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Research article

Hormonal dynamics during recovery from drought in two *Eucalyptus globulus* genotypes: From root to leaf

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Drought is a limiting environmental stress that represents a growing constraint to the forestry sector. *Eucalyptus globulus* is a widely planted coppice species, which capacity to cope with water deficit has already been described. However, the capacity of this species to recover is still poorly understood. In this study, we aimed to investigate the changes in abscisic acid (ABA), ABA-glucose ester (ABA-GE) and jasmonic acid (JA) content in leaves, xylem sap and roots of two genotypes (AL-10 and AL-18) during rewatering (2 h, 4 h, 24 h, and 168 h), after a drought stress period (0 h). We wished to clarify the role of these hormones in the recovery from drought and to determine whether these hormonal relations were related to specific genotype metabolisms. Our results showed that drought caused an increased in ABA and ABA-GE levels in all analysed plant parts, while JA content decreased in leaves, increased in xylem sap and did not change in roots. Some of these responses were genotype specific. During rewatering, ABA and ABA-GE content decreased in both genotypes and all plant parts, but at different time scales, and JA levels did not greatly change. Again, the genotypes responded differently. Altogether, our results characterised the response pattern of clone AL-10 as more responsive and defended that leaf should be used in preliminary screening methods of stress tolerance. The hormonal dynamics were related to the previously documented responses of these genotypes and sustain further physiological and molecular studies of water stress in this and other tree species.

1. Introduction

Since their early transition from aquatic to terrestrial environments, plants have coped with periodic and unpredictable environmental stresses (Zhang et al., 2006). The exposure to these variable and often potentially damaging environmental conditions over a long evolutionary time scale has led plants to evolve complex systems of defence (Dobra et al., 2010). Also, plants have acquired mechanisms by which they can sensitively perceive incoming stresses and regulate their physiology accordingly (Zhang et al., 2006). Among environmental factors, water availability is probably the most limiting abiotic stress affecting terrestrial plants (Cornic and Massacci, 2004). This is a growing concern for agriculture and forestry activities, considering the increasingly unpredictable nature of rainfall (Allen et al., 2010; Roche et al., 2009; Zhang et al., 2006), which translates into important economic losses.

Eucalyptus has been extensively grown around the world, essentially for pulp production, due to the quality of its fibre, its pulp yield, and its high growth rate (Luger, 2003). Eucalypt plantations are a successful example of a fast growing coppice species in several European countries, relying almost exclusively on a single species (*Eucalyptus globulus*), used for the production of industrial biomass (Luger, 2003). Many studies have reported the capacity of this important species to cope with water deficit (Bedon et al., 2011; Costa e Silva et al., 2004; Dutkowski and Potts, 2012; Navarrete-Campos et al., 2012). However, many aspects of eucalypt forestry still need research (Luger, 2003). One of such aspects is the capacity

Abbreviations: ABA, abscisic acid; ABA-GE, abscisic acid glucose ester; UPLC, ultra performance capillary electrophoresis; JA, jasmonic acid; LC, liquid chromatography; MS/MS, tandem mass spectrometry; PCA, principal components analysis; two-way ANOVA, two-way analysis of variance; VPD, vapour pressure deficit; WS, water stressed; WW, well watered.

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to recover after a period of drought, an important and poorly understood topic (Chaves et al., 2003). We have successfully shown that recovery from water deficit involves many physiological modifications in eucalypt plants and is genotype-specific (Correia et al., 2014). However, the mechanisms or processes that mediate the plants' response to water stress and recovery still need to be clarified.

Perception of stress signals often results in a mass production of chemical compounds, including a variety of hormones, to adapt and respond to the environmental challenges (Aimar et al., 2011). Among others, two phytohormones – ABA (abscisic acid) and JA (jasmonic acid) – are known to play major roles in regulating plant defence responses against various abiotic stresses, by mediating a wide range of adaptive responses (Hirayama and Shinozaki, 2010; Peleg and Blumwald, 2011; Santner et al., 2009). The key phytohormone involved in the response to dehydration is ABA (Dobra et al., 2010) and its involvement in mediating drought stress has been extensively explored (Zhang et al., 2006). ABA mediates fast responses associated with regulation of the plant water status through guard cells and growth (Dobra et al., 2010), but it is also involved in slower metabolic changes, coinciding with activation of cellular dehydration tolerance pathways (Dobra et al., 2010; Zhang et al., 2006). Moreover, ABA acts as a long-distance water stress signal (Hartung et al., 2002) as it is synthesised in dehydrated roots and transported via the xylem, thereby regulating stomatal opening/closure and leaf growth in the shoots (Zhang et al., 2006).

On the basis of some studies, other substances can also be involved in drought response (Schachtman and Goodger, 2008). ABA may be conjugated with glucose, thereby forming a glucose ester (ABA-GE) in xylem sap, which has been suggested to serve as a transported form of the hormone and a stress signal (Sauter et al., 2002; Schachtman and Goodger, 2008). Works reporting on the relation between drought and increased ABA-GE concentrations have already been published (López-Carbonell et al., 2009; Sauter et al., 2002). Jasmonic acid is involved in diverse plant developmental processes, such as root growth, leaf senescence or stomatal opening, and plays crucial roles in defence responses against different plant pathogens (Zhang et al., 2006). Moreover, there is increasing evidence that jasmonic acid (JA) and jasmonates are also crucial signalling molecules involved in many plant responses to abiotic stress (Balbi and Devoto, 2008; Devoto and Turner, 2003; Wasternack, 2007). De Ollas et al. (2013) reported JA as a possible precursor in the signal transduction cascade in case of drought stress, providing increased levels of ABA. Considering the pivotal importance of *E. globulus* plantations, it is essential to understand the underlying mechanisms in drought tolerance and recovery in order to select suitable clonal collections for sustainable plantations in a Mediterranean climate. Our early work showed that two different *E. globulus* genotypes (AL-10 and AL-18) coped differently with drought and rewatering: genotype AL-10 exhibited a dynamic and responsive physiological profile, while genotype AL-18 showed a slower and less reactive metabolism (Correia et al., 2014). These same profiles were maintained with respect to ABA response in leaves. However, little is known about how other plant hormones are involved, how they are regulated in the different plant parts and whether they could be used as selective markers. Assessing water stress through analysis of cultured plant tissue or specific isolated organs may offer potential for a quick evaluation as a preliminary screening method of stress tolerance (Naik and Widholm, 1993). Therefore, there is great interest to investigate stress response through the analysis of different plant parts.

Bearing this in mind, the objective of this study was to investigate the changes in ABA, ABA-GE and JA content in leaves, xylem sap and roots of the two different genotypes during rewatering (2 h, 4 h, 24 h and 168 h), following a water deficit stress of three weeks (0 h). Our specific aims were 1) to clarify the role of these hormones in the response and recovery of *Eucalyptus* plants to drought, and 2) to determine whether these hormonal relations are related to specific genotype metabolisms.

2. Materials and methods

2.1. Plant material

Two *E. globulus* Labill. genotypes (AL-10 and AL-18) were obtained from Altri Florestal SA (Portugal). We selected these genotypes, used in Portuguese forest plantations, because of their different physiological response profiles during recovery from drought, as already described in a previous report (Correia et al., 2014).

One hundred and fifty rooted cuttings of each genotype, grown in plastic containers filled with 3:1 (w/w) vermiculite:peat, with an initial height of 30 cm and six months old, were transplanted to 2 L plastic pots filled with equal weight of a 3:2 (w/w) peat:perlite mixture and transferred to a greenhouse, with daily records of temperature and humidity and VPD (vapour pressure deficit) determination.

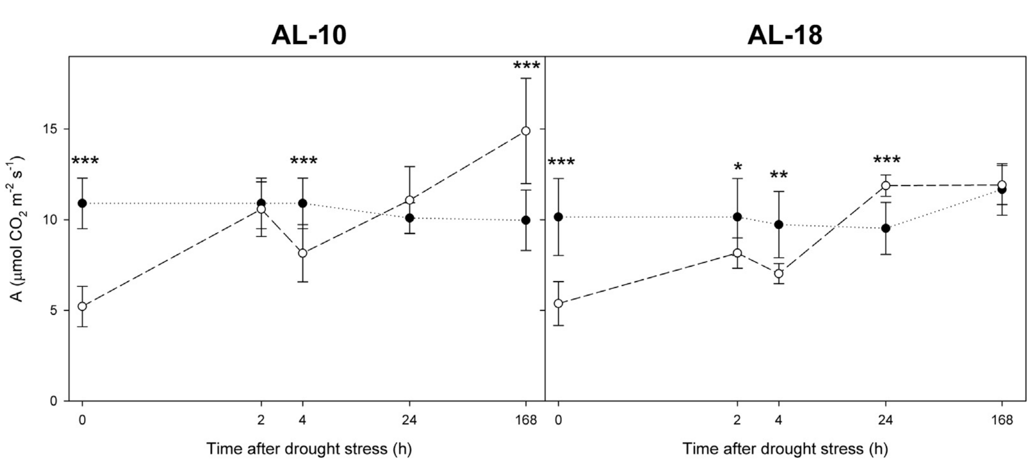

Fig. 1. Foliar net CO_2 assimilation rate (A) of water stressed (WS, dashed lines and open circles) and well-watered plants (WW, dotted line and filled circles) throughout a one-week post-stress rewatering period (i.e., recovery period). Data are shown as mean ± standard deviation; asterisks indicate significant differences between WW and WS plants ($^{***}p \leq 0.001$; $^{**}p \leq 0.01$; $^{*}p \leq 0.05$).