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Nutritional Efficiency of Eucalyptus Clones Under Water Stress

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Abstract: Plant health and nutrition are strongly influenced by the availability of water. The objective of this study was to evaluate the different patterns of initial growth and nutritional efficiency of ten drought-stressed *Eucalyptus* clones. Water stress conditions were induced by osmotic stress, by gradual addition of polyethylene glycol 6000 over a 20-day period. Drought stress significantly reduced the dry matter of leaves, roots, and the whole plant as well as the nutritional efficiency of most clones. Clone PL040 was the least efficient in nutrient uptake and use and the most sensitive to drought stress. Interestingly, under drought stress, the drought-tolerant clones generally had high AE (absorption efficiency), but low nutrient UE (use efficiency), whereas the sensitive clones had low AE and low UE for root formation and high AE for leaf formation. In a combined evaluation of growth and nutrient use efficiency, the clones vc865, i182, i144, and gg157 were grouped as drought-tolerant; 1528 and i224 as moderately tolerant; and 1641, 3367, i042, and PL040 as drought-sensitive.

Keywords: tree, growth, nutrient use efficiency, drought stress

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INTRODUCTION

Forest productivity in Brazil is regarded as one of the highest in the world (Stape et al., 2010; Ibá, 2016). However, due to the high demand for *Eucalyptus* products, plantations expanded into areas with low annual rainfall, which has directly affected the plant use efficiency of resources such as nutrients, CO_2, and water to support continued growth (Stape et al., 2004).

Reductions in water supply can greatly impact plant nutrition by interfering with nutrient uptake and absorption capacities (Hu et al., 2008), and also reducing nutrient availability by limiting the microbial activity (Borken and Matzner, 2009). Prolonged drought periods can also inhibit root growth (Maseda and Fernández, 2016) and affect plant metabolism (Valdés et al., 2013).

Macronutrients and micronutrients play important roles in plant responses to drought stress (Waraich et al., 2011). For example, Ca is involved in the regulation of various processes in response to abiotic stresses, e.g., of stomatal regulation mediated by the hormone ABA (Song et al., 2008), antioxidant enzyme activities (Jiang and Huang, 2001) and photosynthetic rates (Xu et al., 2013). Calcium also plays a fundamental role in the resumption of tree cambium growth (Fromm, 2010) and in dry matter increases of *E. camaldulensis* (Oliva et al., 1995) during water stress.

Phosphorus was mentioned as the main limiting nutrient for *Eucalyptus* growth in Brazil (Barros and Novais, 1990), since it affects CO_2 uptake and assimilation (Yong-fu et al., 2006) and root growth (Jin et al., 2005). Similarly, adequate magnesium supply stimulates root growth, increasing water and nutrient uptake (Makkonen-Spiecker and Spiecker, 1997), and reduces photo-oxidative damage by enhancing plant drought tolerance (Guo et al., 2016). Potassium and boron are also important nutrients for drought-stressed *Eucalyptus* trees. Potassium controls stomatal closure and K deficiency can decrease photosynthetic rates (Hawkesford et al., 2012), negatively affecting the production and accumulation of assimilates. Under drought, K nutrition increases plant tolerance to water stress by making soil moisture use more efficient than in K-deficient plants and also by maintaining the pH in stroma, reducing photo-oxidative damage to chloroplasts (Cakmak and Kirby, 2008). Similarly, Battie-Laclau et al. (2013) showed that K and Na ion accumulation in *E. grandis* leaves in response to K and Na supply, contributes to increasing leaf turgor by reducing the osmotic potential.

Boron is important for structural reinforcement (Matoh et al., 1996), and is found mostly in the cell wall, connected to pectin (O'Neill et al., 2004). However, the role of B in increasing drought tolerance has also been observed in different species (Möttönen et al., 2005; Hodecker et al., 2014; Pita-Barbosa et al., 2016). Our previous studies confirmed the great influence of B on the increase of root growth and water use efficiency in *Eucalyptus urophylla* (Hodecker et al., 2014).

The relationship between nutrient supply, water stress, and *Eucalyptus* plant growth was highlighted in several studies (Oliva et al., 1995; Teixeira et al., 2008; Valdés et al., 2013; Hodecker et al., 2014; Maseda and Fernández, 2016; Pita-Barbosa et al., 2016; Santos and Schumacher, 2016). However, since a reduced water supply can hamper nutrient uptake due to the interrelatedness of water and nutrient supply (Brouder and Volenec, 2008), knowledge about nutrient efficiency, as well as nutrient uptake, transport, and use in different *Eucalyptus* genotypes is still scarce. This information could be useful in the investigation of additional and important strategies used by plants to develop highly drought-resilient genotypes in low-rainfall regions. Adopting a more holistic consideration of water and nutrients should be a better strategy to identify the intrinsic link between these two drivers of tree growth and productivity.

We used a methodology of comparison of *Eucalyptus* genotypes at the seedling stage, for drought-stress tolerance and nutrient use. A clustering strategy is presented, based on clone efficiency of nutrient uptake and use under sufficient or insufficient water supply. The questions addressed in this study were: (1) Are nutrient uptake and use efficiency affected by water availability? (2) Do drought-tolerant genotypes have a higher nutrient uptake and use efficiency? (3) Can potentially drought-tolerant *Eucalyptus* genotypes under water stress be detected by their nutrient uptake and use efficiency patterns?

Answering these questions by the identification of differential patterns of initial growth, biomass accumulation, and nutritional use efficiency of ten *Eucalyptus* clones subjected to drought stress will provide an efficient methodology for use in plant breeding programs.

MATERIALS AND METHODS

Plant material and experimental conditions

Ten *Eucalyptus* clones, bred mostly in dry regions, where selected for the experiment (1528, 1641, 3367, gg157, i042, i144, i182, i224, PL040, and vc865). Of these, clones i042 and i144 were confirmed, respectively, as drought-sensitive and tolerant in the field (Barros Filho, 2014; Nunes et al., 2016), and used as a reference for comparison with other genotypes in this study. *Eucalyptus* seedlings were grown in a greenhouse in plastic pots (8 L) containing intermittently aerated Clark's nutrient solution (Clark, 1975), adapted by Locatelli et al. (1984), consisting of 1.30 mmol L^{-1} $CaCl_2$, 0.30 mmol L^{-1} K_2SO_4, 0.30 mmol L^{-1} $MgSO_4$, 2.93 mmol L^{-1} NH_4NO_3, 0.53 mmol L^{-1} KCl, 0.138 mmol L^{-1} $K(H_2PO_4)_2$, 7 µmol L^{-1} $MnCl_2$, 19 µmol L^{-1} H_3BO_4, 2 µmol L^{-1} $ZnSO_4$, 0.086 µmol L^{-1} Na_2MoO_4, 0.50 µmol L^{-1} $CuSO_4$, 38 µmol L^{-1} Fe.EDTA, and 24.9 µmol L^{-1} $FeSO_4$. The pH (5.5) was adjusted every 2 days with NaOH or HCl. The nutrient solution was renewed weekly. After approximately 30 days of acclimation, the plants were exposed to two different osmotic conditions to simulate water restrictions: well-watered (WW) treatment, in a nutrient solution with water potential of -0.036 MPa; and water stress (WS) treatment, by the addition of polyethylene glycol 6000 (PEG 6000) until reaching a water potential of -1.00 MPa. The PEG 6000 doses were added every five days to gradually reduce the water potential of the solution (to -0.16, -0.32, -0.65, and -1.0 MPa) (Michel and Kaufmann, 1973). The experiment was performed in randomized blocks, with four replicates, in a 10 x 2 factorial design. Each experimental unit consisted of three plants per pot. Five days after applying a PEG 6000 dose to reduce the water potential of the solution to -1 MPa, the predawn water potential (Ψw_{pd}) was measured using a pressure chamber (Scholander bomb).

Biomass determination and stress tolerance index

Root length was measured and subsequently, the plants were separated into old leaves, new leaves, stems, and roots, packed in paper bags, oven-dried, and weighed to obtain the dry matter of the leaves (LDM, including fully expanded and young leaves), old leaves (below the fully expanded leaves), stem, root dry matter (RDM), and total dry matter (TDM), from which the RDM:TDM ratio was calculated.

The stress tolerance index (STI) was computed from the TDM values, according to Fernandez (1992):

$$STI = \frac{TDM_{control} \ x \ TDM_{stress}}{(\ TDM_{stress})^2}$$

Nutrient absorption and use efficiency and diagrams

To quantify the nutrient contents in leaves and roots, the dry plant material was ground in a Wiley mill, ashed in a muffle and the minerals extracted with HCl (0.1 mol L^{-1}). The nutrients P, Ca, and Mg were analyzed by plasma emission spectrometry; K by a flame emission photometer; and B with a spectrophotometer, by the method of Azometina-H. (Wolf, 1974). Nutrient absorption efficiency (AE) (Swiader et al., 1994), translocation efficiency (TE) (Li et al., 1991) and use efficiency for leaf (UEL) and root production (UER) (Siddiqi and Glass, 1981), were calculated with the equations:

$$AE = \frac{\text{Nutrient Content (shoot and root) (mg)}}{\text{Root Dry Matter (g)}}$$

$$TE = \frac{\text{Shoot Nutrient Content (mg)}}{\text{Total Nutrient Content (g)}}$$

$$UEL = \frac{[\text{Total Leaf Dry Matter (g)}]^2}{\text{Leaf Nutrient Content (mg)}}$$

$$UER = \frac{[\text{Root Dry Matter (g)}]^2}{\text{Root Nutrient Content (mg)}}$$

Diagrams were used to group the clones in terms of nutritional efficiency. The diagram axes were generated by the means of all clones and the values of each clone distributed into quadrants.

Statistical analysis

Data were subjected to analysis of variance, followed by the Scott Knott clustering test at 5 % probability using software SAEG 9.1.

RESULTS

Growth and nutrient use efficiency

The gradual increase of PEG in the hydroponic solution reduced the leaf water potential in *Eucalyptus* plants and affected the growth and water and nutrient-use efficiency differentially (Tables 1, 2, and 3), with a mean of -0.26 and -1.36 MPa, respectively in WW (well-watered) and WS (water-stressed) seedlings (Table 1). The LDM (leaf dry matter) was severely affected by drought stress, mainly for clones i182, 1641, i042, and 3367 (Table 1). No statistical differences in LDM between WW and WS plants were observed for clones 1528, i144, i224, and PL40.

The RDM (root dry matter) and TDM (total dry matter) were significantly reduced, mainly for clones 1641, 3367, i042, and PL40. On the other hand, the RDM of clone vc865 increased 20 % under drought stress and no differences in root growth were observed for clones gg157 and i144 (Table 1).

Drought stress did not affect the TDM of clones 1528, i144, i224, gg157, and vc865. The RDM:TDM ratio increased in most of the clones, and reached 79 % in gg157 (Table 1). Therefore, the Stress Tolerance Index (STI), based on TDM, indicated a higher stress tolerance potential of clone vc865 (0.85) and lower stress tolerance potential for clone PL40 (0.35), compared to the mean stress tolerance level of all clones (Table 1).

Based on the distribution of clones in terms of variation in LDM and RDM compared to the control (Figure 1), a diagram was constructed, showing the separation of the clones into four groups of differential growth under drought stress. Clones i144, i182, i224, and gg157 were clustered in Group 1, which contains the clones with least significant reduction in LDM and increase in RDM, which are potentially most drought-tolerant (i144), reference of drought tolerance, based on growth traits. Group 2 composed by the genotypes vc865 and 1528, characterized by smaller reductions in RDM and LDM. Group 3 contained clone PL40, due to the negative effect on LDM when grown under

Table 1. Predawn water potential (Ψw_{pd}), leaf dry matter (LDM), root length (RL), root dry matter (RDM), total dry matter (TDM), root:shoot ratio (RDM:TDM), and Stress Tolerance Index (STI) of ten *Eucalyptus* clones under well-watered (WW) or water stress (WS) conditions. Data indicate the mean ± SE (n =4). Means followed by the same letter in a column did not differ significantly by the Scott Knott test ($p \geq 0.05$)

Clone	**Treat**[(1)]	**Ψw_{pd}**	**LDM**	**RL**	**RDM**	**TDM**	**RDM:TDM**	**STI**
		-MPa	g per plant	cm per plant	g per plant	g per plant		
1528	WW	0.26 ± 0.02c	1.30 ± 0.10c	20.25 ± 1.67b	2.48 ± 0.18b	8.94± 1.07c	0.277 ± 0.014c	0.78
	WS	1.58 ± 0.13a	0.85 ± 0.13c	20.38 ± 7.30b	2.67 ± 0.40b	6.96 ± 1.16c	0.384 ± 0.007a	
1641	WW	0.20 ± 0.04c	2.58 ± 0.36a	28.50 ± 6.74b	3.56 ± 0.55a	16.29 ± 1.93a	0.216 ± 0.012e	0.55
	WS	1.25 ± 0.09b	1.49 ± 0.47b	24.31 ± 7.54b	2.40 ± 0.41b	8.93 ± 1.81c	0.271 ± 0.009c	
3367	WW	0.25 ± 0.08c	1.70 ± 0.14b	33.75 ± 3.86a	2.59 ± 0.30b	11.03 ± 0.83b	0.236 ± 0.024d	0.54
	WS	1.38 ± 0.22a	0.84 ± 0.07c	36.13 ± 6.36a	1.51 ± 0.05c	5.97 ± 0.47c	0.256 ± 0.013d	
gg157	WW	0.15 ± 0.07c	1.49 ± 0.08b	22.44 ± 1.92b	1.55 ± 0.13c	7.96 ± 0.48c	0.193 ± 0.007e	0.61
	WS	0.96 ± 0.16b	1.05 ± 0.10c	31.69 ± 4.38a	1.67 ± 0.12c	4.84 ± 0.33c	0.345 ± 0.020b	
i042	WW	0.18 ± 0.09c	1.63 ± 0.25b	37.63 ± 4.38a	2.69 ± 0.23b	11.78 ± 1.45b	0.231 ± 0.013d	0.52
	WS	1.59 ± 0.07a	0.99 ± 0.20c	41.75 ± 3.95a	1.83 ± 0.32c	6.13 ± 1.08c	0.303 ± 0.023c	
i144	WW	0.30 ± 0.12c	1.15 ± 0.10c	22.63 ± 1.43b	1.87 ± 0.05c	7.53 ± 0.33c	0.249 ± 0.011d	0.66
	WS	1.27 ± 0.10b	0.90 ± 0.20c	18.33 ± 1.48b	1.65 ± 0.24c	5.01 ± 1.02c	0.341 ± 0.018b	
i182	WW	0.44 ± 0.06c	2.43 ± 0.18a	29.44 ± 1.82a	2.72 ± 0.19b	14.45 ± 1.23a	0.189 ± 0.005e	0.57
	WS	1.53 ± 0.14a	1.79 ± 0.30b	28.38 ± 3.31b	2.33 ± 0.34b	8.30 ± 1.09c	0.281 ± 0.013c	
i224	WW	0.30 ± 0.06c	1.70 ± 0.21b	34.88 ± 3.82a	2.49 ± 0.46b	10.54 ± 2.01b	0.237 ± 0.013d	0.74
	WS	1.25 ± 0.02b	1.37 ± 0.24b	35.63 ± 5.92a	2.24 ± 0.48c	7.75 ± 1.32c	0.284 ± 0.019c	
PL40	WW	0.36 ± 0.04c	1.91 ± 0.26b	31.00 ± 6.06a	3.37 ± 0.63a	16.70 ± 3.19a	0.203 ± 0.005e	0.35
	WS	1.36 ± 0.12a	1.38 ± 0.37b	26.13 ± 3.36b	1.65 ± 0.26c	5.91 ± 0.69c	0.277 ± 0.016c	
vc865	WW	0.20 ± 0.07c	3.07 ± 0.64a	31.50 ± 2.61a	2.81 ± 0.42b	14.60 ± 2.93a	0.197 ± 0.010e	0.85
	WS	1.40 ± 0.12a	1.91 ± 0.18b	26.50 ± 3.36b	3.36 ± 0.35a	12.48 ± 1.03b	0.268 ± 0.016c	

(1) Treatment.

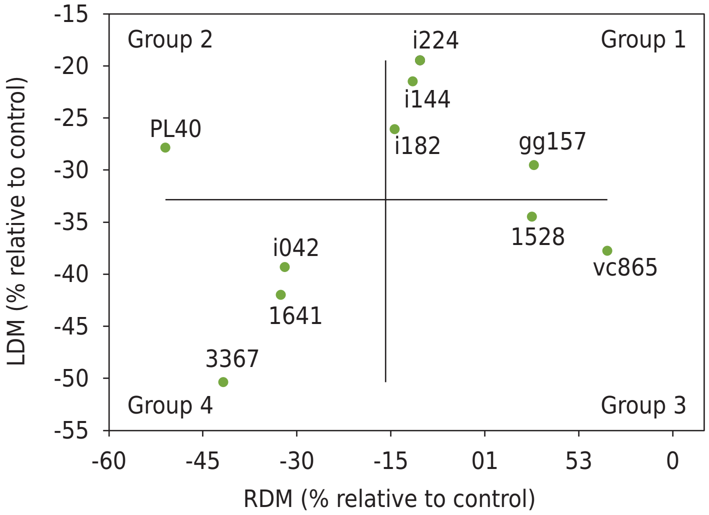

Figure 1. Distribution diagram of ten *Eucalyptus* clones according to the variation in leaf dry matter (LDM) and root dry matter (RDM) in response to drought stress, compared to LDM and RDM of well-watered plants.

drought stress. The reductions in the traits studied were most drastic for clones i042, 3367, and 1641, of Group 4, characterizing them as most sensitive to water stress (i042, as reference of drought sensitivity).

Under well-watered (control) conditions (WW), the absorption efficiency (AE) of the analyzed nutrients varied among genotypes, with highest values for gg157, vc865, i144, and i182 (Table 2). For clones gg157 and vc865 under drought stress, the reductions in

AE were generally highest for all evaluated nutrients, probably due to their higher RDM. For clones gg157 and vc865 under drought stress, the reductions in AE were generally highest for all evaluated nutrients, probably due to their higher RDM (Table 2).

The nutrient translocation efficiency (TE) for Ca, P, and B was similar under sufficient water supply (Table 2). Under water stress, however, TE declined for most nutrients, mainly for Ca (~28 %) in clones 1528, gg157, and i042. The greatest reduction in TE of B to shoots was observed in clone i042 (Table 2). For Ca, Mg, and P, the TE was highly affected in drought-stressed clones gg157, i042, and i182.

Table 2. Absorption efficiency (AE) and translocation efficiency (TE) of nutrients in ten *Eucalyptus* clones under well-watered (WW) or water stress (WS) conditions. Data indicate the mean ± SE (n =4). Means followed by the same letter in a column did not differ significantly by the Scott Knott test (p ≥ 0.05)

Clone	Treat(1)	K	B	Ca	Mg	P
				AE ($mg\ g^{-1}$)		
1528	WW	45.11 ± 6.3c	0.17 ± 0.01b	8.01 ± 0.2b	3.81 ± 0.2c	16.69 ± 1.2c
	WS	23.84 ± 3.9d	0.15 ± 0.01b	7.43 ± 1.1b	2.40 ± 0.5c	10.85 ± 1.5c
1641	WW	59.09 ± 6.3b	0.25 ± 0.03a	6.84 ± 0.8b	4.74 ± 0.7b	22.32 ± 3.4b
	WS	45.42 ± 4.0c	0.22 ± 0.01a	6.95 ± 0.3b	3.69 ± 0.4c	17.91 ± 1.6c
3367	WW	65.35 ± 9.5b	0.23 ± 0.03a	5.12 ± 0.6b	3.74 ± 0.5c	21.72 ± 3.1b
	WS	51.13 ± 4.8c	0.20 ± 0.02b	6.48 ± 0.6b	2.98 ± 0.3c	18.45 ± 2.2c
gg157	WW	79.80 ± 6.5a	0.30 ± 0.02a	10.95 ± 1.0a	5.21 ± 0.4b	29.70 ± 2.1a
	WS	30.83 ± 3.7d	0.16 ± 0.02b	7.07 ± 0.7b	2.16 ± 0.3c	13.06 ± 1.5c
i042	WW	68.60 ± 4.2b	0.19 ± 0.02b	6.99 ± 0.3b	4.29 ± 0.4b	18.18 ± 1.6c
	WS	41.58 ± 8.2c	0.16 ± 0.01b	6.43 ± 0.4b	2.77 ± 0.3c	13.02 ± 1.2c
i144	WW	59.60 ± 3.2b	0.22 ± 0.01a	9.76 ± 0.6a	5.28 ± 0.1b	21.18 ± 0.9b
	WS	33.36 ± 4.2d	0.14 ± 0.01b	8.63 ± 0.2a	3.50 ± 0.2c	14.69 ± 0.8c
i182	WW	81.35 ± 2.6a	0.24 ± 0.02a	7.98 ± 0.4b	6.20 ± 0.3a	27.44 ± 0.9a
	WS	47.01 ± 2.4c	0.20 ± 0.01b	6.70 ± 0.7b	3.31 ± 0.3c	18.67 ± 1.2c
i224	WW	60.96 ± 2.9b	0.21 ± 0.03b	7.71 ± 0.8b	4.67 ± 0.6b	23.00 ± 3.0b
	WS	43.69 ± 5.0c	0.17 ± 0.03b	7.85 ± 0.9b	3.15 ± 0.6c	15.89 ± 2.5c
PL40	WW	67.93 ± 4.5b	0.25 ± 0.01a	7.49 ± 1.1b	4.74 ± 0.4b	23.46 ± 1.3b
	WS	48.45 ± 3.9c	0.17 ± 0.03b	7.02 ± 0.3b	2.67 ± 0.3c	16.10 ± 1.3c
vc865	WW	75.84 ± 8.0a	0.25 ± 0.02a	9.28 ± 0.7a	5.85 ± 0.5a	24.40 ± 1.8b
	WS	48.98 ± 3.6c	0.15 ± 0.01b	6.64 ± 0.4b	3.08 ± 0.4c	15.49 ± 1.7c
				TE ($mg\ g^{-1}$)		
1528	WW	0.83 ± 0.02a	0.75 ± 0.04a	0.85 ± 0.02a	0.84 ± 0.02b	0.78 ± 0.02a
	WS	0.84 ± 0.03a	0.63 ± 0.02b	0.62 ± 0.03c	0.77 ± 0.02c	0.75 ± 0.01b
1641	WW	0.83 ± 0.01a	0.80 ± 0.03a	0.88 ± 0.01a	0.88 ± 0.01a	0.80 ± 0.02a
	WS	0.81 ± 0.01a	0.73 ± 0.01a	0.76 ± 0.01b	0.85 ± 0.01b	0.76 ± 0.02b
3367	WW	0.81 ± 0.02a	0.79 ± 0.04a	0.91 ± 0.01a	0.87 ± 0.02a	0.78 ± 0.04a
	WS	0.85 ± 0.02a	0.74 ± 0.03a	0.74 ± 0.01b	0.85 ± 0.02b	0.79 ± 0.01a
gg157	WW	0.87 ± 0.01a	0.77 ± 0.02a	0.89 ± 0.01a	0.89 ± 0.01a	0.83 ± 0.01a
	WS	0.84 ± 0.01a	0.67 ± 0.02b	0.63 ± 0.04c	0.81 ± 0.03b	0.75 ± 0.03b
i042	WW	0.83 ± 0.01a	0.78 ± 0.05a	0.88 ± 0.01a	0.89 ± 0.01a	0.79 ± 0.01a
	WS	0.78 ± 0.02b	0.52 ± 0.06c	0.66 ± 0.05c	0.84 ± 0.02b	0.72 ± 0.01b
i144	WW	0.81 ± 0.01a	0.78 ± 0.04a	0.86 ± 0.01a	0.87 ± 0.01a	0.82 ± 0.00a
	WS	0.72 ± 0.03c	0.70 ± 0.02b	0.70 ± 0.01b	0.76 ± 0.00c	0.71 ± 0.00b
i182	WW	0.86 ± 0.01b	0.81 ± 0.02a	0.94 ± 0.01a	0.91 ± 0.01a	0.83 ± 0.02a
	WS	0.81 ± 0.02b	0.69 ± 0.00b	0.72 ± 0.02b	0.84 ± 0.01b	0.76 ± 0.01b
i224	WW	0.82 ± 0.01b	0.81 ± 0.03a	0.84 ± 0.04a	0.82 ± 0.02b	0.79 ± 0.02a
	WS	0.82 ± 0.03b	0.70 ± 0.04b	0.67 ± 0.04c	0.82 ± 0.01b	0.80 ± 0.01a
PL40	WW	0.85 ± 0.01b	0.80 ± 0.00a	0.92 ± 0.01a	0.90 ± 0.02a	0.79 ± 0.02a
	WS	0.87 ± 0.00b	0.69 ± 0.05b	0.71 ± 0.04b	0.86 ± 0.01b	0.77 ± 0.01a
vc865	WW	0.84 ± 0.01b	0.85 ± 0.02a	0.89 ± 0.01a	0.86 ± 0.00b	0.79 ± 0.02a
	WS	0.81 ± 0.02b	0.79 ± 0.03a	0.75 ± 0.03b	0.82 ± 0.01b	0.73 ± 0.04b

(1) Treatment.

The nutrient use efficiency for leaf (UEL) and root (UER) production, under well-watered conditions, was generally highest for clones PL40, vc865, and 1641. Under drought, UEL was reduced in most of the clones by around 50 %, compared to the control plants (Table 3). For UER, the clones differed significantly. For Ca, Mg, and P, UER in clones

Table 3. Efficiency of nutrient use of leaf (UEL) and root production (UER) in ten *Eucalyptus* clones under well-watered (WW) or water stress (WS) conditions. Data indicate the mean ± SE (n =4). Means followed by the same letter in a column did not differ significantly by the Scott Knott test ($p \geq 0.05$)

Clone	Treat$^{(1)}$	K	B	Ca	Mg	P
UEL (g^2 mg^{-1})						
1528	WW	0.27 ± 0.02b	65.7 ± 5.37b	1.79 ± 0.36c	2.63 ± 0.40b	0.90 ± 0.06b
	WS	0.21 ± 0.03b	32.4 ± 4.17b	1.11 ± 0.14c	1.85 ± 0.24b	0.63 ± 0.06b
1641	WW	0.46 ± 0.05a	86.9 ± 6.19a	4.49 ± 0.49a	5.12 ± 0.59a	1.31 ± 0.14a
	WS	0.25 ± 0.03b	47.4 ± 5.99b	1.98 ± 0.29c	2.41 ± 0.31b	0.68 ± 0.09b
3367	WW	0.30 ± 0.02b	68.2 ± 11.7b	3.16 ± 0.25b	4.13 ± 0.50a	0.96 ± 0.10b
	WS	0.18 ± 0.02b	42.2 ± 7.07b	1.83 ± 0.25c	2.59 ± 0.26b	0.64 ± 0.05b
gg157	WW	0.21 ± 0.02b	47.3 ± 4.70b	1.58 ± 0.16c	2.51 ± 0.22b	0.67 ± 0.08b
	WS	0.12 ± 0.02b	25.3 ± 2.40b	0.86 ± 0.15c	1.37 ± 0.18b	0.41 ± 0.05b
i042	WW	0.29 ± 0.05b	98.0 ± 15.7a	2.96 ± 0.61b	3.76 ± 0.45a	1.16 ± 0.20a
	WS	0.17 ± 0.04b	51.9 ± 10.2b	1.69 ± 0.46c	2.02 ± 0.53b	0.68 ± 0.19b
i144	WW	0.21 ± 0.01b	43.9 ± 4.84b	1.45 ± 0.18c	1.92 ± 0.19b	0.59 ± 0.05b
	WS	0.14 ± 0.02b	29.7 ± 8.35b	0.73 ± 0.18c	1.09 ± 0.26b	0.38 ± 0.09b
i182	WW	0.39 ± 0.04a	125.2 ± 8.85a	4.10 ± 0.27a	4.58 ± 0.61a	1.16 ± 0.15a
	WS	0.21 ± 0.02b	55.1 ± 9.01b	2.24 ± 0.42c	2.70 ± 0.47b	0.64 ± 0.12b
i224	WW	0.28 ± 0.05b	70.6 ± 11.8b	2.64 ± 0.38c	3.33 ± 0.49b	0.81 ± 0.11b
	WS	0.22 ± 0.04b	52.4 ± 9.15b	1.88 ± 0.35c	2.65 ± 0.39b	0.73 ± 0.14b
PL40	WW	0.51 ± 0.12a	115.8 ± 21.5a	4.93 ± 1.20a	6.26 ± 1.21a	1.77 ± 0.32a
	WS	0.15 ± 0.01b	42.3 ± 9.49b	1.56 ± 0.21c	2.15 ± 0.22b	0.59 ± 0.05b
vc865	WW	0.43 ± 0.10a	105.1 ± 23.4a	3.98 ± 1.43a	5.39 ± 2.25a	1.59 ± 0.67a
	WS	0.32 ± 0.03b	82.0 ± 9.52b	2.85 ± 0.37b	4.02 ± 0.67a	1.17 ± 0.15a
UER (g^2 mg^{-1})						
1528	WW	0.34 ± 0.01b	64.2 ± 12.0b	2.18 ± 0.41c	4.27 ± 0.66a	0.70 ± 0.08a
	WS	0.79 ± 0.14a	48.2 ± 4.30c	0.98 ± 0.11c	5.12 ± 0.62a	1.00 ± 0.09a
1641	WW	0.37 ± 0.05b	73.1 ± 6.78b	4.59 ± 0.83b	6.73 ± 1.38a	0.84 ± 0.16a
	WS	0.28 ± 0.03b	42.3 ± 4.69c	1.46 ± 0.19c	4.21 ± 0.09a	0.59 ± 0.08b
3367	WW	0.22 ± 0.04b	61.7 ± 7.61b	6.17 ± 1.19a	5.79 ± 0.70a	0.59 ± 0.05b
	WS	0.20 ± 0.00b	32.0 ± 6.47c	0.91 ± 0.09c	3.53 ± 0.42a	0.39 ± 0.02b
gg157	WW	0.15 ± 0.02b	22.9 ± 2.11c	1.29 ± 0.13c	2.78 ± 0.20a	0.31 ± 0.03b
	WS	0.37 ± 0.06b	34.0 ± 7.63c	0.66 ± 0.05c	4.33 ± 0.52a	0.53 ± 0.07b
i042	WW	0.24 ± 0.02b	76.9 ± 21.3b	3.49 ± 0.55b	5.72 ± 0.48a	0.73 ± 0.06a
	WS	0.24 ± 0.07b	26.5 ± 7.17c	0.87 ± 0.15c	4.79 ± 1.59a	0.53 ± 0.13b
i144	WW	0.17 ± 0.02b	41.5 ± 7.67c	1.43 ± 0.15c	2.73 ± 0.20a	0.48 ± 0.03b
	WS	0.18 ± 0.02b	41.7 ± 9.20c	0.64 ± 0.10c	1.98 ± 0.18a	0.38 ± 0.04b
i182	WW	0.24 ± 0.02b	62.3 ± 2.99b	5.55 ± 0.68a	4.93 ± 0.24a	0.58 ± 0.04b
	WS	0.26 ± 0.02b	39.8 ± 7.56c	1.34 ± 0.35c	4.69 ± 0.93a	0.52 ± 0.09b
i224	WW	0.23 ± 0.05b	71.5 ± 20.7b	2.29 ± 0.61c	3.15 ± 0.63a	0.54 ± 0.11b
	WS	0.31 ± 0.07b	48.3 ± 8.85c	0.90 ± 0.17c	5.17 ± 2.21a	0.86 ± 0.35a
PL40	WW	0.36 ± 0.11b	66.9 ± 10.6b	6.05 ± 1.83a	8.17 ± 2.84a	0.72 ± 0.20a
	WS	0.27 ± 0.06b	32.9 ± 5.42c	0.81 ± 0.07c	4.59 ± 0.84a	0.45 ± 0.08b
vc865	WW	0.23 ± 0.04b	76.7 ± 15.7b	2.73 ± 0.31c	3.60 ± 0.73a	0.56 ± 0.07b
	WS	0.38 ± 0.06b	107.9 ± 6.30a	2.05 ± 0.22c	6.79 ± 1.39a	0.82 ± 0.06a

(1) Treatment.

PL40 and 3367 was negatively affected by drought. For K, B, and Mg, UER of the clones gg157, vc865, and i224 increased. In clone i144, UER for K, Ca, P, Mg, and B were not affected by drought (Table 3).

Nutrient efficiency groups

Based on the analysis of the mean nutrient AE and UE for leaf and root production, according to the water supply level, the *Eucalyptus* clones were clustered in four groups (Figures 2 to 6): clones with high nutrient AE and UE (Group 1); clones with low AE and high UE (Group 2); clones with high AE and low UE (Group 3); and clones with low AE and UE (Group 4).

Clones PL040, vc865, and i182, under adequate water supply, were characterized as nutritionally efficient for K (Group 1) for leaf formation. Even under drought stress, clones vc865 and i182 remained in Group 1, due to invariably high AE and UE for K (Figure 2a and 2c).

Clone 1641, with high UEK and low AEK for leaf formation under WW conditions (Group 2), increased AEK under drought stress (Group 1). Similar results were observed for AEK of clones 3367 and 1528.

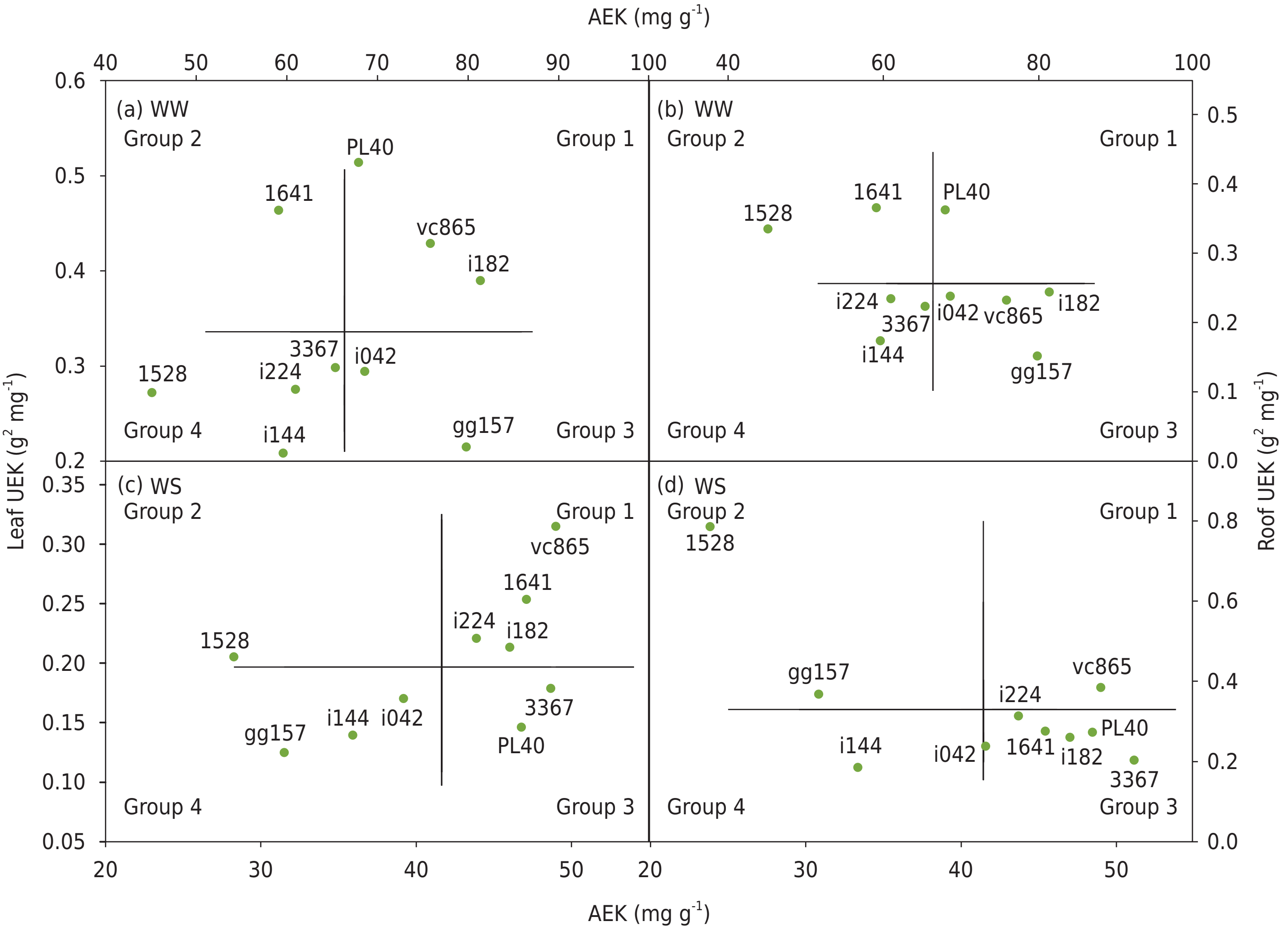

Figure 2. Distribution diagram of K absorption efficiency (AEK) and K use efficiency in leaves (leaf UEK) (a) and roots (root UEK) (b) of well-watered (WW) plants; and of the absorption efficiency (AEK) and K use efficiency in leaves (leaf UEK) (c) and roots (root UEK) (d) of water-stressed (WS) seedlings of ten *Eucalyptus* clones.

Drought stress reduced UE of the clones PL040 and gg157 for leaf and root production, had no effect on AEK of PL040, and increased AE for the other clones (Group 3; Figure 2c and 2d). Clone i144, our reference for drought tolerance, was characterized as inefficient in AEK and UEK for leaf and root production (Group 4), regardless of the water supply level (Figure 2).

Clone PL040 had high B and UECa for both leaf and root production under sufficient water supply. However, AE and UE decreased drastically under drought stress, compared to the other clones (Figures 3 and 4). In clones i182, 3367, 1641, i144, gg157, and PL40 under drought stress, AEB was also reduced for root formation (Figure 3d).

Clones 1641 and 3367, for leaf production and clones 1641 and i182, for root production, were grouped and characterized as low AECA and high UECa, independent of the water supply level (Group 2; Figure 4). In clones i042 and vc865, an increment in Ca- UE was observed under drought stress, for leaf (Group 2; Figure 4c) and root formation (Group 2; Figure 4d), respectively. Water-stressed clones i224 and 1528 showed increased UECa for the formation of both organs (Figure 4).

In clones i224, i182, and vc865, the UEMg for leaf and root production was highest under drought stress (Figure 5). On the other hand, clones i042 (reference for drought

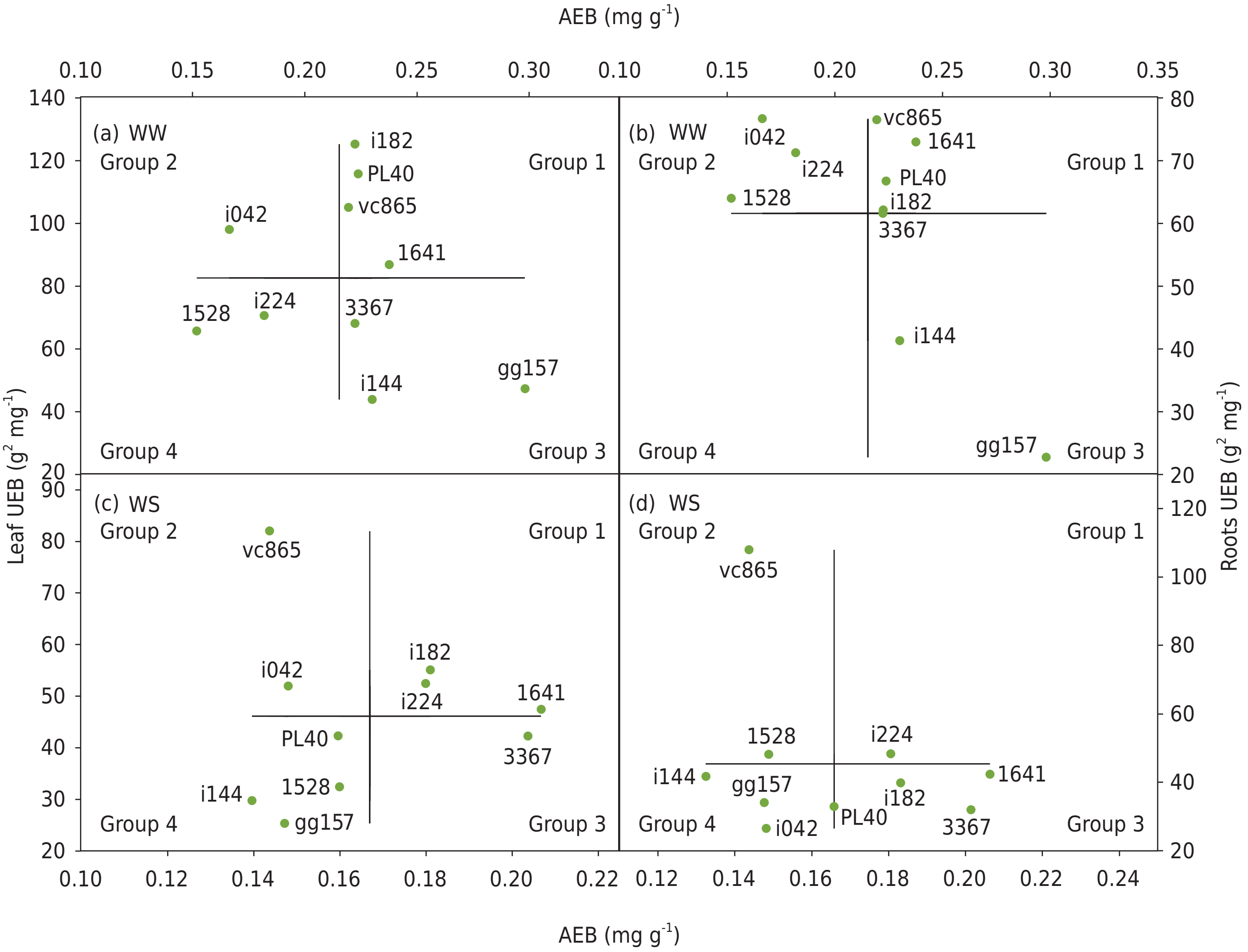

Figure 3. Boron absorption efficiency (AEB) and B use efficiency in leaves (leaf UEB) (a) and roots (root UEB) (b) of well-watered (WW) plants; and B absorption efficiency (AEB) and B use efficiency in leaves (leaf UEB) (c) and roots (root UEB) (d) of water-stressed (WS) seedlings of ten *Eucalyptus* clones.

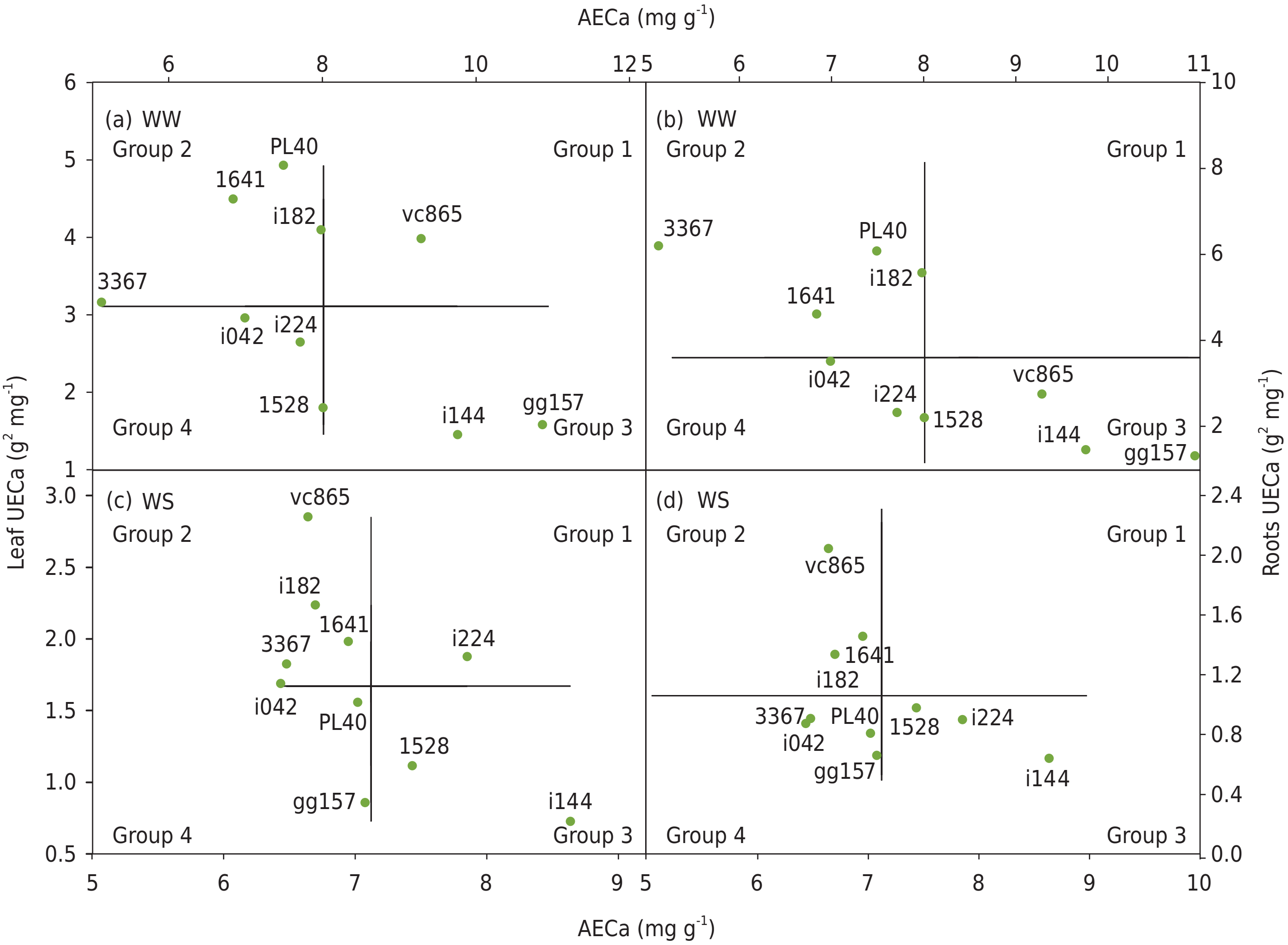

Figure 4. Calcium absorption efficiency (AECa) and Ca use efficiency in leaves (leaf UECa) (a), and roots (root UECa) (b) of well-watered (WW) plants; and Ca absorption efficiency (AECa) and Ca use efficiency in leaves (leaf UECa) (c) and roots (root UECa) (d) of water-stressed (WS) seedlings of ten *Eucalyptus* clones.

sensitivity), 1528, PL040, and 3367 were considered the least efficient in Mg uptake and use for leaf formation during drought stress (Figure 5).

Clone PL040 was the most efficient in P-UE for leaf and root formation under sufficient water supply, whereas under drought stress, clones i224 and vc865 were more efficient in this regard (Figure 6).

DISCUSSION

Responses in growth traits as indicators of drought tolerance level

During drought periods, the water content in plant tissues is usually reduced, promoting cell contraction and loss of turgor (Cosgrove, 1997), leading to slower leaf expansion (Pita and Pardos, 2001). Thus, one of the responses to water stress is a reduction in leaf area formation and expansion, and decreased plant biomass, as observed for clones i042, 3367, and 1641. The growth of drought-tolerant plants, however, can be maintained or, at least, less affected, due to different physiological adaptations. For example, a lower impact of LDM (leaf dry matter) and RDM (root dry matter) reduction was observed for the clones i144, i182, i224, and gg157.

Investment in the root system (Matos et al., 2016) and turgor maintenance through the exploration of a larger soil volume (Granda et al., 2014) was previously described

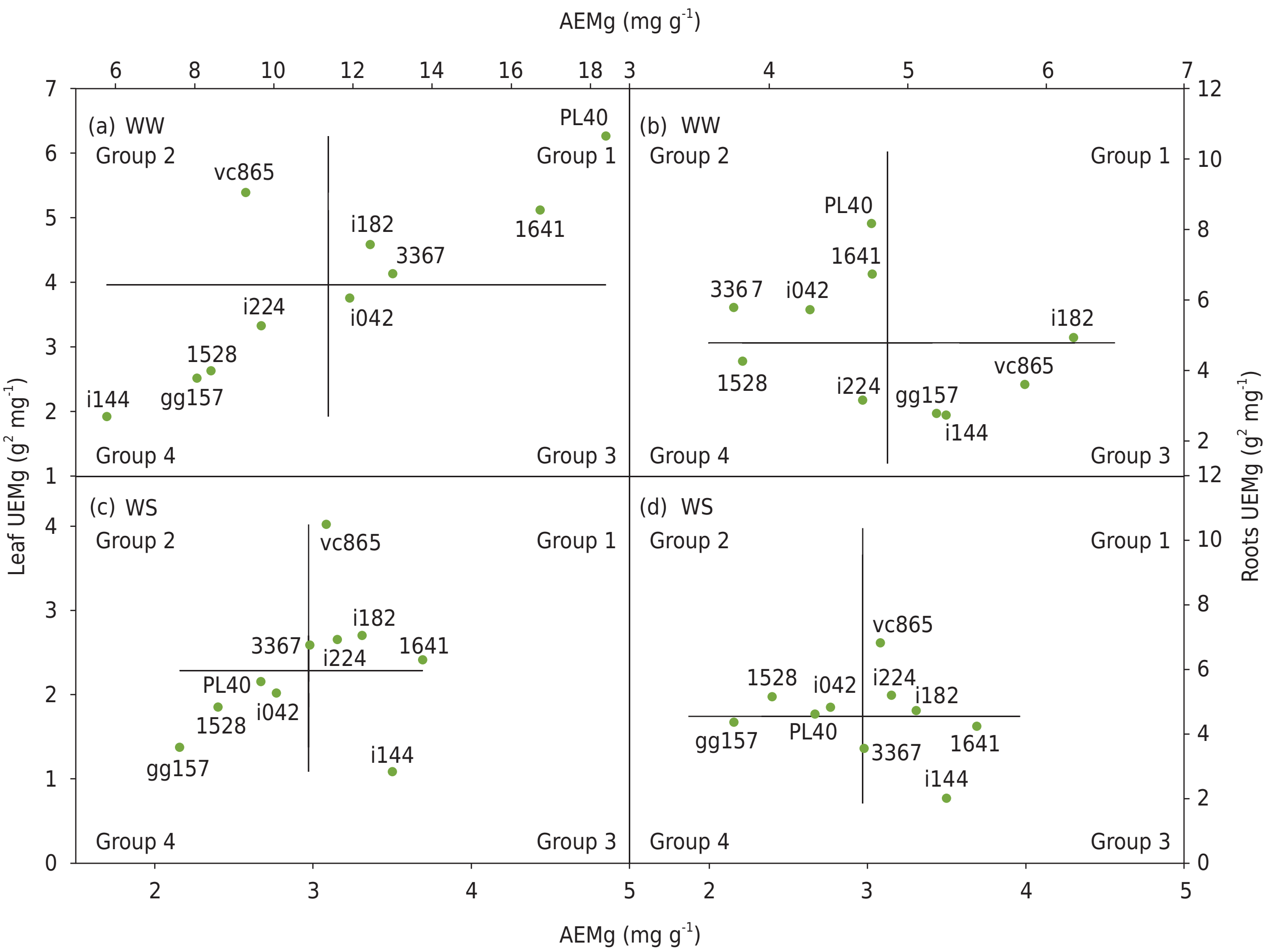

Figure 5. Magnesium absorption efficiency (AEMg) and Mg use efficiency in leaves (leaf UEMg) (a) and roots (root UEMg) (b) of well-watered (WW) plants; and Mg absorption efficiency (AEMg) and Mg use efficiency in leaves (leaf UEMg) (c) and roots (root UEMg) (d) of water-stressed (WS) seedlings of ten *Eucalyptus* clones.

as part of an overall sequence of drought-tolerance mechanisms in *Eucalyptus* trees. In our study, the amount of RDM was more sensitive to drought stress than root length. In clone vc865, despite an unchanged root length, RDM increased.

The significant increase in RDM (vc865) or root length and RDM/TDM ratio (gg157) of drought-stressed plants indicated that, these genotypes allocate photosynthates to root growth in response to drought, as previously observed by Merchant et al. (2006). In addition, cell walls in the apical part of the root were described as more extensible and less drought-sensitive than in the shoot (Wu and Cosgrove, 2000).

Carbon allocation to the root system (Galvez et al., 2011), root hydraulic conductance (Silva et al., 2004), and root cell wall changes (Cosgrove, 1993) become important for plant development, for expanding the exploited area of the soil profile allowing increases in nutrient and water uptake. In this study, the ability of clones vc865 and gg157 to increase root biomass under drought stress most likely contributed to their tolerance to water stress.

Drought-tolerant clones with generally high AE, but low nutrient UE under contrasting water supply.

Roots regulate water and nutrient uptake to sustain plant growth. During restrictions in resource supply, these processes become critical. Higher AE and UE resulted in higher biomass yield (Rosim et al., 2016), allowing maintenance of productivity, mainly on nutrient-poor soils. Consequently, genotypes with greater capacity of nutrient absorption

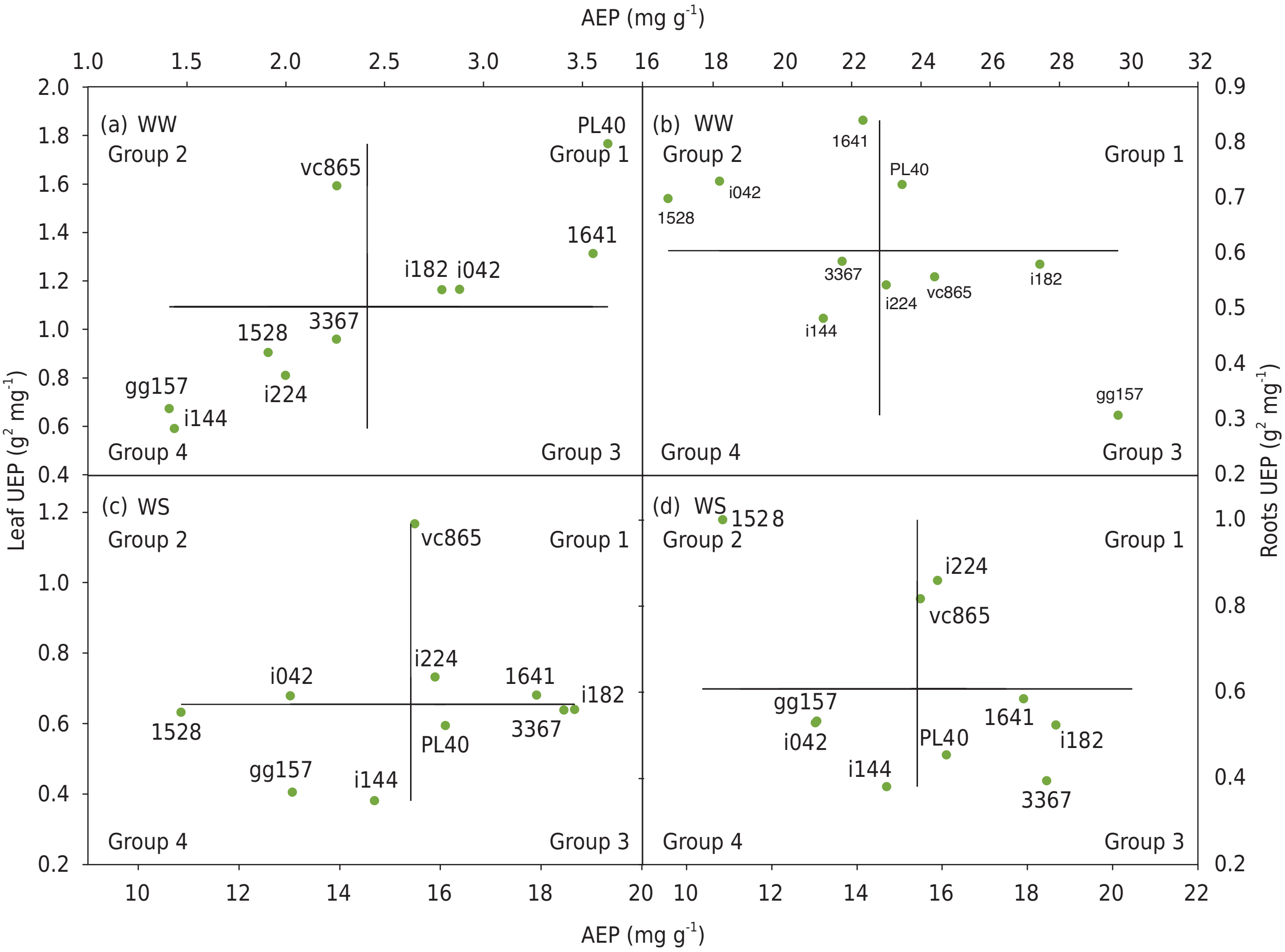

Figure 6. Phosphorus absorption efficiency (AEP) and P use efficiency in leaves (leaf UEP) (a) and roots (root UEP) (b) of well-watered (WW) plants; and P absorption efficiency (AEP) and P use efficiency in leaves (leaf UEP) (c) and roots (root UEP) (d) of water-stressed (WS) seedlings of ten *Eucalyptus* clones.

and/or use are more desirable (Godoy and Rosado, 2011), because, in addition to a better exploitation of the fertilized nutrients, smaller amounts of nutrients are required for growth (increased efficiency).

Comparing all genotypes and analyzing the UE and AE for K, the studied clones had surprising responses to different levels of water availability. The clones vc865 and i182, under sufficient water supply, were characterized as efficient for leaf formation. Clone 1528, on the other hand, inserted in the group with low AE and UE for leaf production under appropriate water conditions, showed a significant increase in UE for leaf formation under water stress, characterized by low AE, but high UEK. The K use for leaf and root formation of the drought-tolerant clone (i144) was characterized as ineffective, both under sufficient water supply and drought stress.

Clones with higher UEK can develop in soils with low K availability, without reductions in biomass production (Pinto et al., 2011), but to support dry spells, higher K contents in the leaves are required (Wang et al., 2013). In this way, the nutritional economy, reflected in higher leaf production per unit of nutrient, may not be advantageous in these adverse situations, but rather a higher K content, which will prevent stomatal inefficiency due to lack of K

During the dry season, stomatal aperture is reduced to avoid excessive water loss and the photosynthetic capacity of plants is restricted. Aside from stomatal limitations, water stress can cause oxidative damage, leading to chlorophyll degradation, as described for *Eucalyptus* (Coscolin et al., 2011). Therefore, lower UEK would probably be advantageous for drought tolerance, since K affects the stomatal control directly, and K deficiency causes a reduction in the osmotic pressure of the guard cells, reducing their swelling and leading to partial stomatal closure (Battie-Laclau et al., 2014). These mechanisms establish a tighter control over leaf water loss and a more conservative strategy of water use in plants.

As important as K, nutrients such as Mg are critical for plant health, due to their incorporation in the chlorophyll and other cellular structures involved in primary metabolism. Deficiency in Mg leads to carbohydrate accumulation in leaves (Kobayashi et al., 2013), as a result of inhibition of carbon metabolism and restriction of CO_2 fixation (Cakmak and Kirby, 2008). Thus, this nutrient may contributed to the maintenance of photosynthetic pigments in plants under drought stress, which could explain the fact that Mg, even indirectly, promotes root growth and increases in specific root area to facilitate water and nutrient acquisition (Makkonen-Spiecker and Spiecker, 1997). The clones i042, 1528, PL040, and 3367 were considered least efficient in Mg uptake and use for leaf formation and clones i224, i182, and vc865 were most efficient during drought stress. Unlike in the case of UEK, the low Mg use efficiency of these genotypes may have decreased their photosynthetic capacity, thus resulting in reductions in TDM, as observed in this study. Associated to plant physiological responses, the clones with most efficient P use for leaf and root formation during drought stress were i224 and vc865. Phosphorus is reported to be involved in the maintenance of the leaf water potential, stomatal conductance (Brück et al., 2000), photosynthesis (Warren, 2011), and root growth (Gonçalves and Passos, 2000) of drought stressed plants.

Similarly, to other nutrients, the importance of Ca and/or B for drought tolerance of plants has been shown in several studies (Möttönen et al., 2005; Hassan et al., 2011; Barros Filho, 2014; Hodecker et al., 2014; Pita-Barbosa et al., 2016). For clone PL040, although efficient in Ca and B use under sufficient water supply, AE and UE were strongly affected under drought stress, compared to the other genotypes. In clones i182, 3367, 1641, gg157, and PL40 under drought stress, UEB for root production declined. Thus, the lower UE of this nutrient in these clones may have been due to the lower B absorption and, consequently, B content, especially in the root system, resulting in a drastic reduction of root growth observed in this study.

Clone vc865 showed an increase in UECA for leaf and root formation in response to drought stress, whereas AE-Ca increased in clones i224 and 1528. Due to its low mobility in the plant, Ca translocation is extremely dependent on the transpiration rate (White, 2001), which is strongly reduced under water stress. In addition, this nutrient promotes increased growth under water stress, especially of roots (Matos et al., 2012). The greater UECa for root production may be an important strategy of drought tolerance, since the expansion of the root surface allows greater water and nutrient uptake. However, we emphasize that a high growth rate of organs with low transpiration rates increases the risk that the content of these nutrients required for cell wall stabilization and membrane integrity would drop below the critical level (Hawkesford et al., 2012).

In this study, K, Ca, and B were the most important nutrients that differentiated the genotypes in groups of sensitive or tolerant to drought in young eucalyptus plants. The high absorption efficiency during water stress periods provides nutrients to the plants, limiting the physiological changes and favoring the plant development. However, several questions arise from these observations, for example: To what extent are higher UE for all nutrients important to cope with unfavorable climatic conditions such as water stress? Interestingly, the drought-tolerant clone (i144) under water stress generally had high AE, however low nutrient UE, while the drought-sensitive clones, mainly PL40 and i042, under water stress had low AE, low UE for root formation, and high AE for leaf formation. The higher AE and lower UE observed in the tolerant clone under drought stress may be an adaptive strategy of nutrient accumulation in an unfavorable soil-climate situation and subsequently increased nutrient translocation and use under favorable growth conditions.

Additionally, clones with high nutrient UE are promising regarding cost reductions for fertilization on plantations.

In general, clones with high nutrient AE and UE under sufficient water supply could not maintain this efficiency under drought stress and can therefore be planted in regions with low nutrient availability, but high water supply. It should be emphasized that a single clone will not have the highest AE and UE for all nutrients and, in general, clones i224, vc865, and i182 were considered the most efficient in nutrient absorption and use for leaf and root formation during water stress. Analyzing AE and EU, especially for K, B, and Ca, allowed an effective separation and characterization of eucalyptus clones for drought tolerance. The rapid identification and strategic distribution of the clones most susceptible to drought stress and nutrient limitation is extremely important, mainly for forest companies that plant hundreds of clones per year. In this sense, the methodology of genotype separation and characterization used in this experiment amplified the indication of *Eucalyptus* genotypes suited for areas with restricted water and nutrient supply.

CONCLUSION

The nutrient uptake and use efficiency of drought-sensitive clones was drastically reduced in trees under drought stress.

Drought-tolerant clones under drought stress generally had high AE, however low nutrient UE, while the drought-sensitive clones under stress had low AE, low UE for root formation, and high AE for leaf formation.

Potassium, Ca, and Mg were the most discriminating nutrients to separate the studied clones. The clones vc865, i182, i144, and gg157 can be grouped as drought-tolerant (lower growth reduction and higher AE); 1528 and i224 as moderately drought-tolerant; and 1641, 3367, i042, and PL040 as drought-sensitive (higher growth reduction and lower STI and AE).

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