CARLA DA SILVA DIAS

LEAF GAS EXCHANGE AND CHLOROPHYLL a FLUORESCENCE IMAGING OF SOYBEAN LEAVES INFECTED WITH *Colletotrichum truncatum*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, para obtenção do título de Magister Scientiae.

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APROVADA: 19 de fevereiro de 2015.

Wilka Messner da Silva Bispo
Wilka Messner da Silva Bispo

Fábio Murilo DaMatta

Fabrício Ávila Rodrigues
Fabrício de Ávila Rodrigues
(Orientador)

Aos meus pais, Elaine e Carlos,

A meu irmão Matheus,

A meu namorado Renan

Aos meus amigos e demais familiares

DEDICO

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BIOGRAFIA

CARLA DA SILVA DIAS, filha de Elaine Ladeira da Silva Dias e Carlos Antônio Dias, nasceu em 22 de outubro de 1990, em Viçosa, Minas Gerais.

Iniciou o curso de Agronomia no ano de 2008 na Universidade Federal de Viçosa (UFV) e em março de 2013, graduou-se Engenheira Agrônoma. Durante a graduação, teve oportunidade de trabalhar como bolsista de Iniciação Científica nos Departamento de Microbiologia e de Engenharia agrícola.

Em Abril de 2013, iniciou o curso de Mestrado no Programa de Pós-Graduação em Fisiologia Vegetal da UFV, atuando na área da Interação Patógeno-Hospedeiro, sob orientação do Prof. Fabrício de Ávila Rodrigues.

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RESUMO

DIAS, Carla da Silva, M. Sc., Universidade Federal de Viçosa, Fevereiro de 2015. **Trocas gasosas e fluorescência da Clorofila a em plantas de soja infectadas por Colletotrichum truncatum.** Orientador: Fabrício de Ávila Rodrigues.

A Anthracnose, causada pelo fungo Colletotrichum truncatum, é uma das doenças de soja mais importantes no mundo, mas não há estudos avaliando as alterações fisiológicas neste patossistema. Portanto, uma abordagem para avaliar os eventos que ocorrem no local da infecção e perto da área infectada na folha, ao longo do tempo, contribuirá para uma melhor compreensão da interação planta-hospedeiro e atividade fotossintética. Assim, o presente estudo buscou investigar parâmetros de fluorescência da clorofila a (Chl a) na área da lesão e uma área adjacente, associando os ás trocas gasosas e avaliação de pigmentos fotossintéticos em plantas de soja inoculadas ou não inoculadas com C. truncatum. O parâmetros de trocas gasosas não foram alterados em plantas inoculadas. No entanto, ocorreu redução da concentração de Chl a, Ch b e da Chl total (a + b) nas plantas inoculadas as 72 e 144 horas após a inoculação (hai), com redução máxima à 144 hai de 24% para Chl a, que demonstrou ser mais sensível que a Chl b, ocasionando, portanto, a redução da razão Chl a/ Chl b. Também foi encontrado queda em valores de fluorescência da clorofila a como, Fluorescência inicial (F_o), Fluorescência máxima (F_m), Eficiencia quântica máxima do fotossistema II (F_v/F_m), Rendimento quântico de dissipação de energia regulada Y(NPQ) e coeficiente não-fotoquímico (q_N), e um acréscimo no Rendimento quântico efetivo do PSII Y(II), Rendimento quântico de dissipação de energia não regulada (NO) e coeficiente fotoquímico (q_P) nas área sintomática de plantas inoculadas. Entretanto esses parâmetros sofreram pequenas alterações nas áreas adjacentes das plantas inoculadas,

diferindo apenas em alguns tempos. Demonstrando, dessa forma, um menor efeito do patógeno nas áreas adjacentes.

ABSTRACT

DIAS, Carla da Silva, M. Sc., Universidade Federal de Viçosa, February 2015. **Leaf Gas Exchange and Chlorophyll a Fluorescence Imaging of Soybean Leaves Infected with Colletotrichum truncatum.** Advisor: Fabrício de Ávila Rodrigues.

Anthracnose, caused by Colletotrichum truncatum, is one of the most important soybean diseases worldwide. However, there are no studies evaluating the physiological changes affecting this pathossystem. Therefore, one approach to evaluating events that occur at the site of infection and near the infected area on the leaf, over time, will contribute to a better understanding of the host-plant interaction and photosynthetic activity. The present study aimed to investigate chlorophyll a fluorescence parameters at injured and adjacent areas and the related changes in gas exchange and evaluation of photosynthetic pigments in soybean plants inoculated or non-inoculated with C. truncatum. There were no significant differences regarding gas exchange parameters for inoculated plants. However, there was a reduction in the concentration of Chl a, Chl b e Chl total (a + b) of inoculated plants in the 72 and 144 hours after inoculation (hai). Reduction in chlorophyll a fluorescence parameters to as initial fluorescence (F_o), maximal fluorescence (F_m), maximal photosystem II quantum yield (F_v/ F_m), quantum yield of regulated energy dissipation Y (NPQ) and coefficient non-photochemical (q_N), and an increase in the Effective PSII quantum yield Y (II), quantum yield of non-regulated energy dissipation (NO) and photochemical coefficient (q_P) in the symptomatic area plants inoculated. However, these parameters have undergone minor adjacent areas of inoculated plants, differing only in a few days. Demonstrating a smaller effect of the pathogen in adjacent.

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1. INTRODUCTION

Soybean Anthracnose, caused by the hemibiotrophic fungus Colletotrichum truncatum [(Schw.) Andrus and W.D. Moore], is one of the most important diseases affecting soybean production worldwide, especially in regions with high temperatures and intense rainfall (Manandhar and Hartman, 1999). The symptoms of anthracnose include irregular brown spots that develop in a random pattern on leaf veins, stems and pods; brown cankers on petioles and stems and premature defoliation are also observed (Gizlice et al., 1993; Manandhar and Hartman, 1999). And because of this, anthracnose has been managed with the application of fungicides, crop rotation, use of certified seeds and resistant cultivars when available for the growers (Manandhar and Hartman, 1999).

Several abiotic and biotic types of stresses that are imposed on plants may cause changes in their growth (Berger, 2007). Plants infected by pathogens show reduced photosynthetic performance generally associated with direct damage on photosynthetic apparatus and reductions in healthy leaf area (Bastiaans, 1991; Berger 2007; Chou 2000). For different host-pathogen interactions, the reduction in pigments concentration, structural damage to the chloroplast sand impairments in energy dissipation via chlorophyll (Chl) a fluorescence are the most notable negative effects that result from pathogens infection (Petit et al., 2006; Resende et al., 2012; Zhao et al., 2011). Pathogen infection can also cause leaf damage at the cuticular and stomatal levels, which can lead to changes in transpiration and plant water balance (Ayres,1980).

Non-invasive methods such as the chlorophyll fluorescence imaging combined with gas exchange measurements are considered efficient indicators of the photosynthetic apparatus (Baker and Rosenquvist ,2004; Berger et al., 2007). The use of the Chl a fluorescence imaging has bringing information on how the biotrophic,

hemibiotrophic and necrotrophic fungal pathogens affect their host´s photosynthesis, based on the mapping of changes in parameters associated with the photosynthetic performance (Rolfe and Scholes, 2010). The maximum photosystem (PS) II photochemical quantum efficiency, often assessed by variable-to-maximum Chl a fluorescence ratio (F_v/F_m), is one of the most important parameters, since it helps to contrast non-infected vs. infected host tissue (Iqbal et al., 2012; Rousseau et al., 2013). For F_v/F_m the ratio is close to 0.8 in healthy leaves and falls progressively below as the damage to PSII reaction centers increase (Krause and Weis, 1991). By comparison, the energy absorbed by PSII reaction centers can be divided between the fraction used in photochemistry [Y(II)] and that lost non-photochemically, which can be further divided into two competing non-photochemical pathways: the yield induced by down-regulatory processes [Y(NPQ)] associated with controlling thermal dissipation and the yield for other energy losses [Y(NO)] (Kramer et al., 2004).

Considering the importance of anthracnose to decrease soybean yield, and whereas the physiological responses of plants to fungal infection is almost unknown, this study aimed to examine the photosynthetic performance of soybean leaves during the infection process of C. truncatum using a combination of gas exchange and Chl a fluorescence measurements.

2. MATERIAL AND METHODS

Plant cultivation

Soybean seeds (cv. TMG 132) were sown in plastic pots containing 2 kg of Plantmax® (Eucatex, São Paulo, Brazil). After seedlings emergence, thinning was made, leaving three plants per pot. The plants were fertilized weekly with 50 mL of nutrient solution containing, in mg L^{-1},192 KCl; 104.42 K_2SO_4; 150.35 $MgSO_4.7H_2O$; 61 urea; 100 NH_4N_2O, 0.27 $(NH_4)_6MO_7)_{24}.4H_2O$; 1,61 H_3BO_3; 6.67 $ZnSO_4.7H_2O$; 1.74 $CuSO_4.5H_2O$; 4.10 mg $MnCl_2.4H_2O$; 4.08 $FeSO_4.7H_20$ and 5 EDTA disodium.

Inoculum production and inoculation procedure

The isolate UFV-DFP Ct 06 of C. truncatum, obtained from symptomatic soybean leaves, was used in this study. The isolate was preserved on strips of filter paper placed into glass tubes containing silica gel at 4ºC. Pieces of filter paper with fungal mycelia were transferred to Petri dishes containing potato-dextrose-agar (PDA). Inoculum preparation proceeded as previously reported on Polanco et al. (2012). The suspension was filtered and adjusted to a concentration of 1.2×10^6 conidia mL^{-1}using a Neubauer-counting chamber. Plants were allowed to grow for 21 days (V4 growth stage) and then inoculated with a conidial suspension of C. truncatum applied as a fine mist to the adaxial and abaxial leaves of a each plant until run off with an atomizer (Paasche Airbrush Co., Chicago). Immediately after inoculation, the plants were transferred to a growth chamber with temperature of 25±2ºC and relative humidity of 90±5% for 24 h. After this period, plants were transferred to a growth chamber with temperature of 28±2ºC and relative humidity of 80 ± 5%.

Assessment of anthracnose severity

The third trifoliate leaf, (from the base to the apex), of each replication per treatment (5 leaves per treatment, 10 leaves per each evaluation time, 70 leaves total) were marked and collected to evaluate anthracnose severity at 24, 36, 40, 48, 72, 96, 120 hours after inoculation (hai). The collected leaves were scanned at 300 dpi resolution and the obtained images were processed using QUANT software (Vale et al., 2003) to obtain severity values.

Photosynthetic measurements

Leaf gas exchange parameters

The net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) were determined on the third leaf, from the base to the apex, of each replication per treatment at 36, 60, 84, 108 and 132 hai by using a portable open-flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA) equipped with a circular leaf chamber that enclosed 2 $cm^2$of leaf area. The measurements were performed from 09:00 to 10:30 hours (solar time), under artificial photosynthetically active radiation 1,000 µmol photons m^{-2} s^{-1} and 400 µmol CO_2 mol^{-1} air. All of the measurements were performed at 25°C, the vapor pressure deficit was maintained at approximately 1.0 kPa and the amount of blue light was set to 10% of the photosynthetic photon flux density to optimize the stomatal aperture.

Chlorophyll (Chl) a fluorescence imaging

Images of the parameters of Chl a fluorescence were obtained on the third leaf, from the base to the apex, of each replication per treatment at 24, 36, 48, 72, 96 and 120 hai using Imaging-PAM (MAXI version) and the imaging fluorometer software Win (Heinz Walz GmbH, Effeltrich, Germany). In order, to obtain the images, (resolution of 640 × 480 pixels) of the Chl a fluorescence parameters, the leaves of each plant were individually fixed in a holder at a distance of 18.5 cm from the recording camera CCD

("charge-coupled device") coupled to fluorescence device. The 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 PS II reaction centers are "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 PS II reaction centers were "closed". From these initial measurements, the maximum PS II photochemical efficiency of the dark-adapted leaves was estimated through the variable-to-maximum Chl fluorescence ratio, $F_v/F_m = [(F_m - F_0)/F_m)]$. The leaf tissues were subsequently exposed to actinic photon irradiance (185µmol 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. (Kramer, 2004), the energy absorbed by PS II 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 non-photochemical (non-regulated) losses [$Y(NO) = F_s/F_m$]. Additionally, the photochemical quenching coefficient (q_P) was calculated as $q_P = (F_m' - F_s)/(F_m' - F_0')$ (Krause, 1991).The parameters of Chl a fluorescence were determined selecting the rectangular option on the Imaging Win software (area of ≈0.07 cm^2) on the main leaf vein and also on the lateral leaf vein of non-inoculated and inoculated leaves.

Determination of photosynthetic pigments

The first three trifoliate leaves of non-inoculated and inoculated plants were collected at 24, 48, 72, 96, 120 and 144 hai, kept in liquid nitrogen were during sampling and stored at -80°C. Leaves were lyophilized (- 48°C) and ground in a cell

disruptor using metal balls of 3.2 mm diameter (Mini-Bead beater-96, Bio Spec Products, Bartlesville, OK, USA). The chlorophylls a and b were determined by a hot ethanol extraction in ELISA microplate reader (Porra et al., 1989).

Experimental design and data analysis

An experiment consisting of two treatments (non-inoculated and inoculated plants) was arranged in a completely randomized design with five replications to evaluate gas exchange measurements and Chl fluorescence imaging (non destructive analysis). Another experiment, consisting of non-inoculated and inoculated plants arranged in a completely randomized design with five replication, was performed to evaluate anthracnose severity and to obtain leaves for the determination of pigments (destructive analysis). Each experimental unit corresponded to a plastic pot with three plants. A total de 120 plants were used in each experiment, with 20 plants for each sampling time. The data were subjected to an by analysis of variance, and the treatment means were compared by t-test ($P \leq 0.05$) using the SAS software (SAS Institute, Inc., Cary, NC,USA).

3. RESULTS

Anthracnose severity

The severity increased from 0.1% to 8.3% from 36 to 120 hai (Fig. 1).

Leaf gas exchange parameters

There were no significant difference for A, g_s and E between non-inoculated and inoculated plants regardless of the evaluation time. For C_i, significant difference between non-inoculated and inoculated plants occurred only at 36 hai (Fig. 2).

Concentrations of pigments

The concentrations of Chl a and Chl b as well as the Chl a/ Chl b ratio were significantly higher (23, 20 and 7%, respectively) for the inoculated plants in comparison to the non-inoculated ones at 24 hai. The concentrations of Chl a, Chl b and the Chl a+b ratio were significantly lower by 21, 17 and 19% at 72 hai and by 24, 22 and 23% at 144 hai, respectively, for the non-inoculated plants in comparison to the inoculate ones. The Chl a/ Chl b ratio was significantly lower by 5 and 3% at 72 and 144 hai, respectively, for the non-inoculated plants in comparison to the inoculate ones (Fig. 3).

Imaging of Chl a fluorescence

The first visual changes in Chl a fluorescence images on leaves of inoculated plants occurred at36 hai and became more notable as the disease developed (Figs. 4 and 5). For F_0, the orange color observed on the leaf surface of non-inoculated leaves indicates that the chlorophylls and the leaf optical properties were maintained intact (healthy areas) (Fig. 4a1). As the disease developed, the main and the lateral veins became dark in color reflecting the destruction of photosynthetic apparatus and loss of the optical properties of leaf tissue. A similar trend was noted for F_m, where the green color represents the non-infected leaf tissue (Fig. 4b1) while the yellowish red is the

result of the massive necrosis of the main and lateral veins (Figs. 4 b2-b6). The F_v/F_m was also negatively impaired on the main and lateral veins as indicated by their dark color in contrast to the bright dark color of the main and lateral veins in the leaves of non-inoculated plants (Figs 4c1-c6). For Y(II), Y(NPQ) and Y(NO), the main and lateral veins on leaves of non-inoculated plants were bright green (Figs. 5 a1, b1 and c1) while the main and lateral veins on leaves of inoculated plants became dark green (Figs 5 a2-a6, b2-b6 and c2-c6). A quantitative examination of the images on the main leaf vein indicated that F_o, F_m and F_v/F_m significantly decreased from 36 to 120 hai for the inoculated plants in comparison to the non-inoculated ones (Fig. 6A, C and E). On the area adjacent to the main leaf vein, F_o and F_m significantly decreased at 72 and 120 hai for the inoculated plants in comparison to the non-inoculated ones (Fig. 6B and D). The Y(II) and q_P were significantly higher on the main vein of inoculated leaves in comparison to the main vein of non-inoculated ones from 36 to 120 hai (Fig. 7A and G). The Y(NO) was significantly higher on the main vein of inoculated leaves in comparison to the main vein of non-inoculated ones from 36 to 72 hai (Fig. 7E). The Y(NPQ) and q_N were significantly higher on the main vein of non-inoculated leaves in comparison to the main vein of inoculated ones from 36 to 120 hai (Fig. 7C and I). On the area adjacent to the main vein of inoculated leaves in comparison to the main vein of non-inoculated ones, Y(II) and Y(NO) were significantly higher at 36 and at 36 and 72 hai, respectively (Fig. 7B and F). The Y(NPQ) and q_N were significantly higher at 36 and 72 hai on the area adjacent to the main vein of inoculated leaves in comparison to the main vein of non-inoculated ones (Fig. 7D and J).

4.DISCUSSION

The present study is, to the best the authors' knowledge, the first to report that photosynthetic performance, herein investigated by examining key parameters related to leaf gas exchange and Chl a fluorescence imaging, was not impaired during the infection process of C. truncatum even at latter stage of fungal infection. It has been reported that the infection of plants by fungi of the genus Colletotrichum reduces the Rubisco activity besides causing stomatal closure, reduces photosynthetic rate and transpiration (Meyer et al., 2001, Resende et al., 2012 and Polanco et al., 2014). However, in the present study, infection by C. truncatum on soybean leaves did not caused effect on photosynthetic gas exchange, in contrast to wheat- Pyricularia oryzae (Aucique-Perez et al., 2014), bean-Urumyces appendiculatus; bean-Phaeoisario psisgriseola; bean- Colletotrichum lindemuthianum (Bassanezi et al, 2002); rice-Pyricularia oryzae (Debona et al, 2014); rice-Monographella albescens interactions (Tatagiba, 2014). Bassanezi et al. (2001) found that A was reduced by more that 50% in the leaves of beans with 10% of Anthracnose severity and was reduced by almost 100% when the severity was above 20%. In the bean-C.lindemuthianum interaction, the production of lytic enzymes and non-specific toxins and their diffusion into the leaf tissues, particularly the veins, which are not yet colonized by the fungus, contribute greatly to the impairment of A. Nevertheless, these negative impacts have not been demonstrated in soybean infection by C. truncatum.

The pigments concentrations decreased during infection process of C. truncatum as the result of the vein necrosis that often occurs as the anthracnose progress leading, therefore, to reduced photosyntensis leaf area. Chla is more prone to photobleaching than Chl b, thus resulting in a difference in the ratio Chl a / Chl b, as described by Murchie and Horton (1997). The pigment concentration in leaves and membrane

integrity are critical to the maintenance of the photosynthesis in plants (Matsuda et al., 2004). Reduction in the concentration of pigments in leaves caused by various pathogens such as corn Exsorohilum turcicum (Chauhan et al., 1997) caused a reduction in photosynthetic rate.

The inoculated plants exhibited a decrease in the values F_0, which may be caused by structural changes in photosynthetic pigments from the infection by C. truncatum, compromising the efficiency of excitation energy from the light collecting. Trend similar to the results for F_m, where lower values took place during the expansion of the lesions. Maximum efficiency at which light is absorbed by PSII to reduce QA is represented by F_v/F_m, is a sensitive indicator of photosynthetic performance of plants with optimal values close to 0.8 for most plant species (Krause, 1991). Values for F_v/F_m found in diseased areas were less than 0.8 indicating damage in the photosynthetic apparatus due to infection by C. truncatum. However, the damage was limited to the diseases region because the surrounding areas the F_v/F_m ratio was similar to that found in non-inoculated plants. Furthermore, the increased photooxidative damage to leaf tissues could also be depicted from the progressive increases in Y(NO), which suggest that the regulation mechanisms of protection become ineffective (Klughammer and Schreiber, 2008).

Y (NPQ) refers to controlled thermal dissipation that occurs in the reaction centers of the PSII via xanthophyll cycle pigments being induced by light and proton gradient in thylakoid membranes (Kramer et al., 2004). The Y (NPQ) suggests the occurrence of dissipation of excess excitation energy as heat, being a physiological mechanism of photoprotection. On the other hand, the lowest values obtained for Y (NPQ), assume that infection with C. truncatum affected the energy dissipation mechanism, disabling this area to protect against light stress. A similar observation was made by Staet el. al

(2012) and Stael et al. (2014) which is likely an NPQ decreased by a reduction in the PSII sub–unitprotein S (PsbS) which is crucial for NPQ. This result correlates with increased chlorophyll fluorescence yield (YII), which is a relative measure of performance of the PSII. Consequently, this increase in PSII function and a lowered NPQ lead to an increased amount of energy at PSII that can react with O_2 to form $^1O_2$, a kind of ROS, which the increase could be, damaged for PSII (Bonfig et al, 2006; Mur et al, 2010).

The harmful effects of the pathogen were insignificant in physiology at the level of whole plant, valued at vegetative stage, demonstrating a restriction of the harmful effects on the symptomatic area located in the leaf vein. Therefore it is unnecessary strict control of the pathogen in this vegetative stage, being necessary only in the early and reproductive stages.

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6.LIST OF FIGURES

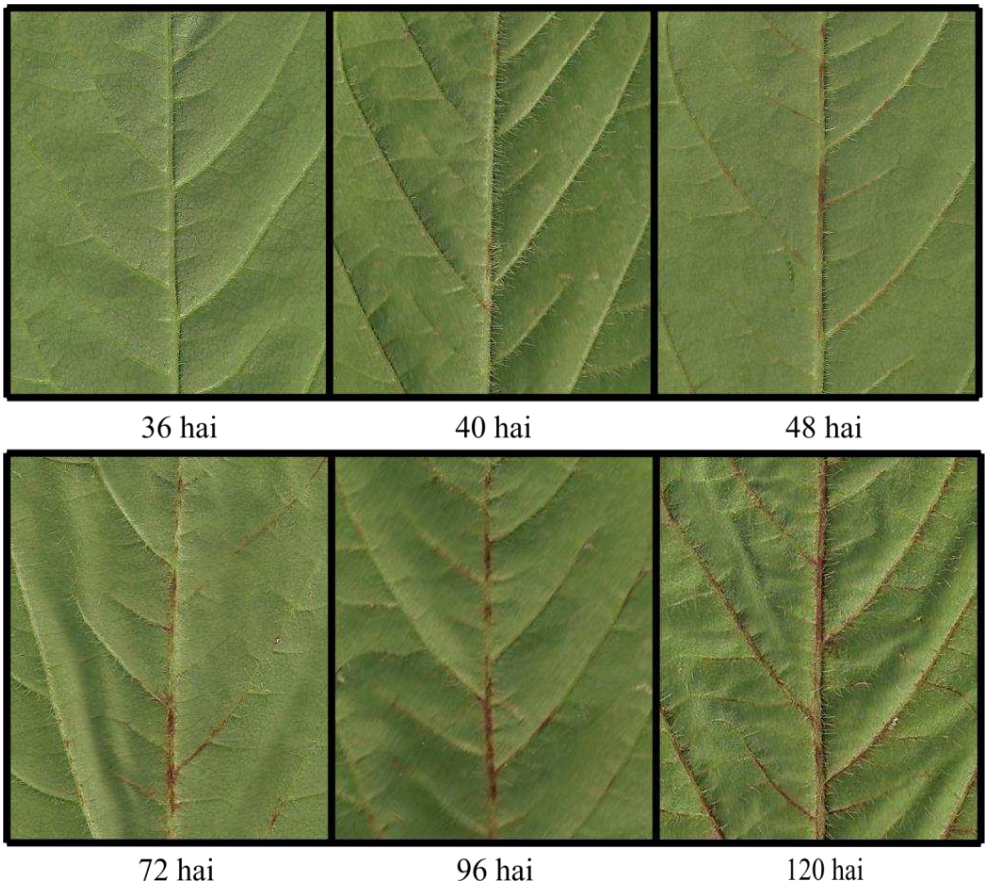
Figure 5. Parameters of chlorophyll a fluorescence: effective photosystem II quantum yield (Y(II)) (a1-a6), quantum yield of regulated energy dissipation (Y(NPQ)) (b1-b6) and quantum yield of non-regulated energy dissipation(Y(NO)) (c1-c6) determined on the leaves of soybean plants non-inoculated or inoculated with Colletotrichum truncatum. n = 12. Bar = 0.5 cm.

Figure 6. Initial fluorescence (F_0) (A and B), maximal fluorescence (F_m) (C and D) and maximal photosystem II quantum yield (F_v/F_m) (E and F) determined in the main leaf vein (A,C and D) and in the adjacent area of the main leaf vein (B, D and E) of soybean leaves non-inoculated (NI) or inoculated (I) with Colletotrichum truncatum. The means for NI and I treatments followed by an asterisk (*) for each evaluation time were significantly different according to the t-test ($P \leq 0.05$). The bars represent the standard error of the means. n = 5.

Figure 7. Effective PS II quantum yield (Y(II)) (A and B), quantum yield of regulated energy dissipation (Y(NPQ)) (C and D), quantum yield of non-regulated energy dissipation (Y(NO)) (E and F), photochemical quenching coefficient (q_P) (G and H) and non photochemical quenching coefficient (q_N) (I and J) determined on the main leaf vein (A, C, E, G and I) and on adjacent area of the main leaf vein (B,D,F, H and J)of soybean leaves non-inoculated (NI) or inoculated (I) with Colletotrichum truncatum. The means for the NI and I treatments followed by an asterisk (*) for each evaluation time were significantly different according to the t-test ($P \leq 0.05$). The bars represent the standard error of the means. n = 5.

FIGURES

A



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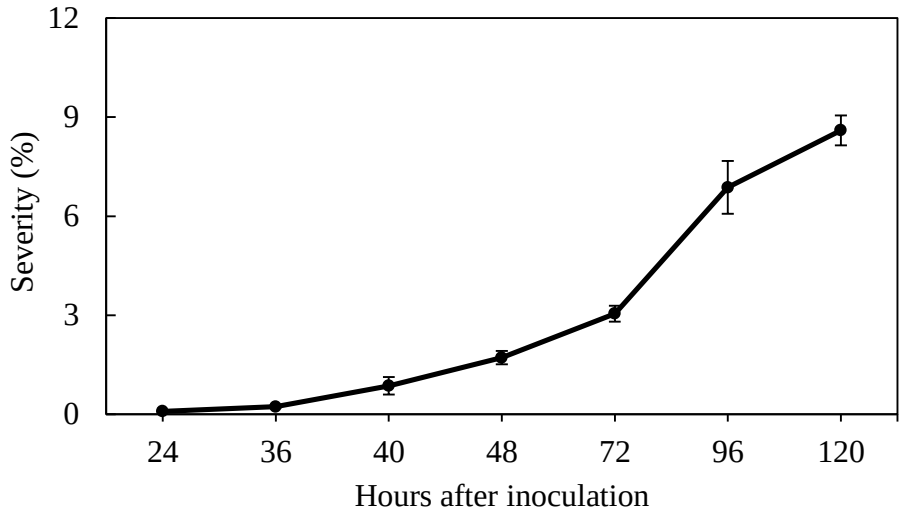

Figure 1

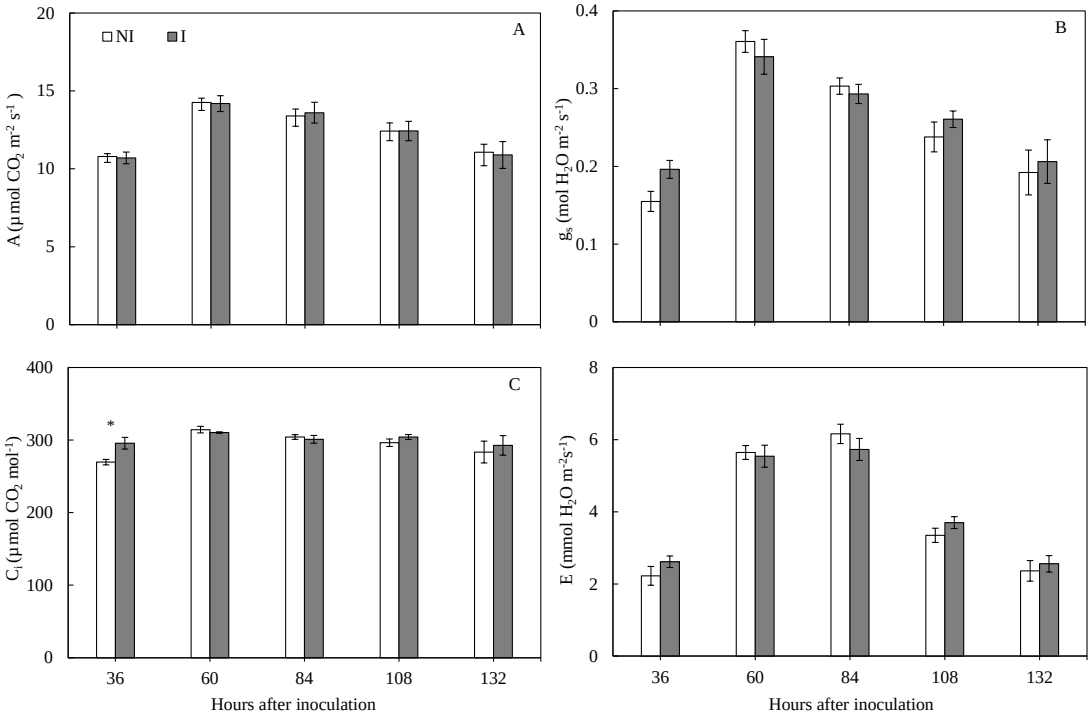
A
NI
I
A (µmol CO2 m-2 s-1)
B
gs (mol H2O m-2 s-1)
C
Ci (µmol CO2 mol-1)
*
E (mmol H2O m-2s-1)
Hours after inoculation
Hours after inoculation

Figure 2

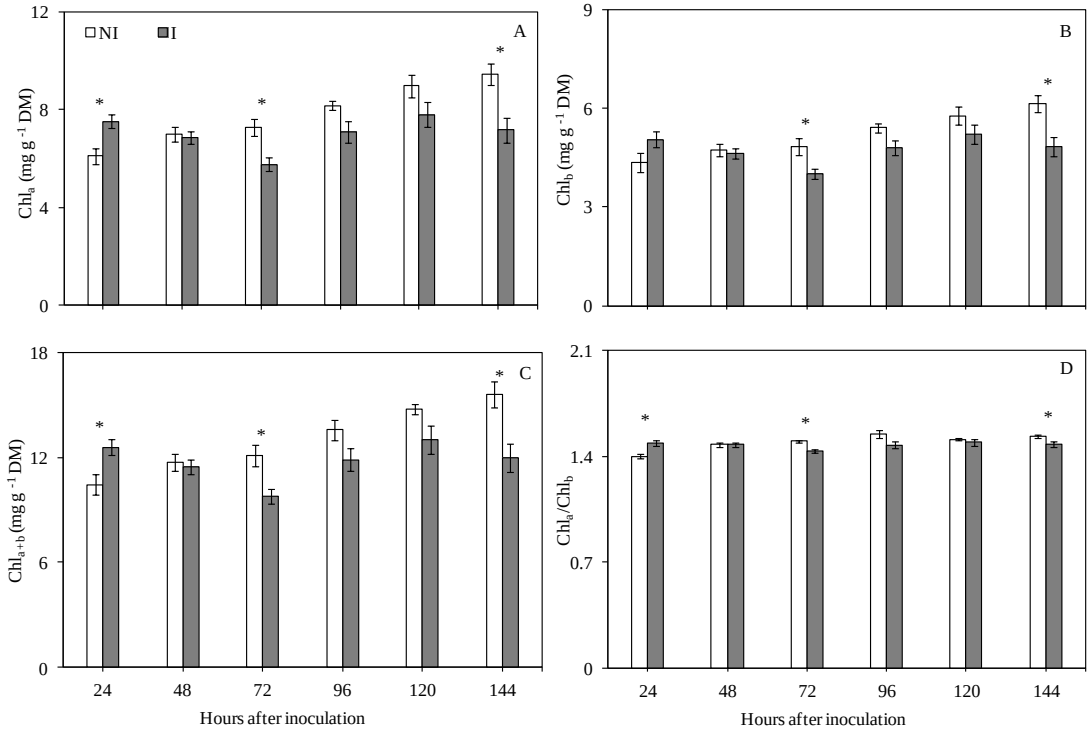
NI
I
A
B
C
D
Chla (mg g-1 DM)
Chlb (mg g-1 DM)
Chla+b (mg g-1 DM)
Chla/Chlb
Hours after inoculation
Hours after inoculation

Figure 3

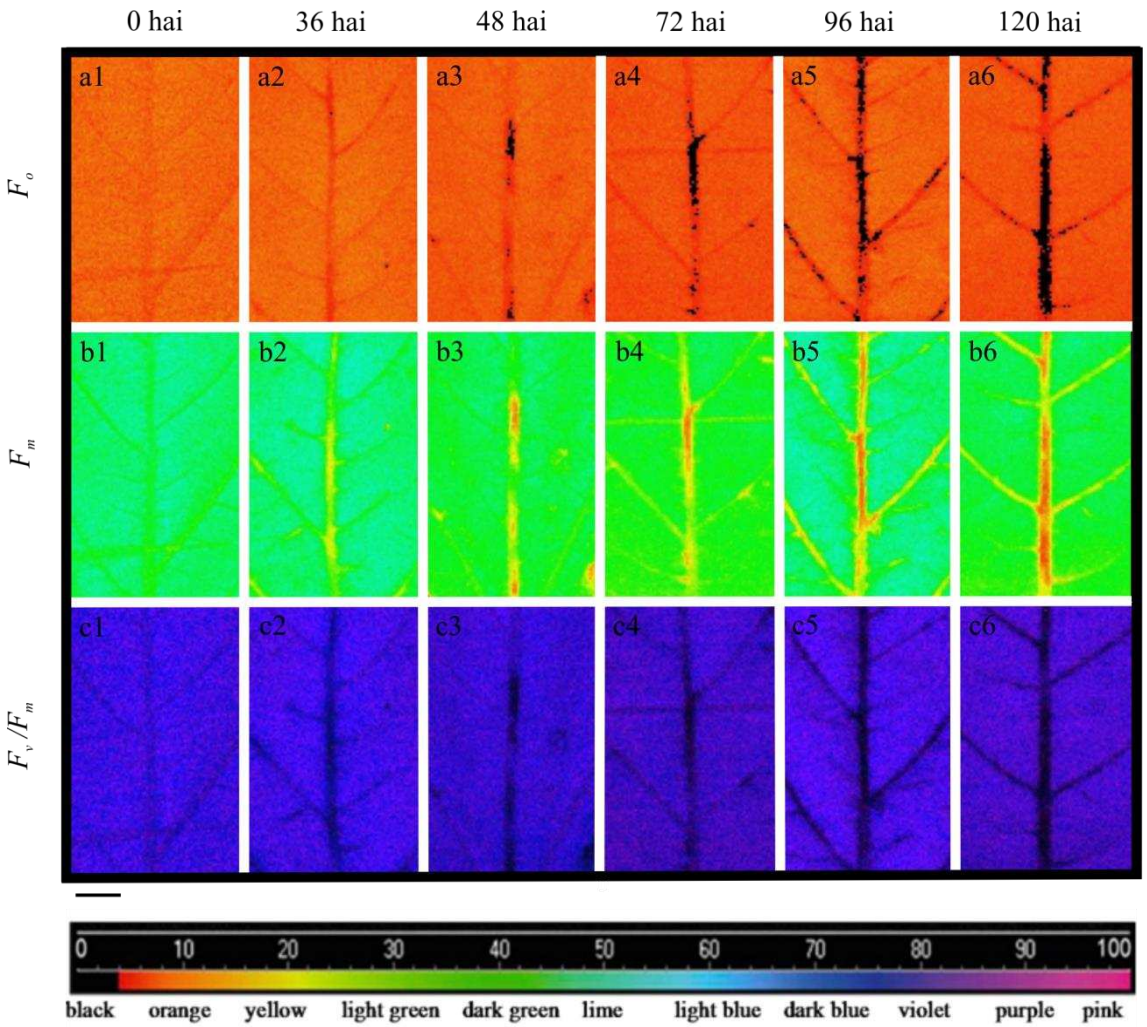
0 hai
36 hai
48 hai
72 hai
96 hai
120 hai
F_o
F_m
F_v/F_m
a1
a2
a3
a4
a5
a6
b1
b2
b3
b4
b5
b6
c1
c2
c3
c4
c5
c6
0
10
20
30
40
50
60
70
80
90
100
black
orange
yellow
light green
dark green
lime
light blue
dark blue
violet
purple
pink

Figure 4

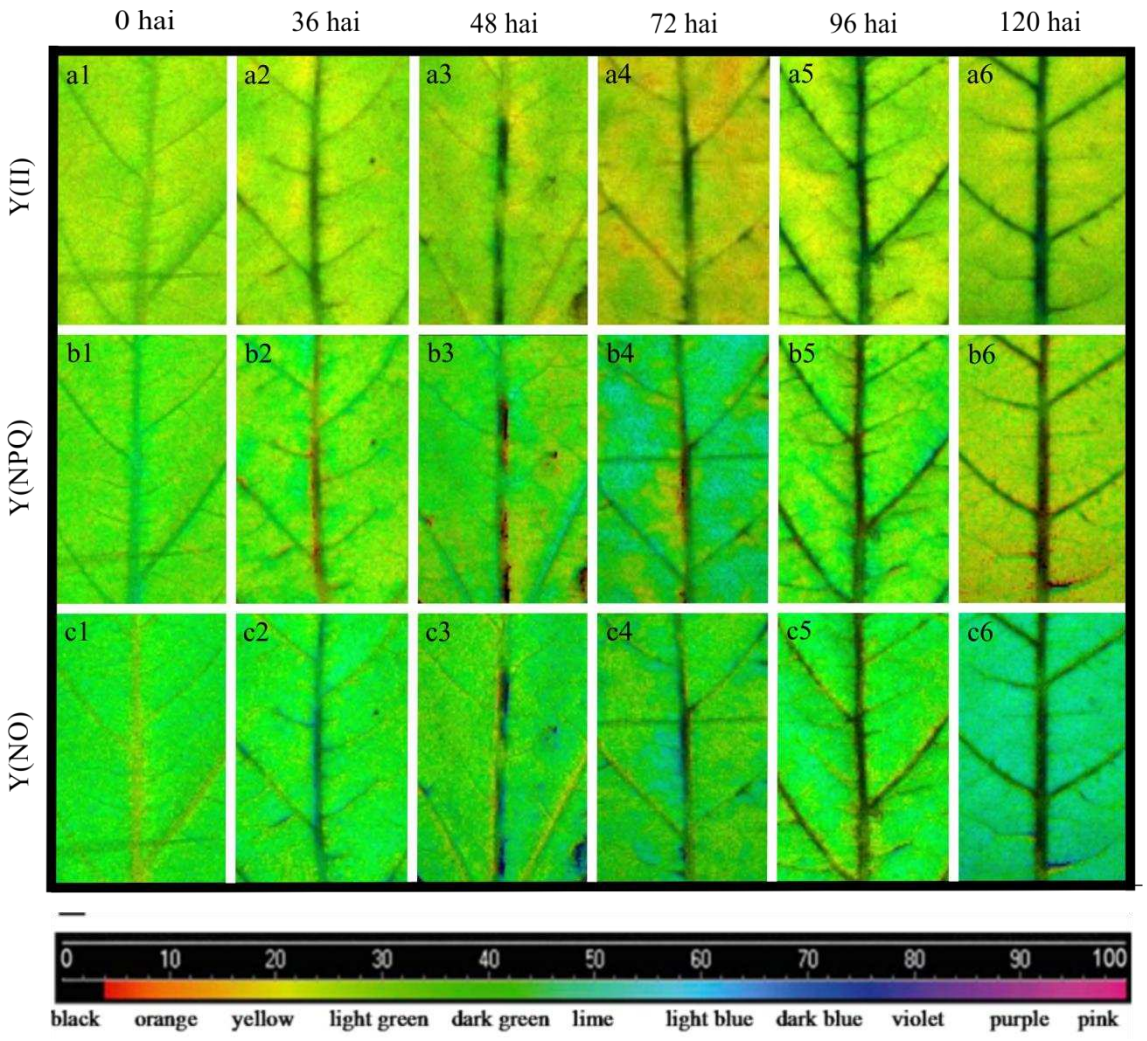
0 hai
36 hai
48 hai
72 hai
96 hai
120 hai
Y(II)
Y(NPQ)
Y(NO)
a1
a2
a3
a4
a5
a6
b1
b2
b3
b4
b5
b6
c1
c2
c3
c4
c5
c6
0
10
20
30
40
50
60
70
80
90
100
black
orange
yellow
light green
dark green
lime
light blue
dark blue
violet
purple
pink

Figure 5

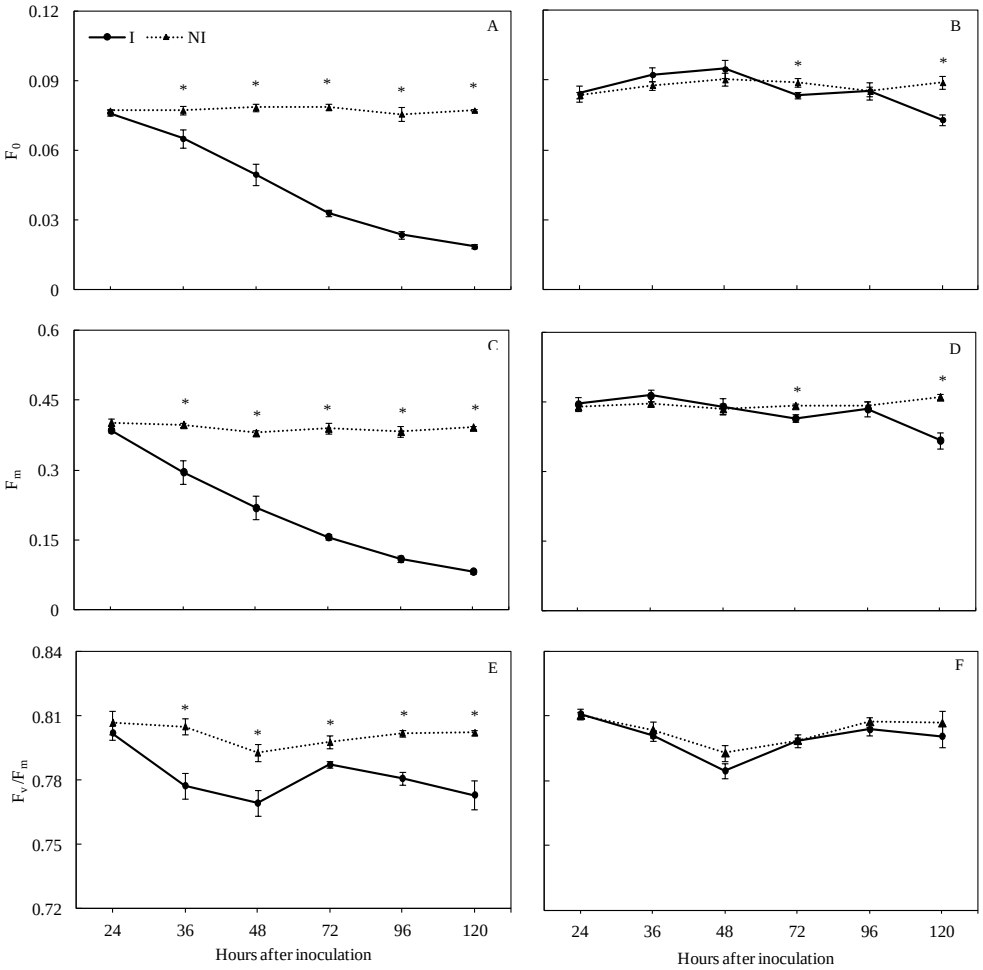
A
B
C
D
E
F
I
NI
F_0
F_m
F_v/F_m
0.12
0.09
0.06
0.03
0
0.6
0.45
0.3
0.15
0
0.84
0.81
0.78
0.75
0.72
24
36
48
72
96
120
Hours after inoculation
Hours after inoculation

Figure 6

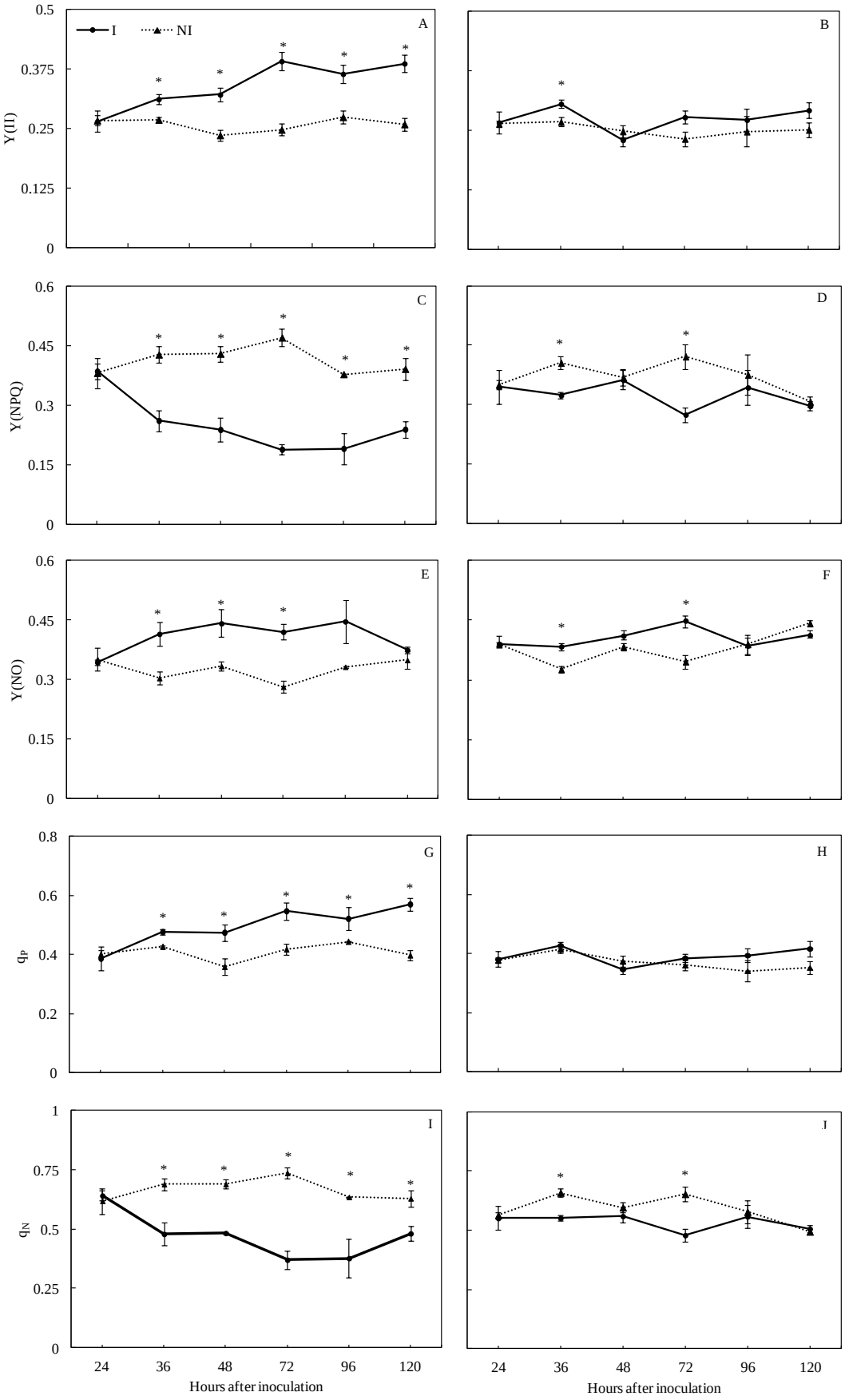
I
NI
A
B
C
D
E
F
G
H
I
J
Y(II)
Y(NPQ)
Y(NO)
qP
qN
0.5
0.375
0.25
0.125
0
0.6
0.45
0.3
0.15
0.8
0.2
1
0.75
24
36
48
72
96
120
Hours after inoculation

Figure 7