

RESEARCH ARTICLE

Photosynthetic performance in *Eucalyptus* clones cultivated in saline soil

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ABSTRACT

The aim of this study was to evaluate the photosynthetic performance of *Eucalyptus* clones with the aim of identifying their tolerance to soil saline stress conditions. The experiment was arranged in a randomized block design with a 3 × 4 factorial arrangement: in five replications, totaling 60 plots, three *Eucalyptus* clones were used, VC865, I224, and I144, which were exposed to four concentrations of NaCl (0, 1, 2 and 3 g NaCl kg⁻¹ of soil). Clone I144 shows mechanisms of salinity tolerance as smaller reductions in the stomatal conductance, transpiration, photosynthesis, photochemical efficiency of PSII, photosynthetic pigment content and total dry mass. On the other hand, clone I224 presents greater physiological damage, indicating high susceptibility to salt stress, while VC865 shows moderate sensitivity to salinity. Variables related to photosynthetic performance, such as gas exchange, photochemical efficiency of PSII and photosynthetic pigments are potentially reliable physiological indicators for selecting of tolerant *Eucalyptus* clones to salt stress.

Keywords: Forestry species; Hybrids; Physiology; Salt stress; Tolerance

INTRODUCTION

The genus *Eucalyptus* has been of increasing interest in the production of fast-growing and high-yield forest feedstock (Tonello and Teixeira Filho, 2011; Silva et al., 2014). Furthermore, *Eucalyptus* species that are managed as short-rotation crops for bioenergy are of increasing interest in many parts of the world (Albaugh et al., 2013). For decades *Eucalyptus* has been planted in Brazil (Neves et al., 2013), where it occupies of 5.7 million hectares of the area of total trees planted (IBÁ, 2017).

Energy demand has been increasing with population growth and the world economy, while at the same time there is a tendency for reduction in the petroleum products supply, resulting in a greater demand for new alternative energy sources that will replace fossil fuels (Vieira et al., 2014a). Among these, biomass energy has gained a prominent position (Goldemberg, 2009). Therefore, *Eucalyptus* wood

appears a good alternative of renewable energy, since Brazil has edaphoclimatic conditions favorable for the cultivation of this species.

The use of degraded areas considered unfit for agriculture may result in a greater number of planted forests, which could increase the industrial economy and the development of crops more resistant to abiotic stress (Lopes et al., 2015). In this context, the search for higher productivity in the forestry sector has encouraged research that identifies genetic material with greater production and greater adaptability to environmental variations (Vellini et al., 2008; Lopes et al., 2011; Harfouche et al., 2014).

Among these variations, salinized areas are increasing in all parts of the world as a consequence of irrigation practices, especially in arid and semi-arid regions. Semi-arid regions are characterized by a negative hydric balance, which requires the practice of irrigation, which in turn is

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oftentimes performed with water of inferior quality. This in itself results in the excessive accumulation of salts in the soil surface, mainly NaCl, that have harmful effects on plant growth and development (Pinheiro et al. 2008; Silva et al., 2012; Plazek et al., 2013; Brito et al., 2018).

Salinity is the main abiotic factor that limits the growth and productivity of crops worldwide (Plazek et al., 2013; Munns and Gilliam, 2015). The excess salts in the soil reduce the amount of available water for plants, leading to ion toxic effects on the metabolic processes, which in turn lead to rupture of membranes and organelles causing osmotic imbalance in the cells (Munns and Tester, 2008; Sá et al., 2015). Furthermore, besides the direct effects of salinity on plants, this causes the induction to water stress, because high concentrations of sodium increases osmotic concentration in soil, thereby inhibiting water absorption by the plant (Plazek et al., 2013).

The increase of solutes in the root zone promotes the water deficit which, consequently, causes an array of morphological, physiological and biochemical changes in plant yield (Prisco and Gomes Filho, 2010). In plants under drought stress, the stomatal closure helps reduce water loss from transpiration (Medeiros et al., 2013). Photochemical damage to photosystem II can be induced under such conditions. Consequently, there is a reduction in photosynthetic capacity and plant growth (Sengar et al., 2013; Kumar et al., 2014). Under these conditions, restriction on the entry of CO₂ occurs due to the relationship between stomatal conductance, transpiration, and photosynthesis (Brito et al., 2018).

In different *Eucalyptus* genotypes cultivated in saline solutions presenting an electrical conductivity (EC) of 7 dS m⁻¹ to 14 dS m⁻¹, decrease in photosynthesis, stomatal conductance, and biomass production were found (Sixto et al. 2016), while higher salt concentrations (EC 20 dS m⁻¹ to 40 dS m⁻¹) were lethal to the plants (Bhargava et al., 2014). Other studies involving different species such as *Ricinus communis* L. (Pinheiro et al., 2008) and *Jatropha curcas* L. (Cavalcante et al., 2018) also found reductions due to salinity of stomatal conductance, photosynthesis, photosynthetic pigments, maximum photochemical efficiency of photosystem II, and total dry matter.

Thus, a simple way to reduce the problem of soil salinity is to study the physiological behavior of the plants that are exposed to such conditions, in order to identify salt-tolerant genotypes (Munns, 2005; Ahmed et al., 2015). The most commonly used method to set the sensitivity to salinity of tree species is saline irrigation water (Campos et al., 2012; Hishida et al., 2014). However, studies on the physiological responses of *Eucalyptus* to soil salinity are still scarce.

Therefore, our goal was to evaluate the photosynthetic performance of *Eucalyptus* clones in order to identify their tolerance to soil saline stress conditions.

MATERIAL AND METHODS

Study area and experimental design

The experiment was conducted in a greenhouse in the Laboratory of Plant Physiology at the Federal University of Alagoas, located in the municipality of Rio Largo-AL (09 ° 28 'S, 35 ° 49' W, at an altitude of 127 m).

Three *Eucalyptus* clones were chosen due to their importance in the Alagoas State plantations. Thus, clones VC865, I224, and I144 were selected, which resulted from the hybridization process between *E. grandis* and *E. urophylla*, classified as tolerant, tolerant and moderately tolerant to drought, respectively (Muller et al., 2017).

The clones were cultivated in plastic pots containing 20 kg of sandy loam soil, which was salinized with NaCl addition on a dry weight basis at four different salt concentrations: 0 (control), 1, 2 and 3 g NaCl kg⁻¹ of soil. The material remained incubated and was maintained at field capacity for four months, increasing the electrical conductivity (EC) of the soil of 2 (0 g of NaCl kg⁻¹ of soil-control) for 6, 10 and 12 dS m⁻¹ at the concentrations of 1, 2 and 3 g of NaCl kg⁻¹ of soil, respectively. The EC of the soil was estimated according to Richards (1954).

The *Eucalyptus* seedlings were acquired in a commercial nursery, were produced in tubes by the cutting method, and were age of 120 days and homogeneous sizes. The seedlings were then transplanted into plastic bags with a soil capacity of 750 g, and stayed in the bags for 30 days, at which time they were transplanted into pots containing 20 kg of salinized soil. The soil was fertilized following the recommendation of Novais et al. (1991), for basic soil fertilization. Each pot was irrigated with distilled water on a daily basis during the entire plant growth period to keep rates close to field capacity. During this period, the drained water was collected and replaced by collectors present in the pot to maintain the EC of the soil.

The experiment design was randomized block design with factorial arrangement 3x4 (3 clones and 4 saline concentrations in the soil), with five replications totaling 60 plots, consisting of one plant per pot.

Environmental conditions were monitored during the experiment at an automatic weather station (model WSGP1; DELTA-T Devices, Cambridge, England) located inside the greenhouse. Temperature and relative air humidity mean were recorded every 5 min; solar radiation was measured

every 10 s. Vapor pressure deficit (VPD) was calculated based on temperature and relative humidity (Fig. 1).

Physiological analysis

The evaluations were made at 30 and 60 days after transplanting (DAT). Gas exchange, maximum photochemical efficiency of PSII (Fv/Fm), and relative index of chlorophyll (SPAD) were performed on completely expanded leaves of the middle third of the plants, after which, at 60 DAT, photosynthetic pigments were measured from leaves collected from the same plants in which the gas exchange was evaluated.

Gas exchange measurements were performed with a gas analyzer model Li-6400XT (Infra Red Gas Analyzer - IRGA, Licor, USA) in the period between 8 am and 10 am. The variables stomatal conductance (g_s), transpiration (E), internal CO_2 concentration (C_i), liquid photosynthesis (A) and leaf temperature (LT) were obtained. The vapor pressure deficit between the leaf and the air, VPD (leaf-air) and instantaneous carboxylation efficiency (A/C_i) correlated liquid photosynthesis with the internal CO_2 concentration.

The measurements of the maximum quantum efficiency of PSII (Fv/Fm) were made at predawn (4:30 am) and at midday (12:00 pm) using a portable modulated light fluorometer (PAM 2500, WALZ), according to method described by Maxwell and Johnson (2000). Completely expanded leaves of the middle third of the plants were adapted to the dark for 30 min with the aid of special leaf clips placed in the intermediate part of the leaf.

The estimated chlorophyll content, the SPAD index, was performed by a portable device model SPAD-502 (Minolta, Japan), and averages obtained from five readings were measured on fully expanded leaves of the middle third of the plant.

The analysis of chlorophylls *a*, *b*, total and carotenoids was made according to the method described by Lichtenthaler (1987), where the extract was obtained using five leaf disks with 0.6 cm^2 that were immersed in threaded test tubes protected from light with aluminum foil and containing 5 mL of 80% acetone for 72 h with regular shaking. The reading of absorbance was subsequently taken by a spectrophotometer at wavelengths of 480, 645 and 663 nm.

At the end of the experiment, plants were collected after drying in a forced air circulation oven for 72 h at 65°C until constant weight, obtaining the total dry mass (TDM).

Statistical analysis

The experimental data were submitted to the analysis of variance (F test), the soil salinity factor being analyzed by means of polynomial regression and the clone factor being analyzed by the Tukey test, with $P < 0.05$. Pearson's (r) correlation was used to verify the relationship between the physiological variables analyzed within each saline condition.

RESULTS

Physiological analysis

Gas exchange variables were affected by soil salinity in the *Eucalyptus* clones, both at 30 and 60 days after transplanting (DAT). The foliar temperature (LT) at 30 DAT increased in all clones studied with the increase of soil salinity (Fig. 2A). In clones VC865, I224 and I144 increased leaf temperature at very high salt concentrations (3 g NaCl kg^{-1} of soil), when compared with control, in order of 3.32%, 3.97% and 2.84%, respectively (Fig. 2A). It was observed at 60 DAT that clone VC865 maintained the leaf temperature constant, while concentrations of I224 and I144 increased by 2.63% and 2.59%, respectively, for the highest concentration of NaCl in the soil (Fig. 2B).

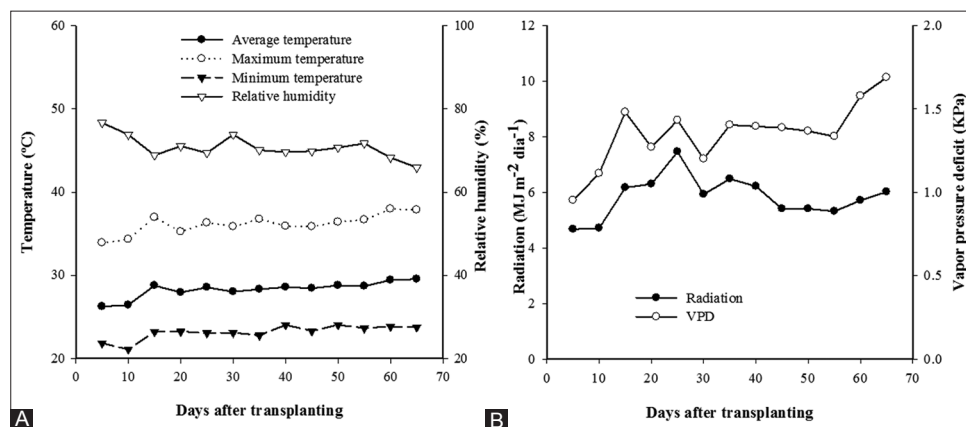


Fig 1. Maximum, minimum and average air temperatures and mean relative humidity (A), mean incident radiation and vapor pressure deficit (B) inside the greenhouse during the period of salt stress.

Similar behavior was observed for leaf-to-air vapor pressure deficit (VPD leaf-air), where at 30 DAT the clones VC865, I224 and I144 showed increases of 21.56%, 16.70% and 27.69%, respectively, when they were compared with control plants for the highest salinity level of 3 g NaCl kg⁻¹ of soil (Fig. 2C). Already at 60 DAT, clones VC865, I224, and I144 presented more moderate increases of DPV (leaf-air) of 7.05%, 7.50% and 14.06%, respectively, in function of the salinity (Fig. 2D). At 60 DAT, the DPV (leaf-air) showed an inverse correlation with the transpiration in all saline conditions studied (Table 1). Besides that, in the same period, the VPD (leaf-air) presented an inverse correlation with g_s in the plants traded with the saline concentrations of 1 and 3 g NaCl kg⁻¹ of soil.

The clones presented reduction of stomatal conductance and transpiration with the increase of salt concentration in the soil in both evaluated periods. At 30 DAT, clones VC865, I224, and I144 observed reductions of g_s of 30.33%, 42.76%, and 46.59%, respectively, with increasing soil salinity up to concentration of 3 g NaCl kg⁻¹ of soil (Fig. 3A). Already at 60 DAT, clone I144 increased the stomatal conductance up to the value of 1.67 g NaCl kg⁻¹ of soil, presenting a lower stomatal closure at the concentration of 3 g NaCl kg⁻¹ of soil when compared with the other clones. By contrast, clones VC865 and I224 showed decreases of g_s of 38.26% and 40.52%, respectively, at the highest level of soil salinity (Fig. 3B).

Regarding transpiration, it was observed that at 30 DAT, the clones VC865, I224 and I144 presented decreases of 20.51%, 33.39% and 49.68%, respectively, as the concentration of NaCl in the soil was increased (Fig. 3C). At 60 DAT, there was increase of E in clone I144 with increase of salinity up to 1.67 g NaCl kg⁻¹ of soil, decreasing from this concentration, similarly to g_s . On the other hand, clones VC865 and I224 showed no changes in this evaluation period in E with the increase of soil salinity (Fig. 3D). Between zero concentration and 2 g NaCl kg⁻¹ of soil, a positive correlation of g_s and E (Table 1) was found.

With the increase of soil salinity, the internal CO₂ concentration at 30 DAT increased at 8.08% and 09.65%, respectively, in clones VC865 and I144, with the increase of soil salinity, while clone I224 was not affected by salinity (Fig. 4A). Already at 60 DAT, clones VC865, I224, and I144 under maximum salt stress showed a more significant increase of C_i of the order of 16.62%, 19.77% and 12.96%, respectively (Fig. 4B).

Similar to g_s and E , the photosynthetic rate of clones decreased with increasing soil salinity. Clones VC865, I224 and I144 at 30 DAT presented reductions of 16.58%, 22.76% and 33.36%, respectively (Fig. 4C). Already at 60 DAT, clone I144 showed an increase in the photosynthetic rates in the concentrations of moderate salt stress (up to 0.96 g NaCl kg⁻¹ of soil), but higher levels of salt concentration caused their decrease. On the other hand,

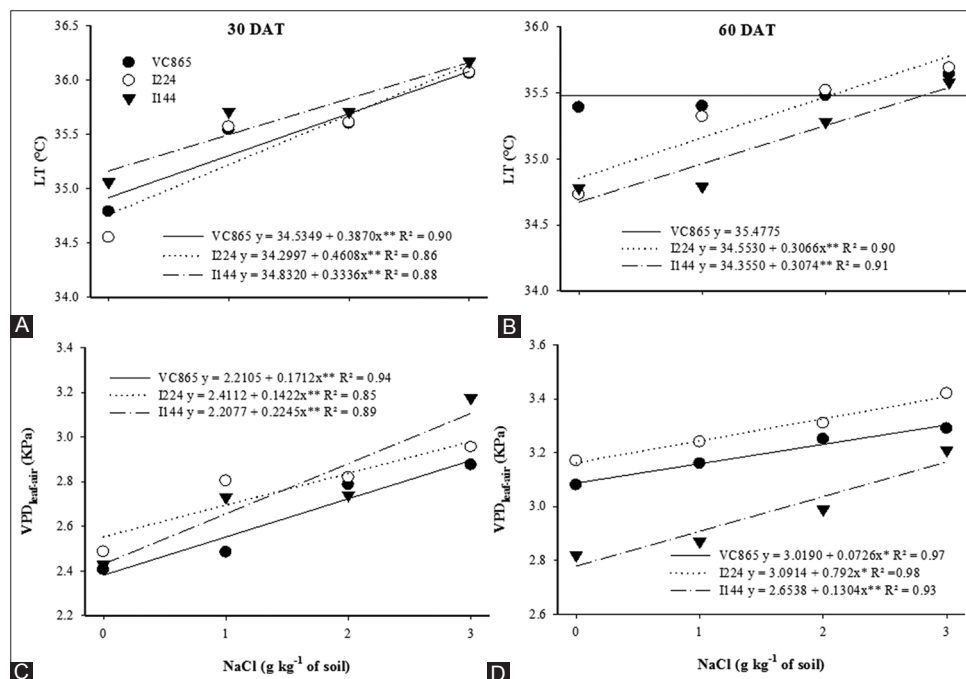


Fig 2. Leaf temperature - LT (A and B) and leaf-to-air vapor pressure deficit - VPD (air-leaf) (C and D) at 30 and 60 days after transplanting in *Eucalyptus* clones grown at different soil salinity levels.

Table 1: Pearson correlation between physiological characteristics at 60 days after transplanting in *Eucalyptus* clones cultivated at different soil salinity levels

S	Var	VPD	gs	E	Ci	A	A/Ci	Fv/Fm	Chl a	Chl b	Chl T	Car	TDM
0	LT	0,12	-0,26	-0,08	0,10	0,24	0,24	-0,51	-0,26	-0,07	-0,24	-0,32	-0,69*
	VPD		-0,69*	-0,90*	-0,19	-0,62*	-0,61*	0,20	-0,21	0,41	0,08	-0,63*	-0,28
	gs			0,75*	-0,06	0,75*	0,78*	-0,20	0,15	-0,50	-0,18	0,57*	0,49
	E				0,04	0,69*	0,72*	-0,24	0,11	-0,29	-0,09	0,46	0,30
	Ci					0,33	0,20	-0,11	0,03	0,21	0,15	0,00	-0,39
	A						0,98*	-0,35	0,03	-0,26	-0,12	0,41	-0,09
	A/Ci							-0,33	0,02	-0,28	-0,15	0,41	-0,06
	Fv/Fm								0,23	0,48	0,46	-0,01	0,05
	Chl a									0,10	0,80*	0,55*	0,15
	Chl b										0,67*	-0,41	-0,40
	Chl T											0,16	-0,12
	Car												0,23
1	LT	0,68*	-0,75*	-0,73*	-0,58*	-0,70*	-0,65*	-0,44	-0,42	0,15	-0,28	-0,39	-0,50
	VPD		-0,86*	-0,68*	-0,61*	-0,70*	-0,67*	-0,51*	0,05	0,53*	0,21	-0,21	-0,19
	gs			0,86*	0,62*	0,84*	0,82*	0,61*	0,04	-0,38	-0,08	0,47	0,43
	E				0,62*	0,98*	0,97*	0,63*	0,12	-0,05	0,08	0,57*	0,43
	Ci					0,66*	0,54*	0,76*	0,03	-0,25	-0,05	0,23	0,24
	A						0,98*	0,63*	0,00	-0,12	-0,03	0,45	0,31
	A/Ci							0,56*	-0,00	-0,08	-0,03	0,46	0,29
	Fv/Fm								0,23	0,01	0,19	0,57*	0,48
	Chl a									0,57*	0,96*	0,52*	0,60*
	Chl b										0,76*	0,27	0,21
	Chl T											0,49	0,54*
	Car												0,89*
2	LT	0,45	-0,36	-0,38	0,18	-0,42	-0,42	-0,10	-0,29	-0,31	-0,31	-0,46	-0,68*
	VPD		-0,50	-0,77*	0,37	-0,54*	-0,54*	-0,55*	-0,52*	-0,39	-0,51	-0,62*	-0,66*
	gs			0,67*	-0,10	0,65*	0,62*	0,58*	0,20	0,26	0,22	0,78*	0,60*
	E				-0,29	0,76*	0,74*	0,64*	0,21	0,21	0,22	0,72*	0,52*
	Ci					-0,44	-0,55*	-0,30	-0,33	-0,25	-0,32	-0,31	-0,36
	A						0,99*	0,66*	0,35	0,38	0,38	0,74*	0,62*
	A/Ci							0,65*	0,37	0,39	0,40	0,74*	0,62*
	Fv/Fm								0,09	0,21	0,13	0,74*	0,53*
	Chl a									0,78*	0,98*	0,30	0,62*
	Chl b										0,88*	0,34	0,61*
	Chl T											0,33	0,65*
	Car												0,83*
3	LT	0,44	-0,23	-0,21	-0,05	-0,13	-0,12	0,02	-0,34	0,20	-0,17	-0,08	-0,06
	VPD		-0,69*	-0,53*	0,04	-0,59*	-0,57*	-0,27	-0,48	-0,12	-0,41	-0,27	-0,56*
	gs			0,42	-0,29	0,79*	0,78*	0,53*	0,43	0,32	0,45	0,57*	0,77*
	E				0,02	0,38	0,35	0,10	0,06	-0,20	-0,03	0,13	0,46
	Ci					-0,45	-0,52*	-0,35	0,14	-0,29	-0,01	-0,35	-0,39
	A						0,99*	0,62*	0,43	0,35	0,46	0,64*	0,89*
	A/Ci							0,63*	0,41	0,36	0,45	0,65*	0,89*
	Fv/Fm								0,57*	0,68*	0,70*	0,78*	0,76*
	Chl a									0,45	0,93*	0,67*	0,58*
	Chl b										0,75*	0,49	0,42
	Chl T											0,70*	0,60*
	Car												0,83*

S: soil salinity; Var: variables; LT: leaf temperature; VPD_(air-leaf): Leaf-air vapor pressure deficit; gs: stomatal conductance, E: perspiration; Ci: internal CO₂ concentration; A: photosynthesis; EiC: carboxylation efficiency; Fv/Fm: Maximum PSII efficiency at noon; Chl a: chlorophyll a; Chl b: Chlorophyll b; Chl t: total chlorophyll; Car: carotenoids; TDM: total dry mass. *significant at P<0.05.

A was progressively reduced by salinity in clones VC865 and I224 at 23.47% and 32.10%, respectively (Fig. 4D). With increase of the salinity of the soil until concentration

2 g NaCl kg⁻¹ of soil, there was a positive correlation of A with gs and E, but no correlation was observed at the highest salinity level (Table 1).

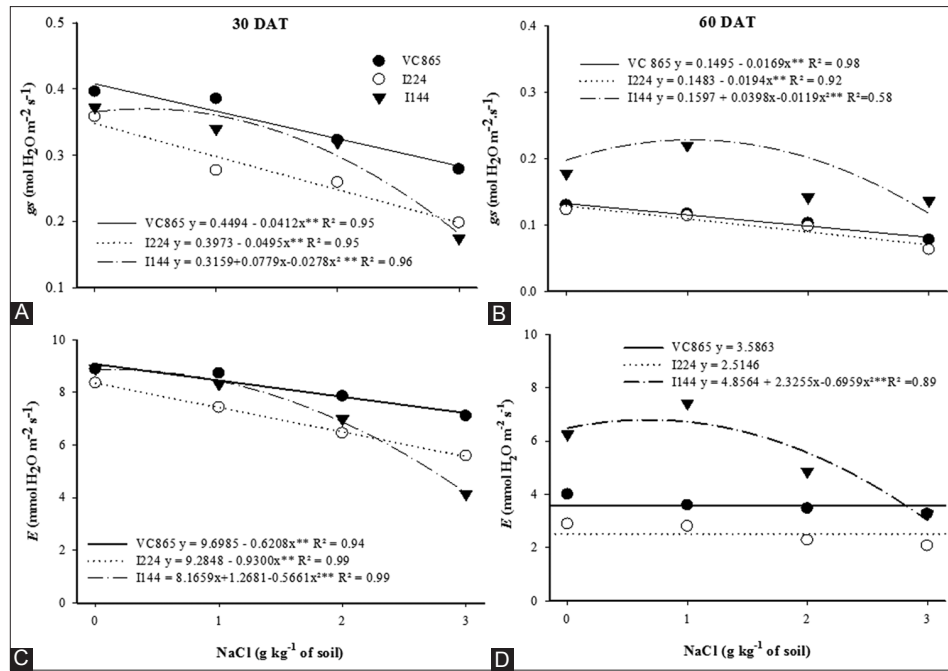


Fig 3. Stomatal conductance - g_s (A and B) and transpiration - E (C and D) at 30 and 60 days after transplanting in *Eucalyptus* clones grown at different soil salinity levels.

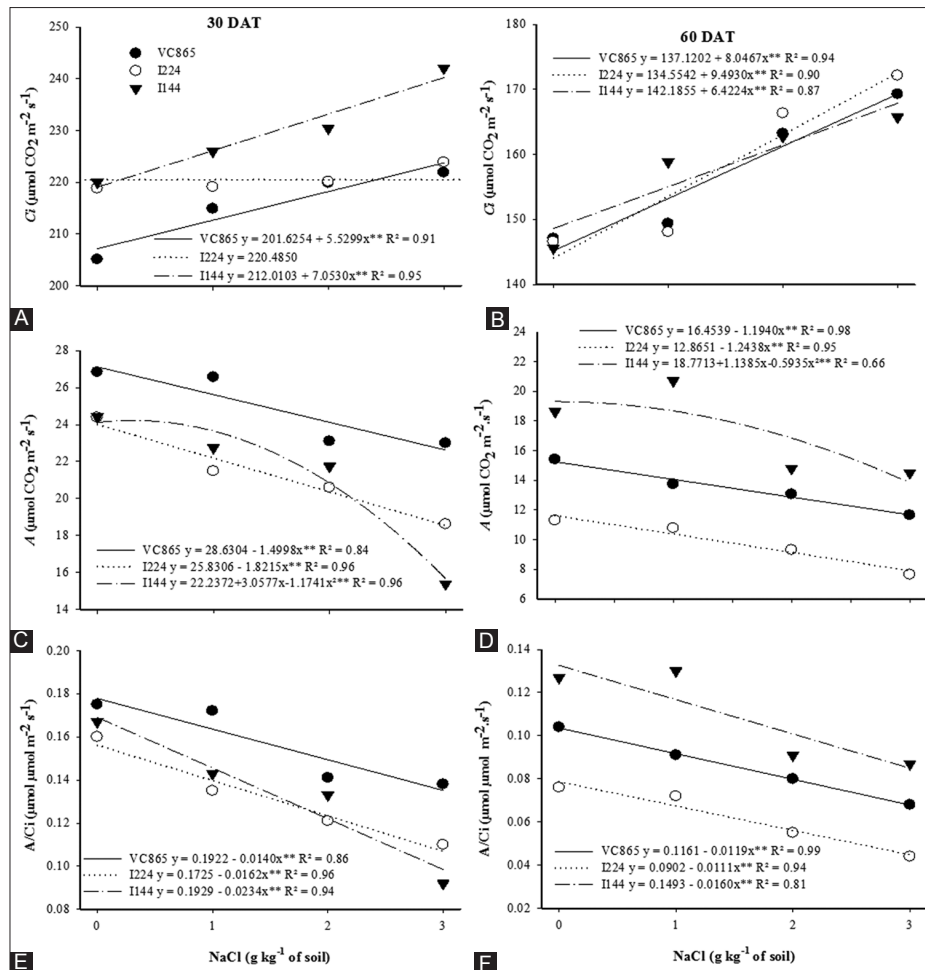


Fig 4. Internal CO₂ concentration - C_i (A and B), photosynthesis - A (C and D) and Efficiency of carboxylation - A/C_i (E and F) at 30 and 60 days after transplanting in *Eucalyptus* clones grown at different salinity levels.

The carboxylation efficiency (A/C_i) was reduced in all clones with increasing soil salinity, in both studied periods. Clones VC865, I224 and I144 at 30 DAT showed decreases of A/C_i of 23.68%, 31.15% and 41.41%, respectively (Fig. 4E), while at 60 DAT, clones VC865, I224 and I144 showed decreases of 34.35%, 42.35%, and 33.15%, respectively (Fig. 4F). A/C_i showed a strong positive correlation with A at all levels of soil salinity (Table 1).

The maximum quantum efficiency of PSII (F_v/F_m) in the *Eucalyptus* clones at predawn reduced as the soil salinity increased, both at 30 and 60 DAT, being the case that, in the first evaluation period, clones VC865, I224 and I144 showed decreases of 1.13%, 1.12% and 0.87%, respectively, from the lowest to the highest level of soil salinity (Fig. 5A), while at 60 DAT, these decreases in clones VC865, I224 and I144 were 0.98%, 1.92% and 0.77%, respectively (Fig. 5B).

At midday, the decreases of F_v/F_m in the *Eucalyptus* clones that were caused by salinity, were slightly larger. Clones VC865, I224, and I144 at 30 DAT showed a reduction of the order of 3.49%, 4.38% and 2.86%, respectively (Fig. 5C). Already at 60 DAT, the decreases were 2.58%, 4.27% and 2.14% for the respective clones (Fig. 5D).

It can also be verified that clone I144 presented higher values at the 60 DAT in the F_v/F_m ratio, of 0.826 at 4:30 am and 0.788 at 12:00 pm, when compared with the other clones in the higher salt concentration level. The F_v/F_m showed a positive correlation with Chl *a*, Chl *b*, Chl *T* and Car in plants at 3 g NaCl kg^{-1} of soil (Table 1).

The estimated chlorophyll content (SPAD index) in clones VC865, I224 and I144 at 30 DAT reduced with increasing saline concentration in the soil. If there are decreases of 12.12%, 14.04% and 7.79%, respectively, comparing the

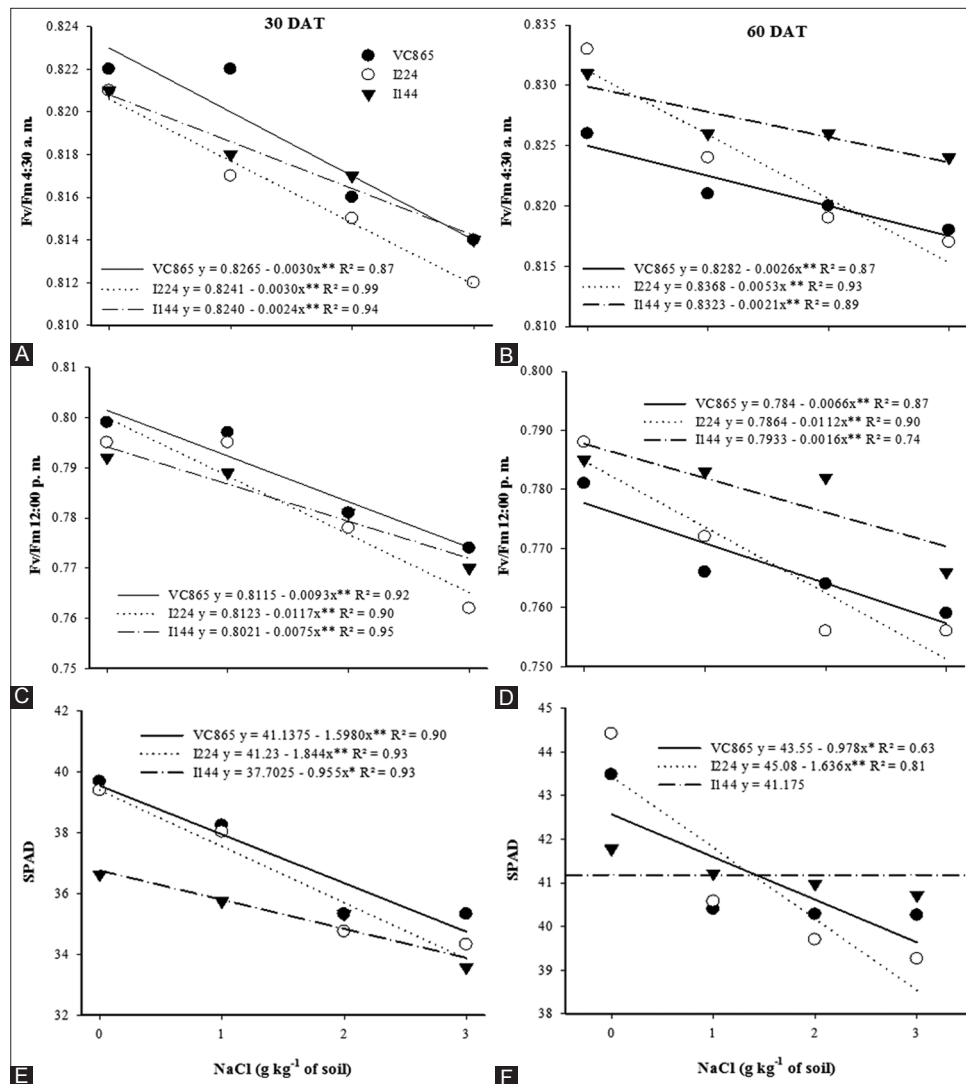


Fig 5. Maximum photochemical efficiency of PSII - F_v/F_m , at predawn (A and B) and midday (C and D) and Chlorophyll estimated content (SPAD index) (E and F) at 30 and 60 days after transplanting in clones of *Eucalyptus* cultivated at different soil salinity levels.

lowest with the highest salinity (Fig. 5E). At 60 DAT, the salinity caused decreases of SPAD in VC865 and I224 of 6.89% and 11.29%, respectively, while I144 was not affected (Fig. 5F).

Photosynthetic pigments, determined only at 60 DAT, were affected by soil salinity, which varied among the clones. Chlorophyll *a* reduced 33.74% in I224, from the lowest to the highest level of soil salinity, while the remaining clones were not affected (Fig. 6A). The chlorophyll content *b* in clones VC865 and I224 decreased to 25.85% and 43.80%, respectively, from the lowest to the highest salinity level, while it remained stable at clone I144 (Fig. 6B), which also presented little variation with increased salinity in the contents of Chl T. However, there were reductions in clones VC865 and I224 of 19.56% and 36.50%, respectively (Fig. 6C).

The carotenoids reduced with the increase of the salinity in VC865, I224 and I144, in the order of 17.19%, 28.74% and 12.58%, respectively (Fig. 6D). Besides that, it may be noted that clone I144 presented higher levels of Chl T and carotenoids when compared with the other clones in the highest concentration of salinity in the soil. There was high correlation between chlorophylls *a* and *b* with Chl T on all saline levels (Table 1). In the same way, positive correlation between Chl T and carotenoids in the concentration of 3 g NaCl kg⁻¹ of soil can be observed.

Total dry mass (TDM) was also affected by increased soil salinity in all clones (Fig. 7). Reductions were observed in

VC865, I224 and I144 of 70.71%, 75.38% and 69.68%, respectively. This variable presented an inverse correlation with the VPD (leaf-air), as well as positive correlation with *g_s*, *A*, *EiC*, *Fv/Fm*, Chl *a*, Chl T and carotenoids in the two largest saline concentrations in the soil.

DISCUSSION

In this study, we verified increases of VPD (leaf-air) and foliar temperature with the increase from soil salinity, in particular in clone I224. The lowest rates of VPD (leaf-air) and LT were observed in clone I144, indicating possible acclimatization of these plants to saline stress. According to Vieira et al. (2014b), leaf temperature reduces the extent to which transpiration increases in the function of energy dissipation in the form of latent heat, contributing to leaf cooling.

Besides that, the *Eucalyptus* clones submitted to salt stress showed an inverse correlation between VPD (leaf-air) with stomatal conductance and transpiration. In that the increase of the VPD (leaf-air) possibly influenced in the stomatal closure and the reduction of the transpiration probably due to the low water availability present in the saline soil (Pinheiro et al., 2008; Santos et al., 2017).

In the most C3 species, high values of VPD (leaf-air) are related to stomatal closure as well as high values of VPD (leaf-air) reducing transpiration, especially when developed in environments with low soil water availability (Santos et al., 2017).

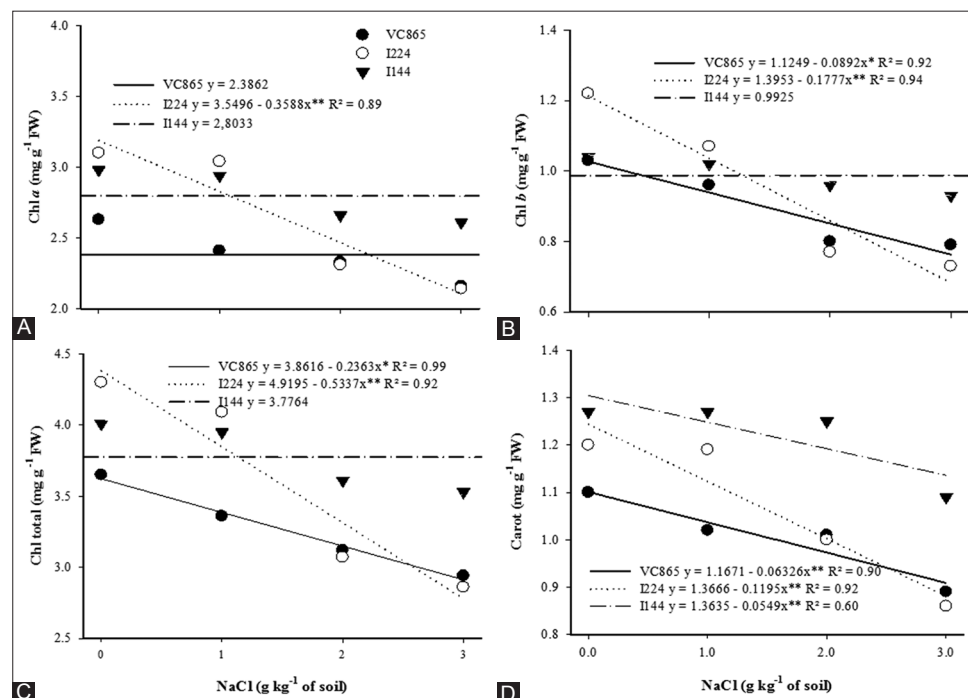


Fig 6. Chlorophyll *a* (A), *b* (B), total (C) and carotenoids (D) at 60 days after transplanting in *Eucalyptus* clones grown at different soil salinity levels.

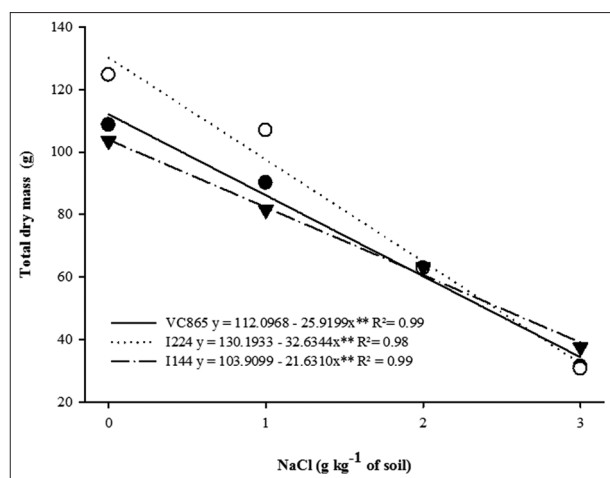


Fig 7. Total dry mass (TDM) at 60 days after transplanting in *Eucalyptus* clones grown at different soil salinity levels.

The positive correlation between stomatal conductance, transpiration, and photosynthesis that was found among the clones in this study confirms that the plants were under water stress in response to high soil salinity, which led to the partial stomata closure, decreased transpiration and led to sharp decline in photosynthetic rates. Salt stress is usually associated with water stress, since high concentrations of sodium in soil promote the restriction of water absorption by plants (Plazek et al., 2013). Under these conditions, a reduction in stomata opening occurs in order to avoid excessive loss of water from plant leaves, thus restricting their photosynthetic capacity by reduction in CO_2 acquisition (Coscolin et al., 2011; Kumar et al., 2014).

The photosynthetic responses of plants to salinity are complex, since these are dependent on the time and salt concentration to which they are subjected (Chaves et al., 2009). As was observed in this study, clone I144 increased its stomatal conductance, transpiration and photosynthesis when exposed to low salinity ($1.67 \text{ g NaCl kg}^{-1}$ of soil), mainly at 60 DAT, whereas the same clone showed superior photosynthetic performance to the other clones, although it was also reduced with increasing soil salinity. This condition probably indicates the possible acclimatization of I144 to salt stress, since there were more pronounced reductions of g_s , E and A , in the other clones, notably in I224, suggesting that this clone is more sensitive to salt stress.

Bhargava et al. (2014) and Sixto et al. (2016), in working with *Eucalyptus* under salt stress, observed that the salinity promoted the reduction of gas exchange in plants. In a similar way, studies done with *Anacardium occidentale* (Bezerra et al., 2003), *Ricinus communis* L. (Pinheiro et al., 2008), and *Jatropha curcas* L. (Cavalcante et al., 2018) cultivated in saline soil have also confirmed that gas exchanges are negatively affected by salinity, as observed among *Eucalyptus* clones.

Reductions in gas exchange, caused by salt stress, contributed to reduction in biomass production, which can be confirmed by the correlations of g_s , A and A/C_i with the TDM, in the largest salt concentrations. Indeed, biomass production is reduced as a consequence of less CO_2 assimilation, as has been verified also in *Eucalyptus* plants (Sixto et al. 2016) and in *Jatropha curcas* L. (Cavalcante et al. 2018) under salt stress.

Increase in the internal CO_2 concentration due to increased salt stress in the *Eucalyptus* clones is possibly related to the non-stomatal factors such as reduction of rubisco activity and concentration and reduction in the final products of the photochemical phase of photosynthesis, ATP and NADPH (ZIVCAK et al., 2013). In this stress situation, the plant cannot consume saturated CO_2 once reduction of the internal CO_2 concentration with decreases in stomatal conductance is expected, if the photosynthetic apparatus is not damaged by salinity (Kalaji et al., 2011).

The carboxylation efficiency reduced according to the fall of photosynthesis in *Eucalyptus* clones under high concentrations of salinity, as verified in the positive correlation between these variables and highlighting clone I224, that presented more reductions of A/C_i and A . The reduction of A/C_i means the occurrence of metabolic damage, which may be related to decrease in activity of the enzyme Rubisco, involved in the CO_2 fixation process, and other enzymes related to photosynthesis (Ashraf and Harris, 2013). However, the clone I144 was more efficient in carboxylation, in other words, that was what lower losses in fixing CO_2 by salt stress.

F_v/F_m decreased in all clones with increased soil salinity, however, these reductions were expressively small. Stressed plants had F_v/F_m values higher than 0.75, suggesting that the photosynthetic apparatus was not damaged by the salinity of the soil, since the variation of F_v/F_m between 0.75 and 0.85 indicates the integrity of the photosynthetic apparatus, with absence of photoinhibitory damage in photosystem II (Bolh ar-Nordenkamp et al., 1989). However, clone I144 stood out with the highest values of F_v/F_m , which suggests for it to be more tolerant to soil salinity, indicated by the greater integrity of the photosynthetic apparatus in this stress situation.

In a study with barley cultivars under saline conditions, the F_v/F_m ratio decreased with increasing salinity, with lower intensity in the more tolerant cultivar (Kalaji et al., 2011), also found in *Jatropha curcas* in which soil salinity reduced F_v/F_m , especially in the hottest hours of the day (Cavalcante et al., 2018). In this sense, the maximum photochemical efficiency of PSII is quite efficient as an indicator of disturbances in the photosynthetic apparatus

caused by soil salinity (Kalaji et al., 2011; Cavalcante et al., 2018), since low values of Fv/Fm are considered a stress sensitivity response (Sá et al., 2018).

The *Eucalyptus* clones presented reduction in the SPAD index and in photosynthetic pigments as a negative effect of soil salinity. However, clone I144 was less affected, because it was able to maintain higher levels of chlorophyll even under salt stress, indicating greater tolerance to this stress condition. On the other hand, clones VC865 and I224 presented decreases of chlorophylls and SPAD index with the increase of NaCl in the soil. These results can be attributed to the ionic effect of Na, which promotes deficiency of some nutrients essential for the metabolism of chlorophyll (Sá et al., 2015).

Chlorophyll degradation also indicates photochemical disturbances in the light collecting complex and in the PSII reaction centers caused by salinity (Bhargava et al., 2014; Cavalcante et al., 2018), which may have occurred in clones VC865 and I224. The reduction in chlorophyll levels caused by stress becomes one of the main limiting factors of photosynthetic activity by directly reflecting the maximum efficiency of PSII (Santos and Silva, 2015), as observed in *Jatropha curcas*, in which the salinity of the soil reduced the levels of chlorophyll, as well as reducing photosynthesis and the maximum efficiency of photosystem II (Cavalcante et al., 2018). Therefore, chlorophylls have often been used to identify damages in the photosynthetic system of plants caused by soil salinity, being that its degradation is an indication of blocking or reduction of photochemical activity (Santos and Silva, 2015; Cavalcante et al., 2018).

Clone I144 also showed lower reduction of carotenoid content in relation to VC865 and I224 with the increase of the salinity of the soil, indicating that the higher stability of Fv/Fm in that clone may be due to photoprotection promoted by carotenoids, as was observed by the correlation between these variables. It's emphasized that the degradation of these pigments interferes in the photoprotection exerted by the plant, increasing the chances of photo-oxidation under stress conditions (Silva et al., 2014; Santos and Silva, 2015), therefore, plants that maintain carotenoids are more tolerant to stress. Besides that, the reduction of pigments along with PSII disorders, by the reduction of Fv/Fm, leads to a decrease in TDM, considering the correlations between these variables, especially when the plants were stressed.

CONCLUSIONS

In conclusion, clone I144 presents mechanisms of soil salinity tolerance, such as reduction of stomatal

conductance, transpiration, photosynthesis, photochemical efficiency of PSII, photosynthetic pigment contents and TDM. On the other hand, clone I224 presents greater physiological damage, which indicates greater sensitivity to salt stress, while clone VC865 demonstrates moderate sensitivity. Variables related to photosynthetic performance, such as gas exchanges, photochemical efficiency of PSII and photosynthetic pigments are important as criteria of tolerance to salt stress, and can be used to identify *Eucalyptus* clones tolerant to this condition.

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Author's contributions

All authors contributed extensively to the work presented in this article. J.R.A. and S.O.M.J.: designed and performed research, wrote and revised the manuscript. C.M.S., V.M.F. and L.E.: contributed to revise the manuscript. A.F.S.S., V.M.S., L.T.B. and J.R.R.S.: carried out the experiments.

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