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Physiological and biochemical responses of *Theobroma cacao* L. genotypes to flooding

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Abstract

Flooding is common in lowlands and areas with high rainfall or excessive irrigation. A major effect of flooding is the deprivation of O_2 in the root zone, which affects several biochemical and morphophysiological plant processes. The objective of this study was to elucidate biochemical and physiological characteristics associated with tolerance to O_2 deficiency in two clonal cacao genotypes. The experiment was conducted in a greenhouse with two contrasting clones differing in flood tolerance: TSA-792 (tolerant) and TSH-774 (susceptible). Leaf gas exchange, chlorophyll (Chl) fluorescence, chemical composition and oxidative stress were assessed during 40 d for control and flooded plants. Flooding induced a decrease in net photosynthesis, stomatal conductance and transpiration of both genotypes. In flood conditions, the flood-susceptible clone showed changes in chlorophyll fluorescence, reductions in chlorophyll content and increased activity of peroxidase and polyphenol oxidase. Flooding also caused changes in macro- and micro-nutrients, total soluble sugars and starch concentrations in different plant organs of both genotypes. Response curves for the relationship between photosynthetically active radiation (PAR) and net photosynthetic rate (P_N) for flooded plants were similar for both genotypes. In flood conditions, the flood-susceptible clone exhibited (*1*) nonstomatal limitations to photosynthesis since decreased in maximum potential quantum yield of PSII (F_v/F_m) values indicated possible damage to the PSII light-harvesting complex; (*2*) oxidative stress; (*3*) increased leaf chlorosis; and (*4*) a reduction in root carbohydrate levels. These stresses resulted in death of several plants after 30 d of flooding.

Introduction

Theobroma cacao L. (Malvaceae*)* is a preferentially allogamous perennial woody species native to tropical America (Bartley 2005). The species is cultivated in the American, African, and Asian continents and many countries worldwide are used for cocoa production, marketing and consumption. Under natural conditions, the tree can reach 20 to 25 m in height, whereas under cultivation, it varies from 3 to 5 m. It is cropped under the shade of forest trees or as a monocrop without shade. Seedlings initially show an orthotropic growth with leaf development relatively independent of climate. The maturity phase begins with the development of plagiotropic branches that form the tree crown. At this stage, environmental factors exert a large influence on plant development. Growth and development of cacao are highly dependent on temperature, which mainly affects vegetative growth, flowering and fruit development (Almeida and Valle 2007, 2009).

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Abbreviations: C_i – intercellular CO_2 concentration; C_i/C_a – ratio between CO_2 in the intercellular mesophyll spaces and atmospheric CO_2; Chl(s) – chlorophyll(s); DM – dry matter; DMSO – dimethyl sulfoxide; E – transpiration rate; F_0 – minimum fluorescence of the dark-adapted state; F_m – maximum fluorescence of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximum potential quantum yield of PSII; g_s − stomatal conductance to water vapor; PAR – photosynthetically active radiation; PODs – peroxidases; P_N – net photosynthetic rate; PPO – polyphenol oxidases; PS – photosystem; R_D – the dark respiration; ROS – reactive oxygen species; TSS – total soluble sugars; WUE – instantaneous water-use efficiency; WUE_i – intrinsic water-use efficiency; α - apparent quantum efficiency of photosynthesis; Γ_{PAR} – compensation irradiance.
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Cacao produces caulescent flowers, which begin dehiscing in late afternoon and are completely open at the beginning of the following morning releasing pollen to a receptive stigma. Nonpollinated flowers abscise 24–36 h after anthesis. The percentage of flowers setting pods ranges from 0.5 to 5%. The cacao tree is commercially used for production of seeds, which are fermented, dried, and ground to produce liquor and fat. These two products are mixed with sugar, milk and other ingredients to produce the most popular derivative of cacao: chocolate (Almeida and Valle 2007).

The cacao plant grows in areas with annual rainfall between 1,500 and 2,000 mm, being sensitive to both water shortage and flooding, thus requiring soil with good drainage (Gomes and Kozlowski 1986). Flooding is a common phenomenon in lowlands and areas with high rainfall or excessive irrigation (Kozlowski 1997). Under natural conditions, flooding changes many physical and chemical soil properties through impairment of biological processes resulting from the depletion of available oxygen (O_2), increased soil P, Mn, and Fe availabilities and decreased Zn and Cu availabilities and formation of hydrogen sulfide and organic acids (Campbell *et al*. 1999). Flooded soils are also characterized by showing high concentrations of CO_2 (Jackson 2004) and increased decomposition of organic matter (Kozlowski 1997).

One major effect of flooding is the deprivation of O_2 in the root zone (anoxia), attributed to: the slow diffusion of O_2 in water-saturated soil, which is about 10,000 times slower than O_2 diffusion in air, and O_2 consumption by soil microorganisms (Armstrong 1979, Folzer *et al*. 2005). In the aerobic respiration pathway, oxygen is a final electron acceptor in oxidative phosphorylation and in several crucial biosynthetic pathways, including synthesis of Chl, fatty acids and sterols (Dennis *et al*. 2000). Under anoxia, glycolysis and fermentation can exceed the aerobic metabolism and become the only route of energy production (Souza and Sodek 2002).

Flooding affects several morphophysiological plant characteristics (Kozlowski 1997, 2002; Drew 1997). Morphologically, hypertrophied lenticels, aerenchyma and adventitious roots often form, which increases the availability of O_2 in the tissues. In addition to their function in uptake and diffusion of O_2 to the root system, hypertrophic lenticels serve as excretory sites for potentially toxic volatile products such as ethanol, acetaldehyde, and ethylene (Medri 1998). Physiological responses to hypoxia or anoxia of the root zone include (*1*) a decline in photosynthesis, mainly attributed to the decrease in CO_2 uptake as a result of stomatal closure; (*2*) changes in source-sink relationships; (*3*) a decrease in nutrient absorption; and (*4*) changes in hormonal balance, with increased synthesis of ethylene and abscisic acid, among others (Kozlowski 1997, Pezeshki 1993).

Evidence for variability in survival and physiological responses under anoxic soil conditions in cloned genotypes of *T. cacao* has been demonstrated by Bertolde *et al*. (2010). However, reasons for tolerance to O_2 deficiency in the soil are not yet sufficiently understood. The objective of this study was to elucidate biochemical and physiological characteristics associated with tolerance to O_2 deficiency in two cacao clonal genotypes previously identified by Bertolde *et al.* (2010) as flood-tolerant (TSA-792) and flood-susceptible (TSH-774).

Materials and methods

Plant material and cultivation conditions: The experiment was conducted in a greenhouse at the Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil (14°47'S, 39°16'W, 55 m a.s.l.). Micrometeorological variables in the greenhouse were monitored through a climatological *Hobo Micro Station Data Logger* (*Onset, Computer Corp*., Bourne, Massachusetts, USA) with internal sensors that record in *Windows* environment. Data collection and storage was automated with subsequent transfer *via* a serial port to a microcomputer. The maximum and minimum temperatures observed during the experimental period were 28 and 21°C, respectively. The minimum relative humidity recorded was 73% and the maximum 95%. The average value of PAR above the canopy was 946.1 μmol(photon) m^{-2} s^{-1}.

We evaluated two *T. cacao* genotypes with contrasting flood tolerance (flood-tolerant clone TSA-792 and flood-susceptible clone TSH-774), which are highly productive and resistant to cacao witches' broom disease (Bertolde *et al.* 2010). These two genotypes were obtained from CEPEC/CEPLAC's Cacao Germplasm Bank, localized about six kilometers north of UESC. They were multiplied through stem cuttings taken from the tip of plagiotropic branches of five-year-old plants. Cuttings were rooted in 285 cm^3 conical plastic tubes containing organic substrate (peat and milled pine bark + shredded coconut fiber, 1:1) enriched with macro- and micronutrients in accordance with the crop requirements (Souza 2007). Five months after rooting, the plants were transplanted to 25-L plastic pots with an Oxisol fertilized according to cacao nutrient demands, and placed in a greenhouse at UESC. Soil water content was maintained near field capacity for six months. After this period, a flooding treatment was initiated by sealing the lower end of 20 pots of each genotype and filling them with tap water up to 20 mm above the soil surface for 40 d. In the control treatment, plants of each genotype remained in pots with the bottom perforated for drainage of excess irrigation water.

Chl fluorescence: After treatments were initiated, Chl fluorescence was measured weekly in 10 plants of each genotype (5 waterlogged and 5 control plants). The measurements were made on the second fully expanded

mature leaf from the apex of the orthotropic axis, with a leaf chamber fluorometer (*LI 6400-40*, *LI-COR Bioscience*, *Inc.*, Lincoln, NE, USA), an LED-based fluorescence accessory for the portable photosynthesis system *LI-6400* (*LI-COR Bioscience, Inc*., Lincoln, Nebraska, USA). To assess the Chl fluorescence of dark-adapted leaves, a cuvette was placed for 30 min on each leaf prior to each making each measurement. The maximum quantum yield of photosystem II (F_V/F_m) was calculated as [$F_V/F_m = (F_m - F_0)/F_m$)], where F_0 is the basal fluorescence yield measured after the illumination of a dark-adapted sample with a modulated light (0.25 kHz, < 0.1 μmol m^{-2} s^{-1}, 630 nm) and F_m is the maximum fluorescence yield of a dark-adapted sample obtained following a saturating light (modulated) pulse (20 kHz; 6,000 μmol m^{-2} s^{-1}, 630 nm, 0.8 s).

Leaf gas-exchange variables and Chl fluorescence measurements were simultaneously made with *LI-6400*. For light-response curves net photosynthetic rate (P_N) was measured at seven photosynthetic active radiation (PAR) levels [800, 600, 300, 100, 50, 25 and 0 μmol(photon) m^{-2} s^{-1}], with the sequence always starting in descending order of PAR values. To save each reading, the minimum pre-established time for reading stabilization at each PAR was 60 s and the maximum 120 s. Also, the reading was saved if the coefficient of variation (CV) for the measurements was less than 0.3%. In addition to PAR, temperature and atmospheric CO_2 within the leaf chamber were maintained constant at 26°C and 380 μmol(CO_2) mol^{-1}, respectively. The rates of P_N and transpiration (E) per unit leaf area, stomatal conductance to water vapor (g_s), the ratio between internal and atmospheric concentrations of CO_2 (C_i/C_a), the intrinsic water-use efficiency (WUE_i) and instantaneous water-use efficiency (WUE) were estimated from values of CO_2 and air humidity inside the chamber, determined by the internal software of the infrared gas analyzer at PAR > 300 μmol(photon) m^{-2} s^{-1} (von Caemmerer and Farquhar 1981). Light-saturation curves were obtained by adjustments to the model $P_N = \{P_{max} [1 - \exp(-\alpha\ PAR)/P_{max}]\} - R_D$, where P_{max} [μmol(CO_2) m^{-2} s^{-1}] corresponding to the maximum photosynthetic rate at saturation irradiance, α [μmol(CO_2) $μmol^{-1}$(photon)], the apparent quantum efficiency of photosynthesis and R_D [μmol(CO_2) m^{-2} s^{-1}] the rate of dark respiration. From the adjusted values, we calculated the compensation irradiance (Γ_{PAR}).

Photosynthetic pigments were determined in the second and third mature leaf from the apex of flooded and control plants of both genotypes. Leaves were collected from flooded and control plants at regular intervals of 0, 10, 20, 30, and 40 d after waterlogging using methodology described by Hiscox and Israelstam (1979), with some modifications. After incubation of three leaf discs (0.5 cm^2) with 4 mL of dimethylsulfoxide (DMSO) saturated with $CaCO_3$ at 65°C for 24 h, absorbance of the extracts was determined in a microplate spectrophotometer (*VersaMax*, *Molecular Devices*, CA, USA) at wavelengths of 480, 649, and 665 nm. Chl *a*, Chl *b*, total Chl, and carotenoid concentrations were determined using equations described by Wellburn (1994) for DMSO extracts.

Peroxidases and polyphenol oxidases activities: To analyze the activity of peroxidases (PODs; EC1.11.1.7) and polyphenol oxidases (PPOs, EC 1.10.3.1), samples were collected from the second and third mature leaves at 0, 3, 6, 12, 24, 48, and 96 h of control and flooded plants of both genotypes. Enzyme activities were determined using methodology similar to that described by Pirovani *et al*. (2008). Peroxidase activity was expressed with the increased consumption of guaiacol in μmol g^{-1}(DM) h^{-1}. The conversion of data from absorbance values, at 470 nm g^{-1}(DM) min^{-1} to guaiacol consumption in μmol g^{-1}(DM) h^{-1}, was done using the equation: y = 0.1284 x + 0.0189 (R^2 = 0.99) obtained from a standard guaiacol-POD curve. The reading for polyphenol oxidase activity was performed at a wavelength of 444 nm. The polyphenol oxidases activity was expressed as the increased consumption of epicatechin in mg g^{-1}(DM) h^{-1}, based on the equation y= 50.657 x + 0.091 (R^2 = 0.99) obtained from a standard PPO-epicatechin curve.

Mineral macro- and micronutrients determination: Macro- and micronutrient contents were determined in the dry biomass of roots, stems, and leaves. After nitro-perchloric digestion, Ca, Mg, Fe, Zn, Cu, and Mn concentrations were quantified with an atomic absorption spectrophotometer *CG7000-SBC* (*Perkin Elmer*, MA, USA). Concentrations of P were determined colorimetrically utilizing the vitamin C approach (Braga and Defelipo 1974) and a *B572-A* spectrophotometer (*Micronal*, Ludwigshafen, Germany), K content was determined by flame emission photometry using a flame photometer *B462* (*Micronal*) (Isaac and Kerber 1971), and N concentration was determined by the Kjeldahl method using a micro Kjeldahl *TE0363* system (*Tecnal*, Piracicaba, Brazilia) after sulphosalicylic digestion of the samples (Jackson 1958).

Total soluble sugars (TSS) and starch were determined in the dry biomass of roots, stems, and leaves of flooded and control plants of both genotypes. TSS were quantified by the anthrone method (Clegg 1956) and starch with the method described by McCready *et al*. (1950).

Statistical analysis: The experiment was arranged as 2 × 2 factorial design, with two genotypes (flood-tolerant clone TSA-792 and flood-susceptible clone TSH-774) × two water regimes (flooded and control). Each treatment combination was replicated five times and each experimental unit had four plants. Mean differences were determined by two-way *ANOVA*. Linear regressions for the evaluated physiological parameters were also done.

Differences between parameters of the linear regression equations were determined using the test of homogeneity of slopes (Steel and Torrie 1980).

Results

Morphological changes: Hypertrophic lenticels on submerged portions of stems and roots appeared as early as 3 d after flooding in the flood-tolerant clone and 20 d after flooding in the flood-susceptible clone. After 15 d of flooding, leaf chlorosis was observed in flooded plants, mainly on the flood-susceptible clone, which showed a higher degree of chlorosis than the flood-tolerant clone. Furthermore, 30 d after initiation of the flooding treatment, 5 plants of the flood-susceptible clone died, whereas no flood-tolerant plants died.

Leaf gas exchange and Chl fluorescence emission: Differences between treatments by the test of homogeneity of slopes (Steel and Torrie 1980) were only detected for response curves of P_N to increasing PAR of both genotypes. The PAR responses curves for P_N of flooded plants showed decline of P_N after 10 d of flooding for both genotypes (Fig. 1). There were no significant differences ($p>0.05$) between treatments for R_D values of both genotypes. Furthermore, the α and Γ_{PAR} values were significantly different ($p<0.05$) between treatments for both genotypes, after 20 d of waterlogging (Table 2).

Significant decreases ($p<0.05$) in g_s and E of flooded plants were observed for both genotypes (Table 1) after 10 d of flooding. Also, in both clones E was about 80% lower in flooded plants relative to nonflooded controls after 20 d of waterlogging (Table 1). Additionally, C_i/C_a was higher in flooded than nonflooded plants ($p<0.05$) for both genotypes, after 30 d of flooding (Table 1). However, no significant differences ($p>0.05$) were observed for both genotypes in relation to WUE_i between control and flooded plants in the first weeks of flooding (Table 1). After 40 d of flooding, a significant increase ($p<0.05$) was found for WUE_i of flooded plants of the flood-tolerant clone (48%) and significant decrease ($p<0.05$) for flooded plants of the flood-susceptible clone (80%) compared to nonflooded plants. For both genotypes no significant difference between flooding treatments were observed ($p>0.05$) for the WUE during the 40 d of flooding.

Significant differences ($p<0.05$) between treatments were observed in the flood-susceptible clone for F_o, F_m and F_v/F_m parameters (Table 2), with higher in F_o and lower in F_m and F_v/F_m values in the flooded plants compared to the control ones. The control plants of both genotypes showed F_v/F_m values close to 0.8, while the flooded ones of the flood-susceptible clone showed significant lower values ($p<0.05$) after 20 d of flooding (Table 2).

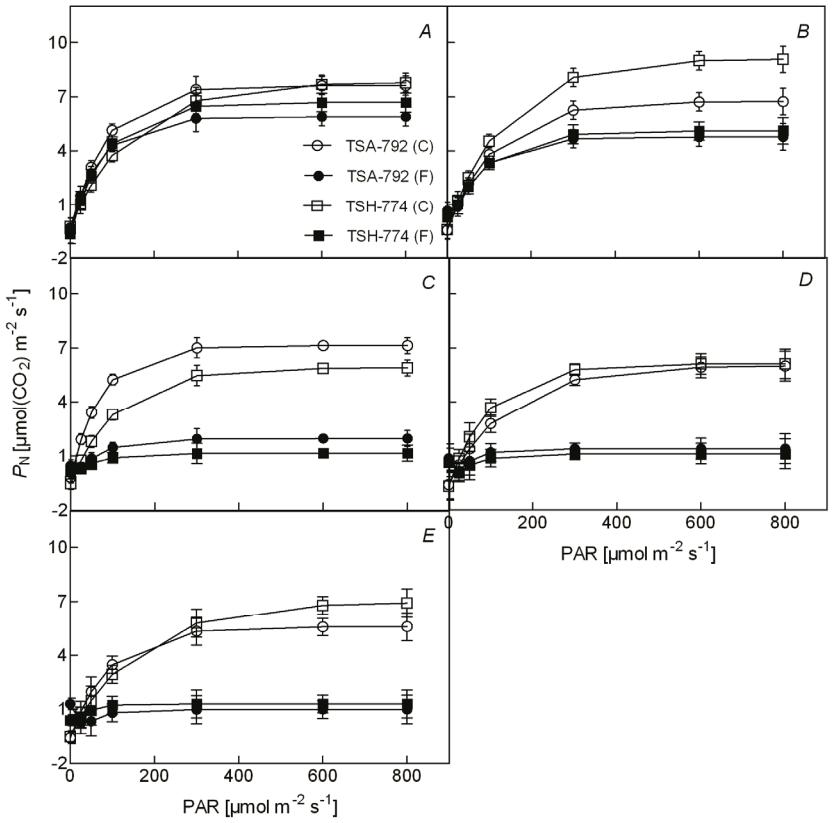

Fig. 1. Response curve of leaf net photosynthetic rate (P_N) as a function of photosynthetically active radiation (PAR) in flood-tolerant (TSA-792) and flood-susceptible (TSH-774) *Theobroma cacao* clones after 0 (*A*), 10 (*B*), 20 (*C*), 30 (*D*) and 40 (*E*) days of flooding. C – control and F – flooded. Symbols indicate mean ± SE, $n = 5$. $P_N = P_{max}\,[1 - \exp(-\alpha\,PAR/P_{max})] - R_D$.

Table 1. Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration (E), and ratio between internal (C_i) and atmospheric (C_a) CO_2 (C_i/C_a), and intrinsic water-use efficiency (WUE_i) of flood-tolerant (TSA-792) and flood-susceptible (TSH-774) *Theobroma cacao* clones after 0, 10, 20, 30, and 40 d of flooding. Variables estimated at PAR > 400 μmol(photon) m^{-2} s^{-1}. Means of five replications ± SE. *Uppercase letters* compare differences between flooding treatments within genotypes and *lowercase letters* compare differences between genotypes. Mean comparisons were made using a *t*-test (p<0.05).

Genotype	Day	Treatment	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	WUE_i	C_i/C_a
TSA-792	0	Control	7.3 ± 0.4^{Aa}	0.078 ± 0.001^{Aa}	1.8 ± 0.3^{Aa}	93 ± 15^{Aa}	0.6 ± 0.1^{Aa}
		Flooded	5.7 ± 0.5^{Aa}	0.058 ± 0.003^{Aa}	1.6 ± 0.1^{Aa}	98 ± 15^{Aa}	0.6 ± 0.1^{Aa}
	10	Control	6.6 ± 0.4^{Aa}	0.07 ± 0.002^{Aa}	1.7 ± 0.3^{Aa}	94 ± 12^{Aa}	0.7 ± 0.1^{Aa}
		Flooded	4.7 ± 0.2^{Ba}	0.05 ± 0.004^{Aa}	1.4 ± 0.2^{Aa}	95 ± 2^{Aa}	0.7 ± 0.1^{Aa}
	20	Control	5.8 ± 0.3^{Aa}	0.072 ± 0.001^{Aa}	1.6 ± 0.1^{Aa}	81 ± 12^{Aa}	0.6 ± 0.1^{Aa}
		Flooded	1.0 ± 0.6^{Ba}	0.012 ± 0.001^{Ba}	0.3 ± 0.0^{Ba}	83 ± 9^{Aa}	0.6 ± 0.1^{Aa}
	30	Control	6.0 ± 0.2^{Aa}	0.069 ± 0.004^{Aa}	1.1 ± 0.2^{Ab}	86 ± 5^{Aa}	0.9 ± 0.1^{Aa}
		Flooded	1.4 ± 0.1^{Ba}	0.017 ± 0.003^{Ba}	0.5 ± 0.2^{Ba}	84 ± 1^{Aa}	0.7 ± 0.2^{Aa}
	40	Control	5.4 ± 0.5^{Aa}	0.055 ± 0.002^{Aa}	1.2 ± 0.1^{Ab}	98 ± 9^{Bb}	0.6 ± 0.1^{Ba}
		Flooded	1.1 ± 0.1^{Ba}	0.008 ± 0.001^{Ba}	0.2 ± 0.0^{Ba}	137 ± 9^{Aa}	1.1 ± 0.2^{Aa}
TSH-774	0	Control	7.1 ± 0.8^{Aa}	0.093 ± 0.001^{Aa}	1.8 ± 0.3^{Aa}	77 ± 2^{Aa}	0.6 ± 0.1^{Aa}
		Flooded	6.9 ± 1.1^{Aa}	0.085 ± 0.002^{Aa}	1.5 ± 0.4^{Aa}	82 ± 9^{Aa}	0.6 ± 0.1^{Aa}
	10	Control	8.2 ± 0.4^{Ab}	0.089 ± 0.004^{Aa}	1.6 ± 0.1^{Aa}	93 ± 24^{Aa}	0.6 ± 0.1^{Aa}
		Flooded	4.9 ± 1.2^{Ba}	0.052 ± 0.002^{Ba}	0.9 ± 0.4^{Bb}	95 ± 25^{Aa}	0.5 ± 0.3^{Aa}
	20	Control	5.7 ± 0.1^{Aa}	0.073 ± 0.003^{Aa}	1.6 ± 0.3^{Aa}	78 ± 3^{Aa}	0.6 ± 0.1^{Ba}
		Flooded	1.0 ± 0.2^{Ba}	0.015 ± 0.001^{Ba}	0.3 ± 0.1^{Ba}	69 ± 16^{Aa}	0.8 ± 0.1^{Aa}
	30	Control	5.8 ± 0.5^{Aa}	0.09 ± 0.004^{Aa}	1.7 ± 0.4^{Aa}	65 ± 7^{Aa}	0.7 ± 0.1^{Ba}
		Flooded	1.6 ± 0.1^{Ba}	0.025 ± 0.003^{Ba}	0.5 ± 0.4^{Ba}	65 ± 10^{Aa}	0.9 ± 0.1^{Aa}
	40	Control	6.7 ± 0.9^{Aa}	0.065 ± 0.002^{Aa}	1.5 ± 0.3^{Aa}	103 ± 7^{Aa}	0.7 ± 0.1^{Ba}
		Flooded	1.1 ± 0.3^{Ba}	0.019 ± 0.004^{Ba}	0.2 ± 0.1^{Ba}	58 ± 28^{Ab}	1.0 ± 0.3^{Aa}

Photosynthetic pigments for all treatments at the beginning of the experimental period were not significantly different (p>0.05) for both genotypes (Table 3). However, 30 days after flooding treatments were initiated, Chl *a* and total Chl were lower in the flooded plants compared to the control ones in the flood-susceptible clone (Table 2).

Peroxidases and polyphenol oxidases activities: In the flood-tolerant clone, there was significantly less activity of peroxidases in the flooded plants compared to the control ones after 12 h of flooding. In the flood-susceptible clone, POD activity tended to be higher in the flooded than in the nonflooded plants after 48 h of flooding (Fig. 2).

In the flood-tolerant clone, the activity of polyphenol oxidases was not significantly different (p>0.05) to that of PODs (Fig. 3). Activity of PPOs in leaves of flooded plants was significantly lower (p<0.05) compared to the control plants after 6 h of flooding, whereas the flooded plants of the flood-susceptible clone had higher PPO activity compared to the control plants after 12 h of flooding.

Mineral nutrients: Flooding altered the macro- and micronutrient contents in different plant organs of both genotypes (Table 4). There was a tendency for N concentration to be higher in roots and stems, and lower in leaves of the flooded plants compared to the nonflooded ones. There was a significantly higher N concentration (p<0.05) in roots and stems of the flooded plants than in those of the nonflooded plants in the flood-tolerant clone. In contrast, in the flood-susceptible clone, there was a significantly lower leaf N concentration (p<0.05) in the flooded plants compared to the nonflooded ones (Table 4). There was a slight decrease in P concentration in all organs of both clones as a result of flooding, but only in leaves of the flood-susceptible clone was the difference statistically significant (p<0.05). Concentrations of K and Mg were higher in the flooded plants than in the nonflooded ones for both clones. The highest decreases in K concentration were found in leaves of the flooded plants of the flood-tolerant clone (29%) and in roots and stems of the flooded plants of the flood-susceptible clone (41%) compared to controls (Table 4).

The Ca, Zn, and Mn concentrations in the different organs of both genotypes were significantly different (p<0.05) between treatments. A higher concentration of these minerals in roots and a decrease in stems and leaves of the flooded plants compared to the control ones was observed (Table 4). Additionally, there was a higher Fe concentration in several organs of the flooded plants of both genotypes compared to the control. The highest Fe

Table 2. Variables of leaf chlorophyll fluorescence emission and compensation irradiance (Γ_{PAR}) and apparent quantum efficiency of photosynthesis (α) of flood-tolerant (TSA-792) and flood-susceptible (TSH-774) *Theobroma cacao* clones after 0, 10, 20, 30, and 40 d of flooding. Means of five replications ± SE. Uppercase letters compare differences between flooding treatments within genotypes and lowercase letters compare differences between genotypes. Mean comparisons were made using a *t*-test (p<0.05).

Genotype	Day	Treatment	F_o	F_m	F_v/F_m	Γ_{PAR} [μmol(photon) m^{-2} s^{-1}]	α [μmol(CO_2) μmol(photon)$^{-1}$]
TSA-792	0	Control	327 ± 37^{Aa}	$1{,}463 \pm 19^{Aa}$	0.79 ± 0.01^{Aa}	6.2 ± 1.7^{Aa}	0.10 ± 0.01^{Aa}
		Flooded	296 ± 12^{Aa}	$1{,}454 \pm 13^{Aa}$	0.78 ± 0.01^{Aa}	3.9 ± 2.1^{Aa}	0.08 ± 0.01^{Ba}
	10	Control	321 ± 26^{Aa}	$1{,}513 \pm 17^{Aa}$	0.80 ± 0.02^{Aa}	5.9 ± 2.7^{Aa}	0.06 ± 0.01^{Aa}
		Flooded	326 ± 20^{Aa}	$1{,}494 \pm 53^{Aa}$	0.80 ± 0.01^{Aa}	8.9 ± 1.4^{Aa}	0.07 ± 0.01^{Aa}
	20	Control	295 ± 8^{Aa}	$1{,}518 \pm 13^{Aa}$	0.77 ± 0.01^{Aa}	10.6 ± 1.9^{Aa}	0.10 ± 0.01^{Aa}
		Flooded	315 ± 7^{Aa}	$1{,}500 \pm 40^{Aa}$	0.77 ± 0.01^{Aa}	13.6 ± 3.7^{Aa}	0.04 ± 0.01^{Ba}
	30	Control	328 ± 34^{Aa}	$1{,}449 \pm 21^{Aa}$	0.78 ± 0.02^{Aa}	12.0 ± 4.6^{Aa}	0.05 ± 0.01^{Aa}
		Flooded	342 ± 20^{Aa}	$1{,}435 \pm 33^{Aa}$	0.77 ± 0.01^{Aa}	15.6 ± 2.1^{Aa}	0.05 ± 0.01^{Aa}
	40	Control	338 ± 10^{Aa}	$1{,}512 \pm 41^{Aa}$	0.79 ± 0.01^{Aa}	8.4 ± 1.5^{Aa}	0.07 ± 0.01^{Aa}
		Flooded	340 ± 23^{Aa}	$1{,}500 \pm 14^{Aa}$	0.78 ± 0.02^{Aa}	21 ± 1.8^{Ba}	0.06 ± 0.01^{Aa}
TSH-774	0	Control	318 ± 11^{Aa}	$1{,}443 \pm 10^{Aa}$	0.8 ± 0.02^{Aa}	5.0 ± 2.2^{Aa}	0.06 ± 0.01^{Aa}
		Flooded	314 ± 10^{Aa}	$1{,}450 \pm 39^{Aa}$	0.8 ± 0.02^{Aa}	10.2 ± 3.4^{Aa}	0.09 ± 0.01^{Aa}
	10	Control	304 ± 10^{Aa}	$1{,}498 \pm 19^{Aa}$	0.79 ± 0.01^{Aa}	5.3 ± 2.2^{Aa}	0.07 ± 0.01^{Aa}
		Flooded	301 ± 9^{Aa}	$1{,}492 \pm 37^{Aa}$	0.78 ± 0.02^{Aa}	4.8 ± 2.3^{Aa}	0.06 ± 0.01^{Aa}
	20	Control	301 ± 6^{Aa}	$1{,}481 \pm 12^{Aa}$	0.80 ± 0.01^{Aa}	8.1 ± 2.9^{Aa}	0.06 ± 0.01^{Aa}
		Flooded	365 ± 14^{Ba}	$1{,}297 \pm 36^{Ba}$	0.76 ± 0.01^{Bb}	6.6 ± 3.8^{Aa}	0.02 ± 0.01^{Ba}
	30	Control	303 ± 5^{Aa}	$1{,}427 \pm 21^{Aa}$	0.80 ± 0.01^{Aa}	9.0 ± 1.9^{Aa}	0.07 ± 0.01^{Aa}
		Flooded	388 ± 26^{Ba}	$1{,}154 \pm 52^{Ba}$	0.75 ± 0.01^{Bb}	17 ± 0.8^{Ba}	0.03 ± 0.01^{Ba}
	40	Control	300 ± 13^{Aa}	$1{,}512 \pm 18^{Aa}$	0.79 ± 0.01^{Aa}	9.7 ± 3.3^{Aa}	0.05 ± 0.01^{Aa}
		Flooded	489 ± 34^{Bb}	$1{,}053 \pm 43^{Ba}$	0.54 ± 0.02^{Bb}	7.5 ± 1.3^{Bb}	0.05 ± 0.01^{Aa}

concentration was found in roots, which showed an increase of almost 200% in the flood-tolerant clone and 318% in the flood-susceptible one.

TSS and starch: Flooding resulted in significantly lower (p<0.05) TSS and starch levels (Table 5) in the stems of both genotypes compared to the nonflooded plants. Furthermore, there was significantly lower concentration of carbohydrates (p<0.05) in roots of the flooded plants compared to the nonflooded ones of the flood-susceptible clone. Whereas, for the flooded plants of the flood-tolerant clone, root starch levels were significantly higher (p<0.05) compared to the flooded plants of the flood-susceptible clone (Table 5).

Discussion

In subtropical and tropical fruit trees such as *T. cacao*, symptoms of flooding include leaf chlorosis, lenticel formation and adventitious roots development (Gomes and Kozlowski 1986, Bertolde *et al.* 2010). Leaf chlorosis can be attributed to the accumulation of toxic substances, hormonal dysfunction leading to senescence, an increase in the concentration of reactive oxygen species (ROS) or to lack of nutrients (Pezeshki *et al.* 1996, Kozlowski 1997, Drew 1997). Lenticel formation is usually associated with tolerance to flooding because they allow the influx and diffusion of O_2 to the submerged roots and the release of toxic compounds associated with anaerobiosis (Chirkova and Gutman 1972). In addition to these symptoms, flooding may promote irreversible damages, leading to plant death (Schaffer *et al.* 1992).

Flooding significantly decreases P_N of many woody plant species (Kozlowski 1997). Initially, the decrease in P_N is due to stomatal closure resulting in decreased absorption of CO_2 by leaves (Pezeshki 1993, Kozlowski 1997). Stomata closuring under flooded conditions may be related to a decrease in root hydraulic conductivity (Andersen *et al.* 1984, Davies and Flore 1986), promoting a decrease in g_s. This is a common response in tolerant- and nontolerant woody plants to this type of stress (Kozlowski 1997, Bertolde *et al.* 2010). In some circumstances, g_s of flood-tolerant species has a tendency to return to values of control plants (Mielke *et al.* 2005). The reopening of stomata is usually related to the development of hypertrophic stem lenticels or adventitious root formation (Lopez and Kursar 1999). In the present study, despite the appearance of hypertrophic lenticels on the stem and in portions of submerged roots, there were no stomata reopening in flooded plants of both clones (Table 1). Similar results were found in studies of other

Table 3. Chlorophyll (Chl) *a*, Chl *b*, total Chl, and carotenoids (Car) concentrations of flood-tolerant (TSA-792) and flood-susceptible (TSH-774) *Theobroma cacao* clones after 0, 10, 20, 30, and 40 d of flooding. Means of five replications ± SE. *Uppercase letters* compare differences between flooding treatments within genotypes and *lowercase letters* compare differences between genotypes. Mean comparisons were made using a *t*-test ($p<0.05$).

Genotype	Day	Treatment	Chl *a* [mg dm^{-2}]	Chl *b* [mg dm^{-2}]	Total Chl [mg dm^{-2}]	Car [mg dm^{-2}]
TSA-792	0	Control	8.4 ± 0.3^{Aa}	2.8 ± 0.1^{Aa}	11.2 ± 0.5^{Aa}	1.8 ± 0.1^{Aa}
		Flooded	8.0 ± 0.2^{Aa}	2.9 ± 0.1^{Aa}	10.9 ± 0.3^{Aa}	1.7 ± 0.1^{Aa}
	10	Control	7.5 ± 0.5^{Aa}	2.5 ± 0.2^{Aa}	10.0 ± 0.8^{Aa}	1.6 ± 0.1^{Aa}
		Flooded	7.3 ± 0.8^{Aa}	2.5 ± 0.3^{Aa}	10.0 ± 1.0^{Aa}	1.6 ± 0.1^{Aa}
	20	Control	7.5 ± 0.4^{Aa}	2.7 ± 0.2^{Aa}	10.3 ± 0.6^{Aa}	1.6 ± 0.1^{Aa}
		Flooded	7.3 ± 0.2^{Aa}	2.5 ± 0.5^{Aa}	10.0 ± 2.6^{Ab}	1.5 ± 0.1^{Aa}
	30	Control	8.2 ± 0.1^{Aa}	2.9 ± 0.1^{Aa}	11.0 ± 0.2^{Aa}	1.8 ± 0.01^{Aa}
		Flooded	7.8 ± 0.2^{Aa}	2.7 ± 0.1^{Aa}	9.1 ± 0.3^{Aa}	1.6 ± 0.01^{Ba}
	40	Control	6.8 ± 0.3^{Aa}	2.4 ± 0.1^{Aa}	9.2 ± 0.4^{Aa}	1.6 ± 0.1^{Aa}
		Flooded	6.0 ± 1.0^{Aa}	2.7 ± 0.5^{Aa}	8.7 ± 1.5^{Aa}	1.6 ± 0.2^{Aa}
TSH-774	0	Control	7.6 ± 0.4^{Aa}	2.7 ± 0.2^{Aa}	10.3 ± 0.5^{Aa}	1.6 ± 0.01^{Aa}
		Flooded	7.4 ± 0.7^{Aa}	2.5 ± 0.1^{Aa}	9.8 ± 0.8^{Aa}	1.7 ± 0.2^{Aa}
	10	Control	6.6 ± 0.8^{Aa}	2.2 ± 0.7^{Aa}	8.8 ± 1.1^{Aa}	1.4 ± 0.2^{Aa}
		Flooded	6.2 ± 1.1^{Aa}	2.6 ± 0.4^{Aa}	8.8 ± 1.4^{Aa}	1.5 ± 0.2^{Aa}
	20	Control	7.1 ± 1.2^{Aa}	2.9 ± 0.3^{Aa}	10.0 ± 1.5^{Aa}	1.5 ± 0.2^{Aa}
		Flooded	6.0 ± 0.3^{Bb}	3.0 ± 0.2^{Aa}	9.0 ± 0.2^{Aa}	1.7 ± 0.1^{Aa}
	30	Control	8.0 ± 0.3^{Aa}	3.1 ± 0.3^{Aa}	11.1 ± 0.6^{Aa}	1.7 ± 0.1^{Aa}
		Flooded	6.6 ± 0.7^{Ba}	2.7 ± 0.2^{Aa}	9.3 ± 0.9^{Aa}	1.8 ± 0.2^{Aa}
	40	Control	7.8 ± 0.6^{Aa}	2.7 ± 0.2^{Aa}	10.5 ± 0.8^{Aa}	1.8 ± 0.1^{Aa}
		Flooded	3.3 ± 1.2^{Bb}	2.3 ± 0.3^{Aa}	5.6 ± 0.5^{Ba}	1.5 ± 0.2^{Aa}

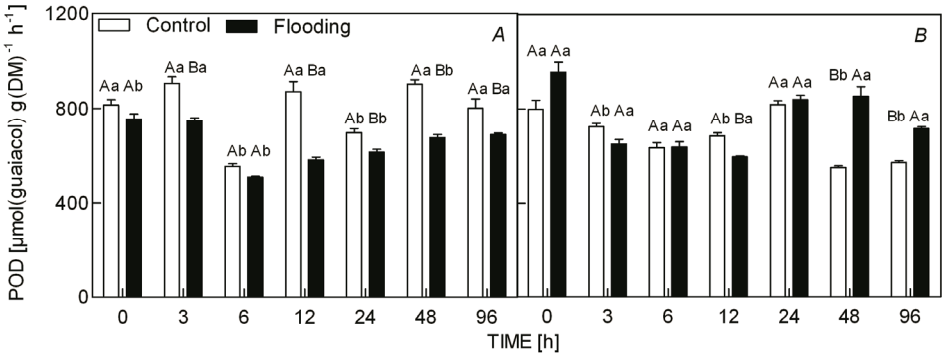

Fig. 2. Peroxidases (PODs) activity in leaves of flood-tolerant (TSA-792) (*A*) and flood-susceptible (TSH-774) (*B*) *Theobroma cacao* clones at 0, 3, 6, 12, 24, 48, and 96 h after flooding. Means of five replications ± SE. *Uppercase letters* compare differences between flooding treatments within genotypes and *lowercase letters* compare differences between genotypes. Mean comparisons were made using *t*-test ($p<0.05$).

tree species by Carvalho and Ishida (2002) and Mielke *et al*. (2003, 2005). In fruit trees, the decline in transpiration rates in response to flooding is probably due to a decrease of g_s, since the deficiency of O_2 did not significantly decrease the xylem water potential (Schaffer *et al*. 1992).

The ratio of C_i/C_a is considered an appropriate indicator of stomatal limitation of photosynthesis (Farquhar and Sharkey 1982). This was corroborated by the fact that C_i/C_a values of flooded plants were higher than those of control plants of both clones (Table 1). Working with neotropical species, Mielke *et al.* (2005) demonstrated that there is an increase in WUE_i of flooded plants, similar to that observed for the flood-tolerant clone in the present study, indicating greater use of water in such condition. In contrast, Mielke *et al*. (2003) observed no significant effects of flooding on WUE of *Genipa americana*. Similar results were also observed for the two evaluated cacao clones in the present study.

The sharp decrease in P_N of woody plants subjected to

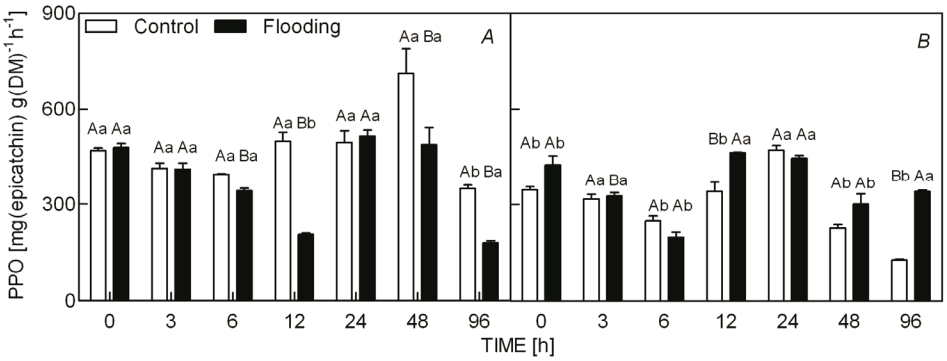

Fig. 3. Polyphenoloxidases (PPOs) activity in leaves of flood-tolerant (TSA-792) (*A*) and flood-susceptible (TSH-774) (*B*) *Theobroma cacao* clones 0, 3, 6, 12, 24, 48, and 96 h after flooding. Means of five replications ± SE. *Uppercase letters* compare differences between flooding treatments within genotypes and *lowercase letters* compare differences between genotypes. Mean comparisons were made using *t*-test ($p<0.05$).

Table 4. Macro- and micronutrient concentrations in flood-tolerant (TSA-792) and flood-susceptible (TSH-774) *Theobroma cacao* clones after 40 d of flooding. Means of five replications ± SE. *Uppercase letters* compare differences between flooding treatments within genotypes and *lowercase letters* compare differences between genotypes. Mean comparisons were made using a *t*-test ($p<0.05$).

Genotype	Organ	Treatment	Macronutrient [g kg^{-1}(DM)] N	P	K	Ca	Mg
TSA-792	Root	Control	14.2 ± 0.5^{Ba}	1.6 ± 0.1^{Aa}	9.5 ± 0.7^{Ab}	4.2 ± 0.2^{Ba}	2.4 ± 0.1^{Ab}
		Flooded	17.0 ± 0.4^{Aa}	1.7 ± 0.1^{Aa}	7.0 ± 0.8^{Ba}	6.1 ± 0.2^{Aa}	2.4 ± 0.2^{Aa}
	Stem	Control	6.0 ± 0.1^{Bb}	1.8 ± 0.1^{Aa}	18.8 ± 0.9^{Ab}	8.8 ± 0.5^{Ab}	2.8 ± 0.1 ^{Ab}
		Flooded	9.0 ± 0.6^{Ab}	1.8 ± 0.1^{Aa}	13.2 ± 0.7^{Ba}	6.5 ± 0.5^{Bb}	1.5 ± 0.1^{Bb}
	Leaf	Control	17.4 ± 0.9^{Aa}	2.0 ± 0.1^{Aa}	20.3 ± 0.6^{Aa}	9.4 ± 0.3^{Aa}	5.5 ± 0.1^{Aa}
		Flooded	16.2 ± 0.7^{Aa}	1.7 ± 0.0^{Ba}	14.5 ± 0.6^{Bb}	6.1 ± 0.3^{Ba}	4.4 ± 0.1^{Ba}
TSH-774	Root	Control	14.2 ± 1.0^{Aa}	2.0 ± 0.1^{Aa}	13.5 ±0.5^{Aa}	4.6 ± 0.2^{Ba}	3.2 ± 0.1^{Aa}
		Flooded	16.4 ± 0.4^{Aa}	1.8 ± 0.1^{Aa}	6.6 ± 0.9^{Ba}	6.6 ± 0.3^{Ab}	2.4 ± 0.1^{Aa}
	Stem	Control	8.2 ± 0.6^{Aa}	2.5 ± 0.2^{Aa}	25.6 ± 0.3^{Aa}	14.3 ± 0.8^{Aa}	4.0 ± 0.1^{Aa}
		Flooded	9.4 ± 0.5^{Aa}	1.8 ± 0.1^{Ba}	15.2 ± 0.6^{Ba}	12.2 ± 0.4^{Aa}	2.3 ± 0.1^{Ba}
	Leaf	Control	21.5 ± 0.8^{Aa}	2.5 ± 0.1^{Aa}	22.8 ± 1.0^{Ab}	8.8 ± 0.4^{Aa}	5.0 ± 0.1^{Aa}
		Flooded	18.1 ± 0.8^{Ba}	1.9 ± 0.0^{Ba}	17.7 ± 0.6^{Ba}	5.6 ± 0.6^{Ba}	3.8 ± 0.2^{Ba}

Genotype	Organ	Treatment	Micronutrient [mg kg^{-1}(DM)] Fe	Zn	Cu	Mn
TSA-792	Root	Control	4,330 ± 243^{Ba}	32 ± 0.7^{Ba}	20 ± 0.5^{Aa}	102 ± 2.9^{Ba}
		Flooded	9,917 ± 323^{Aa}	40 ± 0.9^{Aa}	20 ± 1.8^{Aa}	251 ± 4.7^{Aa}
	Stem	Control	153 ± 7^{Bb}	50 ± 0.8^{Ab}	8 ± 0.6^{Aa}	122 ± 2.2^{Ab}
		Flooded	284 ± 45^{Aa}	39 ± 0.3^{Ba}	7 ± 0.8^{Aa}	63 ± 3.6^{Bb}
	Leaf	Control	130 ± 8^{Ba}	61 ± 1.8^{Aa}	9 ± 0.7^{Aa}	523 ± 5.1^{Aa}
		Flooded	214 ± 6^{Aa}	52 ± 1.9^{Aa}	8 ± 0.8^{Aa}	296 ± 1.4^{Ba}
TSH-774	Root	Control	2,862 ± 81^{Bb}	36 ± 0.8^{Ba}	16 ± 1.6^{Ba}	96 ± 2.7^{Ba}
		Flooded	11,476 ± 257^{Aa}	43 ± 1.3^{Aa}	23 ± 1.0^{Aa}	250 ± 3.7^{Aa}
	Stem	Control	215 ± 15^{Aa}	66 ± 1.9^{Aa}	10.2 ± 0.5^{Aa}	166 ± 5.1^{Aa}
		Flooded	263 ± 9^{Ba}	48 ± 0.9^{Ba}	7.8 ± 0.8^{Ba}	80 ± 4.6^{Ba}
	Leaf	Control	178 ± 13^{Ba}	55 ± 2.4^{Ab}	10.2 ± 0.5^{Aa}	520 ± 7.2^{Aa}
		Flooded	249 ± 9^{Aa}	44 ± 1.6^{Bb}	8.2 ± 0.8^{Aa}	258 ± 6.1^{Ba}

Table 5. Starch and total soluble sugars (TSS) concentrations in flood-tolerant (TSA-792) and flood-susceptible (TSH-774) *Theobroma cacao* clones after 40 d of flooding. Means of five replications ± SE. *Uppercase letters* compare differences between flooding treatments within genotypes and *lowercase letters* compare differences between genotypes. Mean comparisons were made using a *t*-test ($p<0.05$).

Genotype	Organ	Treatment	Starch [mg g^{-1}(DM)]	TSS [mg g^{-1}(DM)]
TSA-792	Root	Control	20 ± 0.7^{Ba}	28 ± 5^{Aa}
		Flooded	22 ± 0.4^{Aa}	26 ± 6^{Aa}
	Stem	Control	50 ± 2^{Ba}	34 ± 7^{Bb}
		Flooded	84 ± 12^{Aa}	62 ± 7^{Ab}
	Leaf	Control	61 ± 8^{Aa}	21 ± 3^{Ba}
		Flooded	54 ± 9^{Aa}	32 ± 8^{Aa}
TSH-774	Root	Control	23 ± 1^{Aa}	31 ± 2^{Aa}
		Flooded	20 ± 1^{Ba}	28 ± 3^{Ba}
	Stem	Control	33 ± 3^{Bb}	44 ± 5^{Ba}
		Flooded	63 ± 16^{Ab}	84 ± 13^{Aa}
	Leaf	Control	36 ± 4^{Ab}	21 ± 0.6^{Ba}
		Flooded	38 ± 6^{Ab}	30 ± 3^{Aa}

flooding can be attributed to stomatal closure (Kozlowski 1997). However, after prolonged periods of flooding, decreases in carbon fixation rates may be related to inhibitory effects on the photosynthetic processes. Alterations in enzymes of the Calvin cycle and degradation of photosynthetic pigments may reduce the carboxylation efficiency as well as the apparent quantum efficiency (α) in flooded plants, which correspond to nonstomatal limitations to photosynthesis (Pezeshki 2001). The decrease in α can be directly linked to the limitations of photosynthesis, which in turn are related to the degradation of photosynthetic pigments (Pezeshki 2001). Values of α are usually constant under favorable conditions, but very sensitive to stressful situations and may undergo changes (Pachepsky and Acock 1996), as observed in this study.

The ratio F_v/F_m is considered to be an important indicator of the effects of environmental stresses on photosynthesis (Maxwell and Johnson 2000), including soil flooding (Mielke *et al*. 2003, Bertolde *et al*. 2010). F_v/F_m values are almost constant (around 0.832 ± 0.004) for most plants species under nonlimiting conditions (Björkman and Demmig 1987); however, in stressed plants, lower values indicate occurrence of photoinhibition or other injury to the light-harvesting complex of PSII (Krause and Weis 1991). Furthermore, stressed plants show an increase of F_0, indicating a decreased flow of electrons through PSII (Oliveira *et al*. 2002). Bertolde *et al*. (2010), working with 35 clones of *T. cacao* under flooded conditions showed decreases in the F_v/F_m ratio and increases in F_0 values for some clonal varieties. This performance was also observed in the present study with the flood-susceptible clone under waterlogging (Table 2), indicating high nonstomatal limitations of photosynthesis.

Other factors such as low levels of Chl and/or its degradation contribute to reduction in leaf photosynthetic capacity (Pezeshki 2001). The decrease in concentrations of photosynthetic pigments has been interpreted as a long-term response to flooding (Smethurst and Shabala 2003) as was observed for plants of the flood-susceptible clone after 30 d of flooding (Table 3). Low concentration of photosynthetic pigments limits the photochemical process, since radiation absorption depends on its content (Pezeshki *et al.* 1996).

Some authors have suggested that flooding can induce oxidative stress, causing an increased production of ROS, like superoxide ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) (Yu and Rengel 1999, Yordanova *et al*. 2004). These oxygen species, which levels are controlled by antioxidant enzymes such as peroxidases (Foyer *et al*. 1994), can cause severe damage to cell membranes, DNA, and proteins. Yordanova *et al*. (2004) found a significant increase in POD activity in leaves of *Hordeum vulgare* L. after 72 h of flooding, which was also observed in this study on leaves of the flood-susceptible clone in the flood conditions (Fig. 2), indicating occurrence of oxidative stress. In contrast, there was a decrease in POD activity in the flooded plants of the flood-tolerant clone, suggesting a probable activation of fermentative pathways, reducing mitochondrial activity. This would imply in a decrease in electron transport, reduction in peroxide and superoxide formation and, consequently, reduction in production and activation of peroxidases.

The activity of oxidative enzymes such as PPOs has been studied in plants under stress or as apart of defense mechanisms (Siegel 1993, Sánchez *et al*. 2000). Most PPOs activity takes place in chloroplasts and their occurrence in leaf tissues cells depends on the developmental stage, species and age, while its activity depends on light (Dogan *et al*. 2005). PPOs oxidize a broad group of phenols and their activity can be enhanced or inhibited in some plants by biotic and abiotic stresses (Vaughn and Duke 1984, Sánchez *et al*. 2000). This justifies the increase in PPO activity in the flooded plants of the flood-susceptible clone when compared to the control plants (Fig. 3).

Flooding promotes the disruption of root growth, which is associated with the impairment of aerobic respiration at the cellular level and causes a decrease in the absorption and transport of nutrients to the shoots (Barrett-Lennard *et al*. 1999, Smethurst and Shabala 2003). In nontolerant species, usually the reduction of leaf N, P, and K concentrations, caused by flooding, coincides with the appearance of chlorosis in this organ (Drew 1997), normally attributed to N deficiency. In this study, we observed a decrease in N and Chl concentrations in the flooded plants of the flood-susceptible clone (Table 4). In this case, the decrease in N concentration greatly contributed to the reduction in Chl content and thus with the appearance of leaf chlorosis (Carvalho and Ishida 2002).

Magnesium is an important component of the Chl molecule and is involved in the activation of several photosynthetic enzymes and carbohydrates partitioning (Laing *et al*. 2000). Its decrease observed in leaves of flooded plants of both cacao genotypes may also be associated with leaf chlorosis (Smethurst and Shabala 2003). In contrast, the concentrations of Fe and Mn in plant vegetative parts often increase due to flooding, which was the case in all organs of the flooded plants of both cacao genotypes (Table 4). These elements are found in soluble forms in flooded soils (Ponnamperuma 1972), which facilitates their absorption.

The deficiency of O_2 in the soil induces the replacement of aerobic- by anaerobic respiration in roots. The latter is less efficient than the aerobic production of ATP per molecule of glucose used; therefore, root tissues require large amounts of sugars to generate enough ATP to keep the cells functioning under anoxic conditions (Vartapetian 1996, Vartapetian and Jackson 1997). On the other hand, flooding reduces g_s and causes chlorosis of leaves, thus decreasing photosynthesis (Carvalho and Ishida 2002). Also, translocation of TSS from shoots to roots is affected by flooding, and is drastically reduced in many nontolerant species (Kozlowski and Pallardy 1984, Pezeshki 1994, Kozlowski 1997). Thus, TSS produced by photosynthesis tend to accumulate in the shoot and are not translocated to the roots, as observed in this study, where the flooded plants of both genotypes showed higher levels of TSS and starch in the stem (Table 5). However, only plants of the flood-susceptible clone showed significant decreases in carbohydrates levels in the roots, suggesting that the flood-tolerant clone, even with increased concentrations of carbohydrates in the shoots of flooded plants, was able to maintain the translocation of photoassimilates to the roots. The greater availability of respiratory substrates, such as glucose, can be decisive in the survival of root tissues under anoxic environments (Crawford and Braendle 1996, Drew 1997, Vartapetian and Jackson 1997).

In summary, *T. cacao* genotypes showed changes in several physiological and biochemical variables in response to flooding. However, the flooded plants of the flood-susceptible clone showed: (*1*) nonstomatal limitations to photosynthesis, since the decrease in F_v/F_m values indicated possible damage to the PSII light-harvesting complex; (*2*) oxidative stress; (*3*) increase leaf chlorosis; (*4*) decreased roots carbohydrates concentrations. These factors resulted in death of five plants after 30 d of flooding. The low incidence of leaf chlorosis associated with the absence of damage to the PSII light-harvesting complex allows carbon fixation by photosynthesis, assuring the supply of assimilates to plants under anoxic conditions. These assimilates, once translocated to the roots, are consumed by the anaerobic metabolism, providing the necessary energy for survival under O_2 deficiency. Further studies will elucidate the role of anaerobic respiration and other regulatory processes in the tolerance to soil flooding of *T. cacao* genotypes.

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