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Morphophysiological responses of *Theobroma cacao* L. rootstocks to flooding and post-flooding conditions

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Abstract

This study investigates how cocoa rootstocks respond to flooding and post-flooding conditions, offering insights for cocoa plantation sustainability in flood-prone areas due to climate change. We studied *Theobroma cacao* L. rootstocks for 60 d of flooding and 30 d post-flooding, grafting PS-1319 scions onto five rootstocks (TSH-1188, Cepec-2002, Pará, Esfip-02, SJ-02). Photochemical performance remained stable across rootstocks, while flooding progressively reduced electron transport efficiency. Photochemical damage emerged after 7 d, worsening occurred at 19 d. Although post-flooding efficiency improved, recovery time was insufficient for full restoration. Stem diameter increased less in Esfip-02. TSH-1188 had the highest stem dry mass during flooding and the most root and total dry mass during post-flooding. SJ-02 had the lowest stem dry mass and post-flooding total dry mass. Principal component analysis revealed stem and root development as a key for recovery. SJ-02 and Esfip-02 showed lower flooding tolerance and recovery, while TSH-1188 and Pará exhibited higher resilience.

Keywords: cocoa; photosynthesis; plasticity; water stress.

Introduction

Cocoa beans (*Theobroma cacao* L.) are economically important and marketed worldwide for the manufacturing of chocolate. In several regions of the world, including Brazil, cocoa is grown in areas subjected to intermittent flooding, such as on the banks of rivers and in shallow or poorly drained soils (Almeida and Valle 2007, Delgado

Highlights

- Photochemical performance of cocoa plants was reduced after 7 d and recovered in 14-d post-flooding
- Root development was reduced after flooding and a compensatory increase was found in stem diameter
- Tolerance was lower in SJ-02 and Esfip-02 and higher in TSH-1188 and Pará

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et al. 2016). Due to the increased intensity of rainfall caused by climate change, more flooding events have occurred in cocoa-growing areas (Lahive *et al.* 2019).

Flooding tolerance in cocoa depends on its genotype, developmental stage, and duration of stress. The juvenile phase of cocoa development is considered relatively sensitive to flooding, with a mortality rate close to 100% observed in two-year-old plants (Delgado *et al.* 2016). In a study involving 35 genotypes subjected to 45 d of flooding, the survival rate ranged from 30 to 96%, with several morphophysiological alterations observed (Bertolde *et al*. 2010). However, no study on flooding has been conducted with grafted cocoa seedlings, despite this being the primary method of propagating the species.

The effects of flooding on the development of cocoa plants have been carefully characterized for different genotypes and indicated a reduction in the growth of leaves, stems, and roots, with the severity varying according to the genotype (Rehem *et al.* 2009, Bertolde *et al.* 2010) and stress period (Sena Gomes and Kozlowski 1986). Molecular (enzymes), physiological (photosynthesis), and biochemical (carbohydrates) mechanisms have been characterized in studies of genotypes that are both sensitive and tolerant to flooding (Bertolde *et al.* 2012, 2014), with the incidence of leaf chlorosis being suggested as a sensitivity marker (Bertolde *et al.* 2010).

Flooding damages the photosynthetic apparatus and changes Chl*a*F in susceptible cocoa clones (Bertolde *et al.* 2012, Silva Branco *et al.* 2017). However, in studies by Bertolde *et al.* (2010) and De Almeida *et al.* (2016), no significant relationship was found between Chl*a*F and flood tolerance. These studies were limited to the evaluation of only a few Chl*a*F parameters, indicating the need for further exploration of the results. Chl*a*F data can be better interpreted using the JIP-test obtained with a non-modulated fluorimeter (Strasser and Strasser 1995). This evaluator test is based on the theory of energy flow in biomembranes and enables the visualization of energy flow through PSII (Strasser and Strasser 1995).

The ability of plants to recover after the removal of stress limits their ability to develop a tolerance to it. The resumption of growth and development after flooding is essential for plant survival; however, knowledge of the responses in different genotypes during the post-flooding phase is limited (Yeung *et al.* 2019). Evaluating the plasticity of genotypes during their recovery and restoration of development after the stress of flooding can be useful in determining the plants' recovery capacity following periods of inundation and guiding farmers in proper management practices to assist in the recovery process of seedlings. Some studies have investigated the recovery capacity of post-flooding in cocoa plants. Sena Gomes and Kozlowski (1986) verified that the Catongo genotype partially recovered development 11 d after a 30-d flood and prioritized the recovery of its root system. De Almeida *et al.* (2016) found that cocoa hybrids flooded for 35 d regained a photosynthetic capacity between 78–100% after 10 d of recovery.

Although there are studies on the response of cocoa genotypes to flooding, we are not aware of any research that characterizes the effects of rootstocks on the canopy under flooding conditions. The majority of cocoa plantations are established using grafted seedlings and understanding the effects of flooding and post-flooding conditions would aid farmers in selecting tolerant rootstocks. These findings have significant implications for the renewal or cultivation of improved seedlings and stress-tolerant rootstocks. Additionally, as with most crops, waterlogging is not persistent, making it essential to assess the plasticity of genotypes during their recovery and restoration of development after stress. Therefore, the objective of this study was to evaluate the development, dry mass accumulation, and Chl*a*F of *Theobroma cacao* L. rootstocks cultivated under flooding and post-flooding conditions.

Understanding the effects of flooding and post-flooding on cocoa rootstocks, which are widely used in cocoa production – a globally significant product for chocolate manufacturing – holds both theoretical and practical relevance. The theoretical importance lies in the fact that no study has assessed the effects of rootstocks on the canopy under flooding conditions. Gaining insight into these effects can provide valuable information for farmers in selecting rootstocks more tolerant to flooding stress, thereby enhancing the productivity and resilience of cocoa plantations. Moreover, from a practical perspective, the results of this study may have important implications for renewing plantations with stress-resistant rootstocks. This is particularly relevant in regions where intermittent flooding is frequent due to climate change and increased rainfall intensity, as previously mentioned. Identifying rootstocks more tolerant to flooding can help ensure the sustainability of cocoa plantations in such flood-prone areas.

Materials and methods

Experimental timing and seedling preparation: The experiment was conducted from May to July 2019, on the Experimental Farm of Linhares owned by INCAPER

Abbreviations: ABS – absorption; ABS/RC – specific absorption flux per active reaction center; APDM – shoot dry mass; APDM/RDM – ratio between shoot dry mass and root dry mass; Chl*a*F – chlorophyll *a* fluorescence; Chl index – chlorophyll index; DI_0/RC – specific dissipated energy flux per active reaction center; DMAP – dry mass of aerial part; ET_0/RC – specific electron transport flux per active reaction center; F_0 – minimum fluorescence of the dark-adapted state; F_m – maximum fluorescence of the dark-adapted state; F_v – variable fluorescence; LDM – leaf dry mass; NL – number of leaves; $PI_{(ABS)}$ – performance index on an absorption basis; RC – reaction center; RDM – root dry mass; RL – longest root length; SD – stem diameter; SDM – stem dry mass; SL – stem length; SL/RL – ratio between stem and root length; SL/SD – ratio between stem length and diameter; TDM – total dry mass; TR_0 – trapped energy flux; TR_0/RC – trapped energy flux per active reaction center; V_J – relative variable fluorescence at J-step; φD_0 – quantum yield of energy dissipation; φE_0 – electron transport quantum yield; φP_0 – maximum quantum yield of primary photochemistry.

(Capixaba Institute of Research, Technical Assistance and Rural Extension) located at 19°25'0.1"S and 40°4'35.3"W in the municipality of Linhares, in the Northern Espírito Santo State. During the experiment, climatic data were obtained from an INCAPER automatic weather station (Fig. 1).

Seedlings were produced at the Filogônio Peixoto Experimental Station, owned by the Cocoa Research Center, a research body of the Executive Committee of the Cocoa Crop Plan (CEPLAC), located in the municipality of Linhares/ES. The genotypes evaluated in this experiment were TSH-1188, Cepec-2002, Pará, Esfip-02, and SJ-02, which were used as rootstocks; and genotype PS-1319 was used as the crown. Seedlings were produced from propagules obtained from stock plants provided by the Active Germplasm Bank of CEPLAC. The genealogy and agronomic descriptors of the six cocoa genotypes used in the experiment are described below.

Full cleft top grafting was performed five months after sowing the rootstock. Two months after grafting, the seedlings were transplanted into black polyethylene pot (25 × 35 cm), with one plant per pot that contained underground earth as a substrate. The substrate was analyzed by the Laboratory of Agronomic, Environmental Analysis, and Chemical Solution Preparation (FULLIN) in Linhares/ES and classified as sandy loam containing 0.008 kg(phosphorus) m^{-3}, 0.024 kg(potassium) m^{-3}, 0.007 kg(sulfur) m^{-3}, 0.222 kg(iron) m^{-3}, 0.0026 kg(zinc) m^{-3}, 0.0006 kg(copper) m^{-3}, 0.015 kg(manganese) m^{-3}, 0.00024 kg(boron) m^{-3}, and 0.005 kg(sodium) m^{-3}. It also contained 0.0486 kg(magnesium) m^{-3}, 0.44088 kg(calcium) m^{-3}, and 9 g(organic matter) kg^{-1}.

The plants were then transferred to masonry tanks lined with canvas to prevent water infiltration. A cover was built with a shading screen under 50% incident radiation attached to the tanks at a height of 3 m. Plants were acclimatized for 60 d before treatment and irrigation during acclimatization and in the control treatment group was performed daily every 4 h for 45 min using a sprinkler system. Foliar nitrogen fertilization and pest control were performed when necessary.

Nine-month-old plants were subjected to flooding for 60 d, maintaining the water level at the height of the collection. Water was replaced when necessary to avoid column shrinkage. The dissolved oxygen content was maintained close to 0.00898 kg m^{-3}. After 60 d of flooding, the tank was emptied and the plants were irrigated for 30 d under the same conditions as the control treatment to evaluate plant recovery post-flooding.

Chl*a*F and Chls index (SPAD units) were evaluated during flooding and recovery. These factors were monitored during flooding on days 3, 5, 7, 9, 19, 29, and 55; whereas during recovery, they were monitored on days 7, 14, and 21. Evaluations were performed between 07:00 and 10:00 h on a fully expanded leaf located at the third node of the middle portion of the plant. Two plants

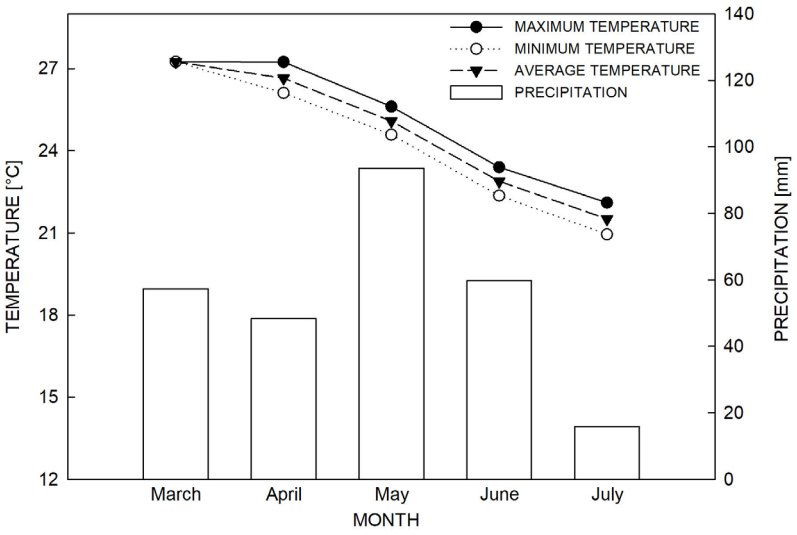

Fig. 1. Total precipitation [mm] and maximum, minimum, and average temperatures [°C], recorded at the Linhares Meteorological Station in the state of Espírito Santo from May to July 2019 during the acclimation and evaluation process of the experiment.

Genotype	Origin	Parent	Ancestry	Pollination	Fruit color and formation
Pará	Bahia	Undefined	Forastero	SC	Y/AM
TSH-1188	Trinidad and Tobago	IMC67, ICS1, SCA6, and P18	Amazônico/Trinitário	SI	R/EL
Esfip-02	Espírito Santo Region	TSH-565 and IMC-67	Trinitário/Forastero	SI	R/AL
Cepec-2002	Brasileira Farm, Uruçuca-BA	Sca-6 and Comum**	Amazônico/Amazônico	SC	YAM
SJ-02	São José Farm, Itajuipe-BA	IMC-67 and ICS-01	Amazônico/Trinitário	SC	Y/AL
PS-1319	Porto Seguro Farm, Ilhéus-BA	ICS-01 and PA-150	Amazônico/Trinitário	SC	Y-V/AM

SC – self-compatibility; SI – self-incompatibility; Y – yellow; R – red; AM – amelonado; EL – elongated. **Catongo in its composition.

per plot were evaluated. Growth, development, and dry mass accumulation were measured after 60 d of flooding and 30 d of recovery. The survival rate was calculated by analyzing mortality at the end of the experiment.

Chl index and Chl*a*F measurements: Analysis of the Chl index was determined using a portable chlorophyll meter (*SPAD-502*, *Minolta*®, Japan). Chl*a*F parameters were measured using a portable fluorimeter (*Handy-PEA*, *Hansatech*, UK) in leaves dark-adapted using leaf clips (*Hansatech*, UK) for 30 min, which is a period of complete oxidation of the photosystem. After, a flash of saturating light of 3,000 μmol(photon) m^{-2} s^{-1} (650 nm) was emitted and the fast fluorescence kinetics (F_0 to F_m) was recorded from 10 μs to 1 s. The Chl*a*F parameters established by the JIP-test were calculated according to Strasser and Strasser (1995).

Growth, development, and biomass evaluation: The number of leaves (NL), stem length (SL), stem diameter (SD), and the longest root length (RL) were evaluated in cocoa plants subjected to flooding and post-flood recovery. NF was determined by manual counting, SL was measured with a tape measure, SD was measured with a digital caliper, and RL was measured using a ruler. Based on these measurements, the relationships between stem length and diameter (SL/SD) and between stem and root length (SL/RL) were calculated.

For dry mass evaluation, the plants were separated into roots, stems, and leaves, placed in paper bags, and dried in a forced circulation oven at 70°C for 7 d until they reached a constant mass. Weighing was carried out on an analytical balance model *QUINTIX3102-10BR* with an accuracy of 0.01 g. Leaf (LDM), root (RDM), and stem (SDM) dry mass were quantified and used to calculate shoot dry mass (APDM), total dry mass (TDM), and the ratio of shoot dry mass to root dry mass (APDM/RDM).

Statistical analysis: The experimental design used randomized blocks with four replicates per treatment in a 5 × 3 factorial scheme using five rootstocks and three flood durations (non-flooded, flooded, and recovered). Each plot consisted of ten plants, and two plants per block were used to measure Chl*a*F and Chl index. Plant development, growth, and dry mass accumulation were evaluated using all ten plants per plot.

Statistical analysis was performed using *SISVAR* software (Ferreira 2011). After analysis of variance (*ANOVA*), the means were analyzed using the *Tukey*'s test or *Scott-Knott* cluster test at a significance level of 5%. The polynomial fit for the data for the flooding and recovery periods was checked using regression analysis. Data were subjected to principal component analysis (PCA) and score plots using *R* software.

Results

Effect of environment on cocoa survival and photosynthetic apparatus: No statistical differences were observed in the survival of the evaluated genotypes; the mean values obtained in the flooded plants were 98% for TSH-1188, 96% for Pará, 93% for Esfip-02, 95% for Cepec-2002, and 85% for SJ-02. Flooding promoted a small reduction in the survival rate, regardless of the genotype, with 93% in the flooded environment and 99% in the non-flooded environment.

Chl*a*F was not associated with a significant interaction between genotype and environment, but it was associated with the environment in isolation (Table 1). Flooding increased the F_0, V_J, and TR_0/RC and reduced F_m and φE_0 values. In the post-flooding treatment, the F_0, V_J, TR_0/RC, F_m, and φE_0 values were recovered in comparison with the flooded treatment but did not return to the values of the non-flooded treatment. The $PI_{(ABS)}$, ET_0/RC, and Chl index values decreased with flooding and did not recover after the stress was removed.

Effect of flooding duration on the photosynthetic apparatus: A significant interaction was found between the environment and treatment duration for all Chl*a*F and Chl index parameters, regardless of genotype (Table 2). Genotype did not influence the response to the duration of treatment. Therefore, the average of the five genotypes was used in the analysis. Flooding promoted a linear decrease in F_m, F_v, φP_0, φE_0, $PI_{(ABS)}$, and the Chl index. In contrast, F_0, V_J, φD_0, ABS/RC, TR_0/RC, and DI_0/RC values increased with the plant's exposure to flooding conditions.

After 5 d of flooding, the rootstocks did not show significant differences between treatments (Table 2). After 7 d of flooding, a reduction in F_m, F_v, and φP_0, and an increase in φD_0 were observed. Nine days after flooding, increased F_0, V_J, and ABS/RC and reduced φE_0 and $PI_{(ABS)}$ values were observed. After 19 d of flooding, TR_0/RC and DI_0/RC values increased while a reduction in the Chl index was observed.

Recovery capacity of the photosynthetic apparatus after stress interruption: No significant interaction was observed between the genotype and recovery period, and thus the average of the five genotypes was used for the experimental analyses (Table 3). The recovery capacity of the photosynthetic apparatus showed a linear change with an inverse pattern to that observed in the flooded conditions. After the flooding condition was removed, we observed that F_0, V_J, φD_0, ABS/RC, TR_0/RC, and DI_0/RC values decreased, while F_m, F_v, φP_0, φE_0, ET_0/RC, and $PI_{(ABS)}$ values increased. The Chl index did not change between the flooded and recovery periods.

After 7 d of recovery, the Chl*a*F parameters were improved compared to the last day of flooding, except for F_m, F_v, φE_0, and $PI_{(ABS)}$, which showed differences only after 14 d (Table 3). Although the recovery process started on the last day of flooding (day 0), only ET_0/RC and DI_0/RC values were statistically similar to those reported to the non-flooded plants.

Effect of environment and genotype on development and biomass parameters: The interaction between genotype and environment was observed by measuring

Table 1. Chl*a*F and Chl index (SPAD unit) of five cocoa genotypes subjected to flooding and non-flooding conditions (evaluated at 55 d) and after recovery (recovered at 21 d). Minimal fluorescence (F_0); maximum fluorescence (F_m); relative variable fluorescence in J-step (V_J); quantum yield of electron transport (φE_0); specific trapped energy flux per active reaction center (TR_0/RC); relative absorption performance index [$PI_{(ABS)}$]; specific electron transport flux per active reaction center (ET_0/RC). Means followed by *the same letter* are not different from each other by the *Tukey*'s test at 5% probability. *Uppercase letters* are used to compare the environments in the columns for each variable analyzed.

Environment	Genotype					Means of genotypes
	TSH-1188	Pará	Esfip-02	Cepec-2002	SJ-02	
F_0						
Non-flooded	625.38	619.88	604.00	584.63	759.25	638.63^{C}
Flooded	989.13	1,115.38	978.13	1,161.25	892.50	1,027.28^{A}
Recovery	825.13	762.75	792.00	795.63	798.25	794.75^{B}
F_m						
Non-flooded	3,308.38	3,186.38	3,273.25	2,966.13	3,300.88	3,207.00^{A}
Flooded	2,480.00	2,633.63	2,709.25	2,499.25	2,541.00	2,572.63^{C}
Recovery	2,863.50	2,841.50	2,722.00	3,026.38	2,820.75	2,854.90^{B}
V_J						
Non-flooded	0.54	0.56	0.50	0.54	0.54	0.53^{C}
Flooded	0.75	0.75	0.72	0.76	0.72	0.74^{A}
Recovery	0.66	0.69	0.68	0.66	0.63	0.66^{B}
φE_0						
Non-flooded	0.36	0.34	0.40	0.36	0.35	0.36^{A}
Flooded	0.14	0.14	0.17	0.12	0.17	0.15^{C}
Recovery	0.22	0.21	0.21	0.23	0.25	0.22^{B}
TR_0/RC						
Non-flooded	1.65	1.69	1.60	1.65	1.80	1.67^{C}
Flooded	2.48	2.53	2.40	2.57	2.42	2.48^{A}
Recovery	2.19	2.14	2.13	2.18	2.16	2.16^{B}
$PI_{(ABS)}$						
Non-flooded	14.63	12.60	19.24	14.26	16.24	15.39^{A}
Flooded	1.31	1.81	1.83	0.93	2.14	1.60^{B}
Recovery	3.27	3.23	3.74	3.69	4.85	3.76^{B}
ET_0/RC						
Non-flooded	0.76	0.74	0.81	0.77	0.80	0.77^{A}
Flooded	0.61	0.59	0.67	0.61	0.67	0.63^{B}
Recovery	0.75	0.66	0.66	0.73	0.77	0.71^{AB}
Chl index						
Non-flooded	42.90	41.91	43.30	42.20	50.43	44.15^{A}
Flooded	33.70	33.93	33.83	34.28	31.56	33.46^{B}
Recovery	31.94	30.49	31.68	29.76	32.51	31.28^{B}

development and biomass parameters (Tables 4, 5). NL and SL were less influenced by the environment, with differences observed only in some cocoa genotypes. Esfip-02 showed a reduction in NL and an accumulation of LDM after flooding. LDM was also lower in the SJ-02 flooded plants but NL was unchanged in response to flooding. The SL of Pará increased after the flooding condition was stopped, while there was no effect in SDM.

SD increased in all genotypes after the recovery stage, reaching values higher than those reported to the control Cepec-2002 and SJ-02 plants (Table 4). The SDM values of TSH-1188 and Cepec-2002 increased after stress interruption and were higher than those of the other treatments (Table 5). SJ-02 had a lower SDM but did not differ from the control during the recovery period. The SDM and DMAP of the flooded plants were higher in TSH-1188 and lower in SJ-02 when compared to the control conditions.

As shown in Table 4, the SL/SD ratio differed between treatments (non-flooded, flooded, and recovery

Table 2. Chl*a*F and Chl index (SPAD unit) of the plants were evaluated at 3, 5, 7, 9, 19, 29, and 55 d in flooded and non-flooded conditions. The genotype did not influence the response to the duration of treatment and, thus, the average of the five genotypes was considered. Minimal fluorescence (F_0); maximum fluorescence (F_m); variable fluorescence (F_v); relative variable fluorescence in J-step (V_J); maximum quantum yield of primary photochemistry (φP_0); quantum yield of electron transport (φE_0); quantum efficiency of energy dissipation (φD_0); specific absorption flux per active reaction center (ABS/RC); specific trapped energy flux per active reaction center (TR_0/RC); specific dissipation energy per active reaction center (DI_0/RC); relative absorption performance index [$PI_{(ABS)}$]; chlorophyll index (SPAD unit). Means followed by *the same letter* are not different from each other by the *F* test at 5% probability. *Uppercase letters* are used to compare the environments in the columns.

Environment	Evaluation period [d]							Equation	R^2
	3	5	7	9	19	29	55		
F_0									
Flooded	786.47^A	749.70^A	767.52^A	851.82^A	907.95^A	893.32^A	1,027.27^A	y = 4.9052 x + 765.87	87%
Non-flooded	748.95^A	694.20^A	696.97^A	711.15^B	629.50^B	676.40^B	638.62^B	y = –1.5871 x + 713.91	51%
F_m									
Flooded	2,977.87^A	3,119.07^A	3,115.62^B	2,843.21^B	2,815.20^B	3,057.10^A	2,572.62^B	y = –7.8468 x + 3,071.00	54%
Non-flooded	3,058.95^A	3,247.42^A	3,300.67^A	3,094.07^A	3,058.30^A	3,218.20^A	3,207.00^A	-	-
F_v									
Flooded	2,191.40^A	2,369.37^A	2,348.10^B	1,991.39^B	1,907.25^B	2,163.77^B	1,545.35^B	y = –12.752 x + 2,305.20	68%
Non-flooded	2,310.00^A	2,553.22^A	2,603.70^A	2,382.92^A	2,428.80^A	2,541.80^A	2,568.37^A	-	-
V_J									
Flooded	0.66^A	0.63^A	0.64^A	0.68^A	0.68^A	0.67^A	0.73^A	y = 0.0016 x + 0.6453	78%
Non-flooded	0.64^A	0.60^A	0.60^A	0.61^B	0.60^B	0.56^B	0.53^B	y = –0.0018 x + 0.6294	89%
φP_0									
Flooded	0.68^A	0.70^A	0.69^B	0.62^B	0.61^B	0.64^B	0.52^B	y = –0.0029 x + 0.6945	80%
Non-flooded	0.69^A	0.73^A	0.73^A	0.72^A	0.74^A	0.74^A	0.76^A	y = 0.0008 x + 0.7190	60%
φE_0									
Flooded	0.24^A	0.26^A	0.25^A	0.21^B	0.20^B	0.21^B	0.14^B	y = –0.0019 x + 0.2533	80%
Non-flooded	0.25^A	0.29^A	0.29^A	0.27^A	0.30^A	0.32^A	0.36^A	y = 0.0017 x + 0.2694	88%
φD_0									
Flooded	0.31^A	0.29^A	0.30^A	0.37^A	0.38^A	0.35^A	0.47^A	y = 0.0029 x + 0.3055	80%
Non-flooded	0.30^A	0.26^A	0.26^B	0.28^B	0.25^B	0.25^B	0.23^B	y = –0.0008 x + 0.2810	60%
ABS/RC									
Flooded	3.08^A	2.98^A	2.95^A	3.50^A	3.90^A	3.61^A	5.78^A	y = 0.0504 x + 2.7767	90%
Non-flooded	3.02^A	2.72^A	2.58^A	2.72^B	2.44^B	2.58^B	2.29^B	-	-
TR_0/RC									
Flooded	1.99^A	2.01^A	1.96^A	2.06^A	2.23^A	2.22^A	2.47^A	y = 0.00960 x + 1.9658	94%
Non-flooded	2.06^A	1.97^A	1.86^A	1.91^A	1.79^B	1.89^B	1.67^B	y = –0.0057 x + 1.9870	70%
DI_0/RC									
Flooded	1.08^A	0.97^A	0.98^A	1.44^A	1.66^A	1.38^A	3.31^A	y = 0.0408 x + 0.8105	86%
Non-flooded	0.95^A	0.75^A	0.71^A	0.81^A	0.64^B	0.69^B	0.61^B	-	-
$PI_{(ABS)}$									
Flooded	6.40^A	7.38^A	6.54^A	4.41^B	3.54^B	4.12^B	1.60^B	y = –0.0959 x + 6.5963	79%
Non-flooded	5.72^A	8.11^A	8.59^A	7.34^A	9.94^A	10.85^A	15.39^A	y = 0.1611 x + 6.5134	93%
Chl index									
Flooded	38.10^A	38.43^A	38.33^A	37.53^A	37.16^B	36.89^B	33.45^B	y = –0.0896 x + 38.757	94%
Non-flooded	37.07^A	39.25^A	37.22^A	36.62^A	41.67^A	43.35^A	44.14^A	y = 0.1470 x + 37.239	76%

conditions). Flooding resulted in an increased SL/SD ratio in Esfip-02 and SJ-02 and reduced values in both TSH-1188 and Cepec-2002 after stress interruption. In the flooded and recovered plants, the ratio was higher

Table 3. Chl*a*F and Chl index (SPAD unit) of the plants were evaluated at 7, 14, and 21 d after stress interruption. No interaction was observed between the genotype and the recovery period, therefore, the average of the five genotypes was considered. Means followed by *the same letter* do not differ from each other by the test of *Scott-Knott*, at 5% probability. *Lowercase letters* on the row are used to compare recovery periods and control without flooding. *Regression analysis was performed only with plant data during the recovery period, where zero corresponds to 55 d of flooding.

Parameter	Non-flooded	Recovery [d]				Equation*	R^2*
		0	7	14	21		
F_0	638.62^{d}	1,027.27^{a}	934.55^{b}	869.17^{b}	794.75^{c}	y = –11.344 x + 1,028.4	99%
F_m	3,207.00^{a}	2,572.62^{c}	2,671.90^{c}	2,868.72^{b}	2,854.90^{b}	y = 15.499 x + 2,575.4	86%
F_v	2,568.37^{a}	1,545.35^{c}	1,737.35^{c}	1,999.55^{b}	2,060.15^{b}	y = 26.842 x + 1,547	95%
V_J	0.53^{c}	0.73^{a}	0.76^{a}	0.67^{b}	0.66^{b}	y = –0.0046 x + 0.7591	74%
φP_0	0.76^{a}	0.52^{d}	0.58^{c}	0.63^{b}	0.66^{b}	y = 0.0068 x + 0.5278	98%
φE_0	0.36^{a}	0.14^{c}	0.14^{c}	0.20^{b}	0.22^{b}	y = 0.0044 x + 0.1324	85%
φD_0	0.23^{d}	0.47^{a}	0.41^{b}	0.36^{c}	0.33^{c}	y = –0.0068 x + 0.4722	98%
ABS/RC	2.29^{c}	5.78^{a}	4.02^{b}	3.70^{b}	3.30^{b}	y = –0.1142 x + 5.4343	81%
TR_0/RC	1.67^{c}	2.47^{a}	2.27^{b}	2.30^{b}	2.16^{b}	y = –0.0137 x + 2.4526	81%
ET_0/RC	0.77^{a}	0.62^{b}	0.54^{c}	0.73^{a}	0.71^{a}	y = 0.0068 x + 0.5812	46%
DI_0/RC	0.61^{b}	3.31^{a}	1.74^{b}	1.40^{b}	1.14^{b}	y = –0.1005 x + 2.982	80%
$PI_{(ABS)}$	15.39^{a}	1.60^{c}	1.41^{c}	2.85^{b}	3.75^{b}	y = 0.1196 x + 1.12	88%
Chl index	44.14^{a}	33.45^{b}	32.26^{b}	31.72^{b}	31.27^{b}	ns	-

Table 4. Development and growth of seedlings of five cocoa genotypes subjected to three environments: flooded and non-flooded (evaluated at 60 d) and recovered 21 d after stress suspension. NL – number of leaves, SL – stem length in cm, SD – stem diameter in mm, SL/SD – stem length/stem diameter ratio, RL – root length in cm, SL/RL – stem length/root length ratio. Means followed by *the same letter* are not different from each other by the *Tukey*'s test at 5% probability. *Uppercase letters* are used to compare the environments in the columns and *lowercase letters* compare the genotypes in the rows.

Environment	Genotype				
	TSH-1188	Pará	Esfip-02	Cepec-2002	SJ-02
NL					
Non-flooded	26.94^{Aa}	20.03^{Aa}	27.18^{Aa}	20.87^{Aa}	24.75^{Aa}
Flooded	17.17^{Aa}	15.84^{Aa}	13.15^{Ba}	11.54^{Aa}	16.58^{Aa}
Recovery	19.63^{Aa}	15.13^{Aa}	21.00^{ABa}	19.13^{Aa}	14.88^{Aa}
SL [cm]					
Non-flooded	49.86^{Aa}	47.89^{Ba}	49.88^{Aa}	50.74^{Aa}	48.13^{Aa}
Flooded	45.54^{Aa}	49.93^{ABa}	45.00^{Aa}	45.19^{Aa}	45.58^{Aa}
Recovery	47.81^{Ab}	56.20^{Aa}	50.66^{Aab}	51.81^{Aab}	47.08^{Ab}
SD [mm]					
Non-flooded	14.92^{ABab}	13.11^{ABab}	15.54^{Aa}	12.65^{Bb}	14.38^{Bab}
Flooded	13.69^{Ba}	12.65^{Ba}	12.38^{Ba}	11.24^{Ba}	12.24^{Ba}
Recovery	18.14^{Aa}	15.09^{Ab}	17.50^{Aab}	15.47^{Aab}	15.09^{Ab}
SL/SD					
Non-flooded	33.43^{Ab}	36.55^{Aab}	32.11^{Bb}	40.17^{Aa}	33.41^{ABb}
Flooded	33.27^{Ab}	39.46^{Aa}	36.74^{Aab}	40.22^{Aa}	37.72^{Aab}
Recovery	26.44^{Bc}	37.52^{Aa}	29.05^{Bbc}	33.54^{Bab}	31.60^{Bbc}
RL [cm]					
Non-flooded	43.40^{Aa}	39.46^{Aa}	44.05^{Aa}	43.09^{Aa}	38.94^{Aa}
Flooded	34.87^{Ba}	33.39^{Aa}	31.05^{Ba}	29.75^{Ba}	34.98^{Aa}
Recovery	41.88^{ABa}	36.13^{Aa}	40.91^{Aa}	44.06^{Aa}	37.01^{Aa}
SL/RL					
Non-flooded	1.15^{Aa}	1.21^{Ba}	1.14^{Ba}	1.18^{Ba}	1.25^{Aa}
Flooded	1.32^{Aa}	1.51^{Aa}	1.46^{Aa}	1.52^{Aa}	1.31^{Aa}
Recovery	1.15^{Ab}	1.56^{Aa}	1.24^{Bb}	1.18^{Bb}	1.28^{Ab}

Table 5. Dry mass allocation and partition of seedlings of five cocoa genotypes subjected to three environments: flooded and non-flooded evaluated at 60 d, and recovered at 21 d after stress suspension. Dry mass is expressed in grams. APDM/RDM – ratio between shoot dry mass and root dry mass; DMAP – dry mass of aerial part; LDM – leaf dry mass; TDM – total dry mass; RDM – root dry mass; SDM – stem dry mass. Means followed by *the same letter* are not different from each other by the *Tukey*'s test at 5% probability. *Uppercase letters* are used to compare the environments in the columns and *lowercase letters* compare the genotypes in the rows.

Environment	Genotype				
	TSH-1188	Pará	Esfip-02	Cepec-2002	SJ-02
LDM [g]					
Non-flooded	12.54^{Aa}	10.07^{Aa}	12.61^{Aa}	9.84^{Aa}	12.19^{Aa}
Flooded	8.98^{Aa}	8.16^{Aa}	6.31^{Ba}	6.58^{Aa}	2.99^{Ba}
Recovery	11.45^{Aa}	8.37^{Aa}	9.23^{ABa}	10.14^{Aa}	8.07^{ABa}
SDM [g]					
Non-flooded	14.17^{Ba}	11.97^{Aa}	14.16^{Aa}	10.36^{Ba}	13.07^{ABa}
Flooded	16.53^{Ba}	15.98^{Aab}	12.71^{Aab}	11.57^{Bab}	9.52^{Bb}
Recovery	22.79^{Aa}	17.56^{Aa}	18.23^{Aa}	17.49^{Aa}	16.87^{Aa}
RDM [g]					
Non-flooded	9.05^{Ba}	6.77^{ABa}	8.51^{Ba}	6.08^{Ba}	6.73^{ABa}
Flooded	6.56^{Ba}	5.62^{Ba}	5.49^{Ba}	3.72^{Ba}	5.66^{Ba}
Recovery	16.53^{Aa}	10.39^{Ab}	13.27^{Aab}	11.43^{Ab}	9.59^{Ab}
DMAP [g]					
Non-flooded	26.70^{Aa}	22.05^{Aa}	26.78^{Aa}	20.20^{Aa}	25.26^{Aa}
Flooded	25.51^{Aa}	24.14^{Aab}	19.02^{Aab}	18.14^{Aab}	12.50^{Bb}
Recovery	34.24^{Aa}	25.93^{Aa}	27.46^{Aa}	27.64^{Aa}	24.94^{Aa}
TDM [g]					
Non-flooded	35.75^{Ba}	28.82^{Aa}	35.29^{ABa}	26.29^{ABa}	31.99^{Aa}
Flooded	32.08^{Ba}	29.76^{Aa}	24.51^{Ba}	21.87^{Ba}	18.17^{Ba}
Recovery	50.77^{Aa}	36.31^{Aab}	40.73^{Aab}	39.07^{Aab}	34.53^{Ab}
APDM/RDM					
Non-flooded	2.95^{ABa}	3.21^{ABa}	3.17^{Aa}	3.37^{Ba}	3.82^{Aa}
Flooded	3.85^{Aab}	4.33^{Aab}	3.44^{Abc}	5.10^{Aa}	2.15^{Bc}
Recovery	2.11^{Ba}	2.57^{Ba}	2.21^{Aa}	2.46^{Ba}	2.63^{ABa}

in Pará and Cepec-2002 than in the other genotypes, while TSH-1188 exhibited the lowest SL/SD ratio after stress interruption.

Flooding reduced the RL of TSH-1188, Esfip-02, and Cepec-2002, and the values did not recover after flooding (Table 5). All cocoa genotypes accumulated more dry mass in the roots after stress interruption, but the values were higher than those observed in the control plants for TSH-1188, Esfip-02, and Cepec-2002. SJ-02 had lower total dry mass accumulation after recovery (Table 5). TSH-1188 had the highest capacity to allocate dry mass to roots and accumulated more TDM after flooding was stopped.

Pará had the highest SL/RL ratio among all genotypes after recovery (Table 4). In Pará, this ratio was higher after flooding and recovery, while Esfip-02 and Cepec-2002 had the highest SL/RL ratios among the plants in the flooded condition. The APDM/RDM ratio decreased in TSH-1188 and Pará during recovery (Table 5). However, under flooding conditions, Cepec-2002 had a higher APDM/RDM ratio than the SJ-02 cocoa genotype.

Principal component analysis: For the analyzed variables, two principal components (PC) explained 76.7% of the data variance: 54.0% for PC1 and 22.7% for PC2 (Fig. 2). PC1 (54.0%) was responsible for the variation in ET_0/RC, φP_0, and NL, which included more genotypes from the non-flooded environment and was inversely proportional to them. PC1 also influenced F_0, φD_0, ABS/RC, and DI_0/RC, contributing more to the genotypes in flooded environments. PC2 (22.7%) was responsible for the variation in SDM and RDM and contributed the most to the genotypes in the recovered environment. Based on the growth, development, and Chl*a*F characteristics used in the PCA, three groups were formed that distinguished the environment and contained all cocoa genotypes in each treatment (Fig. 2). The DI_0/RC, ABS/RC, F_0, and φD_0 were positively correlated with flooding, while survival, LDM, and RL were negatively correlated with flooding (Fig. 2). In the control plants, positive correlations were found for NL, F_V, ET_0/RC, φP_0, F_m, and φE_0. Therefore, these variables were positively correlated with flooding and negatively correlated with non-flooding conditions.

Discussion

Effects of environment on cocoa plant survival and photosynthetic apparatus: Previous research has indicated that cocoa rootstock can modify the Chl*a*F parameters, gas exchange, and antioxidant metabolism of the grafts and promote tolerance to abiotic stress (Ribeiro *et al.* 2016). The cocoa genotypes evaluated in this study exhibited changes in Chl*a*F parameters and Chl index between the flooded and non-flooded plants (Table 1). However, as no genotype–environment interaction was observed, we concluded that the rootstock did not influence the photochemical performance of the graft during or after flooding.

According to Delgado *et al.* (2016), the juvenile stage in cocoa plants is relatively sensitive, with a mortality rate close to 100% in two-year-old plants exposed to flooded conditions. Therefore, survival may be associated with the age of the trees (Lahive *et al.* 2019) rather than their tolerance to flooding stress (Bertolde *et al.* 2010). In the present study, cocoa plants subjected to flooded conditions had a similar mortality rate to those grown under control conditions.

A study evaluating 35 non-grafted cocoa genotypes reported the presence of genetic variability, with differences in the JIP-test parameters as F_0, F_m, and φP_0, among the clones grown under both control and flooding conditions (Bertolde *et al.* 2010). The genotypes SJ-02, Cepec-2002, and TSH-1188 increased F_0 values after 45 d of flooding but without affecting F_m (Bertolde *et al.* 2010). These data do not corroborate the findings in the present study, as all rootstocks showed an increase in F_0 and a reduction in F_m with flooding (Table 1). The longer duration of flooding (55 d) may have contributed to these differences, as well as the use of the same genotype as the scion.

High F_0 values associated with reduced F_m values have also been reported in flooded cocoa genotypes (Bertolde *et al.* 2010, De Almeida *et al.* 2016, Silva Branco *et al.* 2017). Initial fluorescence occurs when Q_A is oxidized, and the reaction centers are open (Baker and Rosenqvist 2004). The increase in F_0 indicates that plants are under stress due to the decreased electron flow through PSII (Oliveira *et al.* 2002) and damage to the D1 protein (Dias and Marenco 2006), which further indicates nonstomatal limitations of photosynthesis and oxidative damage (Baker 2008). The observed reduction in F_0 values after 21 d post-flooding indicated only partial recovery in the electron flow, as they were still higher than those in the control (Table 1).

Our data showed a decreased electron transport rate per active RC (ET_0/RC) in flooded plants, which was associated with higher energy dissipation (DI_0/RC) (Shamshiri and Fattahi 2016). The lowest ET_0/RC in the flooded plants confirmed the interruption of electron flow beyond Q_A^-, but the post-flooding values did not differ between treatments, indicating that the plants had not recovered (Table 1).

The increase in V_J in the flooded plants and the reduction in the post-flooding period demonstrated

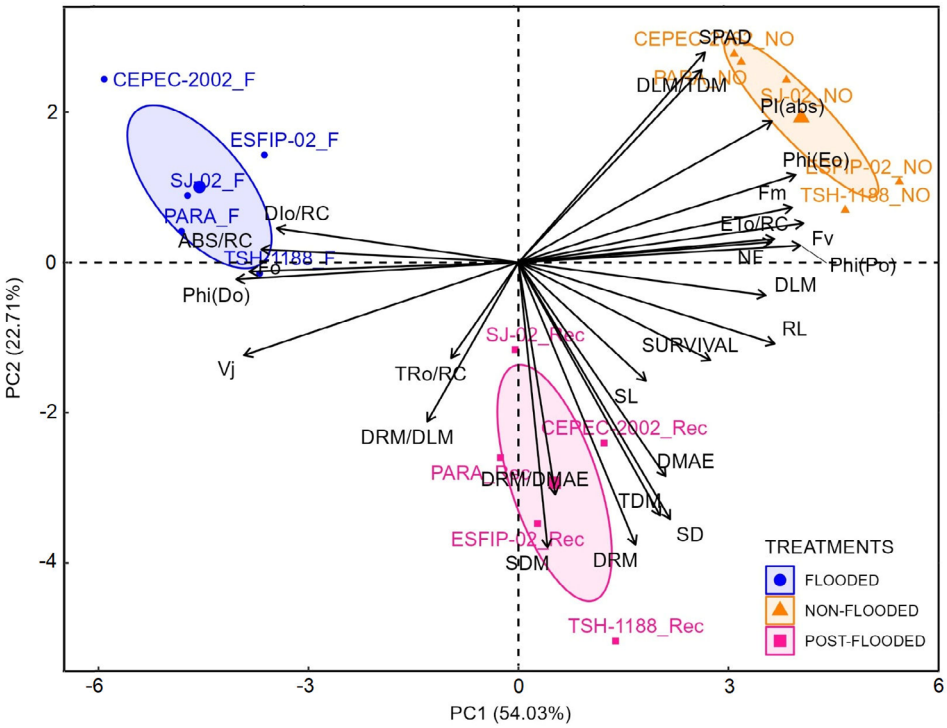

Fig. 2. Principal component analysis (PCA) score chart considering the 26 factors analyzed in three environments: non-flooded, flooded, and recovery (post-flooded). PC1 corresponds to initial fluorescence (F_0), the quantum efficiency of energy dissipation (φD_0), the specific flux of absorption per active reaction center (ABS/RC), the specific flux of dissipation per active reaction center (DI_0/RC), specific electron transport flux per active reaction center (ET_0/RC), maximum quantum yield of primary photochemistry (φP_0), and number of leaves (NF). PC2 corresponds to stem dry mass (SDM) and root dry mass (RDM).

the recovery of photochemical capacity. However, as the values were higher than those in the control, they confirmed that the recovery period was insufficient for the plants to return to pre-flooding levels (Table 1). Increases in V_J are indicative of a reduction in the capacity for plastoquinone reoxidation (Shamshiri and Fattahi 2016) and in this study, slow Q_A reoxidation was reported for all cocoa genotypes subjected to flooding. This result can also be explained by the increase in the TR_0/RC values, as this parameter indicates inhibited Q_A reoxidation, preventing efficient electron transfer from Q_A to Q_B (Rathod *et al.* 2011).

The $PI_{(ABS)}$ parameter measures the capture of light energy by RC and reflects the state of electron transport in PSII, and its reduction demonstrates reduced photochemical reactions (Zhang *et al.* 2018) and relates the density of the reaction centers to the probability of electrons going beyond Q_A (Gonçalves *et al.* 2007). Therefore, when analyzed along with other parameters, it can be inferred that the cocoa plants under flooding stress were less efficient in energy capture and transport in PSII than plants under non-flooded conditions. All genotypes presented photochemical damage during flooding and did not recover after flooding (Table 1). Compared to the control, $PI_{(ABS)}$ was ten times lower in flooding and four times lower in the recovery period for the tested genotypes, indicating it is the most sensitive variable to flooding stress.

Furthermore, leaf chlorosis is a characteristic used to identify flood tolerance among cocoa genotypes (Lahive *et al.* 2019). Bertolde *et al.* (2010) suggested that the absence of leaf chlorosis may be a suitable feature to identify cocoa clones with higher survival rates under flooding conditions. We analyzed three of the 35 cocoa genotypes and observed that SJ-02 showed low chlorosis, while TSH-1188 and Cepec-2002 exhibited medium chlorosis after 45 d of flooding treatment. The Chl index decreased during flooding and did not recover in the post-flooding period, indicating that this recovery time was insufficient for the plant to return to the pre-flooding conditions (Table 1).

Effect of the flooding period on the photosynthetic apparatus: Plants did not show significant differences in Chl*a*F parameters until 5 d after flooding, but on the seventh day, F_m, F_v, and φP_0 decreased and φD_0 increased (Table 2). Only after 9 d of flooding, did V_J and ABS/RC increase, while φE_0 and $PI_{(ABS)}$ decreased. TR_0/RC increased and the Chl index decreased after 19 d of flooding. The decrease in F_m impairs the reduction of electron acceptors, affecting the energy capture by the photosynthetic apparatus. As suggested by Demmig-Adams and Adams (1992), this may indicate a photoprotective mechanism associated with dissipation energy in the form of heat, which corresponds to the increase in DI_0/RC observed after 19 d of flooding (Table 2). Additionally, the reduction in F_v may have occurred due to interference in water oxidation and electron transport, as this parameter measures the electron flow from F_0 to F_m, or from oxidized Q_A to reduced Q_A (Oukarroum *et al.* 2009).

The increase in φD_0 after 7 d of flooding was associated with the increase in DI_0/RC, indicating that the plant dissipated more energy in the form of heat, which may result in photoprotection (Table 2). Thus, this increase in φD_0 is a strategy of energy dissipation used by plants to avoid photoinhibitory damage (Kalaji *et al.* 2018a). The reduction in φP_0 associated with the increase in DI_0/RC is related to photoinhibition in stressed plants, as φP_0 is reduced when PSII is impaired (Souza *et al.* 2004, Martins *et al.* 2015, Hazrati *et al.* 2016).

The values of φP_0 in plants under ideal growing conditions ranged from 0.75 to 0.85 (Bolhàr-Nordenkampf *et al.* 1989). The reduction in the maximum quantum yield of primary photochemistry (φP_0) indicates a decrease in the capacity to reduce Q_A, further indicating damage to PSII during stress (Lin *et al.* 2009). Thus, it is important to assess its impact on plant photosynthesis (Maxwell and Johnson 2000). These ideal values were not observed when cocoa plants were subjected to flooding stress (Table 2). Parameters of quantum yield are used as indicators of abiotic stress because they reflect the lower efficiency of excitation energy use and dissipation by the thylakoid membrane (Dąbrowski *et al.* 2019). As φP_0 was affected early in the stress, it can be used as a sensitive and efficient parameter to detect PSII damage.

Recovery capacity of the photosynthetic apparatus after stress removal: The recovery capacity of plants after stress interruption limits their survival because they cannot adapt to that stress. When the environment changes, plants need to induce molecular, biochemical, and physiological mechanisms to adjust their metabolism and structure to optimize resource capture and ensure survival and reproductive success under new environmental conditions. Phenotypic plasticity is the ability of plants to adjust to environmental changes (Zotz *et al.* 2011). Studies on the ability of the photosynthetic apparatus of cocoa plants to recover from flooding are limited. The analysis of the photosynthetic apparatus recovery of the plants in this study showed that 53% of the photochemical parameters evaluated changed 7 d after stress removal (55 d flooded), with only ET_0/RC and DI_0/RC values being restored to the non-flooded control levels after stress removal (Table 3).

When the plants were removed from the flooding conditions, there was an increase in the electron transport rate per active reaction center (ET_0/RC). Liu *et al.* (2019) reported that reduced ET_0/RC values were associated with reduced energy flow for electron transport. Therefore, the increase in ET_0/RC observed during the recovery from flooding indicates that cocoa plants re-established their electron transport capacity per active reaction center through PSII. Additionally, a reduction in DI_0/RC suggested less oxidative damage and increased photoprotection (Kalaji *et al.* 2014).

The low recovery capacity of the photosynthetic apparatus may be associated with the reduction in the Chl index. At 19 d after flooding, the Chl index was 2.7% lower than the control and decreased by 12.2% at 55 d after flooding (Table 2). Furthermore, at 21 d after

flooding, the Chl index did not recover to control levels (Table 3). Maintenance of the Chl pool is essential for the absorption of light energy and its subsequent conversion into biochemical energy. Thus, nonstomatal factors play an important role in the response to stress after flooding.

Although $PI_{(ABS)}$ increased post-flooding and may be used to detect signs of plant recovery, the values were four times lower than the control after 21 d of recovery (Table 3). The reduction in $PI_{(ABS)}$ suggests a decrease in overall photosynthetic performance associated with reduced electron transport capacity (Kalaji *et al.* 2018b). Therefore, the post-flooding period was not sufficient to restore the general photosynthetic performance and leaf electron transport capacity to non-flooding conditions.

Environmental effect on the development and biomass parameters: Flooding causes a sequence of events that affect photoassimilate production and plant development. Reduced oxygen supply and acidosis in the roots from waterlogging decrease cellular energy and inhibit the activity of aquaporins, interrupting hydraulic flow through the roots and reducing stomatal conductance and photosynthesis (Domec *et al.* 2021). Furthermore, when plants are stressed, they tend to save energy to ensure survival and to maintain a balance in the distribution of photoassimilates until the flooding condition is lifted (Colmer and Voesenek 2009).

After 60 d of flooding, the cocoa plants showed reduced dry mass accumulation, development, and growth (Tables 4, 5). A reduction in cocoa tree development in flooded environments was also reported by Bertolde *et al.* (2010) and may be related to the reduced metabolic activity from the blockage of electron transport in the flooded environment, which also reflected in decreased carbon fixation (Mielke *et al.* 2003, Fritz *et al.* 2004). Therefore, plants enhance the fermentation process by producing lactate and ethanol, which can reach toxic concentrations depending on their tolerance levels (Li *et al.* 2021).

Leaf development was less affected by flooding, and only the rootstocks Esfip-02 and SJ-02 showed reduced leaf developmental parameters under flooding compared to the control (Tables 4, 5). Esfip-02 reduced NL and LDM, while SJ-02 only presented a reduction in LDM. The decrease in shoot growth occurred due to the reduced leaf expansion rate caused by reduced leaf turgor and induction of early leaf senescence, evidenced by chlorosis and leaf abscission (Silva Branco *et al.* 2017). Reductions in chlorophyll content and shoot development can decrease plant photosynthetic capacity (Bangar *et al.* 2019, Nasrullah *et al.* 2022). However, a reduction in leaf development and stomatal closure protects the integrity of the entire hydraulic system of plants and saves energy (Pivovaroff *et al.* 2014, Pires *et al.* 2018, Domec *et al.* 2021).

The rootstock Esfip-02 also reduced SD and RL, SL to SD ratio (Table 4), and the TDM of the plants during flooding (Table 5). Flooding led to a reduction in the accumulation of dry mass in the leaves, stems, and roots of SJ-02, which contributed to a lower dry mass in the aerial parts and whole plant (Table 5). TSH-1188 and Pará were less affected by flooding, and although they reduced root development, they maintained total plant dry mass accumulation (Tables 4, 5). The development of the shoots and roots of the cocoa tree affects the production of cocoa; therefore, because the Esfip-02 and SJ-02 rootstocks showed reductions in several parameters in response to flooding (Tables 4, 5), they should not be used for cocoa cultivation in flood-prone areas. However, the TSH-1188 and Pará rootstocks seemed to tolerate flooding for 60 d, indicated by the maintenance of the total dry mass accumulation, suggesting that they could be promising rootstocks for cocoa-growing regions prone to flooding.

Considering the survival strategies, the reduction in dry mass accumulation and development of plants exposed to flooding for 60 d (Tables 4, 5) and energy dissipation after 7 d of flooding showed that the cocoa trees entered quiescence (Table 2). As a flood survival strategy, plants that enter a state of quiescence accumulate less biomass and reduce activation of energy dissipation events (Zhang *et al.* 2021).

Development and dry mass accumulation of plants during recovery after flooding: Flooding is a sequential stress, and the ability of plants to resume post-flooding molecular, physiological, and developmental processes is an important aspect of tolerance development (Yeung *et al.* 2019). We report that cocoa rootstocks responded differently to flooding and identified different tolerance strategies (Tables 4, 5). The NL produced by the test plants did not change in response to the growth environment, which was similar to the findings reported in the Catongo cocoa genotype (Sena Gomes and Kozlowski 1986), and most rootstocks induced root development instead of shoot development in response to flooding (Table 4). Photoassimilate allocation promoted the recovery of the root system, which proved to be more affected by flooding (Kang *et al.* 2019). Pará was the only rootstock whose root development was not affected by growth conditions; however, it showed higher SL, SD, and SDM values after flooding (Tables 4, 5).

The reduction in growth during flooding can be reversed after water drainage (Ishida *et al.* 2002). Although the photochemical capacity of the cocoa tree did not recover after flooding (Table 1), the rootstocks resumed plant growth, prioritizing root growth in SD (Table 5). Dry mass is an important parameter for assessing the ability of a species to tolerate flooding (Nascimento *et al.* 2015). The flooding condition affects the root system's ability to transport nutrients, contributing to reduced plant growth (Ronchi *et al.* 2006). Sena Gomes and Kozlowski (1986) studied the Catongo genotype and reported that, after the removal of flooding stress, plants did not absorb enough water to resume shoot growth as all their energy was directed towards root growth. During recovery, priority is given to root system development over the aerial parts to restore normal development (Kolb *et al.* 1998, Parad *et al.* 2016).

SD of all rootstocks was higher after flooding (Table 4), but only TSH-1188, Cepec-2002, and SJ-02 showed higher SDM (Table 5). The compensatory increase in

growth under SD conditions may be associated with the need to increase water transport to sustain growth after flooding (Mozo *et al.* 2021). The SD is a good indicator of cocoa tree tolerance to flooding (Prawoto *et al.* 2005) and we found TSH-1188 had the highest SD after flooding, whereas SJ-02 and Pará had the lowest SD of the genotypes tested (Table 4). SJ-02 exhibited the lowest RDM accumulation. TSH-1188 had the largest SD and dry mass accumulation in the roots and in the whole plant, as well as the best performance in flooding conditions (Tables 4, 5). Therefore, TSH-1188 is the recommended rootstock for plants cultivated in flood-prone areas, while SJ-02 should be avoided in these areas.

PCA analysis: The PCA analysis allowed the visualization of the formation of three groups, which were influenced by the growth environment because the groups included the same genotypes but under different growth conditions (Fig. 2). The key variables that most influenced the results of the non-flooded group were ET_0/RC, φP_0, and NL. As previously shown, these variables represent the normal behavior of plants not under stress. The formation of the flooded genotype group was more influenced by Chl*a*F, F_0, φD_0, ABS/RC, and DI_0/RC, indicating that flooding negatively influenced the photosynthetic apparatus, which is consistent with previous studies (Bertolde *et al.* 2010, De Almeida *et al.* 2016, Silva Branco *et al.* 2017). However, the environmental effects on plants in the recovered environment were determined by development characteristics, such as SD and dry mass accumulation of stems, roots, plants, and roots. Additionally, we found that RL correlated negatively with flooding, while SD correlated positively with TDM in the post-flooding condition. The results from the environmental condition groups confirm that the cocoa tree is negatively affected by flooding but can recover, suggesting that it is a species with great plasticity and adaptability to changes in environmental conditions (Zimmermann *et al.* 2019). Our results also confirm that the rootstock has little influence on the photosynthetic apparatus in flooded plants, but is essential for the recovery of development in post-flooded plants.

Conclusion: During flooding, the photosynthetic apparatus in cocoa plants is impaired. After flooding and when normal soil water conditions resumed, the plants showed signs of recovery, indicating that the damage caused by flooding was reversible. TSH-1188 and Pará rootstocks were the least affected by flooding and maintained the total dry mass accumulation of the plants after stress, indicating that they can be planted in cropping areas at risk of flooding.

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