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Chlorophyll *a* fluorescence imaging of soya bean leaflets infected by *Corynespora cassiicola*

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Abstract

Considering the importance of target spot, caused by *Corynespora cassiicola*, to impact soya bean yield, this study investigated the *C. cassiicola*-induced perturbations in the photosynthesis of soya bean plants from cultivars Fundacep 59 (moderately resistant) and TMG 132 (susceptible) by examining the chlorophyll (Chl) *a* fluorescence parameters and imaging and the photosynthetic pigment pools. The area under disease progress curve decreased by 38% for plants from cultivar Fundacep 59 in comparison with plants from cultivar TMG 132. The concentration of total Chl_{a+b} significantly increased by 16% and the Chl_{a+b}/carotenoids ratio significantly decreased by 11% for plants from cultivar Fundacep 59 in comparison with plants from cultivar TMG 132. Significant decreases of 31% and 8%, respectively, for the concentration of Chl_{a+b} and the Chl_{a+b}/carotenoids ratio occurred for inoculated plants in comparison with noninoculated ones regardless of the cultivar. As target spot developed, the values of maximal photosystem II quantum yield (F_v/F_m), effective photosystem II quantum yield [Y(II)], quantum yield of regulated energy dissipation [Y(NPQ)] and quantum yield of nonregulated energy dissipation [Y(NO)] increased, especially for plants from cultivar TMG 132. There were positive correlations of area under disease progress curve (AUDPC) with both Y(NPQ) and Y(NO) regardless of the cultivar. For cultivar TMG 132, the correlation between AUDPC and the electron transport rate was negative. Based on the analysis of the Chl *a* fluorescence, it was possible to elucidate the enormous potential of target spot to impair the functionality on the photosynthetic apparatus that may help the plants to invest in metabolic pathways involved in host defence responses.

KEYWORDS

Corynespora cassiicola, foliar disease, necrotrophic fungus, photosynthesis, target spot

1 | INTRODUCTION

Target spot, caused by the necrotrophic fungus *Corynespora cassiicola* (Berk and MA Curtis) CT Wei., is one of the most serious threats to soya bean [*Glycine max* (L.) Merrill] production in Brazil (Godoy et al., 2012; Teramoto et al., 2013). The symptoms of target spot on soya bean leaflets are characterized as roughly circular to irregular necrotic lesions, which have alternating light and dark rings surrounded by a dull green or yellowish-green halo (Almeida et al., 2005; Sinclair, 1999). Severe target spot epidemics occur in regions with temperature ranging from 25 to 28°C and high relative humidity (Godoy et al., 2012). The absence of resistant cultivars and the report of isolates of *C. cassiicola* resistant to benzimidazole make target spot control very difficult to achieve (Teramoto et al., 2013; Xavier, Canteri, Barros, & Godoy, 2013).

As a necrotrophic pathogen, *C. cassiicola* relies on the action of nonhost selective toxins and hydrolytic enzymes to facilitate host

penetration and colonization causing remarkable decrease on the amount of photosynthetically leaf area as the lesions coalesce to form a zonate pattern (Barthe et al., 2007; Onesirosan, Mabuni et al., 1975). The main effects caused by pathogens infection on the photosynthetic apparatus of their hosts consist of reductions in gas exchange rates, impairment in energy dissipation via chlorophyll (Chl) *a* fluorescence, increases in foliar temperature, structural damage to the chloroplasts that contributes to reduced pigments concentration, disruption of carbon and nitrogen metabolism, limitations in mesophyll conductance and biochemical constraints (Barón, Flexas, & Delucia, 2012; Berger, Sinha, & Roitsch, 2007). These changes occurring in asymptomatic leaf tissue may be equal, proportionally greater or proportionally smaller than the corresponding infected leaf tissue (Owera, Farrar, & Whitbread, 1981; Shtienberg, 1992) and can dramatically impact other physiological processes especially the activation of some mechanisms of defence (Rohácek, 2002; Scholes & Rolfe, 2009).

The Chl *a* fluorescence is a sensitive, nondestructive and noninvasive technique that provides invaluable information regarding the physiological state of infected plants (Berger et al., 2007). Moreover, it is a quantitative measure of both photochemical and nonphotochemical energy dissipation processes occurring on leaves exposed to any stress (Kramer, Johnson, Kirats, & Edwards, 2004; Rohácek, 2002). The composition of the pigment systems, excitation energy transfer, physical changes in pigment-protein complexes, primary photochemistry and rate of electron transfer reactions in photosystem II are the most critical information obtained based on the intensity of Chl *a* fluorescence in the chloroplasts (Govindjee, 2004). For the barley-*Rhynchosporium secalis* (Martin, 1986), bean-*Colletotrichum lindemuthianum* (Lopes & Berger, 2001), coffee-*Hemileia vastatrix* (Honorato Júnior, Zambolim, Duarte, Aucique-Pérez, & Rodrigues, 2015), rice-*Monographella albescens* (Tatagiba, Neves, Bitti, & Rodrigues, 2016) and soya bean-*Colletotrichum truncatum* (Dias, Araujo, Chaves, DaMatta, & Rodrigues, 2018) interactions, photosynthesis rates were reduced due to an impairment in the functional leaf area and reduction in the photosynthetic efficiency of the remaining amount of green leaf tissue. On the other hand, barley leaves infected by *Puccinia hordei* displayed a reduced number of functional chloroplasts and lowered chlorophylls concentration (Owera et al., 1981).

The present study aimed to examine the *C. cassiicola*-induced perturbations in photosynthesis in soya bean leaflets by using the Chl *a* fluorescence imaging technique and determining the concentration of photosynthetic pigment pools.

2 | MATERIAL AND METHODS

2.1 | Plant material and growing conditions

A total of ten soya bean seeds from cultivars TMG 132 and Fundacep 59, susceptible and moderately resistant to target spot, respectively (Fortunato, Debona, Bernardeli, & Rodrigues, 2015; Godoy et al., 2012) were sown into plastic pots (Ecovaso, Jaguariúna, SP, Brazil) containing 2 kg of Tropstrato® (Vida Verde, Mogi Mirim, SP, Brazil) substrate composed of an 1:1:1 mixture of pine bark, peat and expanded vermiculite. Five days after seedlings emergence, each pot was thinned to two seedlings, which were fertilized weekly with 50 ml of a nutrient solution, prepared using deionized water, containing: 40 mM KNO_3, 10 mM $NH_4H_2PO_4$, 10 mM $MgSO_4.7H_2O$, 15 mM $Ca(NO_3)_2.4H_2O$, 2.4 mM $ZnSO_4.7H_2O$, 3 mM H_3BO_3, 10 mM K_2SO_4, 3.3 mM CH_4N_2O and 7.5 mM urea (Dallagnol, Rodrigues, Tanaka, Amorim, & Camargo, 2012). Plants were watered with deionized water as needed and kept in a greenhouse (temperature of 30 ± 5°C and relative humidity of 65 ± 5%) until 35 days after sowing (V4 growth stage) (Fehr, Caviness, Burmood, & Pennington, 1971).

2.2 | Inoculation procedure

The isolate UFV-DFP *Cc*22 of *C. cassiicola* was used to inoculate the plants. This isolate was preserved using the Castellani's method, in which five plugs of potato-dextrose-agar (PDA) medium containing fungal mycelium (5 mm diameter) were placed in a flask containing 5 ml of distilled sterilized water (Dhingra & Sinclair, 1995). At 14 days before inoculation, plugs of mycelium from cultures grown on PDA were placed in Petri dishes containing carrot leaf-pea-dextrose-agar (CL-PeDA) medium (Fortunato et al., 2015). Three days old fragments of fungal mycelia were transferred and homogenously spread onto a fresh plate containing CL-PeDA medium and placed in a growth chamber with a 12-hr photoperiod at 25°C for 4 days. After this period, fungal mycelia were carefully removed using a Drigalski spatula in a laminar flow chamber to induce sporulation (Onesirosan, Arny, & Durbin, 1975). The plates were maintained in a growth chamber under continuous white light (40 W lamps alternately distributed to provide a light intensity of 165 μmol s^{-1} m^{-2}) for 6 days until conidia were produced. Conidia were carefully removed from the Petri dishes with a soft-bristle brush using water containing gelatin (1% w/v). Plants were inoculated with a conidial suspension of *C. cassiicola* (5×10^4 conidia/ml) (10 ml/plant), which was applied as a fine mist using a VL airbrush atomizer (Paache Airbrush Co., Chicago, IL) to both adaxial and abaxial leaf surfaces of each plant. After inoculation, plants were maintained in a plastic mist growth chamber inside a greenhouse (day length of 12.5 hr and maximum natural photon flux density at plant canopy of 975 μmol m^{-2} s^{-1}) for the duration of the experiments. The relative humidity was maintained at 90 ± 5% using a misting system that sprayed mist from nozzles (model NEB-100, KGF Co., São Paulo, Brazil) above the plants' canopies for 15 s every 30 min. The temperature and relative humidity were measured with a thermohygrograph (TH-508, Impac, Brazil).

2.3 | Disease assessment

Target spot severity was evaluated on the three leaflets of the fourth leaf of each plant per replication of each treatment at 4, 6, 8 and 10 days after inoculation (dai) using a diagrammatic scale proposed by Soares, Godoy, and Oliveira (2009). The area under disease

progress curve (AUDPC) for each leaflet in each plant was computed using the trapezoidal integration of the target spot progress curve (Shaner & Finney, 1977).

2.4 | Determination of chlorophyll (Chl) *a* fluorescence

Images and parameters of Chl *a* fluorescence were determined on the three leaflets of the fourth leaf, from base to apex, at 4, 6, 8 and 10 dai using the MAXI version of the Imaging-PAM fluorometer and the Imaging Win software (Heinz Walz GmbH, Effeltrich, Germany). The Chl *a* fluorescence emission transients were captured by a CCD (charge-coupled device) camera with a resolution of 640 × 480 pixels in a visible sample area of 24 × 32 mm on each leaf. Initially, the leaves were dark-adapted for 60 min after which they were carefully and individually fixed in support at a distance of 18.5 cm from the CCD camera. Leaf tissues were then exposed to a weak, modulated measuring beam (0.5 μmol m^{-2} s^{-1}, 100 μs, 1 Hz) to determine the initial fluorescence (F_0) when all the PSII reaction centres were "open." Next, a saturating white light pulse of 2,400 μmol m^{-2} s^{-1} (10 Hz) was applied for 0.8 s to ensure the maximum fluorescence emission (F_m) when all the PSII reaction centres were "closed". From these initial measurements, the maximum PSII photochemical efficiency of the dark-adapted leaves was estimated through the variable-to-maximum Chl *a* fluorescence ratio as follows: $F_v/F_m = [(F_m - F_0)/F_m)]$ (Osório, Osório, Correia, Varennes, & Pestana, 2014; Rolfe & Scholes, 2010; Scholes & Rolfe, 2009). The leaf tissues were subsequently exposed to actinic photon irradiance (185 μmol m^{-2} s^{-1}) for 300 s to obtain the steady-state fluorescence yield (F_s), after which a saturating white light pulse (2,400 μmol m^{-2} s^{-1}; 0.8 s) was applied to achieve the light-adapted maximum fluorescence (F_m'). The light-adapted initial fluorescence (F_0') was estimated according to Oxborough and Baker (1997). Following the calculations of Kramer et al. (2004), the energy absorbed by the PSII for the following three yield components for dissipative processes were determined: the yield of photochemistry [Y(II) = $(F_m' - F_s)/F_m'$)], the yield for dissipation by down-regulation [Y(NPQ) = $(F_s/F_m') - (F_s/F_m)$] and the yield for other nonphotochemical (nonregulated) losses [Y(NO) = F_s/F_m]. Additionally, the electron transport rate (ETR) was calculated as ETR = Y(II) × PAR × 0.5 × absorptivity and assuming an equal light distribution between PSII and PSI (Krall & Edwards, 1992; Rolfe & Scholes, 2010). The parameters of Chl *a* fluorescence were determined to select the square option on the Imaging Win software (area of ≈ 0.50 cm^2) on noninoculated and inoculated leaves. Monochrome images of noninoculated and inoculated leaves at 4, 6, 8 and 10 dai were obtained using the camera of the fluorometer PAM Image to highlight their contrast regarding colour.

2.5 | Determination of the concentration of photosynthetic pigments

The concentrations of Chl *a*, Chl *b* and carotenoids were determined using dimethyl sulfoxide (DMSO) as an extractor (Wellburn, 1994). Five leaf discs (10 mm in diameter) were punched from each leaflet of the fourth leaf in each noninoculated and inoculated plant at 4, 6, 8 and 10 days after inoculation (dai). The collected discs were immersed in glass tubes containing 6 ml of a saturated DMSO solution and calcium carbonate ($CaCO_3$) (5 g/L) (Santos, Cruz, Iarema, Kuki, & Otoni, 2008) and kept in the dark for 48 hr. The absorbances of the extracts were read at 480, 649 and 665 nm using a saturated solution of DMSO and $CaCO_3$ as a blank. Data from the concentrations of Chl *a*, Chl *b* and carotenoids were used to calculate the total Chl concentration (Chl_{a+b}) and the Chl_{a+b}/carotenoids ratio.

2.6 | Experimental design and statistical analysis

Two experiments (Experiments 1 and 2), consisting of two treatments (two cultivars) and arranged in a completely randomized design with six replications, were performed to evaluate disease severity. Two other experiments (Experiments 3 and 4) were performed to determine the Chl *a* fluorescence parameters and to obtain the leaflets to determine the concentration of photosynthetic pigments, which consisted of a 2 × 2 factorial (two cultivars and noninoculated or inoculated plants) and was arranged in a completely randomized design with six replications. Each experimental unit corresponded to a plastic pot containing two plants. Data from AUDPC from Experiments 1 and 2 as well as data from the Chl *a* fluorescence parameters and the concentration of photosynthetic pigments from Experiments 3 and 4 were analysed using the MIXED procedure of the SAS software (Release 8.02 Level 02M0 for Windows, SAS Institute, Inc., 1989, Cary, NC, USA) to determine whether data from Experiments 1 and 2 and Experiments 3 and 4 could be combined (Moore & Dixon, 2015). For AUDPC, only the two cultivars were considered in the ANOVA. For the Chl *a* fluorescence parameters, the ANOVA was considered a 2 × 4 factorial experiment consisting of two cultivars and four sampling times (4, 6, 8 and ten dai). For the Chl_{a+b} concentration and the Chl_{a+b}/carotenoids ratio, the ANOVA was considered a 2 × 2 factorial experiment consisting of two cultivars and noninoculated or inoculated plants. Data from all variables were analysed by ANOVA and means from the treatments were compared by the *F*-test ($p \leq 0.05$) using SAS (version 6.12; SAS Institute, Inc., Cary, NC).

3 | RESULTS

3.1 | AUDPC

The factor cultivar (C) was significant for AUDPC ($p \leq 0.001$) and the factor sampling time (ST) and the interaction C × ST were not significant. For cultivar Fundacep 59, the AUDPC was significantly lower by 20% in comparison with cultivar TMG 132 (AUDPC values of 46.8 and 58.8, respectively from combined experiments).

3.2 | Concentration of photosynthetic pigments

The factors C and plant inoculation (PI) were significant for the concentration of Chl_{a+b} and the Chl_{a+b}/carotenoids ratio ($p \leq 0.001$)

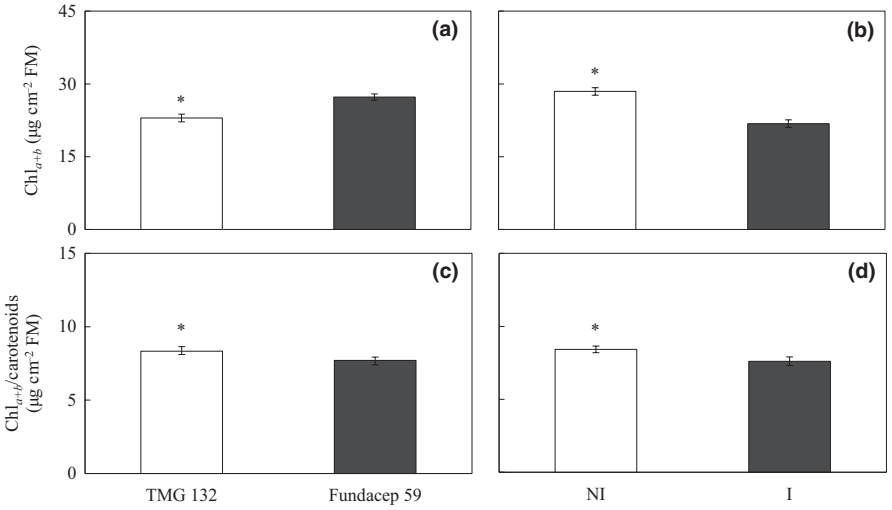

FIGURE 1 Concentration of total chlorophylls (Chl_{a+b}) (a and b) and total chlorophylls/carotenoids ratio (Chl_{a+b}/carotenoids) (c and d) in the leaflets of soya bean plants from cultivars TMG 132 and Fundacep 59 (a and c) noninoculated (NI) or inoculated (I) (b and d) with *Corynespora cassiicola*. Means from cultivars TMG 132 and Fundacep 59 and the NI and I treatments followed by an asterisk (*) are significantly different ($p \leq 0.05$) according to F-test. Bars represent the standard errors of the means. FM = fresh matter

while the interaction C × PI was not significant. The concentration of Chl_{a+b} was significantly higher by 16% for cultivar Fundacep 59 in comparison with cultivar TMG 132 while the Chl_{a+b}/carotenoids ratio was significantly lower by 11% for cultivar Fundacep 59 in comparison with cultivar TMG 132 (Figure 1A,C). There were significant decreases of 31% and 8% for the concentration of Chl_{a+b} and for the Chl_{a+b}/carotenoids ratio, respectively, for inoculated plants in comparison with the noninoculated ones (Figure 1B,D).

3.3 | Parameters and imaging of Chl *a* fluorescence

The parameters F_v/F_m, Y(II), Y(NPQ), Y(NO) and ETR were significantly influenced by at least one of the factors studied as well as by some of the two-way interactions (Table 1). First visual changes in the images of Chl *a* fluorescence were noticed at 4 dai in inoculated leaflets of plants from both cultivars (Figures 2 and 3) but were more prominent for plants of cultivar TMG 132. As target spot developed, changes in F_v/F_m, Y(II), Y(NPQ) and Y(NO) increased for inoculated leaflets, especially for plants of cultivar TMG 132. Indeed, decreases in the Y(NPQ) values coupled with increases in Y(NO) values started at six dai in the necrotic leaf tissue. Progressive loss of photosynthesis was started at 4 dai as indicated by the black dots in the images. In addition to the semi-quantitative assessment of photosynthesis, a quantitative examination of the images based on the leaflets from noninoculated and inoculated leaflets was also determined (Table 2). Values for F_v/F_m and Y(NO) obtained from leaflets of plants from cultivar TMG 132 were significantly higher in comparison with leaflets of plants from cultivar Fundacep 59. The values of F_v/F_m, Y(II) and ETR were significantly lower for inoculated leaflets in comparison with noninoculated ones regardless of the cultivar (Table 2). On the other hand, the Y(NO) values were significantly higher for inoculated leaflets in comparison with noninoculated ones regardless of the cultivar. The values of F_v/F_m, Y(II) and ETR were significantly higher for inoculated leaflets of plants from cultivar Fundacep 59 in comparison with inoculated leaflets of plants from cultivar TMG 132. The Y(NO) values were significantly higher for inoculated leaflets of plants from cultivar TMG 132 in comparison with inoculated leaflets of plants from cultivar Fundacep 59 (Table 2).

3.4 | Pearson correlation

For cultivar TMG 132, the correlations of AUDPC with Y(NPQ) and Y(NO) were significant and positive (r = 0.87 and 0.90, respectively) but significant and negative between AUDPC and ETR (r = −0.95). For cultivar Fundacep 59, the correlations of AUDPC with F_v/F_m, Y(NPQ) and Y(NO) were significant and positive (r = 0.88, 0.90 and 0.93, respectively). There was no correlation of AUDPC with F_v/F_m and Y(II) for cultivar TMG 132 and of AUDPC with Y(II) and ETR for cultivar Fundacep 59.

4 | DISCUSSION

To the best of the authors' knowledge, the present study is the first to report that the photosynthetic performance, denoted by examining

TABLE 1 Analysis of variance of the effects of cultivars (C), sampling times (ST), and their interaction on maximal photosystem II quantum yield (F_v/F_m), effective photosystem II quantum yield [Y(II)], quantum yield of regulated energy dissipation [Y(NPQ)], quantum yield of nonregulated energy dissipation [Y(NO)] and electron transport rate (ETR) determined in the leaflets of soya bean plants from cultivars TMG 132 and Fundacep 59 inoculated with *Corynespora cassiicola*

Parameters[a]	C	ST	C × ST
F_v/F_m	**	**	*
Y(II)	**	**	ns
Y(NPQ)	ns	ns	ns
Y(NO)	**	ns	ns
ETR	**	ns	ns

[a]Levels of probability: ns = nonsignificant, *0.05 and **<0.001.

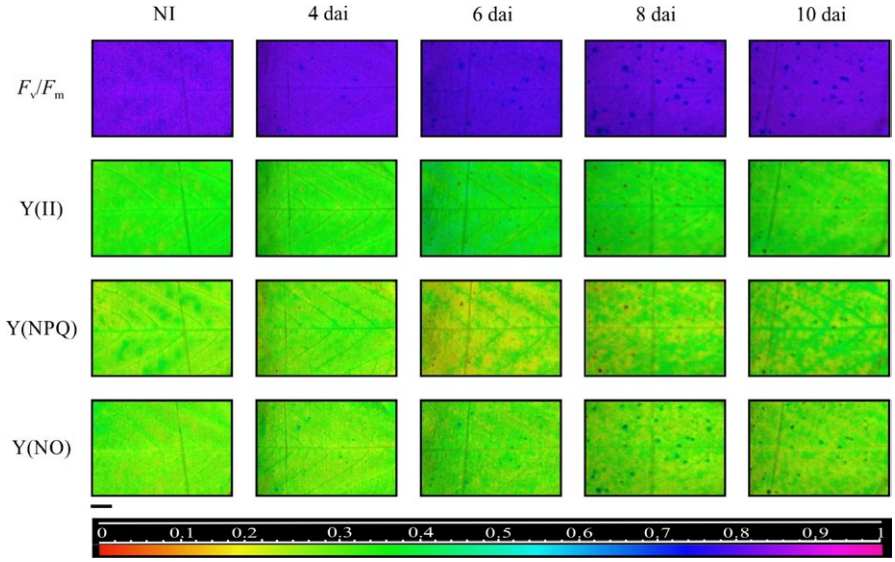

FIGURE 2 Parameters of chlorophyll *a* fluorescence quantum efficiency or yield (F_vF_m), effective PSII quantum yield [Y(II)], quantum yield of regulated energy dissipation[Y(NPQ)] and quantum yield of regulated energy dissipation [Y(NPQ)] determined in leaflets of soya bean plants from cultivar TMG 132 noninoculated (NI) or at 4, 6, 8 and 10 days after inoculation (dai) with *Corynespora cassiicola* at. Bar = 0.5 cm [Colour figure can be viewed at wileyonlinelibrary.com]

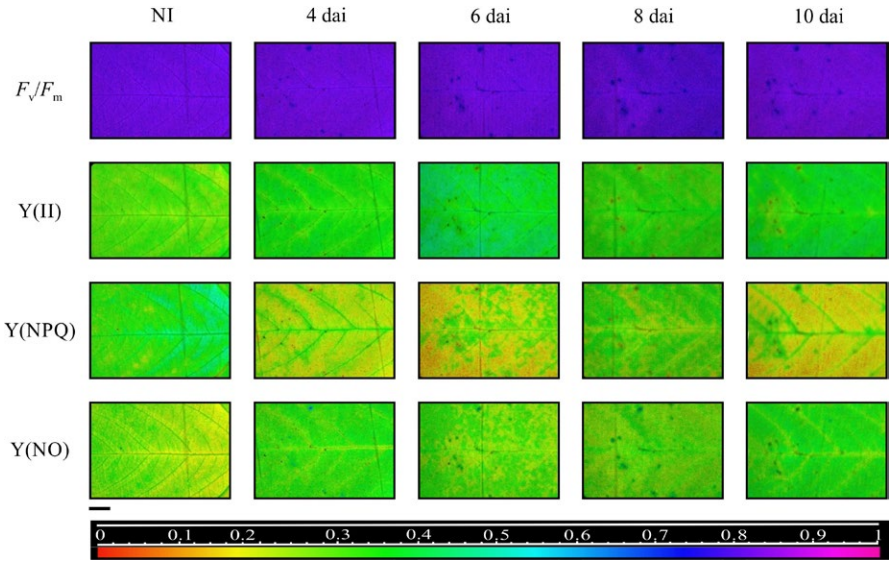

FIGURE 3 Parameters of chlorophyll *a* fluorescence quantum efficiency or yield (F_vF_m), effective PSII quantum yield [Y(II)], quantum yield of regulated energy dissipation [Y(NPQ)] and quantum yield of regulated energy dissipation [Y(NPQ)] determined in leaflets of soya bean plants from cultivar Fundacep 59 noninoculated (NI) or at 4, 6, 8 and 10 days after inoculation (dai) with *Corynespora cassiicola*. Bar = 0.5 cm [Colour figure can be viewed at wileyonlinelibrary.com]

key parameters related to Chl *a* fluorescence imaging, was impaired during the infection process of *C. cassiicola* on the leaflets of soya bean plants, especially from the susceptible cultivar TMG 132.

Changes in photosynthesis rates in many host-pathogen interactions have been intensively investigated using the Chl *a* fluorescence imaging technique (Scholes & Rolfe, 2009; Tatagiba, DaMatta, & Rodrigues, 2015). In general, the damage caused by pathogens of different lifestyles (biotrophics, hemibiotrophics and necrotrophics) on the leaf can be detected due to an decrease on *A*, g_s, *E* and F_v/F_m values (Debona et al., 2014; Dias et al., 2018; Honorato Júnior et al., 2015; Lopes & Berger, 2001; Rolfe & Scholes, 2010; Tatagiba et al., 2015, 2016). For reflecting the quantum efficiency of the PSII, the F_v/F_m parameter has been used as an excellent indicator of how the plants can support a certain level of stress (Maxwell & Johnson, 2000). In nonstressed plants, the F_v/F_m can fluctuate between 0.8 and 0.83, but it sharply decreases on stressed plants due to photoinhibition and damage to the PSII (Björkman & Powles, 1984). In the present study, the F_v/F_m values decreased in the inoculated leaflets confirming the damage caused by *C. cassiicola* infection on the photosynthetic machinery, especially for plants from the susceptible cultivar. Meanwhile, the F_v/F_m values obtained from leaflets of noninoculated plants were close to 0.8. On the other hand, the Y(II) and ETR values decreased while the values of Y(NPQ) and Y(NO) increased on the leaflets of plants from cultivar TMG 132 in comparison with cultivar Fundacep 59. In general, these changes were more evident on

TABLE 2 Maximal photosystem II quantum yield (F_v/F_m), effective PSII quantum yield [Y(II)], quantum yield of regulated energy dissipation [Y(NPQ)], quantum yield of nonregulated energy dissipation [Y(NO)], and electron transport rate (ETR) determined in the leaflets of soya bean plants from cultivars TMG 132 and Fundacep 59 noninoculated or inoculated with *Corynespora cassiicola*

		Cultivars	
Parameters	Plants	TMG 132	Fundacep 59
F_v/F_m	NI	0.83 Aa	0.83 Aa
	I	0.67 Ba	0.71 Bb
Y(II)	NI	0.38 Aa	0.40 Aa
	I	0.21 Ba	0.28 Bb
Y(NPQ)	NI	0.30 Aa	0.28 Aa
	I	0.17 Aa	0.20 Aa
Y(NO)	NI	0.32 Aa	0.32 Aa
	I	0.52 Ba	0.46 Bb
ETR	NI	30.79 Aa	32.68 Aa
	I	20.50 Ba	25.42 Bb

Note. For each cultivar, within each parameter, means in each column followed by the same uppercase letter (comparing noninoculated (NI) and inoculated (I) plants and between cultivars, within each NI and I treatment, followed by the same lowercase are not significantly different according to Tukey's test at 5% of probability.

the inoculated leaflets at the advanced stage of fungal infection in contrast to noninoculated leaflets. In the inoculated leaflets, mainly from plants from the susceptible cultivar, the reductions in the Y(II) and Y(NPQ) values were generally coupled to an increase on Y(NO) values indicating inhibition of photosynthesis. Changes in the values of Y(II) and Y(NPQ) show clear adjustments in light capture and dissipation to prevent photodamage once lower Y(II) values reflect decreases in the apparent electron transport activity while high Y(NPQ) values indicate heat dissipation (Krause & Weis, 1991).

Therefore, the high Y(NPQ) values are evidence of the high capacity of photoprotection for plants submitted to stress (Anjos, Oliva, & Kuki, 2012). According to Su et al. (2017), high and low Y(NPQ) and Y(NO) values, respectively, indicate that the excess of excitation energy is safely dissipated at the antenna level meaning that photosynthetic energy fluxes are well regulated. However, in the present study, the increase in Y(NPQ) values was not sufficient to avoid the photoinhibition of the photosynthesis once the values F_v/F_m dramatically decreased.

The reduction of photosynthesis rates in the leaves infected by pathogens of different lifestyles can occur due to reduction or destruction of chlorophyll molecules or chloroplasts results in chlorotic and necrotic leaf tissue (Bassanezi, Amorim, Bergamin Filho, & Berger, 2002; Dallagnol, Rodrigues, Martins, Cavatte, & DaMatta, 2011; Debona et al., 2014). The coalescence of lesions and a progressive yellowing of the leaf tissue at the infection sites of *C. cassiicola*, especially on the leaflets of plants from cultivar TMG 132, were most likely associated with a lower concentration of Chl_{a+b} and, to a lesser extent, to the Chl_{a+b}/carotenoids ratio. The carotenoids are involved in the capture of light energy and constitute the first step in the initiation of photochemical events (Murchie & Horton, 1997; Taiz & Zeiger, 2009). The photooxidative conditions caused by pathogens in the infected leaf tissue are closely associated with the reduced concentration of photosynthetic pigments (Murchie & Horton, 1997). Considering that chlorotic haloes reflect the macroscopic symptoms of chloroplasts degradation (Han et al., 2007), in the present study, infection by *C. cassiicola* may have compromised the structural integrity of the thylakoid network and reducing the capacity for electron transport in the leaflet tissue.

Furthermore, the increased photooxidative damage to inoculated leaflets of plants from cultivar TMG 132 could also be depicted from the progressive increase in the Y(NO) values in comparison with cultivar Fundacep 59. This finding suggests, therefore, that both the excitation energy directed to photochemical conversion and the regulatory mechanisms of protection were ineffective as reported by Klughammer and Schreiber (2008). High Y(NO) values are the result of chronic photoinhibition related to damage of chlorophylls and carotenoids as well as to the low resilience of the D1 protein which together protects the reaction centres coupled with losses in the cycling xanthophyll (Demmig-Adams, Ebbert, Zarter, & Adams, 2006). Taken together, all of these changes culminated in the complete loss of the optical properties of the inoculated leaflets and ultimately the complete destruction of the photosynthetic apparatus. Indeed, the target spot lesions rapidly expanded over time to a state with extensive chlorotic and necrotic areas in which no local photosynthetic activity could be detected as indicated by the black zones in the images. The changes in the values of the F_v/F_m, Y(II), ETR, Y(NPQ) and Y(NO) parameters in the inoculated leaflets may be explained, at least in part, by the nonhost selective toxins and hydrolytic enzymes released by *C. cassiicola* which contribute to cause intense leaf tissue disorganization and further necrosis (Fortunato et al., 2015). For the *Arabidopsis thaliana-Pseudomonas syringae*, *Nicotiana benthamiana-P. syringae* and rice-*Monographella albescens* interactions, the F_v/F_m parameter significantly decreased on diseased leaf tissue compared to nondiseased ones (Bonfig, Schreiber, Gabler, Roitsch, & Berger, 2006; Iqbal, Goodwin, Leonardos, & Grodzinsli, 2012; Tatagiba et al., 2015).

Based on the results from the present study, it can be concluded that Chl *a* fluorescence imaging was an excellent tool to describe the loss of functionality of the photosynthetic apparatus of soya bean leaflets infected by *C. cassiicola*.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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