Silicon improves the photosynthetic performance of black pepper plants inoculated with *Fusarium solani* f. sp. *piperis*

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Abstract

This study evaluated the effect of silicon (Si)-supplementation in controlling the fusariosis in two *Piper nigrum* cultivars (Bragantina and Kottanadan). We hypothesized that susceptible cultivar improves the photosynthetic performance with Si-supplementation. Under greenhouse conditions, six-month-old plants were treated with silicon 30 and 15 d before inoculation with *Fusarium solani* f. sp. *piperis* and compared with untreated plants and with healthy control plants. All samples remained photosynthetically active after infection. Chlorophyll content, chlorophyll *a* fluorescence, and leaf gas-exchange parameters were less affected in Si-supplemented plants as well as healthy control plants. The net carbon assimilation, stomatal conductance, associated with higher internal carbon values suggest that the lower CO_2 influx in inoculated plants of Bragantina cultivar was caused by photochemical and biochemical limitations. Si-supplementation reduced the disease severity improving protection, apparently associated with the preservation of photosynthetic performance, especially for Bragantina cultivar.

Additional key words: photosynthesis; photosystem; plant defense; plant disease; plant nutrition; resistance responses.

Introduction

Root rot or fusariosis, caused by *Fusarium solani* f. sp. *piperis*, is the main disease for black pepper. This disease is restricted to Brazil (Tremacoldi 2010) reducing the reproductive cycle from 12 to 5–6 years (Lemos *et al*. 2011). The high yield losses caused by *F. solani* in black pepper, combined with the absence of efficient fungicides and resistant pepper cultivars (Tremacoldi 2010), makes *F. solani* one of the most important pathogens affecting black pepper yield in Brazil.

In the last years, many authors have demonstrated that silicon induces protection against several pathogens, as previously described for rice plants (*Oryza sativa* L.) infected by *Bipolaris oryzae* (Dallagnol *et al.* 2013), in soybean (*Glycine max* L.) infected by *Diaporthe phaseolorum* f. sp. *meridionalis*, which causes the stem canker (Debona *et al.* 2017), and in soybean infected by *Phakopsora pachyrhizi* and *Phytophthora sojae*, which cause the Asian soybean rust and root rot, respectively (Rasoolizadeh *et al.* 2018).

Overall, it appears that the Si influences the metabolic,

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Abbreviations: ABS/RC – absorption flux per reaction center of PSII; AUDPC – area under the curve of disease progress; BRA – Bragantina; C_a – ambient CO_2 concentration; CE – carboxylation efficiency; Chl – chlorophyll; Chl_{total} – total chlorophyll index; C_i – internal CO_2 concentration; C_i/C_a – intra/extracellular CO_2 concentration; DAI – day after inoculation; DI_0/RC – dissipated energy flux per reaction center of PSII; E – transpiration rate; F_0 – initial fluorescence; F_m – maximum fluorescence; F_v/F_m – maximum photochemical quantum yield of PSII; g_s – stomatal conductance; KOT – Kottanadan; OEC – oxygen-evolving complex; OJIP transient – fluorescence induction transient defined by the names of its intermediate steps; P_{700} – the primary electron donor of photosystems; PC – plastocyanin; PI_{total} – photosynthetic performance index; P_N – net CO_2 assimilation rate; PQ – plastoquinone; Q_A and Q_B – primary and secondary quinone electron acceptors of photosystem II, respectively; RC – reaction center; Si – silicon; V_J – relative variable Chl *a* fluorescence at the J-step; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency; φ_{D0} – quantum yield (at t = 0) of energy dissipation; φ_{E0} – quantum yield for electron transport (ET); φ_{R0} – quantum yield for reduction of the end electron acceptors at the PSI acceptor side; ψ_{E0} – efficiency with which a trapped exciton can move an electron into the electron transport chain.
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physiological, and/or structural activity of higher plants infected by pathogens (Fauteux *et al.* 2005, Datnoff *et al.* 2007, Meharg and Meharg 2015, Rodrigues *et al.* 2015). Physiologically, changes in the membrane permeability, degradation of cell membranes and photosynthetic pigments, protein synthesis, water potential, and absorption of nutrients and water occur, causing plant wilting and death (Wheeler and Rush 2001). Water relations of diseased plants can be affected by disturbances in stomatal functioning, increased resistance to flow and root water uptake, increasing the sensibility of host plants to water deficiency (Syvertsen *et al*. 1980). In addition, pathogen infection usually leads to the development of chlorotic and necrotic areas and a decreased photoassimilate production (Schreiber 2004), resulting from reduced leaf gas exchange and low performance of photochemical reactions associated to PSII (Berger *et al*. 2007). Also, the protection conferred to plants by Si might be related to its accumulation and polymerization in the cells, forming a mechanical barrier that hampers the development and growth of pathogens (Datnoff *et al.* 2007). However, mechanical barriers are not the only defense mechanism against pathogens stimulated by Si. Also, the accumulation of soluble phenolic compounds, lignin, phytoalexins, and other antimicrobial compounds in response to Si has been reported (Rodrigues *et al*. 2005, Fortunato *et al*. 2012). Cucumber (*Cucumis sativus* L.) plants inoculated with *Pythium ultimum* showed increased chitinase activity (Chérif *et al.* 1991). Moreover, Fawe *et al*. (1998) demonstrated that cells attacked either by *P. ultimum* or *Sphaerotheca fuliginea* accumulated phenolic material toxic to fungal structures, similar to the action described for phytoalexins.

Thus, as previously reported by Aucique-Pérez *et al*. (2014) and Rios *et al.* (2014), treatment with Si improves the net photosynthesis, increases the chlorophyll (Chl) content and improves the performance of photochemical reactions associated with PSII, which can be evaluated using *in vivo* Chl *a* fluorescence (OJIP transient and JIP-test), reducing the intensities of several diseases (damping off, leaf blights, leaf spots, galls, powdery mildews, root rots, rusts, and wilts) caused by different genera of bacteria, fungi, nematodes, and oomycetes as well as viruses in many economically important crops (Fortunato *et al.* 2012, Rodrigues *et al.* 2015). Also, silicon has unique biochemical properties that may explain its bioactivity as a regulator of plant defense mechanisms, in addition to acting as a modulator influencing the timing and extent of plant defense responses in a manner reminiscent of the role of secondary messengers in induced systemic resistance (Fauteux *et al.* 2005). In rice plants (*Oryza sativa* L.) infected by *Pyricularia oryzae*, the Si-supplementation improved protection of the photosynthetic apparatus against chronic photoinhibition and indirectly protected the photosystems against photodamages, which frequently result in a state of chronic hyperexcitation (Domiciano *et al.* 2015). In the literature, few studies report the use of OJIP transients and the JIP-test to quantify changes in photochemical activity caused by fungal diseases or their pathogens (Ajigboye *et al.* 2016).

The present study aimed to evaluate the photosynthetic performance of black pepper plants, cv. Bragantina (susceptible) and Kottanadan (resistant), in the *Piper nigrum* × *Fusarium solani* f. sp. *piperis* pathosystem, in order to evaluate the effect of Si-supplementation in controlling the fusariosis. We hypothesized that the black pepper plants susceptible to fusariosis improve the photosynthetic performance with Si-supplementation.

Materials and methods

Plant material and cultivation: Four-month-old plants of black pepper cv. Kottanadan (KOT) and Bragantina (BRA) were planted in 5-L plastic pots (one plant per pot) containing 5 kg of sterilized soil [*Haplic Planosol*, sandy clay loam, previously solarized according to Ghini (2004)] and maintained in greenhouse conditions under approximately 800–1,000 μmol(photon) m^{-2} s^{-1}, air temperature and relative humidity ranging from 24–30°C and 70–80%, respectively. Previously, chemical analysis of soil was performed to correct nutrient and pH levels.

Plants were fertilized every 2 d with 100 mL of a modified nutrient solution (Fernandes *et al.* 2002) for six months until the onset of treatment. Then, plants were supplemented with 1.0 g(calcium silicate) kg^{-1}(soil) (*Aldrich Chemistry*®) 30 and 15 d before inoculation (ISi+) and compared with nonsupplemented plants (Isi–) and with healthy control plants (NISi–). The calcium silicate contained 62% SiO_2 and 18% CaO. All sampling and measurements were conducted using completely expanded third or fourth leaf from the apex, 180 d after inoculation (DAI), except the gas-exchange measurements, which were performed on 150 DAI. Ten measurements were made per treatment. After the application of silicon, the plants were fertilized every 3 d with 50 ml of nutrient solution until the end of the experiment.

Inoculum production, spore suspension and inoculation procedure: *Fusarium solani* f. sp. *piperis* CML 2466, obtained from the Lavras Mycological Collection of Federal University of Lavras, Minas Gerais, was maintained in Petri dishes containing PDA (potato dextrose agar) medium at 4°C. For inoculation, 5-mm discs from the culture were transferred to Petri dishes containing the same medium. The plates were incubated at 25°C in a biochemical oxygen demand (BOD) chamber, with 12-h photoperiod for 15 d. The spore suspension was prepared by the addition of 20 ml of sterile distilled water to each plate. The spore count was performed in a Neubauer chamber and the suspension was adjusted to a concentration of 10^8 spores per ml. To each vessel, 10 ml of the spore suspension was added. The Koch postulates were observed.

Area under the curve of disease progress (AUDPC): The AUDPC was calculated according to Campbell and Madden (1990).

Chl *a* fluorescence and total Chl index: The Chl *a* fluorescence induction kinetics was measured at room temperature using a plant efficiency analyzer (*Handy-PEA*, *Hansatech*, King's Lynn, Norfolk, UK). Before the measurements, the leaves were dark-adapted for 30 min using a leaf clip (*Hansatech*). Light intensity reaching the leaf was 3,000 μmol(photon) m^{-2} s^{-1}, which was sufficient to generate maximal fluorescence for all the treatments. The fast fluorescence kinetics (F_0 to F_m) were recorded from 10 ms to 1 s with 120 fluorescence points. The fluorescence intensity at 50 μs (considered as F_0), 100 μs, 300 μs, 2 ms (F_J), 30 ms (F_I), and maximum fluorescence or F_m were collected and used to obtain the parameters for the JIP-test (Strasser and Strasser 1995). The total Chl index (Chl_{total}) content was obtained using a *ClorofiLOG* model *CFL 1030* chlorophyll meter.

Leaf gas-exchange variables: The net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were estimated from 7 to 11 h (solar time) using a portable open-system infrared gas analyzer (*LCpro-SD*, *ADC BioScientific*) under an external CO_2 concentration of 400 μmol mol^{-1}(air). All the measurements were conducted under artificial saturating photon irradiance [1,000 μmol(photon) m^{-2} s^{-1}]. The intrinsic water-use efficiency [$WUE_i = P_N/g_s$, μmol(CO_2) $mmol^{-1}$(H_2O)], water-use efficiency [$WUE = P_N/E$, μmol(CO_2) mol^{-1}(H_2O)], carboxylation efficiency ($CE = P_N/C_i$, μmol $mmol^{-1}$ ppm^{-1}) and ratio between intra/extra-cellular CO_2 concentrations (C_i/C_a) were calculated.

Experimental design and data analysis: The experiment was performed using a randomized block design with plants noninoculated and inoculated with *Fusarium solani* f. sp. *piperis* treated and nonsupplemented with silicon (ISi+, Isi–, and NISi–). Each replication consisted of one plastic pot with one plant. The significance of treatment effects on all variables analyzed was tested with analysis of variance (*ANOVA*) and the mean treatment values for each cultivar were compared by the *Scott-Knott*'s test ($p<0.05$) using the software *Genes 3.1* (Cruz 2016). The *Pearson*'s linear correlation technique was used to determine the relationships among the photosynthetic traits and AUDPC (*see* Table 1).

Results

Area under the curve of disease progress (AUDPC): The Si-supplementation reduced the area under the curve of disease progress in both pepper cultivars infected by *Fusarium* (ISi+). However, AUDCP values were lower in Kottanadan than that in Bragantina (–58% and –33%, respectively) (Fig. 1).

Chl *a* fluorescence and total Chl index: Noninoculated (NISi–) and inoculated plants with and without Si-supplementation (ISi+ and Isi–, respectively) showed OJIP curves with typical polyphasic behavior, with well-defined J and I intermediate steps (Fig. 2). For Kottanadan pepper cultivar, no change was observed at J, I, and P steps in all treatments (Fig. 2*A*). In contrast, Si-supplementation resulted in increased J, I, and P steps in Bragantina pepper cultivar, which was similar to NISi– (Fig. 2*B*). There was a significant difference for F_0, F_m, F_v/F_m, V_J, DI_0/RC, φ_{E0}, φ_{R0}, PI_{total}, Chl_{total}, ABS/RC, and φ_{D0} (Table 2). F_0, ABS/RC, DI_0/RC, and φ_{D0} values were higher in BRA independently of treatment (Table 2). The Si+ increased F_m, φ_{E0}, and ψ_{E0} values in inoculated Bragantina plants. φ_{R0} values increased in Kottanadan plants inoculated and Si-supplemented (ISi+), which was similar to NISi–. Also, Si-supplementation resulted in changes of PI_{total}. For Kottanadan and Bragantina pepper cultivars, there were increases of PI_{total} values by approximately 49.8 and 34.2%, respectively. Increases of ≈ 24 and 11.7% in Chl_{total} occurred in Kottanadan and Bragantina cultivars, respectively, after Si-supplementation (ISi+) compared to ISi– (Table 2).

Leaf gas-exchange variables: The results of the present study show that photosynthesis was impaired by inoculation in those plants nonsupplemented with Si (ISi–). However, P_N, g_s, WUE, WUE_i, E, and CE values increased in ISi+ for both pepper cultivars, compared with the corresponding control, ISi– and NISi– (Fig. 3*A*,*B*,*D*–*G*). Si-supplementation reduced C_i in Bragantina pepper cultivar and increased C_i/C_a (Fig. 3*B*,*H*).

Discussion

The use of Si for resistance against plant diseases has been studied for several decades and several researchers have reported that Si can increase the resistance of plants against pathogenic fungi (Chérif *et al.* 1994, Fauteux *et al.* 2005, Datnoff *et al.* 2007, Resende *et al.* 2012, Debona *et al.* 2014, Tatagiba *et al.* 2016). In this study, in order to evaluate the efficiency of Si for the control of fusariosis caused by *F. solani* f. sp. *piperis*, a detailed photosynthetic analysis was carried out, comparing two black pepper cultivars, Kottanadan and Bragantina. Overall, pepper plants with Si+ showed improved photosynthetic capacity and lower disease progress. To the best of our knowledge, this study is the first to evaluate the efficiency of Si for the control of fusariosis in black pepper infected by *F. solani* f. sp. *piperis* based on photosynthetic traits.

It was observed that soil Si-supplementation (Si+) influenced the incidence of disease in both pepper cultivars, with reduction of AUDCP values for Kottanadan (≈ 58%) and for Bragantina (≈ 33%) infected by *Fusarium* compared with the ISi– plants (Fig. 1). These results allow us to attribute higher resistance to the Kottanadan compared with ISi– and NISi– plants, as well as increased resistance in Bragantina, which is considered by farmers susceptible to the presence of the fungus. Differences in the incubation period reflect differences in the pathogen growth rate in the host plants and, consequently, in the epidemic progress rate, being an important component of resistance (Spósito *et al*. 2004). In this context, it was observed that cv. Kottanadan had longer incubation periods than that of Bragantina, indicating higher resistance when evaluated by this parameter. In this study, these results are supported by negative correlation between incubation period and AUDCP values ($r = -0.8747$, $p=0.0013$). According to

Table 1. *Pearson*'s correlation coefficients among the photosynthesis, JIP-test parameters, total chlorophyll index (Chl_{total}), and area under the curve of disease progress (AUDCP) of black pepper plants cultivars Kottanadan and Bragantina, healthy control plants (NISi–), inoculated plants with *Fusarium solani* f. sp. *piperis* Si-supplemented (ISi+) or nonsupplemented (ISi–). Significant at 1% (**) and at 5% probability (*) according to the *Student*'s *t* test; ns = not significant. Values are the means ± SE, $n = 10$. F_v/F_m – maximum photochemical quantum yield of PSII; ABS/RC – absorption flux per reaction center of PSII; DI_0/RC – dissipated energy flux per reaction center of PSII; PI_{total} – photosynthetic performance index; F_0 – initial fluorescence; F_m – maximum fluorescence; C_i – internal CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; P_N – net CO_2 assimilation rate; CE – carboxylation efficiency; WUE – water-use efficiency; WUE_i – intrinsic efficiency of water use; C_i/C_a – intra/extracellular CO_2 concentration; V_J – relative variable Chl *a* fluorescence at the J-step; φ_{E0} – quantum yield for electron transport; φ_{D0} – quantum yield (at t = 0) of energy dissipation; φ_{R0} – quantum yield for reduction of the end electron acceptors at the PSI acceptor side; ψ_{E0} – efficiency with which a trapped exciton can move an electron into the electron transport chain.

	AUDCP	F_0	F_m	F_v/F_m	V_J	ABS/RC	DI_0/RC	φ_{E0}	φ_{D0}	φ_{R0}	ψ_{E0}	PI_{total}	Chl_{total}	P_N	C_i	g_s	E	WUE	WUE_i	CE	C_i/C_a
AUDCP	1																				
F_0	0.429^{ns}	1																			
F_m	–0.677**	–0.629**	1																		
F_v/F_m	–0.535*	–0.740**	0.480**	1																	
V_J	0.046*	–0.104^{ns}	–0.253^{ns}	0.041*	1																
ABS/RC	0.160**	0.687**	–0.633**	–0.573**	0.344^{ns}	1															
DI_0/RC	0.130*	0.595*	–0.642*	–0.507*	0.326^{ns}	0.932**	1														
φ_{E0}	–0.283**	–0.546*	0.689*	0.508**	–0.668^{ns}	–0.813*	–0.791*	1													
φ_{D0}	0.373**	0.762**	–0.795**	–0.674*	0.293^{ns}	0.935**	0.871**	–0.868**	1												
φ_{R0}	–0.394*	–0.713**	0.468*	0.876**	–0.066^{ns}	–0.691^{ns}	–0.635**	0.534**	–0.685**	1											
ψ_{E0}	0.092**	–0.107^{ns}	0.289^{ns}	0.092^{ns}	–0.896^{ns}	–0.548**	–0.505*	0.813^{ns}	–0.499^{ns}	0.201^{ns}	1										
PI_{total}	–0.390**	–0.614*	0.467^{ns}	0.784**	–0.402^{ns}	–0.655**	–0.568^{ns}	0.702*	–0.691*	0.781*	0.506^{ns}	1									
Chl_{total}	–0.488**	–0.769**	0.445**	0.842**	0.164^{ns}	–0.536**	–0.513**	0.350**	–0.561**	0.905^{ns}	–0.049**	0.723**	1								
P_N	–0.488**	–0.751**	0.521^{ns}	0.912**	0.094^{ns}	–0.690**	–0.673**	0.500**	–0.730**	0.791**	0.033^{ns}	0.679**	0.802**	1							
C_i	0.473**	0.591**	–0.394**	–0.542*	0.209^{ns}	0.346*	0.327**	–0.407**	0.381**	–0.496*	–0.149^{ns}	–0.561*	–0.590**	–0.495*	1						
g_s	–0.483*	–0.655^{ns}	0.479^{ns}	0.825**	0.263^{ns}	–0.510**	–0.515**	0.338**	–0.608**	0.761**	–0.118^{ns}	0.566**	0.787**	0.836^{ns}	–0.188^{ns}	1					
E	–0.180**	–0.340^{ns}	0.429^{ns}	0.423^{ns}	0.262^{ns}	–0.402^{ns}	–0.493*	0.197^{ns}	–0.449^{ns}	0.473^{ns}	–0.182**	0.124^{ns}	0.466^{ns}	0.504*	0.047^{ns}	0.714*	1				
WUE	–0.318*	–0.637**	0.559*	0.627*	–0.252^{ns}	–0.591*	–0.563*	0.693*	–0.692*	0.400*	0.363^{ns}	0.500^{ns}	0.351**	0.678**	–0.533**	0.345*	0.172^{ns}	1			
WUE_i	–0.393**	–0.492**	0.251**	0.666**	–0.103^{ns}	–0.339**	–0.266**	0.362**	–0.382**	0.457**	0.113^{ns}	0.565**	0.505**	0.648**	–0.780**	0.210**	–0.119^{ns}	0.722**	1		
CE	–0.613**	–0.760**	0.621**	0.860**	0.092^{ns}	–0.645**	–0.619*	0.476*	–0.710**	0.750**	–0.007^{ns}	0.658**	0.820**	0.949**	–0.598**	0.727**	0.420^{ns}	0.680**	0.736**	1	
C_i/C_a	0.407**	0.543^{ns}	–0.421**	–0.637**	0.081^{ns}	0.493**	0.430**	–0.465**	0.545**	–0.439**	–0.144^{ns}	–0.513*	–0.477^{ns}	–0.699**	0.685**	–0.256**	–0.047^{ns}	–0.778**	–0.936**	–0.804**	1

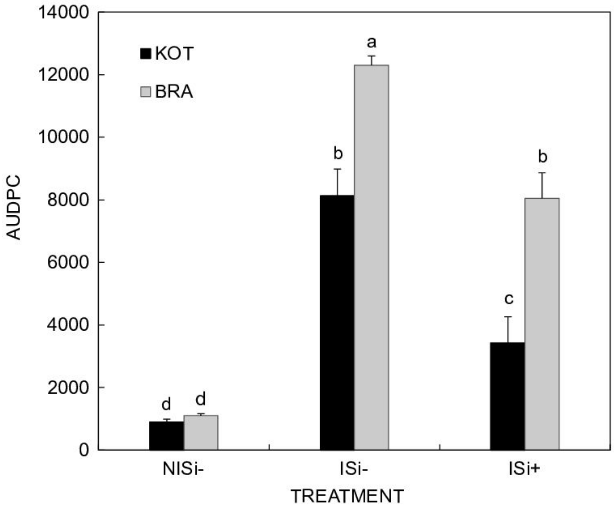

Fig. 1. Area under the curve of disease progress (AUDPC). evaluated at 180 d after inoculation, in two black pepper cultivars (KOT – Kottanadan and BRA – Bragantina), in healthy control plants (NISi–), Si-supplemented (ISi+) or nonsupplemented (ISi–). All plants were inoculated with *Fusarium solani* f. sp. *piperis* except the control. Values are the means ± SE, $n = 10$. Columns followed by *the identical letter* are not statistically different according to the *Scott-Knott*'s test at 1% probability ($p<0.01$).

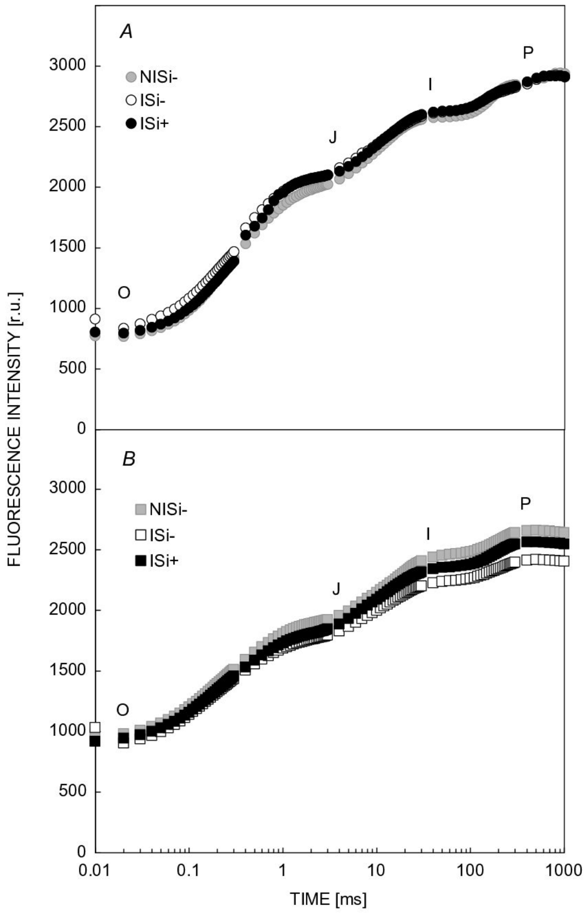

Fig. 2. Chlorophyll *a* fluorescence transient curve (OJIP) in Kottanadan (KOT) (*A*) and Bragantina (BRA) (*B*) cultivars, healthy control plants (NISi–), inoculated with *Fusarium solani* f. sp. *piperis* Si-supplemented (ISi+) or nonsupplemented (ISi–). The leaves were previously dark-adapted for 30 min ($n = 10$).

Chérif *et al.* (1994), two hypotheses for the Si-enhanced resistance to diseases and pests have been proposed. One is that Si deposited on the tissue surface forms physical and biochemical barriers, which, consequently, reduces the effects of pathogens (Bélanger *et al.* 2003). It prevents physical penetration and/or makes the plant cells less susceptible to enzymatic degradation by fungal pathogens. This mechanism is supported by the positive correlation between the Si content and the degree of suppression of diseases and pests (Ratnayake *et al.* 2016). The other one is that Si functions as a signal to induce the production of phytoalexin. According to Berhow and Vaughn (1999), phytoalexins are produced by plants in response to invasion or contact with pathogenic microorganisms. Phytoalexins may be flavones, flavonoids, and isoflavonoids that inhibit the growth of various fungal species. The impact of Si on host resistance components can partially explain why the resistance of susceptible cultivars can be increased (Resende *et al.* 2013). These positive effects of Si have traditionally been associated with its role in alleviating abiotic and biotic stresses in addition to improving resistance to lodging and increasing the erectness of leaves, which allows better light transmittance through rice canopies and thereby potentially enhances whole-plant photosynthesis (Tamai and Ma 2008).

For both pepper cultivars, the OJIP transients showed a typical polyphasic behavior with the fluorescence signal rising from the initial fluorescence level (F_0) to the maximum level (F_m) with well-defined intermediate J and I step. These results demonstrate that all samples were photosynthetically active after infection by *Fusarium* (Fig. 2). The relative stability of OJIP curves observed in Kottanadan pepper cultivar indicates higher homogeneity of samples (Fig. 2*A*), which can be associated with higher ability to balance the photosynthetic electron transport under *Fusarium* infection. Furthermore, the Si increased the fluorescence yield in Bragantina pepper cultivar, evidenced by increases mainly of I and P steps of the OJIP curves (Fig. 2*B*), which refer to the processes of reduction of the final electron acceptors at the PSI acceptor side, such as ferredoxin (Fd) and NADP (Yusuf *et al.* 2010), indicating higher pool of the final electron acceptors of PSI in ISi+ plants. This reflects more efficient electron transfer to the PSI acceptor side. A positive increase in the I–P phase may indicate enhanced capacity of the final acceptor of PSI to receive electrons and form NADPH (Stirbet and Govindjee 2011). According to Gill and Tuteja (2010), the electron flow from the excited photosystem centers is directed to NADP, which is reduced to NADPH. However, it remains unclear whether the activity of the final electron acceptors of PSI (ferredoxin) increases with stress conditions or whether there is another mechanism increasing the apparent efficiency of PSI in receiving electrons from PSII, because not all of the electrons

Table 2. JIP-test parameters and total chlorophyll index (Chl_{total}) of black pepper plants cultivars Kottanadan and Bragantina, healthy control plants (NISi–), inoculated plants with *Fusarium solani* f. sp. *piperis* Si-supplemented (ISi+) or nonsupplemented (ISi–). evaluated at 180 d after inoculation. In the line, means followed by *the identical letter* are not statistically different according to by *Scott-Knott*'s test. Values are the means ± SE, *n* = 10. ** – significant at 1% probability ($p<0.01$); * – significant at 5% probability ($p<0.05$). F_0 – initial fluorescence; F_m – maximum fluorescence; F_v/F_m – maximum photochemical quantum yield of PSII; V_J – relative variable Chl *a* fluorescence at the J-step; ABS/RC – absorption flux per reaction center of PSII; DI_0/RC – dissipated energy flux per reaction center of PSII; φ_{E0} – quantum yield for electron transport; φ_{D0} – quantum yield (at t = 0) of energy dissipation; φ_{R0} quantum yield for reduction of the end electron acceptors at the PSI acceptor side; ψ_{E0} – efficiency with which a trapped exciton can move an electron into the electron transport chain; PI_{total} – photosynthetic performance index.

	Kottanadan			Bragantina		
	NISi–	ISi–	ISi+	NISi–	ISi–	ISi+
F_0	772.33 ± 9.00[d**]	912.06 ± 47.62[c**]	802.70 ± 16.87[d**]	982.83 ± 22.49[b**]	1034.42 ± 54.16[a**]	919.40 ± 14.66[c**]
F_m	2.940.27 ± 16.23[a**]	2.914.71 ± 38.05[a**]	2.909.31 ± 36.78[a**]	2.647.74 ± 18.61[b**]	2.398.15 ± 22.25[c**]	2.565.27 ± 23.62[b**]
F_v/F_m	0.74 ± 0.00[a**]	0.68 ± 0.03[b**]	0.73 ± 0.03[a**]	0.63 ± 0.04[b**]	0.56 ± 0.03[c**]	0.65 ± 0.02[b**]
V_J	0.58 ± 0.56[a**]	0.59 ± 0.00[a**]	0.57 ± 0.00[a**]	0.54 ± 0.03[b**]	0.57 ± 0.00[a**]	0.53 ± 0.00[b**]
ABS/RC	3.30 ± 0.09[c*]	3.59 ± 0.53[c*]	3.08 ± 0.31[c*]	5.70 ± 1.45[a*]	4.89 ± 0.42[b*]	5.08 ± 0.06[a*]
DI_0/RC	1.10 ± 0.04[b**]	1.42 ± 0.05[b**]	1.04 ± 0.04[b**]	3.35 ± 1.34[a**]	2.48 ± 0.21[c**]	2.82 ± 0.20[b**]
φ_{E0}	0.30 ± 0.07[a**]	0.28 ± 0.00[a**]	0.30 ± 0.00[a**]	0.27 ± 0.05[a**]	0.25 ± 0.00[b**]	0.27 ± 0.01[a**]
φ_{D0}	0.32 ± 0.05[c*]	0.36 ± 0.02[b*]	0.33 ± 0.01[c*]	0.44 ± 0.06[a*]	0.44 ± 0.02[a*]	0.43 ± 0.00[a*]
φ_{R0}	0.12 ± 0.00[a**]	0.09 ± 0.00[b**]	0.13 ± 0.01[a**]	0.09 ± 0.06[b**]	0.09 ± 0.00[b**]	0.09 ± 0.00[b**]
ψ_{E0}	0.43 ± 0.01[b**]	0.43 ± 0.03[b**]	0.44 ± 0.02[b**]	0.43 ± 0.04[b**]	0.43 ± 0.04[b**]	0.46 ± 0.02[a**]
PI_{total}	2.36 ± 0.11[b**]	1.79 ± 0.18[c**]	3.58 ± 0.11[a**]	1.90 ± 0.64[c**]	1.41 ± 0.23[c**]	2.13 ± 0.30[b**]
Chl_{total}	55.47 ± 0.48[b**]	51.74 ± 0.68[b**]	67.37 ± 0.57[a**]	46.65 ± 0.60[c**]	40.97 ± 0.59[d**]	46.44 ± 0.23[c**]

that reach ferredoxin result in the formation of NADPH (Shikanai 2007). The electrons originating from the reduced ferredoxin connected with PSI can interact with ferredoxin-dependent enzymes to assimilate inorganic nitrogen, sulfur to fix molecular nitrogen and regulate the CO_2-assimilation cycle, lowering the electron flux toward production of NADPH (Fukuyama 2004). Also, considering the cyclic flow of electrons, which depends on the electron transfer reactions of PSI, the electrons are recycled from ferredoxin to the plastoquinone pool, which results in ATP synthesis (Shikanai 2007). Thus, it can be assumed that the enhanced I–P phase in Bragantina cultivar in ISi+ and NISi– reflects the increased electron flux toward the cyclic flow in order to improve metabolic energy (ATP) in plants infected by *Fusarium*. Similar results were observed by Oukarroum *et al.* (2009), Gomes *et al.* (2012) and Guha *et al*. (2013).

In this study, the increase of F_0 observed in both pepper cultivars inoculated without Si (ISi–) is attributed by Schreiber and Armond (1978) as being a perturbation of thylakoid membranes leading to the separation of the light-harvesting complex associated with the PSII core complex (LHCII) and a block of the PSII reaction center. Other authors further attribute the increase of inactive CR number or the inactivation of OEC as cause of increased F_0 values (Kalaji *et al.* 2011). This result is based on the higher ABS/RC, DI_0/RC, and φ_{D0} values reported for the Bragantina cultivar, independently of Si-supplementation (Table 2). Also, increased F_m values were observed for Bragantina plants in ISi+, which was similar to those values described for the healthy control plants (NISi–) (Table 2), indicating increases the efficiency of light use by Si-supplementation as previously reported by Nwugo and Huerta (2008). Furthermore, a sharp decrease of F_m values occurred in ISi–, indicating functional alterations on photosystems followed by drastic reductions of Chl_{total}, which ultimately affected the efficiency of excitation energy from the light-harvesting capacity (Rios *et al.* 2017).

Although with lower extension for Bragantina pepper cultivar, the increase of F_v/F_m in ISi+ and healthy control plants (NISi–), compared with ISi– plants, suggests that photosynthetic electron transport was not fully blocked during the *F. solani* infection process and that Si presence increases the photosynthetic efficiency. According to Oukarroum *et al.* (2015) and Khan *et al*. (2017), Si has a role in enhancing detoxification of reactive oxygen species under stress conditions and, consequently, Chl_{total} increases, which leads to higher F_v/F_m (Table 2). Tatagiba *et al.* (2016) reported that Si maintain the photochemical performance of rice plants infected by *Monographella albescens*. According to Maxwell and Johnson (2000), increased F_v/F_m under Si-supplementation reduced the occurrence of photo-inhibition, where F_v/F_m has a significant positive correlation with P_N and Chl values, as observed in our results (Table 1). The F_v/F_m ratio indicates the potential photochemical efficiency of PSII and is calculated as the proportional photosynthetic rate in intact leaves (Björkman and Demmig 1987).

Furthermore, a marked increase of PI_{total} values occurred in Kottanadan and Bragantina pepper cultivars when Si-supplemented, confirming the role of Si in improving the electron transport chain until reduction of the final acceptors of PSI. This positive role of Si has been reported in other studies (Chen *et al.* 2011, Habibi and Hajiboland 2013, Aucique-Pérez *et al.* 2014). PI_{total} represents the energy flow efficiency of the photosynthetic transport

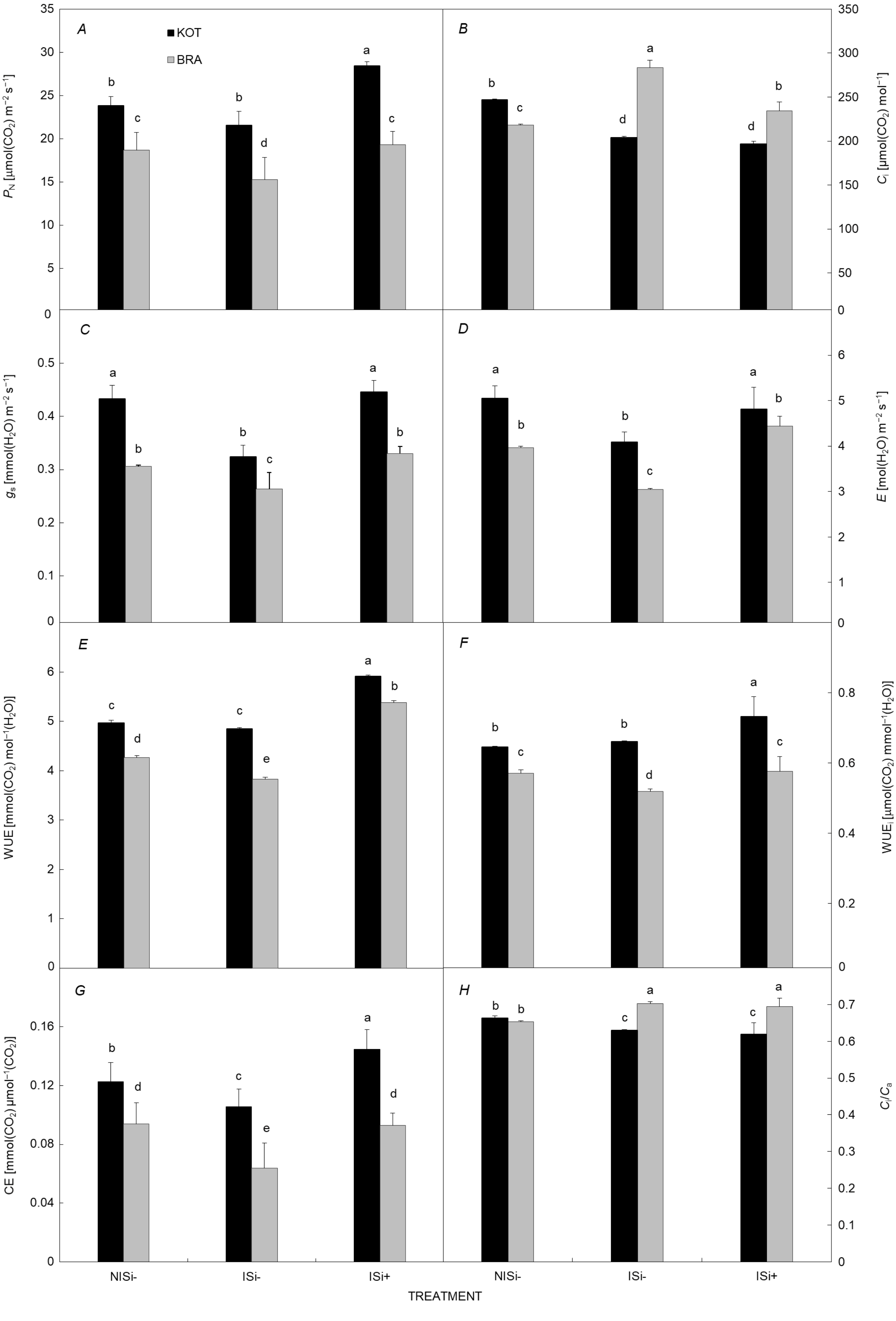

Fig. 3. Net photosynthetic rate (P_N) (*A*), internal CO_2 concentration (C_i) (*B*), stomatal conductance (g_s) (*C*), transpiration rate (*E*) (*D*), water-use efficiency (WUE) (*E*), intrinsic water-use efficiency (WUE_i) (*F*), carboxylation efficiency (CE) (*G*), and intra/extracellular CO_2 concentrations (C_i/C_a) (*H*) of two black pepper cultivars (KOT – Kottanadan. BRA – Bragantina), healthy control plants (NISi–), supplemented and nonsupplemented with silicon (ISi+ and ISi–, respectively). All plants were inoculated with *Fusarium solani* f. sp. *piperis* except the control. Values are the means ± SE, *n* = 10. Columns followed by *the identical letter* are not statistically different according to the *Scott-Knott*'s test at 5% probability (p<0.05).

chain (Yusuf *et al.* 2010) and is closely related to the overall growth and survival of plants under stress conditions and has been described as a very sensitive parameter for the JIP-test.

Finally, we observed that φ_{E0} and ψ_{E0} decreased in Bragantina plants infected by *Fusarium* (Table 2). φ_{E0} and ψ_{E0} express the quantum yield of electron transport and the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (or yield of Q_A^- reoxidation), respectively. These results suggest fast accumulation of Q_A^- in PSII reaction centers, showing a rise of V_J due to the blockage of PSII electron flow beyond Q_A^-, inactivating PSII RCs (Chen *et al.* 2015). Similar results were found by Mehta *et al.* (2010) and Kalaji *et al*. (2018). In contrast, increases in φ_{R0} were observed for Kottanadan plants in ISi+ and NISi–, indicating efficient electron transfer towards the end electron acceptors of PSI (Kalaji *et al.* 2018).

To better explore the role of Si improving the resistance in pepper cultivars against infection by *F. solani*, a detailed gas-exchange analysis was carried out (Fig. 3). Regardless of cultivar, Si-supplementation improved the gas-exchange performance of plants inoculated with *F. solani*. Resende *et al.* (2012) reported that fungal infections negatively affect P_N due to the physical limitations of CO_2 influx into leaves. In the *Piper nigrum* × *Fusarium solani* pathosystem, the presence of enzymes and toxins produced by the fungus can prevent the functioning of xylem vessels to transport water and photoassimilates (Leslie and Summerell 2006), triggering the closure of the stomata. The Si application (ISi+) resulted in significant (p<0.05) increases in P_N and g_s, which were associated with decreases in C_i in the Bragantina cultivar (Fig. 3*A*–*C*). These results suggest that the higher influx of CO_2 into leaves caused by stomatal opening was a prominent factor in the increase of P_N in both pepper cultivars treated with Si. In contrast, in Si– plants (ISi–), the lower P_N, g_s, and E values (Fig. 3*A*,*C*,*D*), associated with higher C_i values (Fig. 3*B*), suggest that photosynthesis of the Bragantina cultivar was limited by nonstomatal factors. Additional support for this conclusion comes from the nonsignificant correlation between P_N and g_s (r = 0.83), the negative correlation between P_N and C_i (r = 0.49, p<0.01), and the positive correlation between blast severity and C_i (r = 0.47, p<0.01). Similar results have been found in other pathosystems and these results have usually been interpreted as an indication of biochemical, rather than diffusive, limitations of photosynthesis (Pinkard and Mohammed 2006, Dallagnol *et al.* 2011).

According to Alves *et al*. (2011), reductions of P_N values in infected plants are unlikely to be only associated with lower CO_2 entry into the leaves, but also with some biochemical limitation in CO_2 fixation within the chloroplasts. Frequently, alterations in leaf photochemistry and carbon metabolism are related to lower Rubisco activity and changes in the capacity for ribulose-1,5-bisphosphate regeneration (Hiscox and Israelstam 1979). Reductions of E in soybean leaves infected by *Phakopsora pachyrhizi* can be associated with reduced g_s, and therefore associated with stomatal closure (Rios *et al.* 2017). Reductions of E maintain a favorable water status in the infected leaves (Resende *et al.* 2012). Reductions in the values of both E and g_s have been also reported by Alves *et al.* (2011). According to Debona *et al.* (2014), decreases in E were mainly governed by g_s, which was further corroborated by the positive and significant correlation between E and g_s, supporting our results (Table 1).

In despite of the reductions in g_s, fungus-induced changes in photosynthesis were largely related to the light capture capacity and the reduced capacity of the mesophyll to fix CO_2 (Dallagnol *et al.* 2011). Thus, the decrease of photosynthetic productivity in response to the disease resulted in a lower amount of healthy leaf area, mainly for Bragantina cultivar in ISi–. Furthermore, the reduction of photosynthetic activity in diseased leaves was related to decomposition of Chl (Table 2), as indicated by continuous yellowing of photosynthetic foliar tissues. Similar results have been found in other pathosystems (*Oryza sativa* × *Bipolaris oryzae*; *Sorghum* × *Colletotrichum sublineolum*; *Triticum aestivum* × *Pyricularia oryzae*) and were interpreted as an indication of biochemical limitations of photosynthesis (Pinkard and Mohammed 2006, Dallagnol *et al.* 2011, Resende *et al.* 2012, Aucique-Pérez *et al.* 2017, 2019). Finally, the results of CE (Fig. 3*G*) indicate that the decreased P_N values were mainly associated with lower apparent Rubisco activity (Debona *et al.* 2014) supporting the biochemical limitations of photosynthesis as previously described above. All leaf gas-exchange variables were less affected in those more resistant pepper cultivars infected by *F. solani* (Fig. 3).

In conclusion, the chlorophyll content, chlorophyll *a* fluorescence, and leaf gas-exchange parameters were less affected in Si-supplemented plants (ISi+) as well as healthy control plants (NISi–). Although the two pepper cultivars had shown high disease severity compared with healthy control plants (NISi–), the analysis of the OJIP phases showed that the plants inoculated with *F. solani* remained photosynthetically active after the infection. Damages on the photosynthesis and chlorophyll content were significantly minimized in both cultivars with Si-supplementation. Finally, the results of this study provide further evidence that black pepper's photosynthetic machinery can be greatly protected in susceptible plants supplied with Si before infection by *F. solani*. This protection is apparently associated with the preservation of photosynthetic performance.

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