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Early selection in *Theobroma grandiflorum*, aiming at tolerance to hypoxia

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Abstract - Cupuassu (*Theobroma grandiflorum*)-based agroforestry system (AFS) can be used for restoration of vegetation around springs and riparian forests of water sources (rivers, streams, lakes etc.), subject to constant flooding. The objectives of this work were to study the relations between morphophysiological traits when flooding occurs, determining the most important to assess genotypes' tolerance, and evaluate the ability of cupuassu genotypes to express alternative phenotypes and grow efficiently, in order to select materials capable of sustaining hypoxic conditions. The trial was carried out in a greenhouse, in a completely randomized design with a 16 x 2 (16 progenies and two water regimes) factorial scheme and four replications. Treatments started six months after the seedlings were transplanted. 15 traits related to vegetative development, biomass accumulation and physiology were measured for the experimental evaluation. Correlations and principal components were estimated to study the traits' interrelationships and identify the most important to assist progeny selection. The results revealed that, among the evaluated traits, ten were sufficient to explain the variability within the population and four were elected as the most relevant to select the best progenies. 75% of the genotypes showed tolerance to partial flooding and based on performance and plasticity, we selected G174, G48 and G56 for a broader spectrum and G44, G47 and G51 for hypoxic conditions only. The promising evidence pointed out here will need to be corroborated with field tests, so that the cupuassu tree can be recommended in the recovery of environments subject to hypoxia.

Index terms: Flooding, recovery of degraded areas, path analysis, principal components.

Seleção precoce em *Theobroma grandiflorum*, visando tolerância à hipóxia

Resumo - O sistema agroflorestal (SAF) à base de cupuaçu (*Theobroma grandiflorum*) pode ser utilizado para a recomposição da vegetação no entorno de nascentes e de matas ciliares de mananciais (rios, córregos, lagos, etc.), sujeitos a inundações constantes. Os objetivos deste estudo foram estudar a relação entre caracteres

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morfofisiológicos sob regime de alagamento, determinando quais os mais importantes para estudar a tolerância dos genótipos e para avaliar a habilidade de genótipos de cupuaçuzeiro em expressar fenótipos alternativos e crescer de forma eficiente, para seleção de materiais capazes de suportar condições de hipóxia. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, em esquema fatorial 16 x 2 (16 progênies e dois regimes hídricos) e quatro repetições. Os tratamentos iniciaram seis meses após o transplante das mudas. Para a avaliação experimental, foram medidos 15 caracteres relacionados ao desenvolvimento vegetativo, ao acúmulo de biomassa e às variáveis fisiológicas. Correlações entre características e componentes principais foram estimadas para estudar as inter-relações das características e identificar as mais responsivas para auxiliar na seleção de genótipos. Os resultados revelaram que, dentre as características utilizadas, dez foram suficientes para explicar a variabilidade dentro da população e quatro foram escolhidas para selecionar as melhores progênies. 75% dos genótipos apresentaram tolerância ao alagamento parcial e, com base no desempenho e na plasticidade, foram selecionados G174, G48 e G56 para um espectro mais amplo, e G44, G47 e G51 apenas para condições de hipóxia. As evidências promissoras aqui levantadas precisarão ser corroboradas, com a realização de testes de campo, para que o cupuaçuzeiro possa ser recomendado na recuperação de ambientes sujeitos à hipóxia.

Termos para indexação: Alagamento, recuperação de áreas degradadas, análise de trilha, componentes principais.

Introduction

Low floodplains in the Amazon estuary areas are daily flooded. This occurs due to the influence of the tide, alternating a period of flood and another of ebb. High floodplain land is only flooded during the high tides of March and September. In the lower and middle Amazonas River, the regime of floods and inundations is different. This is because the river spends six months in flood and six months in ebb (CORRÊA et al., 2017). On riverbanks there are floodplain areas that can spend a large part of the flood period inundated and others, a short period (SANDERS et al., 2017). Variation in flood regimes is an important factor in maintaining species diversity in a plant community (THOM et al., 2018; THOM et al., 2020). However, the balance of these areas is constantly at risk due to landscape transformation, whether for the construction of dams that permanently flood these places (LATRUBESSE et al., 2017), or for the removal or replacement of vegetation by agricultural undertakings or the establishment of urban areas (RENO; NOVO, 2019). Therefore, the recovery or restoration of these areas must be prioritized, using native species that are already adapted to the local edaphoclimatic conditions and re-establishing the previously existing balance (FERNANDES et al., 2018). This highlights the need to know the behaviour of such species under hypoxic conditions.

The partial or total flooding of the soil is harmful to the development of most plants, as it inhibits their growth, which can lead to plant death. Nevertheless, some species have a remarkable ability to tolerate these conditions, being able to grow vigorously even when subjected to flooding. Such species have adaptive strategies to this stress, which allow them to occupy a diverse range of floodable soils in different ecosystems (MUSTROPH, 2018). The morphophysiological changes for adaptation to hypoxia vary from species to species, some of which induce quiescence and others develop a series of escape strategies (LORETI et al., 2016). Some plant responses to flooding may include leaf senescence, hyponasty, increased stem and root growth, adventitious root development, aerenchyma formation, lenticel hypertrophy, and changes in leaf pigments (VOESENEK; BERRES, 2015; YAMAUCHI et al., 2018). Also, there may be decreased photosynthetic rates and increased synthesis of reactive oxygen species (SASIDHARAN et al., 2018). Physiologically, plants reduce energy-consuming processes due to ethylene hormone signalling, which induces a reaction chain in association with other hormones that will culminate in the aforementioned processes (LORETI et al., 2016). These phenotypic responses are a systemic result emerging from the effect of the genotype, the environment, and the genotype–environment interaction (XU, 2016). Given this strong connection between plants and their surrounding environment, morphophysiological responses depend on the plant's ability to perceive and react to environmental change. This ability to express alternative phenotypes in response to changes in the environment is known as phenotypic plasticity (VALLADARES et al., 2006; VALLADARES et al., 2007), and it is suggested that the plasticity of some functional traits can benefit plant growth under adverse environmental conditions (MATESANZ et al., 2010). Under excessive water availability, species tolerant to soil flooding are recognized for their ability to show morphophysiological changes to overcome the stressful condition.

Cupuassu [*Theobroma grandiflorum* (Willd. ex Spreng.) Schum., Malvaceae] emerges as one of the most suitable native fruit-bearing trees for the development of agriculture in the Brazilian Amazon, as there is a market with potential for expansion (ALVES et al., 2014). Pará is the state with the largest planted area of cupuassu trees in the country, with 8,500 hectares, with the municipality of Tomé Açu as the largest producer, followed by Mojú, Acará and Bujarú, responsible for approximately 71% of Pará's production (PARÁ, 2022). Embrapa Eastern Amazon has promoted the genetic breeding of this species and obtained varieties with traits that guarantee excellent fruit production capacity and good resistance to witches' broom [*Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora], a disease that causes a major reduction in cupuassu production in the State of Pará (CHAVES et al., 2021). The cultivar BRS Carimbó, released by Embrapa Eastern Amazon in 2012, has these traits. It is estimated that, at eight years after planting, orchards with this cultivar produce about 11 tons of fruits per hectare (ALVES; FERREIRA, 2012). It is important that BRS Carimbó and other breeding materials be evaluated in areas subject to flooding, as it will make it possible to identify and select appropriate materials for this type of environment. In the context of frequent climate changes and increase in water levels year by year, identifying genotypes tolerant to this situation is of paramount importance to provide materials with high performance, resistance to diseases and pests and tolerance to abiotic stresses. This is especially important for perennial species such as cupuassu, which can produce on a commercial scale for several decades (NIETHER et al., 2020).

Since cupuassu trees can occur naturally in areas subjected to flooding, we tested the hypothesis that Embrapa's breeding material holds alleles of tolerance to hypoxia, with primary or lesser consequences for phenotypic expression, depending on the trait. Therefore, our objectives were i) to study the relations between morphophysiological traits when flooding occurs, determining which are the most important to assess genotypes' tolerance; and ii) evaluate the ability of cupuassu genotypes to express alternative phenotypes and grow efficiently, in order to select materials capable of enduring hypoxic conditions.

Material and Methods

The trial was carried out at Embrapa Eastern Amazon in Belém, Pará State, Brazil (01º 27' 21" S and 48º 30' 16" W), under controlled cultivation conditions. According to Köppen's classification, the predominant climate in the region is Afi, with an average annual temperature of 26 °C and average annual rainfall of 2,754.4 mm (NECHET, 1993). The soil used as substrate for filling the seedling bags was classified as *Latossolo Amarelo Distrófico* (Oxisol) (EMBRAPA, 2016).

Sixteen half-sib progenies from BRS Carimbó cultivar were used. Although there is no information on the level of tolerance of these genotypes to flood stress, these are high-performance materials, regarding both yield and resistance (ALVES; FERREIRA, 2012).

The seedlings were grown from open pollinated seeds, extracted from mature fruits, obtained in an orchard at Embrapa Eastern Amazon in Tomé Açu-PA (2° 32' 54.4'' S and 48 ° 15' 50.4'' W). Up to three fruits per plant were collected. The seeds were extracted, and the pulp that surrounds them was manually removed. Afterwards, they were mixed to compose a sample of 50 seeds per clone. Seed germination was carried out using a seedbed containing aged sawdust as substrate.

The trial followed the completely randomized design, with a 16 x 2 (16 progenies and two water regimes) factorial scheme, comprising 32 treatments. These were distributed in four repetitions, totalling 128 seedlings. Each sampling unit consisted of one plant. The cultural practices applied to the seedlings were those normally used for the species (SOUZA, 2007).

In the first month after sowing, they were transplanted from the seedbed into perforated plastic bags, which had dimensions of 45 x 20 cm. These were filled with 8 kg of a mixture of soil and manure (poultry litter) in the proportion of 3:1, respectively. Afterwards, the seedlings were arranged on benches inside a greenhouse. Irrigation was done manually, by daily applying 300 ml of water per seedling, to maintain soil moisture close to field capacity.

Six months after transplanting (180 days), the seedlings were subjected to two types of water regimes: i) with flooding; and ii) without flooding (control). Seedlings subjected to flooding were placed in plastic buckets of 20 L, which received 15 L of water, so that a 4-cm-deep layer of water remained above the level of the substrate. This level was kept constant throughout the experiment, by constant replacement of water to compensate for the loss through evapotranspiration. In the control treatment, the seedlings remained in plastic bags and were placed in buckets without water like those used in non-control treatments. These seedlings continued to be irrigated daily.

The measurements were taken right before the flooding treatment (180 days after transplanting) and after 87 days of flooding (267 days after transplanting). We measured plant height (in cm), from the stem to the apical bud of the seedling, stem diameter (in cm) at 5 cm from the soil, number of leaves and the length and width of two leaves per plant. The average leaf area per seedling was multiplied by the number of leaves to determine the total leaf area (cm²), which was adjusted by a correction factor, based on the model proposed by Conceição et al. (1997):

$$y = [1.5959 + (0.6687L \times W)]NL$$

Where L is leaf length, W is leaf blade width and NL is the number of leaves. Those traits were classified as "measurable", as their measurement is done easily, in a non-destructive way.

Another set of traits are the "destructible" traits. These are root length (in cm), the dry masses of leaves, stem, and roots (in g) and the total dry mass (in g). The dry masses were obtained by drying the parts in different paper bags in an oven with forced air circulation, until a constant mass was observed. Afterwards, they were weighed on a semi-analytical balance to obtain the dry biomass value of each part and the total of each sample.

From the obtained data of total dry mass of the seedling and its respective leaf area, the following parameters were determined for each progeny: Dry Matter Accumulation (DMA, in g plant^{-1}), Relative Growth Rate (RG, in g g^{-1}d^{-1}), Absolute Growth Rate (AG, in

$g\ g^{-1}d^{-1}$) and Leaf Area Ratio (LAR, in $mm^2\ g^{-1}$) (HUNT, 1990; MAGALHÃES, 1985; RADFORD, 1967; RICHARDS, 1969):

$$DMA = w_1 - w_0$$

$$RG = \frac{\ln w_1 - \ln w_0}{(t_1 - t_0)}$$

$$AG = \frac{w_1 - w_0}{(t_1 - t_0)}$$

$$LAR = \frac{S_1}{TDM}$$

Where w_0 is total biomass at 180 days after planting (t_0), w_1 is total biomass at 267 days after planting (t_1), S_0 is total leaf area at t_0, S_1 is total leaf area at t_1, TDM is the Total Dry Mass, and ln is the Napierian Logarithm. DMA, RG, AG, and LAR are secondary traits, i.e., derived from traits that are measured directly. Thus, they were classified as the "Rates and indexes".

Finally, leaf transpiration rate (in $\mu g\ cm^{-2}\ s^{-1}$) and stomatal conductance (in $\mu mol\ m^{-2}\ s^{-1}$) were determined using a portable infrared gas analyser (LI-6200 – Li-Cor), using a fully expanded leaf from the second or third node of each plant of the referred treatments, starting at 09:00 a.m. Both were labelled as "physiological" traits.

Statistical procedures

Data were analysed following a completely randomized design, using a fixed linear model. The pairwise correlation between traits was estimated, and the significance of the coefficients was tested using Student's t-test. Significant correlations were represented in a correlation network. Subsequently, they were divided into direct and indirect effects using the path analysis methodology (WRIGHT, 1921). Path analysis is an important complementary tool which allows visualizing the cause-and-effect relationship between a set of explanatory traits in relation to a main trait (CRUZ et al., 2012). In our case, total dry mass and root length were the main traits, and the measurable traits were the explanatory. We chose these traits because they cannot be measured without discarding the material afterwards. Thus, observing the relationship between these traits and others of easier measurement is an important outcome for the breeding program.

Path analysis' accuracy depends on the level of multicollinearity between the explanatory traits. Multicollinearity was assessed by the Variance Inflation Factor (VIF), obtained by multiplying the diagonal element of the **X'X** matrix by the residual variance (MARQUARDT, 1970). Multicollinearity will be more severe as the VIF increases, reaching impending values when VIF ≥ 10 (NETER, 1974). This did not occur in our study, as the VIFs of the measurable traits were 1.18, 1.49, 3.11 and 4.03 for plant height, stem diameter, number of leaves, and total leaf area, respectively.

To assist in the selection of genotypes, visualization of the traits' relationship and trait discarding, Principal Component Analysis was used. Principal components were obtained from the eigenvalues and eigenvectors of the correlation matrix, after the standardization of the traits. To discard traits, we considered the criterion of Jolliffe (1972, 1973). After defining the most representative traits, we performed the Scott-Knott method for grouping the progenies. With these traits, we also calculated the relative distance plasticity index for each genotype ($RDPI_i$), proposed by Valladares et al. (2006), given by:

$$RDPI_i = \frac{\sum \frac{d_{ij \to i'j'}}{x_{i'j'} + x_{ij}}}{n}$$

in which $d_{ij \to i'j'}$ is the relative distance between trait values of a plant of a given genotype (j) under hypoxic conditions (i) and other plant of the same genotype (j') under control conditions (i'). This distance is given by the absolute value of $x_{i'j'} - x_{ij}$, where $\boldsymbol{x}$ is the trait value. Finally, $\boldsymbol{n}$ is the number of distances. The index ranges from 0 to 1. Here, we considered that genotypes with RDPI$_i$ closer to 0 are better, as they are less influenced by the water regime. The Scott-Knott and the RDPI$_i$ outputs were used as criteria for selection.

All analyses were performed in the R statistical environment (R CORE TEAM, 2021), using the packages “ExpDes.pt” (FERREIRA et al., 2021), “Hmisc” (HARREL JÚNIOR, 2022), “lavaan” (ROSEEL, 2012), “mctest” (IMDAD; ASLAM, 2018) and “factoextra” (KASSAMBRA; MUNDT, 2020). The plots were constructed using the packages “qgraph” (EPSKAMP et al., 2012), “semPlot” (EPSKAMP et al., 2022), “ggplot2” (WICKHAM, 2016), and “factoextra”.

Results and discussion

The effects of genotype, water regime and their interaction were not significant on only two of the fifteen analysed traits: relative growth rate and leaf area ratio. The effect of genotypes was significant on 11 of the 15 traits, showing that genetic control is relevant in the expression of flood tolerance. For eight traits, the genotype x water regime interaction was significant, indicating that there is differential expression of genes related to the traits according to the water regime to which the plant is subjected (Table 1).

Table 1 - Summary of Analysis of Variance for 15 traits evaluated in cupuassu genotypes, subjected to different water regimes.

Traits	Mean Squares			C.V. (%)
	Genotypes (G)	Water regime (RH)	G x RH	
Plant height (cm)	265.43 *	3.40	181.65 *	16.34
Stem diameter (cm)	0.05 **	0.80 **	0.02 *	10.35
Number of leaves	125.14 **	41.60	26.50	25.45
Total leaf area (cm^2)	10406692 **	8018897	2385452	27.83
Leaf dry mass (g)	113.29 **	54.40	41.75	29.49
Stem dry mass (g)	83.10 **	659.44 **	55.48 *	28.13
Root dry mass (g)	27.93 **	12.44	15.69 *	31.32
Total dry mass (g)	546.75 **	554.40	291.48 *	26.35
Root length (cm)	139.78 **	650.30 **	107.73 *	21.64
Relative growth rate (g g^{-1} day^{-1})	0.00 †	0.00†	0.00 †	44.3
Absolute growth rate (g g^{-1} day^{-1})	0.06 **	0.06	0.04	43.58
Leaf Area Ratio (cm^2 g^{-1})	0.00 †	0.00 †	0.00 †	20.05
Dry matter accumulation (g plant^{-1})	441.61 **	424.30	265.35	43.58
Leaf transpiration rate (μg cm^{-2} s^{-1})	2370794	61161800**	3329772	24.28
Stomatal conductance (μmol m^{-2} s^{-1})	0.01	0.12**	0.01 **	23.89

* and **: Significant effect at 5% and 1% probability, respectively. †Values lower than 0.001

Most of the analysed traits have a strong positive relationship with each other, except for leaf area ratio and stomatal conductance, which have a negative correlation with some other traits (Figure 1). This was expected due to the presence of primary and secondary traits. The former is directly collected from the plants and the latter derive from the former. Among the primary traits, the correlations between dry masses of root and leaf (0.71), root and stem (0.72), leaf and stem (0.74), and transpiration rate and stomatal conductance (0.87) were the highest. Several environmental and genetic events can lead to the correlation, such as pleiotropy, when the cause is permanent, or gene linkage, a transitory cause (CRUZ et al., 2012). For this reason, the behaviour observed in this study has yet to be proven with further tests in populations with different genetic structures.

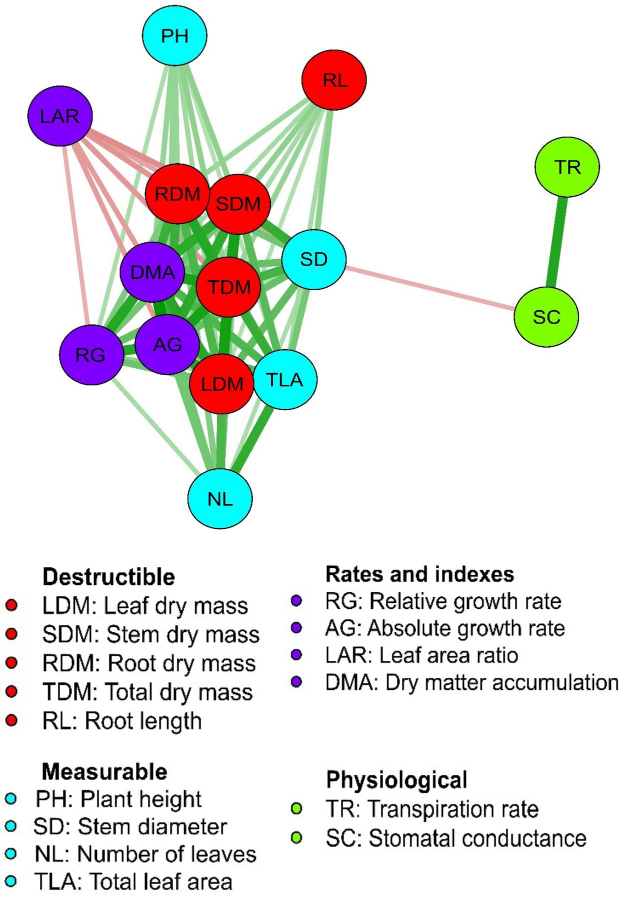

Figure 1 - Correlation network between the 15 traits analysed. The thickness of the line connecting the traits represents the magnitude of the correlation, while the colour represents the direction, being green for positive and red for negative.

The vigour evaluation of the root system under adverse conditions is fundamental for the breeding aiming at tolerance to abiotic stresses, such as hypoxia (TANDONNET et al., 2018). Assessments of this nature not only provide more complete information about the genotypes, but also open the possibility of a new breeding branch: the rootstock improvement, an important step towards promoting the sustainability of cultivation in species that can be grafted, such as cupuassu. This branch is consolidated in other fruit trees, such as apple (*Malus domestica* Borkh.), citrus (*Citrus spp.*), grapevine (*Vitis spp.*), among others (FORNER-GINER et al., 2020; TANDONNET at al., 2018; WANG et al., 2019). Nevertheless, root assessments are destructive, making it difficult to quickly generate results, which makes indirect selection an interesting option (DINIZ; OLIVEIRA, 2019). In this study, such analysis proved that at least half of the root dry mass behaviour can be explained by measurable traits (Figure 2). Among the measurable traits, total leaf area and stem diameter are those that have the greatest direct effect on root dry mass. Even so, half of the behaviour of this trait is due to causal effects of uncontrolled traits in the experiment, which decreases the accuracy in cases of indirect selection via measurable traits. In cacao (*Theobroma cacao* L.) subjected to drought, Santos et al. (2018) found significant cause-and-effect relationships between the leaf biomass and stem diameter variables and root volume, a result close to that found in the present study.

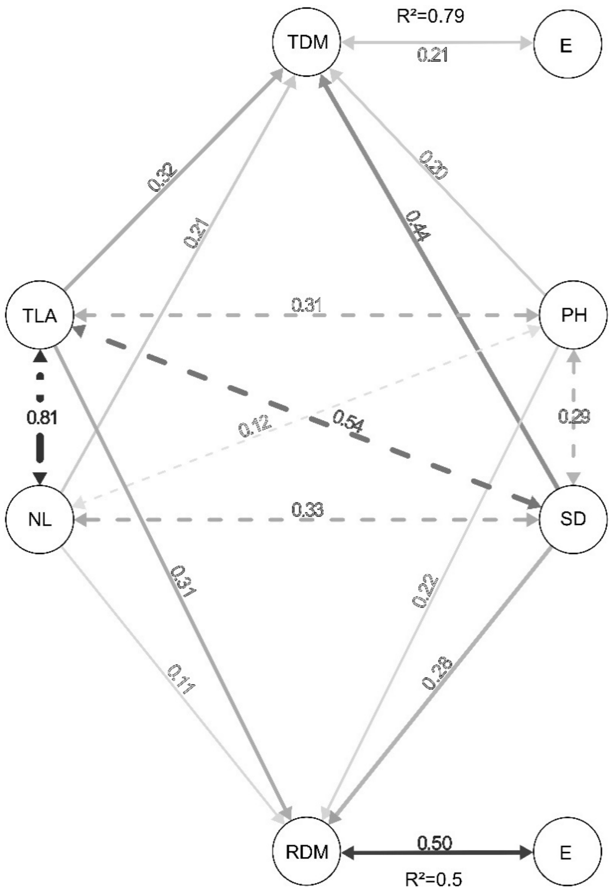

Figure 2 - Diagram of path analysis between the measurable traits plant height (PH), stem diameter (SD), total leaf area (TLA) and number of leaves (NL) and the destructive traits root dry mass (RDM) and total dry mass (TDM). The letter E represents the residual effects of random variables not predicted in the model. The intensity of grayscale represents the magnitude of the correlation. Dashed lines represent correlations between measurable traits and solid lines represent the direct effects of such traits on RDM and TDM.

Despite the high correlation between the total dry mass and the measurable traits (Figure 1), the direct effects were low (Figure 2). In other words, to have the effect on the total dry mass seen in Figure 1, the measurable traits interact with each other, making the cause-and-effect relationship explicit. In the breeding context, this behaviour implies that a single measurable trait cannot be used for indirect selection purposes, but the use of a selection index containing such traits can be an interesting alternative. Setyawan et al. (2016) also used path analysis to propose selection indices for cacao production components. Omotayo et al. (2018) used the same technique to study the cause-and-effect relationships between the traits of the cocoa bean. Therefore, the efficiency of the technique for the purposes foreseen in this study is attested.

In principal component analysis (PCA), the number of components is equivalent to the number of traits used. Generally, most of the variation can be explained by the first two components (CRUZ et al., 2012). In the present study, the use of these components explained a total of 78.9% of the variation (60.8% in component 1 and 18.1% in component 2). The traits dispersion, considering their interrelationships, can be observed in the Biplot (Figure 3).

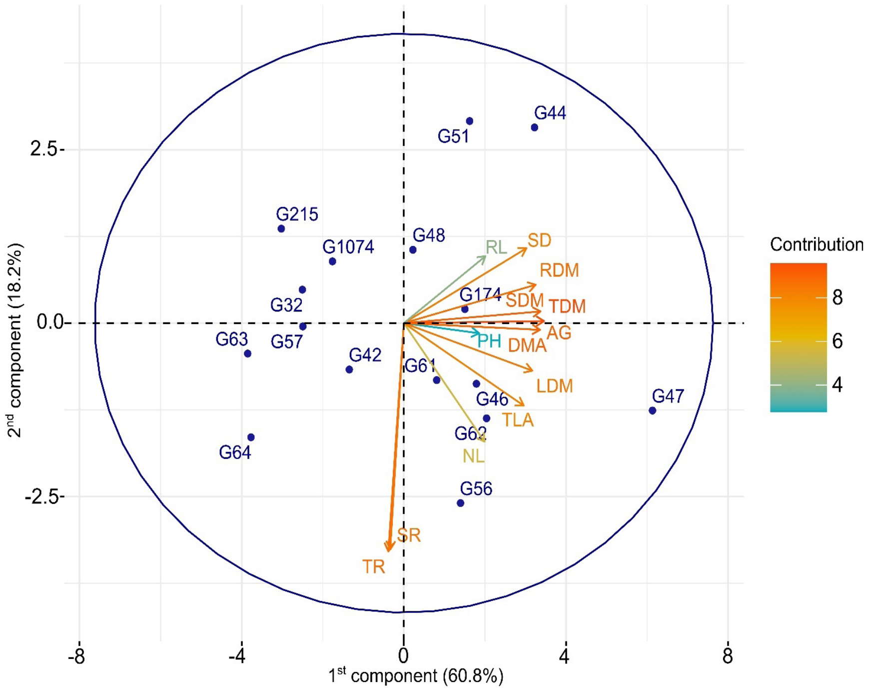

Figure 3 - Biplot obtained from principal component analysis, showing the distribution of genotypes in the four quadrants and the relative contribution of each trait.

When analysing Figures 1 and 3 together, one can visualize that the distribution of the traits is a part of the correlation between them, evidenced by the difference in the direction of the arrows. For instance, the physiological traits' arrows are in a completely different direction compared to the other traits, a fact also observed in the correlation (Figure 1).

PCA also highlights the importance of these traits, i.e., their contribution to the formation of the principal components. According to the analysis, total dry mass, stem dry mass, dry matter accumulation, absolute growth rate, root dry mass, stem diameter and total leaf area are the most important traits in the first component, the one with the greatest relevance. Adenuga and Ariyo (2020) used this same criterion to select/classify traits in cacao diversity analysis. On the other hand, height, root length and number of leaves contribute little to the differentiation between genotypes. Therefore, such traits can be discarded.

The dispersion of genotypes in Biplot shows both their similarity and their performance with respect to component variables. G47, G44 and G51 genotypes stand out from the others regarding growth traits, showing greater adaptation to the hypoxic conditions imposed by the experiment. G47 and G44 share a common ancestor, from whom they probably inherited alleles for flood tolerance. Despite being more closely related to G44, G51 does no share any ancestor with G44 nor G47. In other words, the phenotypic expression of G51 is probably originated from a different set of alleles than G44 and G47. Ofori et al. (2014) used PCA to distinguish the more susceptible and tolerant cacao genotypes in a drought situation and reached this conclusion to explain the overlapping genotypes in the groups formed.

Based on the analysis of variance, correlations and principal components, four traits were selected: i) Total dry mass, which is an indicative of vegetative vigour, has relationships of great magnitude with most of the measurable and destructible traits, and is the trait with the greatest contribution to the formation of the principal components; ii) stem diameter, which is an easy-to-measure trait, has significant relationships with most of the traits, and explains a good part of variation; iii) total leaf area, which represents the vegetative surface suitable for carrying out photosynthesis, vital for the plant's survival and adaptation to the most varied situations, including hypoxia; and iv) stomatal conductance, a representative of the physiological traits, which are highly correlated, being of great importance for the formation of the principal components, especially the second.

The four chosen traits were used to calculate the relative plasticity of the genotypes (Figure 4). Note how the environment strongly influences most of the genotypes, causing major changes in their performance when there is a shift in the water regime. The previously highlighted G47, for example, was unstable for all the traits. G174, on the other hand, was amongst the top five genotypes that were least influenced by the environment in all four traits. Other genotypes that deserve attention in terms of plasticity are G48 and G56. These genotypes may be prioritized for generating progenies that perform consistently in a broader spectrum.

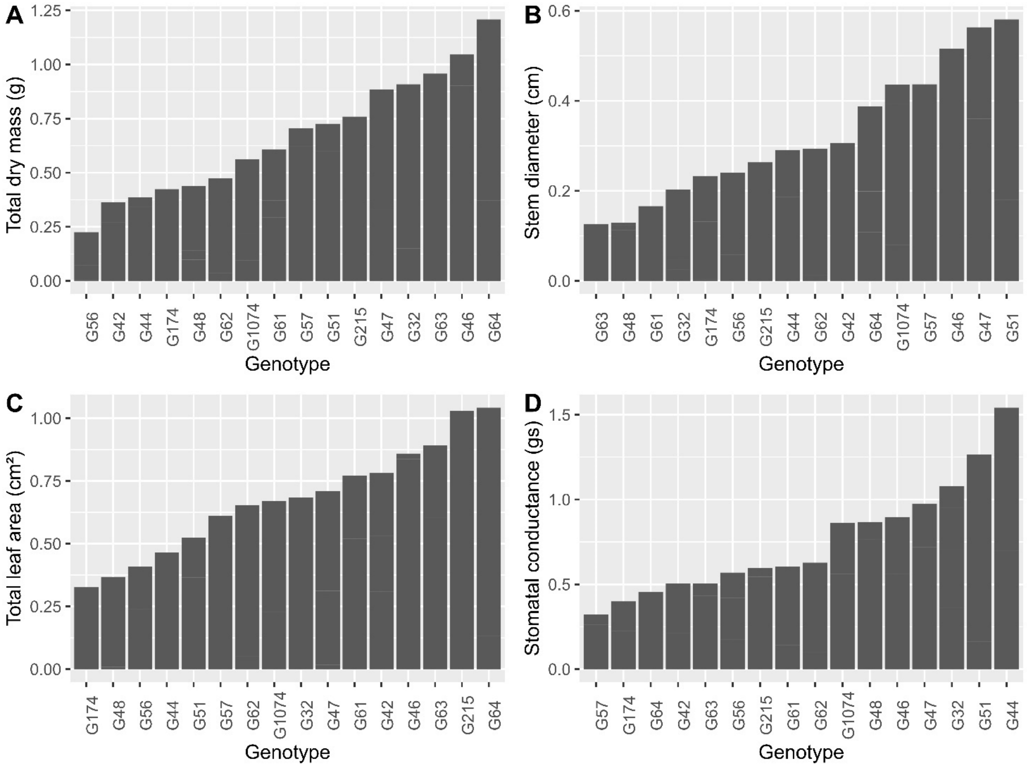

Figure 4 - Relative distance plasticity index (RDPI) of the 16 evaluated genotypes for the most informative traits: total dry mass (A), stem diameter (B), total leaf area (C), and stomatal conductance (D).

Nevertheless, be aware that the relative distance plasticity index (RDPI) considers only the relative difference between the performance under hypoxic and regular-watered conditions. In other words, the performance itself is not considered and still needs to be checked. For this purpose, we used the Scott-Knott test. The genotypes G47 and G44, which were previously indicated as top-performing by the PCA analysis, were also grouped in the high-performance progenies in all four traits by the Scott-Knott method under hypoxic conditions (Table 2). G51 was also in the top-performing group in three of the four chosen traits (total dry mass, stem diameter and stomatal conductance). These genotypes may be prioritized when the aim is to develop materials specifically for areas subjected to flooding. G174 has favourable alleles for total dry mass and stomatal conductance under hypoxia. G48 and G56 had a complementary performance for total dry mass and stem diameter and had good performance for total leaf area and stomatal conductance.

Table 2 - Average performance† of 16 cupuassu genotypes, regarding the four selected traits: total dry mass, stem diameter, total leaf area and stomatal conductance.

Genotype	Total dry mass (g)		Stem diameter (cm)		Total leaf area (cm^2)‡	Stomatal conductance (gs)	
	Hypoxia	Control	Hypoxia	Control		Hypoxia	Control
G32	39.23 Bb	59.74 Aa	1.156 Ab	1.187 Aa	6879 a	0.198 Ba	0.351 Aa
G42	44.52 Ab	41.44 Ab	1.169 Ab	0.995 Bb	6115 b	0.226 Aa	0.289 Aa
G44	59.55 Aa	58.91 Aa	1.346 Aa	1.165 Ba	8096 a	0.131 Ba	0.296 Aa
G46	54.02 Aa	34.19 Bb	1.223 Ab	0.939 Bb	6090 b	0.243 Ba	0.376 Aa
G47	72.55 Aa	46.40 Bb	1.462 Aa	1.102 Ba	8134 a	0.253 Aa	0.293 Aa
G48	47.27 Ab	58.89 Aa	1.280 Aa	1.203 Aa	7385 a	0.174 Ba	0.267 Ab
G51	56.13 Aa	38.94 Bb	1.382 Aa	1.031 Bb	5877 b	0.159 Ba	0.314 Aa
G56	52.93 Aa	56.20 Aa	1.246 Ab	1.104 Aa	8061 a	0.271 Aa	0.227 Ab
G57	40.67 Ab	29.00 Ab	1.18 Ab	0.949 Bb	4516 b	0.241 Aa	0.256 Ab
G61	49.24 Aa	45.82 Ab	1.193 Ab	1.096 Aa	7263 a	0.215 Aa	0.223 Ab
G62	56.47 Aa	46.47 Ab	1.202 Ab	1.053 Ab	7299 a	0.222 Aa	0.222 Ab
G63	36.08 Ab	34.88 Ab	1.112 Ab	1.047 Ab	5029 b	0.235 Aa	0.232 Ab
G64	32.92 Ab	36.02 Ab	1.017 Ab	0.985 Ab	5377 b	0.261 Aa	0.289 Aa
G174	54.72 Aa	43.71 Ab	1.204 Ab	1.067 Aa	5810 b	0.218 Aa	0.234 Ab
G215	36.11 Ab	43.83 Ab	1.149 Ab	1.099 Aa	5573 b	0.184 Aa	0.242 Ab
G1074	41.83 Ab	33.17 Ab	1.140 Ab	0.917 Bb	59257 b	0.198 Ba	0.308 Aa
Mean	**48.388**	**44.226**	**1.216**	**1.059**	**6464.84**	**0.214**	**0.276**

†Means followed by the same letter, uppercase horizontally and lowercase vertically, are statistically different at $P < 0.05$, according to Scott-Knott's method. ‡Only the overall means were presented because the progenies x water regimes interaction was not significant for this trait.

Differential performances of the genotypes regarding total dry mass and stem diameter were observed when the plants were subjected to hypoxia. For most genotypes, there was a greater vegetative vigour under the flooding conditions, when compared to the control. The progenies that expressed the phenotypes with this pattern have a greater indication of adaptation to hypoxic conditions. Hence, the fact that the cupuassu tree can maintain the functioning of the photosynthetic apparatus in a flooded environment may be an indication of the greater capacity of this species to survive in flooded or transitional environments, such as the Amazon floodplains. Similar results were obtained by Bertolde et al. (2010), Branco et al. (2017) and Niether et al. (2020), with moderate reduction in the increase in height, number of leaves and survival rate in cacao plants subjected to soil flooding. This slight reduction is a consequence of the decrease in photosynthetic processes occurring in plants subjected to water stress.

Stomatal conductance for the control treatment was higher than that for plants under hypoxia, indicating that the gas exchanges were occurring, and consequently their physiological and metabolic processes were also in full operation. The high intensities of photosynthetically active radiation and the high temperatures during the test period, causing water losses by evapotranspiration, may have affected the performance of the control plants, due to the lower vegetative vigour.

Despite the greater vegetative vigour under hypoxia, stomatal conductance was lower under this water regime (Table 2). Decreased stomatal conductance is common in plants subjected to drought stress, aiming at less water loss from the leaves to the external environment. In plants subjected to flooding, hypoxia triggers such a physiological response, culminating in stomatal limitation of photosynthesis (KREUZWIESER; RENHENBERG, 2014).

The photosynthetic process stands out in plants as a driving force for the reactions that occur in their metabolism, responsible for converting light energy into chemical energy (OLIOSI et al., 2017). Photosynthetic capacity is progressively reduced under conditions of water stress, resulting in a decrease in the use of light in the photosynthetic process. When a field is flooded, adaptive mechanisms arise, such as hypertrophic stem lenticels that can serve as openings through which toxic compounds associated with anaerobiosis are released in the roots, thus preventing their translocation to the leaves. The increment of stem diameter was greater under stagnant water than under control conditions (Table 2). This could also be a result of an adaptation to soil waterlogging, as stem diameter is positively correlated with root biomass. This soil flooding tolerance mechanism may have influenced the greater vegetative vigour of cupuassu plants. (MARTINAZZO et al., 2013; TAIZ; ZEIGER, 2017; SANTOS et al. 2018).

By studying the responses of young cupuassu plants subjected to soil flooding, several morphophysiological traits were evaluated and reported in this study. This large dataset provided insight into how materials express alternative phenotypes under hypoxic conditions. This trait, known as phenotypic plasticity, is something that benefits plants and has been used to study the process of acclimation of materials to environmental changes and to understand the ability to grow under limiting conditions (VALLADARES et al., 2006). In general, our data indicate that the relative plasticity of the genotypes does not always represent an effective advantage to maintain the morphophysiological apparatus of the plants under soil flooding conditions (Figure 4). As our study was carried out with young plants, further research is needed to evaluate whether morphological and physiological responses found herein are sustained under field conditions.

Conclusion

The four most important traits to explain the variation in the studied population were identified (total dry mass, stem diameter, total leaf area and stomatal conductance). Based on performance and plasticity, we selected G174, G48 and G56 for a broader spectrum and G44, G47 and G51 for hypoxic conditions only.

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