Acta Physiol Plant (2010) 32:729–737
DOI 10.1007/s11738-009-0454-5

ORIGINAL PAPER

Ecophysiology parameters of four Brazilian Atlantic Forest species under shade and drought stress

Bruna D. Souza · Bruno M. Rodrigues ·
Laurício Endres · Mauro Guida Santos

Received: 3 September 2009 / Revised: 15 December 2009 / Accepted: 30 December 2009 / Published online: 9 January 2010

Abstract The Brazilian Atlantic Forest has experienced a reduction in its original area since the discovery of Brazil. Over the last 30 years, studies and techniques for forest recovery have advanced. Establishing a self-sustainable reforested area with adequate biodiversity is the main parameter for any reforestation program. Thus, knowledge of the ecophysiological behavior of the species to be used is crucial. Our hypothesis is that certain tools are efficient in determining the ecophysiological characterization of native species within different functional groups. Filling group plants show fast growth, intense gas exchange, present mechanisms of water deficit tolerance and show high efficiency in radiation capture, so they are first planted in a reforestation area. While plants pertaining to the diversity group do not exhibit these characteristics, thus are plants after the establishment of the first group of species. To test this hypothesis, two experiments were installed using young plants of four species native to the Atlantic Forest, grown in 9-L pots. Leaf water potential, gas exchange, chlorophyll fluorescence and certain biochemical parameters of leaf metabolism were evaluated. In the first experiment, plants were maintained under two forms of light availability for 15 days, full light (control) and shaded (shade). The species *Inga* sp. and *Brosimum guianensis* presented the most contrasting responses on day 15, principally in the variables leaf water potential, gas exchange, leaf soluble sugar content, F'_v/F'_m and F_v/F_m. In the second experiment, plants were divided into two groups: a well-hydrated group (control) and one that underwent irrigation suspension for 7 days (drought); measurements were performed on day 8 of drought. Again, *Inga* sp. and *Brosimum guianensis* plants showed responses characterizing them as pertaining to distinct functional groups for the experimental parameters previously described. Thus, *Inga* sp. was classified as pertaining to the filling group and *B. guianensis* to the diversity group. The performance of the species *Cinnamomum zeylancium* and *Tapirira guianensis* under the conditions studied suggests that these are intermediate species with potential for use as filling group species.

Keywords Chlorophyll fluorescence · Conservation biology · Functional group · Gas exchange · Leaf water potential · Restoration practices

Introduction

The Atlantic Forest is the fourth hottest biodiversity hotspot and houses more than 60% of the terrestrial species on the planet, although it only covers 11.7% of the surface of the globe. Moreover, the forest houses 21,361 species of vascular plants, amphibians, reptiles, birds and mammals, and among those 8,567 are endemic species (Myers et al. 2000). The high level of endemism in this ecosystem demonstrates that many species have become extinct and that others will also meet the same fate due to intense fragmentation (Brooks and Balmford 1996; Rodrigues et al. 2009; Silva and Tabarelli 2000).

Communicated by J. Zwiazek.

B. D. Souza · B. M. Rodrigues · M. G. Santos (✉)
Laboratório de Ecofisiologia Vegetal, Departamento de Botânica, Universidade Federal de Pernambuco,
Recife, PE 50670-901, Brazil
e-mail: mauroguida@yahoo.com.br

L. Endres
Laboratório de Fisiologia Vegetal, Centro de Ciências Agrárias, Universidade Federal de Alagoas, Maceió, AL 57100-000, Brazil

These endemic species are not distributed at random but occur in particular portions of the Atlantic Forest. The species are preferentially distributed in at least six centers of endemism, including the Pernambuco Center for Endemism (Silva and Casteleti 2003). The center includes the coastal forests situated north of the River San Francisco, or between the states of Alagoas and Rio Grande do Norte. Although the original area of the Pernambuco Center for Endemism represents just over 4% of the original area of the entire Atlantic Forest, this region hosts about 8% of vascular plant flora of this forest (Porto et al. 2006). Currently, a few stretches of forest north of the River San Francisco have unique characteristics, since the block was reduced to forest archipelagos of small forest fragments (Silva and Tabarelli 2000). Pernambuco is one of the most degraded areas of the entire Atlantic Forest (CI-Brasil 1993) and, as a consequence, several species endemic to this region are on the verge of global extinction (Brooks and Rylands 2003). This is one of the regions of the planet where conservation efforts are most urgently required (Rodrigues et al. 2004). However, reforestation must follow the strategies established by Alves-Costa et al. (2008). Among their criteria, the authors stated that the appropriate proportion between diversity and filling species must be observed. Filling species are those that have rapid growth and high canopy cover, permitting rapid shading of the planted area (Chazdon et al. 1996; Souza et al. 2004). Species classified as pertaining to the diversity group do not show rapid growth and generally have low tree canopy coverage (Ribeiro et al. 2004; Souza et al. 2004; Swaine and Whitmore 1988); however, they are essential to ensure the perpetuation of the planted area. Species from this group will gradually replace the filling species when they come into senescence, finally occupying the area restored and ensuring its continuity.

Native species are widely used and perform well, even in highly disturbed environments (Rodrigues et al. 2009). Restoration takes time, the costs of monitoring are high and success is often hard to assess (Parker 1997; Rodrigues et al. 2009). Moreover, global climate changes are altering the natural characteristics of many ecosystems around the world (Intergovernmental Panel on Climate Change 2007). To the northeast of Brazil the most optimistic forecast is an increase of 2.0°C in the mean temperature, in the worst situation 4.0°C. However, in both cases is expected to uneven rainfall with dry period of about greater than today. Thus, plants need to adjust their metabolism to the changing scenario, such that those with greater plasticity will most likely be able to establish successfully. Within this scenario of global climate change, several studies have used plant ecophysiology to understand the behavior of species in different ecosystems (Gulías et al. 2009; Ogaya and Peñuelas 2003).

A study involving two species from different functional groups indicated that, in the absence of water constraints, the higher CO_2 assimilation rates of filling group species are supported by the efficiency of the entire dissipative structure, involving both degradation and the dissipative process (Souza et al. 2004). Thus, the higher photosynthetic performance of filling group species was associated with higher values for stomatal conductance and transpiration rate (Ribeiro et al. 2004; Vincent 2001).

In this study, the behavior of four native species of Brazilian Atlantic Forest was evaluated under different light and water availability schemes. Species plasticity in relation to gas exchange, chlorophyll fluorescence and certain aspects of leaf metabolism was investigated. The main goal was to determine whether the species presented filling or diversity characteristics using ecophysiological tools.

Materials and methods

Study site, plant species and growth conditions

The species studied were from the Cucaú, which is private property located in the city of Rio Formoso, Pernambuco State, northeastern Brazil. The seeds were collected at this location, which has argil soils, a tropical climate and an annual rainfall of 2,788 mm, with the wettest period between May and June. The annual mean temperature ranges between 24 and 29°C. However, in some years of severe drought, the current annual rainfall is concentrated within a few days of the year.

The study included only species endemic to the Brazilian Atlantic Forest: *Inga* sp. of the family Leguminosae and subfamily Mimosaceae; *Cinnamomum zeylancium* Blume of the family Lauraceae, *Brosimum guianensis* Aubl., which is included in the family Moraceae and *Tapirira guianensis* Aubl. of Anacardiaceae.

The seeds of the species studied were collected in fragments of local Atlantic Forest. After germination and the acclimatization period of 4 months, the plants were transplanted into 9-L pots and maintained under greenhouse conditions for 70 days. During this period the mean temperature alternated between 24 and 32°C. The pots contained 7 kg of soil, a mixture of soil and washed sand (3:1), respectively.

Water treatment

Irrigation was suspended for 7 days during stress treatment (drought) and remained continuous in the control treatment. Measurements were performed on day 7 of treatment. Before the suspension of irrigation and during treatment, the control pots, was added 2.0 L of water to keep the substrate fully moistened.

Light treatment

This experiment was conducted during the same period as the water-deficit experiment. All the plants were well watered the entire time. Half of them were kept under 10% of natural light (shade treatment) and the remainder under natural light (control). Photosynthetic active radiation (PAR) ranged from 1,500 to 2,000 μmol m^{-2} s^{-1} during this period. Measurements were taken after 20 days of acclimation, on the same day as the measurements performed on the plants under water deficit.

Leaf water status

Under water deficit, water tension in the xylem was measured after 8 days without irrigation and on the same day in the shade and light treatments, using a Scholander pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) on a fully expanded leaf from the shoot middle of the plant, a mature but not senescent leaf. These measurements were assumed to determine the leaf water potential (Ψ_l).

Gas exchange

Gas exchange measurements were performed on day 7 of drought, from 10:00 to 11:30 h, and on the same day in the shade and light treatments, using a Portable Infrared CO_2 Gas Analyser (IRGA), ADC, model Lci (Hoddesdon, UK). Leaf gas exchange was measured on a mature but not senescent leaf in the middle of plant with a maximum PAR of 1,080 μmol m^{-2} s^{-1} in the leaf cuvette of the IRGA. The local PAR at the time of measurement was 1,500 μmol m^{-2} s^{-1}, and mean temperature ranged from 27.1 to 29.2°C. The vapor pressure deficit (VDP) was approximately 2.1 kPa. The intrinsic water use efficiency (IWUE) was calculated by the relationship A/g_s (Osmond et al. 1980).

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured using an ADC fluorometer (Hoddesdon, UK). Maximal (F_m) and basal (F_o) fluorescence yields were measured in dark-adapted (30 min) leaves, and steady-state (F_s) and maximal (F'_m) fluorescence yields were determined in a light-adapted state (Schreiber et al. 1994; Van Kooten and Snel 1990). The following parameters were assessed (Schreiber et al. 1994): the maximal quantum yield of PSII $[F_v/F_m = (F_m - F_o)/F_m]$ and the excitation capture efficiency of open centers $[F'_v/F'_m = (F'_m - F'_o)/F'_m]$.

Leaf soluble sugar, free amino acid, protein and chlorophyll contents

Leaf samples were collected in the middle of the afternoon, from 14:00 to 15:00 h. Sample leaves were immediately frozen in liquid nitrogen and stored at −80°C in humidity proof containers until analysis.

Soluble carbohydrates were determined according to Dubois et al. (1956), using D(+)-glucose as standard. For free amino acid determination, 0.5 mL of 10% trichloroacetic acid was added to an aliquot of 0.5 mL of the water extract, and the mixture was maintained at 25°C for an hour. This mixture was then centrifuged at 12,000×*g* for 5 min, and the supernatant was used for amino acid determination (Yemm and Cocking 1955), using L-leucine as standard. Leaf protein content was determined according to Bradford (1976). Chlorophyll content was determined according to Lichtenthaler and Wellburn (1983).

Experimental design and statistical analysis

The experimental design was completely randomized with two water levels (water-deficit experiment), two light levels (light availability experiment) and three replicates. The data were subjected to analysis of variance (ANOVA) and means were compared and segregated by the Student–Newman–Keuls test ($p < 0.05$), when significance was detected.

Results

Effect of light availability

Leaf water potential (Ψ_l) was higher ($p < 0.05$) in the treatment under low light availability (shade) in the species *Inga* sp. and *T. guianensis*. However, *B. guianensis* showed a lower Ψ_l value in plants under shade ($p < 0.05$) (Table 1).

The values of stomatal conductance (g_s), CO_2 assimilation (A) and transpiration (E) determined for *B. guianensis* were lower ($p < 0.05$) compared with the other three species. Moreover, *Inga* sp. showed high values of g_s, A and E, especially in the control treatment (light) compared with *B. guianensis* and *T. guianensis* (Fig. 1A, C). In general, IWUE was higher in the shade treatment, except for *Inga* sp. (Fig. 1D).

The values of fluorescence parameters changed under shade, showing diminished basal fluorescence (F_o) ($p < 0.05$) in all species (Fig. 2). Furthermore, except for

Table 1 Leaf water potential (Ψ_l, MPa) in young plants of tree species under different light availability of full light (control) and shaded (shade) or water deficit (drought)

Species	Treatments					
	Control		Shade		Drought	
Inga	−1.40	Aa	−0.37	Bb	−1.68	Bb
C. zeylancium	−1.65	Aa	−1.38	Aa	−2.07	Aa
B. guianensis	−0.42	Bb	−1.75	Aa	−0.57	Cc
T. guianensis	−1.19	Aa	−0.44	Bb	−1.23	Bb

The measurements were realized at the moment of gas exchanges (10:00 h), after 15 days of treatment (shade) or 7 days (drought)

Values represent the mean of three replications. Values followed by different capital letters in the line and followed by different letter in the column differ by test average Student–Neuman–Keuls ($p \leq 0.05$)

Inga sp., all species showed an increase ($p < 0.05$) in maximum quantum yield of PSII, as determined by the ratio F_v/F_m. The species *B. guianensis* and *T. guianensis* showed a reduction ($p < 0.05$) in the values of excitation capture efficiency of open centers F'_v/F'_m, under control conditions. For the fluorescence parameters, significant differences among species ($p < 0.05$) occurred only when exposed to full light, except for the species *C. zeylancium* which showed a difference in relation to the others when under shade (Fig. 2).

The determination of leaf soluble sugar, amino acid and protein (Table 2) in plants under light and shade revealed that only minimal alterations occurred between the treatments. *B. guianensis* showed the highest value of soluble sugars under shade.

Effect of water deficit

Water deficit induced by 7 days of irrigation suspension reduced the Ψ_l in all the species studied ($p < 0.05$) (Table 1), except *C. zeylancium*. This finding was not accompanied by the rates of g_s, A, E and IWUE ($p > 0.05$) (Fig. 3), except for *B. guianensis* which showed an A rate of zero (Fig. 3B). The gas exchange values obtained tended to be lower in plants under stress; however, *T. guianensis* plants under drought showed a high value for g_s ($p < 0.05$) and A (Fig. 3A, B). No significant differences were observed for E and IWUE ($p > 0.05$), except for *B. guianensis*, which showed high g_s and E values with a low A rate; thus, the IWUE for this species was the lowest determined ($p < 0.05$) (Fig. 3C, D).

After 7 days under drought, the fluorescence parameters F_o, F_v/F_m and F'_v/F'_m showed no significant difference between the treatments (Fig. 4A, C); however, *B. guianensis* showed the lowest values of F_v/F_m and F'_v/F'_m among the species studied.

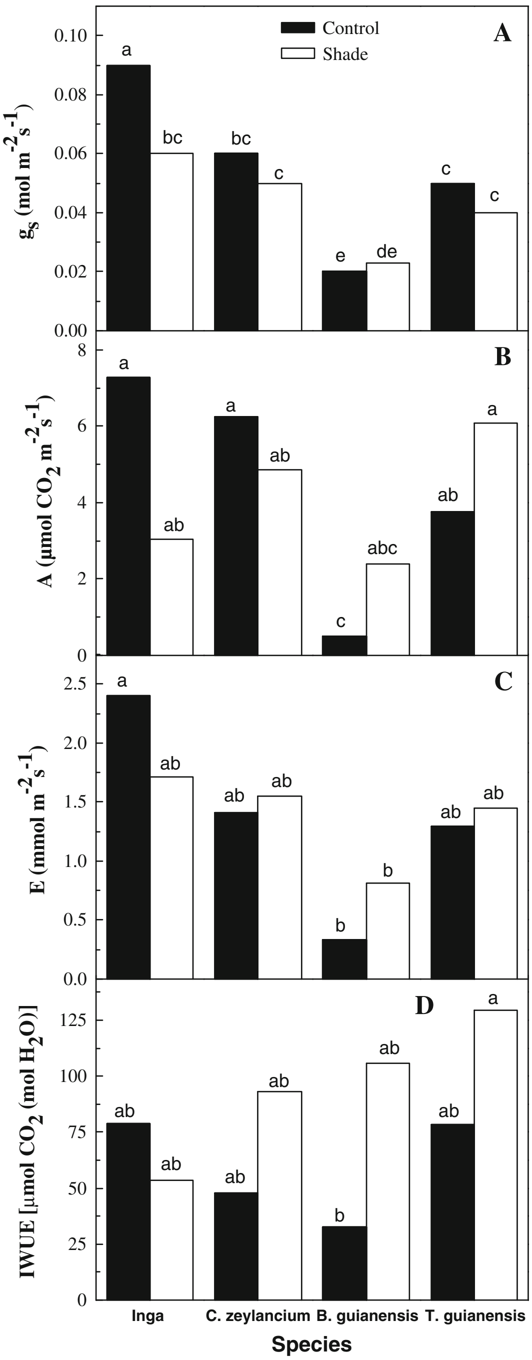

Fig. 1 Changes in stomatal conductance, g_s (**A**), net photosynthesis, A (**B**), transpiration, E (**C**) and intrinsic water use efficiency, IWUE (**D**) of young plants of tree species after 15 days of treatment: full light 1,080 µmol m^{-2}s^{-1}(*control*) and shaded 167 µmol m^{-2} s^{-1} (*shade*). Values followed by *different letters* in each column differ by test average Student–Neuman–Keuls ($p \leq 0.05$). Values represent the mean of three replications

Leaf metabolism parameters showed no significant difference between the treatments; however, *Inga* sp. and *T. guianensis* showed lower values compared with the other two species (Table 3).

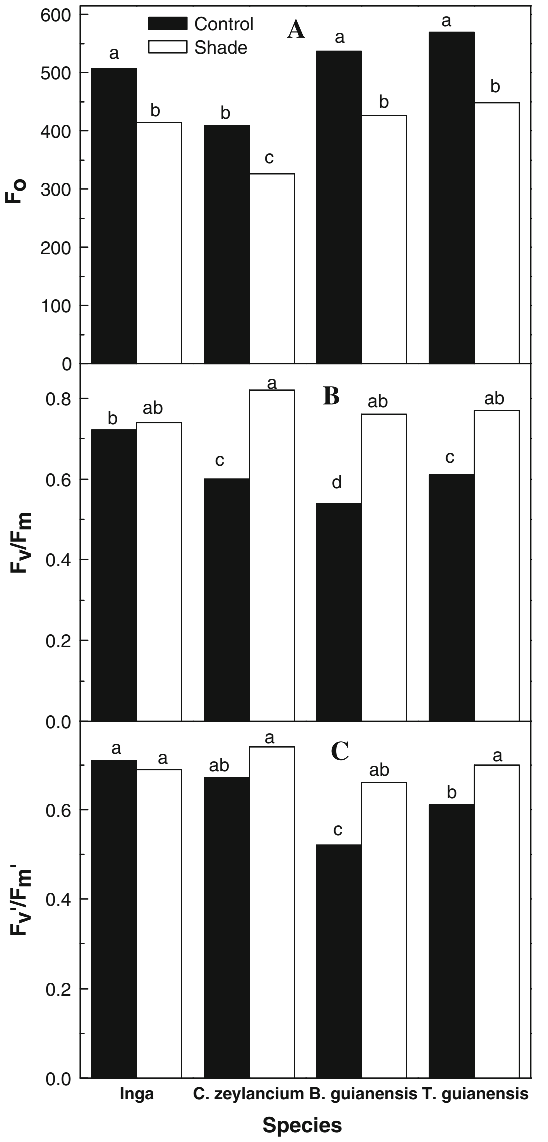

Fig. 2 Fluorescence parameters responses of young plants of tree species after 15 days of treatment: full light 1,080 μmol m^{-2} s^{-1} (*control*) and shaded 167 μmol m^{-2} s^{-1} (*shade*). F_o, fluorescence basal; F_v/F_m, PSII maximum quantum yield; F'_v/F'_m, excitation capture efficiency of open centers. Values followed by *different letters* in each column differ by test average Student–Neuman–Keuls ($p \leq 0.05$). Values represent the mean of three replications

Discussion

Gas exchange and chlorophyll fluorescence are two good parameters for understanding the behavior of different species (Gulías et al. 2009; Ogaya and Peñuelas 2003; Ribeiro et al. 2004; Souza et al. 2004) and are used worldwide. This study aimed to determine the role that ecophysiological tools can play in the difficult task of restoring areas of the Brazilian Atlantic Forest, where many species have become extinct (Rodrigues et al. 2009; Silva and Tabarelli 2000).

Analysis of the results showed that the four species behaved differently both under distinct light availabilities and under water deficit. Under shade, both *Inga* sp. and *T. guianensis* showed a high value for leaf water potential (Ψ_l) ($p < 0.05$) (Table 1). Such behavior may have been a consequence of lower stomatal conductance (g_s) (Fig. 1A) when exposed to low light, which may be indicative of a low tolerance to shade, typical of the filling species group (Alves-Costa et al. 2008). Furthermore, lower values of gas exchange were obtained for *B. guianensis* in both treatments compared to the other species, especially to *Inga* sp. (Fig. 1A, C). Plant response to light availability is an important characteristic for growth, directly affecting the forest succession process. Thus, species range from the early successional light demanding species (filling group) to the very shade tolerant (diversity group) (Chazdon et al. 1996). The results obtained support the argument that under different light availabilities, *Inga* sp. and *B. guianensis* can be characterized as species pertaining to different functional groups.

After 15 days under distinct light availabilities, the basal fluorescence (F_o) was significantly reduced ($p < 0.05$) in all species under shade treatment (Fig. 2A); this variable is related to excitation energy loss during its transfer from the pigment bed to reaction centers (Yordanov et al. 1997). This result verified that low light availability was sufficient to reduce the photochemical reactions of shaded plants, on the other hand, was not enough to modify the content of chlorophyll *a*, *b* and carotenoids ($p > 0.05$) (data not shown). However, the maximum quantum efficiency of PSII photochemistry (F_v/F_m) was increased in all species under shade (Fig. 2B), except *Inga* sp. The largest difference between treatments was determined in *B. guianensis*, followed by *C. zeylancium*. Similar behavior was observed for *B. guianensis* when evaluating PSII maximum efficiency, which estimates the maximum quantum efficiency of PSII under given light conditions (Fig. 2B, C). These data again suggest the same division between functional groups: *Inga* sp. pertaining to the filling group and *B. guianensis* to the diversity group, as suggested by the gas exchange data.

Soluble sugar, amino acid and protein contents (Tables 2, 3) were evaluated in this study; however, it was not possible to determine any typical behavior among species using these variables; i.e., separation into different functional groups could not be achieved using these data. However, the contrasting behavior between *Inga* sp. and *B. guianensis* was further emphasized by the determination of the lowest and the highest ($p < 0.05$) contents of leaf soluble sugars, respectively, among species studied. Data for amino acids and protein showed no distinct pattern among these species (Tables 2, 3), in disagreement with results obtained for the common bean, in which it was

Table 2 Leaf soluble sugars, amino acids and protein of young plants of tree species under different light availability of full light (control) and shaded (shade), after 15 days of treatment

Species	Treatment	Soluble sugars (μmol g^{-1} FW)		Amino acids (μmol g^{-1} FW)		Protein (mg g^{-1} FW)	
Inga sp.	Control	43	c	69	cd	0.53	c
	Shade	38	c	37	cd	0.68	c
C. zeylancium	Control	108	bc	136	b	2.21	a
	Shade	110	bc	220	a	1.74	ab
B. guianensis	Control	136	b	91	bc	0.41	c
	Shade	266	a	85	bc	0.88	bc
T. guianensis	Control	66	bc	68	d	0.36	c
	Shade	53	c	12	d	1.31	bc

Values represent the mean of three replications. Values followed by different letters in each column differ by test average Student–Neuman–Keuls ($p \leq 0.05$)

shown that the daily balances of leaf soluble sugars and free amino acids were good indicators for evaluating the stage of water deficit and metabolic capacity (Santos and Pimentel 2009). In addition, Ennajeh et al. (2009) showed the role of sugars in osmoprotection and osmoregulation in leaf cell of two olive cultivars under severe drought stress.

After 7 days without irrigation, lower Ψ_l values ($p < 0.05$) were obtained for all species compared with controls (Table 1). Among the four species, the lowest values were presented by *C. zeylancium* followed by *Inga* sp. and the greatest potential was shown by *B. guianensis*. Thus, as occurred when exposed to different light availability (Fig. 1A), *B. guianensis* showed the lowest g_s value ($p < 0.05$) among the four species, regardless of treatment, and *Inga* sp. presented the highest ($p < 0.05$) value. Seven days without water supply was sufficient to reduce the g_s of *Inga* sp. and *C. zeylancium*, but was not sufficient to reduce the g_s of *B. guianensis* and *T. guianensis*. Stomatal closure and the consequent decrease in *E* responses to water deficit are expected in plants to prevent excessive loss of water (Kramer and Boyer 1995; Souza et al. 2004). However, these species showed no significant difference in *A* and *E* between treatments (Fig. 3B, C), except for the IWUE of *B. guianensis*, which showed a reduction ($p < 0.05$) when exposed to severe drought (Fig. 3D). IWUE is a good parameter for assessing drought tolerance (Pimentel et al. 1999). However, it must be evaluated along with other variables, as g_s is dependent on several factors, including root activity, when it remains high under low soil water potential provides a high g_s, thus decreasing the IWUE (Fig. 3A, D).

Moreover, without any interfering environmental factor (control treatment), *Inga* sp. showed higher g_s, *A* and *E* than *B. guianensis* (Figs. 1A, C, 3A, C), supporting its classification as a filling group species (Alves-Costa et al. 2008). The gas exchange performance of the majority of the filling plant group can be affected under adverse environmental conditions, such as water deficit in the soil (Ribeiro et al. 2004; Souza et al. 2004) and low light conditions (Vincent 2001).

The stomatal behavior of the species supported the changes in *A* values (Figs. 1A, B, 3A, B), except for the pattern presented by *B. guianensis* under water deficit (Fig. 3A, B). High g_s value was obtained for this species compared to controls, even though it showed *A* equal to zero. In tropical regions, plant species often experience substantial daily and seasonal changes in light availability (Chazdon et al. 1996). For some species, such as the diversity group, high light availability works as a stress factor rather than as a resource that can be used by the leaves (Chazdon et al. 1996; Ribeiro et al. 2004; Souza et al. 2004). This seems to be the case with *B. guianensis*, as revealed by the contrasting behavior between the control and stressed plants (Figs. 1A, B, 3A, B), showing high g_s and low *A*. This indicates distinctive behavior in photochemical reactions (Baker and Rosenqvist 2004; Björkman and Powles 1984), including low photochemical efficiency of PSII, which was confirmed by lower values ($p < 0.05$) of F'_v/F'_m and F'_v/F'_m for *B. guianensis* compared to other species under the environmental conditions studied (Figs. 2B, C, 4B, C).

The present results confirm that certain ecophysiology parameters help to classify plant species within functional groups, as reported by Ribeiro et al. (2004) and Souza et al. (2004). The importance of correct classification is essential for reforestation programs, especially in northeastern Brazil. In this region, most of the Atlantic Forest area has been converted into agricultural land, with only 2% of the original forest remaining (Silva and Tabarelli 2000). Forest remnants are dispersed as small patches surrounded by open fields; thus, protected areas in this region are mostly small and isolated (Rodrigues et al. 2009). Determining the functional group to which a species belongs may increase the chance of success to establish reforested areas.

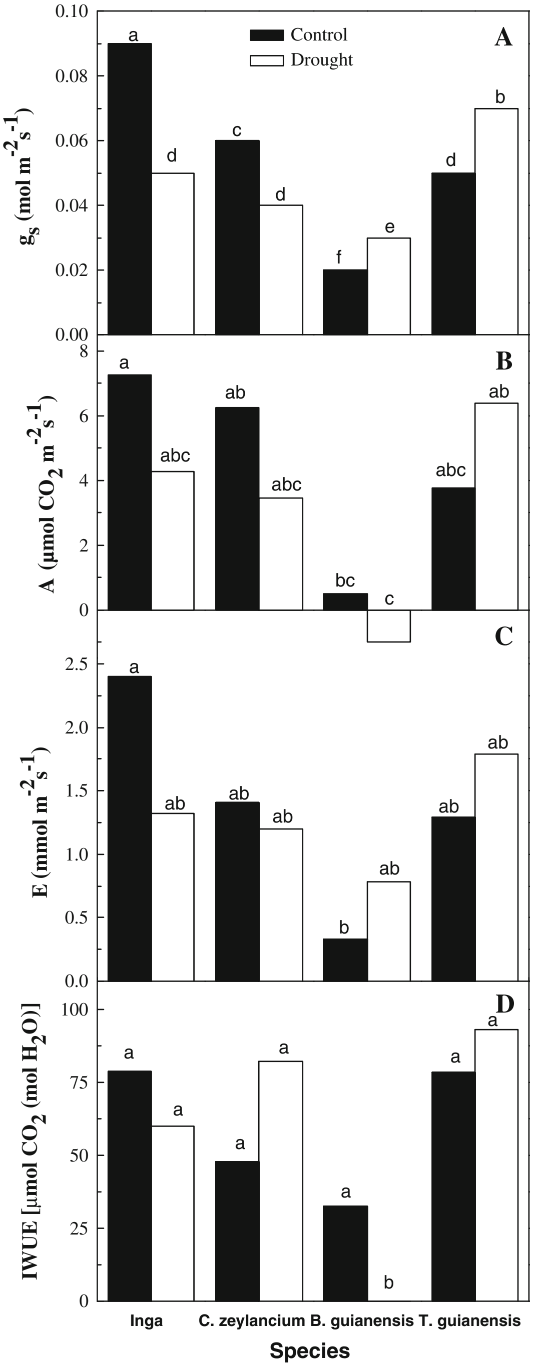

Fig. 3 Changes in stomatal conductance, g_s (**A**), net photosynthesis, A (**B**), transpiration, E (**C**) and intrinsic water use efficiency, IWUE (**D**) of young plants of tree species after 7 days of treatment: well hydrated (*control*) and water deficit (*drought*). Values followed by *different letters* in each column differ by test average Student–Neuman–Keuls ($p \leq 0.05$). Values represent the mean of three replications

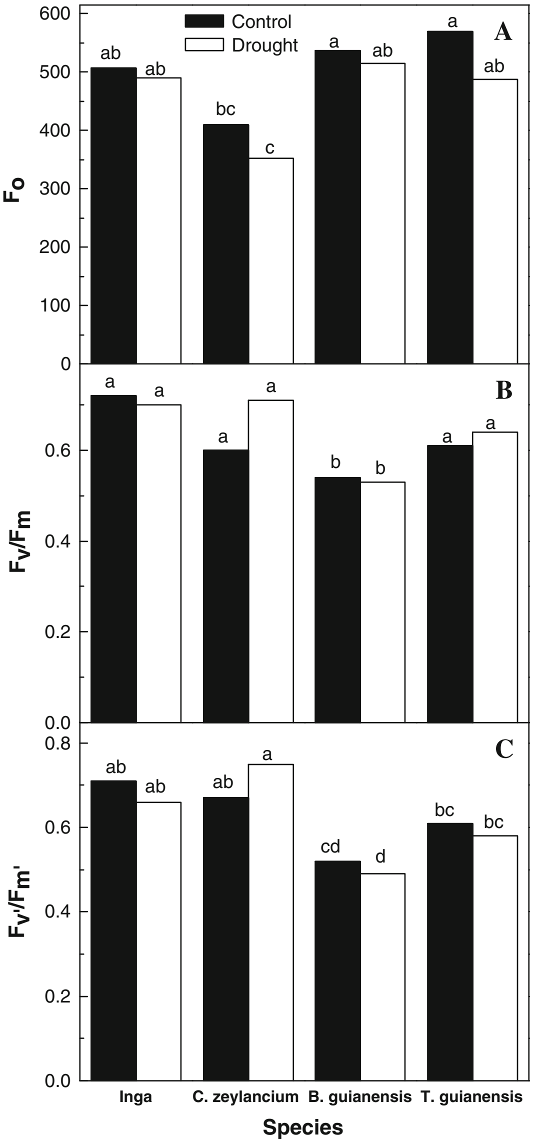

Fig. 4 Fluorescence parameters responses of young plants of tree species after 7 days of treatment: well-hydrated plants (*control*) and water deficit (*drought*). F_o fluorescence basal; F_v/F_m PSII maximum quantum yield; F'_v/F'_m excitation capture efficiency of open centers. Values followed by *different letters* in each column differ by test average Student–Neuman–Keuls ($p \leq 0.05$). Values represent the mean of three replications

Reforestation with high diversity is a chance to maintain or recover environmental functions, species and ecological interactions that occur or originally occurred in the northeastern Atlantic Forest. This is one way to definitively reduce the chances of extinction of several plant and animal species that are disappearing in this region and the world (Alves-Costa et al. 2008).

Among the species studied, two species can be characterized as clearly as pertaining to different functional groups: *Inga* sp., filling group; and *B. guianensis*, diversity group. Under the conditions determined in this study, *C. zeylancium* and *T. guianensis* showed characteristics of species of both groups, suggesting the possibility of their widespread use in reforestation programs.

Table 3 Leaf soluble sugars, amino acids and protein of young plants of tree species under water-deficit (drought) and well-hydrated conditions (control), after 7 days of treatment

Species	Treatment	Soluble sugars (μmol g^{-1} FW)		Amino acids (μmol g^{-1} FW)		Protein (mg g^{-1} FW)	
Inga sp.	Control	43	b	69	ab	0.53	b
	Drought	29	b	47	ab	0.73	b
C. zeylancium	Control	108	ab	136	ab	2.21	a
	Drought	136	ab	146	a	2.58	a
B. guianensis	Control	136	ab	91	ab	0.41	b
	Drought	210	a	80	ab	0.23	b
T. guianensis	Control	66	b	68	b	0.36	b
	Drought	60	b	17	ab	0.61	b

Values represent the mean of three replications. Values followed by different letters in each column differ by test average Student–Neuman–Keuls ($p \leq 0.05$)

Acknowledgments B. D. Souza is grateful to the FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) and B. M. Rodrigues is grateful to the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) for fellowships granted to them.

References

Alves-Costa CP, Lôbo D, Leão T, Brancalion PHS, Nave AG, Gandolfi S, Santos AMM, Rodrigues RR, Tabarelli M (2008) Implementando reflorestamentos com alta diversidade na zona da mata nordestina: Guia prático. Recife, 220 pp

Baker NR, Rosenqvist E (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J Exp Bot 55:1607–1621. doi:10.1093/jxb/erh196

Björkman O, Powles SB (1984) Inhibition of photosynthetic reaction under water stress: interaction with light level. Planta 161:490–504. doi:10.1007/BF00407081

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Brooks T, Balmford A (1996) Atlantic forest extinctions. Nature 380:115. doi:10.1038/380115a0

Brooks T, Rylands AB (2003) Species on the brink: critically endangered terrestrial vertebrates. In: Galindo-Leal C, Câmara IG (eds) The Atlantic Forest of South America: biodiversity status, threats and outlook. Center for Applied Biodiversity Science and Island Press, Washington, DC, pp 360–371

Chazdon RL, Pearcy RW, Lee DW, Fetcher N (1996) Photosynthetic responses of tropical forest plants to contrasting light environment. In: Mulkey SS, Chazdon RL, Smith AP (eds) Tropical forest plant ecophysiology. Chapman & Hall, London, pp 5–55

CI-Brasil (Conservation International do Brasil) (1993) Fundação Biodiversitas & Sociedade Nordestina de Ecologia. In: Workshop "Áreas prioritárias para a conservação da Mata Atlântica do Nordeste", Pernambuco 1993. Mapa de remanescentes, Belo Horizonte

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 29:350–356. doi:10.1021/ac60111a017

Ennajeh M, Vadel AM, Khemira H (2009) Osmoregulation and osmoprotection in the leaf cells of two olive cultivars subjected to severe water deficit. Acta Physiol Plant 31:711–721. doi:10.1007/s11738-009-0283-6

Gulías J, Cifre J, Jonasson S, Medrano H, Flexas J (2009) Seasonal and inter-annual variations of gas exchange in thirteen woody species along a climatic gradient in the Mediterranean island of Mallorca. Flora 204:169–181. doi:10.1016/j.flora.2008.01.011

Intergovernmental Panel on Climate Change (2007) http://www.ipcc.ch. Accessed 25 October 2007

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, San Diego

Lichtenthaler HK, Wellburn AR (1983) Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem Soc Trans 603:591

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:845–853. doi:10.1038/35002501

Ogaya R, Peñuelas J (2003) Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holy Oak forest. Flora 198:132–141. doi:10.1078/0367-2530-00085

Osmond CB, Winter K, Powles SB (1980) Adaptive significance of carbon dioxide cycling during photosynthesis in waterstressed plants. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. Wiley, New York, pp 139–154

Parker VT (1997) The scale of successional models and restoration objectives. Rest Ecol 5:301–306. doi:10.1046/j.1526-100X.1997.00031.x

Pimentel C, Laffray D, Louguet P (1999) Intrinsic water use efficiency at the pollination stage as a parameter for drought tolerance selection in *Phaseolus vulgaris*. Physiol Plant 106:184–198. doi:10.1034/j.1399-3054.1999.106206.x

Porto K, Cortez JA, Tabarelli M (2006) Diversidade biológica no Centro de Endemismo Pernambuco: sítios prioritários para a conservação. Coleção Biodiversidade. Ministério do Meio Ambiente, Brasília

Ribeiro RV, Santos MG, Souza GM, Ribeiro HL, Oliveira RF (2004) Gas exchange of two species from different successional status under greenhouse condition. Sci Forestalis 65:30–39

Rodrigues ASL, Akçakaya HR, Andelman SJ, Bakarr MI, Boitani L, Brooks TM, Chanson JS, Fishpool LDC, Fonseca GAB, Gaston KJ, Hoffmann M, Marquet PA, Pilgrim JD, Pressey RL, Schipper J, Sechrest W, Stuart SN, Underhill LG, Waller RW, Watts MEJ, Yan X (2004) Global gap analysis: priority regions for expanding the global protected-area network. Bioscience 54:1092–1100. doi:10.1641/0006-3568(2004)054[1092:GGAPRF]2.0.CO;2

Rodrigues RR, Lima RAF, Gandolfi S, Nave AG (2009) On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. Biol Conserv 142:1242–1251. doi:10.1016/j.biocon.2008.12.008

Santos MG, Pimentel C (2009) Daily balance of leaf sugars and amino acids as indicators of common bean (*Phaseolus vulgaris* L.) metabolic response and drought intensity. Physiol Mol Biol Plants 15:23–30. doi:10.1007/s12298-009-0002-1

Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze ED, Caldwell MM (eds) Ecophysiology of photosynthesis. Ecological studies, vol 100. Springer, Berlin, pp 49–70

Silva JMC, Casteleti CHM (2003) Status of the biodiversity of the Atlantic Forest of Brazil. In: Galindo-Leal C, Câmara IG (eds) The Atlantic Forest of South America: biodiversity status, threats and outlook. CABS & Island Press, Washington, DC, pp 43–59

Silva JMC, Tabarelli M (2000) Tree species impoverishment and the future flora of the Atlantic Forest of northeast Brazil. Nature 404:72–74. doi:10.1038/35003563

Souza GM, Ribeiro RV, Santos MG, Ribeiro HL, Oliveira RF (2004) Functional groups of Forest succession as dissipative structures: an applied study. Braz J Biol 64:707–718. doi:10.1590/S1519-69842004000400020

Swaine MD, Whitmore TC (1988) On the definition of ecological species groups in tropical rain forests. J Veg Sci 75:81–86

Van Kooten O, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosyn Res 25:147–150. doi:10.1007/BF00033156

Vincent G (2001) Leaf photosynthetic capacity and nitrogen concent adjustment to canopy openness in tropical forest tree seedling. J Trop Ecol 17:495–509. doi:10.1017/S0266467401001377

Yemm EW, Cocking EC (1955) The determination of amino-acids with ninhidrin. Analyst 80:209–213

Yordanov I, Tsonev T, Goltsev V, Kruleva L, Velikova V (1997) Interactive effect of water deficit and high temperature on photosynthesis of sunflower and maize plants. 1. Changes in parameters of chlorophyll fluorescence induction kinetics and fluorescence quenching. Photosynthetica 33:381–402