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RESEARCH ARTICLE

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Coordinated effects of root autotoxic substances and *Fusarium oxysporum* Schl. f. sp. *fragariae* on the growth and replant disease of strawberry

Abstract Effects of hydroxybenzoic acid, an important autotoxic substance in roots, on plant growth, photosynthesis and *Fusarium oxysporum* occurrence in succession cropping of strawberry were evaluated in this paper. It was found that plant growth was negatively regulated by hydroxybenzoic acid or inoculation with *F. oxysporum*. Compared with these single factor treatments, the combination of the hydroxybenzoic acid treatment and *F. oxysporum* inoculation caused more severe inhibition in plant growth, greatly enhanced the occurrence of disease symptoms, and significantly decreased the chlorophyll content, net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration. In the meantime, the chlorophyll fluorescence parameters in strawberry were also significantly affected. After the application of hydroxybenzoic acid, the original chlorophyll fluorescence rapidly increased, resulting in a combined corresponding decrease in the maximum chlorophyll fluorescence and the chlorophyll fluorescence transformation efficiency. The effects of hydroxybenzoic acid treatment on the above chlorophyll fluorescence parameters from inoculation were delayed. Similarly, the coordination of hydroxybenzoic acid and *F. oxysporum* showed an elevated negative effect on the degree of inhibition of leaf photosynthesis more than the single factor treatments.

Keywords strawberry, autotoxic substance, *Fusarium oxysporum*, hydroxybenzoic acid, replant disease

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1 Introduction

Continuous cropping is currently widely practiced in strawberry production in China, owing to the limitations of arable fields and protective production frequently adopted in strawberry cultivation (Hoestra, 1988). However, this cropping system, which may result in worse plant growth and development, deterioration of root diseases, and low yield and quality, usually produces continuous cropping obstacles or replant disease (Fehrmann, 1988; Harris, 1990; Harris, 1991). Previously, we found that plants in the continuous cropping system showed a decrease in disease resistance, and a severe occurrence of soil-borne diseases, especially due to *Fusarium* wilt, was also observed. In the mean time, plant roots were found to be senesced earlier than usual. Other studies also noted that extrudes and putrid substances from plants were poisonous. More autotoxic substances accumulated in the soil, and harm to the strawberry became more severe in the continuous cropping system. One of the autotoxic substances with strong effects on strawberry is 4-hydroxybenzoic acid (Zhen et al., 2004c). In this paper, the effects of 4-hydroxybenzoic acid on plant growth, photosynthesis and *F. oxysporum* occurrence in the continuous cropping system of strawberry were evaluated, with their roots inoculated with *F. oxysporum*. The purpose of this study was to understand the disorder mechanism in the continuous cropping system of strawberry production.

2 Materials and methods

2.1 Materials

The disease strains of *Fusarium oxysporum* Schl. f. sp. *fragariae* (hereinafter the "FO") were isolated from infected strawberry roots. The virus-free seedlings of cultivar Toyonoka were grown in pots filled with 1.5 L sterilized

growing medium in each pot and with 2 plants per pot. 4-Hydroxybenzoic acid (hereinafter the "PA") was purchased from SCRC National Medicine Chemical Reagent Ltd..

2.2 Methods

2.2.1 Experimental treatment

Seedlings with 3–4 leaves were selected and assigned to four different treatments. Each treatment had three replicates, with eight pots per replicate. These treatments were as follows:

Treatment A (0.3 mmol·L^{-1} PA treatment): Plants were irrigated twice with as much as 0.3 mmol·L^{-1} PA per day and the irrigation amount was 250 mL.

Treatment B (0.3 mmol·L^{-1} PA + FO treatment): During the first 6 days, plants were irrigated with 0.3 mmol·L^{-1} PA with the same method as in Treatment A, after which they were inoculated with FO by irrigating them with 100 mmol·L^{-1} solution containing 1.0×10^6 FO spores per milliliter.

Treatment C (FO treatment): Plants were irrigated with tap water, with the amount and frequency the same as those in Treatment A. The plants were then inoculated with FO following the method in Treatment B.

Treatment D (CK): Plants were normally cultivated without application of PA or FO.

2.2.2 Measurements of traits

Growth performance of strawberry plants including root number, root length, dry weight of root and stem, diameter of stem and leaf area were measured using the method of Zhen et al. (2004b) on the 50th day after inoculation.

Photosynthetic parameters and the chlorophyll content were assayed by spectrophotometric methods (Cui et al., 2006). The full-expanded third leaf was selected to be the representative sample for measurement of the following parameters: net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci). The equipment used for measuring the above parameters was a photosynthesis analytic system (Ciras-1, PPsystem, UK). The original fluorescence (Fo), maximum fluorescence (Fm), maximal quantum efficiency of PSII (Fv/Fm) and ΦPSII of the third leaf were assayed using a portable fluorescence analyzer in combination with the photosynthesis analytic system (Liu et al., 2006).

F. oxysporum occurrence after inoculation in plants was evaluated every 10 d, using 5 levels which were classified based on the degree of disease occurrence (Zhen et al., 2004b). The disease index (DI) was calculated with the following formula:

$$DI = \sum(Xi \times Si)/(\sum Xi \times S\max) \times 100,$$

where Xi stands for root numbers with the corresponding disease index, Si means the disease index, and Smax stands for the highest disease index in the diseased roots.

2.2.3 Data statistics and analysis

The significance levels in the assayed traits and parameters of the experimental treatments were calculated with statistical software DPS (Data processing system).

3 Results

3.1 Growth performance of strawberry plants regulated by 4-Hydroxybenzoic acid and *F. oxysporum*

The growth performance of strawberry plants when treated with PA and FO independently, as well as with PA plus FO (0.3 mmol·L^{-1} PA + FO), are listed in Table 1. After 50 days of FO inoculation, the root number, root dry weight and leaf area per plant in 0.3 mmol·L^{-1} PA + FO treatment were all significantly lower than those in the FO treatment. The dry weight of stem and leaf in plant and root length in 0.3 mmol·L^{-1} PA + FO treatment were also decreased compared to FO, but the differences in traits were not significant between the two treatments. The stem and leaf dry weight and leaf area per plant in 0.3 mmol·L^{-1} PA + FO treatment were also significantly lower than those in 0.3 mmol·L^{-1} PA treatment.

3.2 The photosynthetic parameters regulated by 4-Hydroxybenzoic acid and *F. oxysporum*

3.2.1 Chlorophyll content under different treatments

The chlorophyll content of strawberry plants in various treatments is shown in Fig. 1. On the 30th day after FO

Table 1 Effects of PA and *F. oxysporum* on the growth of strawberry

treatment	root length/cm	root number per plant	root dry weight/g	stem and leaves dry weight/g	stem diameter/cm	leaf area per plant/cm^2
FO	13.3 c	22.1 c	1.471 c	1.743 ab	1.01 ab	64.5 c
0.3 mmol·L^{-1} PA	14.5 b	25.3 b	2.175 b	1.804 a	1.04 b	78.1 b
0.3 mmol·L^{-1} PA + FO	12.4 c	18.8 d	1.225 d	1.377 b	0.91 b	56.9 d
CK	16.1 a	28.2 a	2.520 a	1.998 a	1.11 a	99.7 a

Note: Each datum in the table was an average of three replicates in a treatment; Different letters in the same column stand for significant difference at 0.05 level.

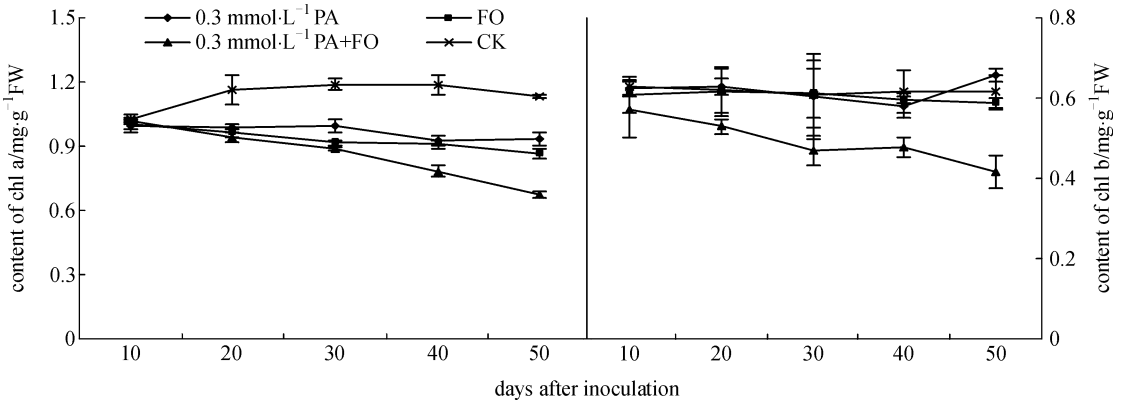

Fig. 1 Effect of PA and *F. oxysporum* on the contents of chlorophyll in strawberry leaves

spore inoculation, the chlorophyll content in the 0.3 mmol·L^{-1} PA + FO treatment dramatically decreased compared to that in 0.3 mmol·L^{-1} PA treatment and FO treatment; and on the 50th day after inoculation, the chlorophyll content in the 0.3 mmol·L^{-1} PA + FO treatment decreased by 0.26 mg·g^{-1} FW and 0.19 mg·g^{-1} FW compared to that in 0.3 mmol·L^{-1} PA treatment and FO treatment, respectively. The differences in chlorophyll content between the treatments with 0.3 mmol·L^{-1} PA + FO and 0.3 mmol·L^{-1} PA, as well as FO, were all significant at 5% statistical level.

3.2.2 Net photosynthetic rate and transpiration rate under different treatments

The diurnal variations of the net photosynthetic rate (Pn) and transpiration rate (Tr) in leaves of strawberry plants under different treatments are shown in Fig. 2 and Fig. 3. The above parameters all show a pattern of increasing from 9:00 to 11:00, with the highest values appearing at 11:00. However, the 0.3 mmol·L^{-1} PA + FO treatment increased more slowly than the 0.3 mmol·L^{-1} PA treatment and FO treatment. During the whole day, the Pn of the 0.3 mmol·L^{-1} PA + FO treatment was obviously lower than that of the other treatments from 10:00 to 12:00. The Tr of the 0.3 mmol·L^{-1} PA + FO treatment was significantly lower ($P = 0.05$) than that of other two treatments at all assayed time points of the whole day.

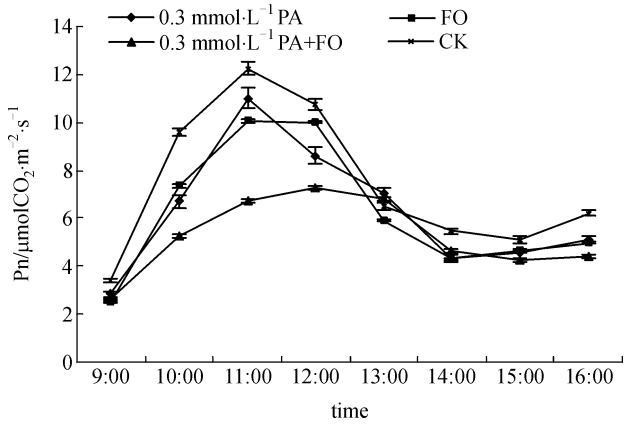

Fig. 2 Effect of PA and *F. oxysporum* on Pn of strawberry leaves

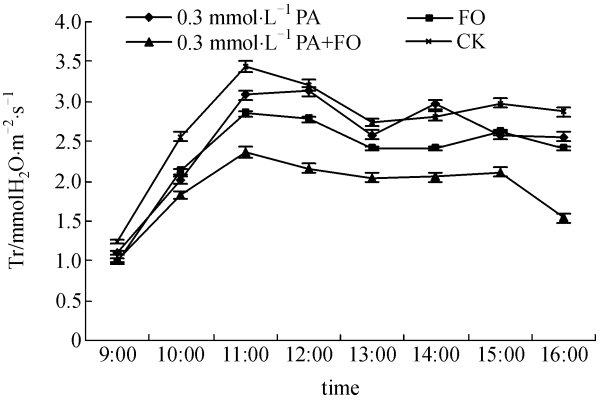

Fig. 3 Effect of PA and *F. oxysporum* on Tr of strawberry leaves

3.2.3 Stomatal conductance and intercellular CO_2 concentration under different treatments

The diurnal variations of stomatal conductance (Gs) in different treatments are shown in Fig. 4. Similar to Pn, the values of Gs at all the assayed time points during one whole day in the 0.3 mmol·L^{-1} PA + FO treatment were all lower than those in the 0.3 mmol·L^{-1} PA treatment and FO treatment, and those of treatments 0.3 mmol·L^{-1} PA and FO were lower than the Gs values of CK. For the PA treatment, the values of Gs decreased more significantly than those of CK at the assayed time points in one whole day except at 9:00, 10:00, and 15:00. The diurnal variations of intercellular CO_2 concentration (Ci) in different treatments are shown in Fig. 5. Obviously, the values of Ci in treatment 0.3 mmol·L^{-1} PA + FO, 0.3 mmol·L^{-1} PA, and FO were all higher than those of CK from 10:00 to 15:00. Among the three treatments, the values of Ci in one whole day were highest in the

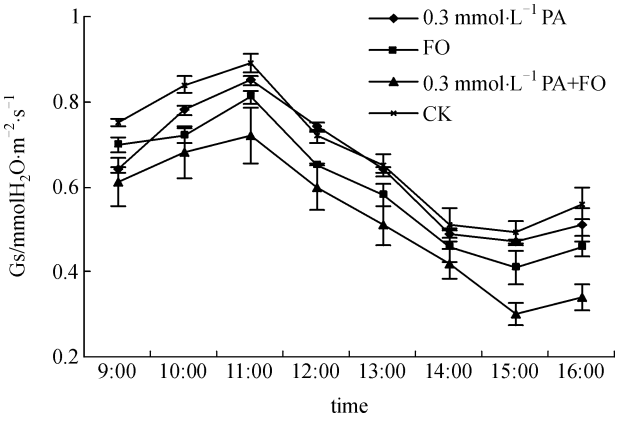

Fig. 4 Effect of PA and *F. oxysporum* on Gs of strawberry leaves

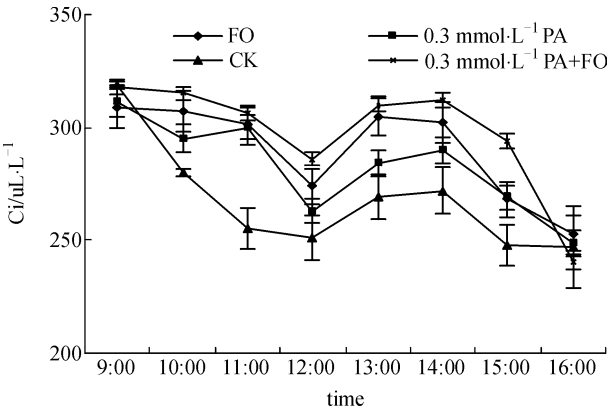

Fig. 5 Effect of PA and *F. oxysporum* on Ci of strawberry leaves

0.3 mmol·L^{-1} PA + FO treatment, followed by the FO treatment and the 0.3 mmol·L^{-1} PA treatment (the lowest). The differences in Ci between treatments 0.3 mmol·L^{-1} PA + FO and FO at the assayed time points in one whole day were significant.

3.2.4 Chlorophyll fluorescence parameters under different treatments

The chlorophyll fluorescence parameters in different treatments are shown in Fig. 6 (a)–(d). Except for CK, the Fo values were all enhanced with the progress of the treatments, with the highest percentage increase seen in the treatment 0.3 mmol·L^{-1} PA + FO. Twenty days after FO spore inoculation, Fo was significantly higher in the 0.3 mmol·L^{-1} PA + FO treatment than in the 0.3 mmol·L^{-1} PA treatment and the FO treatment. The Fm values remained unchangeable in CK, and gradually dropped in other treatments, with the highest percentage decrease in the 0.3 mmol·L^{-1} PA + FO treatment. Further, 25 days after the inoculation, the Fv/Fm ratio in the 0.3 mmol·L^{-1} PA + FO treatment was obviously decreased compared to treatment 0.3 mmol·L^{-1} PA and FO. On the 30th day after FO spore inoculation, the Fv/Fm ratios decreased by 27.2%, 17.1%

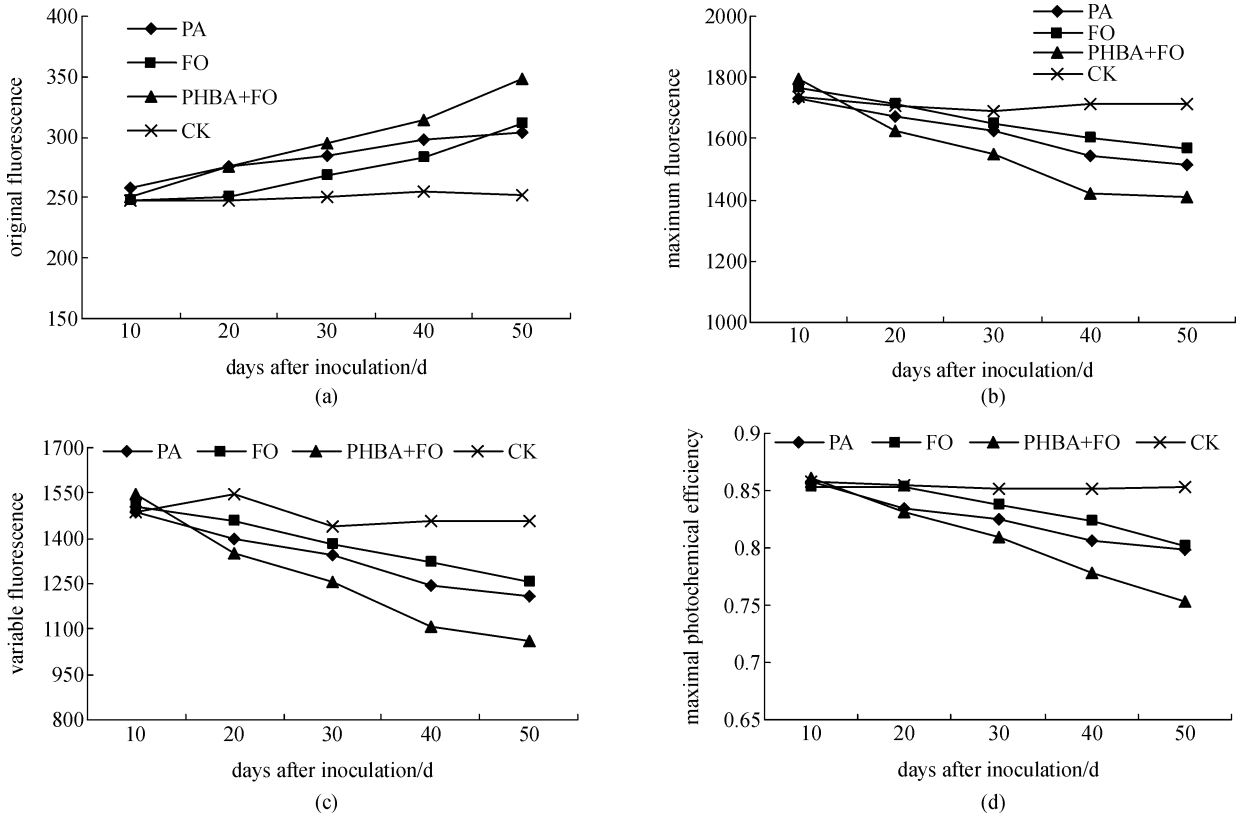

Fig. 6 Effects of 4-Hydroxybenzoic acid and *F. oxysporum* on characteristics of chlorophyll fluorescence of strawberry leaves

and 16.2% in treatments 0.3 mmol·L^{-1} PA + FO, 0.3 mmol·L^{-1} PA, and FO, respectively, compared to CK. In one whole day, ΦPSII showed a similar pattern with Fm. The 0.3 mmol·L^{-1} PA + FO treatment had the lowest ΦPSII values among the treatments, showing significant differences with those in the 0.3 mmol·L^{-1} PA and FO treatments 20 days after FO spore inoculation.

3.3 Disease occurrence regulated by 4-Hydroxybenzoic acid and *F. oxysporum*

The disease occurrences under different treatments are shown in Fig. 7. The disease symptoms were found some days after FO spore inoculation in treatments 0.3 mmol·L^{-1} PA + FO and FO. Up to 40 days after inoculation, the disease index in treatment 0.3 mmol·L^{-1} PA + FO rapidly elevated, and reached 39.3 on the 50th day after inoculation. Meanwhile, the FO treatment had a slower increase in disease index than the former treatment, with the disease index being only 15.0 at the 50th day after inoculation. The difference in disease indexes between the two treatments was found to be statistically significant (P = 0.05).

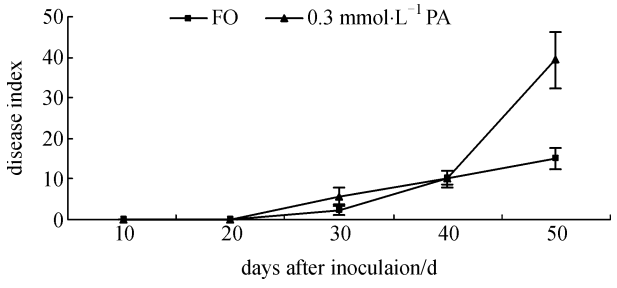

Fig. 7 Effect of 4-hydroxybenzoic acid and *F. oxysporum* on the disease index of strawberry roots

4 Conclusions

4.1 Strawberry growth was inhibited and disease occurrence was aggravated by the coordinated action of autotoxic substance 4-Hydroxybenzoic acid and *F. oxysporum*

A lot of reports have noted that continuous cropping could release a series of autotoxic substances into the soil through root exudation and decomposition of the crop residue. *F. oxysporum*, a soil-borne disease pathogen, frequently induces wilt disease in crops, and the disease could be aggravated with continuous cropping. Meanwhile, the *F. oxysporum* pathogen can remain alive in the soil for several years and its germination and growth could be promoted by some specific amino acids and oligosaccharides exuded from the plant roots (Rice, 1984; Yu and Matsui, 1994; Yu and Matsui, 1997; Gao and Zhang, 1998). In our study, it was found that the growth performances of strawberry plants were reversely regulated by autotoxic substance PA and inoculation of FO. There existed coordinated negative effects due to PA and FO after interaction. In our previous study, we had noted that the root physiological functions and cellular protection enzyme activities of the strawberry seedlings were inhibited by PA, which was released from the roots, resulting in the decrease of disease resistance in plants (Zhen et al., 2004a). Therefore, the negative coordinated effects of PA and FO may constitute one of the important reasons for strawberry replant disease. Whether either the type or number of the extruded amino acids and oligosaccharides is affected by autotoxic substances of strawberry roots needs further future studies.

4.2 Photosynthesis in strawberry was inhibited by the coordinated action of autotoxic substance 4-hydroxybenzoic acid and *F. oxysporum*

As an action mode of the autotoxic substances in the continuous cropping system, photosynthetic inhibition was demonstrated in tomato and cucumber, in which the replant disease also frequently occurs. The inhibition effects of autotoxic substances were quickly shown when they acted on roots (Balke, 1985; Einhellig, 1986; Blum et al., 1999; Jia and Zhang, 1999). One report noted that photo inhibition after *F. oxysporum* inoculation was done did not result from the disease, but the decrease in photosynthesis of plants was mainly due to the reduced water acquisition in roots and further lowering of enzyme activities in the Calvin cycle after *F. oxysporum* inoculation. The disease symptoms in roots could also be identified some time later (Yu et al., 2003). In our present study, compared to single factor PA or FO, there exists a coordinated effect of PA and FO on chlorophyll content, net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration in the double-factor treatment with PA and FO (0.3 mmol·L^{-1} PA + FO). The values of the above parameters were obviously (or significantly) decreased on treatment with 0.3 mmol·L^{-1} PA + FO compared to the other single-factor treatments. Similarly, PA treatment caused a dramatic elevation in origin fluorescence (Fo) and a drop in maximum fluorescence (Fm), maximum fluorescence transforming efficiency and quantum efficiency of electron transfer in photosynthesis. It was clear that Fo was the fluorescence yield when photo reaction system II was fully opened. The increase in Fo indicated that the PSII reaction center was being destroyed or reached by the reversible time point. On the other hand, the Fv/Fm ratio is commonly used as the criteria to evaluate the potential activity and original photo-transformation efficiency in PS II. The ΦPS II is an index to relatively evaluate the transfer speed of photo-electrons in leaves (Poeles, 1984; Gagues et al., 2002; Xu et al., 2005). Our study indicated

that the PSII activity could also be further negatively regulated by the coordination and interaction of PA and FO.

The inhibition on photosynthesis from the coordinated interaction of PA and FO was possibly due to the limitation in shortage of photosynthetic substrates, resulting from the destruction of the vascular bundle after infection of FO in plants. Therefore, blockage of the xylem after disease infection may create an obstacle in the transportation of water and inorganic nutrients in roots, which finally induces the negative effects regulated by the adverse factors, PA and FO.

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