INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20F–035/2020/24–3–531–537
DOI: 10.17957/IJAB/15.1469
http://www.fspublishers.org

Full Length Article

Photosynthetic Rate, Chlorophyll Fluorescence in Anthracnose Infected Tea Plants

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Received 24 February 2020; Accepted 31 March 2020; Published 11 July 2020

Abstract

Anthracnose is one of the most common diseases that threaten tea plants (*Camellia sinensis*) by negatively affecting the tea quality and yield. Photosynthesis related to tea plants growth is very important in disease defense management. Few reports exist on the effect of anthracnose disease on the photosynthetic functions of tea plant. The susceptible and resistant tea plants Longjing 43 and Yingshuang were chosen, and a pot experiment was carried out. Healthy (CK), slight (D1), moderate (D2) and serious (D3) anthracnose infected treatments with three replicates were set up. Percent disease index (PDI), leaf photosynthesis, chlorophyll fluorescence and pigment contents were measured. The results showed that the PDIs in anthracnose infected treatments were higher than those in healthy plants and reached the highest value of 43.69 and 13.57 in D3 of Longjing 43 and Yingshuang plants. Compared with the healthy plants, the total of chlorophyll *a* and chlorophyll *b* content reduced 32.3 and 39.6% in anthracnose infected Longjing 43 and Yingshuang. In further measurements, the photosynthetic rate in anthracnose infected Longjing 43 and Yingshuang decreased 73.8 and 53.8% in comparison of healthy plants. OJIP curves and parameters F_m, F_v/F_m, Φ_{PSII} images could differentiate CK, D1, D2 and D3 anthracnose infected plants in the experiment. These suggested that anthracnose disease may inhibit the PSII photochemical activity across the two tea plants after they were infected by anthracnose. The findings might contribute to the knowledge of the disease stress effect on photosynthesis in tea plants and the valuable application to monitor the disease. © 2020 Friends Science Publishers

Keywords: Percent disease index;·OJIP curves;·PSII photochemical activity; Pigment content

Introduction

Anthracnose, caused by *Colletotrichumtheae sinensis*, is one of the most severe diseases that can afflict many crops such as pepper, grape, and tea, etc. (Liu *et al.* 2015; Wang *et al.* 2016; Waskow *et al.* 2016, Mishra *et al.* 2017). The disease could attack any parts of a plant at any growth stage. It causes leaf spots, blotches, defoliation, twig cankers and dieback on many different plants. The disease can decrease the plant's vigor, weakening the plant growth and decreasing the quality and yield of the host crop (Chowdhury and Rahim 2009; Ali *et al.* 2014; Cota *et al.* 2017).

Photosynthesis plays an important role in plant disease defense responses (Dong *et al.* 2016). In general, disease has a strong negative influence on plant photosynthesis (Prokopová *et al.* 2010; Zhao *et al.* 2011; Hu *et al.* 2018), while resistance to disease has a positive influence on plant photosynthesis. The highly disease-resistant cultivar usually exhibits a high rate of photosynthesis and may obtain high yields in regions of widespread disease. In, Hu *et al.* 2018 found that bacterial leaf blight infection had a higher maximal photosynthetic rate and dark respiration rate in resistant rice than in susceptible one. Debona *et al.* (2014) also reported that, for two wheat cultivars infected with *Pyricularia oryzae*, the susceptible cultivar (BR 18) exhibited a drastically reduced net photosynthesis rate compared with the partially resistant cultivar.

Chlorophyll (*Chl*) fluorescence, which is released in plant photosynthesis, is a rapid, non-destructive detection method for abiotic and biotic stress in plants. In 2002, Bassanezi *et al.* found that the minimal fluorescence (F_0) was remarkably reduced in bean leaves after infection by an angular leaf spot. Zhori *et al.* (2015) also reported that the rapid fluorescence kinetics was used *in situ* in the field to

To cite this paper: Hu H, MJ Tang, HW Guo, GZ Zhang, Q Gu, YW Yuan, L Sheng, HK Zhou, Z Liu (2020). Photosynthetic rate, chlorophyll fluorescence in anthracnose infected tea plants. *Intl J Agric Biol* 24:531–537

monitor the healthy and *Uromyces*-infected plants of *Euphorbia cyparissias*. It proved to be useful for differentiating between the infected and healthy plants and may be used in the investigation on the disease severity.

Tea plants have been widely cultivated in most areas and tea is considered an important beverage around the world (Paiano *et al.* 2014). The fungicide and pesticide residues greatly affect the quality of raw tea products and may pose many risks to consumer's health (Jaggi *et al.* 2001; Gupta *et al.* 2008). Photosynthesis related to tea plant growth plays an important role in disease defense management. There is a close relationship between plant photosynthesis and leaf chlorophyll status. The disease may affect the photosynthesis by destroying the chlorophyll. Limited information has been reported on the effect of anthracnose disease on the photosynthetic characteristics and the related chlorophyll changes of tea plants. Monitoring of tea anthracnose incidence based on photosynthesis using *Chl* fluorescence has also seldom been reported. It is known that planting resistant varieties is an efficient, reliable, and cheap way to manage disease (Silva *et al.* 2018); however, the effects of the disease resistance on tea plant photosynthesis have not been reported.

This study aims to research the leaf photosynthetic rate and the *Chl* fluorescence parameters which may be involved in these changes after the tea plant was infected by anthracnose. It was hypothesized that photosynthetic rate is damaged by anthracnose infection and the damage may differ between resistant and susceptible cultivars. Moreover, the reduction in photosynthesis may be related to stomata closure and decreased pigment. To evaluate these hypotheses, anthracnose disease severity, photosynthetic traits, and feasibility of some *Chl* fluorescence parameters which may be used to monitor the anthracnose disease was discussed in two tea cultivars with variable resistance to anthracnose.

Materials and Methods

Plant materials

Glasshouse experiments were conducted at the Zhejiang Academy of Agricultural Sciences (ZAAS), Zhejiang province, P.R. China in the 2017 growing season. Two-year seedlings of anthracnose susceptible and resistant tea plants Longjing 43 and Yingshuang were planted in 30 L polyvinyl chloride pots (one plant each pot) in the glasshouse. The physical and chemical properties of the cultivated soil were N content 0.835 $g.kg^{-1}$, pH 6.21, organic matter content 13.63 $g.kg^{-1}$, available phosphorus and potassium 25.28 and 49.66 $g.kg^{-1}$.

Experimental design and inoculation

The experiment consisted of four treatments that are healthy (CK), slight (D1), moderate (D2) and serious (D3) disease grade. Each treatment had seven tea plants. The trial was set up as a completely randomized block arrangement with three replications. Anthracnose inoculations were performed manually. The youngest fully expanded leaves (the second or third leaf from the top) were selected and inoculated by a hypodermic syringe needle method on June 15, 2017, and CK was inoculated in deionized water. The anthracnose infected treatments D1, D2, D3 were created and inoculated in 10^5, 10^6 and 10^7 conidia/mL anthracnose suspension. Inoculated and control plants were seal-covered with polyvinyl chloride and maintained at 25°C and 90% relative air humidity with a 12-h photoperiod for 5 days. Disease incidence, leaf photosynthesis rate and *Chl* fluorescence were measured at 30 days after inoculation.

Percent disease index

Three plants of each variety were selected to assess disease incidence on the leaves. According to visual observations, the disease severity degree was measured by a 5 grade scale based on the percent area of leaf affected (0, 1–10%, 11–25%, 26–50%, 51–75% and above 75%). The percent disease index (PDI) was calculated by the method of Sridhar and Sohi (1970).

$$\mathrm{PDI} = \frac{S}{T \times M} \times 100$$

where S represents the sum of numerical leaves, T represents the total number of leaves, and M represents the maximum disease scale.

Leaf Gas exchange measurement

The net photosynthesis rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*Gs*) and intercellular carbon dioxide concentration (*Ci*) in leaves were measured and recorded on fully developed clean leaf using a portable photosynthesis system (Ciras-II, PP Systems, UK). The photosynthetic chamber with irradiance levels of 1000 μmol m^{-2} s^{-1} provided a leaf area of 1.7 cm^2, relative air humidity of 70%, and a CO_2 concentration of 400 μmol mol^{-1}.

Chlorophyll fluorescence transient measurements

Transient *Chl* fluorescence (OJIP) was measured using Multi-Function Plant Efficiency Analyser (Hansatech, UK) after the leaves adapted to the dark for about 30 min. A fluorescence intensity recorded at 20 μs, 2 ms, 30 ms and the maximum represented the *O*, *J*, *I* and *P* point. The biophysical parameters, such as absorption, trapping, electron transport, and dissipation per active reaction center (*ABS/RC*, TR_0/RC, ET_0/RC, DI_0/RC), were calculated from the obtained data.

Chlorophyll fluorescence imaging system

The tea plant leaves were measured by the *Chl* fluorometer imaging system (Open FluorCam, Photon Systems

Instruments, Brno, Czech Republic) after they adapted to the dark. In this system, a charge-coupled device camera was used, which produces a pseudo-color 512×512 pixel fluorescence images. The imaging fluorometer was controlled and the images were analyzed by the FluorCam software (Photon Systems Instruments, Brno, Czech Republic). The minimum fluorescence (F_0), maximal fluorescence (F_m), the maximum quantum efficiency of PSII (F_v/F_m), the effective photochemical quantum yield of PSII (Φ_{PSII}), and non-photochemical quenching (NPQ) were auto measured and stored in the computer. The above fluorescence parameter images were shown randomly and the mean of all fluorescence values was given.

Pigment contents

The pigment content in the leaf was collected with 80% acetone. Absorption at 663, 645 and 470 nm was measured using an ultraviolet-visible (UV) spectrophotometer (Unico, UV-3802, China). *Chl a*, *Chl b* and carotenoid (*Car*) content were calculated as previously described (Arnon 1949). The unit of the photosynthesis pigment content was $mg.g^{-1}$ based on fresh mass.

Data analyses

Data analyses were performed by SPSS 17.0 (SPSS Inc., Chicago, USA). Differences in parameters within various treatments (CK, D1, D2, and D3) were assessed with one-way analysis of variance with multiple comparisons of means using Fisher's LSD test at the 0.05 level. All of the measurements were performed three times, and calculated standard errors (SE) were reported.

Results

Percent disease index

PDI of healthy and anthracnose infected plants of Longjing 43 and Yingshuang was shown in Table 1. In Longjing 43, PDI increased almost linearly during evaluation treatments, ranging on average from 8.55 in D1 to 43.69 in D3. Compared with Longjing 43, Yingshuang had smaller fleck symptoms with PDI 4.32, 8.18 and 13.57 in D low, D med and D sev anthracnose infected treatment. Across the two tea plants, significant differences in PDI were found among all the treatments and PDI increased significantly with the anthracnose development. Comparably, Yingshuang had lower PDI than Longjing 43 when they were infected by anthracnose disease.

Pigment contents

The pigment of Longjing 43 and Yingshuang following infection by anthracnose is shown in Table 2. Compared with the healthy plants (CK), the total of Chl *a* and Chl *b* content reduced 32.3 and 39.6% in anthracnose infected Longjing 43 and Yingshuang. Significant differences in *Chl a*, *Chl b* and the total of *Chl a and Chl b* in Longjing 43 and Yingshuang between CK and D1 or D2 treatments were found. There were no significant differences between D2 and D3 treatments for *Chl a* and *Chl a+Chl b* across the two tea plants. The statistically significant difference in *Car* between CK and the infected treatments in Longjing 43 was observed.

Table 1: Percent disease index (PDI) of CK, D1, D2 and D3 treatments in Longjing 43 and Yingshuang tea plants

Treatment	Longjing 43	Yingshuang
CK	0d	0d
D1	8.55c	4.32c
D2	21.33b	8.18b
D3	43.69a	13.57a

Note: CK, D1, D2 and D3 represent healthy, slight, moderate and serious anthracnose disease treatments. Mean values for various treatments followed by the same letter are not significantly different ($P<0.05$) according to the LSD test. CK was inoculated with deionized water. D1, D2 and D3 were inoculated with 10^5, 10^6 and 10^7 conidia/ml anthracnose suspension, the same as below

Table 2: Pigment contents of CK, D1, D2 and D3 treatments in Longjing 43 and Yingshuang tea plants

Cultivar	Treatment	*Chl a*	*Chl b*	*Car*	*Chl a+Chl b*
Longjing 43	CK	2.62a	0.87a	1.21a	3.50a
	D1	2.00b	0.64b	1.13b	2.64b
	D2	1.63c	0.51c	1.07c	2.13c
	D3	1.70c	0.64b	1.08c	2.34bc
Yinshuang	CK	2.65a	0.87a	1.16	3.52a
	D1	1.94b	0.46b	1.09	2.40b
	D2	1.58bc	0.41b	1.09	1.99b
	D3	1.48c	0.50b	1.03	1.98b

Note: *Chl a*, *Chl b* and Car represent chlorophyll a, chlorophyll b and carotenoid content

Leaf gas exchange

Fig. 1 shows the changes of *Pn*, *Tr*, *Gs* and *Ci* of CK, D1, D2 and D3 treatments in Longjing43 and Yingshuang plants. Compared with healthy plants (CK), *Pn*, *Gs* in the two anthracnose infected tea plants decreased significantly. *Pn* in anthracnose infected Longjing 43 and Yingshuang decreased 73.8 and 53.8% in comparison with healthy plants. On the whole, Longjing 43 had low *Pn* than Yingshuang. With the development of anthracnose infection, *Tr* in longjing43 deceased gradually. No significant changes for *Ci* in Longjing 43 and Yingshuang were found after infection with anthracnose disease.

Chlorophyll fluorescence transient

Fig. 2 (A, B) shows that the differences in transient *Chl* fluorescence existed between CK, D1, D2 and D3 treatments for Longjing 43 and Yingshuang tea plants. It revealed characteristic fluorescence transient curve shape (OJIP curve) with differences in healthy and anthracnose-infected tea plants. Collectively, there was lower *Chl* fluorescence in anthracnose-infected treatments (D1, D2 and D3) compared to CK across the two tea plants.

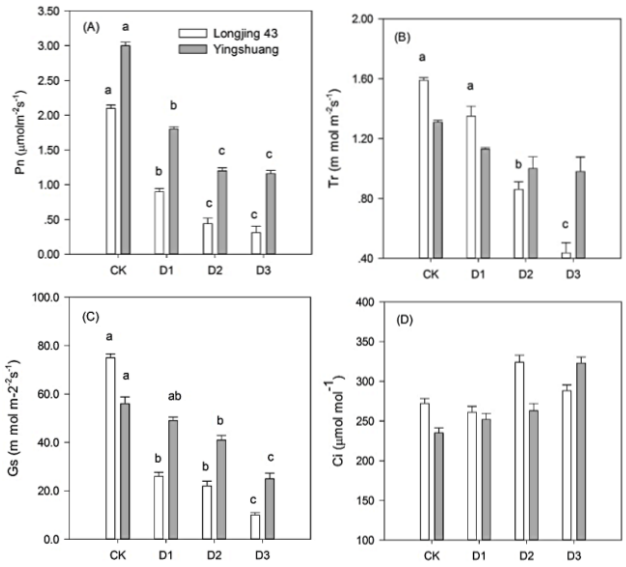

Fig. 1: Changes of net photosynthesis rate (*Pn*). (A): transpiration rate (*Tr*); (B): stomatal conductance (*Gs*); (C): intercellular carbon dioxide concentration (*Ci*); (D) CK, D1, D2 and D3 treatments in Longjing 43 and Yingshuang tea plants

Note: CK, D1, D2 and D3 represent healthy, slight, moderate and serious anthracnose disease treatments, respectively. Mean values for various treatments followed by the same letter are not significantly different ($P< 0.05$) according to the LSD test. CK was inoculated with deionized water. D1, D2 and D3 were inoculated with 10^5, 10^6 and 10^7 conidia/ml anthracnose suspension. The means and calculated standard errors are reported, the same as below

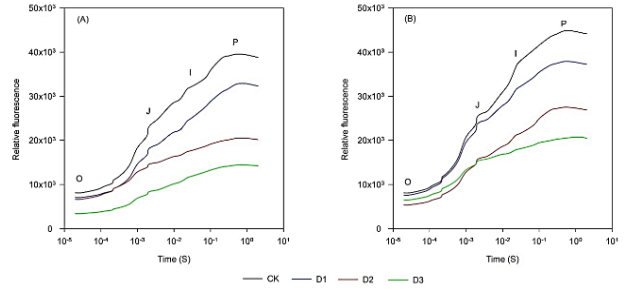

Fig. 2: Chlorophyll fluorescence OJIP transient curves of CK, D1, D2 and D3 treatments in Longjing 43 (A) and Yingshuang (B) tea plants

Among the treatments, small differences in *O* point and great differences in *P* point were observed. The differences in *J*, *I*, *P* points in the OIJP curves increased gradually from CK to D3 treatments.

Fig. 3 (A, B) shows the parameters indicating the absorption (*ABS/RC*), trapping (*TRo/RC*), electron transport (*ETo/RC*) and dissipation (*DIo/RC*) per reaction center of PSII calculated from the fluorescence transient curve in CK, D1, D2, and D3 treatments in Longjing 43 and Yingshuang plants. Collectively, *ABS/RC*, *DIo/RC*, *ETo/RC*, *TRo/RC* increased after infection by anthracnose across the two tea plants. Comparably, the differences in the above parameters between CK and D1 were smaller than CK with D2 and D3 treatments.

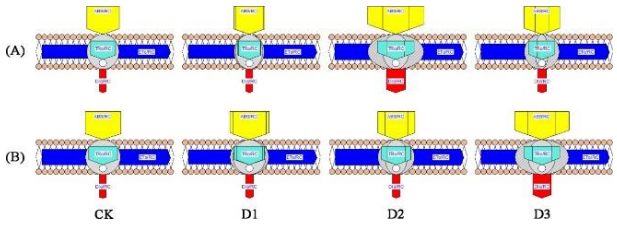

Fig. 3: Energy pipeline models of specific fluxes per reaction center (RC) of CK, D1, D2 and D3 treatments in Longjing 43 (A) and Yingshuang (B) tea plants. ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC indicate absorption, trapping, electron transport and dissipation per active RC

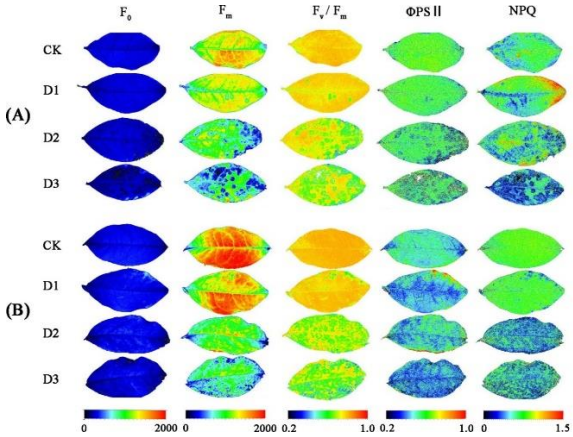

Fig. 4: Changes in chlorophyll fluorescence parameters F_0, F_m, F_v/F_m, Φ_{PSII} and *NPQ* images of CK, D1, D2 and D3 treatments in Longjing43 (A) and Yingshuang (B) tea plants. F_0, F_m, F_v/F_m, Φ_{PSII} and *NPQ* indicate the minimum fluorescence, maximal fluorescence, maximum quantum efficiency of PSII, effective photochemical quantum yield of PSII, and non-photochemical quenching

Chlorophyll *a* fluorescence image

Dark and light adapted *Chl* fluorescence parameters F_0, F_m, F_v/F_m, Φ_{PSII}, and *NPQ* of CK, D1, D2, D3 treatments in Longjing 43 and Yingshuang are shown in Fig. 4. The average values of the above parameters are shown in Table 3. Across the two tea plants, the reductions of F_m, F_v/F_m and Φ_{PSII} in infected treatments in comparison with CK were observed. F_0 in Longjing 43 decreased significantly after infection by anthracnose. No significant difference in F_0 between CK and the infected treatments in Yingshuang plants, while *NPQ* reached the maximum in D1, while the transition from D1 to D3 exhibited a decrease.

Discussion

To date, the study of tea plant disease has been concentrated on the chemical composition and the quality of tea affected by the disease and disease control (Gulati *et al.* 1999; Sanjay *et al.* 2008; Pallavi *et al.* 2012). The effects of

Table 3: Chlorophyll fluorescence parameters F_0, F_m, F_v/F_m, Φ_{PSII} and *NPQ* of CK, D1, D2 and D3 treatments in Longjing 43 and Yingshuang tea plants

Cultivar	Treatment	F_0	F_m	F_v/F_m	Φ_{PSII}	NPQ
Longjing 43	CK	274.2a	1281.8a	0.78a	0.60a	0.63b
	D1	265.63a	1171.8a	0.77a	0.57ab	0.72a
	D2	241.36b	865.7b	0.70b	0.51bc	0.61b
	D3	214.89c	703.8c	0.68b	0.48c	0.39c
Yingshuang	CK	296.4	1542.4a	0.81a	0.61a	0.56a
	D1	303.1	1391.9b	0.78ab	0.58a	0.57a
	D2	293.4	958.5c	0.68bc	0.43b	0.61a
	D3	253.8	750.6d	0.64c	0.45b	0.40b

Note: F_0, F_m, F_v/F_m, Φ_{PSII}, *NPQ* indicate the minimum fluorescence, maximal fluorescence, maximum quantum efficiency of PSII, effective photochemical quantum yield of PSII, and non-photochemical quenching

disease infection on photosynthetic characteristics in tea plants were closely related to the quality and quantity of tea production (Gulati *et al.* 1999). To our knowledge, this is the first time that the impact of anthracnose infection on tea leaf photosynthesis has been reported. Tea leaves were damaged gradually as the anthracnose disease developed. In the experiments presented here, anthracnose caused PDI values of 13.57 in Yingshuang and 43.69 in Longjing 43. This suggested that Yingshuang was highly resistant to anthracnose infection while Longjing 43 was susceptible.

Pigments are important parts of plant photosynthesis. Reduction of *Chl a*, *Chl b* and *Car* was observed after the two tea plants infected by anthracnose disease in the experiment. The result was similar to the report by Lobato *et al.* (2010). Scarpari *et al.* (2005) also found that *Chl a* and *Chl b* reduced in *Theobroma cacao* plants infected by the pathogen *Crinipellis perniciosa*. The significantly decreased amount of *Chl*s in anthracnose infected plants may be due to decreased leaf photosynthetic area promoting less light absorption, and chloroplast damages during disease infection (Radwan *et al.* 2008).

Limited photosynthesis was observed in two tea varieties after anthracnose infection, and *Chl*s and stomatal conductance may be considered to explain. Decreased Chls may cause the depressed photosynthesis rate as mentioned above due to the close relationship between photosynthesis and Chls. A lower *Gs* is one of the major constraints to photosynthesis in disease infected plants by limiting CO_2 influx into leaves (Erickson *et al.* 2003). *Gs* were greater in healthy plants and decreased with increased disease severity. The lower *Gs* in tea plants resulted in lower *Tr*. da Silva *et al.* (2018) also reported that a partial closure of the stomata results in the reduction of photosynthesis and the transpiration rate in eucalyptus plants after infection with *Ceratocystis fimbriata*. Interestingly, almost no great changes were found in *Ci* in the two tea plants with the development of anthracnose disease, suggesting that the reduction of *Chl*s was the main factor for the impaired photosynthesis in tea plants caused by the anthracnose infection.

The transient OJIP indicates the response of plants to environmental stress. Disease stress could change the special sites of the transient OJIP and reduce fluorescence intensity at the *J*, *I*, and *P* steps (Baker 2008). In our experiment, higher *Chl* fluorescence values in healthy plants than anthracnose infected ones were found in two tea plants. There were great differences in *P* point and the differences at the *J*, *I* and *P* point in the OIJP curves increased gradually with the development of anthracnose disease. The decreased *P* point caused by anthracnose in the OIJP test provided an explanation for the reduction of F_v/F_m in the *Chl* fluorescence images. Some parameter values in D1 treatments were similar or even lower than healthy ones. It may be related to the response of the plant itself to the disease.

Electron transport is the first stage of photosynthesis that produces chemical energy (Trebst 2003). It may mainly occur in PSII and some electron transport parameters such as *ABS*/*RC*, TR_0/RC, ET_0/RC and DI_0/RC indicating the activity of PSII reaction center were analyzed. In general, damage to PSII RCs under anthracnose disease was observed in this study. Almost all the *ABS/RC*, DI_0/RC, TR_0/RC, and ET_0/RC in infected treatments were higher than those in healthy plants. These results suggested that PSII RCs may be inactive and the efficiency per RC may be enhanced during the electron transport process. Compared with the other parameters, a sharp increase in DI_0/RC was observed. It indicated that most energies in *RC* dissipated in a form of heat due to self-protection when a plant was in disease conditions (Monneveux *et al.* 2003). Interestingly, the *ABS/RC*, DI_0/RC, TR_0/RC and ET_0/RC of Longjing 43 in D1 (slight disease) were lower than or even similar to those in healthy plants at the first stage of the infection treatment and reached the maximum in D2. While in Yingshuang, those parameters ET_0/RC increased and reached the maximum in D3, suggesting that Longjing 43 might be more susceptible infected from anthracnose disease than in Yingshuang. to maintain growth by inhibiting electron transport and decreasing PSII photochemical activity during the earlier stage of the disease stress.

The anthracnose infection induced decreased F_m, F_v/F_m, Φ_{PSII} across the resistant and sensitive tea plants. It suggested that these parameters have the potential to be used to differentiate and monitor the anthracnose disease in the application. On the parameter F_0, Bassanezi *et al.* (2002)

found that the value remarkably reduced in bean leaves after infection by an angular leaf spot. In our experiment, the decreased F_0 was observed in susceptible Longjing 43 after infection by anthracnose, but not in resistant Yingshuang. This result showed that anthracnose resistance of tea plants had a significant effect on F_0. , On F_v/F_m, Tung *et al.* (2013) reported that the parameters F_v/F_m would be appropriate for the detection of foliar plant infections. But some researchers found that F_v/F_m value did not change in *Eupatorium makinoi* infected by geminivirus (Funayama *et al*. 1997) but was significantly lower in *Nicotiana tabacum* (Ryšlavà *et al*. 2003), and in *Oncidium* (Chia and He 1999) after virus infection. On Φ_{PSII}, Brabandt *et al*. (2014) found that Φ_{PSII} proved to be appropriate for early and objective detection of susceptible butterhead lettuce eight days after inoculation under laboratory conditions. Whether the above parameters could be appropriate for objective detection of the other disease infection, need to be researched further.

Conclusion

Anthracnose decreased photosynthesis in leaves of susceptible and resistant tea plants mainly by the reduced *Chl*s. The electron transport chain and PSII photochemical activity were inhibited after tea plants were infected by anthracnose. The OJIP curves and the parameters F_m, F_v/F_m, and Φ_{PSII} might be used to differentiate and monitor the anthracnose disease. The result of this study might contribute to the knowledge of the disease's effect on photosynthesis in the leaves of tea plants and provide a valuable application for disease monitoring.

Acknowledgments

This research was supported by the Public Projects of Zhejiang Province, China (No. LGN18C140005) and the National Natural Science Foundation of China (No. 31501220, 41601024). Thanks are given to the Beijing EcoTech Company for providing the portable chlorophyll fluorometer (FluorCam).

Author Contributions

Hao Hu, Mei-Jun Tang and Yu-Wei Yuan conceived of the presented idea. Hua-Wei Guo, Guang-Zhi Zhang, Qing Gu, Li Sheng, Hong-Kui Zhou, Zhi Liu performed the experiment. Hao Hu wrote the original manuscript and all authors discussed the results and contributed to the final manuscript.

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