucumber Chlorophyll Fluorescence Parameters under *Corynespora cassiicola* Stress

ABSTRACT

Aims: The paper aimed to clarify the effect of Jingdusha on chlorophyll fluorescence parameters of cucumber seedlings under *Corynespora cassiicola* stress.

Study design: We applied the method of artificial inoculation in the pot and analyzed the changes in the content of chlorophyll and chlorophyll fluorescence parameters.

Place and Duration of Study: In 2018, these experiments were conducted in the College of Bioscience and Biotechnology of Shenyang Agricultural University (Lab 240).

Methodology: The seedlings in the two-leaf period were induced by 2.0 $mL \cdot L^{-1}$ of Jingdusha, then inoculated *C. cassiicola* for 24 h. Cucumber seedlings of each treatment group were randomly selected for the content of chlorophyll and chlorophyll fluorescence parameters determination. The leaves of cucumber seedlings in each treatment group were randomly collected at 1 d, 3 d, 5 d, 7 d and 9 d after inoculation for the determination of the content of chlorophyll and chlorophyll fluorescence parameters.

Results: When *C. cassiicola* infects cucumber, Jingdusha can effectively increase the energy conversion efficiency of the PSII central response and the potential activity of PSII, besides, the photosynthetic reaction activity did not change significantly in the early stage, but increased significantly in the later stage and rescure the degree of photosynthetic structural damage.

Conclusion: In conclusion, under *C. cassiicola* stress, plant immune resistant inducer Jingdusha can effectively increase the capacity of PSII and the efficiency of PSII operation under the light in cucumber seedlings, reduce the damage of photosynthetic structure, increase the ability of PSII to accept and transfer electrons, as well as reduce the degradation of chlorophyll and the dissipation of light energy under *C. cassiicola* stress.

Keywords: Cucumber; Corynespora cassiicola; Jingdusha; Induced resistance; Chlorophyll fluorescence parameter

1. INTRODUCTION

Target Leaf Spot (TLS) caused by *Corynespora cassiicola* in cucumber, which is also called *Rhizoctonia solani* and cercospora leaf spot. In some provinces in China, TLS has a universal prevalence [1]. TLS has a wide host range and mainly affects the leaves, stems and fruits of cucumbers, tomatoes, and some others. The disease on the leaves first appears in gray-purple-black round dots, gradually expanding into star-shaped 0.5 to 1 cm diameter round or irregular-shaped spots, the edge is not neat, the perimeter is purple-black, slightly lighter in the middle, and some spots have whorls, can cause early leaf drop, which has caused yield reductions of up to 20-70% in cucumbers. In recent years, TLS has more serious damage to cucumbers than before [2].

In field production, chemical pesticides are mainly applied to resist TLS. Commonly used fungicides include 45% chlorothalonil, 35% dicyhalothione-tebuconazole, 43% Fluoropyramides-Oxime Esters SC, 35% Phenylfeniconazole-Prochloraz EW, 70% methyl WP and 50% iprodione WP[3]. However, due to the long-term irrational use of chemical pesticides, the resistance of pathogenic bacteria has become seriously increasing[4], which has resulted in environmental pollution and safety hazards of agricultural products. Therefore, it is necessary to take some cost-effective and compatible with the environment methods to solve the current environmental pollution and food safety crisis. Good results have been achieved by using resistance inducers to induce plants to develop disease resistance on their own to control some diseases at present[5].

Jingdusha(JDS), a bio-resistance-inducer produced by China Ocean University Biological Engineering Development Co. has the functional characteristics of anti-virus, growth promotion and pollution-free. It contains chitosan and seaweed organic iodine, which can improve the ability of crops to resist disease and immunity, achieve fertilizer and medicine work together. It has an obvious preventive effect on fungal, bacterial, and viral diseases, such as wilt, yellow wilt, downy mildew, gray mold, epidemic, anthracnose, stripe blight, vertical blight, rice quill, powdery mildew, horn spot, phloem, and so on. It has a significant effect on improving the soil, fertilizing the ground, promoting the root system to develop and grow strong. As the ingredients are all pure natural extracts, have no residue, natural and pollution-free, which can greatly improve the quality of crops[6].

A previous study[6] found that JDS did not kill *C. cassiicola* by itself but had a significant resistance-inducing effect on cucumber seedlings. JDS at 2.0 $mL \cdot L^{-1}$ induced cucumber three times every 7 d interval for 1 d showed the highest efficacy of 68.93% against *C. cassiicola*[7]. To clarify the mechanism of resistance induction of cucumber TLS, we analyzed the changes of physiological and biochemical substances in cucumber plants after the optimal application program and then elucidated the mechanism of resistance induction of JDS to *C. cassiicola*.

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Tested materials

The tested cucumber was “JinKe 1”. The tested plant immune resistance inducer JDS, which is provided by Qingdao Seawin Biotech Group Co., Ltd. *C. cassiicola* was purchased from the Bacterium Conservation Center of the Chinese Academy of Agricultural Sciences.

2.1.2 Materials treatments

After sowing for 14 days, when the cucumber seedlings grew to the stage of two leaves and one heart, the solution was to be sprayed evenly on the leaf surface and leaf back of the cucumber seedlings as the concentration of 2.0 $mL \cdot L^{-1}$ JDS once every 7 d for 3 weeks, while the control group was sprayed with distilled water to the extent that the solution was ready to drip. Both treatments were set up with six replications of 10 seedlings each time, and every three replications were set up as one group. One group of seedlings was selected in each treatment 24 h after the last induction, and the leaves were inoculated with a suspension of *C. cassiicola* at a concentration of 10^3 spores/mL, with the amount of inoculum dripping downward from the leaf surface. Sampling and measuring chlorophyll fluorescence parameters at 2 pm each day at 1 d, 3 d, 5 d, 7 d, and 9 d after inoculation, respectively, at the third euphylla from the bottom up of the plant, with five replicates, and the sampled leaves were used for chlorophyll content determination.

2.2 Methods

2.2.1 Measurement of chlorophyll content

The measurement of chlorophyll content of cucumber seedling leaves was carried out according to the reported method[8]. Euphyllas were weighed 0.1 g and cut, placed in a 10 mL centrifuge tube, added 10 mL of extract (acetone: ethanol: water = 4.5:4.5:1), left at room temperature and protected from light until the leaves turned white, shaken well and then take 3 ml extraction in a colorimetric cup and the light absorption values were measured at 663 nm, 645 nm and 440 nm, and the chlorophyll content was calculated according to the following equation.

Chlorophyll a （$mg \cdot L^{-1}$）=9.784OD663-0.99OD645 as ca

Chlorophyll b （$mg \cdot L^{-1}$）=21.426OD663-4.65OD645 as cb

Chlorophyll （$mg \cdot L^{-1}$）=ca+cb=5.134OD663+20.436OD645

Carotenoids （$mg \cdot L^{-1}$）=4.695OD440-0.268(ca+cb)

Pigment content （$mg \cdot g^{-1}$）= Concentration （$mg \cdot L^{-1}$）* Total volume of extraction (L)/samples weight (g)

2.2.2 Measurement of chlorophyll fluorescence parameters

We use FMS–2 pulse-modulated fluorometer produced by Hansatech, UK to test chlorophyll fluorescence parameters. The measurement of chlorophyll fluorescence parameters of cucumber seedlings was carried out according to the reported method[9]. Five seedlings were selected from each treatment group, and the plants were clamped with the third euphylla from the bottom up and treated with darkness for 30 min. The initial fluorescence parameter (Fo) and the maximum fluorescence parameter (Fm) were measured by pushing away the dark treatment disc. By determining the above chloroplast fluorescence parameters, We calculated light energy absorption value per unit area (ABS/CSO), light energy capture per unit area (TRo/CSO), the quantum yield of electron transfer per unit area (ETo/CSO), heat dissipation of per unit area (DIo/CSO), functional index based on light energy absorbance (PIABS), the maximum light-chemical efficiency of PSII [Φ Po = Fv/Fm = TRo/ABS = (Fm-Fo)/Fm], The ratio of excitons captured in the reaction center that is used to drive electron transfer to other electron acceptors in the electron transport chain over the QA (ET) occupied to drive the QA reduction exciton [Φ o = ETo/TRo = (1-VJ)], quantum yields of electron transfer in photosynthetic apparatus [Φ Eo =ETo/ABS = (1-Fo/Fm) • Φ o), maximum quantum yield without chemical quenching (Φ Do =DIo/ABS = 1 - Φ Po), number of reaction centers per unit leaf area (RC/CSO).

2.3 Statistical analysis

Excel statistical software was used to complete the processing of the original data of the experiment, Origin 9 software was used to create the graphs, and the values were expressed as the mean ± standard deviation. The data were analyzed by a two-tailed overall t-test, and the ANOVA method in SPSS 17.0 software was applied to analyze the significance of differences in the experimental data, and the values were the means of five experiments.

3. RESULTS

3.1 Effect of JDS on chlorophyll content of cucumber seedlings under *C. cassiicola*

According to Fig. 1, the content of chlorophyll a, chlorophyll b, chlorophyll and carotenoid have a significant increase at all times when the seedlings under the JDS treatment alone, with no significant changes at other times. Only under *C.cassiicola* stress, the content of chlorophyll a have a sharp decrease at all times. The content of chlorophyll b has an evident decrease at 1 h, 5 h and 9 h. The rest of the time has no sharp changes. The content of chlorophyll has decreased significantly at 1 h, 5 h, 7 h and 9h. There is no evident change at 3 h. The content of carotenoid has decreased at all times. Under *C.cassiicola* stress, the changes of chlorophyll a, chlorophyll b, chlorophyll and carotenoid in cucumber seedlings treated by JDS are as follows. The content of chlorophyll a has a significant decrease at all times. The chlorophyll b content has a sharp decrease at 1 h, 5 h, and 9 h and an

increase at 3 h. The chlorophyll content significantly decreases at 1 h, 5 h, and 9 h. There are no evidently changes at 3 h and 7 h. The content of carotenoid has not significantly changed at 7 h, the rest of the time shows an evident decrease.

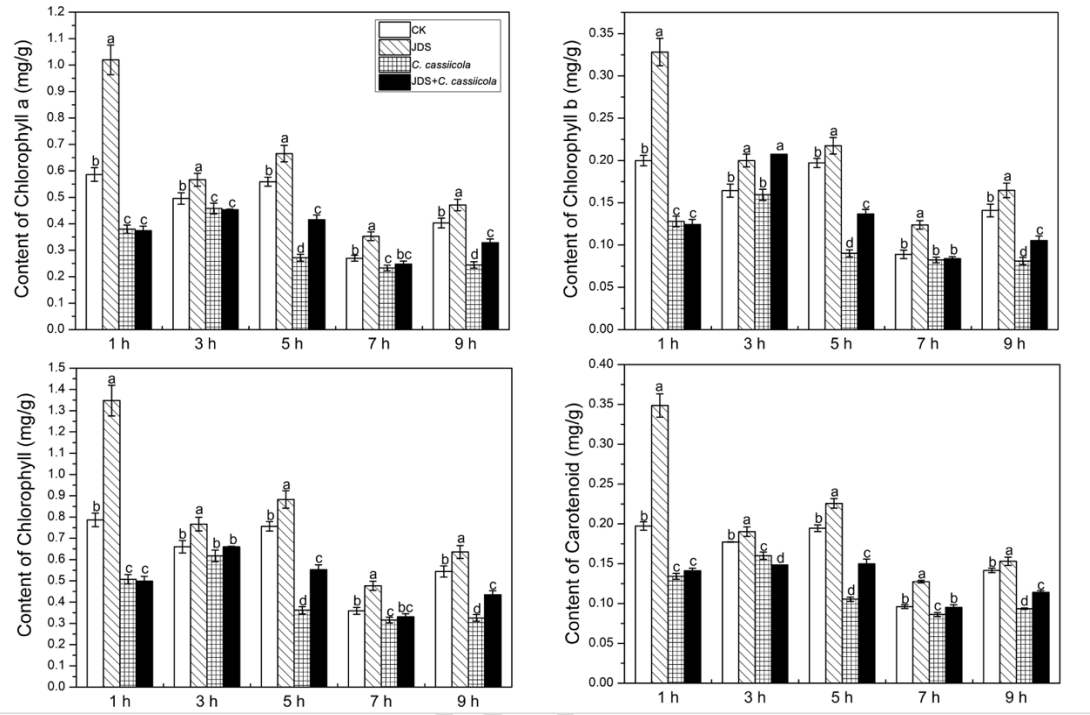


Fig. 1 Effects of JDS on Chl a, Chl b, Chl and Car under *C.cassiicola* stress

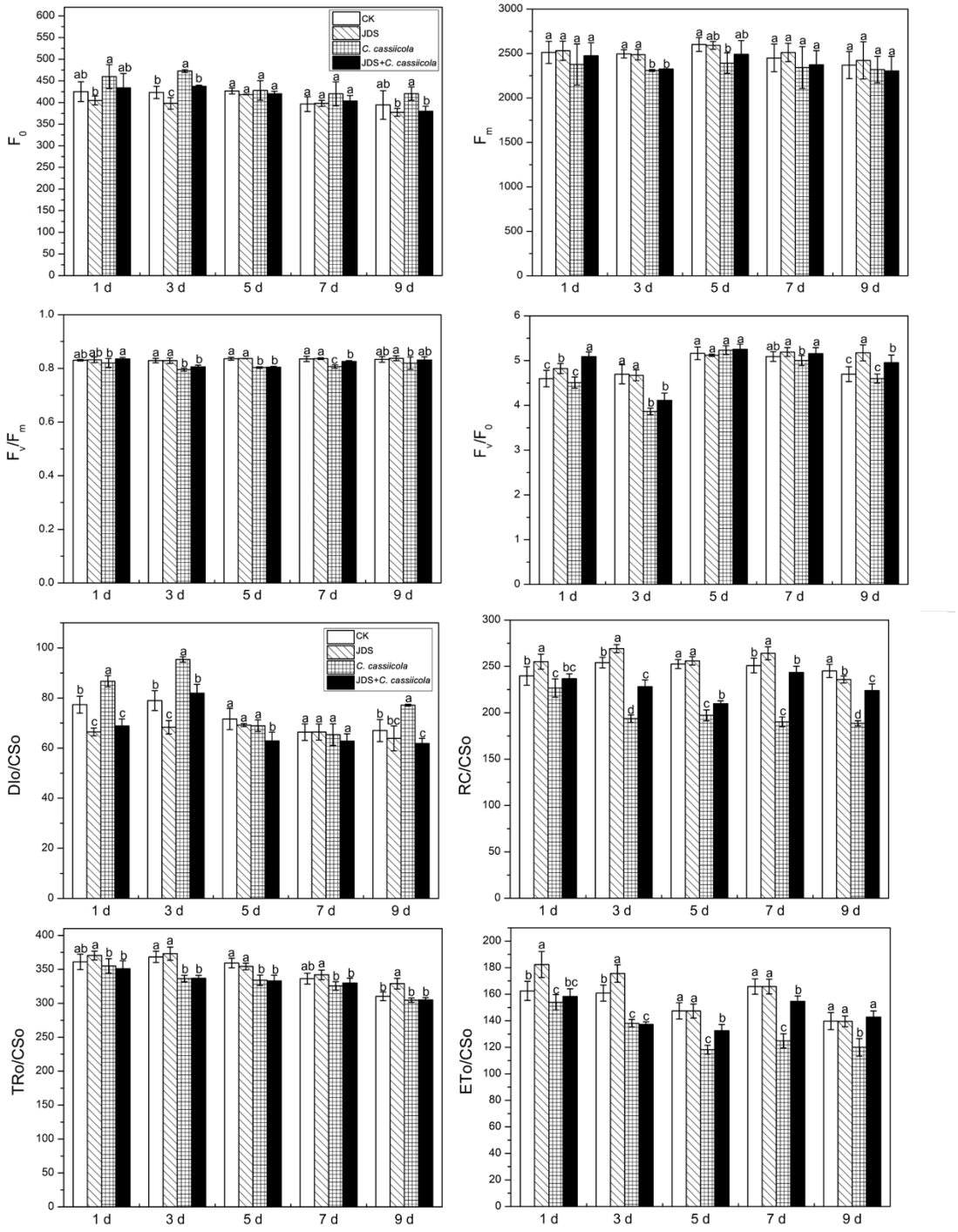
3.2 Effect of JDS on chlorophyll fluorescence parameters of cucumber seedlings under *C. cassiicola* stress

As is shown in Fig. 2, the chlorophyll fluorescence parameters of cucumber seedling leaves decreased significantly in F0 only at 3 d when treated with JDS alone, with no significant changes in the rest of the time; Fm, ϕP and ϕDo have no significant changes. Fv/F0 increases obviously at 1 d and 9 d, the rest time does not change evidently. DIo/CSO decreased significantly at 1 d and 3 d, after which there was no significant change; RC /CSO was significantly raised except for 3 d, where there was no significant change; TRo/CSO did not change significantly from 1 to 7 d and increased significantly at 9 d; ETo/CSO increase sharply at 1 d and 3 d, the other time have no obvious change. PIABS increased at 1d and 3 d and decreased at 7d and 9d significantly, not different at 5 d; φo increased significantly at 1 d, after which there were no obvious changes; φEo increased evidently at 1 d, 3 d and 5 d, after which there was no significant change.

Only under *C.cassiicola* stress, F0 only increased significantly at 3 d, but no significant changes at the rest of the time; Fm and TRo/CSO decreased

sharply at 3 d and 5 d, but no significant changes at the other time; φP did not change obviously at 1 d, then decreased significantly, and no significant changes after 9 d; Fv/F0 decreased significantly at 3 d, but no significant changes at the rest of the time; DIo/CSO increased significantly at 1d and 3d, then did not change significantly, and repeated an evident increase at 9 d; RC/CSO and ETo/CSO decreased significantly; PI_{ABS} decreased sharply at 1 d and 3 d, increased significantly at 5 d, and decreased markably at 7 d and 9 d; φo did not change significantly at 1 d and 3 d, increased significantly at 5 d, and decreased significantly at 7 d and 9 d; φEo increased significantly at 1 d, did not change significantly at 3 d, increased significantly at 5 d, and decreased significantly at 7 d and 9 d; φDo did not change significantly at 1 d, and increased significantly thereafter.

Under *C.cassiicola* stress, the changes in chlorophyll fluorescence parameter in cucumber seedlings treated by JDS are as follow. F0 decreased significantly at 3 d and 9 d, with no significant changes at the rest of the time; Fm and TRo/CSO showed no significant changes; φP increased significantly at 1 d and 7 d, with no significant changes at the rest of the time; Fv/F0 increased significantly at 1 d, 7 d and 9 d, with no significant changes at the rest of the time; DIo/CSO decreased significantly at 1 d, 3 d and 5 d, with no significant changes at 7 d and a significant decrease at 9 d; RC/CSO showed no significant change at 1 d and then increased significantly; ETo/CSO showed no significant change at 1 d and 3 d and then increased significantly; PI_{ABS} showed a significant increase at 1 d and 3 d, a significant decrease at 5 d, and a significant increase at 7 d and 9 d; φo showed no significant change at 1 d, 3 d, and 5 d and then increased significantly; φEo showed no significant change at 1 d and 3 d, a significant decrease at 5 d, and a significant increase at 7 d and 9 d; φDo decreased significantly at 1 d and 7 d, and did not change significantly in the rest of the time.


CK
JDS
C. cassiicola
JDS+C. cassiicola
F0
Fm
Fv/Fm
Fv/F0
DIo/CSo
RC/CSo
TRo/CSo
ETo/CSo
1 d
3 d
5 d
7 d
9 d

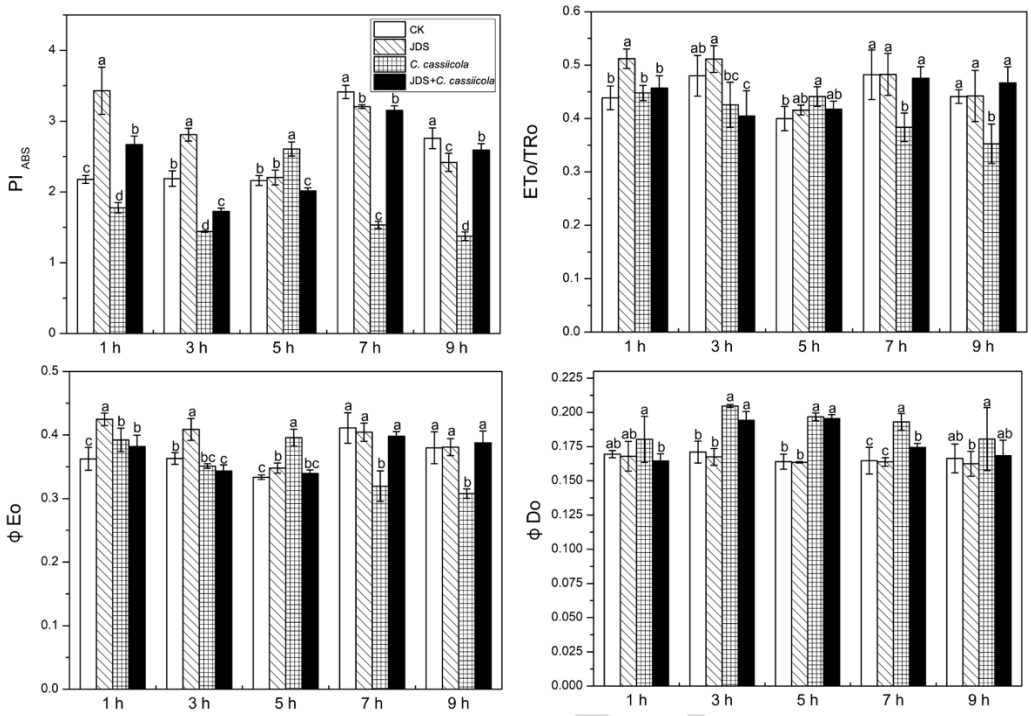


Fig. 2 Effects of JDS on F0、Fm、ΦP、Fv/F0、DIo/CSO、RC/CSO、TRo/CSO、ETo/CSO、PIABS、Φo、ΦEo and ΦDo under the stress of *C.cassiicola*

4. DISCUSSION

Plant disease resistance inducers enhance the ability of preventing itself from diseases and stresses by activating the plant's immune system and regulating plant metabolism[10]. Plant disease resistance inducer itself does not have a direct killing effect on the pathogenic bacteria that is different from the previous chemical pesticides[11]. But after spraying on the plant, which will cause the physiological and biochemical defense response by plants to prevent the infestation of foreign pathogens, to achieve the purpose of disease control[12]. Meanwhile, there are many characteristics of plant disease resistance inducers, such as preventive, systemic disease resistance[13], safe, and boost production[14].

Plant disease damage can directly affect photosynthesis, chlorophyll plays a decisive role in plant photosynthesis, while chlorophyll fluorescence is an important parameter of function and efficiency of the photosystem during the light reaction, and its changes can sensitively reflect the effect of adversity on plants[15], therefore, different chlorophyll fluorescence parameters can diagnose the plant's physiological states and environment stress[16]. For example, F0 reflects the degree of damage to the thylakoid membrane. Fm reflects the electron transfer through PSII. Fv/F0 shows the potential activity

of PSII, reflecting the size of the activity of PSII centers. φP shows the light energy conversion efficiency of PSII centers.

When seedlings were treated with JDS alone there were no significant changes in Fm, φP, and φDo, indicating that there was no significant change in the energy conversion efficiency of the PSII central reaction; F0 decreased while PI_{ABS} increased, indicating that the damage of photosynthetic structure was weakened. The increase of Fv/F0 shows that the potential activity of PSII has increased. The parameters ETo/CSO, RC/CSO and TRo/CSO, reflecting the activity of PSII reaction centers per unit leaf area, increased while DIo/CSO decreased, reflecting an increase in photosynthetic reaction activity. The rise in φo and φEo indicated that the electron transfer efficiency of PSII was promoted, which reduced the rate of the QA electron acceptor being turned off and facilitated the downstream transfer of photosynthetic electrons.

Only under the *C.cassiicola* stresses, Fm and φP decreased while φDo increased significantly, which reduce the energy conversion efficacy of PSII central reactive. TRo/CSO、RC/CSO、ETo/CSO decreased while DIo/CSO increased sharply, indicating the reduction of photosynthetic reaction activity. F0 increased while PI_{ABS} decreased, indicating that the photosynthetic structure was more severely damaged than the control group. The decrease in Fv/F0 indicates a decrease in PSII potential activity. φo and φEo increased significantly in the early stage of stress and started to decrease in the later stage. This trend shows that the seedlings reduces the rate of QA electron acceptor being turned off by increasing the electron transfer efficiency of PSII at the early stage of inoculation and promoting the downstream transfer of photosynthetic electrons, so as to weaken photosynthetic capacity reduction caused by this injury However, with the lengthen of proliferation and diffusion time of the pathogen, the effect is no longer significant, but rather the electron transfer efficiency of PSII is affected and begins to decrease.

Under the stress of *C.cassiicola*, φP significantly increased and φDo significantly decreased in leaves of cucumber seedlings treated with JDS, indicating that the energy conversion efficiency of the PSII central response was increased. The decrease in F0 and the increase in PIABS indicate that the degree of photosynthetic structural damage is diminished. The increase in Fv/F0 indicates that the potential activity of PSII is effectively increased; The parameters ETo/CSO and RC/CSO, reflecting the activity of PSII reaction center per unit leaf area, did not change significantly in the early stage but increased in the later stage, while DIo/CSO decreased significantly, indicating that the photosynthetic reaction activity did not change significantly in the early stage but increased significantly in the later stage. φo and φEo did not change much or even decreased to some extent in the early stage

but increased significantly in the later stage. This trend indicates that the effect of JDS was not significant under *C. cassiicola* stress compared to the negative feedback phenomenon of the organism itself. However, with the prolongation of the disease time, the effect of the organism's autoimmune mechanism weakening. At this time, the effect of the JDS really show that promoting the electron transfer efficiency of PSII.

5. Conclusion

Under the stress of *C. cassiicola*, plant immune resistant inducer JDS can effectively increase the capacity of PSII and the efficiency of PSII operation under the light in cucumber seedlings, reduce the damage of photosynthetic structure, increase the ability of PSII to accept and transfer electrons, as well as reduce the degradation of chlorophyll and the dissipation of light energy under C. cassiicola stress. In addition, it was found through this experiment that the induced resistance of JDS to TLS tended to weaken with the extension of the induction time, which is the same as Sequeira[17] and Liu et al[18] who showed that the induced resistance of plants has the disadvantage of timeliness. However, the previous study[19] indicated that the inducers may have an effect on the activity of defense-related enzymes and the expression of disease-resistance-related protein genes in cucumber after induction. So it is necessary to conduct experiments for the above possibilities in the next step to further elucidate the resistance-inducing mechanism of JDS from more perspectives.

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