UNIVERSIDADE DE SÃO PAULO
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Integrated effects of K and Na application on initial growth of eucalyptus seedlings submitted to water stress: water use efficiency, leaf gas exchange and $\delta^{13}C$ isotopic variation

Piracicaba

2019

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Revised version according to Resolution CoPGr 6018 at 2011

Dissertação apresentada ao Centro de Energia Nuclear na Agricultura da Universidade de São Paulo para obtenção do título de Mestre em Ciências

Área de Concentração: Energia Nuclear na Agricultura e no Ambiente

Orientador: Prof. Dr. José Lavres Junior

Piracicaba

2019

AKNOWLEDGMENTS

"Pensar pequeno ou pensar grande dá o mesmo trabalho"

Jorge Leman

ABSTRACT

MATEUS, N. S. **Integrated effects of K and Na application on initial growth of eucalyptus seedlings submitted to water stress: water use efficiency, leaf gas exchange and δ^{13}C isotopic variation.** 2019. 76 p. Dissertação (Mestrado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2019.

In Brazil, fertilizer application is one of the main factors for achieving high productivity in commercial *Eucalyptus* plantations, since it is frequently carried out in soils with low water availability and low chemical fertility. Potassium (K) is the most required macronutrients by the *Eucalyptus* plant, while sodium (Na), can partially substitute some physiological functions of K, influencing directly the plants water use efficiency (WUE). Thus, the present study aimed to evaluate, in a greenhouse conditions, the nutritional and physiological responses of three eucalypt genotypes contrasting to drought tolerance under combined effects of different water regimes and partial replacement of K by Na. Plant growth, nutrients concentration, gas exchange parameters (A-CO_2 assimilation rate, g_s-stomatal conductance, E-transpiration, WUE), chlorohpyl content, absorption and use efficiency (AE and UE, respectively), stomatal density in adaxial and abaxial surfaces (Std_{AD} and Std_{AB}), leaf water potential (Ψw), leaf area (LA) and leaf carbon isotope composition (δ^{13}C ‰) were measured. The low replacement of K by Na improved total dry matter (TDM) of *Eucalyptus* under different water supply conditions, except for root dry matter (RDM), which decreased with any Na supply. Besides that, there was a higher potential of replacement according to raised drought tolerance levels among genotypes. Water supply was the most limiting factor for the growth, characterizing the plants under drought by lower TDM, LA, Std, A, E, g_s, Ψ_{WN} and UE_K, despite higher δ^{13}C and long time water use efficiency (WUE_L). In general, plants supplied with low Na rate (up to 50 % of K-replacement) reduced the critical level of K without showing symptoms of K deficiency and providing higher TDM than those *Eucalyptus* plants supplied only with K. Also expressed the ability to improve CO_2 assimilation, Std, UE_K and WUE, maintaining leaf turgidity by reducing Ψw_N, with stomata partially closed, indicated by the higher values of leaf carbon isotope composition (δ^{13}C ‰). The exclusive Na supply, which leads to K-deficient plants, besides the lower TDM, are mainly characterized by lower values of δ^{13}C, WUE_L, leaf K content and higher leaf Na content.

Keywords: abiotic stress, forest nutrition, CO_2 assimilation rate, drought tolerance.

RESUMO

MATEUS, N. S. **Efeitos integrados do suprimento de K e Na no crescimento inicial de mudas de eucalipto submetidas ao estresse hídrico: eficiência do uso da água, nas trocas gasosas e na variação isotópica de δ^{13}C**. 2019. 76 p. Dissertação (Mestrado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2019.

No Brasil, a aplicação de fertilizantes é um dos principais fatores para alcançar alta produtividade em plantações comerciais de *Eucalyptus*, realizadas frequentemente em solos com déficit hídrico e baixa fertilidade química. O potássio (K) é o macronutriente mais exigido pela planta de *Eucalyptus*, enquanto o sódio (Na) pode substituir parcialmente algumas funções fisiológicas do K, influenciando diretamente a eficiência do uso da água (WUE) das plantas. Com isso, o presente estudo objetivou avaliar, em casa de vegetação, a resposta de três genótipos de eucalipto contrastantes quanto a tolerância ao déficit hídrico à substituição parcial de K por Na em diferentes regimes hídricos. Foram avaliados o crescimento das plantas, o conteúdo de nutrientes, as trocas gasosas (*A* - taxa de assimilação de CO_2, g_s – condutância estomática, *E* – transpiração, a "WUE", o conteúdo de clorofila, a eficiência de absorção ("AE") e utilização ("UE") de K e Na, a densidade estomática ("Std"), o potencial hídrico foliar (Ψw), a área foliar ("LA") e a composição isotópica de carbono (δ^{13}C ‰) nas folhas. A pequena substituição de K por Na aumentou a matéria seca (TDM) das plantas de *Eucalyptus* sob diferentes condições de água, com exceção da matéria seca de raiz (RDM), que diminuiu com o fornecimento de Na. Além disso, houve maior potencial de substituição de acordo com o aumento da tolerância à seca entre os genótipos. O fornecimento de água foi o fator mais limitante para o crescimento, caracterizando as plantas cultivadas em déficit hídrico por menor TDM, LA, Std, *A*, *E*, g_s, Ψw$_N$ e UE$_K$, além do maior δ^{13}C e eficiência de uso da água em longo prazo ("WUE$_L$"). Em geral, plantas supridas com pequenas doses de Na (até 50 % de substituição de K) reduziu o nível crítico de K, sem acarretar em sintomas de deficiência de K e, por sua vez, proporcionou maior produção de TDM das plântulas de *Eucalyptus* supridas exclusivamente com K. Também expressaram baixo teor foliar de Na em ambas as condições hídricas expressaram maior assimilação de CO_2, Std, UE$_K$ e WUE, mantendo a turgidez foliar e reduzindo o Ψw$_N$, com estômatos parcialmente fechados, indicados pelo maior valor de δ^{13}C. O suprimento exclusivo de Na, que infere na nutrição das plantas em K, além do menor produção de TDM e sintomas de deficiência de K, é caracterizado principalmente por menores valores de δ^{13}C, WUE$_L$, concentração foliar de K e maior concentração foliar de Na.

Palavras-chave: Estresse abiótico. Nutrição florestal. Taxa de assimilação de CO_2. Tolerância à seca.

SUMMARY

1. General introduction

The genus *Eucalyptus* plays an important role in meeting the growing global wood demand (PAQUETTE; MESSIER, 2010). However, it is largely dependent on fertilization (SMETHURST, 2010) and vulnerable to drought, the main limiting factors for plant growth (GONCALVES, 2010; IBA, 2017). Against the background of a changing climate, the intensity and frequency of droughts will increase in the near future (IPCC, 2013). Among the macronutrients, potassium (K) is one of the most required nutrient for *Eucalyptus*, enhancing yields by 50% compared to plants under K deficiency (BATTIE-LACLAU et al., 2013). Despite the well-known effects of nutrients on forest tree species (HE; DIJKSTRA, 2014), the role of potassium in plant drought tolerance is still unclear (BOOTH, 2013). Adequate management strategies to improve tolerance to water deficit (BATTIE-LACLAU et al., 2016), such as enhancing plant water use efficiency (WUE), are necessary to mitigate the adverse impacts of drought.

Sodium (Na), a beneficial element, can partially replace K in some physiological and other unspecific functions, such as in osmotic control (SUTCLIFFE; BAKER, 1989; MATEUS et al., 2019), as already verified in *Eucalyptus* plantations established in areas with low K availability (ALMEIDA et al., 2010; BATTIE-LACLAU et al., 2014a). However, according to these authors, more studies are needed to identify the processes involving Na in nutritional physiology of *Eucalyptus*. A major benefit of replacing K fertilization by Na is the relatively lower cost of NaCl compared to KCl, bringing greater profitability to the forest sector. However, soil salinity is also one of the main causes of abiotic stress, contributing to desertification and progressive yield reduction worldwide (MARTÍNEZ et al., 2005), requiring careful Na application.

Given that drought stress is the most important factor hampering *Eucalyptus* growth, appropriate zenotype selection can avoid overall losses (WHITE et al., 2009). Insights into forestry drought tolerance, besides being essential, are difficult to obtain due to the complex processes of physiological, biochemical, and morphological adjustments, such as hormonal balance (GRANDA et al., 2014), growth and pigment content variations (LIU et al., 2011), osmotic adjustment (MERCHANT et al., 2006a), and photosynthesis adjustment (WARREN; BLEBY; ADAMS, 2007). In addition, different genotypes and/or developmental stages of the same species can behave differently under nutritional stress conditions (PITA-BARBOSA; HODECKER; BARROS, 2016).

2. Literature review

2.1. Functions of K in plants

Potassium ions (K^+) are involved in several physiological and biochemical processes in plants, such as cell growth, transport of sugars in the phloem, cell turgor, enzymatic activation in more than 50 reactions, defense mechanisms, protein synthesis, photosynthesis, resistance to biotic and abiotic stress, and reduction of excessive ion uptake, such as Na, strongly influencing the productivity and quality of the crop (MARSCHNER, 2012; WANG et al., 2013). The K supply improves CO_2 fixation and the synthesis of large biomolecules, such as starch and protein, reducing small molecules, such as free sugars, amino acids, organic acids, and amides, increasing the concentration of phenols, and supporting plant resistance and responses to abiotic stress (WANG; WU, 2010). According to LACLAU et al., (2009), adequate K concentration also prevents leaf senescence, interfering directly with the leaf area and, consequently, with gas exchanges. The increase in chlorophyll content as a function of K fertilization has been observed in cotton (ONANUGA; JIANG; ADL, 2011), wheat (MORICONI et al., 2012), and *Eucalyptus* (BATTIE-LACLAU et al., 2014a), with a positive correlation with dry matter production (MARSCHNER, 2012). In contrast, drought reduces the chlorophyll content by affecting chloroplast membranes, promoting lamellar vesicle swelling and distortion, and influencing lipid droplet formation (KAISER, 1982).

2.2. Symptoms of K deficiency

Since K is highly mobile in the phloem, a visible symptom of K deficiency in plants is marginal chlorosis, initially in old leaves, evolving to necrosis, besides malformation of roots and stems (MARSCHNER, 2012). In addition, K deficiency also reduces plant tolerance to water deficit due to its influence on plant osmoregulation, playing a critical role in stress avoidance and adaptation (TRÄNKNER; TAVAKOL; JÁKLI, 2018). It also reduces the photosynthetic efficiency (JIN et al., 2011), consequently affecting carbon partitioning to wood production, influencing the plant´s anatomical composition (EPRON et al., 2012). Low K concentrations also decrease starch leaf content by inhibiting starch synthase and impairing soluble compound translocation, such as photosynthates, from source to sink organs.

2.3.   Substitution of K by Na application

The use of Na, via NaCl, can partially replace K in osmotic control and enzymatic reactions, since Na supply is essential to activate ATPases (SUTCLIFFE; BAKER, 1989). Absorbed and taken up as Na^+, it replaces K as osmotically active solute and stimulates cell elongation, besides improving stomatal control and contributing to maintain cell turgor (MARSCHNER, 2012), directly affecting plant water use efficiency (WUE). This occurs due to the similarity between the hydrated ionic radii of Na (0.358 nm) and K (0,331 nm) (MARSCHNER, 2012). However, despite the importance of K, the effects of Na application on water balance are not well studied (GATTWARD et al., 2012).

Positive effects of partial NaCl supply have been found for coconut (BONNEAU et al., 1997), sugar beet (WAKEEL; STEFFENS; SCHUBERT, 2010), cocoa (GATTWARD et al., 2012) and *Eucalyptus* (MATEUS et al., 2019). In these studies, NaCl increased crop quality and yield because of the improved water use efficiency (WUE), improving photosynthetic and reducing transpiration rates. The application of NaCl also facilitated the growth of *E. grandis* under low K availability in Brazil (BATTIE-LACLAU et al., 2014a). Osmotic control is not a specific function of K and can be substituted by other cations (SUBBARAO et al., 2003), such as Na, whose function in stomatal regulation is well known, especially at low K concentrations (KRONZUCKER et al., 2013). Some cultivars of sugar beet have 98% of K substitution by Na without growth decrease, due to changes in plant water balance and better stomatal control (MARSCHNER, 2012). A previous study has shown that Na supply stimulates growth and improves the nutritional quality of numerous crop species (SUBBARAO et al., 2003), indicating some practical value of the metabolic effects attributed to Na (MARSCHNER, 2012). However, Na is not as efficient as K in a myriad of biochemical processes. Starch synthesis has a requirement of about 50 mmol L^{-1} of K for normal activity, being reduced by the substitution for other monovalent cations for K (EVANS; SORGER, 1966), such as Na, which is approximately 20% as effective as K at maintaining starch synthetase activity (NITSOS; EVANS, 1969). In Brazil, NaCl only accounts for 18% of the cost for KCl (ALMEIDA et al., 2010); in addition, nutrient interaction may be a strategy to increase K use efficiency (LACLAU et al., 2003).

However, despite its benefit to a certain degree, the excessive abundance of Na is problematic (KRONZUCKER et al., 2013). Large-scale absorption of Na and Cl negatively affects plant physiology by altering metabolic processes (AHANGER et al., 2017), inducing stomatal closure as a mechanism to minimize Na leaf accumulation, and reducing crop transpiration rates (VERY et al., 1998). According to MUNNS (2002), the increase in Na leaf

content results in lower photosynthesis (A) and stomatal conductance (g_s) values. Thus, the control of the tissue K: Na ratio directly influences plant growth and is widely used in breeding programs to select plants resistant to high Na availability (MUNNS; TESTER, 2008). Differences in nutritional efficiency between species and clones of *Eucalyptus* have already been verified (LIMA et al., 2015), allowing the selection of *Eucalyptus* genotypes adapted to the edaphoclimatic site conditions. However, in spite of the great variety of studies involving nutrient application, plant growth, and water deficit (MÜLLER et al., 2017), studies involving K and Na uptake efficiency and the use of different genotypes and water regimes are still scarce.

2.4.   Fertilization on gas exchange and WUE

Among the critical roles of K^+ in plants, stomatal opening and closure are highlighted. This ion provides the necessary driving force for the water influx into guard cell vacuoles, maintaining the turgescence of these cells (PEITER, 2011). Water supply and temperature are limiting factors for the growth of forest plantations in tropical regions (STAPE et al., 2004). According to Cakmak, Hengeler and Marschner (1994), the mitigation of the negative impacts of K in plants under drought is due not only to the regulation of stomatal movement, but also to the positive effects of K on CO_2 assimilation, primary metabolism, phloem loading, and the redistribution of photoassimilates from the source to the sink organs.

The positive relationship between K supply and higher WUE has already been demonstrated for several crops such as cotton (TSONEV et al., 2011), canola (ALI; BAKHT; KHAN, 2014), and eucalyptus (BATTIE-LACLAU et al., 2016). The WUE is a parameter used to evaluate plant yield (LÉVESQUE et al., 2014). However, its measurement is methodologically challenging since it can be determined via several methods, with different spatial and temporal scales (SEIBT et al., 2008). On a short time/leaf level scale, the intrinsic WUE of leaves (WUE_I) can be measured through the ratio of the net assimilation of CO_2 (A) by stomatal conductance (g_s) or instantaneous WUE (WUE_T), through the ratio of A by transpiration (E) (SEIBT et al., 2008). On the long time/whole plant scale, the WUE can also be determined by the relationship between plant dry matter yield and water used throughout the experimental period (WUE_L) (MARTIN; THORSTENSON, 1988). Since the K supply affects the xylem water transport (NARDINI; SALLEO; JANSEN, 2011), the Na supply can improve g_s and mitigate anatomical and biochemical deficiencies of plants caused by low K availability and content, as previously reported (BATTIE-LACLAU et al., 2014a). According to these authors, A and g_s correlate strongly and may be even greater with the simultaneous application

of K and Na. The application of K, supplemented by Na, increased the WUE_I in cacao plants (GATTWARD et al., 2012) and olive trees (EREL et al., 2014). According to EPRON et al. (2012), eucalyptus plantations supplied with Na presented a higher leaf biomass with increased CO_2 assimilation than K-deficient plants.

The WUE measurement at various scales may provide more information on spatial and temporal variations, since there are few studies that combine WUE evaluations at different scales and water conditions in forest plantations (ALBAUGH; DYE; KING, 2013). In addition, the potential role of Na in the stomatal movement, although previously discussed (KRONZUCKER et al., 2013), is still not well determined and may be an appropriate practice to improve the WUE and productivity under K-deficient soils and water deficit conditions.

2.5. Stable isotope composition ($\delta^{13}C$) and WUE correlations

According to Mårtensson et al. (2017), $\delta^{13}C$ is a robust parameter to evaluate genotypic variation in WUE and physiological responses to environmental factors, as well as drought-tolerant crops and shoot dry matter production (FARQUHAR; SHARKEY, 1982). According to Farquhar, Ehleringer and Hubick, (1989), variations in A and g_s, due to water availability, led to changes in $\delta^{13}C$ and WUE (SEARSON et al., 2004). Based on this, $\delta^{13}C$ values can be used as an indicator for the classification of plant WUE, allowing the selection of water stress-tolerant genotypes, improving yield under drought conditions (ROUX et al., 1996). In another study, $\delta^{13}C$ and WUE_T did not correlate in grasslands (ZAVALLONI et al., 2009), since the evaluation at leaf level (A/E) produces accurate data of a specific time (CONDON et al., 2002). However, the correlation between $\delta^{13}C$ and WUE_L was demonstrated in several crops such as wheat, peanut, tomato, and potato genotypes (FARQUHAR; RICHARDS, 1984; HUBICK; FARQUHAR; SHORTER, 1986; MARTIN; THORSTENSON, 1988; VOS; GROENWOLD, 1989), since the WUE_L reflects the conditions of the entire experimental period, also correlating with dry matter production (MÅRTENSSON et al., 2017). Thus, the WUE at the whole plant level becomes more reliable when studying gas exchanges and changes in the environmental conditions that occur during the cultivation period (SEIBT et al., 2008). If a similar relationship occurs for high-yielding *Eucalyptus* under water deficit, the $\delta^{13}C$ values will be confirmed as a useful tool for suitable species/clone/genotype selection under drought conditions.

3. Hypothesis

Based on the knowledge presented, the following hypotheses are tested:

- *Eucalyptus* seedling genotypes respond positively to the partial replacement of K by Na due to the increase in photosynthetic efficiency - CO_2 assimilation rate, stomatal conductance, and transpiration -, contributing to the increase in water use efficiency, since these elements interfere directly with the anatomical and morphological components of leaf area, providing greater osmotic control and gas exchange;

-The partial Na supply alleviates the potential physiological disorders of K-deficient plants, facilitating plant growth by ameliorating the ionic imbalance and optimizing the leaf water potential of plants;

-There is a correlation between Na leaf concentrations with A and g_s, since the leaf K: Na ratio directly influences gas exchange and, consequently, plant growth;

-The partial replacement of K by Na contributes to attenuate the negative effects of the water deficit on eucalyptus cultivation due to the direct and positive contribution of these elements to plant water balance, allowing a greater regulation of stomatal movement and a reduction of the transpiration rate, resulting in increased WUE and leaf water content;

- The parameters WUE_L, leaf K: Na ratio, and dry matter production are correlated with $\delta^{13}C$, allowing the selection of the most efficient genotype for K use and the percentage of K substitution by Na, which promotes the highest growth for each water regime;

-The plant genotypic variation affects the mechanisms of uptake and use of K and Na in the tissues - especially K use efficiency;

-Higher Na-soil availability and tissue content are detrimental, inducing K deficiency and, consequently, reducing plant growth, besides accelerating the adverse effects of the water deficit.

4. General objectives of the study

This work evaluates the partial replacement of K by Na and its impacts on plant growth and the physiology of leaf gas exchange in different genotypes of *Eucalyptus* plants under different water regimes, investigating whether the partial supply of K by Na may ameliorate the negative effects of droughts compared to the application of only K. In addition, it determined the water use efficiency and the K use efficiency.

4.1. Specific objectives

- To evaluate the impacts of K and Na supply and of different water regimes on plant growth, nutritional status, leaf gas exchanges (A, g_s, and E), WUE, stomatal density (Std), leaf water potential (Ψw), and chlorophyll content in genotypes of *Eucalyptus*;

- To determine the leaf K: Na ratio associated with the highest growth, CO_2 assimilation, K and water use efficiency, and $\delta^{13}C$;

- To assess the relationship between $\delta^{13}C$ and different WUE scales and dry matter production under different water regimes and K: Na rates;

- To evaluate K and Na absorption by *Eucalyptus* genotypes and their use efficiency under different water regimes.

5. Material and Methods

5.1. Experimental design and growth conditions

The experiment was carried out in a greenhouse at the Center for Nuclear Energy in Agriculture (CENA-USP) in Piracicaba, São Paulo State, Brazil, from July to November 2018. Plants were grown at temperatures between 17.1 and 39.8°C (mean of 32.8°C), an average relative humidity of 65%, and a maximum photosynthetic photon flux density of approximately 1.100 µmol m^{-2} s^{-1}. Three *Eucalyptus* genotypes (*E. saligna*, *E. urophylla*, and *E. camaldulensis*) contrasting to drought tolerence, with approximately 90 days and 30 cm of height, were transplanted into individual plastic pots (5 kg) containing a typical Oxisol medium texture and with low natural fertility, collected at the Itatinga Experimental Station (ESALQ - USP), Itatinga-SP. The soil samples were collected from the upper soil layer (0-20 cm), homogenized, air-dried, passed through a 2-mm mesh sieve, and sent to the laboratory for chemical and physical analyses (Table 1).

In the experiment, K was replaced by Na (as NaCl) based on the soil K critic level (1.20 mmol$_c$ dm^{-3} of K) for *Eucalyptus*, representing a substitution of 0/100, 25/75, 50/50, 75/25, and 100/0 (% of Na/% of K), for 150 days. Thus, the treatments consisted of five combinations of Na and K application rates (0/0.90, 0.22/0.67, 0.44/0.44, 0.67/0.22, and 0.90/0 mmol$_c$ dm^{-3} of Na/mmol$_c$ dm^{-3} of K), which, added to the soil K content, reached the soil K critical level (1.20 mmol$_c$ dm^{-3} of K). The rates 0 and 0.90 mmol$_c$ dm^{-3} of Na represented the control (solely K-supplied plants) and the K deficiency treatments, respectively. The plants were exposed to two water regimes, starting 75 days after the onset of the treatments: well-watered (WW) and water stress (WS) condition, simulating adequate water availability and

drought conditions. The soil relative water content (SRWC) of both water conditions was controlled daily by the gravimetric method, using irrigation with deionized water up to 80% of the water-holding capacity (WHC) under WW and up to 35% of the WHC under WS. Watering and weighing were conducted on a daily basis at dusk until the pots reached their corresponding target SRWC (Eq. 1) (XU; ZHOU; SHIMIZU, 2009). Monthly, one plant as harvested and weighed to maintain the desired SRWC in the pots. The water use of each plant (by transpiration) was obtained by evaluating soil evaporation from the weight loss of a plantless pot, using the following equation:

$$\text{SRWC: } \frac{(\text{Wsoil} - \text{Wpot} - \text{DWsoil} - \text{TDMplant})}{(\text{WFC} - \text{Wpot} - \text{DWsoil} - \text{TDMplant})} * 100 \qquad (1)$$

where Wsoil is the current soil weight (soil + pot + water), Wpot is the weight of the empty pot, DWsoil is the dry soil weight, TDMplant is the total dry matter of the plant, and WFC is the soil weight at field capacity (soil + pot + water).

The experiment was performed in randomized blocks, with four replications, in a 5 x 2 factorial design, totaling 120 experimental units with one plant each.

In addition to the treatments with K and Na, all samples received the following complementary fertilization: 135 mg dm^{-3} of N plus 20 mg dm^{-3} of N in coverage at 2 months after the onset of the treatments, 300 mg kg^{-1} of P, 92 mg kg^{-1} of Ca, 7.2 mg kg^{-1} of Mg (reaching 7 mmol$_c$ dm^{-3} in a Ca^{+2}: Mg^{+2} ratio of 4:1), 45 mg kg^{-1} of S, 0.82 mg kg^{-1} of B, 4.0 mg kg^{-1} of Zn, 3.66 mg kg^{-1} of Mn, 1.55 mg kg^{-1} of Fe, 1.39 mg kg^{-1} Cu, and 0.20 mg kg^{-1} of Mo, facilitating adequate plant development (NOVAIS; NEVES; BARROS, 1991). The sources used of the elements were as follows: $(NH_4)H_2PO_4$, $CaCO_3$, $MgCO_3$, elementary S $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, H_3BO_3, $MnSO_4.H_2O$, and $MoO_3.H_2O$.

5.2. Leaf gas exchange and leaf water potential

The youngest fully expanded leaf of each plant was used to evaluate gas exchange in the late morning (9 to 11 am), using an infrared gas analyzer (IRGA, Li-6400XT, LICOR Inc., Lincoln, NE, USA) at environmental humidity and temperature; external CO_2 concentration (Ca) was fixed at 400 μmol and photosynthetically active radiation (PAR) flux density at 1.200 μmol m^{-2} s^{-1}. Photosynthesis (A), stomatal conductance (g$_s$), and transpiration (E) were measured. Intrinsic water use efficiency (WUE$_i$) and instantaneous water use efficiency

(WUE$_T$) in the leaf were determined by dividing the values of *A* by *g$_s$* and *A* by *E*, respectively (FARQUHAR; RICHARDS, 1984; MARTIN; THORSTENSON, 1988). Long-term water use efficiency (WUE$_L$) was calculated by dividing the total dry matter value (belowground plus aboveground) by water use throughout the experiment (g dry matter/kg H$_2$O) (MARTIN; THORSTENSON, 1988). Mean leaf temperature during the measurement was 32ºC.

In the same leaves, the predawn (3 a.m.) and noon (12 p.m.) leaf water potential (Ψw$_{PD}$ and Ψw$_N$ respectively) were also measured, using a Scholander pressure chamber (TURNER, 1981). All these evaluations were realized prior to harvesting.

Table 1- Physical and chemical attributes of the Oxisol medium texture for *Eucalyptus* growth

Attributes		Value	Attributes		Value
Sandy	g kg^{-1}	798	H+Al	mmol$_c$ dm^{-3}	25
Silt	g kg^{-1}	50	Al	mmol$_c$ dm^{-3}	3
Clay	g kg^{-1}	152	BS	mmol$_c$ dm^{-3}	2
Text	-	md-ar	CEC	mmol$_c$ dm^{-3}	27
pH	CaCl$_2$	4.2	V	%	8
OM	g dm^{-3}	5	m	%	57
P	mg dm^{-3}	2	Cu (DTPA)	mg dm^{-3}	0.6
K	mmol$_c$ dm^{-3}	0.3	Fe (DTPA)	mg dm^{-3}	33
S-SO$_4$	mg dm^{-3}	6	Zn (DTPA)	mg dm^{-3}	0.8
Ca	mmol$_c$ dm^{-3}	1	Mn (DTPA)	mg dm^{-3}	0.8
Mg	mmol$_c$ dm^{-3}	1	B	mg dm^{-3}	0.14
Na	mmol$_c$ dm^{-3}	-			

P, Na, K, Fe, Zn, Mn, Cu (extracted by Mehlich 1), Ca, Mg, Al (extracted by KCl, 1 *M*) H + Al (extracted by calcium acetate 0.5 *M*), B (extracted by hot water), S (extracted by monocalcium phosphate in acetic acid). SB, sum of bases; CEC, cation exchange capacity (pH 7.0); V, base saturation; m, Al saturation; OM, organic matter = Org C. $\times$ 1.724.

5.3.   Chlorophyll content, stomatal density (Std), and leaf area (LA)

Leaf chlorophyll content (Chl) was indirectly evaluated using a portable Dualex-DX4 (FORCE-A, Orsay, France). Stomatal density (std; stomates mm^{-2}) was calculated using the two youngest fully expanded leaves per plant (MATEUS et al., 2019), in abaxial and adaxial surface, applying the software package ImageJ (https://imagej.nih.gov/ij/). Complementary micrograph material of Std was obtained by scanning electron microscopy (JEOL JSM-IT300 LV, Tokyo-Japan) at 20 kV, and digital images were recorded (LAVRES et al., 2019). Plants were harvested 150 days after of the start of the treatments, and their leaves, stems,

branches, and roots were separated. Leaf area (LA) was obtained by passing all leaves through a leaf area integrator (LI-3100). The SLA (m² kg^{-1}) was obtained by division LA (m² plant^{-1}) by leaf dry matter (kg plant^{-1}).

5.4.   Dry matter production and nutritional status

After drying in a forced air ventilation oven at 60°C for 72 h, each plant part was weighed to determine dry matter. Subsequently, the plant material was ground in a Wiley type mill and forwarded to nitric-perchloric digestion (MALAVOLTA; VITTI; OLIVEIRA, 1997) to quantify P, K, Na Ca, Mg, S, Cu, Fe, Al, Mn, Zn, Ni, and B by inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 7000 SERIES, Thermo Fisher Scientific, Waltham, USA). The accumulation of individual elements was obtained by multiplying the concentration of each element in the tissue by the dry matter production of the respective tissue (root, stems, and leaves). Based on the leaf K and Na content, we calculated the K: Na ratio, which was correlated with the estimated rate of maximum dry matter production (the critical level of 90 % of maximum yield) of each genotype, obtained by equaling the equation to zero. The accumulation of K and Na was used to determine the absorption efficiency (AE) (SWIADER; CHYAN; FREIJI, 1994) and the use efficiency (UE) (SIDDIQI; GLASS, 1981), according to Equations (2 and 3):

$$ AE: \frac{\text{nutrient in plant (mg)}}{\text{root dry matter (g)}} \qquad (2) $$

$$ UE: \frac{(\text{plant dry matter})^2 \text{ (g)}}{\text{nutrient in plant (mg)}} \qquad (3) $$

where nutrient refers to K or Na (beneficial element) accumulation.

5.5.   Leaf carbon isotope composition ($\delta^{13}C$)

The same samples used for leaf dry matter determination were also used to assess the carbon isotope composition, determined using a mass spectrometer (ANCA-GSL Hydra 20–20 model, SERCON Co., Crewe, GBR) coupled to a C automatic analyzer (BARRIE; PROSSER, 1996), and the isotope values (‰) were calculated via Equation (4) (FARQUHAR; SHARKEY, 1982):

$$\delta13C(‰): \left(\frac{\text{Rsample}}{\text{Rpdb}} - 1 \right) x \ 1000 \qquad\qquad (4)$$

where R is the ratio of $^{13}C/^{12}C$. The reference material is the Vienna Pee Dee Belemnite (PDB).

6. Statistical procedures

The data were analyzed by the F test ($p < 0.05$), and the significant differences among means were determined via Tukey's post hoc test ($p < 0.05$) to compare WW and WS conditions as well as treatments to elucidate details on the differences within Na rates. The significant effects of Na application were described by linear, quadratic, and square root regression models, in which the significant model ($p < 0.05$) with the highest determination coefficient (R^2) was selected. The original data were standardized to be analyzed via Pearson´s correlation ($p < 0.05$), principal components analysis (PCA), and cluster analysis, correlating the measured variables in each genotype and water condition. In cluster analysis, treatments were grouped into functional units by their similarity; for the PCA, we used the treatments with Na supply for the first two main components (PC1 and PC2) and 95 % confidence ellipses to visualize the multivariate trends of Na application under WW and WS conditions.

Statistical analyses were performed using the software packages SAS version 9.1 (SAS INSTITUTE, 2004; 2012) and R version 3.5.1 (R DEVELOPMENT CORE TEAM, 2018). Data variability was indicated with standard error and shown graphically using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

7. Results

7.1. Dry matter production

Root dry matter production (RDM) of *E. saligna* plants and its root: shoot ratio were negatively influenced by Na and water stress (WS), decreasing simultaneously with increasing Na supply (Figure 1a and d). Total dry matter yield (TDM) was affected by Na, WS, and Na*WS, increasing with low Na rates under WW and decreasing with increased Na supply under WS (Figure 1g). An Na rate of 0.22 $\text{mmol}_c \ \text{dm}^{-3}$, besides providing the highest TDM under WW, also was the most affected by the negative impacts of WS, reducing by 32% its TDM. The K-deficient plants (0.9 $\text{mmol}_c \ \text{dm}^{-3}$ of Na) decreased their RDM levels by 55 and 70% under WW and WS, respectively, and their TDM levels by 35% under both water conditions compared to the control (0 $\text{mmol}_c \ \text{dm}^{-3}$ of Na). Plants of *E. saligna* subjected to

WS decreased their RDM and TDM values by 20 and 15%, respectively. Under WW condition, the Na rate estimated to give the maximum TDM was 0.048 mmol$_c$ dm^{-3} of Na and 0.852 mmol$_c$ dm^{-3} of K, corresponding to 5.3% of substitution and to 48.9 g plant^{-1}.

In *E. urophylla*, the RDM was influenced by Na and WS, the root: shoot ratio by Na and Na*WS, and the TDM by Na, WS, and Na*WS (Figure 1b, e, and h). Despite higher TDM levels, plants under low Na rates (0.22 mmol$_c$ dm^{-3}) were also most significantly affected by drought, with a decrease by 20%. The RDM and TDM of K-deficient plants decreased by 50% in WW and by 30% in WS, relative to the control (0 mmol$_c$ dm^{-3} of Na). The mean RDM and TDM levels of all Na rates of *E. urophylla* under WS decreased by 20 and 15% of the mean Na rates, respectively, compared to WW. Under WW, the estimated Na rate to give the maximum TDM was 0.06 mmol$_c$ dm^{-3} of Na and 0.84 mmol$_c$ dm^{-3} of K, which correspond to 6.7 % of substitution reaching 69.9 g plant^{-1}. Under WS, the estimated Na rate was 0.085 mmol$_c$ dm^{-3} of Na and 0.815 mmol$_c$ dm^{-3} of K, corresponding to 9.3% of substitution and to 55.13 g plant^{-1}.

The parameters root dry matter yield, root: shoot ratio, and total dry matter yield of *E. camaldulensis* were influenced by Na, WS, and Na*WS (Figure 1c, f, and i). The RDM decreased with increasing soil Na availability under both conditions. The root: shoot ratio, under WS, decreased according to Na rates, while under WW, it was lower in the intermediary rates, indicating higher shoot dry matter and lower RDM values. Similar to the other genotypes, plants with higher TDM values were observed under low and intermediary Na rates (0.44 mmol$_c$ dm^{-3}), under both water conditions, and were also most significantly affected by drought, decreasing their TDM values by 40%. The K-deficient plants decreased their RDM values by 67% under both conditions, and the TDM of plants supplied with Na at a rate of 0.9 mmol$_c$ dm^{-3} decreased by 55 and 35%, respectively, under WW and WS, compared to the control (0 mmol$_c$ dm^{-3} of Na). Water stress decreased the TDM average by 25% compared to WW. The rates estimated to obtain the maximum TDM under WW was 0.27 mmol dm^{-3} of Na and 0.63 mmol$_c$ dm^{-3} of K, corresponding to a substitution level of 30% and 69.81 g plant^{-1}. Under WS, the estimated rates were 0.09 mmol$_c$ dm^{-3} of Na and 0.81 mmol$_c$ dm^{-3} of K, corresponding to 10.2% of K replacement by Na and 46.4 g plant^{-1}.

Figure 1- Root (a-c), root: shoot ratio (d-f) and total (g-i) dry matter production of *E. saligna* (a, d and g), *E. urophylla* (b, e and h) and *E. camaldulensis* (c, f and i) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4)
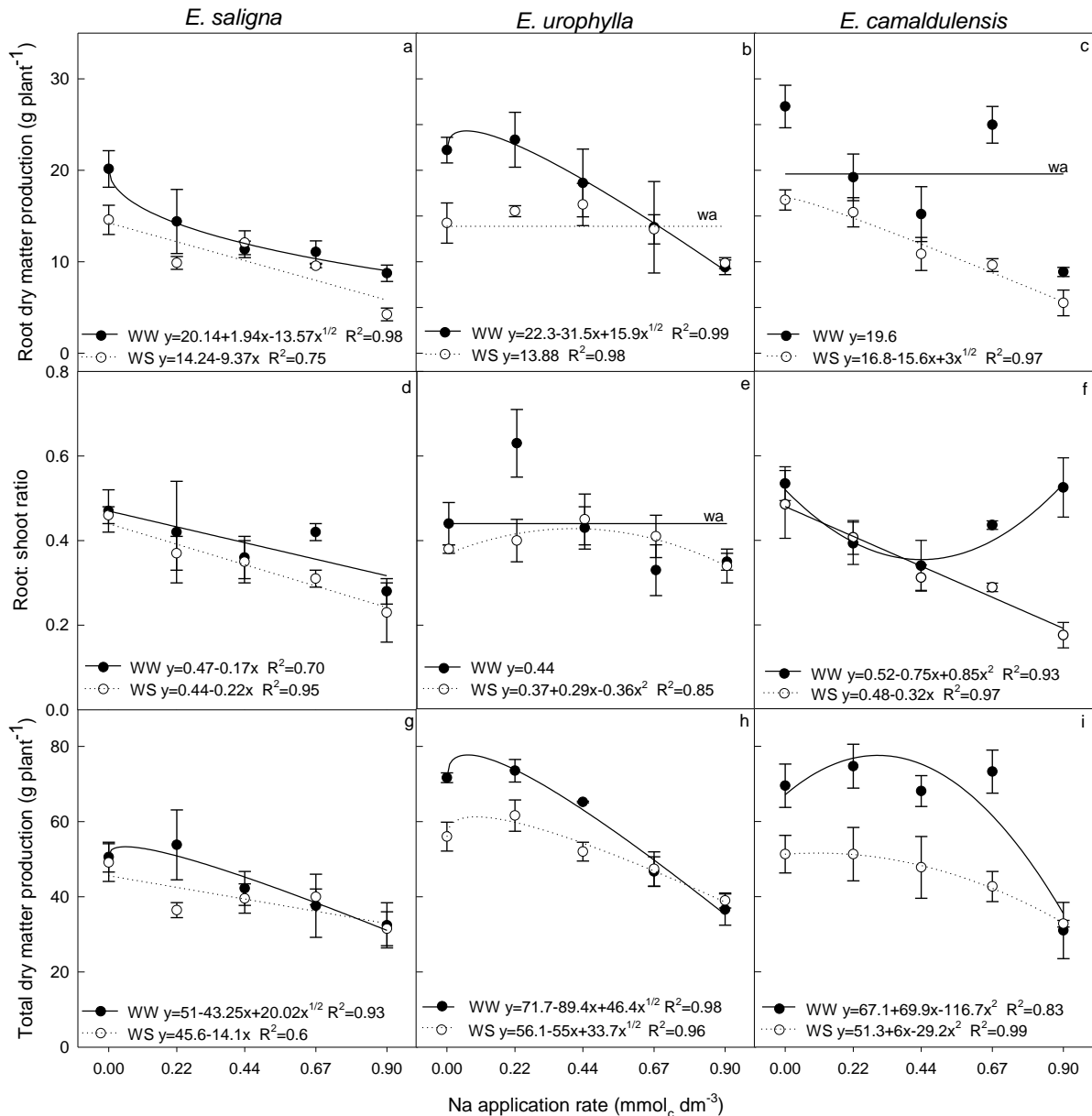
7.2. Leaf area (LA) and specific leaf area (SLA)

The LA was influenced by Na, WS, and Na*WS in *E. saligna* (Figure 2a). Under WW, the LA decreased by 60% with Na supply, while under WS, the intermediary rates of Na (0.44 and 0.67 mmol$_c$ dm^{-3}) increased the LA nearly by 10%. Plants with higher LA levels (supplied with 0 mmol$_c$ dm^{-3} of Na) drastically decreased their LA by 55% under drought conditions. The LA of *E. urophylla* and *E. camaldulensis* was affected by Na and WS (Figure 2b and c). Low to intermediary Na rates (0.22 and 0.44 mmolc dm^{-3}) increased the LA

of *E. urophylla* by up to 60 and 25% under WW and WS, respectively, compared to the control (0 mmol$_c$ dm^{-3} of Na). In *E. camaldulensis*, the same rates provided similar results for LA values, increasing by 6%, while the higher Na rates decreased the LA by 40%. Water stress decreased the LA by 15, 35, and 36% in *E. saligna, E. urophylla*, and *E. camaldulensis*, respectively, according to the mean of all Na rates.

The SLA was influenced by Na, WS, and Na*WS in *E. saligna* and *E. urophylla*, whereas in *E. camaldulensis*, it was affected by Na and WS (Figure 2d, e, and f). In *E. saligna*, with intermediate Na rates, the SLA was 50 and 15% lower under WW and WS, respectively, compared to K supply only. The SLA of *E. urophylla* increased linearly with increasing Na supply under WW, with higher levels at low Na rates under WS, relative to the plants receiving only K. Therefore, in *E. camaldulensis*, the SLA increased up to an Na rate of 0.67 mmol$_c$ dm^{-3}. All genotypes under WS showed decreased SLA levels by around 30%, according to the mean of all Na rates.

Figure 2- Leaf area (a-c) and specific leaf area (d-f) of *E. saligna* (a, d), *E. urophylla* (b, e) and *E. camaldulensis* (c, f) seedlings, respectively, under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4)
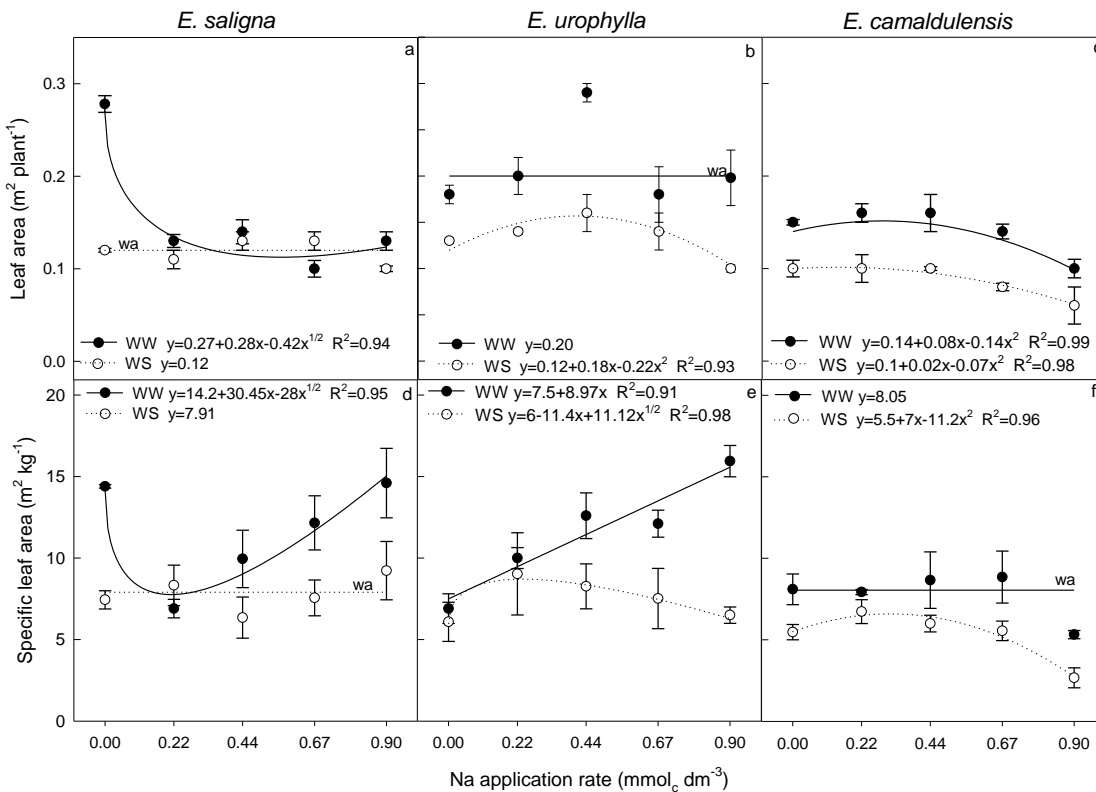
7.3.   Adaxial and abaxial stomatal density (Std$_{AD}$ and Std$_{AB}$)

The Std$_{AD}$ was influenced by Na, WS, and Na*WS in *E. saligna*, *E. urophylla*, and *E. camaldulensis* (Figure 3a, b, and c). Under WW, the low Na supply (up to 0.22 mmol$_c$ dm^{-3}) increased the Std$_{AD}$ nearly to 30% in *E. saligna* and *E. camaldulensis*, while was decreased in *E. urophylla*, reaching the highest values in 0.67 and 0.9 mmol$_c$ dm^{-3} of Na. Under WS, the Na application increased the Std$_{AD}$ of *E. saligna* and *E. urophylla* compared to only K supply. In *E. camaldulensis*, a similar response was observed at low Na doses, decreasing with increasing rates, as observed for 0.9 and 0.67 mmol$_c$ dm^{-3} of Na under WW and WS, respectively. Water stress increased the Std$_{AD}$ by 20% in *E. saligna* and reduced it by nearly 10 and 15% in *E. saligna* and *E. camaldulensis*, respectively.

The Std$_{AB}$ was influenced by Na and WS in *E. saligna* and *E. urophylla*, whereas in *E. camaldulensis*, it was affected by Na application (Figure 3d, e, and f). The *E. saligna* seedlings grown under WW and WS regimes and *E. urophylla* subject to WW showed increased Std$_{AB}$ levels up to Na rates of 0.22 mmol$_c$ dm^{-3}. Besides that, the highest Na rate (0.9 mmol$_c$ dm^{-3} of Na) led to decreased Std$_{AB}$ levels by 50, 30, and 20% in *E. saligna, E. urophylla*, and *E. camaldulensis*, respectively. Water stress also decreased the mean Std$_{AB}$ by 15 and 10% in *E. saligna* and *E. urophylla*, respectively.

Figure 3- Stomatal density of adaxial - Std_{AD} (a-c) and abaxial- Std_{AB} (d-f) leaf surface of *E. saligna* (a and d), *E. urophylla* (b and e) and *E. camaldulensis* (c and f) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa)
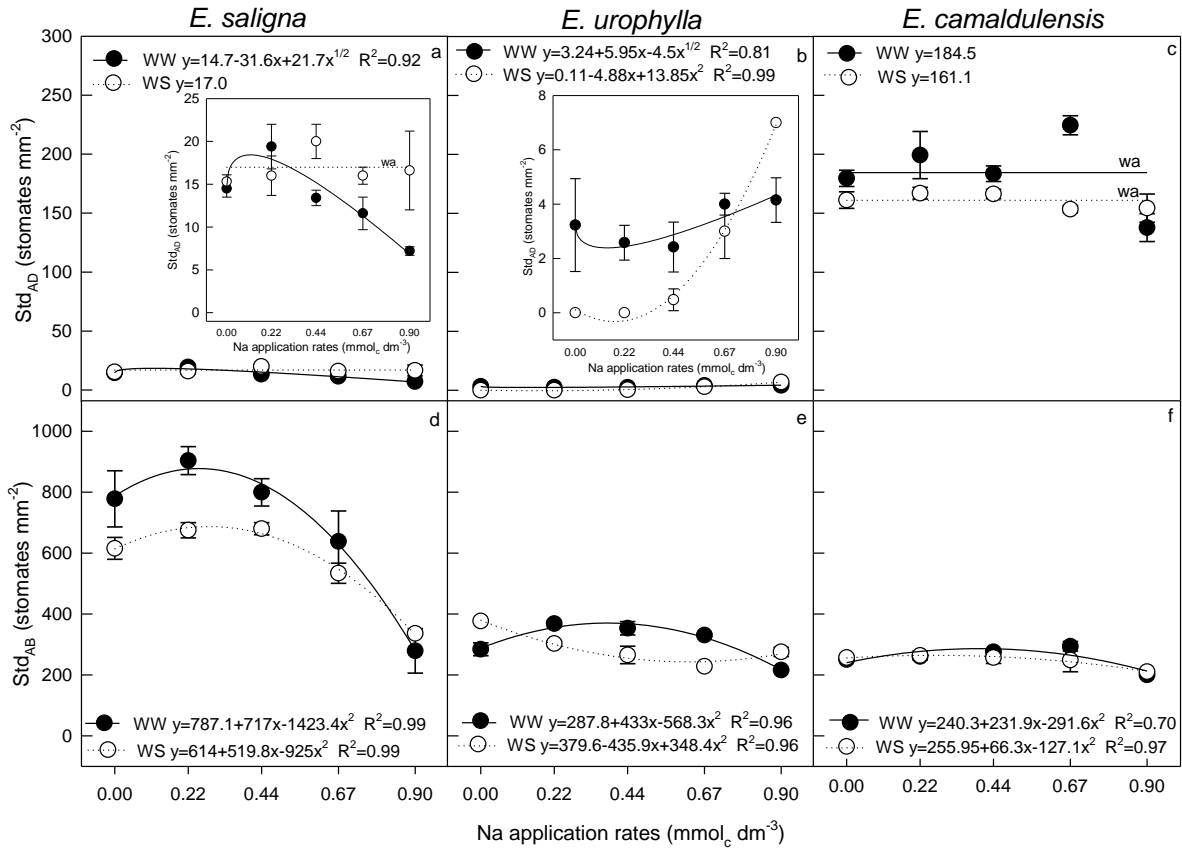
Figure 4- Micrograph augmented 500x of scanning electron microscopy in adaxial (A-C) and abaxial (D-F) of *E. saligna* (A and D), *E. urophylla* (B and E) and *E. camaldulensis* (C and F) supplied with 0 $mmol_c$ dm^{-3} of Na under WW condition
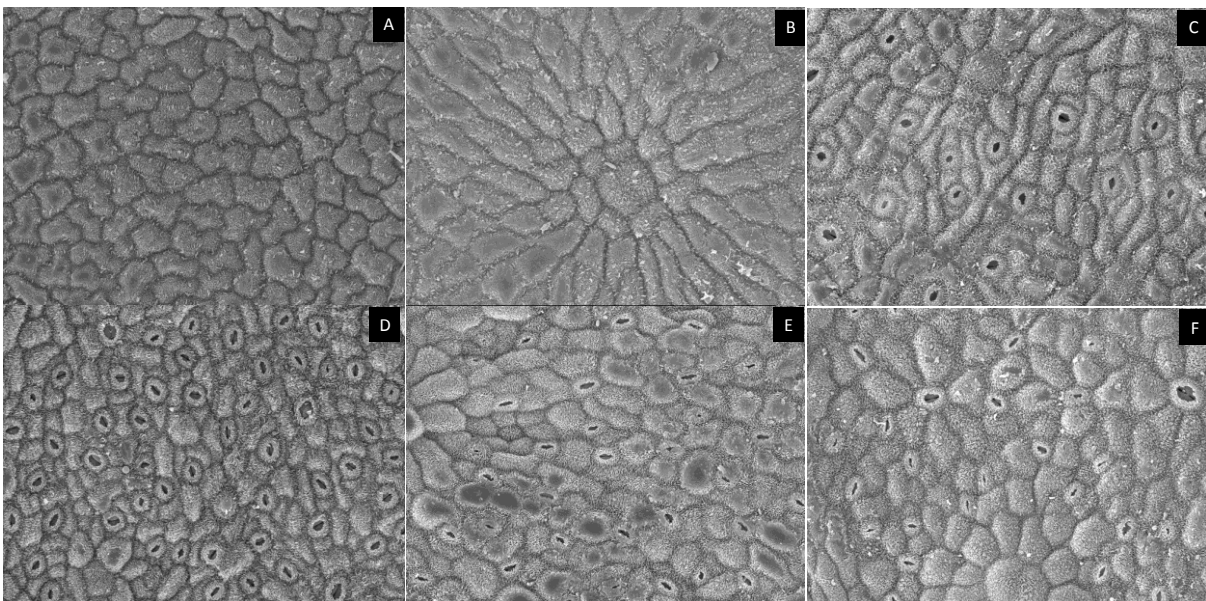
7.4. Leaf gas exchange

The parameter A was influenced by Na rate and water regime in all genotypes (Figure 5a, b and c). The application of Na had negative impacts at high Na doses, but was beneficial at low Na application rates (0.22 and 0.44 $mmol_c$ dm^{-3}), increasing A by 77 and 40% under WW and WS, respectively, compared to the control. A similar response was observed in *E. urophylla* and *E. camaldulensis*, with an increase by 50 and 20%, respectively, in both conditions, compared to the control. The Na rate of 0.9 $mmol_c$ dm^{-3} resulted in a significantly decreased photosynthetic rate in all genotypes. Water stress reduced the mean CO_2 assimilation rate of all rates in all genotypes by 15% compared to plants under WW.

The parameter E was influenced by Na rate and WS in all genotypes (Figure 5d, e, and f). In *E. saligna*, the Na rate of 0.44 $mmol_c$ dm^{-3} increased E by 220 and 200% under WW and WS, respectively, compared to the control. In *E. urophylla*, E was around 50% higher at intermediary Na rates (0.44 and 0.67 $mmol_c$ dm^{-3} of Na) under both water conditions. In *E. camaldulensis*, plants subjected to WW showed lower E values with exclusive K supply, although, under WS, the E decreased until intermediary Na rates were reached, increasing significantly with the highest Na supply. Compared to WW, WS decreased the mean E values of all Na rates by 45, 35, and 75% in *E. saligna*, *E. urohyplla*, and *E. camaldulensis*, respectively.

The g_s values were influenced by Na rate and WS in all genotypes (Figure 5g, h, and i). In *E. saligna*, the Na rate of 0.44 $mmol_c$ dm^{-3} increased the g_s by 350 and 250% under WW and WS conditions, respectively. In *E. urophylla*, the g_s increased by up to 50% with an Na rate of 0.44 $mmol_c$ dm^{-3}, followed by a decrease by 20% at a rate of 0.9 $mmol_c$ dm^{-3} under WS conditions. In *E. camaldulensis*, the g_s increased by up to 45% at an Na rate of 0.44 $mmol_c$ dm^{-3}, under both conditions, followed by a decrease by 15 and 80% at the highest Na rate (0.9 $mmol_c$ dm^{-3} of Na). Specimens of of *E. saligna, E. urohyplla*, and *E. camaldulensis* grown under WS decreased their g_s values by 50, 30, and 55%, respectively, compared to those under WW, irrespective of the Na rate.

Water stress significantly influenced the WUE_I of *E. saligna* and *E. urophylla*, whereas the WUE_I of *E. camaldulensis* was affected by Na and WS (Figure 6a, b, and c). The WUE_I of *E. camaldulensis* grown at Na rates of 0.22 and 0.44 $mmol_c$ dm^{-3} increased by 10 and 50%, respectively, while the highest Na rate decreased the WUE_I by 30 and 40% in plants under WW and WS conditions, respectively. Water stress increased the WUE_I by around 90, 45, and 30% compared to WW in *E. saligna, E. urophylla*, and *E. camaldulensis,* respectively, at all Na rates.

The WUE$_T$ was also affected by WS in *E. saligna* and *E. urophylla*, whereas in *E. camaldulensis* it was affected by Na application and WS regime (Figure 6d, e, and f). The low Na applications (0.22 and 0.44 mmol$_c$ dm^{-3}) significantly increased the WUE$_T$ of *E. camaldulensis* by 15 and 25% in WW as well as in WS, respectively, compared to the control, followed by a decrease by 20 and 35% at higher Na rates. Water stress increased the transpiration rate WUE$_L$ by 200, 60, and 20% in *E. saligna, E. urophylla*, and *E. camaldulensis*, respectively, compared to WW, for all Na rates.

The WUE$_L$ was influenced by Na, WS, and Na*WS in *E. saligna* (Figure 6g) and *E. camaldulensis* (Figure 6i), whereas in *E. urophylla* (Figure 6h), it was affected by Na and WS. In *E. saligna* and *E. urophylla* under WS, Na supply decreased the WUE$_L$, while under WW, the low Na supply resulted in a higher WUE$_L$, decreasing its values with higher Na application. In *E. camaldulensis*, a small increase by 6 and 2% was observed at low Na rates under WW and WS conditions, respectively. Drought stress increased the WUE$_L$ by 33, 35, and 17% in *E. saligna, E. urophylla*, and *E. camaldulensis*, respectively, irrespective of the Na rate.

Figure 5- Photosynthetic rate-*A* (a-c), leaf transpiration rate-*E* (d-f) and stomatal conductance-g_s (g-i), in leaves of *E. saligna* (a, d and g) *E. urophylla* (b, e and h) and *E. camaldulensis* (c, f and i) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4)
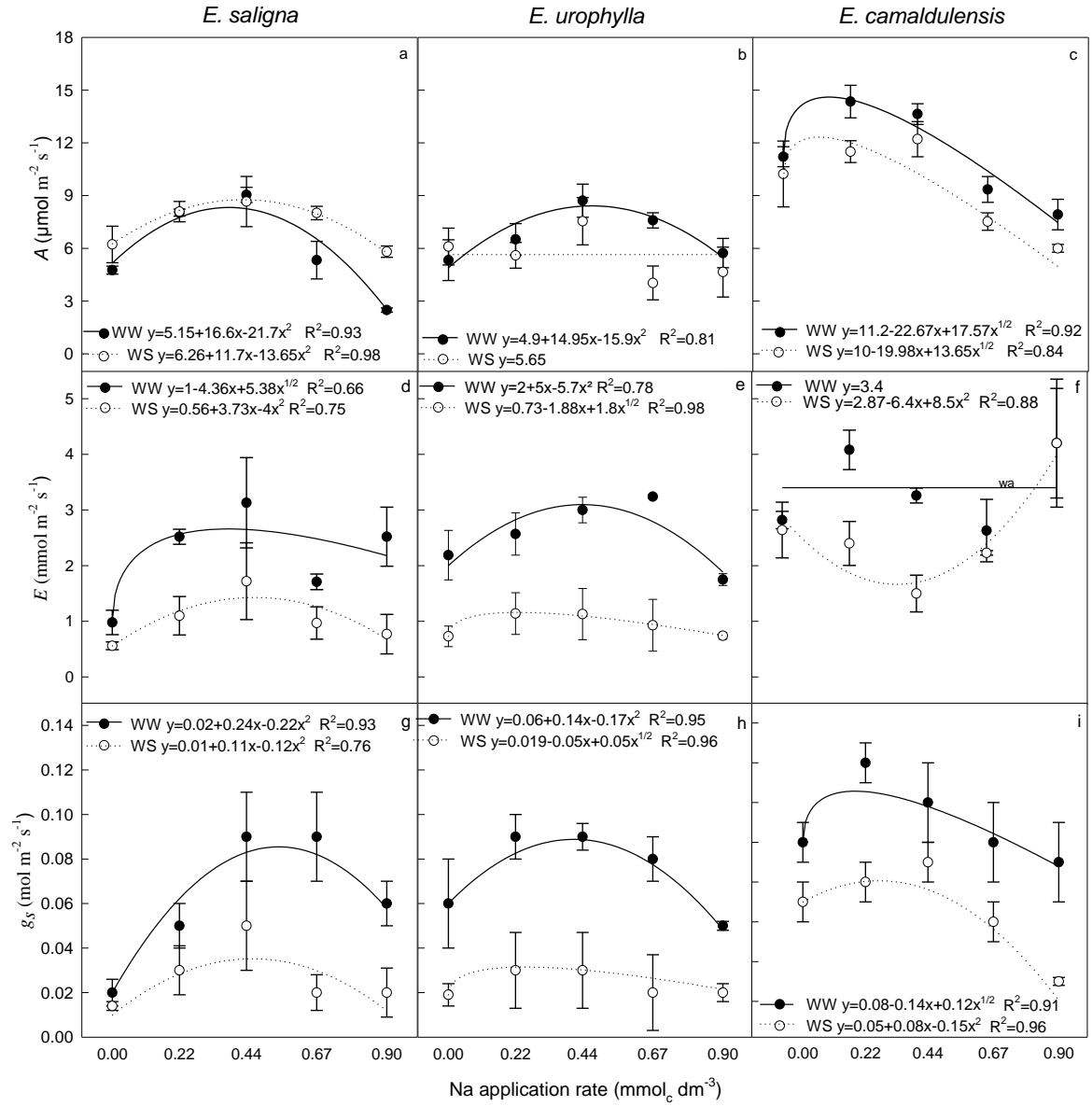
Figure 6- Intrinsic (a-c), instantaneous (d-f) and long-term (g-i) water-use efficiencies in leaves of *E. saligna* (a, d and g) , *E. urophylla* (b, e and h) and *E. camaldulensis* (c, f and i) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa)
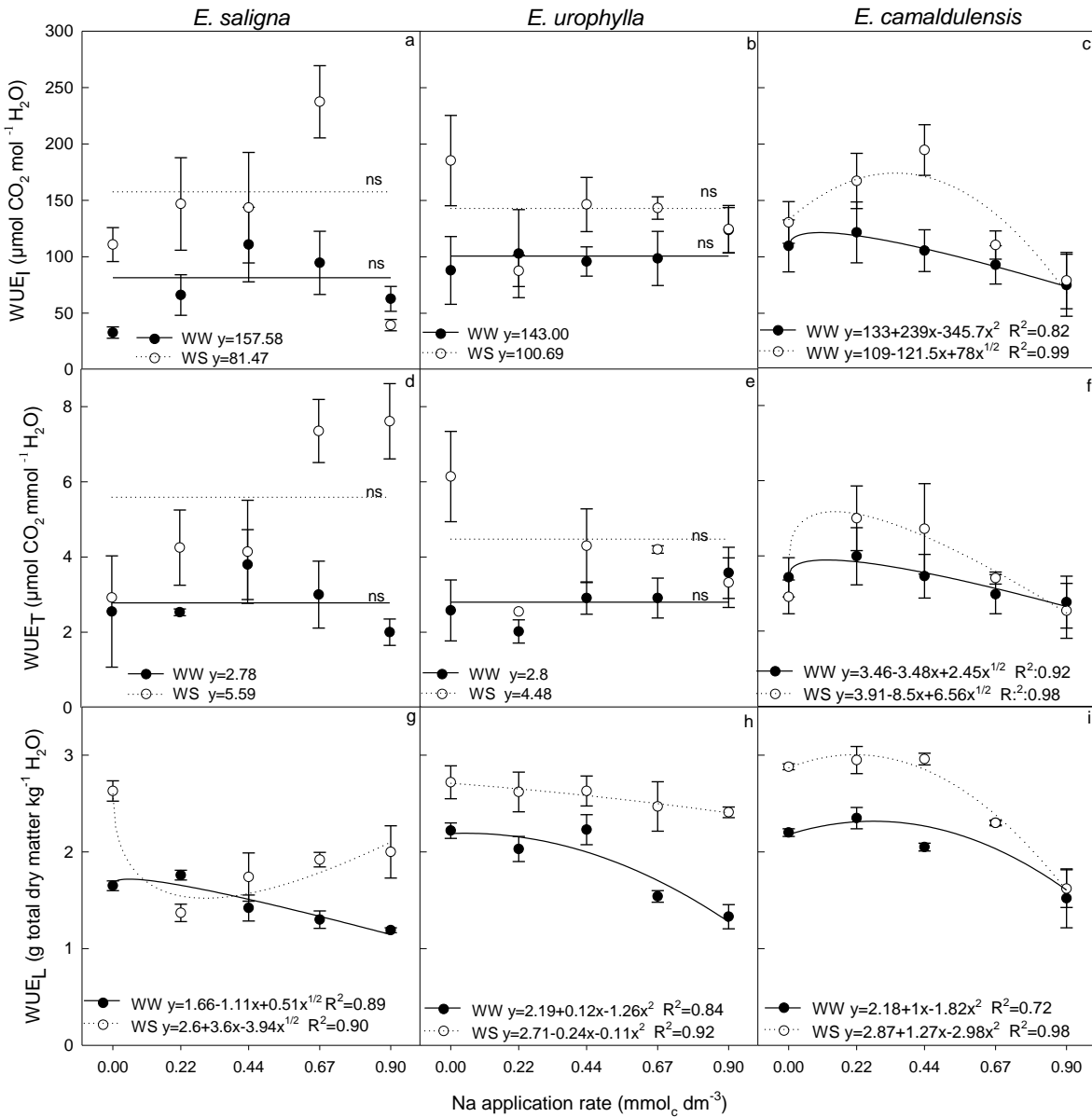

7.5. Pre-dawn and noon leaf water potential (Ψ_{WPD} and Ψ_{WN}, respectively)

In *E. saligna*, both Ψ_{WPD} and Ψ_{WN} were affected by Na rate, WS, and Na*WS (Figure 7a and d); while in *E. urophylla* (Figure 7b and e) and *E. camaldulensis* (Figure 7c and f), they were affected by Na rate and WS treatment. The Ψ_{WPD} of all genotypes was lower under WS than under WW. Specimens of *E. saligna* under both water supply conditions and o *E. urophylla* under WW decreased their Ψ_{WPD} values at low to intermediate Na rates, reaching the highest Ψ_{WPD} value at the highest Na rate. The Ψ_{WPD} in *E. urophylla* plants grown under WS decreased

with increasing Na application rates. The Ψw_{PD} of *E. camaldulensis* increased by up to 0.67 $mmol_c\ dm^{-3}$ of Na in both water regimes. As expected, the opposite occurred in Ψw_N, with lower values observed under WS than under WW. For *E saligna* seedlings grown under WW as well as for *E. urophylla* subjected to both water supply conditions, the lowest Ψw_N values were found at the low Na rates (0.22 and 0.44 $mmol_c\ dm^{-3}$). Compared to the other genotypes, the Na application rates linearly decreased the Ψw_N in *E. camaldulensis*. The Ψw_N values under WS conditions decreased by 25, 10, and 55% for *E. saligna, E. urophylla*, and *E. camaldulensis*, respectively, at all Na rates.

Figure 7- Pre-dawn- Ψw_{PD} (a-c) and noon- Ψw_N (d-f) leaf water potential of *E. saligna* (a and d) *E. urophylla* (b and e) and *E. camaldulensis* (c and f) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4)
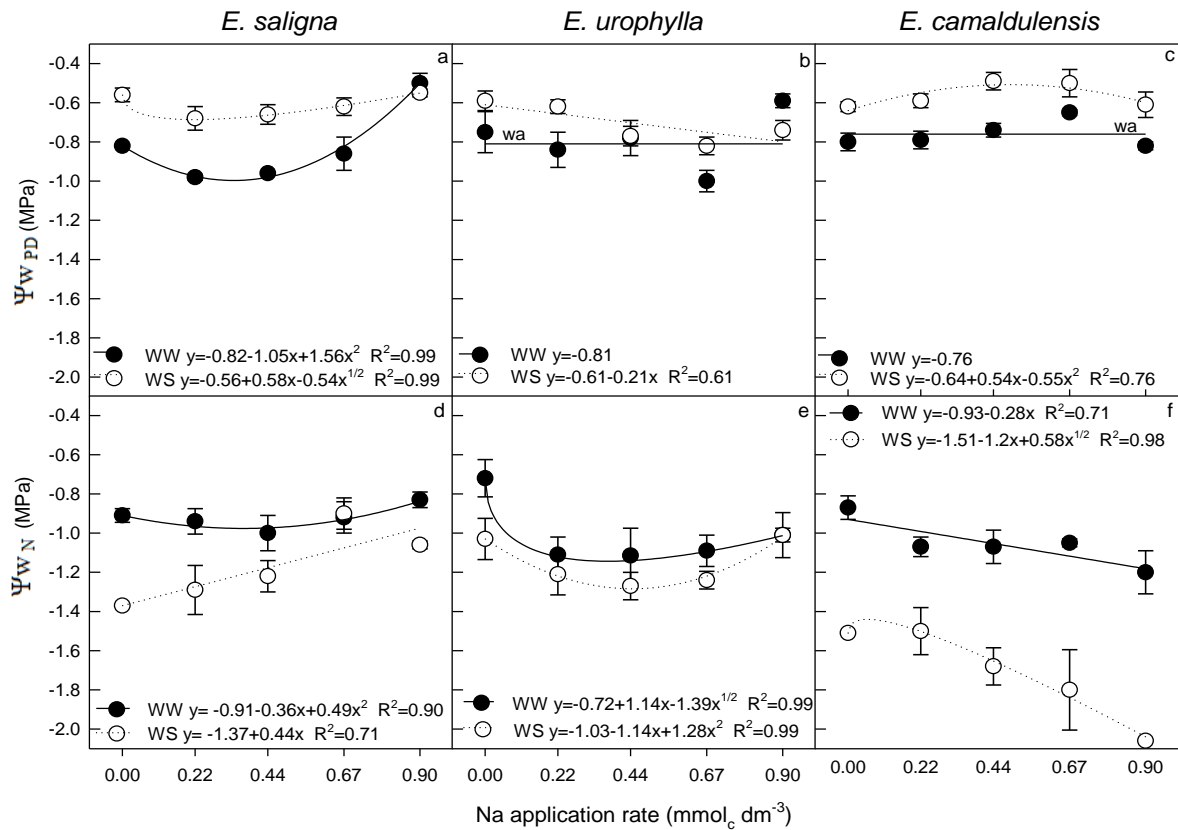
7.6. Leaf carbon isotope composition ($\delta^{13}C$ ‰)

The factors Na, WS, and Na*WS significantly influenced leaf carbon isotopic composition ($\delta^{13}C$ ‰) of *E. saligna* and *E. urophylla*, whereas for *E. camaldulensis*, it was affected by Na and WS (Figure 8a, b, and c). Under WW, for *E. saligna* and *E. camaldulensis*, the $\delta^{13}C$ increased with low Na application rates (0.22 $mmol_c\ dm^{-3}$ of Na) and decreased with

increasing Na rates. In contrast, the values for *E. urophylla* were lower at low rates (0.22 mmol$_c$ dm^{-3} of Na). In all genotypes, the Na rate of 0.9 mmol$_c$ dm^{-3} led to the lowest δ^{13}C values. Under WS, in *E. saligna*, the δ^{13}C decreased with intermediate Na rates (0.44 and 0.67 mmol$_c$ dm^{-3} of Na) and increased at the highest Na rate. In *E. urophylla* and *E. camaldulensis*, the δ^{13}C decreased with increasing Na rates. Drought stress led to 1.25, 0.47, and 0.91‰ higher δ^{13}C values of, respectively, *E. saligna*, *E. urophylla*, and *E. camaldulensis* when compared to WW.

Figure 8- δ^{13}C of *E. saligna*, *E. urophylla* and *E. camaldulensis* seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa).Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa)
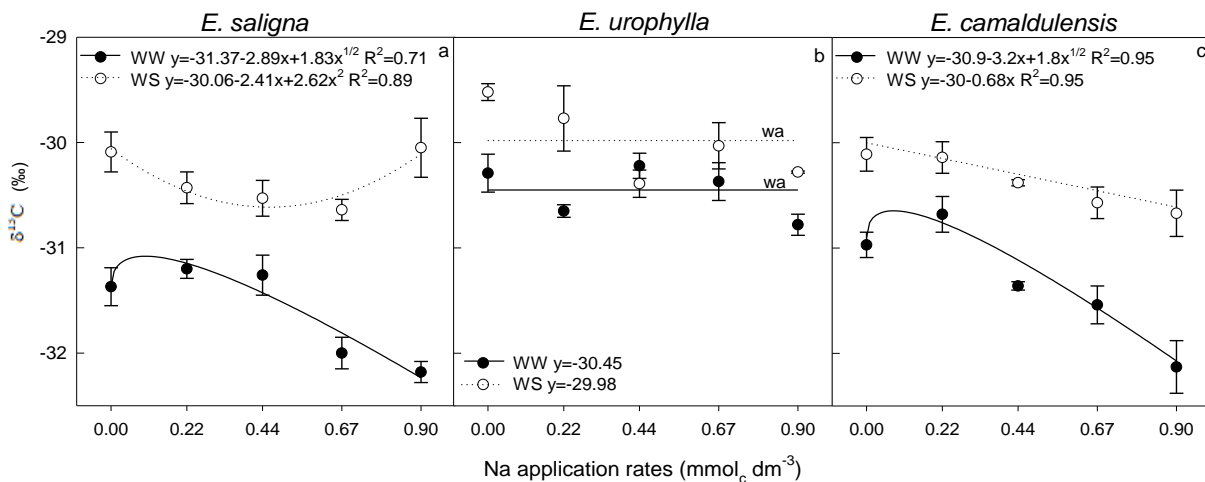
7.7.   Chlorophyll content (Chl)

The Chl was only influenced by WS in *E. saligna* and by Na and WS in *E. urophylla* and *E. camaldulensis* (Figure 9 a, b, and c). Overall, the Chl content decreased with increasing Na rates, being lower at intermediate Na rates (0.22 and 0.44 mmol$_c$ dm^{-3} of Na), under both water conditions. The WS regime led to a 25, 20, and 15% lower Chl value in *E. saligna, E. urophylla*, and *E. camaldulensis*, respectively, for all Na rates.

Figure 9- Chlorophyll content of *E. saligna* (a), *E. urophylla* (b) and *E. camaldulensis* (c) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa)
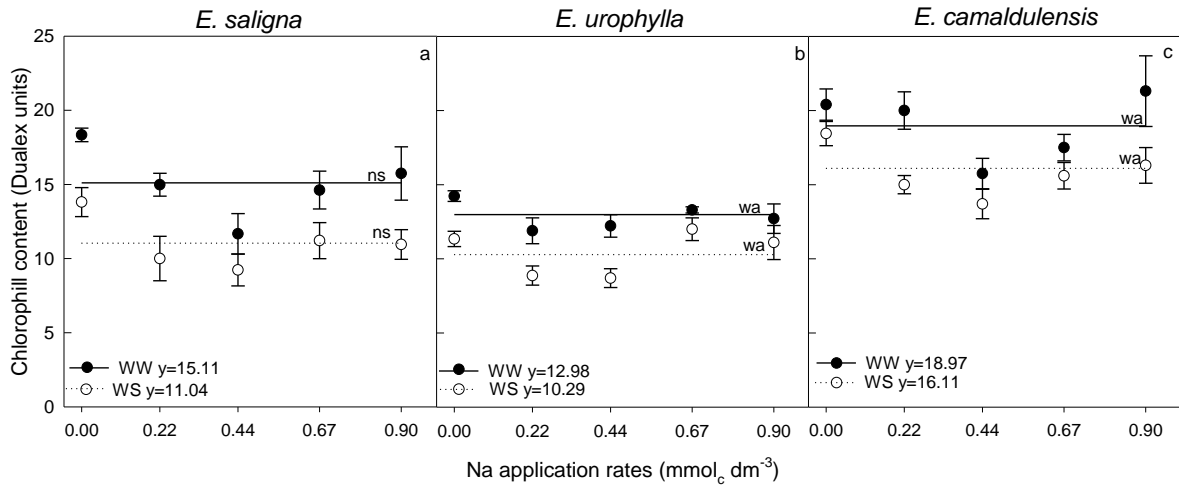
7.8.   Leaf K: Na ratio, K and Na accumulation and efficiency of absorption and use

According to the estimated rate of Na to achieve the maximum total dry matter of *E. saligna*, *E. urophylla*, and *E. camaldulensis* under WW and WS conditions the optimal leaf K: Na ratios were 1.71 and 2.61, 2.94 and 2.28, and 2.13 and 3.20, respectively (Figure 10a, b, and c). The accumulation of Na and K was influenced by Na in *E. saligna* (Figure 11a and d), while in *E. urophylla*, Na accumulation was influenced by Na, WS, and Na*WS (Figure 11b); K accumulation was influenced by Na and WS (Figure 11e). In *E. camaldulensis*, K and Na accumulation was influenced by Na and WS (Figure 11c and f). Overall, K accumulation decreased and Na accumulation increased with increasing Na levels; under WW, the accumulation levels were higher than under WS.

In *E. saligna*, AE_{Na} and AE_K were affected by Na application and WS condition, respectively (Figure 12a and d), while UE_{Na} and UE_K were affected by Na, WS, and Na*WS (Figure 13a and d). In *E. urophylla*, AE_{Na} and AE_K were affected by Na application (Figure 12b and e) and UE_{Na} and UE_K by Na and WS (Figure 13b and e). In *E. camaldulensis*, EA_K was influenced by Na and WS (Figure 12f), while AE_{Na} (Figure 12c), UE_{Na}, and UE_K (Figure 13c and f) were affected by Na, WS, and Na*WS. Overall, AE_{Na} increased with higher Na supply, while AE_K decreased in *E. saligna* and *E. camaldulensis* and increased in *E. urophylla*, except for low Na application, which resulted in a lower AE_K under WS. Water stress decreased the AE of both K and Na compared to WW. The UE_{Na} decreased with increasing Na levels, while the opposite occurred for UE_K. Water stress decreased the UE of both K and Na compared to WW.

Figure 10- Leaf K: Na ratio of *E. saligna* (a), *E. urophylla* (b) and *E. camaldulensis* (c) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4)
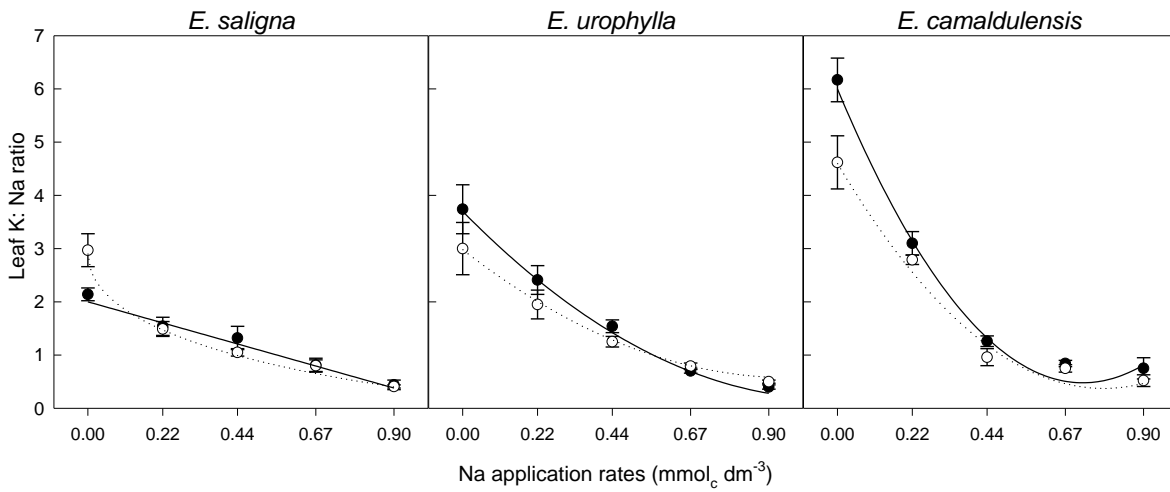

Figure 11- Na accumulation (a-c) and leaf K accumulation (d-f) of *E. saligna* (a and d), *E. urophylla* (b and e) and *E. camaldulensis* (c and f) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4)
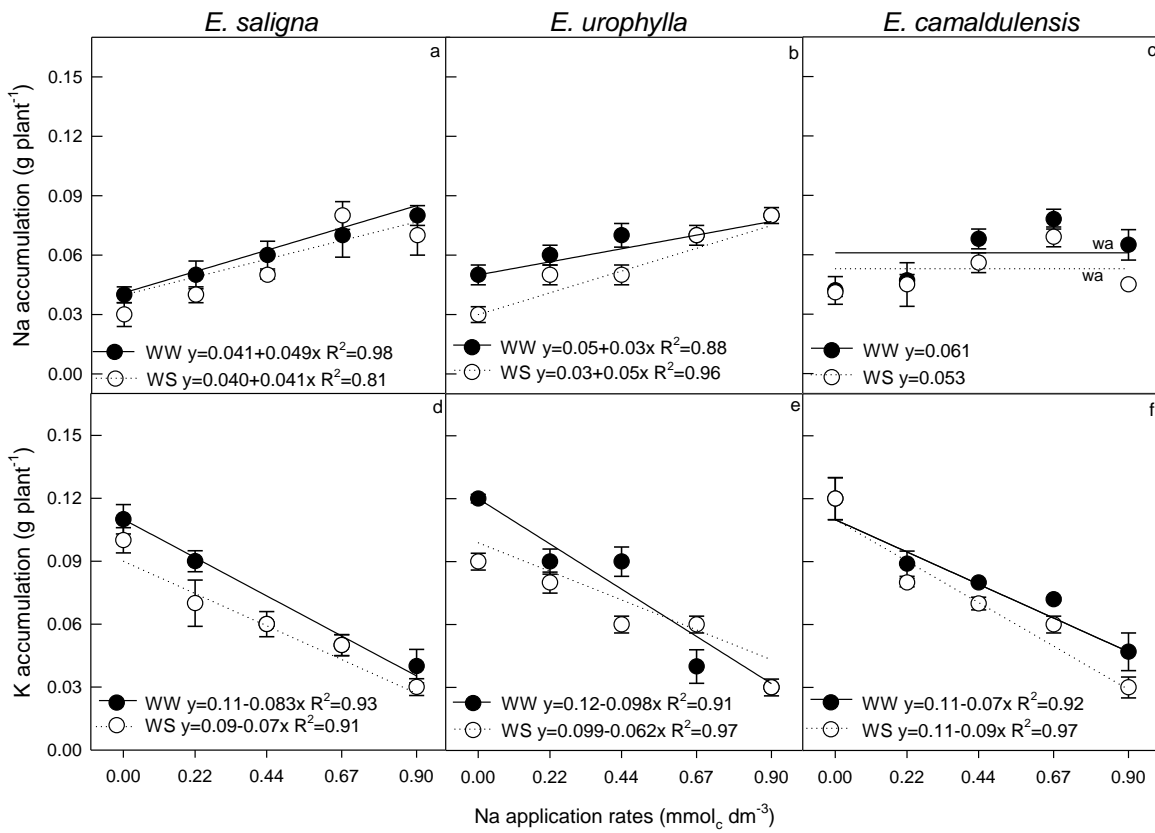
Figure 12- Na (a-c) and K (d-f) absorption efficiency of *E. saligna* (a and d) *E. urophylla* (b and e) and *E. camaldulensis* (c and f) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa)
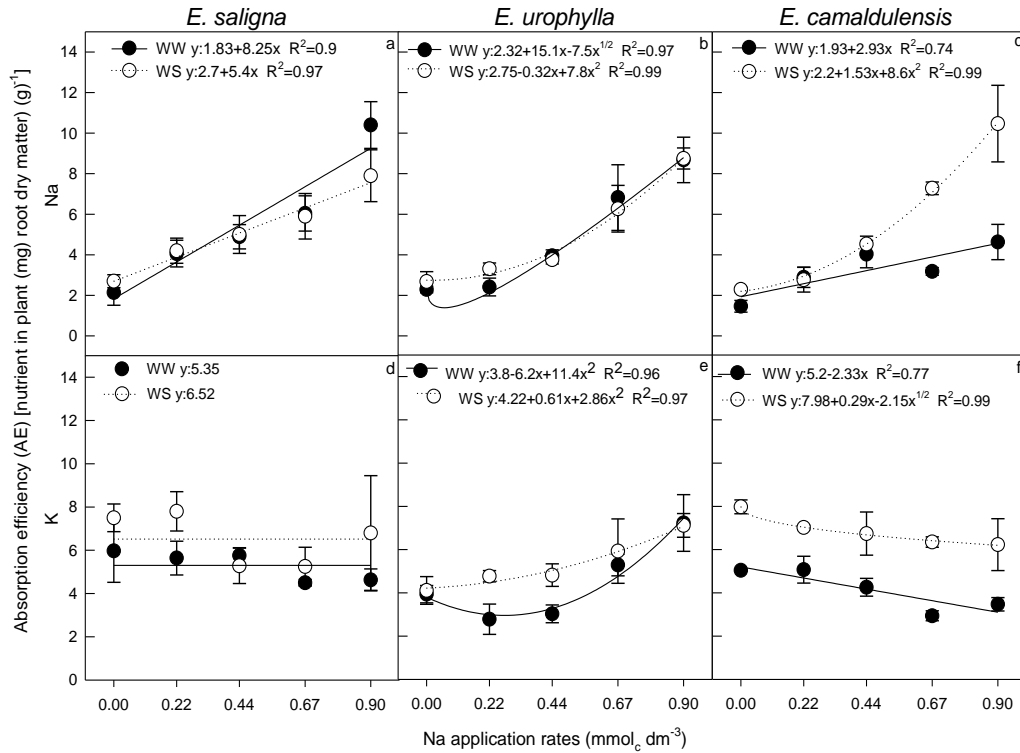

Figure 13- Na (a-c) and K (d-f) use efficiency of *E. saligna* (a and d) *E. urophylla* (b and e) and *E. camaldulensis* (c and f) seedlings under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition. Vertical bars indicate standard errors between blocks (n=4). The adjustment model is indicated by not significant (ns) and without suitable adjustment (wa)
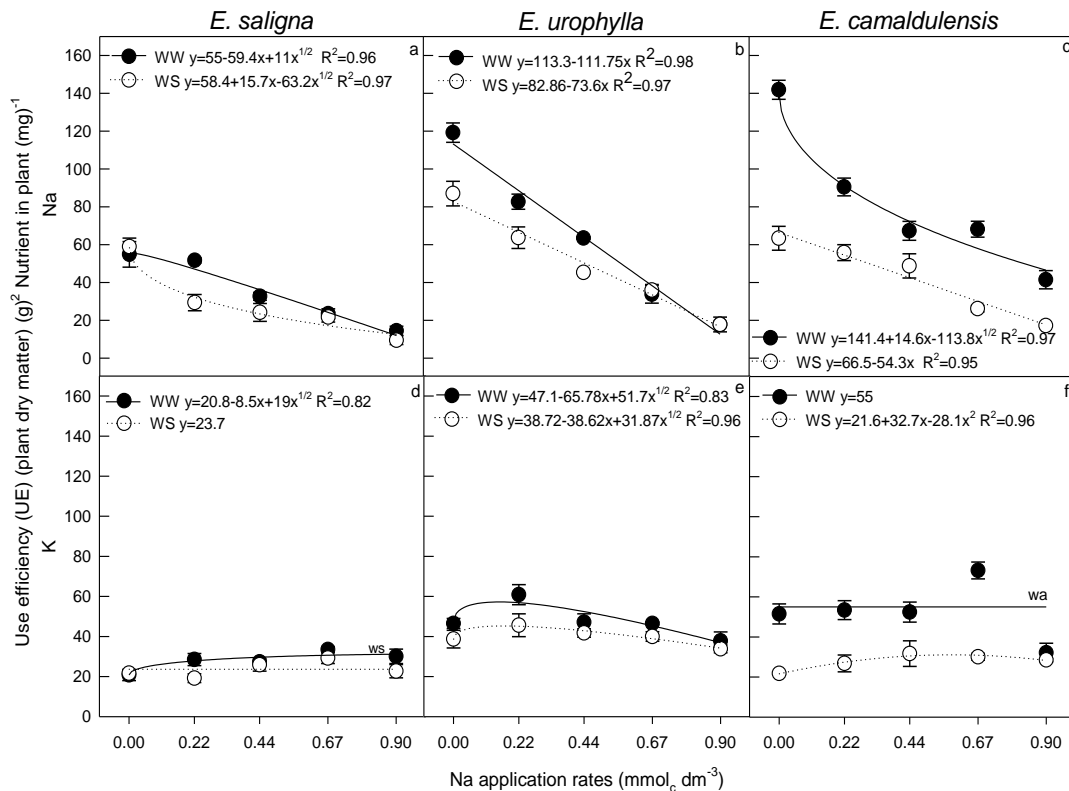
7.9. Characterization among genotypes

According to the Pearson's correlation coefficients, we highlight the positive correlation between $\delta^{13}C$ and WUE_L in all genotypes (Figure 14). The K leaf concentration [K] was negatively related to Na leaf concentration [Na], which also had a negative correlation with the TDM of all genotypes. Furthermore, the TDM of *E. urophylla* (Figure 14b) and *E. camaldulensis* (Figure 14c) was positively correlated to UE_K, which also had a positive correlation with g_s in all genotypes. Considering the 95% confidence ellipse analysis, *E. saligna* had a negative correlation among the mean vectors of variables under WW and WS, with a low correlation between water conditions (WW and WS) and a higher variance in WW (Figure 15a). In *E. urophylla*, there was a positive correlation among the mean vectors, besides the low correlation of WW and WS, as well as higher variance in WW (Figure 15b). In *E. camaldulensis* it was found a positive correlation under both conditions with a similar variance (Figure 15c).

In *E. saligna* (Figure 16a), the total variance was explained by 64% (PC1 + PC2), with PC1 being explained by Ψw_N, UE_K, [K], and $\delta^{13}C$, while PC2 was explained by TDM, Std_{AB}, and [Na]. The parameters *A*, WUE_L, LA, Chl, *E,* and g_s contributed with average weights to explain the data variance in PC1 and PC2. Under WW, low to intermediate Na rates (up 0.44 $mmol_c$ dm^{-3}) were characterized by higher values of Chl, TDM, and Std_{AB} and lower values of [Na] and $\delta^{13}C$. Plants under lower Na rates and WS showed higher WUE_L and $\delta^{13}C$ values. The Na rate of 0.9 $mmol_c$ dm^{-3} resulted in higher [Na] levels and lower [K], TDM, and Std_{AB} values. In *E. urophylla* (Figure 16c), the total variance was explained by 66 % (PC1 + PC2), with PC1 being explained by g_s and *E* and PC2 by WUE_L, $\delta^{13}C$, [K], TDM, and [Na]. The parameters Std_{AB}, UE_K, *A*, Ψw_N, LA, and Chl contributed with average weights to explain the data variance in PC1 and PC2. Under WW, low to intermediate Na rates (0 to 0.44 $mmol_c$ dm^3) were characterized by higher values of TDM, Std_{AB}, UE_K, *A*, LA, g_s, and *E*.

The higher Na rates were identified by higher [Na] and lower TDM, [K], WUE_L, and $\delta^{13}C$ levels. Under WS, Na rates of 0 and 0.22 $mmol_c$ dm^{-3} resulted in higher WUE_L and $\delta^{13}C$ and lower LA*, E*, and g_s levels. In *E. camaldulensis* (Figure 16e), the total variance was explained by 77% (PC1 + PC2), with PC1 being explained by TDM, g_s, LA, Ψw_N, and [Na] and PC2 by WUE_L, $\delta^{13}C$, and *E*. The parameters [K], Std_{AB}, *A*, EU_K, and Chl contributed with average weights to explain the data variance in PC1 and PC2. Under WW, low to intermediate Na rates were characterized by higher values of TDM, g_s, LA, Ψw_N, and EU_K and by lower values of [Na]. Moreover, the Na rate of 0.9 $mmol_c$ dm^{-3} resulted in higher *E* and [Na] and lower $\delta^{13}C$, WUE_L, and [K] values. Plants under WS with low to intermediate Na supply were characterized by higher values of [K], WUE_L, and $\delta^{13}C$ and by lower *E* values.

Cluster analysis showed the formation of three main groups among the treatments in all genotypes. In *E. saligna* (Figure 16b), group 1 was represented by Na rates of 0, 0.22, and 0.44 $mmol_c$ dm^- under WW, group 2 by the rate of 0.67 $mmol_c$ dm^{-3} under WW and the rates of 0, 0.22, 0.44, and 0.67 $mmol_c$ dm^{-3} under WS, while group 3 contained plants which only received Na Na (0.9 $mmol_c$ dm^{-3} of Na) under both conditions. In *E. urophylla* (Figure 16d), group 1 was comprised of plants receiving no Na under WW and 0.22, 0.44, and 0.9 $mmol_c$ dm^{-3} of Na under WS, while group 2 contained plants receiving 0.67 and 0.9 $mmol_c$ dm^{-3} of Na under WS. In group 3, the plants received 0.22, 0.44, and 0.67 $mmol_c$ dm^{-3} of Na under WW and no Na under WS. In *E. camaldulensis* (Figure 16f), group 1 was composed of plants receiving 0, 0.22, 0.44, and 0.67 $mmol_c$ dm^{-3} of Na under WW, while in group 2, plants received 0, 0.22, 0.44, and 0.67 $mmol_c$ dm^{-3} of Na under WS. In group 3, plants received only 0.9 $mmol_c$ dm^{-3} of Na under both water regimes.

Figure 14- Pearson correlations of *E. saligna* (a), *E. urophylla* (b) and *E. camaldulensis* seedlings (c) under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition

a — *E. saligna*

	TDM	LA	SLA	Std_{AB}	Std_{AD}	A	E	g_s	WUE_I	WUE_T	WUE_L	Ψ_{WPD}	Ψ_{WN}	$\delta^{13}C$	Chl	[Na]	[K]	UE_K
TDM																		
LA	0.51																	
SLA	-0.2	0.49																
Std_{AB}	0.79**	0.35	-0.29															
Std_{AD}	0.4	-0.03	-0.82**	0.5														
A	0.22	-0.21	-0.77**	0.61	0.71*													
E	0.03	-0.08	0.17	0.26	-0.27	0.11												
g_s	-0.16	-0.23	0.24	0.21	-0.37	0.1	0.86**											
WUE_I	-0.12	-0.32	-0.55	0.04	0.24	0.59	-0.15	-0.1										
WUE_T	-0.45	-0.32	-0.41	-0.37	0.35	0.33	-0.44	-0.39	0.44									
WUE_L	0.4	-0.05	-0.55	0	0.49	0.12	-0.61	-0.67*	0.12	0.29								
Ψ_{WPD}	-0.56	-0.25	0	-0.85**	-0.2	-0.42	-0.54	-0.55	0.16	0.35	0.32							
Ψ_{WN}	-0.07	0.29	0.6	-0.13	-0.46	-0.42	0.45	0.36	-0.22	-0.07	-0.53	-0.28						
$\delta^{13}C$	0.03	-0.23	-0.74*	-0.02	0.70*	0.49	-0.64*	-0.68*	0.31	0.61	0.74*	0.36	-0.75*					
Chl	0.47	0.62	0.71*	0.12	-0.47	-0.69*	0.1	0.02	-0.61	-0.64*	-0.11	-0.24	0.53	-0.63				
[Na]	-0.87***	-0.5	-0.81**	-0.2	-0.28	-0.14	-0.01	-0.04	0.64*	-0.16	0.52	0.21	0.08	-0.37				
[K]	0.44	0.17	-0.36	0.59	0.42	0.51	-0.32	-0.3	0.19	-0.17	0.24	-0.21	-0.73*	0.47	-0.18	-0.63*		
UE_K	-0.16	-0.32	0.17	-0.1	-0.36	-0.15	0.57	0.67*	0.14	-0.13	-0.4	-0.25	0.65*	-0.67*	0.14	0.21	-0.71*	

b — *E. urophylla*

	TDM	LA	SLA	Std_{AB}	Std_{AD}	A	E	g_s	WUE_I	WUE_T	WUE_L	Ψ_{WPD}	Ψ_{WN}	$\delta^{13}C$	Chl	[Na]	[K]	UE_K
TDM																		
LA	0.41																	
SLA	-0.22	0.67*																
Std_{AB}	0.61	0.27	-0.14															
Std_{AD}	-0.47	-0.08	0.16	-0.34														
A	0.44	0.76*	0.4	0.44	-0.39													
E	0.38	0.79**	0.59	0.39	0.16	0.67*												
g_s	0.49	0.83**	0.58	0.45	0.13	0.66*	0.97***											
WUE_I	-0.4	-0.45	-0.37	-0.04	-0.25	-0.33	-0.66*	-0.64*										
WUE_T	-0.39	-0.39	-0.34	-0.03	-0.33	-0.23	-0.61	-0.65*	0.95***									
WUE_L	0.29	-0.42	-0.82***	0.19	-0.5	-0.13	-0.64*	-0.59	0.39	0.4								
Ψ_{WPD}	-0.09	-0.22	-0.06	-0.17	-0.32	-0.29	-0.61	-0.53	0.34	0.4	0.29							
Ψ_{WN}	0.2	0.05	-0.09	0.05	0.39	-0.12	0.21	0.2	-0.24	-0.16	-0.27	0.13						
$\delta^{13}C$	0.14	-0.4	-0.61	0.33	-0.53	-0.18	-0.49	-0.54	0.39	0.54	0.74*	0.34	-0.14					
Chl	0.08	0.36	0.26	0.03	0.51	-0.03	0.57	0.51	-0.27	-0.22	-0.62	-0.33	0.71*	-0.33				
[Na]	-0.67*	0.01	0.32	-0.44	0.83**	-0.18	0.03	-0.01	-0.16	-0.21	-0.38	-0.18	-0.02	-0.47	0.1			
[K]	0.77**	0.14	-0.27	0.34	-0.53	0.21	0.1	0.15	-0.15	-0.06	0.23	0.31	0.53	0.26	0.18	-0.80**		
UE_K	0.79**	0.48	0.06	0.46	-0.17	0.42	0.66*	0.72*	-0.65*	-0.70*	-0.07	-0.57	-0.05	-0.18	0.19	-0.36	0.33	

c — *E. camaldulensis*

	TDM	LA	SLA	Std_{AB}	Std_{AD}	A	E	g_s	WUE_I	WUE_T	WUE_L	Ψ_{WPD}	Ψ_{WN}	$\delta^{13}C$	Chl	[Na]	[K]	UE_K
TDM																		
LA	0.91***																	
SLA	0.9***	0.92***																
Std_{AB}	0.81**	0.6	0.79**															
Std_{AD}	0.89***	0.73*	0.78***	0.81**														
A	0.72*	0.78**	0.71*	0.59	0.48													
E	-0.12	0.08	-0.21	-0.54	-0.1	-0.18												
g_s	0.77**	0.93***	0.85**	0.51	0.6	0.84**	0.08											
WUE_I	0.1	-0.03	0.11	0.38	0.01	0.53	-0.74*	0.13										
WUE_T	0.2	0.1	0.23	0.43	0.05	0.63	-0.62	0.26	0.95***									
WUE_L	0.28	0.05	0.24	0.6	0.2	0.48	-0.79***	0.12	0.89***	0.89***								
Ψ_{WPD}	-0.36	-0.63*	-0.39	0.17	-0.19	-0.27	-0.70*	-0.58	0.54	0.38	0.54							
Ψ_{WN}	0.75*	0.91***	0.86***	0.41	0.58	0.57	0.15	0.84**	-0.18	-0.04	-0.08	-0.78**						
$\delta^{13}C$	-0.03	-0.32	-0.26	0.2	-0.12	0.16	-0.48	-0.31	0.69*	0.68*	0.75*	0.63	-0.52					
Chl	0.16	0.36	0.15	-0.33	0.04	-0.04	0.62	0.33	-0.58	-0.45	-0.47	-0.84**	0.6	-0.5				
[Na]	-0.68*	-0.74*	-0.70*	-0.46	-0.4	-0.71*	0.11	-0.68*	-0.25	-0.41	-0.41	0.53	-0.79**	-0.03	-0.44			
[K]	0.24	0.12	0.09	0.26	-0.04	0.42	-0.36	0.06	0.53	0.6	0.70*	0.08	0.06	0.70*	0.03	-0.64*		
UE_K	0.80**	0.79**	0.79**	0.59	0.89***	0.35	0.11	0.65*	-0.3	-0.27	-0.19	-0.44	0.73*	-0.52	0.25	-0.32	-0.34	

Figure 15- 95 % Confidence ellipse of *E. saligna* (a), *E. urophylla* (b) and *E. camaldulensis* seedlings (c) under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition.
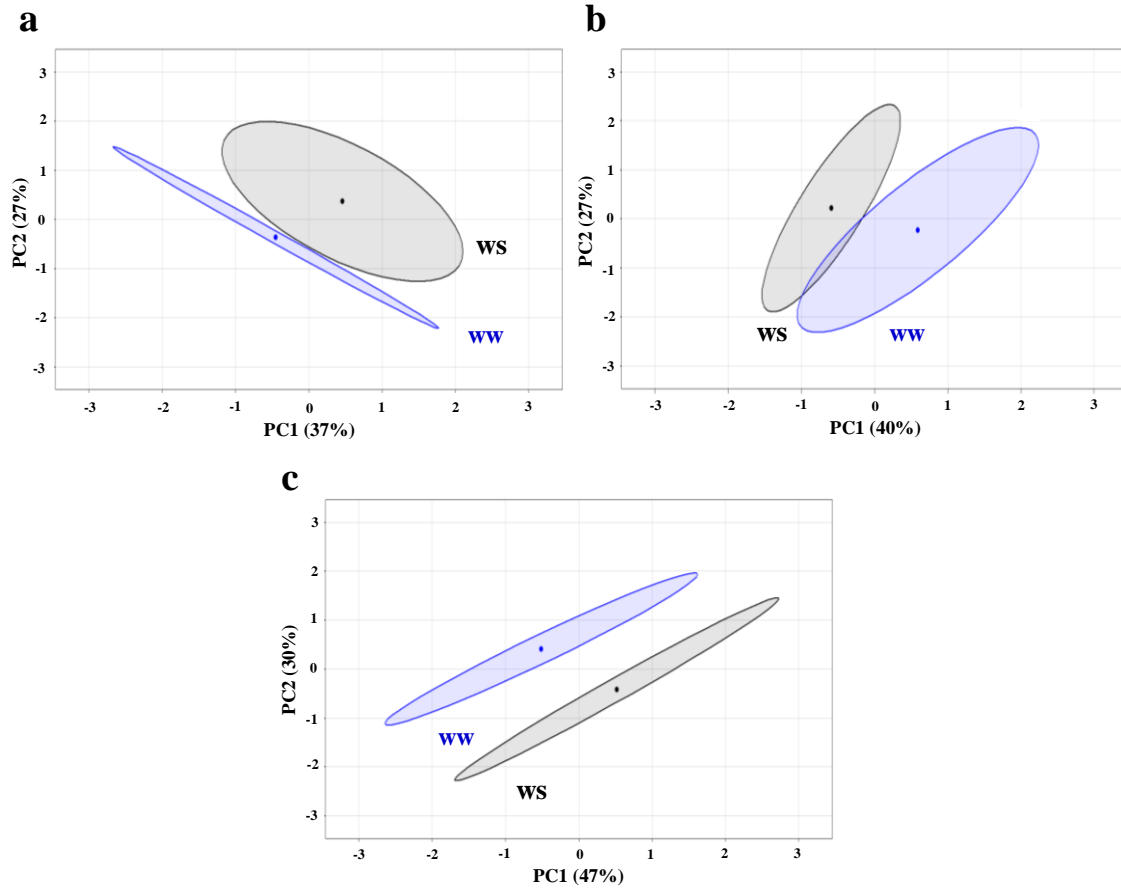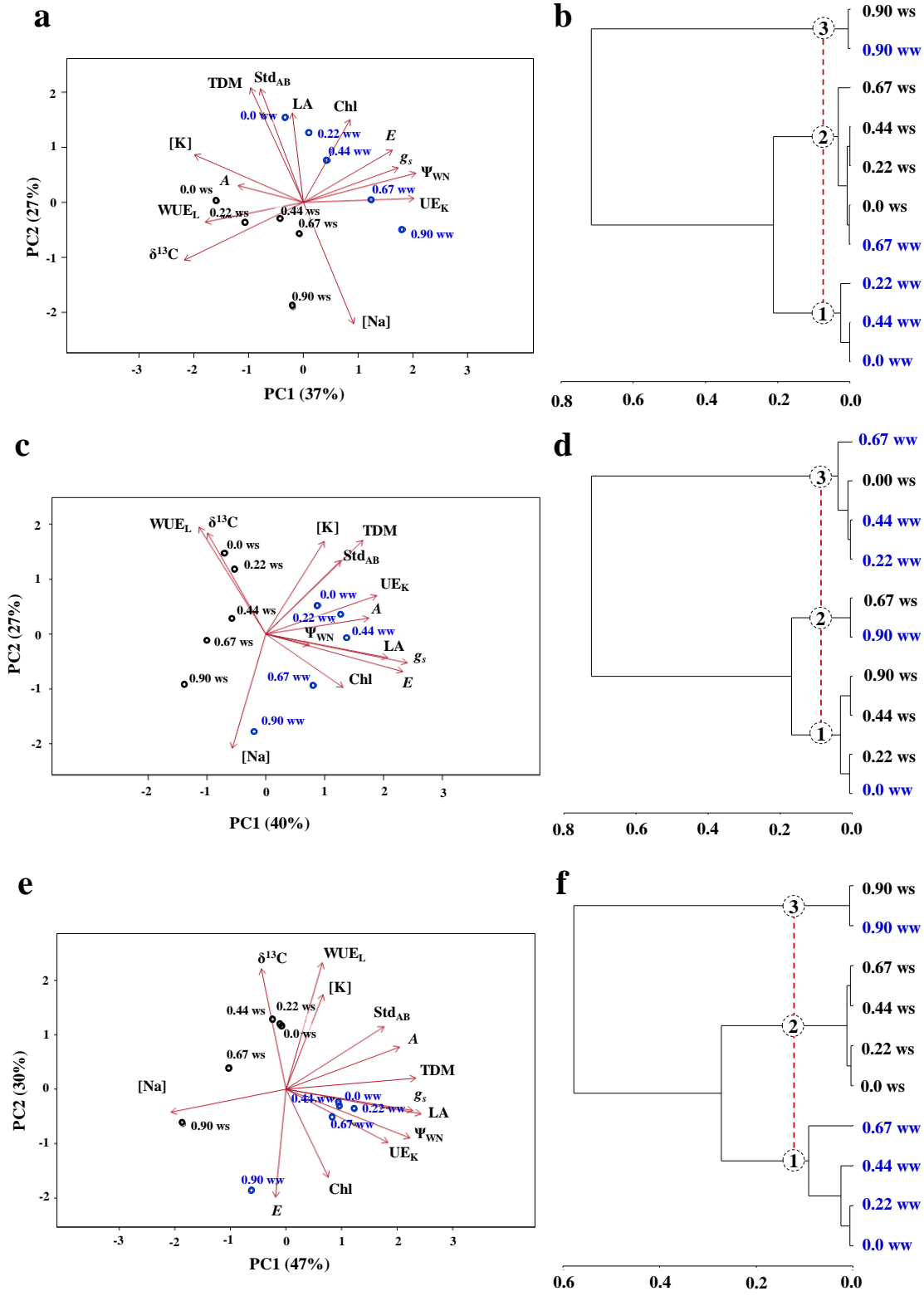
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Figure 16- Principal component analysis- PCA (a, c and e) and hierarchical cluster analyses (b, d and f) of *E. saligna* (a and b), *E. urophylla* (c and d) and *E. camaldulensis* seedlings (e and f), under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition

8.   Discussion

8.1.   Dry matter production

Root growth can be inhibited during prolonged drought periods (MASEDA; FERNÁNDEZ, 2016), hampering nutrient uptake and, consequently, greatly impacting plant nutrition (HU; BURUCS; SCHMIDHALTER, 2008). As observed in this study, plants grown under WS significantly decreased their RDM compared to plants under water stress, albeit only for a short period. The RDM of *E. saligna* (Figure 1a) and *E. camaldulensis* (Figure 1c) dropped due to Na supply, demonstrating the function of adequate K supply in root surface area development and water uptake (RÖMHELD; KIRKBY, 2010). In *E. urophylla* (Figure 1b), the low Na supply increased the RDM, resulting in a higher root: shoot ratio, indicating the allocation of photosynthates to root growth, as occurs in plants grown under drought condition (MERCHANT et al., 2006b), since carbon allocation to the root system (GALVEZ; LANDHAUSSER; TYREE, 2011), root hydraulic conductance (SILVA et al., 2004), and root cell wall changes (COSGROVE, 1997) are mechanisms for plants to expand the exploited area, allowing increases in nutrient and water uptake. In addition, the decrease in root: shoot ratio values, highlighted in *E. saligna* (Figure 1d) and *E. camaldulensis* (Figure 1f) under higher Na rates, indicates a higher reduction in shoot than in root mass. Overall, the TDM increased with low Na supply, as observed to a higher extent in *E. camaldulensis* (Figure 1i), confirming Na as a beneficial element in shoot dry matter, except for *E. saligna* under WS (Figure 1g); this species is considered less tolerant to drought, since Na impaired plant growth. Drought conditions greatly reduced plant growth, mainly at low to moderate Na application rates, which showed higher growth compared to the highest Na rate (0.9 m*M*).

Furthermore, the estimated ideal percentage of substitution increased according to the drought tolerance of the genotypes, reaching 30% in *E. camaldulensis* under WW, which, although this genotype has a higher drought-tolerance, decreased its TDM by 25% due to drought, the highest decrease among the genotypes. These responses reflect the higher water use of plants receiving low Na supply compared to those only receiving K or of K-deficient plants (0.9 mmol$_c$ dm^{-3} of Na). As also concluded by Schulze et al. (2012), low to moderate Na contents are facilitate the growth of many plant species due to the replacement of K by Na in the vacuole, making more K available to the cytosol (RODRÍGUEZ-NAVARRO; RUBIO, 2006). Similar results suggest the possibility of using Na in the absence of K to promote plant growth, as Na application greatly improved the growth of *E. grandis* compared to the growth of plants in unfertilized sites under WW (EPRON et al., 2012; BATTIE-LACLAU et al., 2013) and WS (BATTIE-LACLAU et al., 2014b).

Despite the similarities between the Na^+ and K^+ hydrated ionic radii, causing a difficult discrimination between the two ions by transport proteins (ADAMS et al., 2005), the substitution of K by Na in plant nutrition is restricted (FAUST; SCHUBERT, 2017), as observed for K-deficient plants (or plants receiving higher Na levels). Under salinity conditions, which affect more than 20% of the global agriculture lands, plants increase Na^+, Cl^-, and proline contents and reduce the accumulation of K^+, Ca^{2+}, and Mg^{2+} in stems and leaves (ALY; AL-BARAKAH; EL-MAHROUKY, 2018). Thus, at high concentrations, Na can increase the osmotic potential and disrupt the membrane integrity of roots (COSKUN et al., 2013) due to the toxicity caused by an excess of Na, which exceeds the cells ability to compartmentalize Na^+ in the vacuole. As a result, it accumulates in the cytoplasm, leading to enzyme activity inhibition (MUNNS; PASSIOURA, 1984), leaf necrosis, and sudden death of entire shoots (ADAMS et al., 2005). Almost 98% of the crop species are glycophytes (intolerant to salt), being inhibited by Na concentrations above 20 $mmol_c$ dm^{-3} (BLUMWALD; AHARON; APSE, 2000), although they can metabolically use Na to some degree, as observed in the current study and in *Eucalyptus* grown in nutrient solution (MATEUS et al., 2019).

Thus, the increase in plant yield under low rates of Na shows the benefits of Na to a certain degree. Plant yield also decreased due to K deficiency, an essential element that cannot be completely replaced (ARNON; STOUT, 1939) due to it specific functions, such as enzyme activation, initiation (SPYRIDES, 1964), elongation (LUBIN; ENNIS, 1964), termination of translation (NÄSLUND; HULTIN, 1971), and conformation of ribosomes (KLEIN, 2004).

8.2. Leaf area (LA) and specific leaf area (SLA)

The LA development is directly affected by the plant mineral nutritional status (MARSCHNER, 2012), especially K nutrition (EGILLA; DAVIES; BOUTTON, 2005; BATTIE-LACLAU et al., 2013; TAVAKOL et al., 2018). As Na can partially substitute K in the vacuole, it also contributes to enhance cell turgor and expansion (WAKEEL et al., 2011), which was observed in the control (0 $mmol_c$ dm^{-3} of Na) and at low Na rates. Under K deficiency, plants exhibit a poor root system, yield reductions, lodging, yellowing in their leaf margins, delayed stomatal closure, and even incomplete pore closure (WANG et al., 2013), as noticed for K-deficient plants (0.9 $mmol_c$ dm^{-3} of Na), dramatically reducing LA and sunlight interception (BEDNARZ; OOSTERHUIS; EVANS, 1998).

Plant water use is strongly associated to LA (ZHU et al., 2015). If its formation and expansion is reduced, biomass is decreased during drought periods as a mechanism to reduce the transpiration rate (HU et al., 2012), avoiding the negative impacts of water limitation. For this, the plant reduces its tissue water content as a coordination of physiological and structural adaptations (MERCHANT et al., 2006b), which promotes cell contraction, turgor loss (COSGROVE, 1997), and slower leaf expansion (PITA-BARBOSA; HODECKER; BARROS, 2016). Our findings clearly suggest that all genotypes are sensitive to drought stress, as indicated by the marked growth reduction. Furthermore, *E. saligna* (Figure 2a), as a drought-sensitive genotype, decreased its LA (15%) to a lower degree than *E. urophylla* and *E. camaldulensis* (Figure 2b and c), which greatly reduced (65%) the LA to cope with drought.

All genotypes have a lower SLA under WS than WW conditions, which increased with increasing Na rates, as noticed in *E. saligna* and *E. urophylla* (Figure 2d and e), suggesting a pronounced drop in leaf dry matter compared to LA. These responses indicate variations in leaf thickness, such as lower leaf dry matter per unit of leaf area, a physiological characteristic in response to nutritional status and stress treatment (VENEKLAAS; SANTOS SILVA; DEN OUDEN, 2002).

8.3. Stomatal density

Contrasting responses of stomatal development among species, water regimes (ZHAO et al., 2015) and nutritional status (MATEUS et al., 2019) have been reported, with Std values ranging from 30 to 1.190 mm^{-2} in trees (WILLMER; FRICKER, 1996). Stomatal patterns are determined by the genetic makeup of each species (JAMES; BELL, 2000) and by environmental conditions (BERTOLINO; CAINE; GRAY, 2019). Modulations resulting from external signals perceived by mature leaves lead to systemic responses that moderate new leaf epidermis stomatal development (QI; TORII, 2018), allowing plants to adjust their stomatal pore area in response to environmental condition, thus affecting leaf gas exchange (BERTOLINO; CAINE; GRAY, 2019).

According to Tanaka et al. (2013) plants with lower Std values have considerably reduced levels of E and are able to grow larger, especially under WS conditions (DOHENY-ADAMS et al., 2012). Converse results were seen in the current study, as *E. saligna* (Figure 3d) had two-fold higher Std$_{AB}$ values than *E. urophylla* and *E. camaldulensis* (Figure 3e and f), which had the highest values of A, E, g_s and TDM. Thus, plants with fewer stomata were capable to increase g_s and, consequently, A and E at the expense of higher WUE values.

The stomata distribution on the leaf surface is also related to the amount of energy used in transpiration (latent heat transfer) (JARVIS; MCNAUGHTON, 1986). In *E. saligna* and *E. urophylla* (Figure 4A and B), stomata occurred on the underside (hypostomatous leaves), which is more common in plants of mesophytic habitats. However, the stomata of *E. camaldulensis* (Figure 4C and F) - the drought-tolerant genotype - occurred on both sides (amphistomatous leaves), which is common in arid environments (PARKHURST, 1978). Hypostomatous leaves have been correlated with leaf thickness (PARKHURST, 1978) and reduced internal diffusion resistance by the lower pathway length of the CO_2 molecules to the carboxylation sites (MOTT; MICHAELSON, 1991). Furthermore, the occurrence of stomata on both sides would be advantageous in plants growing under high light intensity, where the internal CO_2 concentration (C_i) limits photosynthetic rates.

An increased Std was observed with partial replacement of K by Na in *Eucalyptus* under nutrient solution (MATEUS et al., 2019), improving leaf total pore area (FRANKS; BEERLING, 2009) and WUE, followed by a huge decrease in Std_{AB} in K-deficient plants. Regarding water availability, under WS, Std_{AB} decreased by 15 and 10% in *E. saligna* and *E. urophylla*, respectively, without a significant influence in *E. camaldulensis*, leading to a relatively lower g_s, as also observed for *A*, *E*, and TDM, besides significantly enhanced WUE and drought tolerance (WANG et al., 2016), suggesting adaptations to able survival under drought conditions (FRANKS et al., 2015) without negatively impacting nutrient uptake (HEPWORTH et al., 2016). These statements indicate that a lower Std level is an effective tool to improve *Eucalyptus* production during dry seasons.

8.4. Gas exchange

Overall, the g_s was also correlated to the control of *E* and *A* (Figure 5), considering simultaneous processes in *Eucalyptus* plants reported by Battie-Laclau et al. (2016) and Mateus et al. (2019). Higher gas exchange was observed with low Na doses (up to 0.44 mmol$_c$ dm^{-3} of Na), while gas exchange was considerably decreased at higher Na levels (0.9 mmol$_c$ dm^{-3} of Na), as also observed by Mateus et al. (2019).

Drought is considered the main environmental limiting factor of photosynthesis, even in plants well adapted to arid conditions (LAWLOR, 1995), resulting in turgor loss and affecting the hydraulic conductivity of plants (OLIVEIRA et al., 2014). Different adaptation mechanisms of drought tolerance to maintain the preservation of internal moisture have been reported for *Eucalyptus* species (WHITEHEAD; BEADLE, 2004). Morphological adaptations,

as prominent mechanisms to avoid the deleterious impacts of drought, include limiting water loss by reducing LA (MARSCHNER, 2012), improving root surface area (PITA-BARBOSA; HODECKER; BARROS, 2016), and stomatal closure (WARREN; BLEBY; ADAMS, 2007). Biochemical strategies such as osmotic changes (CALLISTER et al., 2008) include compatible solute accumulation, such as of soluble sugars and proline, which have also been considered a defense mechanism to maintain cell turgor and gas exchange (HESSINI et al., 2009), besides protecting cell membranes from rupturing, thereby normalizing the functions and structures of enzymes and proteins (VILLADSEN; RUNG; NIELSEN, 2005). Physiological adjustments, such as quick stomatal closure followed by g_s reduction due to the synthesis of high amounts of abscisic acid (ABA) and, consequently, a drop in E. Synthesized in the roots and transported to the shoots, ABA stimulates the efflux of K from the guard cells into the leaf apoplast, resulting in a loss of turgor pressure and a decrease in g_s (SAUTER; DAVIES; HARTUNG, 2001). As a consequence, plants also limit C_i, decreasing A, which may be driven by a decline in RuBisCO activity (PARRY et al., 2002), resulting from a loss of ATP synthase (TEZARA et al., 1999).

The K-deficient specimens of *E. saligna* and *E. camaldulensis* showed a marked g_s reduction under low Na rates when exposed to WS condition (Figure 5g and i). In contrast, in *E. urophylla*, g_s was more significantly reduced at low Na rates than in K-deficient plants subjected to drought (Figure 5h). Similar results have been observed by Benlloch-Gonzalez et al. (2010), who found a higher ethylene synthesis under K deficiency, combined with a significant decrease in g_s in plants only receiving K. The absence of K stimulates ethylene synthesis, impairing the action of abscisic acid (ABA) on stomata and delaying their closure (TANAKA et al., 2006). In contrast to the other genotypes, the stomata of K-deficient specimens of *E. camaldulensis* (0.9 mmol$_c$ dm^{-3} of Na) cannot function properly, favoring stomatal opening and promoting E (Figure 5f) (BENLLOCH-GONZALEZ et al., 2010). These findings are also in agreement with Teixeira et al. (1995), who stated a better control of E and turgor maintenance in K-supplied *Eucalyptus* plants under water stress. This discrepancy among genotypes may be related to interspecific differences (WANG et al., 2013).

Differences occurred among WUE scales (Figure 6). Regarding water stress, the marked reduction in E (biophysical process) by stomatal closure due to g_s decline was not accompanied, at the same degree, by A (biophysical/biochemical process), increasing WUE (MOINUDDIN et al., 2017; SARABI et al., 2019) at the leaf and whole plant level and enhancing plant drought resistance (EGILLA; DAVIES; BOUTTON, 2005). While this parameter is usually measured at the leaf level (WUE$_I$ and WUE$_T$), more useful results can be obtained at the whole plant level

(WUE$_L$) (MEDRANO et al., 2015), since it indicates the TDM production per unit of water requirement throughout the experimental period.

An adequate K nutritional status of plants promotes tolerance to abiotic stresses (CAKMAK, 2005) and enhances the WUE of trees (BATTIE-LACLAU et al., 2016), since water uptake by roots, stomatal opening, and E are facilitated by K accumulation in root xylem vessels and guard cells, increasing tissue water status and improving tolerance to water stress (MENGEL et al., 2001). These statements are in agreement with our findings regarding WUE$_L$, except for *E. saligna* under WS, which increased under low Na rates, followed by a drop in K-deficient plants (Figure 6g). Furthermore, the low Na supply, which enhanced *A*, WUE, and TDM of *Eucalyptus* and may be associated with high water use and E (EPRON et al., 2012; BATTIE-LACLAU et al., 2016), could lead to reduced water storage in deep soil layers (CHRISTINA et al., 2018), increasing *Eucalyptus* water stress during long periods of drought (WHITE et al., 2009). Besides, *Eucalyptus* species are considered less susceptible to drought, and plants under K deficiency strongly deplete their water use and TDM, increasing drought tolerance (WHITE et al., 2009; CHRISTINA et al., 2018), as observed in our study.

8.5. Leaf water potential

The ion flux in and out of the guard cells mediates stomatal aperture and closure (KIM et al., 2010). Changes in cell turgor involve controlled uptake of K and other ions, mediated by voltage-gated K$^+$ transporters at the cellular plasma membrane, inducing solute accumulation (SHABALA; LEW, 2002) and water uptake from the apoplast and, finally, stomata aperture (AHMAD; MAATHUIS, 2014). Stomatal closure by osmotic adjustment (ODDO et al., 2011) is a key factor to mitigate the negative impacts of droughts, avoiding excessive water loss at the expense of photosynthetic rate restriction (ANJUM et al., 2011) and turgor loss, decreasing cell growth (STEUDLE, 2000)

Tolerant genotypes, such as *E. camaldulensis*, avoid stress by lowering their osmotic potential and adjusting their cell elasticity (WHITE; TURNER; GALBRAITH, 2000), enabling *Eucalyptus* to withstand droughts (MERCHANT et al., 2007). Water stress reduces the Ψw_N in *Eucalyptus* plants, affecting plant growth and WUE$_L$ (MÜLLER et al., 2017). Regardless of the Na application, the Ψw_N in *E. camaldulensis* (Figure 7f), the drought-tolerant genotype, was significantly lower under WS than under WW, with values higher than -1.5 MP, suggesting a higher tolerance to drought stress than in *E. saligna* and *E. urophylla* (Figure 7d and e), which reached values of up to -1.4 MPa. Furthermore, plants grown under WW showed the lowest

decrease from Ψw_{PD} to Ψw_N, demonstrating a reduction in osmotic pressure to maintain cell turgor in WS plants. With low humidity and high irradiance levels, g_s decreases and closes the stomata to prevent Ψw decrease below critical levels, thereby stabilizing the water transport system (MARENCO et al., 2006). The K deficiency also strengthens stomatal closure, further reducing guard cell osmotic pressure and swelling (BATTIE-LACLAU et al., 2014b). Thus, our findings suggest that low Na application rates can augment the ability of *Eucalyptus* to tolerate drought stress by increasing *A* and g_s and decreasing Ψw, indicating that Ψw is an effective indicator for measuring the drought tolerance of plants.

8.6. Stable isotopic composition ($\delta^{13}C$)

The determination of $\delta^{13}C$ provides an integrated tool to measure the physiological properties of plants throughout their cycle. Because of its relation to environmental conditions, mainly due to *A* and g_s variations (ANDERSON et al., 1996), it is being used as a tool to determine drought-tolerant genotypes (FARQUHAR; EHLERINGER; HUBICK, 1989), besides its correlation with WUE (CONDON et al., 2004). Water stress significantly increased the $\delta^{13}C$ values of all *Eucalyptus* genotypes, compared to WW condition, corroborating the results of Ngugi et al. (2003) and Yin, Pang and Chen (2009), who report that plants under drought have higher $\delta^{13}C$ values (less negative) and a lower water content in the leaf tissues. Furthermore, drought-tolerant genotypes have lower $\delta^{13}C$ values than less tolerant genotypes (DAMATTA et al., 2003). In the current study, the drought-tolerant genotype - *E. camaldulendis* - had a lower $\delta^{13}C$ value (-30.37‰; Figure 8c) and lower mean values of Ψw_N, indicating better stomata control and a more conservative water use strategy (LI et al., 2000) by maintaining *A*, concomitantly to a greater decrease in g_s than in *E. saligna* and *E. urophylla*.

Generally, carbon isotope fractionation during photosynthesis in C_3 plants is based on two main discriminating steps: discrimination during CO_2 diffusion from the ambient air into the leaves through the stomata and during its carboxylation by RuBisCO, the primary carboxylating enzyme in C_3 plants (FARQUHAR; SHARKEY, 1982). In the first step, $\delta^{13}C$ is related to the ratio C_i/C_a, in which C_i refers to the CO_2 mole fraction in the leaf intercellular spaces and C_a to the CO_2 mole fraction in the ambient atmosphere (FARQUHAR; RICHARDS, 1984). Changes in environmental conditions affecting g_s, and therefore C_i/C_a, lead to alterations in photosynthetic discrimination in C_3 leaves. In the second step, RuBisCO catalyzes the initial step in the C_3 photosynthetic carbon reduction cycle by adding CO_2 to ribulose 1,5-bisphosphate

(RuBP) to form two molecules of 3-phospho-D-glycerate (3-PGA). Thus, the isotope fractionation by Rubisco catalyzation may be the main source of the large isotope discrimination (kinetic effect) (O'LEARY, 1989). Thus, the carbon isotope signature represents the composition of carbon isotopes in a photosynthesizing system during photosynthetic carbon assimilation (FARQUHAR; EHLERINGER; HUBICK, 1989), providing a reliable estimation of C_i/C_a as a long-term indicator of plant metabolism (SHAHEEN; HOOD-NOWOTNY, 2005).

Under drought stress, the decline in A due to stomatal factors occurs as a consequence of the decrease in g_s, and the expected trend would be an increase in WUE and $\delta^{13}C$ and a decrease in C_i. However, if the decrease in A does not occur due to a decrease in g_s, this will be because of non-stomatal effects (XIA et al., 2017), and the expected consequence would be a decrease in WUE and $\delta^{13}C$ and an increase in C_i (SARABI et al., 2019). Under water stress, g_s had a greater effect than non-stomatal factors, although under WW, a higher $\delta^{13}C$ was observed in plants grown under low Na rates due to the relatively higher biochemical activity, such as a higher A, leading to a higher WUE (BRUGNOLI; FARQUHAR, 2000), decreasing significantly at higher Na rates. According to Jiang et al. (2006) the magnitude of stomatal and/or non-stomatal limitations on A is linked to the severity of the imposed stress; thus, under intermediate drought, stomatal factors dominate the reduction in A, whereas non-stomatal limitations to A dominate under severe drought (RIVELLI; LOVELLI; PERNIOLA, 2002).

According to the above explanations, the assimilation capacity of *Eucalyptus* grown under water stress is closely regulated by stomatal factors, indicated by the marked decrease in g_s and the higher WUE and $\delta^{13}C$ values. Furthermore, when grown under solely Na supply (K-deficient plants), the assimilation capacity of *Eucalyptus* are regulated by non-stomatal factors, indicated by the decrease in C_i/C_a, WUE and $\delta^{13}C$, which may have arisen from increased affinity of RuBisCO in capturing the CO_2 delivered by g_s and mesophyll conductance (g_m) (SARABI et al., 2019). Over longer time periods, a drought-induced increase in $\delta^{13}C$ of about 2‰ has been observed (SARABI et al., 2019). In the current study, plants grown under water stress had higher WUE and $\delta^{13}C$ values when compared to WW. In a short drought period, the $\delta^{13}C$ increase ranged from -0.47 to -1.25‰, reflecting an increase in WUE_L from 17 to 33%, varying with genotype and condition, as also observed by Condon et al. (2004); Sarabi et al. (2019). Therefore, it may be expected that significant increases in $\delta^{13}C$ and WUE_L would be attained for longer drought periods (POU et al., 2008). Based on this, $\delta^{13}C$ could be a useful indicator when selecting these crops for biomass production under stress conditions. It also suggests that the effect of fertilization on $\delta^{13}C$ values may be related to the water status of leaves, confirmed by the positive correlation between $\delta^{13}C$ and WUE_L.

The application of instantaneous gas exchange measurements has some limitations, such as the heterogeneity of stomata opening as a consequence of ABA signaling (BEYSCHLAG; ECKSTEIN, 1998), the time of measurement (ORSINI et al., 2012), light conditions (EISINGER et al., 2000), and changes in the cuticular conductance to vapor pressure (BOYER; WONG; FARQUHAR, 1997), which can affect C_i (MATZNER; RICE; RICHARDS, 2001), leading to variations in leaf photosynthesis. Consequently, the large increases in leaf level WUE (WUE$_I$ and WUE$_T$) were not reflected in significant increases in the long-term whole-plant high WUE values, concealing the long-term adjustments in leaf level. This must carefully be considered when integrating these values to create more accurate and complex models for predicting WUE (ORSINI et al., 2012). As also stated by Farquhar and Richards (1984), the superior genotypes that exhibited higher WUE$_L$ values were reliably exploited through $\delta^{13}C$ (POU et al., 2008), integrating CO_2 assimilation over the life of the plant being analyzed (CONDON et al., 2006).

8.7.   Chlorophyll content

Drought reduces the chlorophyll content, an essential photosynthetic pigment, and decreases *A* (TARIQ et al., 2018) and TDM (ZHANG; KIRKHAM, 1996), potentially resulting in oxidative damage (over-production of reactive oxygen species - ROS). Consequently, chlorophyll and membranes are degraded (WANG et al., 2013) because of the disturbed balance between ROS production and antioxidant defense (REDDY; CHAITANYA; VIVEKANANDAN, 2004), as described for *Eucalyptus* (COSCOLIN et al., 2011).

Plant nutrition also strongly influences premature leaf senescence, as observed for K deficiency (ARMENGAUD; BREITLING; AMTMANN, 2004), which also leads to oxidative stress and photooxidation of chloroplast pigments. The root epidermal cells act as sensors of the cellular K status, accumulating ROS after K starvation and initiating the signal cascades that lead to the activation of K uptake (DEMIDCHIK, 2014). At low levels, ROS are involved in the stress-signaling pathway, triggering defense responses to biotic and abiotic stresses (VRANOVA; INZÉ; VAN BREUSEGEM, 2002). Elevated ROS concentrations harm the cellular membranes and other cellular components, resulting in oxidative stress and cell death (MITTLER, 2002).

The Chl levels were lower at intermediate Na rates (0.22 and 0.44 mmol$_c$ dm^{-3} of Na) under both water conditions in all genotypes (Figure 9). These findings provide an interesting insight: partial Na supply significantly improved *A*, g_s, and WUE$_L$, besides decreasing the

pigment content, possibly for an efficient regulation of the available amount of light and to reduce ROS formation by enhancing antioxidant enzyme activities. Thus, K-deficient plants may show a depressed photosystem activity and electron transport rate (BENDALY et al., 2016), resulting in elevated ROS concentrations and the appearance of leaf chlorosis and necrosis, visual symptoms of K deficiency (Appendix A).

8.8. Leaf K: Na ratio, K and Na accumulation and absorption and use efficiency

The K: Na ratio is commonly used as a predictor of plant performance (MUNNS; TESTER, 2008), varying among *Eucalyptus* genotypes, as also observed by Marcar and Termaat (1990). The appropriate leaf K: Na ratio was found for low Na rates, as observed by the estimated maximum yield, varying from 1.7 to 3.2 among genotypes and water regimes (Figure 10). Since K-deficient plants occurred at Na rates higher than 0.67 $mmol_c$ dm^{-3}, a K: Na ratio of 1: 0 is critical for *Eucalyptus* growth, since lower ratio values significantly decreased plant TDM. A similar leaf K: Na ratio of 3.4 was found by Mateus et al. (2019) in hybrid *Eucalyptus* subjected to K replacement by Na in the nutrient solution. Thus, understanding the K: Na ratio mechanisms may be useful for the development of strategies to reduce K fertilization by replacing it by more cost- and energy-efficient alternatives (BENITO et al., 2014).

Chemical attributes of soil after harvest (Appendix B) and leaf nutrient content also differentiated among genotypes, rates, and water conditions (Appendix C). In general, the drought-sensitive genotype (*E. saligna*) had a higher leaf concentration than *E. urophylla* and *E. camaldulensis*. Consequently, variations in nutritional efficiency among clones and species of *Eucalyptus* have also been observed (PINTO et al., 2011). Because of the lack of direct action of low Na application on the metabolic processes of C_3 plants, the increased Na leaf content suggests that the Na ion is directed toward the vacuole, as an alternative inorganic osmoticum (FLOWERS; LAUCHLI, 1983), including guard cells (TERRY; ULRICH, 1973), promoting a higher *A* (SPEER; KAISER, 1991). Thus, K, whose leaf content decreased concomitantly to the higher Na rates in *Eucalyptus*, may be available in the cytoplasm and released to metabolic pathways, such as in the chloroplast (SPEER; KAISER, 1991). There is no evidence of key cytosolic components being hampered by low Na supply, unlike under salinity conditions with higher Na supply (GATTWARD et al., 2012), although a greater efficiency in the osmotic function of plants supplied with both K and Na is suggested, as observed by the higher g_s and WUE_L values, corroborating with the results of a previous studies (JESCHKE, 1977);

these authors suggested the replacement of K by Na in the process of osmoregulation in vacuoles, improving turgor and cell expansion. However, this parameter is rarely measured, and the leaf content is usually discussed in the literature (KRONZUCKER; BRITTO, 2011). The replacement of K by Na significantly influenced the mineral status of the *Eucalyptus* leaf, partly because of the changes in plant performance and demand, such as higher WUE_L and TDM levels, under an adequate K: Na ratio. The tissue levels of other ions, besides being significant, displayed no marked patterns of response to Na application rates, since some contents were maximized or minimized according to the higher demands. We expected a decrease in Ca^{2+} uptake with higher Na supply (LAZOF; BERNSTEIN, 1998) due to ion competition at the whole-tissue level (SPEER; KAISER, 1991).

Apart from the variation in the leaf nutrient content among genotypes, the K leaf content decreased when replacement by sodium was increased, albeit without any symptoms of K deficiency, agreeing with Besford (1978) and Greenwood and Stone (1998), who suggested that Na can reduce the critical levels of leaf K. In E. *saligna* under WW, the replacement of 5.3%, corresponding to 90% of TDM and reaching rates of 0.041 $mmol_c$ dm^{-3} of Na and 0.85 $mmol_c$ dm^{-3} of K, decreased the K leaf content by 0.06 g kg^{-1}, while the Na leaf content increased by 0.12 g kg^{-1} compared to the application of only K, increasing plant growth. Furthermore, the K leaf content of 2.9 g kg^{-1} was still above the critical level of K, without symptoms of K deficiency. In *E. urophylla* under WW, with 6.7 % of K replacement by Na, reached the rates 0.06 $mmol_c$ dm^{-3} of Na and 0.83 $mmol_c$ dm^{-3} of K; the K leaf content decreased by 0.13 g kg^{-1}, while the Na leaf content increased by 0.018 g kg^{-1} compared to the application of only K. Under WS, with 9.3 % of K replacement by Na, reached the rates 0.085 $mmol_c$ dm^{-3} of Na and 0.815 $mmol_c$ dm^{-3} of K; the K leaf content decreased by 0.07 g kg^{-1}, while the Na leaf content increased by 0.052 g kg^{-1} compared to the application of only K. Conversely, *E. camaldulensis* under WW, with 30% of K replacement by Na, reached the rates 0.27 $mmol_c$ dm^{-3} of Na and 0.63 $mmol_c$ dm^{-3} of K; the K leaf content decreased by 0.46 g kg^{-1}, while the Na leaf content increased by 0.44 g kg^{-1} compared to the application of only K. Despite the drop in K leaf content, as also suggested by Gattward et al. (2012), the K cytoplasm content is maintained near at about 100 mmol L^{-1} of K, which are required for adequate enzyme activities (BRITTO; KRONZUCKER, 2008).

During long periods of drought, plant growth is sustained by root regulation in water and nutrient uptake, becoming critical processes (MÜLLER et al., 2017). Thus, genotypes with greater AE and UE values are more desirable (GODOY; ROSADO, 2011) due to the lower amounts of nutrients required for growth, favoring plant development. In general, low to

intermediate Na application increased the EU_K of all genotypes, allowing the maintenance of productivity, despite the lower K supply. Comparing water regimes, WS increased EA_K (Figure 12) and drastically reduced EU_K in all genotypes (Figure 13), being an adaptive strategy favoring nutrient accumulation in an unfavorable soil-climate situation and subsequently increasing nutrient translocation and use under favorable growth conditions (MÜLLER et al., 2017). Moreover, these findings evidence the interaction among mineral nutrition, nutrient uptake, and use and soil water availability, as also observed previously (TARIQ et al., 2019).

8.9. Characterization among genotypes

Multivariate analyses offer the alternative of processing all parameters together and obtaining an overview of the genotypes´ characterization and adjustment to cope with abiotic stresses (CORREIA et al., 2014). The 95% confidence ellipse graphically indicates the correlation between the variables of each genotype, while principal components analysis (PCA), a statistical technique for information synthesis, allows the identification and visualization of the strategies followed by each genotype under each water condition, providing a reduction of variables, with minimal loss of information. Cluster analysis, an exploratory data technique, groups the treatments (Na supply) and the water conditions applied (WW and WS) into functional units by their similarity (GRANDA et al., 2014).

The responses to Na application varied among genotypes and water conditions, with a higher variance of data under WW in *E. saligna* (Figure 15a), while *E. urophylla* had a higher variance under both conditions, suggesting plasticity (Figure 15b). However, *E. camadulensis* had a similar variance regarding water availability and a low correlation under WW and WS conditions (Figure 15c), showing a higher tolerance to drought under environmental stress, apart from a higher potential response to Na supply, as observed by the higher percentage of replacement of K by Na.

Water supply is the most important factor limiting plant growth (STAPE et al., 2004); plants under drought show lower TDM, LA, Std, A, E, g_s, Ψ_{WN}, and UE_K levels and higher $\delta^{13}C$ and WUE_L levels (Figure 16). Droughts result in A impairment due to a reduction in g_s and g_m (CHAVES; FLEXAS; PINHEIRO, 2009), decreasing C_i and resulting in the fixation of available CO_2 molecules. Thus, under drought, stomatal closure leads to the enrichment in ^{13}C and, consequently, in a higher $\delta^{13}C$ (ROBINSON et al., 2000). In contrast, the decrease in $\delta^{13}C$ (more negative value) and WUE_L indicates higher stomatal aperture (FARQUHAR; EHLERINGER; HUBICK, 1989), as found for the highest Na rates, confirming that the stomata

of K-deficient plants cannot function properly, favoring stomatal opening and promoting *E.* Also, the *A* inhibition in K-deficient plants (JIN et al., 2011) may have occurred due to a down-regulation of RuBisCO activity (ZHU, 2001), disrupting carbon metabolism (HU et al., 2017). Furthermore, the decreases in the K: Na ratio also correlates with decreased photosynthetic performance and biomass (KRONZUCKER et al., 2006), as observed by the close relation between TDM and [Na] (Figure 14).

Therefore, K-deficient plants under both conditions were characterized by lower δ^{13}C, WUE_L, TDM, and [K] levels and by higher [Na] levels, which explains their grouping in the same cluster (0.9 mmol$_c$ dm^{-3} of Na under WW and WS) (Figure 16). The low Na rates, markedly under drought, also resulted in a lower number of open stomata, leading to a higher δ^{13}C (more positive) and WUE_L value, which was associated with a better response to drought, confirming the statement that the richer plants are in δ^{13}C, the greater WUE is, proposed by Farquhar, Ehleringer and Hubick (1989). The results of the partial K replacement by Na confirms that nutrient interaction is a strategy to increase the UE_K under low soil K availability, as proposed by Laclau et al. (2003), increasing plant TDM. Moreover, plants with low replacement levels of K by Na and plants under WW were grouped in same cluster, with similar responses characterized by higher Std, UE_K, δ^{13}C, WUE_L, TDM, and [K] levels and lower [Na] levels.

9.   Final Remarks

The increase in *Eucalyptus* growth in response to the replacement of K by Na shows that the Na ion can act as a beneficial nutritional element in the C$_3$ crop *Eucalyptus*. In our study, a progressive increase in TDM was observed up to the adequate K: Na ratio of each genotype, coincident with the optimum-curve responses observed for LA, Std$_{AB}$, *A*, *E*, g_s, and WUE_L. Plants develop adaptive mechanisms to drought and K deficiency signaling, undergoing a series of biochemical and physiological reactions that include both short, occurring within a few hours, such as phytohormone changes, e.g., ABA and ROS synthesis (MAATHUIS; SANDERS, 1993), which regulate stomatal opening (PEI; KUCHITSU, 2005), and long-term responses (SCHACHTMAN; SHIN, 2007), occurring over days or weeks and resulting in metabolic changes (ARMENGAUD et al., 2009), such as K transporter changes, and morphological changes (HODGE, 2004), such as stomatal density and size alterations.

A comprehensive analysis of the molecular functions, such as K and Na transporters and channels, is required to better understand the mechanisms of their uptake and transport in plants,

since Na competes with K uptake sites, interfering in K translocation from root to shoot (BOTELLA et al., 1997) and its consequence for plant metabolism (WANG et al., 2013). Conversely, some authors suggest that root K uptake is only slightly suppressed by Na supply (RAINS; EPSTEIN, 1967). In response to decreasing external K values at both molecular and functional levels, the K-deficient plants may successfully up-regulate high-affinity transporters WANG 2013. However, under high K rates, normally when the external K+ concentration is higher than 1 mmol L^{-1}, the low-affinity K uptake is predominant (BRITTO; KRONZUCKER, 2008), being of interest to understand whether an increased Na supply can also modulate gene expression to predominantly high-affinity systems, increasing K uptake under low K levels (GATTWARD et al., 2012). Besides, greater attention has been given to the K and Na transporters in saline conditions, without reports in the literature about the mechanisms involved in *Eucalyptus* under partial replacement of K by Na, whose physiological benefits have already been ascertained, justifying the importance of this research field.

Since the range that characterizes the substitution of K by Na as detrimental to beneficial is narrow and varies among genotypes and water availability, more attention should be given to the precursors of such positive responses, such as biochemical, genetic, physiological, and morpho-anatomic adjustments. Thus, providing a better understanding of the mechanisms involved in the metabolic regulation and integration of these different signaling pathways into *Eucalyptus*. In addition, most studies use *Arabidopsis* as a model plant, and in this sense, it is important to transfer this knowledge to other cultivated genera, such as *Eucalyptus*.

10.   Conclusions

- The low replacement level of K by Na improved the TDM of *Eucalyptus* under different water conditions, except for root dry matter, which decreased with Na supply; there was a higher potential of replacement according to drought tolerance increase.

- Water supply was the most limiting factor for plant growth, with plants under drought showing lower TDM, LA, Std, A, E, g_s, Ψ_{WN}, and UE$_K$ levels and higher $\delta^{13}C$ and WUE$_L$ levels.

- Plants supplied with low levels of Na under WW and WS were able to improve CO_2 assimilation, Std and UE$_K$, WUE, maintain leaf turgidity by reducing Ψ_{WN}, with stomata partially closed, indicated by the higher $\delta^{13}C$, expressing the higher dry matter production; Na supply mitigated the negative impacts of drought.

- When only Na was supplied, inferring into K-deficient plants, besides the lower growth, the plants were characterized by lower δ^{13}C, WUE_L, and [K] levels and by higher [Na] levels.

- The positive correlation between δ^{13}C and WUE_L confirms that δ^{13}C is a useful tool to determine stress responses and yield, since the WUE_L represents the long term whole plant WUE, while the large increases in leaf level WUE did not reflect plants growth.

- The ideal leaf K: Na ratio to provide the maximum yield varied from 1.7 to 3.2 among genotypes and water regimes; values below 1:0 are critical for *Eucalyptus* growth, since lower ratio values significantly decreased plant development.

- In general, the substitution of K by Na at a level of 25 to 50% reduced the critical level of K without symptoms of K deficiency and allowed optimum *Eucalyptus* growth.

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APPENDIX

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Appendix A – Figure 17 - Plants supplied with 0 mmol$_c$ dm^{-3} of Na and 0.9 mmol$_c$ dm^{-3} of Na and visual symptom of K deficiency in mature leaves of *E. saligna* (A and D), *E. urophylla* (B and E) and *E. camaldulensis* (C and F) supplied with 0.9 mmol$_c$ dm^{-3} of Na under WW condition, 90 days after onset of treatments.
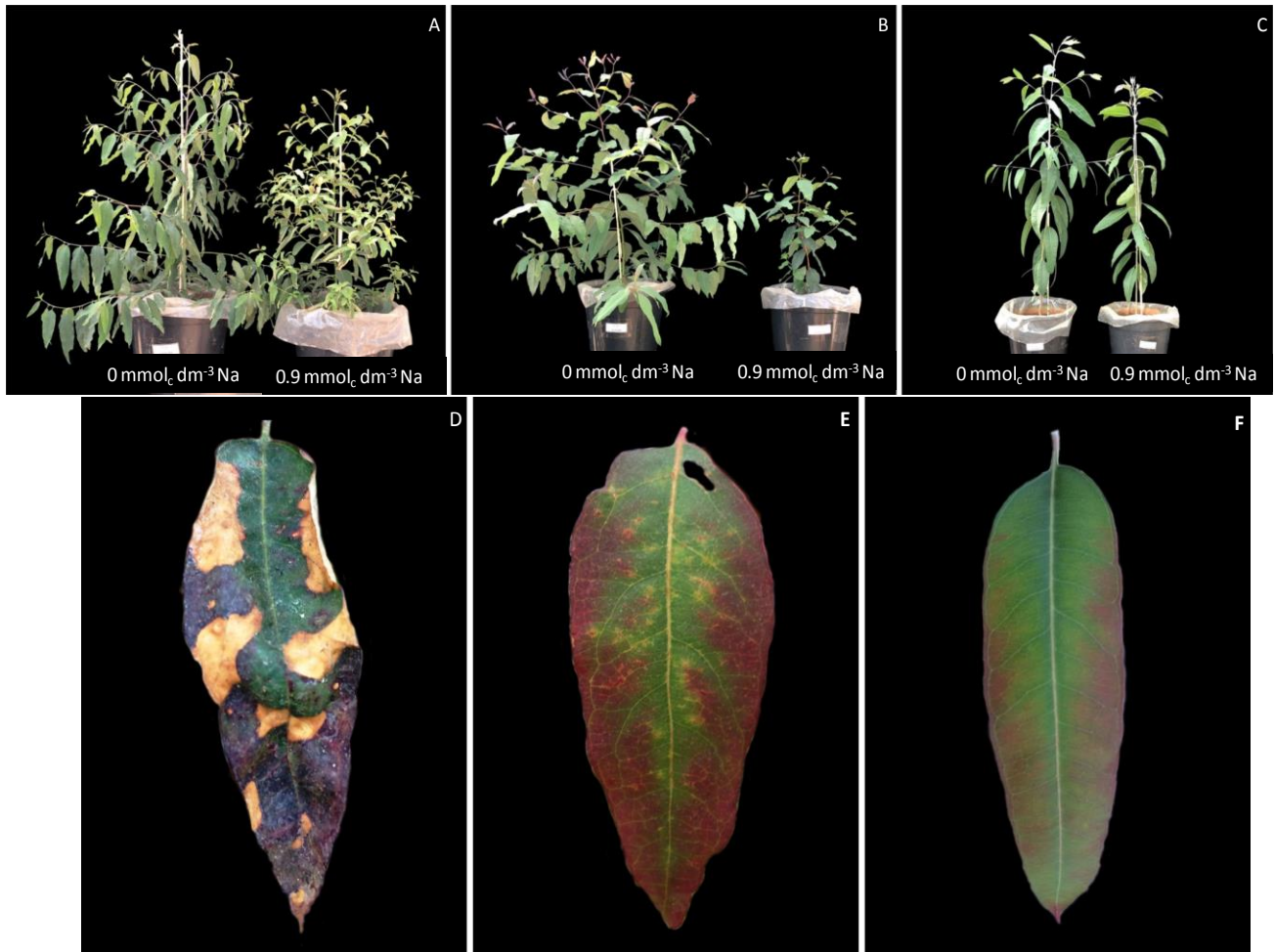
Appendix B - Table 2- Chemical attributes of soil after harvest of *E. saligna*, *E urophylla* and *E. camaldulensis* under partial K replacement by Na.

Na rate (mmol$_c$ dm^{-3})	0		0.22		0.44		0.67		0.9	
Attributes	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
E. saligna										
pH CaCl$_2$	3.7	3.7	3.7	3.8	3.8	3.8	3.8	3.7	3.8	3.7
OM (g dm^{-3})	5	5	5	5	5	5	5	5	5	5
P (mg dm^{-3})	138	90	117	105	105	111	159	132	120	150
K (mmol$_c$ dm^{-3})	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
S-SO$_4$ (mg dm^{-3})	11	11	11	11	7	18	6	14	8	14
Ca (mmol$_c$ dm^{-3})	1	1	1	1	1	1	1	1	1	2
Mg (mmol$_c$ dm^{-3})	1	1	1	1	1	1	1	1	1	1
Na (mmol$_c$ dm^{-3})	0.07	0.03	0.06	0.03	0.06	0.03	0.05	0.03	0.07	0.12
H+Al (mmol$_c$ dm^{-3})	38	42	38	38	34	38	38	47	42	38
Al (mmol$_c$ dm^{-3})	3	2	2	2	1	2	2	3	2	2
BS (mmol$_c$ dm^{-3})	2	2	2	2	2	2	2	2	2	3
CEC (mmol$_c$ dm^{-3})	40	44	40	40	36	40	40	49	44	41
V%	5	5	5	5	6	5	5	4	5	8
m%	59	49	49	49	32	49	49	59	49	39
E. urophylla										
pH CaCl$_2$	3.7	3.7	3.8	3.7	3.9	3.8	3.9	3.9	4.1	3.9
OM (g dm^{-3})	5	5	5	5	5	5	5	5	5	5
P (mg dm^{-3})	126	111	96	108	117	126	108	102	117	108
K (mg dm^{-3})	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
S-SO$_4$ (mg dm^{-3})	11	11	18	14	8	15	14	11	14	21
Ca (mmol$_c$ dm^{-3})	1	1	1	2	1	1	1	2	1	2
Mg (mmol$_c$ dm^{-3})	1	1	1	1	1	1	1	1	1	1
Na (mmol$_c$ dm^{-3})	0.04	0.06	0.06	0.03	0.03	0.04	0.11	0.16	0.08	0.1
H+Al (mmol$_c$ dm^{-3})	42	42	34	42	34	38	31	34	34	38
Al (mmol$_c$ dm^{-3})	3	2	2	2	2	2	2	2	1	2
BS (mmol$_c$ dm^{-3})	2	2	2	3	2	2	2	3	2	3
CEC (mmol$_c$ dm^{-3})	44	44	36	45	36	40	33	37	36	41
V%	5	5	6	7	6	5	6	8	6	8
m%	59	49	49	39	49	49	49	39	32	39
E. camaldulensis										
pH CaCl$_2$	3.7	3.7	3.7	3.7	3.8	3.8	3.7	3.7	3.7	3.8
OM (g dm^{-3})	5	5	5	5	5	5	5	5	5	5
P (mg dm^{-3})	114	123	96	102	96	126	120	111	111	105
K (mg dm^{-3})	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
S-SO$_4$ (mg dm^{-3})	12	8	11	8	12	11	15	14	8	19
Ca (mmol$_c$ dm^{-3})	1	1	1	1	1	1	1	1	1	3
Mg (mmol$_c$ dm^{-3})	1	1	1	1	1	1	1	1	1	1
Na (mmol$_c$ dm^{-3})	0.08	0.04	0.05	0.03	0.07	0.05	0.06	0.04	0.07	0.10
H+Al (mmol$_c$ dm^{-3})	47	47	47	42	42	38	42	38	38	38
Al (mmol$_c$ dm^{-3})	3	3	3	2	2	2	2	2	2	2
BS (mmol$_c$ dm^{-3})	2	2	2	2	2	2	2	2	2	4
CEC (mmol$_c$ dm^{-3})	49	49	49	44	44	40	44	40	40	42
V%	4	4	4	5	5	5	5	5	5	10
m%	59	59	59	49	49	49	49	49	49	33

Appendix C - Table 3- Nutrients leaf content of *E. saligna*, *E. urophylla* and *E. camaldulensis* seedlings, respectively, under K partial replacement by Na, in well-watered (WW) and water-stressed (WS) condition

E. saligna

Na rate (mmol$_k$ dm^{-3})	Na*,**		K*,**,***		Ca*		Mg*,**,***		P*		S**		Fe*		Cu*		Zn**		Al*		Mn*,**		Ni*,***		B*	
Water condition	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
0	1.29±0.57c	1.35±0.58c	2.91±0.78a	3.25±0.58ab	6.46±0.81ab	7±1.68a	1.51±0.17a	1.78±0.32b	1.3±0.14b	1.62±0.09a	1.42±0.1b	1.98±0.23a	256.9±70a	1334±327a	6.47±1.18b	9.75±0.92a	36.74±1.81a	60±5.86a	789±467a	1899±493a	598±77.7b	810±59a	0.87±0.13b	3.92±0.98a	63.5±6.57b	71.69±8.25a
0.22	1.47±0.21bc	2.38±0.9bc	2.56±0.42a	3.76±0.58a	7.45±0.86a	7.44±0.87a	1.86±0.14a	2.26±0.14b	1.65±0.05ab	1.82±0.27a	1.75±0.02ab	2.07±0.14a	1500±390a	1469±263a	10.15±1.21ab	12.34±1.07a	46.53±4.6a	50.9±5.1a	2177±523a	2381±377a	634±17.2ab	898±65a	4.58±0.33a	4.45±0.29a	82.9±2.39a	90.4±3.95a
0.44	1.93±0.77abc	2.52±0.81bc	2.7±0.23a	2.6±0.49bc	7.5±0.08a	7.42±0.97a	1.94±0.32a	2.11±0.31b	1.82±0.1ab	1.93±0.1a	1.71±0.27ab	2±0.14a	1701±424a	1580±305a	11.4±0.35ab	12.11±0.8a	49.26±4.47a	50.6±5.21a	2422±784a	2432±335a	711±36.5ab	949±30a	4.29±0a	4.15±0.61a	93.9±7.33a	92.17±8.71a
0.67	3.03±1.29ab	2.96±1.25a	2.21±0.16ab	2.2±0.56c	6.5±0.71ab	6.52±1.03a	1.82±0.37a	2.19±0.29b	2±0.11ab	2.39±0.3a	2.24±0.01a	2.17±0.34a	1287±606a	1060±382a	13.9±0.51ab	10.5±2.4a	46.52±4a	44.8±6.8a	2685±283a	1807±279a	733±57.7ab	873±75a	3.8±0a	2.76±0.2a	92.42±5.22a	80.2±1.86a
0.9	3.37±1.25a	4.93±0.94a	1.58±0.2b	2±0.55c	5±1.16b	6.22±1.05a	1.57±0.15a	2.98±0.16a	2.09±0.02a	2.45±0.15a	1.67±0.13ab	2.04±0.21a	395±187a	372±154a	15.5±1.97a	13.9±2.35a	49.81±6.19a	53.4±5.67a	673±418a	1078±83.9a	888±54a	1037±45a	3.18±0.68ab	2.74±0.3a	89.7±2a	75.5±7.55a
Means	2.16b	2.83b	2.39b	2.77a	6.56a	7a	1.75b	2.75a	1.73a	1.98a	1.76b	2.04a	1015a	1181a	11.17a	11.73a	45.25b	51.44a	1606a	1970a	701.7b	903.3a	3.34a	3.62a	85.63a	82.6a

E. urophylla

Na rate (mmol$_k$ dm^{-3})	Na*		K*,**		Ca*,**,***		Mg*,**,***		P*		S**		Fe*,***		Cu*,***		Zn		Al**		Mn		Ni**,***		B*,***	
Water condition	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
0	0.52±0.18a	0.4±0.17b	1.65±0.05a	1.29±0.19a	3.62±0.6a	2.95±0.33b	0.27±0.09b	0.31±0.009b	1.25±0.04a	1.26±0.08b	1.11±0.07a	1.47±0.13a	1158±335a	769±180.2b	8.56±1.19b	7.57±1bc	39±1.28a	45.75±3.4a	1726±346a	1833±407a	497±44a	683±47a	2.4±0.9a	3.31±1.3a	58.66±5.9b	6±2a
0.22	0.53±0.25a	0.53±0.05b	1.14±0.1b	1.18±0.25a	3.44±0.59a	3.48±0.38b	0.33±0.09b	0.33±0.06b	1.31±0.22a	1.26±0.14b	1.27±0.08a	1.52±0.13a	1248±303a	1023±38.9a	6.48±1.14b	5.49±0.1c	45.1±2.42a	37.1±5.4a	2169±486a	2127±850a	579±46.7a	662±67.6a	3.1±1.13a	3.37±1a	76.39±3.2ab	73.1±5.9a
0.44	0.76±0.14a	0.63±0.09b	0.96±0.17b	0.94±0.12ab	3.22±0.54a	3.62±0.69b	0.46±0.06ab	0.43±0.11b	1.34±0.04a	1.37±0.02ab	1.09±0.09a	1.37±0.09a	1248±542a	1929±100a	4.63±0.9b	7.4±0.56bc	35±2.24a	34.4±2.33a	764±267a	2808±150a	522±42.8a	557±6a	2.9±1.3a	5.36±0.6a	82±6.48a	76.8±2.1a
0.67	0.73±0.33a	0.66±0.11b	0.76±0.23b	0.76±0.02bc	3.84±0.66a	6.2±0.79a	0.46±0.08ab	0.6±0.14ab	1.71±0.16a	1.43±0.12ab	1.46±0.09a	1.6±0.1a	2227±89.7a	2018±84a	15.2±1.2a	9.8±0.68ab	41.7±7.69a	39.9±3.54a	2399±608a	3039±316a	624±52a	580±29a	4.6±2.82a	5.81±0.3a	89±2.2a	83.6±3.4a
0.9	0.82±0.18a	1.03±0.18a	0.87±0.15b	0.56±0.08c	4±0.98b	6.1±1a	0.59±0.12a	0.97±0.23a	1.96±0.21a	1.82±0.15a	1.29±0.14a	1.59±0.19a	866±99a	2042±136a	6.78±0.6b	11.6±0.3a	38±5.4a	42.7±2.16a	1205±390a	2902±117a	557±32a	546±30a	2.1±0.47a	7.85±0.7a	81±5.26a	82.25±5.9a
Means	0.65a	0.64a	1.05a	0.94a	3.65b	4.46a	0.42b	0.53a	1.54a	1.43a	1.23b	1.5a	1305a	1650a	8.27a	8.55a	40a	39.7a	1625b	2494a	552a	606a	2.89b	5.03a	77.8a	79.4a

E. camaldulensis

Na rate (mmol$_k$ dm^{-3})	Na**,***		K*,**,***		Ca*,**		Mg*,***		P*,**		S*		Fe*,**,***		Cu*,**		Zn*,**		Al*,**		Mn*		Ni*,**,***		B	
Water condition	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
0	0.52±0.22a	0.88±0.42c	2.87±0.18a	3.76±0.53a	5.96±1.04a	7.91±0.8a	1.58±0.45a	2.21±0.24a	1.26±0.09a	1.44±0.1b	1.58±0.28a	1.73±0.2a	1268±346a	1512±315a	6.2±0.5a	9±0.77a	37±6.4a	54±7a	2225±640a	2022±340a	476.8±48.7a	719±34a	3±0.9a	4±0.99a	72.5±3.6a	87±3a
0.22	1.08±0.71a	1.36±0.9c	2.43±0.47ab	2.71±0.52ab	6.45±0.57a	7.54±1.22a	1.45±0.13a	1.55±0.15b	1.32±0.14a	1.46±0.15ab	1.69±0.14a	1.6±0.08a	1321±466a	1059±288a	7.2±1.2a	7.77±1.48a	44.4±0.4a	47.8±10.8a	2033±533a	1818±500a	492±24a	569±59a	4.5±1.3a	3.8±1.4a	75.2±3.8a	71±2.9a
0.44	1.49±0.48a	2.2±0.61bc	2.07±0.17bc	2.38±0.39b	6±0.73a	5.88±0.94a	1.66±0.25a	1.69±0.29ab	1.51±0.02a	1.24±0.12b	2±0.3a	1.4±0.01a	867±209a	617±136a	6±0.7a	8.5±0.65a	51±0.27a	49±8.77a	1326±296a	1145±266a	494±7a	427±77a	1.7±0.6a	3.5±1.6a	83.9±2.6a	66.5±9.3a
0.67	1.78±0.36a	3.17±1.24ab	1.49±0.28c	1.93±0.07b	5.29±0.51a	7.34±1.99a	1.39±0.18a	1.53±0.02b	1.2±0.12a	1.82±0.13a	1.6±0.16a	1.85±0.4a	702±288a	639±159a	6.8±0.89a	8.69±0.75a	43±3.19a	50.7±2a	1090±479a	1348±103a	425±15a	638±71a	3.7±1.3a	3.5±0.6a	75.9±2.5a	81±18a
0.9	2.04±1.27a	3.69±1.77a	1.41±0.07c	1.66±0.1b	5.75±0.96a	5.21±1.69a	1.58±0.27a	1.55±0.2b	1.47±0.16a	1.81±0.12a	1.86±0.26a	2±0.14a	1155±300a	1428±182a	7.3±0.6a	6.5±0.5a	45±4.9a	40.8±2.8a	2058±393a	2012±334a	468±36a	473±85a	3.15±0.49a	2.5±0.3a	76.9±5.5a	95.8±3.2a
Means	1.35b	2.26a	2.05b	2.56a	5.91a	6.72a	1.53b	1.74a	1.35b	1.55a	1.76a	1.75a	1068a	1079a	6.69b	8.12a	44.6a	48.3a	1767a	1670a	471b	560a	3.24a	3.5a	76.9a	80a

Data represent mean values and standard errors between blocks (n=4).*, **, *** Influenced statistically (by F test $p < 0.05$) by Na, WS and Na x WS interaction. Different letters indicate significant differences according to Tukey test ($p < 0.05$).