INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–1286/2020/24–5–1125–1134
DOI: 10.17957/IJAB/15.1541
http://www.fspublishers.org

Full Length Article

Physiological Responses of Cocoa (*Theobroma cacao*) to Variable Supply of Iron under Flooding

Priscila da Conceição de Souza Braga[1], Marcos Góes Oliveira[1*], Marco Antonio Galeas[2] Aguilar, Fábio Luiz Partelli[1] and Weslaine Pinheiro Martins[1]

[1]*Centro Universitário Norte do Espírito Santo, Departamento Ciências Agrárias e Biológicas, Universidade Federal do Espírito Santo, Rod. BR 101 Norte, 29932-540, São Mateus, Brazil*

[2]*Centro de Pesquisas do Cacau, Estação Experimental Filogônio Peixoto, Caixa Postal 102, 29901-970 Linhares, Brazil*

[*]For Correspondence: mgoesoliveira@hotmail.com

Received 15 August 2019; Accepted 02 May 2020; Published 31 August 2020

Abstract

Large amounts of iron (Fe) in flood conditions increase its availability to the plants, generating a double stress condition. The present study evaluated the effects of different concentrations of iron in hypoxic or anoxic condition in different cacao genotypes. Two cacao genotypes, TSH 1188 and SIAL 70, were evaluated with Fe dose of 44.5 μmol L^{-1} (recommended dose); 133.5 μmol L^{-1} (high dose) and 400.5 μmol L^{-1} (very high). In addition, evaluations of gaseous exchanges and indices of chlorophyll *a*, *b* and total were carried out. Excess iron reduced photosynthetic, stomatal conductance, and transpiration rate. The results showed that these physiological changes are dependent on the ambient condition, as well as hypoxic or anoxic condition. The levels of chlorophyll *a* and *b* were also affected by the concentration of iron; however, according to the type of stress, it showed the possibility of later recovery of these chloroplast pigment contents. The present study showed that the two genotypes of *T. cacao* are tolerant to the anoxic condition and excess of Fe, however, showed different responses, indicating involvement of different mechanisms to deal with each type of stress.

Keywords: Gas exchange; Photosynthesis; Anoxia; Interaction

Introduction

Theobroma cacao L. (cocoa) is an economically important crop for tropical (producer) countries and consumers, due to its butter and cocoa, cosmetics, and foods (Almeida and Valle 2007). This has a global production scale of 9.7 million hectares with production of 4.5 million tons in 2017/2018 (FAOSTAT 2016 and ICCO 2018).

Cacao originates from tropical rainforest region from Peru to Mexico, where the climate is hot and humid with an average temperature of about 25°C and has an annual precipitation between 1500 and 2000 mm (CEPLAC 2018). Its cultivation requires deep soils with good water retention capacity, adequate levels of water and nutrients and high organic matter content (Branco *et al.* 2017). However, in many regions of the world the plant is cultivated under irrigation, the soils are easily saturated with water during periods of high precipitation, which promotes flooding and temporary flooding (Almeida and Valle 2007).

During the flood process, excess water replaces the air present in the soil pores, restricting the flow of oxygen to the soil and creating a condition of hypoxia or anoxia (Sauter 2013). Plants intolerant to this condition show a reduction in the photosynthetic activity, due to the low concentration of CO_2 in the leaves caused by the stomatal limitation.

For relatively long periods of flooding, photosynthesis restrictions that do not occur because of stomatal limitation can be attributed to the degradation of the photosynthetic pigments and reduction of leaf water potential, as well as deterioration in the distribution of photosynthetic molecules, due to the low absorption activity (Kreuzwieser and Rennenberg 2014). In addition, changes caused by hypoxia/anoxia can promote a number of metabolic and morpho-physiological changes, modifying the translocation of metabolites from the root to the shoot of the plant (Kreuzwieser and Rennenberg 2014).

Thus, this condition has been a limiting factor for the initial growth and establishment of cocoa in sites subject to periodic flooding, as occurs in some cocoa producing regions of Brazil, Ghana, Nigeria, and Côte d'Ivoire. In these regions, rainfall often exceeds evapo-transpiration, creating conditions of hypoxia in the soil (Gomes and Kozlowski 1986).

The promoted waterlogging causes the exclusion of air from the ground by the drop in oxygen levels, thus creating a reducing environment. Oxygen is rapidly consumed by

soil microorganisms and by root respiration of plants, which leads to varying degrees of molecular oxygen depletion (hypoxia) or absence (anoxia). Under these circumstances, iron is reduced to Fe^{2+} and manganese to Mn^{2+}, which may be toxic to plants (Fageria *et al.* 2002).

Iron (Fe) is an essential element for the physiological development of plants, but in excess, it can have deleterious effects, capable of altering plant metabolism and survival (Müller *et al.* 2017). These effects include: anatomical alterations (Sahrawat 2005), photosynthetic stress (Suh *et al.* 2002), the chlorophyll content in old leaves (Chatterjee *et al.* 2006), and the presence of reactive oxygen species (ROS) in the leaves (Connolly and Guerinot 2002), consequently inhibiting plant growth.

There is little information on the effects of Fe toxicity on tropical plant species, and research on the effects of Fe on the physiological aspects of cacao is scarce. In view of the above, the objective of this study was to evaluate the effects of different concentrations of iron under hypoxia or anoxia in two cocoa genotypes over time.

Materials and Methods

Plant material and experimental design

The experiment was carried out in a greenhouse, located in the municipality of São Mateus-ES, Espírito Santo, Brazil (latitude 18° 43 'S, longitude 39° 51' W) at 39 m altitude in flat terrain. Cacao seeds from the TSH 1188 and SIAL genotypes were used. The mucilage of the seeds was removed by friction with dry saw dust. The surface sterilized was with 0.5% sodium hypochlorite, washed in tap water, selected for size, and placed to pre-germinate for four days in distilled water under constant aeration. After this period, pre-germinated seeds were placed in trays containing sand washed with 5% HCl, rinsed with distilled water, and sterilized by autoclaving.

The seedlings were irrigated daily with distilled water and, after 30 days, selected for size, washed, and transferred, four in number, to externally painted polyethylene vats of aluminum with polystyrene lids coated with aluminum foil. Each lid contained four holes for foaming, which served to support and protection for the plants. The vessels contained 7.0 L of Hoagland and Arnon # 2 (1950) nutrient solution with ¼ ionic strength. An air compressor was used to oxygenate the nutrient solutions. The pH of the solution was monitored every two days and adjusted with NaOH and/or HCl and maintained in the range of 5.5 to 6.0.

During the experiment, the evapotranspiration of each vessel was monitored by the maximum reduction around 30% of the vessel volume. It was measured with a mark made before the addition of the solution, and it was replaced with deionized water. To replace the nutrients, a depletion of up to 20% was allowed, based on the reduction of the electrical conductivity. The solution was renewed every two weeks.

At 80 days after transplantation (DAT), the Hoagland and Arnon # 2 nutrient solution (1950) was modified to contain the following concentrations of Fe ($FeSO_4$): 44.5 (recommended concentration); 133.5 (high) and 400.5 (very high) μmol/L^{-1}. At 81 DAT, part of the experiment was submitted to the absence of aeration (flooding) by suspending the aeration for a period of 21 days.

The physiological evaluations were carried out at 80, 87, 94, 101, 108 and 115 DAT, that is, 0, 7, 14, 21, 28, and 35 days after the application of the iron doses. Suspension of aeration was performed from 81 to 101 DAT with subsequent evaluations corresponding to the recovery period of the plants.

The experiment was conducted in a randomized complete block design, using three replications in the factorial arrangement 2 x 2 x 3 and two cacao genotypes as well as with and without aeration and three iron concentrations.

Gas exchange

The net photosynthesis rates in the leaves (*A*), stomatal conductance to water vapor (*gs*), internal CO_2 concentration (*Ci*) and transpiration (*E*) were obtained under photosynthetic steady-state conditions in completely expanded leaves between 08:00 and 10:00 a.m. A portable open-system infrared gas analyzer (CIRAS-II, PP System, U.K.) was used with the following settings: external [CO_2] supply of (400 μL L^{-1}), irradiance (600 μmol m^{-2} s^{-1}), and temperature (25ºC).

Chlorophyll content

The chlorophyll *a*, chlorophyll *b* and *total* chlorophyll contents of the leaves were determined, with the use of an electronic chlorophyll content meter (Falquer clorofila CFL 1030).

Statistical analysis

The results were submitted to analysis of variance and regression. All the triple interactions were deployed, and the averages compared by the Scott-Knott test at 5%. In the regression analysis, the choice of the model that best fit the data was based on the significance of the regression effect evaluated by the F test at 5% of probability, as well as at the highest coefficient of determination (r2). The coefficients of the regression equations were tested at 5 and 1% probability by the "t" test.

Results

Gas exchange

The net photosynthetic rate per unit leaf area (*A*), stomatal conductance (*gs*), transpiration (*E*), and internal CO_2

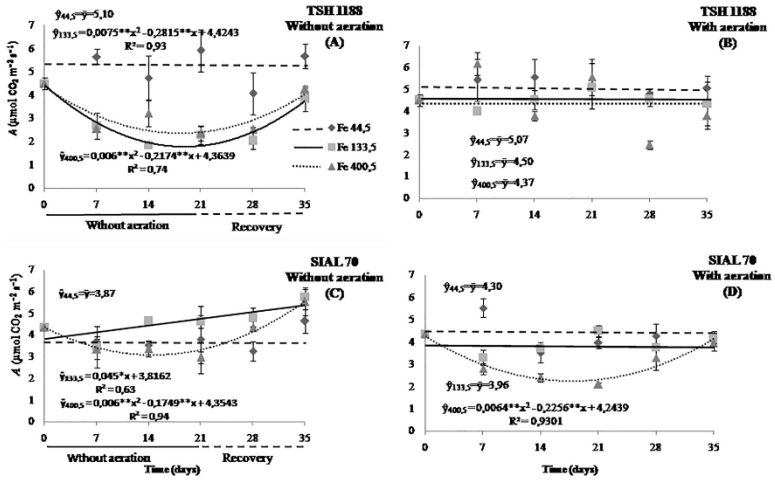

Fig. 1: Photosynthetic rates of cacao genotypes, TSH 1188 (**A** and **B**) and SIAL 70 (**C** and **D**) at different iron concentrations under anoxia or hypoxia. Mean values of three replicates (± SE)

concentration (*Ci*) were significantly influenced in both genotypes (TSH 1188 and SIAL 70) by the effects of iron (Fe) in hypoxic or anoxic condition over time (Fig. 1).

At the recommended concentration of iron (44.5 µmol L^{-1}), the effects were milder on the photosynthetic rates for both genotypes in hypoxic or anoxic condition over time. However, for concentrations high and very high (133.5 and 400.5 μmol L^{-1}), there were reductions in these rates of up to 59.9 and 45.0% at 18.8 and 18.1 days in TSH 1188 without aeration, respectively. Subsequently, at the end of the recovery period, these genotypes restored 85 and 94.1% of the photosynthetic rates found before aeration shutdown (Fig. 1A). In the aerated environment, although the average photosynthetic rate was 11.2 to 13.8% higher at the concentration 44.5 μmol L^{-1}, no significant changes were observed over time in TSH 1188 (Fig. 1B).

For the SIAL 70 genotype in the absence of aeration condition, the intermediate concentration of Fe promoted a linear increase in the photosynthetic rate, reaching a 41.3% higher value in the 35th day, while at the highest concentration, there was a decrease of 29.4% at 14.6 days, followed by an increase that exceeded the initial photosynthetic rate by 28.2% at the end of the experiment (Fig. 1C). In an environment with aeration, the SIAL 70 genotype showed no significant changes in the photosynthetic rates at the concentrations 44.5 and 133.5 µmol L^{-1}. In contrast, there was a reduction of 46.7% at 17.6 days with 400.5 µmol L^{-1}, followed by an increase at 35 days' witch, restored almost the total photosynthetic rate (98.7%) (Fig. 1D).

The lack of aeration affected the photosynthetic rates differently over time for each genotype and dose used (Fig. 2). In hypoxic or anoxic condition, genotype TSH 1188 did not showed significant changes during the 35 days of treatment at each concentrations of Fe used (Fig. 2A, C and E), having the same average photosynthetic rate (5.10 μmol CO_2 m^{-2} s^{-1}) as obtained in anoxic condition in the recommended concentration (Fig. 2A). On the other hand, at the highest concentrations of Fe, the photosynthetic rates of the anoxic condition treatment decreased by 59.9 and 45% at 18.8 and 18.1 days, restoring itself at the end of the recovery period (35th day) by 85 and 94.1% of the photosynthesis measured before aeration shutdown at respective concentrations (Fig. 2C and 2E). On average, in the concentrations 133.5 and 400.5 μmol L^{-1}, TSH 1188 with aeration showed photosynthetic rates 57.3 and 44.2% higher than in anoxic condition treatment, respectively.

In both environments hypoxic or anoxic condition, the SIAL 70 genotype maintained constant photosynthetic rates over time at the concentration of 44.5 μmol L^{-1} (Fig. 2B). At concentrations high and very high of Fe (Figures 2D and 2E), *A* values were on average 15.9 and 26.7% higher in plants without aeration than in aeration, and in the high concentration, there was a linear increase, reaching a photosynthetic rate 41.3% higher than observed before the aeration shutdown (Fig. 2D). At the concentration of 400.5 μmol L^{-1} of Fe, the photosynthesis of the SIAL 70 without aeration reduced 29.4% at 14.6 days and, then, increased by 28.2%, surpassing the photosynthesis measured before the aeration shutdown at the end recovery (Fig. 2F). On the other hand, the aeration treatment reduced by 46.7% at 17.6 days and, then, increased to 98.7% of the initial photosynthesis.

For photosynthetic rates among the genotypes, independent of the environment, the photosynthetic rates were higher in the TSH 1188 genotype. However, these rates were altered depending on the Fe concentration, as demonstrated in the case of the high concentration where the photosynthetic rates were 66% higher in the SIAL 70 genotype when compared to the TSH 1188 genotype.

The stomatal conductance (*gs*) also decreased when the plants were submitted to different concentrations of iron in hypoxic or anoxic condition over time (Figure 3). After the 15th and 17th day, reductions of 52.6 and 67.7% of *gs*

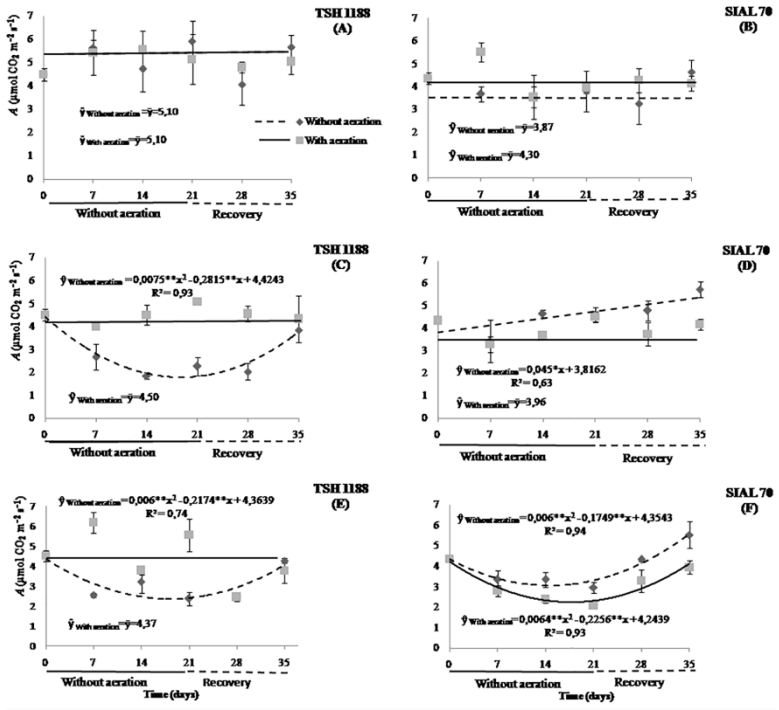

Fig. 2: Photosynthetic rates of cacao genotypes at different iron concentrations 44.5 (**A** and **B**), 133.5 (**C** and **D**), and 400.5 μmol L^{-1} (**E** and **F**) under anoxia or hypoxia. Mean values of three replicates (± SE)

were observed for the TSH 1188 genotype submitted to the concentrations 133.5 and 400.5 μmol L^{-1} of Fe, respectively, except for the concentration of Fe without aeration which did not influence the stomatal conductance. Later, at the end of the recovery period, the plants of this genotype at concentrations high and very high of Fe exceeded the stomatal conductance at 8.2 and 35.6% of the initial value before the aeration shutdown (Fig. 3A). In an environment with aeration, this same genotype showed significant reductions in the three concentrations of Fe, being 79.8; 55.5 and 51.1% at 25.5; 25.6 and 22 days, followed by a 35-day increase of 68.8; 48 and 33.5% of the values found at the beginning of the application of Fe doses at concentrations recommended, high and very high of Fe, respectively (Fig. 3B).

The genotype SIAL 70 did not show changes in stomatal conductance under hypoxic or anoxic condition at concentrations recommended, high and very high of Fe, however, in the concentration recommended at 15 days, it reduced 28% and recovered by exceeding the initial index by 21.8% after 35 days (Fig. 3C). In hypoxic condition, the recommended concentration also did not show changes in the stomatal conductance over time, however, at 17.7 and 13.7 days, this same genotype reduced 34.0 and 41.1% in the concentrations high and very high of Fe, respectively. Then, the stomatal conductance recovered 97.9% and exceeded the initial values by 57.1% after 35 days of application of the treatments in the respective concentrations (Fig. 3D).

In general, hypoxic or anoxic condition caused significant reductions in stomatal conductance in the two genotypes studied, however, different forms of response to this stress were verified over time (Fig. 4). Without aeration, TSH 1188 showed no significant changes during the 35 days for the recommended Fe concentration, however, under aeration conditions, a reduction of 79.8% was observed after 25.5 days. There was a recovery of only 68.8% after 35 days of experiment, while the value of this variable was, on average, 125% higher in plants hypoxic condition than in plants anoxic condition (Fig. 4A). On the other hand, reductions of 52.6 and 55.5% after 15.2 and 25.6 days, respectively, occurred at 133.5 μmol L^{-1} of Fe without and with aeration. Nevertheless, in both environments, this genotype recovered. On average, the aeration environment had higher values than the anoxic condition environment, presenting 31.6% of difference (Fig. 4C). At the highest concentration of Fe, the TSH 1188 genotype showed a reduction of 51.1% with aeration and 67.7% without aeration. As a consequence, the aeration environment had higher mean values (10.3%) than that of the anoxic condition environment (Fig. 4E).

At the recommended dose of Fe, the SIAL 70 genotype did not show changes in stomatal conductance (Fig. 4B). Similarly, at the concentrations high of Fe, the condition did not show alterations in an environment anoxic condition, however, hypoxic condition, there was a reduction of 34.0% at 17.7 days. At the end of 35 days, it recovered almost totally to the initial value (97.9%).

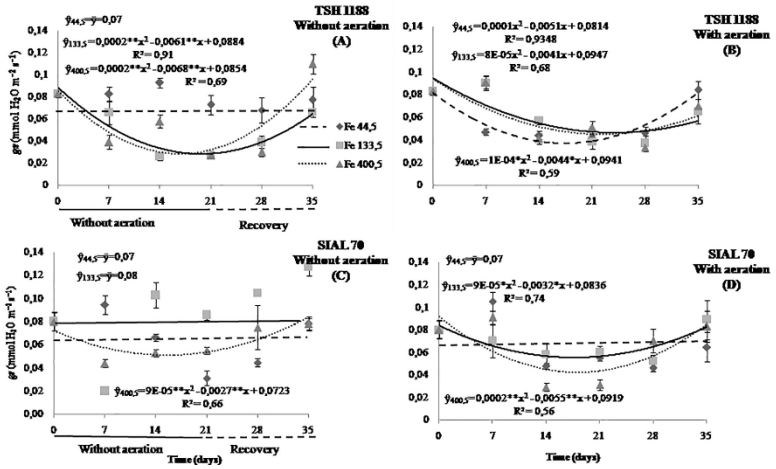

Fig. 3: Stomatal conductance of cacao genotypes, TSH 1188 (**A** and **B**) and SIAL 70 (**C** and **D**) at different iron concentrations under anoxia or hypoxia. Mean values of three replicates (± SE)

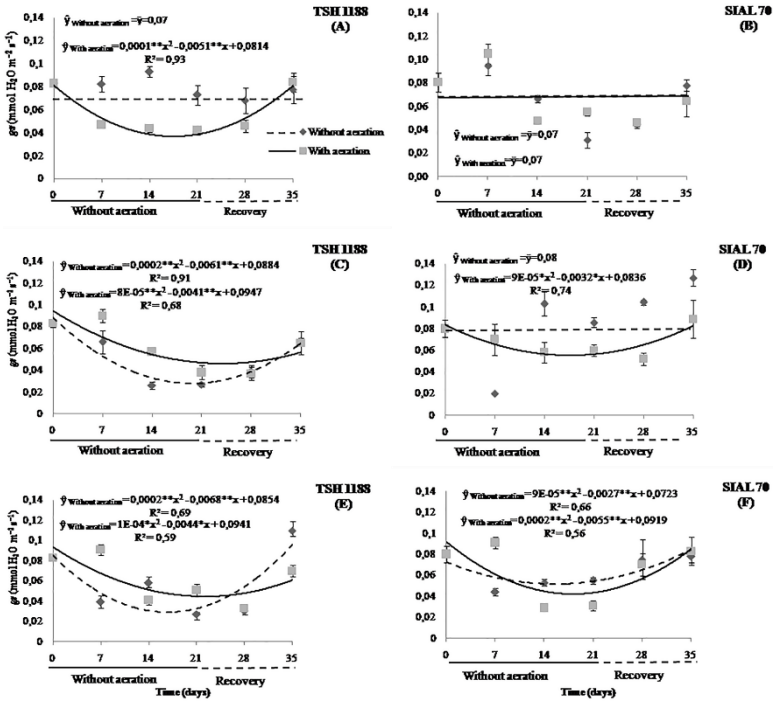

Fig. 4: Stomatal conductance of cocoa genotypes at different iron concentrations, 44.5 (**A** and **B**), 133.5 (**C** and **D**), and 400.5 μmol L^{-1} (**E** and **F**) under anoxia or hypoxia. Mean values of three replicates (± SE)

On average, the non-aerated environment was 33.6% higher than the environment hypoxic condition in stomatal conductance (Fig. 4D). At the concentration of 400.5 μmol L^{-1} of Fe, the stomatal conductance of the SIAL 70 reduced 41.1 and 28% at 13.7 and 15 days, respectively, and then, it rose, surpassing by 57.7 and 21.8% at the end of recovery. On average, with aeration, it was 31.3% higher than hypoxic condition (Fig. 4F).

With aeration under an intermediate concentration of Fe, there was a marked decline of the transpiration rates observed after 14.8 days, presenting reductions above 50%. However, at the end of the experiment, an increase was observed that reached 46.2% of the initial transpiration rate (Fig. 5), which are results similar for this same genotype in non-aerated condition (Fig. 6). Even under the highest concentrations of Fe, the TSH 1188 genotype showed no changes over time (Fig. 5A–B), which was also observed in the non-aerated environment (Fig. 6E).

Under this same environment condition, the genotype SIAL 70 presented reductions in transpiration rates of 49.0; 42.5, and 69.7% on days 13.8; 17.9, and 16.6 for all concentrations of Fe, respectively (Fig. 5C).

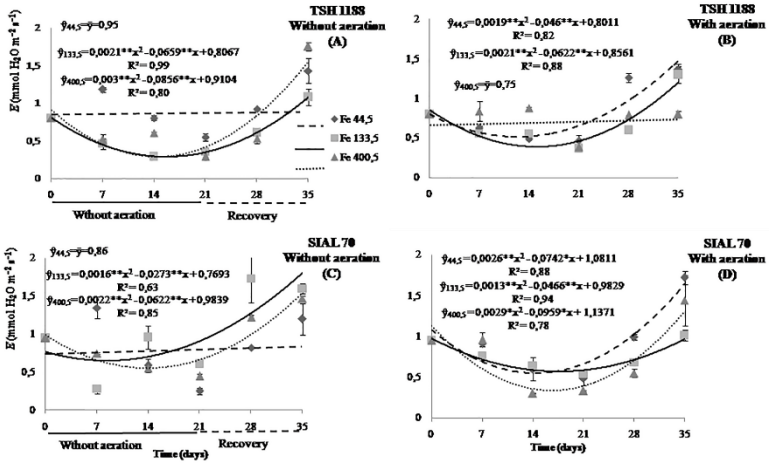

Fig. 5: Transpiration rate of cacao genotypes, TSH 1188 (**A** and **B**) and SIAL 70 (**C** and **D**), at different iron concentrations under anoxia or hypoxia. Mean values of three replicates (± SE)

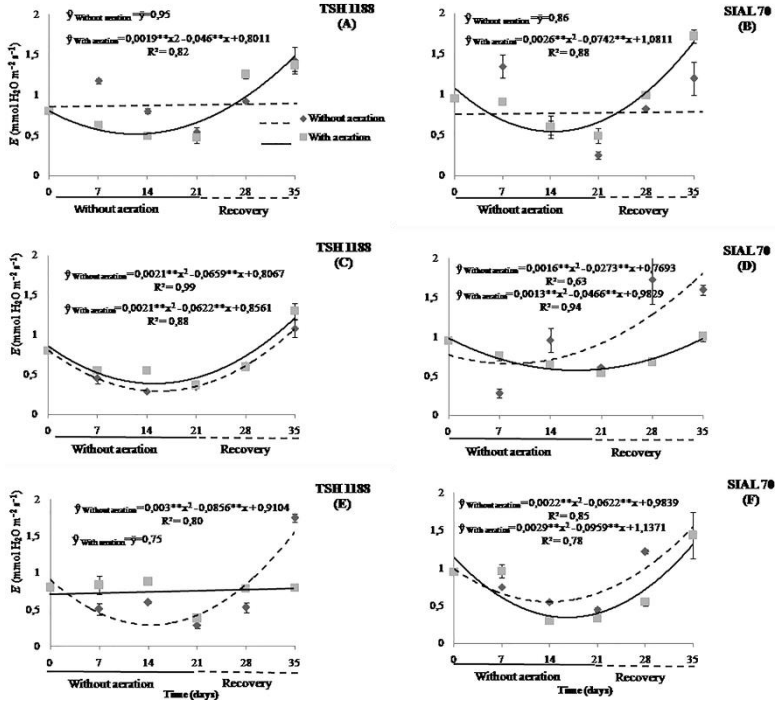

Fig. 6: Transpiration rate of cacao genotypes at three different iron concentrations, 44.5 (**A** and **B**), 133.5 (**C** and **D**), and 400.5 μmol L^{-1} (**E** and **F**) under anoxia or hypoxia. Mean values of three replicates (± SE)

Subsequently, the transpiration rate was restored after 35 days and exceeded the initial values for the concentrations 44.5 and 400.5 μmol L^{-1} of Fe at 54.4 and 17.2%, while at the concentration 133.5 µmol L^{-1} of Fe, it was restored almost completely with 96.1% (Fig. 5D). For non-aeration environment, the transpiration rates remained unchanged when submitted to the lowest concentrations of iron (Fig. 6). On the other hand, in the concentrations 133.5 and 400.5 μmol L^{-1} of Fe, the transpiration rate of SIAL 70 without aeration reduced 15.4 and 44.7% at days 8.5 and 14.1. This was followed by an increase which exceeded 130.5 and 52.6% of the transpiration as measured before aeration cessation at the end of recovery (Fig. 5B, C, D).

Chlorophyll content

The chlorophyll *a*, *b*, and total of the TSH 1188 and SIAL 70 genotypes were similar as a function of time, both hypoxic or anoxic condition, differing only in relation to the concentration of iron used (Fig. 7, 8 and 9).

In the non-aeration environment, there were no significant changes in the three types of chlorophyll over

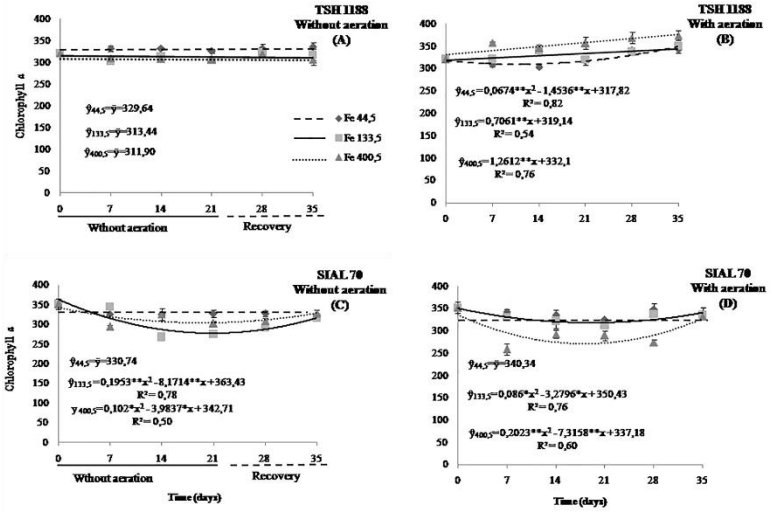

Fig. 7: Chlorophyll *a* content of cacao genotypes, TSH 1188 (**A** and **B**) and SIAL 70 (**C** and **D**), at different iron concentrations under anoxia or hypoxia Mean values of three replicates (± SE)

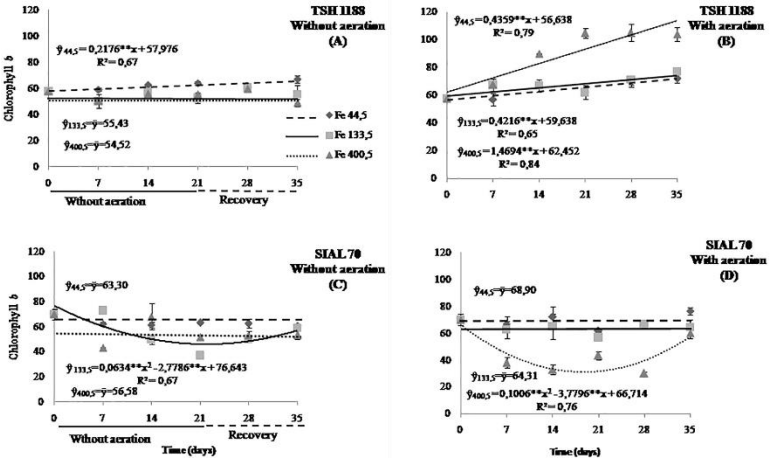

Fig. 8: Chlorophyll *b* content of cacao genotypes, TSH 1188 (**A** and **B**) and SIAL 70 (**C** and **D**), at different iron concentrations under anoxia or hypoxia. Mean values of three replicates (± SE)

time in the TSH 1188 genotype (Fig. 7A, 8A and 9A), except for chlorophyll b at the concentration recommended of Fe. This condition presented a linear increase reaching values 13.1% higher than the initial ones at the end of the recovery period (35th day). In an environment hypoxic condition (Fig. 7B, 8B, and 9B), this same genotype presented linear increases in chlorophyll *a*, *b* and total content as a function of time, except for the lowest concentration for chlorophyll *a* and total, whose increase was quadratic. After 35 days, the values of these indices at concentrations recommended, high and very high of Fe increased 10.0, 7.8, and 13.3% for chlorophyll *a*; 26.9, 24.7, and 82.5% for chlorophyll *b*; and 12.6, 10.4, and 24.2% for total chlorophyll, respectively.

For the genotype SIAL 70, in the concentrations high and very high of Fe, the chlorophyll content decreased by 23.5 and 11.4% at 20.9 and 19.5 days without aeration. Then, an increase in this index was observed, recovering 87.1 and 95.8% of the initial values on the 35th day (Figure 7C). On the other hand, in the concentration high, only the values of chlorophyll *b* and total chlorophylls (Fig. 8C and 9C) showed reductions of 39.7 and 26.4% at 21.9 and 21.2 days, with partial recovery of 74.4 and 84.9% of the initial values after 35 days, respectively. In the environment hypoxic condition, the chlorophyll *a* of the SIAL 70 in the concentration high of Fe decreased by 8.9% at 19.1 days, whereas at concentration high of Fe, the reduction was 19.6% at 18.1 days (Fig. 7D). For the chlorophyll *b* and total chlorophyll indices of the same genotype, there was only a significant reduction of 53.2 and 25.2% in the highest concentration, around 18.5 days. Then, there was again an increase of these indices, reaching 86.4 and 95.5% of the values recorded at the end of the period as compared to time zero (Fig. 8D and 9D).

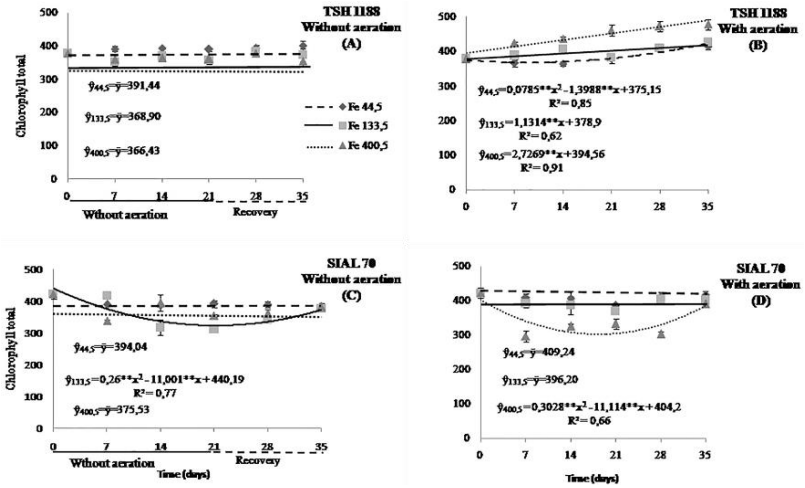

Fig. 9: Total Chlorophyll content of cacao genotypes, TSH 1188 (**A** and **B**) and SIAL 70 (**C** and **D**) at different iron concentrations under anoxia or hypoxia. Mean values of three replicates (± SE)

Discussion

Negative effects of flood stress on cocoa plants are well documented in the literature (Rehem *et al.* 2010; Bertolde *et al.* 2012, 2014; Almeida *et al.* 2016), but there is no report on the effect of iron (Fe) concentration associated with soil flooding on the eco-physiological traits of this species. In the present study, the photosynthetic rates were reduced according to iron concentration, flood time, and genotype evaluated. Generally, these decreases during the flooding period occur due to inhibitory effects on the stomatal and non-stomatal processes (Zhang *et al.* 2018). The non-stomatic limitations of photosynthesis are strongly associated with changes in Calvin cycle enzymes and the degradation of photosynthetic pigments. The decrease in ribulose-1,5-biphosphate carboxylase oxygenase activity (RUBISCO) is one of the initial symptoms of hypoxia stress, which contributes to losses in photosynthetic capacity (Patel *et al.* 2014).

Although a decrease of A for both genotypes was observed, the SIAL 70 plants exposed to higher Fe concentrations showed an increase in the photosynthetic rate, a fact that seems to be related to the physiological response of the species when submitted to this type of stress.

One of the first responses of plants to flood stress is stomatal closure to avoid water loss and tissue dehydration (Pucciariello and Perata 2012). However, in plants which are not tolerant to flooding, stomatal closure is due to the loss of cellular turgor caused by the decrease in hydraulic conductivity, which limits the transport of water to the plant shoots (Rasheed-Depardieu *et al.* 2015; Chaudhary *et al.* 2016). Under these conditions, water loss for transpiration cannot be compensated for by water absorption (Dalmolin *et al.* 2012, 2013), leading to a lower degree of stomatal opening. Similar to what observed in the present study, Rodriguez *et al.* (2015) found a significant relationship between *A* and *gs* during soil flood periods for *Schinus terebinthifolius*, *Rapanea rustinae* and *Populus deltoides*, where both variables were altered by anaerobic stress.

From the 15 days of flooding, the photosynthetic rate was limited by the low stomatal conductance in both cacao genotypes evaluated in this study. In general, the reduction of stomatal conductance also lessens the rate of transpiration, decreasing the absorption of Fe in excess. This can be understood as a strategy to reduce Fe absorption, as it is transported by xylem (Curie and Briat 2003).

In the present study, environments concentration high and very high showed reductions in the stomatal conductance of the cocoa plants over time, suggesting that the closure and/or opening of the stomata was intensified by Fe stress. This was observed more in the TSH 1188 genotype, where hypoxic condition presented higher stomata opening at the higher Fe concentrations. The SIAL 70 genotype responded differently at the intermediate dosage, presenting larger stomatal openings in the non-aeration environment. At the highest Fe concentration, the largest openings were in the aeration environment, suggesting that the excess of Fe increased the closure of the stomata (Xu *et al.* 2016).

The mechanism by which the excess of Fe affects the stomatal movement is still not very clear, but it is likely that the reduction of the stomatal conductance is linked to the H^+-ATPase activity of the cellular membranes, since the excess of Fe can potentiate its depolarization. The activity of H^+-ATPase can reduce by 80–90%, or even cause complete loss of the protein function with free Fe in the cells (Santos-Souza *et al.* 2001). In addition, the stromal closure may also indirectly lead to oxidative stress through reduction of the electron transport chain and photoinhibition, contributing to the effects on photosynthesis (Lin *et al.* 2013; Loreti *et al.* 2016).

The cacao tree seems to tolerate the conditions of hypoxia/anoxia better in the studied condition. However, changes in the stomatal conductance of flooded plants seem

to work as a control mechanism for transpiration, since lower values of *gs* promote the reduction of water absorption in the roots and, consequently, a reduction in the hydraulic conductivity (Lavinsky *et al.* 2007). Thus, lower stomatal opening can be considered a survival mechanism for plants under flood conditions, since flood-tolerant woody species have shown reductions in gas exchange due to flooding (Branco *et al.* 2017).

The transpiration rates are directly linked with stomata opening. In the present study, the excess of Fe reduced the transpiration of the two genotypes as a function of stomatal opening over time, and because Fe is transported via xylem, it can be considered a plant strategy to avoid toxicity (Curie and Briat 2003). According to Kozlowski (1997), the transpiration reduction occurs initially due to the stomatal closure, resulting in the decrease of CO_2 absorption in the leaves. In flooded environments, however, this is confirmed because O_2 deficiency does not significantly decrease the water potential of the xylem. Moreover, if the species is sensitive to flooding, they often exhibit severe reductions in perspiration and stomatal conductance. Thus, these variables become useful in determining the degree of plant tolerance to soil flooding (Gravatt and Kirby 1998).

The present study confirms suggests that this physiological response of the plant can also be influenced by other factors, such as the effect caused by the Fe concentration. This is suggested because there were also reductions of stomatal conductance and transpiration even with aeration (Mohammed *et al.* 2019).

Internal CO_2 concentration values were generally higher in flooded plants. At the recommended and intermediate doses, the TSH 1188 genotype had higher values of the internal CO_2 concentration in reaction to the environment with aeration (data not shown).

According to Ashraf (2003), the reduction of the internal CO_2 concentration is considered normal in stress tolerant tree plants due to flooding. However, normally attenuation of internal CO_2 concentration is reconciled with stomatic limitations of photosynthesis and greater conservation of the plant in relation to water use. This fact was not observed in the present study, since there was no synchronism with the results of photosynthesis and conductance with the internal CO_2 concentration when comparing environments hypoxic or anoxic condition. According to Liao and Lin (1994), when photosynthesis is reduced and CO_2 increases or is unchanged, it is suggested that the CO_2 that reaches the mesophyll cells is not used for the carboxylation phase. This indicates a biochemical limitation, possibly by damage to the structure of Rubisco or reduction in the regeneration of Ribulose 1,5-bisphosphate.

As a consequence of high concentrations of Fe, there was reduction of chlorophyll indices for only the SIAL 70 genotype over time. According to Patel *et al.* (2014), flooding may promote reduction in chlorophyll content without major damage at the beginning. However, at 30 days after flooding, falls in the concentration of photosynthetic pigments in flooded plants compared to non-flooded plants may occur (Bertolde *et al*. 2012). The decrease has been interpreted as a long-term response to flooding (Smethurst and Shabala 2003). This is because its low concentration can limit the photochemical process, since the absorption of radiation depends on its content (Pezeshki *et al.* 1996).

Nonetheless differences between the genotypes respond in the absence of aeration and under Fe excess. Although the photosynthetic pigment contents did not suffer great variations, anoxic condition caused changes in the photosynthetic rates of the two genotypes studied. These results also showed that these specific responses of cacao to flooding may vary depending on various factors, such as species, genotype, age, and plant condition, as well as duration of flooding period.

Conclusion

Excess iron causes reduction of the photosynthetic rate, stomatal conductance, and transpiration under flood conditions. However, this response is dependent on the ambient condition, as well as the presence or absence of aeration. The levels of chlorophyll a, b, and total chlorophyll are also affected by the concentration of iron, however, depending on the stress; there is the possibility of later recovery of these chloroplast pigment contents. The two genotypes of *T. cacao* are tolerant to the absence of aeration and excess of iron (Fe), however, showed different responses, indicating that have different mechanisms to deal with each type of stress.

Acknowledgements

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes - Código de Financiamento 001) and Universidade Federal do Espírito Santo (UFES).

Author Contributions

Braga PCS, MG Oliveira and MAG Aguilar conceived and designed the experiments.; Braga PCS, MG Oliveira, MAG Aguilar, FL Partelli and WP Martins collected and analyzed the data. Braga PCS, MG Oliveira, MAG Aguilar, FL Partelli and WP Martins. wrote the paper.

References

Almeida AAF, RR Valle (2007). Ecophysiology of the cacao tree. *Braz J Plant Physiol* 19:425–448

Almeida J, W Tezara, A Herrera (2016). Physiological responses to drought and experimental water deficit and waterlogging of four clones of cacao (*Theobroma cacao* L.) selected for cultivation in Venezuela. *Agric Water Manage* 171:80–88

Ashraf M (2003). Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (*Panicum antidotale* Retz.) under salinity or waterlogging. *Plant Sci* 165:69–75

Bertolde FZ, AAF Almeida, CP Pirovani (2014). Analysis of gene expression and proteomic profiles of clonal genotypes from *Theobroma cacao* subjected to soil flooding. *PLoS One* 9; Article e108705

Bertolde FZ, AAF Almeida, CP Pirovani, FP Gomes, D Ahnert, VC Baligar, RR Valle (2012). Physiological and biochemical responses of *Theobroma cacao* L. genotypes to flooding. *Photosynthetica* 50:447–457

Branco M CS, AAF Almeida, AC Dalmolin, D Ahnert, VC Baligar (2017). Influence of low light intensity and soil flooding on cacao physiology. *Sci Hortic* 217:243–257

CEPLAC - COMISSÃO EXECUTIVA DO PLANO DA LAVOURA DO CACAUEIRA (2018). Cacau – História e evolução. Disponível em: < http://www.ceplac.gov.br/radar/radar_cacau.htm>. Acesso em: 15 Nov. 2018

Chatterjee C, R Gopal, BK Dube (2006). Impact of iron stress on biomass, yield, metabolism and quality of potato (*Solanum tuberosum* L.). *Sci Hortic* 108:1–6

Chaudhary S, A Kusakabe, JC Melgar (2016). Phytophthora infection in flooded citrus trees reduces root hydraulic conductance more than under non-flooded condition. *Sci Hortic* 202:107–110

Connolly EL, ML Guerinot (2002). Iron stress in plants. *Genom Biol* 3:1–4

Curie C, JF Briat (2003). Iron transport and signaling in plants. *Annu Rev Plant Biol* 54:183–206

Dalmolin AC, HJ Dalmagro, FDA Lobo, MZJ Antunes, CER Ortíz, GL Vourlitis (2013). Photosynthetic light and carbon dioxide response of the invasive tree *Vochysia divergens* Pohl, to experimental flooding and shading. *Photosynthetica* 51:379–386

Dalmolin AC, HJ Dalmagro, FDA Lobo, S Antune, MZ Junior, CER Ortíz, GL Vourlitis (2012). Effects of flooding and shading on growth and gas exchange of *Vochysia divergens* Pohl (Vochysiaceae) of invasive species in the Brazilian Pantanal. *Braz J Plant Physiol* 24:75–84

Fageria NK, VC Baligar, RB Clark (2002). Micronutrients in crop production. *Adv Agron* 77:185–268

FAOSTAT (2016). Food and Agricultural Organization of the United Nation Statistics Data Retrieved July 16, 2016

Gomes ARS, TT Kozlowski (1986). The effects of flooding on water relations and growth of *Theobroma cacao* var. *Catongo* seedlings. *J Hortic Sci* 61:265–276

Gravatt DA, CJ Kirby (1998). Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. *Tree Physiol* 18:411–417

Hoagland DR, DL Arnon (1950). *The Water Culture Methods for Growing Plants Without Soil*, p:32. University of California. (Circular 347), Berkeley, California, USA

ICCO (2018). International Cocoa Organization. Produção mundial de cacau. Disponível em ⟨http://www.icco.org/⟩. Acesso em: 15 Nov. 2018

Kozlowski TT (1997). Responses of woody plants to flooding and salinity. *Tree Physiol* 17:490–518

Kreuzwieser J, H Rennenberg (2014). Molecular and physiological responses of trees to waterlogging stress. *Plant Cell Environ* 37:2245–2259

Lavinsky AO, CDS Sant'ana, MS Mielke, AAF Almeida, FP Gomes, AS Franca, DDC Silva (2007). Effects of light availability and soil flooding on growth and photosynthetic characteristics of *Genipa americana* L. seedlings. *New For* 34:41–50

Liao CT, CH Lin (1994). Effect of flooding stress on photosynthetic activities of *Momordica charantia*. *Plant Physiol Biochem* 32:479–485

Lin KH, WS Kuo, CM Chiang, TC Hsiung, MC Chiang, HF Lo (2013). Study of sponge gourd ascorbate peroxidase and winter squash superoxide dismutase under respective flooding and chilling stresses. *Sci Hortic* 162:333–340

Loreti E, HV Veen, P Perata (2016). Plant responses to flooding stress. *Curr Opin Plant Biol* 33:64–71

Mohammed U, RS Caine, JÁ Atkinson, EL Harricson, D Wells, CC Chater, JE Gray, R Swarup, EH Murchie (2019). Rice plants overexpressing OsEPF1 show reduced stomatal density and increased root cortical aerenchyma formation. *Sci Rep* 9; Article 5584

Müller C, SF Silveira, MD Daloso, D Mendes, GC Merchant, KN Kuki, MA Oliva, ME Loureiro, AM Almeida (2017). Echophysiological responses to excess iron in lowland and upland rice cultivars. *Chemosphere* 189:123–133

Patel PK, A Kumar, NT Singh, D Yadav, A Hemantaranjan (2014). Flooding: Abiotic constraint limiting vegetable productivity. *Adv Plant Agric Res* 1:2–9

Pezeshki SR, JH Pardue, RD Delaune (1996). Leaf gas exchange and growth of flood-tolerant and flood-sensitive tree species under low soil redox conditions. *Tree Physiol* 16:453–458

Pucciariello C, P Perata (2012). Flooding tolerance in plants. *In*: *Plant Stress Physiology*. Shabala, S. (Ed.). CABI, Willingford, UK

Rasheed-Depardieu C, J Parelle, F Tatin-Froux, C Parent, N Capelli (2015). Short-term response to waterlogging in *Quercus petraea* and *Quercus robur*: A study of the root hydraulic responses and the transcriptional pattern of aquaporins. *Plant Physiol Biochem* 97:323–330

Rehem BC, AAF Almeida, MS Mielke, FP Gomes, RR Valle (2010). Photosynthetic and growth responses of *Theobroma cacao* L. clones to Water logging. *J Trop Agric* 48:17–22

Rodriguez ME, FG Achinelli1, VMC Luquez (2015). Leaf traits related to productivity in *Populus deltoids* during the post-flooding period. *Trees* 29:953–960

Sahrawat KL (2005). Managing iron toxicity in lowland rice: The role of tolerant genotypes and plant nutrients. *In: Rice is Life: Scientific Perspectives for the 21st Century*, pp:452–453. Toriyama K, KL Heong, B Hardy (Eds.). International Rice Research Institute, Los Baños, Philippines

Santos-Souza P, RS Ramos, ST Ferreira, PC Carvalho-Alves (2001). Iron-induced oxidative damage of corn root plasma membrane H^+-ATPase. *Biochim Biophys Acta Biomembr* 1512:357–366

Sauter M (2013). Root responses to flooding. *Curr Opin Plant Biol* 16:282–286

Smethurst CF, S Shabala (2003). Screening methods for waterlogging tolerance in lucerne: Comparative analysis of waterlogging effects on chlorophyll fluorescence, photosynthesis, biomass and chlorophyll content. *Funct Plant Biol* 30:335–343

Suh HJ, CS Kim, JY Lee, J Jung (2002). Photodynamic effect of iron excess on photosystem II function in pea plants. *Photochem Photobiol* 75:513–518

Xu Z, Y Jiang, B Jia, G Zhou (2016). Elevated-CO_2 response of stomata and its dependence on environmental factors. *Front Plant Sci* 7; Article 657

Zhang H, P Feng, W Yang (2018) Effects of flooding stress on the photosynthetic apparatus of leaves of two *Physocarpus* cultivars. *J For Res* 29:1049–1059