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Screening *Genipa americana* Progenies for Their Ability to Maintain Leaf Vitality Under Severe Dehydration Using Chlorophyll Fluorescence †

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Abstract: Damage to the photosynthetic apparatus during leaf dehydration is an indicator of the maintenance of leaf vitality and the resilience of tree seedlings to severe drought. *Genipa americana* is a tree widely distributed in the neotropical region but with great ecological and sociocultural importance in the south of the state of Bahia, Brazil, where its fruits are harvested from subspontaneous trees. This study aimed to compare the feasibility of the maximum quantum efficiency of photosystem II (Fv/Fm) and performance indexes derived from the *JIP* test, i.e., performance index on absorption basis (PI_{abs}) and total performance index (PI_{total}), for screening *G. americana* seedlings from different mother plants for leaf damage caused by dehydration. From leaf dehydration curves, we calculated the values of relative water content (RWC) in which Fv/Fm, PI_{abs}, and PI_{total} reach a loss of 10% and 50% in relation to the values of fully hydrated leaves. PI_{total} was the only parameter that revealed consistent significant differences between progenies for RWC at 50% of percentage loss. Significant differences were observed among progenies for leaf traits; however, no correlation was detected between these traits and chlorophyll fluorescence parameters. Monitoring the PI_{total} values during leaf dehydration is a useful tool for screening *G. americana* progenies in relation to their capacity to maintain leaf vitality under occasional severe droughts.

Keywords: extreme weather events; forest restoration; photochemical efficiency; relative water content

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1. Introduction

Tropical trees have been impacted by sudden changes in temperature and water constraints, induced by global climate change [1], with negative impacts on biodiversity and forest ecosystem services worldwide [2]. Rising average surface temperatures on Earth have contributed to an increase in the frequency and intensity of extreme weather events.

One key driver of these changes is the El Niño–Southern Oscillation (ENSO), a climate phenomenon involving periodic variations in ocean temperatures in the central and eastern tropical Pacific. These temperature fluctuations significantly influence global weather patterns and can lead to severe droughts and floods, particularly in tropical regions [3,4]. With the increased frequency and intensity of extreme events, many tropical rainforests are expected to be impacted by the increased occurrence of droughts [5]. For example, studying eight tropical forests in central Panama, [6] reported that the 2014/16 ENSO increased seedling mortality by an average of 11%, with a range of about 2% to 21%. Also, during the last extreme 2014/15 ENSO, the cocoa agroforests in southern Bahia, Brazil, were extremely impacted by the drought, causing an average cocoa tree mortality of 15% and a decrease in cocoa production of 89% [7].

Tree planting has been suggested as a way to mitigate global climate change [8]. However, the increased occurrence of extreme climatic events, such as occasional severe droughts, is an imminent challenge, as it may affect the survival and growth of seedlings after planting. The initial establishment of tree seedlings is a critical phase, especially in the first post-planting year [9]. In recent decades, studies have shown that many tree seedlings are already exposed to upper limits of climatic extremes and resource limitations in planting environments, representing a great challenge for ecological restoration and plantations of tree crops in agroecosystems [10,11]. Compared to adult trees, seedlings have a smaller volume of roots and a smaller stock of carbon reserves. Thus, the seedling phase, which corresponds to the period of full establishment of the new plant, is the phase of greatest vulnerability due to the effects of stress factors, such as unexpected severe droughts [12,13]. Because tree seedlings typically have few leaves, maintaining leaf vitality under low relative water content is important for their survival during extreme drought, as well as for recovery after periods of drought stress. Thus, knowing the limits of maintaining leaf vitality in conditions of low relative water content is important for the selection of tree species [14] or progenies of different mother trees in order to increase the success of plantings in sites prone to occasional severe droughts.

The analysis of chlorophyll fluorescence emission by portable equipment is a non-destructive and fast technique, considered very useful for analyzing the photochemical efficiency of plants under abiotic stress [15]. The maximum quantum yield of photosystem II, or the ratio between variable and maximum fluorescence emission (Fv/Fm), is one of the most applied parameters in plant ecophysiological studies to assess environmental stress in plants, being considered an excellent indicator of photochemical efficiency and leaf vitality [16,17], including water deficit [18,19]. Recent studies have demonstrated that the chlorophyll fluorescence emission technique can be a useful tool to assess the vitality of leaves under continuous dehydration and, consequently, to screen tree species in relation to their capacity to survive periods of unexpected severe droughts [14,20]. Although [20] reported that the relative water content (*RWC*) value at which the Fv/Fm ratio reaches 50% of its initial value is lower than the limiting leaf rehydration capacity for plant species with different tolerances to water deficit, [14] demonstrated that drops in the Fv/Fm ratio in relation to leaf dehydration were effective in separating tree seedlings from nine tropical species due to their susceptibility to severe leaf dehydration.

The fast phase of chlorophyll fluorescence emission is called *OJIP* and reflects the photochemical processes associated with photosystem II (*PSII*) and information about the photosynthetic electron transport chain integrity of the photosynthetic electron transport chain [21–23]. The *OJIP* curve is analyzed by the *JIP* test, which quantifies the characteristics of the structure and vitality of photosystems II (*PSII*) and I (*PSI*) [21,24]. Different parameters can be derived from the *JIP* test, such as performance indices based on absorption and energy conservation (PI_{abs} and PI_{total}), respectively [21]. These indices have been used to effectively quantify photosynthetic sensitivity to various abiotic stresses [17,21,25]. This is because PI_{abs} and PI_{total} integrate the energy conservation for electron transport rate outside the *PSII*, being potentially more sensitive than other fluorescence parameters used in most studies, such as Fv/Fm [21]. The PI_{abs} and PI_{total} performance indices have been used as indicators of drought stress in plants [26,27] and for screening tolerant genotypes [18,28,29]. Despite this, we are unaware of published studies on the impacts of continuous leaf dehydration on these *JIP* test parameters in seedlings of tropical trees, especially in progenies originating from native and sub-spontaneous mother trees.

Genipa americana (Rubiaceae) is a neotropical fruit tree with a wide territorial distribution, being found in the tropical forests of South and Central America [15]. The fruit of the species is popularly known as "*Jenipapo*" and has a high iron content. Furthermore, in its initial state of maturation, the fruit is an excellent natural source of genipin [30]. The species is recognized for its valuable wood and for its ecological role in tropical ecosystems, being used as food for many animals, and mainly for its tolerance to soil flooding [31]. It has great sociocultural importance in the south of the state of Bahia, Brazil, a tropical region with rainfall well distributed throughout the year, where its fruits are harvested from sub-spontaneous trees [32]. In addition, *G. americana* is a rustic species, presenting fast growth and high acclimatization to different environmental conditions [32,33]. These characteristics contribute to the tree being considered an essential tree crop in tropical agroecosystems and indicated for silvicultural management in the Brazilian Atlantic Forest biome [34]. However, although *G. americana* is a species widely recognized for its tolerance to flooding, information about its tolerance to extreme severe drought is still scarce, and it is an interesting model species to test the use of different chlorophyll fluorescence variables to screen progenies in relation to their capacity for leaf vitality maintenance under unexpected severe droughts.

This study aimed to compare the feasibility of the maximum quantum efficiency of photosystem II (Fv/Fm) and variables from the *JIP* test (PI_{abs} and PI_{total}) for screening *G. americana* seedlings originating from different mother plants to their capacity for leaf vitality maintenance under leaf dehydration. As PIabs and PItotal integrate more steps of the electron transport chain and are potentially more sensitive than the Fv/Fm ratio in detecting environmental stress in plants, we tested the hypothesis that these derived *JIP* test parameters are more efficient than the Fv/Fm ratio for screening seedlings of *G. americana* progenies in relation to their leaf vitality maintenance under unexpected severe droughts. We believe that this is a simple methodology that can contribute to advances in the process of selecting mother trees, aiming at the production of seedlings with greater resilience to extreme climate events in a context of tree planting under climate threats.

2. Materials and Methods

2.1. Plant Material

The seeds of *G. americana* used to produce the seedlings were obtained from the fruits of twelve mother plants growing spontaneously in the southern region of Bahia, Brazil. To ensure genetic diversity, the fruits were collected from mother trees located at a minimum distance of 30 m from each sampling individual. According to the Köppen classification [33], the climate in the region is humid tropical with an average monthly temperature of 24–26 °C and annual precipitation greater than 1.500 mm. Fruits were collected from farms within an altitude gradient of 29–400 masl, and only healthy, fully ripened fruits were used. The seedlings were cultivated in containers measuring 21.0 cm in height × 17 cm in diameter with a volume of 5.0 L, in the nursery of the State University of Santa Cruz (UESC), located in Ilhéus, BA, Brazil (39°13′59″ W; 14°45′15″ S). After a growth period of 198 days, analyses of leaf dehydration essays were carried out.

2.2. Leaf Dehydration Essays and Fluorescence Parameters

The methodology used during the leaf dehydration essays was adapted from the methodologies proposed by [14,20]. The leaf dehydration curves were assessed through two independent essays. Initially, leaf dehydration curves were assessed in progenies from six mother plants, always considering nine seedlings per mother plant, totaling 54 seedlings. Before starting the leaf dehydration curves, always at the end of the day, before each test, nine well-irrigated plants of *G. americana* were taken to the laboratory. In the laboratory, the seedlings were sprayed with water and wrapped in black plastic bags. The environmental conditions of the laboratory for the foliar dehydration experiments were rigorously controlled, maintaining the ambient temperature between 23 and 25 °C and the relative humidity between 65% and 70%. Chlorophyll fluorescence was quantified using a Pocket PEA portable fluorometer (Hansatech Instruments, King's Lynn, UK). To ensure complete oxidation of the photosystem II (*PSII*) electron transport chain, one leaf per plant was selected and kept in the dark for a minimum period of 30 min using a specific clip. After this period, the leaves were exposed to a saturating light pulse (3500 µmol photons m^{-2} s^{-1}, wavelength of 650 nm, duration of 1 s). Subsequently, the recorded transient fluorescence data were analyzed through the *JIP* test, allowing the calculation of maximum quantum efficiency of *PSII* (Fv/Fm) and the potential performance indexes for energy conservation from exciton to the reduction of intersystem electron acceptors (PI_{abs}) and for energy conservation from exciton to the reduction of *PSI* end acceptors (PI_{total}). The Fv/Fm ratio was calculated by the formula $Fv/Fm = (Fm - F_0)/Fm$, whereas PI_{abs} and PI_{total} were calculated according to the formulas proposed by [25]: $PI_{abs} = (RC/ABS)\cdot(TR_0/DI_0)\cdot[ET_0/(TR_0 - ET_0)]$ and $PI_{total} = PIabs\cdot[(RE_0/ET_0)/(1 - RE_0/ET_0)]$; where RC/ABS is the density of active reaction centers, TR_0/DI_0 is the ratio between the energy capture and dissipation flux, and $(ET_0)/(TR_0 - ET_0)$ is the efficiency of electron transport after the primary acceptor QA^-.

After the initial measurement of chlorophyll fluorescence, the leaves were detached and digitized using a flatbed scanner. In addition, measurements of leaf thickness (*LT*) were carried out, and the leaves were weighed to obtain the saturated fresh mass (*LMs*). From the digitalized images, the individual leaf area (*LA*) was measured using the ImageJ software version 1.54g [35]. The *LT* was measured with an analog micrometer (Zaas Precision, São Paulo, Brazil). After these procedures, the leaves were placed under the laboratory bench and weighed at intervals of 0, 3, 6, 10, 24, 32, 48, 52, 58, 72, 76, 82, and 96 h to obtain the fresh mass during dehydration (*LMde*). These time intervals were established from previous tests. Right after the leaves were weighed, the chlorophyll fluorescence emission was measured. At the end of 96 h of leaf dehydration, the leaves were placed separately in a forced air circulation oven at 60 °C to obtain the dry mass of individual leaves (*LMd*). The leaf mass per area (*LMA*) was calculated using the formula $LMA = LMd/LA$. To determine the relative water content (*RWC*), the values of *LMs*, *LMde*, and *LMd* were used for each measurement time, through the formula $RWC = (LMde - LMd)/(LMs - LMd)$. The percentage loss for photosystem II maximum quantum yield on dehydration ($PL[Fv/Fm]$) were calculated using the formula $PL[Fv/Fm] = 100 \times (1 - [Fv/Fm]de/[Fv/Fm]s)$; where $[Fv/Fm]$ is the value of the Fv/Fm ratio at each *LMde* value and $[Fv/Fm]s$ is the value of the Fv/Fm ratio of the leaf in water saturation, which corresponds to the fresh saturated mass of the individual leaf before dehydration (*LMs*). Likewise, this same formula was used for the performance index on an absorption basis ($PL[PI_{abs}]$), in this case $PL[PI_{abs}] = 100 \times (1 - [PI_{abs}]de/[PI_{abs}]s)$, and for the performance index for energy conservation ($PL[PI_{total}]$), in this case $PL[PI_{total}] = 100 \times (1 - [PI_{total}]de/[PI_{total}]s)$.

Subsequently, a second essay was conducted using the best chlorophyll fluorescence parameter and the time at which this parameter reaches 50% in relation to time zero (0 h).

For this, we used the same six progenies used for the first essay, in addition to six more independent progenies (twelve mother plants and 108 seedlings in total, one leaf per plant).

2.3. Data Analysis

For data analysis in this study, initially, six progenies were named from A to E. The relative water content (*RWC*) for percentage loss of 10% and 50% of Fv/Fm, PI_{abs}, and PI_{total} for each seedling was determined by fitting the relationships between percentage loss (*PL*) of the parameters over decreasing *RWC* and using the best fit of three differently shaped functions, as reported by [14]: linear, $PL = b \cdot (100 - RWC)$; exponential, $PL = a \cdot e^{(-b \cdot RWC)}$; and sigmoidal, $PL = a / \left\{ 1 + e^{[-(RWC - X_0)/b]} \right\}$ (Table S1, Supplementary Materials). All functions were compared using maximum likelihood, and the best fit was selected according to the lowest Akaike information criterion corrected for small n [36]. The chlorophyll fluorescence parameters derived from the leaf dehydration curves and time course (Table 1) were compared with Fisher's one-way ANOVA or Kruskal–Wallis, and post-hoc Tukey's Honest Significant Difference test (Tukey HSDs test for ANOVA) or Least Significant Difference test (LSDs test for Kruskal–Wallis). After this, the data of leaf traits and the best chlorophyll fluorescence parameter(s) for screening leaf dehydration tolerance of twelve progenies, named from A to L, were submitted to analysis of variance (one-way ANOVA), followed by the Scott–Knott test ($p < 0.05$). Spearman's correlation coefficients of bivariate cross-correlations of leaf trait variables with RWC and the best chlorophyll fluorescence parameter in the first essay were also calculated. All statistical analyses were performed with the programming language *R*, version 4.1.0 [37]. with a level of significance of $\alpha = 0.05$.

Table 1. Abbreviations, definitions, and units of all variables measured.

Abbreviature	Definition	Units
Chlorophyll fluorescence's parameters derived		
Fv/Fm	Maximum quantum yield of photochemical energy conversion in photosystem II	Unitless
PI_{abs}	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors	Unitless
PI_{total}	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end electron acceptors	Unitless
$PL_{[\text{Fv/Fm}]}$	Percentage loss of F_v/F_m	%
$PL_{[\text{PIabs}]}$	Percentage loss of PI_{abs}	%
$PL_{[\text{PItotal}]}$	Percentage loss of PI_{total}	%
$PL_{[\text{Fv/Fm}]10}$	$PL_{[\text{Fv/Fm}]}$ at 10%	%
$PL_{[\text{Fv/Fm}]50}$	$PL_{[\text{Fv/Fm}]}$ at 50%	%
$PL_{[\text{PIabs}]10}$	$PL_{[\text{PIabs}]}$ at 10%	%
$PL_{[\text{PIabs}]50}$	$PL_{[\text{PIabs}]}$ at 50%	%
$PL_{[\text{PItotal}]10}$	$PL_{[\text{PItotal}]}$ at 10%	%
$PL_{[\text{PItotal}]50}$	$PL_{[\text{PItotal}]}$ at 50%	%
Parameters derived from the response of chlorophyll fluorescence to loss of relative water content (RWC) and time-course		
$RWC_{\text{PL[Fv/Fm]10}}$	*RWC* at percentage loss of F_v/F_m at 10%	%
$RWC_{\text{PL[Fv/Fm]50}}$	*RWC* at percentage loss of F_v/F_m at 50%	%
$RWC_{\text{PL[PIabs]10}}$	*RWC* at percentage loss of PI_{abs} at 10%	%
$RWC_{\text{PL[PIabs]50}}$	*RWC* at percentage loss of PI_{abs} at 50%	%
$RWC_{\text{PL[PItotal]10}}$	*RWC* at percentage loss of PI_{total} at 10%	%
$RWC_{\text{PL[PItotal]50}}$	*RWC* at percentage loss of PI_{total} at 50%	%

Table 1. *Cont.*

Abbreviature	**Definition**	**Units**
$Time\_RWC_{\mathrm{PL[Fv/Fm]10}}$	Time at $RWC_{\mathrm{PL[Fv/Fm]10}}$	hour
$Time\_RWC_{\mathrm{PL[Fv/Fm]50}}$	Time at $RWC_{\mathrm{PL[Fv/Fm]50}}$	hour
$Time\_RWC_{\mathrm{PL[PIabs]10}}$	Time at $RWC_{\mathrm{PL[PIabs]10}}$	hour
$Time\_RWC_{\mathrm{PL[PIabs]50}}$	Time at $RWC_{\mathrm{PL[PIabs]50}}$	hour
$Time\_RWC_{\mathrm{PL[PItotal]10}}$	Time at $RWC_{\mathrm{PL[PItotal]10}}$	hour
$Time\_RWC_{\mathrm{PL[PItotal]50}}$	Time at $RWC_{\mathrm{PL[PItotal]50}}$	hour
Leaf traits		
LT	Leaf thickness	µm
LS	Leaf succulence	$\mathrm{g\ m^{-2}}$
LMA	Leaf mass per area	$\mathrm{g\ m^{-2}}$
ILA	Individual leaf area	$\mathrm{cm^2}$

3. Results

The chlorophyll fluorescence parameter most sensitive to leaf dehydration was $PL_{\mathrm{[PItotal]}}$ (90.0–91.0% and 50.1–55.2% *RWC* for $PL_{\mathrm{[PItotal]}}$ at 10% and 50%, respectively; Figure 1, Table 2), followed by $PL_{\mathrm{[PIabs]}}$ (88.7–90.1% and 43.7–50.6% *RWC*), and finally $PL_{\mathrm{[Fv/Fm]}}$ (18.9–27.5% and 7.1–13.8% *RWC*). As for the parameters of chlorophyll fluorescence response to *RWC* loss and time course (Table 3), overall $RWC_{\mathrm{PL[Fv/Fm]}}$ and $RWC_{\mathrm{PL[PIabs]}}$ at 10% and 50% showed no significant differences between progenies (Figure 2A–C), but $RWC_{\mathrm{PL[PItotal]}}$ at 50% presented significant differences between progenies (Figure 2E), where progeny *A* showed the highest resistance to dehydration and the lowest progeny *E*; $Time\_RWC_{\mathrm{PL[Fv/Fm]}}$ at 10% and 50% showed significant differences between progenies (Figure 2B), but $Time\_RWC_{\mathrm{PL[PIabs]}}$ and $Time\_RWC_{\mathrm{PL[PItotal]}}$ at 10% and 50% did not exhibit differences (Figure 2D–F).

Table 2. Model of best fit between the parameters of percentage loss of chlorophyll fluorescence (F_v/F_m, PI_{abs}, and PI_{total}) and relative water content (*RWC*) for each of the *G. americana* progenies.

Progenies	**Model**	R^2	AIC_c	P_m
	Percentage loss of *Fv*/*Fm*			
A	$87.4 \cdot e^{(-0.093\,RWC)}$	0.82	866.1	***
B	$96.4 \cdot e^{(-0.065\,RWC)}$	0.78	947.9	***
C	$157.9 \cdot e^{(-0.125\,RWC)}$	0.80	959.3	***
D	$180.0 \cdot e^{(-0.109\,RWC)}$	0.88	730.1	***
E	$105.6 \cdot e^{(-0.068\,RWC)}$	0.78	974.5	***
F	$137.8 \cdot e^{(-0.097\,RWC)}$	0.82	777.1	***
	Percentage loss of PI_{abs}			
A	$0.893 \cdot (100 - RWC)$	0.84	794.2	***
B	$0.942 \cdot (100 - RWC)$	0.73	986.1	***
C	$0.938 \cdot (100 - RWC)$	0.87	646.4	***
D	$0.997 \cdot (100 - RWC)$	0.85	665.7	***
E	$1.005 \cdot (100 - RWC)$	0.82	569.2	***
F	$1.018 \cdot (100 - RWC)$	0.88	653.9	***
	Percentage loss of PI_{total}			
A	$1.002 \cdot (100 - RWC)$	0.90	988.1	***
B	$1.029 \cdot (100 - RWC)$	0.84	1033.3	***
C	$1.061 \cdot (100 - RWC)$	0.89	825.7	***
D	$1.091 \cdot (100 - RWC)$	0.84	777.9	***
E	$1.110 \cdot (100 - RWC)$	0.87	860.7	***
F	$1.102 \cdot (100 - RWC)$	0.89	944.7	***

R^2: coefficient of determination; AIC_c: Akaike Information Criterion corrected; P_m: model probability; ***: $p < 0.001$. See abbreviation in Table 1.

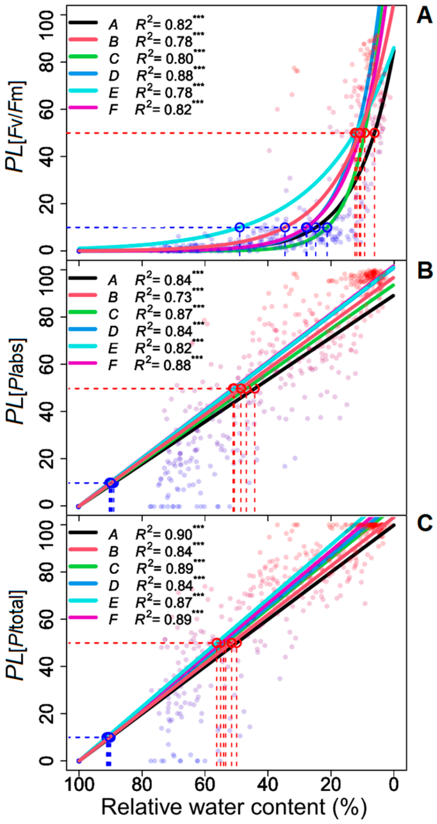

Figure 1. Relationship of the parameters of percentage loss of chlorophyll fluorescence (**A**): *Fv*/*Fm*; (**B**): PI_{abs}; and (**C**): PI_{total} and relative water content (*RWC*) for each of the *G. americana* progenies. Solid regression lines are best-fit models for each progeny. The dashed lines in each plot indicate the *RWC* associated with 10% (blue) and 50% (red) losses of *Fv*/*Fm*, PI_{abs}, or PI_{total}. R^2: coefficient of determination; ***: $p < 0.001$. See statistical analysis in Table 2.

Table 3. Summary of the ANOVA of the comparison among *G. americana* progenies for parameters derived from the response of chlorophyll fluorescence to loss of relative water content (*RWC*) and time-course and leaf trait variables.

Variables (Abbreviature)	***F* or *KW*** $^{\beta}$	R^2	P_{m}
Parameters from the response of chlorophyll fluorescence to loss of RWC and time-course $^{\gamma}$			
$RWC_{\text{PL[Fv/Fm]10}}$	8.53 $^{\beta}$	-	n.s.
$RWC_{\text{PL[Fv/Fm]50}}$	10.87 $^{\beta}$	-	n.s.
$RWC_{\text{PL[PIabs]10}}$	9.77 $^{\beta}$	-	n.s.
$RWC_{\text{PL[PIabs]50}}$	9.75 $^{\beta}$	-	n.s.
$RWC_{\text{PL[PItotal]10}}$	9.21 $^{\beta}$	-	n.s.
$RWC_{\text{PL[PItotal]50}}$	15.31 $^{\beta}$	-	**
$Time\_RWC_{\text{PL[Fv/Fm]10}}$	14.05 $^{\beta}$	-	*
$Time\_RWC_{\text{PL[Fv/Fm]50}}$	4.71	0.29	**
$Time\_RWC_{\text{PL[PIabs]10}}$	9.37 $^{\beta}$	-	n.s.
$Time\_RWC_{\text{PL[PIabs]50}}$	8.61 $^{\beta}$	-	n.s.
$Time\_RWC_{\text{PL[PItotal]10}}$	9.71 $^{\beta}$	-	n.s.
$Time\_RWC_{\text{PL[PItotal]50}}$	10.50 $^{\beta}$	-	n.s.
Leaf traits			
Individual leaf area (*ILA*)	4.41	0.24	**
Leaf thickness (*LT*)	6.07	0.32	***
Leaf mass per area (*LMA*)	8.9 $^{\beta}$	-	n.s.
Leaf succulence (*LS*)	2.73	0.14	*

F: Fisher coefficient's value; *KW*: Kruskal–Wallis's test ($^{\beta}$); R^2: coefficient of determination; P_{m}: model probability; n.s.: not significant; *: $p < 0.05$; **: $p < 0.01$; and ***: $p < 0.001$. $^{\gamma}$: See abbreviation in Table 1.

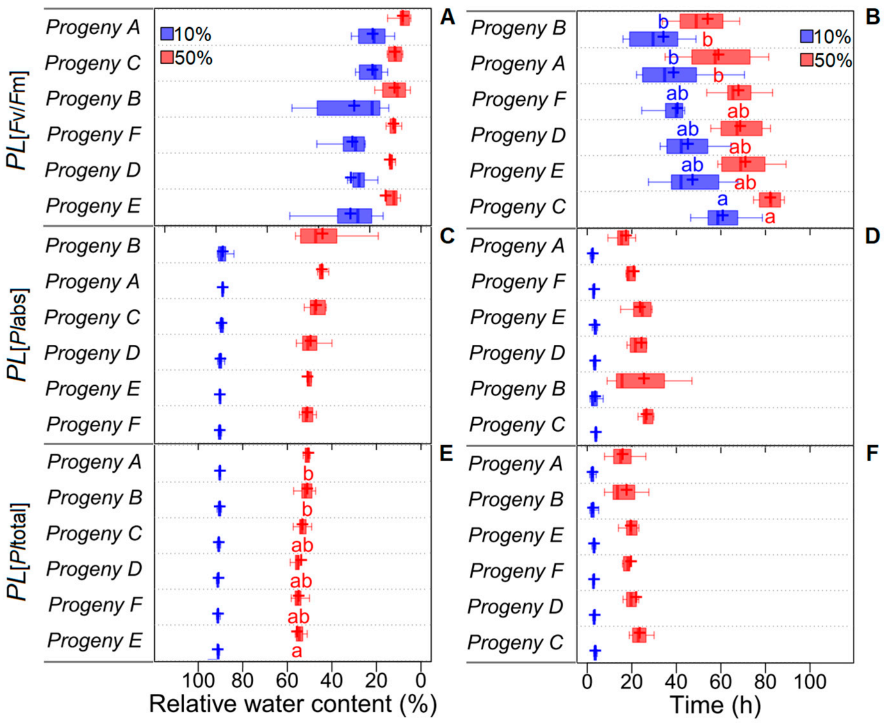

Figure 2. Comparisons of the average relative water content (*RWC*; (**A**,**C**,**E**)) and dehydration time (**B**,**D**,**F**) between *G. americana* progenies for the percentage loss at 10% (blue) and 50% (red) of the parameters of chlorophyll fluorescence for Fv/Fm, PI_{abs}, and PI_{total}. Equal letters indicate no statistically significant difference between the progenies in each percentage loss (10% or 50%; $p > 0.05$). See statistical analysis in Table 3.

Although the progenies came from seeds collected from mother plants of the same region, significant differences among progenies for the leaf traits were observed (Table 4). All leaf traits showed significant differences (*LT*, *LS*, and *LMA*), except for *ILA* Progeny A stood out with the highest *LMA* and highest *LS*. In addition, ten progenies had an average value of $PL[PI_{total}]_{24h}$ above 50% as a function of *RWC*, except for progenies G and L, $PL[PI_{total}]_{24h}$, around 49% (Table 4). In addition, no correlation was observed between the chlorophyll fluorescence parameters and leaf trait variables (Table 5). Except for RWC_{24h}, the only parameter correlated with leaf succulence (*LS*; $r_s = 0.21$) (Table 5).

Table 4. Mean values comparing the variables of leaf characters for *RWC* and $PL_{[PItotal]}$ in the percentage loss of 24 h for twelve progenies of *G. americana*.

	Variables					
Progenies	***LA***	***LT***	***LS***	***LMA***	RWC_{24h}	$PL_{[PItotal]24h}$
A	420.77 ^{a}	20.44 ^{b}	3.88 ^{a}	58.91 ^{a}	53.65 ^{a}	57.22 ^{a}
B	428.88 ^{a}	23.00 ^{a}	3.77 ^{a}	44.44 ^{a}	56.10 ^{a}	57.24 ^{a}
C	358.33 ^{a}	22.11 ^{a}	3.11 ^{a}	57.83 ^{a}	50.21 ^{b}	64.39 ^{a}
D	383.00 ^{a}	24.33 ^{a}	3.82 ^{a}	49.51 ^{a}	58.78 ^{a}	57.55 ^{a}
E	373.44 ^{a}	22.11 ^{a}	3.68 ^{a}	51.59 ^{a}	55.88 ^{a}	54.87 ^{a}
F	390.66 ^{a}	24.00 ^{a}	3.50 ^{a}	48.16 ^{b}	53.50 ^{a}	54.18 ^{a}
G	350.88 ^{a}	21.55 ^{b}	2.74 ^{b}	44.79 ^{b}	53.87 ^{a}	48.51 ^{b}
H	346.55 ^{a}	20.22 ^{b}	3.21 ^{a}	52.17 ^{a}	52.32 ^{a}	51.21 ^{b}
I	412.88 ^{a}	21.55 ^{b}	3.40 ^{a}	51.20 ^{a}	55.10 ^{a}	50.69 ^{b}
J	385.55 ^{a}	19.44 ^{b}	2.46 ^{b}	40.18 ^{b}	50.70 ^{b}	57.14 ^{a}
K	343.33 ^{a}	19.88 ^{b}	2.65 ^{b}	45.75 ^{b}	45.87 ^{b}	57.86 ^{a}
L	323.88 ^{a}	20.66 ^{b}	2.48 ^{b}	41.76 ^{b}	52.97 ^{a}	48.87 ^{b}

Equal letters indicate no statistical difference between the progenies of *G. americana* ($p > 0.05$, Scott–Knott test). ($n = 108$). See abbreviation in Table 1.

Table 5. Correlations between all measured variables of *G. americana* progenies, leaf trait variables, and parameters derived from *RWC* and PL$_{[PItotal]}$ at 24 h percentage loss.

	Leaf Traits Variables			
Parameters	*LA*	*LT*	*LMA*	*LS*
RWC_{24h}	0.04	0.07	−0.00	**0.21** *
$PL_{[PItotal]24h}$	0.06	−0.05	0.03	−0.04

Spearman's correlation coefficients of bivariate cross-correlations (n = 108). Bold values indicate significant correlations (*: $p < 0.05$). See abbreviation in Table 1.

4. Discussion

Global climate change has already led to an increase in extreme weather events, such as heat waves and extreme occasional droughts in tropical regions [4]. Although still under debate, tree death caused by severe drought may occur due to two main mechanisms, that is, by hydraulic failure (when the loss of water through transpiration exceeds the absorption of water by the roots) and carbon starvation (when the stomata close and leaves fall to prevent excessive water loss, resulting in the depletion of carbon reserves necessary to maintain vital physiological processes) [1]. In this study, we compared the capacity of seedlings of a neotropical tree from different mother plants to maintain leaf vitality during dehydration. As observed, the decline in the RWC had a strong influence on the performance indexes, in which the percentages of loss for 10% and 50% of PI_{abs} and PI_{total} were obtained at *RWC* values much higher than those of Fv/Fm. The tree seedlings are more vulnerable to unexpected severe droughts than adult individuals, as their roots have less capacity to exploit soil resources and have less capacity to store carbon reserves. Under such conditions, maintaining leaf vitality under low water content is important for the resumption of vital functions after periods of unexpected severe droughts.

Chlorophyll fluorescence emission is a physicochemical process that occurs in all photosynthetic organisms in an extremely fast time step [17]. So, the analysis of chlorophyll fluorescence parameters allows very rapid and highly accurate inferences to be made about the conversion of physical light energy into chemical energy, as well as the integrity of the photosynthetic electron transport chain [24]. The Fv/Fm ratio is a classic chlorophyll fluorescence parameter related to the transformation of the physical energy of light into chemical energy, indicating the probability of the energy of an absorbed photon reaching the reaction center and triggering a photochemical event [38]. Although it has been well established that the Fv/Fm ratio is an important indicator of environmental stress in plants for a long time [39,40]. It has been reported that this quantitative parameter is not sensitive enough to detect physiological changes to mild water stress [22]. Furthermore, with technological advances in the manufacture of portable fluorescence meters, the *JIP* test, which corresponds to a series of time steps on the curve between the initial (F_0) and maximum (Fm) fluorescence emissions, has allowed a more detailed and efficient assessment of how environmental stress affects the performance of fundamental processes related to the photochemistry of photosynthesis [38]. Thus, other chlorophyll fluorescence variables, originating from the *JIP* test, have been considered more efficient in detecting the response of plants to water stress, such as performance indices (PI_{abs} and PI_{total}) used in this study [16,22,23,41]. The two performance indices used in this study integrate a series of events related to the conservation of the energy absorbed in the antenna pigments of PSII until the reduction of electron acceptors after PSII (PI_{abs}) and after PSI (PI_{total}) [22]. Because the JIP curve performance indexes integrate several stages of the chloroplast electron transport chain, several studies have considered PI_{abs} and PI_{total} as the best predictors to measure photosynthetic efficiency [21,22]. Thus, any alteration in terms of the PI_{abs} and PI_{total} components can be easily observed, mainly in the most sensitive performance index. In some cases, PI_{total} is the most prevalent and most sensitive parameter, as it reflects changes in photosynthetic electron transport activity outside the *PSII* [42].

Our results indicated that PI_{total} was the most responsive parameter related to fluorescence emission in dehydrated leaves of different *G. americana* progenies. Our results suggest a possible limitation of the electron transport chain at the PSI level [43], indicating that leaf dehydration negatively affected the photochemical efficiency of the progenies. Previous studies have also shown similar trends regarding leaf dehydration tolerance for different genotypes [36]. Although we observed that there were no significant differences between progenies for *RWC* at $PL_{[\mathrm{PItotal}]10}$, it was shown that there were consistent significant differences between progenies for *RWC* at $PL_{[\mathrm{PItotal}]50}$. An explanation for this pattern is related to the linear regression model, since it considers the intercept at zero (at the origin). The values of all progenies are very close in 10%; that is, in 10% it was still not possible to observe changes. On the other hand, we observed that statistical differences between progenies increased as *RWC* decreased. This finding is consistent with previous studies on tolerance to leaf dehydration, which showed important declines in *RWC* and loss of photochemical efficiency in tree species [14,20,44].

In the context of global climate change, the increase in average temperature with consequent changes in the water regime brings with it extreme ecological filters that can limit the survival and initial growth of many forest species. Water stress reduces the ability of newly planted seedlings to fix their roots in the soil, with serious impacts on nutrient and water absorption, being even more marked in the first months after planting [45]. In this study, we used *G. americana* as a model species to compare the tolerance to leaf vitality during dehydration of seedlings from different mother trees. Even considering a single species, our results were accurate in indicating that the PI_{total} parameter was the best variable related to the emission of chlorophyll fluorescence capable of separating the progenies. The methodology proposed was effective for the fast screening of native forest trees in relation to leaf dehydration using chlorophyll fluorescence (PI_{total}). We suggest that it can be applied to other tropical tree species as an alternative to reduce seedling mortality after planting in the field. In addition, considering that the increased occurrence of heat waves is one of the main effects of global climate change on plant physiological performance [12,46], we suggest the development of new studies aiming to simultaneously test the effects of high temperatures and leaf dehydration on chlorophyll fluorescence parameters.

5. Conclusions

In summary, the results of Fv/Fm and PI_{abs} showed no consistent significant differences between progenies, while PI_{total} was the only parameter that revealed consistent significant differences between progenies for *RWC* at 50% of percentage loss. Among the chlorophyll fluorescence parameters tested, the PI_{total} was the best for screening *G. americana* genotypes in relation to photosynthetic damage caused by leaf dehydration in the seedlings' ontogenetic stage. Although other approaches at leaf and whole plant scales are highly related to drought tolerance, monitoring the PI_{total} values during leaf dehydration is a useful tool for screening *G. americana* progenies in relation to their capacity to maintain leaf vitality under occasional severe droughts. From a practical point of view, considering ecosystems more prone to the occurrence of severe droughts, or even areas with soils with low water retention capacity, studies comparing the leaf dehydration tolerance of seedlings of tree species in the initial stage of growth can be useful to assist in the selection of mother trees for the production of high-quality seedlings for planting in tropical agroecosystems or forest restoration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16050770/s1, Table S1: Dataset of relative water content (*RWC*) and percentage loss of F_v/F_m, PI_{abs}, and PI_{total} variables measured for six *G. americana* progenies. Table S2: Dataset containing all leaf trait variables for six *G. americana* progenies. Table S3: Model results for the determination of the response of percentage loss of F_v/F_m, PI_{abs}, and PI_{total} to decrease in relative water content (*RWC*) for six *G. americana* progenies.

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References

1. McDowell, N.G.; Sapes, G.; Pivovaroff, A.; Adams, H.D.; Allen, C.D.; Anderegg, W.R.L.; Arend, M.; Breshears, D.D.; Brodribb, T.; Choat, B.; et al. Mechanisms of woody-plant mortality under rising drought, CO_2 and vapour pressure deficit. *Nat. Rev. Earth Environ.* **2022**, *3*, 294–308. [CrossRef]
2. França, F.M.; Benkwitt, C.E.; Peralta, G.; Robinson, J.P.W.; Graham, N.A.J.; Tylianakis, J.M.; Berenguer, E.; Lees, A.C.; Ferreira, J.; Louzada, J.; et al. Climatic and local stressor interactions threaten tropical forests and coral reefs. *Philos. Trans. R. Soc. B Biol. Sci.* **2020**, *375*, 20190116. [CrossRef]
3. Rifai, S.W.; Li, S.; Malhi, Y.S. Coupling of El Niño events and long-term warming leads to pervasive climate extremes in the terrestrial tropics. *Environ. Res. Lett.* **2019**, *14*, 105002. [CrossRef]
4. Yang, H.; Ciais, P.; Wigneron, J.P.; Chave, J.; Cartus, O.; Chen, X.; Fan, L.; Green, J.K.; Huang, Y.; Joetzjer, E.; et al. Climatic and biotic factors influencing regional declines and recovery of tropical forest biomass from the 2015/16 El Niño. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2101388119. [CrossRef]
5. Hartmann, H.; Bastos, A.; Das, A.J.; Esquivel-Muelbert, A.; Hammond, W.M.; Martínez-Vilalta, J.; McDowell, N.G.; Powers, J.S.; Pugh, T.A.; Ruthrof, K.X.; et al. Climate Change Risks to Global Forest Health: Emergence of Unexpected Events of Elevated Tree Mortality Worldwide. *Annu. Rev. Plant Biol.* **2022**, *73*, 673–702. [CrossRef]
6. Dantas, L.G.; dos Santos, C.A.C.; Santos, C.A.G.; Martins, E.S.P.R.; Alves, L.M. Future Changes in Temperature and Precipitation over Northeastern Brazil by CMIP6 Model. *Water* **2022**, *14*, 4118. [CrossRef]
7. Gateau-Rey, L.; Tanner, E.V.J.; Rapidel, B.; Marelli, J.-P.; Royaert, S. Climate change could threaten cocoa production: Effects of 2015-16 El Niño-related drought on cocoa agroforests in Bahia, Brazil. *PLoS ONE* **2018**, *13*, e0200454. [CrossRef] [PubMed]
8. Pita, K.; Wickham, S.B.; Davis, E.L.; Lauriault, P.; Johnson, A.; Le, N.Q.; Trant, A.J. How does restoration ecology consider climate change uncertainties in forested ecosystems? *Restor. Ecology.* **2024**, *32*, e14265. [CrossRef]
9. Grossnickle, S. Seedling Establishment on a Forest Restoration Site—An ecophysiological perspective. *Reforesta* **2018**, *6*, 110–139. [CrossRef]
10. Allen, C.D.; Breshears, D.D.; McDowell, N.G. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* **2015**, *6*, 1–55. [CrossRef]

11. Brodribb, T.J.; Powers, J.; Cochard, H.; Choat, B. Hanging by a thread? Forests and drought. *Science* **2020**, *368*, 261–266. [CrossRef] [PubMed]
12. Kijowska-Oberc, J.; Staszak, A.M.; Kamiński, J.; Ratajczak, E. Adaptation of Forest Trees to Rapidly Changing Climate. *Forests* **2020**, *11*, 123. [CrossRef]
13. Browne, L.; Markesteijn, L.; Engelbrecht, B.M.J.; Jones, F.A.; Lewis, O.T.; Manzané-Pinzón, E.; Wright, S.J.; Comita, L.S. Increased mortality of tropical tree seedlings during the extreme 2015–16 El Niño. *Glob. Change Biol.* **2021**, *27*, 5043–5053. [CrossRef]
14. Mielke, M.S.; Oliveira, L.A.; dos Santos, M.S.; Pérez-Molina, J.P.; Cerqueira, A.F.; Dalmolin, Â.C.; Sousa-Santos, C.; de Brito, C.R. Photochemical efficiency and lethal leaf dehydration in seedlings of nine tropical tree species. *New For.* **2024**, *55*, 505–521. [CrossRef]
15. Zappi, D. *Genipa americana* na Lista de Espécies da Flora do Brasil. 2016. Available online: https://reflora.jbrj.gov.br/reflora/listaBrasil/ (accessed on 12 April 2025).
16. Kalaji, H.M.; Schansker, G.; Ladle, R.J.; Goltsev, V.; Bosa, K.; Allakhverdiev, S.I.; Brestic, M.; Bussotti, F.; Calatayud, A.; Dąbrowski, P.; et al. Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues. *Photosynth. Res.* **2014**, *122*, 121–158. [CrossRef] [PubMed]
17. Stirbet, A.; Lazár, D.; Kromdijk, J.; Govindjee, G. Chlorophyll a fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? *Photosynthetica* **2018**, *56*, 86–104. [CrossRef]
18. Ferraz, T.M.; Júnior, S.O.M.; Souza, G.A.R.; Baroni, D.F.; Rodrigues, W.P.; Sousa, E.F.; Penchel, R.; Loos, R.; Figueiredo, F.A.M.M.A.; Rakocevic, M.; et al. Clonal differences in ecophysiological responses to imposed drought in selected *Eucalyptus grandis* x *Eucalyptus urophylla* hybrids. *Tree Physiol.* **2024**, *45*, tpae160. [CrossRef]
19. Yan, W.; Lu, Y.; Guo, L.; Liu, Y.; Li, M.; Zhang, B.; Zhang, B.; Zhang, L.; Qin, D.; Huo, J. Effects of Drought Stress on Photosynthesis and Chlorophyll Fluorescence in Blue Honeysuckle. *Plants* **2024**, *13*, 2115. [CrossRef]
20. Trueba, S.; Pan, R.; Scoffoni, C.; John, G.P.; Davis, S.D.; Sack, L. Thresholds for leaf damage due to dehydration: Declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. *New Phytol.* **2019**, *223*, 134–149. [CrossRef]
21. Strasser, R.J.; Tsimilli-Michael, M.; Qiang, S.; Goltsev, V. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant Haberlea rhodopensis. *Biochim. et Biophys. Acta BBA Bioenerg.* **2010**, *1797*, 1313–1326. [CrossRef]
22. Tsimilli-Michael, M. Revisiting JIP-test: An educative review on concepts, assumptions, approximations, definitions and terminology. *Photosynthetica* **2020**, *58*, 275–292. [CrossRef]
23. Koutra, E.; Chondrogiannis, C.; Grammatikopoulos, G. Variability of the photosynthetic machinery tolerance when imposed to rapidly or slowly imposed dehydration in native Mediterranean plants. *Photosynthetica* **2022**, *60*, 88–101. [CrossRef] [PubMed]
24. Strasser, R.J.; Tsimilli-Michael, M.; Srivastava, A. Analysis of the fluorescence transient. In *Chlorophyll Fluorescence: A Signature of Photosynthesis*; Papageorgiou, G.C., Govindjee, Eds.; Advances in Photosynthesis and Respiration Series; Springer: Dordrecht, The Netherlands, 2004; pp. 321–362.
25. Strasser, R.J.; Srivastava, A.; Tsimilli-Michael, M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*; Yunus, M., Pathre, U., Mohanty, P., Eds.; Taylor & Francis: London, UK, 2000; pp. 445–483.
26. van Heerden, P.; Swanepoel, J.; Krüger, G. Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO2 assimilation. *Environ. Exp. Bot.* **2007**, *61*, 124–136. [CrossRef]
27. Wang, Z.X.; Chen, L.; Ai, J.; Qin, H.Y.; Liu, Y.X.; Xu, P.L.; Jiao, Z.Q.; Zhao, Y.; Zhang, Q.T. Photosynthesis and activity of photosystem II in response to drought stress in Amur Grape (Vitis amurensis Rupr.). *Photosynthetica* **2012**, *50*, 189–196. [CrossRef]
28. Bano, H.; Athar, H.; Zafar, Z.U.; Kalaji, H.M.; Ashraf, M. Linking changes in chlorophyll *a* fluorescence with drought stress susceptibility in mung bean [*Vigna radiata* (L.) Wilczek]. *Physiol. Plant.* **2021**, *172*, 1244–1254. [CrossRef]
29. Mihaljević, I.; Vuletić, M.V.; Šimić, D.; Tomaš, V.; Horvat, D.; Josipović, M.; Zdunić, Z.; Dugalić, K.; Vuković, D. Comparative Study of Drought Stress Effects on Traditional and Modern Apple Cultivars. *Plants* **2021**, *10*, 561. [CrossRef]
30. Neves, M.I.L.; Silva, E.K.; Meireles, M.A.A. Natural blue food colorants: Consumer acceptance, current alternatives, trends, challenges, and future strategies. *Trends Food Sci. Technol.* **2021**, *112*, 163–173. [CrossRef]
31. Santos, C.S.; Dalmolin, A.C.; Schilling, A.C.; Santos, M.S.; Schaffer, B.; Mielke, M.S. Root deformation affects mineral nutrition but not leaf gas exchange and growth of *Genipa americana* seedlings during the recovery phase after soil flooding. *Braz. J. Biol.* **2022**, *82*, e234018. [CrossRef]
32. Santos, C.S.; Dalmolin, Â.C.; dos Santos, M.S.; dos Santos, R.B.; Lima, T.M.; Pérez-Molina, J.P.; Mielke, M.S. Morphometry of the fruits of *Genipa americana* (Rubiaceae): A case study from the southern coast of Bahia, Brazil. *Rodriguesia* **2021**, *72*, e00652020. [CrossRef]
33. Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.d.M. Modeling monthly mean air temperature for Brazil. *Theor. Appl. Clim.* **2013**, *113*, 407–427. [CrossRef]

34. Rolim, S.G.; Piña-Rodrigues, F.C.M.; Piotto, D.; Batista, A.; Freitas, M.L.M.; Brienza, J.S.; Zakia, M.J.B.; Calmon, M. Research Gaps and Priorities in Silviculture of Native Species in Brazil. 2019. Available online: https://doi.org/10.13140/RG.2.2.31264.61445 (accessed on 13 June 2023).
35. Abramoff, M.D.; Magalhães, P.J.; Ram, S.J. Image Processing with ImageJ. *Biophotonics Int.* **2004**, *11*, 36–42.
36. Vuletić, M.V.; Horvat, D.; Mihaljević, I.; Dugalić, K.; Šimić, D.; Čupić, T.; Jurković, V.; Lepeduš, H. Photosynthetic Variability of Oblačinska Sour Cherry Ecotypes under Drought. *Plants* **2022**, *11*, 1764. [CrossRef]
37. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2023; Available online: https://www.R-project.org/ (accessed on 26 June 2023).
38. Banks, J.M. Continuous excitation chlorophyll fluorescence parameters: A review for practitioners. *Tree Physiol.* **2017**, *37*, 1128–1136. [CrossRef]
39. Meng, L.; Zhou, Y.; Gu, L.; Richardson, A.D.; Peñuelas, J.; Fu, Y.; Wang, Y.; Asrar, G.R.; De Boeck, H.J.; Mao, J.; et al. Photoperiod decelerates the advance of spring phenology of six deciduous tree species under climate warming. *Glob. Change Biol.* **2021**, *27*, 2914–2927. [CrossRef] [PubMed]
40. Zhuang, J.; Wang, Y.; Chi, Y.; Zhou, L.; Chen, J.; Zhou, W.; Song, J.; Zhao, N.; Ding, J. Drought stress strengthens the link between chlorophyll fluorescence parameters and photosynthetic traits. *PeerJ* **2020**, *8*, e10046. [CrossRef]
41. Murchie, E.H.; Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J. Exp. Bot.* **2013**, *64*, 3983–3998. [CrossRef] [PubMed]
42. Niu, S.; Luo, Y.; Li, D.; Cao, S.; Xia, J.; Li, J.; Smith, M.D. Plant growth and mortality under climatic extremes: An overview. *Environ. Exp. Bot.* **2014**, *98*, 13–19. [CrossRef]
43. Tsimilli-Michael, M.; Strasser, R.J. In vivo Assessment of Stress Impact on Plant's Vitality: Applications in Detecting and Evaluating the Beneficial Role of Mycorrhization on Host Plants. In *Mycorrhiza*; Springer: Berlin/Heidelberg, Germany, 2008; pp. 679–703.
44. Rane, J.; Babar, R.; Kumar, M.; Kumar, P.S.; Singh, Y.; Nangare, D.D.; Wakchaure, G.C.; Minhas, P.S. Desiccation tolerance of Photosystem II in dryland fruit crops. *Sci. Hortic.* **2021**, *288*, 110295. [CrossRef]
45. Ji, W.; Hong, E.; Chen, X.; Li, Z.; Lin, B.; Xia, X.; Li, T.; Song, X.; Jin, S.; Zhu, X. Photosynthetic and physiological responses of different peony cultivars to high temperature. *Front. Plant Sci.* **2022**, *13*, 969718. [CrossRef]
46. Preece, N.D.; van Oosterzee, P.; Lawes, M.J. Reforestation success can be enhanced by improving tree planting methods. *J. Environ. Manag.* **2023**, *336*, 117645. [CrossRef]