Journal of Plant Physiology 188 (2015) 64–71

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Metabolites and hormones are involved in the intraspecific variability of drought hardening in radiata pine

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ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 30 July 2015
Accepted 3 August 2015
Available online 25 September 2015

Keywords:
Amino acids
Growth
Hardening
Phytohormones
Pinus radiata
Polyamines

ABSTRACT

Studies of metabolic and physiological bases of plant tolerance and hardening against drought are essential to improve genetic breeding programs, especially in productive species such as *Pinus radiata*. The exposure to different drought cycles is a highly effective tool that improves plant conditioning, but limited information is available about the mechanisms that modulate this process. To clarify this issue, six *P. radiata* breeds with well-known differences in drought tolerance were analyzed after two consecutive drought cycles. Survival rate, concentration of several metabolites such as free soluble amino acids and polyamines, and main plant hormones varied between them after drought hardening, while relative growth ratio and water potential at both predawn and dawn did not. Hardening induced a strong increase in total soluble amino acids in all breeds, accumulating mainly those implicated in the glutamate metabolism (GM), especially L-proline, in the most tolerant breeds. Other amino acids from GM such as γ-aminobutyric acid (GABA) and L-arginine (Arg) were also strongly increased. GABA pathway could improve the response against drought, whereas Arg acts as precursor for the synthesis of spermidine. This polyamine showed a positive relationship with the survival capacity, probably due to its role as antioxidant under stress conditions. Finally, drought hardening also induced changes in phytohormone content, showing each breed a different profile. Although all of them accumulated indole-3-acetic acid and jasmonic acid and reduced zeatin content in needles, significant differences were observed regarding abscisic acid, salicylic acid and mainly zeatin riboside. These results confirm that hardening is not only species-dependent but also an intraspecific processes controlled through metabolite changes.

1. Introduction

Radiata pine (*Pinus radiata* D. Don) is a species widely distributed in Northern Spain, particularly in the Basque Country. Its fast growth has stimulated an exhaustive study of its wood production and a heavy requirement for developing breeding programs (Espinel et al., 1995; Codesido and Fernández-López, 2009). However, keeping in mind the future climate change scenario, the survival is still considered one of the main bottleneck processes for forest productivity. In this sense, plant survival and distribution strongly depend on their ability to adjust to environmental variations (Klein et al., 2011, 2013a,b), being drought the major limiting factor that conditions plant growth (Mena-Petite et al., 2004). The exposure of seedlings to different cycles and intensities of water stress before transplanting them into the forest can improve their

Abbreviations: ABA, abscisic acid; Arg, L-arginine; GABA, γ-aminobutyric acid; Glu, L-glutamic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; Pro, L-proline; Put, putrescine; RdGR, relative diameter growth ratio; RhGR, relative aerial height growth ratio; SA, salicylic acid; Spd, spermidine; Spm, spermine; Z, zeatin; ZR, zeatin riboside.

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http://dx.doi.org/10.1016/j.jplph.2015.08.006
0176-1617/

Fig. 1. Scheme of experimental design. First drought cycle of four weeks, rewatering for a week, and a second drought cycle until half of the plants from each breed presented external symptoms such as epinasty and apical curvature [C2.*i*, where *i* represents each breed (from O1 to O6)].

survival during subsequent drought, a process traditionally called plant conditioning or hardening (Edwards and Dixon, 1995; Villar-Salvador et al., 2004). Response and tolerance of pines to short drought periods has been widely studied (De Diego et al., 2012, 2013b) but there are limited references that have studied plant drought-hardening in depth.

In our preliminary studies, we demonstrated that exposure to a short drought period of four weeks induced a different osmotic response in each *P. radiata* breed (De Diego et al., 2013b). In addition, although all plants recovered their osmotic potential after rewatering, some solutes, especially amino acids, were maintained at high levels, including L-proline (Pro) and γ-aminobutyric acid (GABA) as the most abundant ones. Controversially, amino acids contributed in less extent to plant osmotic adjustment, pointing out their possible role as signal molecules that activate protection mechanisms to mitigate the effects of water stress (Kinnersley and Turano, 2000; Vendruscolo et al., 2007), and suggesting their possible implication in improving tolerance against future water stress situations (De Diego et al., 2013b).

Meanwhile, the short-time drought response of *P. radiata* plants also included hormonal changes, mainly for abscisic acid (ABA) and indole-3-acetic acid (IAA), although jasmonic acid (JA), salicylic acid (SA) and some cytokinins (CKs) might also be involved (De Diego et al., 2012). JA, SA and, especially CKs have been reported to show antioxidant activity that confers more tolerance against stress (Ghanem et al., 2008; Wang et al., 2010). Finally, preliminary studies of acclimation in *P. radiata* also showed that this process is conditioned by the genotype and is regulated by changes in physiological process and phytohormone concentration (De Diego et al., 2013a).

Hardening has been described as one of the most useful process for increasing plant drought tolerance and improve plantation success (Fernández et al., 2003), making its comprehension essential. Therefore, in this work we focused on the study of drought hardening at the metabolic level to clarify the possible implication of free soluble amino acids and plant growth regulators in this process. In this respect, as the most drought tolerant breeds kept accumulating Pro and GABA after rewatering (De Diego et al., 2013b) and altered the concentration of several phytohormones during first drought cycle and rewatering (De Diego et al., 2012), we hypothesized that these molecules are strongly implicated in plant tolerance and drought hardening. Moreover, we intended to clarify whether this response is species-dependent.

2. Material and methods

2.1. Plant material and experimental design

2.1.1. Plant material

Seeds from different geographical and climatological breeds were used in this work:

- O1—*P. radiata* var. radiata × *P. radiata* var *binata*: provided by Proseed and collected from a seed orchard located in Amberley (New Zealand).
- O2—*P. radiata* var. *radiata*: provided by Servicio de Material Genético del Ministerio de Medio Ambiente and collected from open-pollinated trees grown in the Basque coastline (Spain).
- O3—*P. radiata* var. *radiata*: provided by Australian Tree Seed Centre (CSIRO Forestry and Forest Products) and collected from a seed orchard located in Billapaloola (Australia).
- O4—*P. radiata* var. *radiata* × *Pinus attenuate*: provided by Proseed and collected from a seed orchard located in Amberley (New Zealand). This hybrid species was used as a tolerance marker.
- O5—*P. radiata* var. *radiata* × *P. radiata* var. *cedrosensis*: provided by Proseed and collected from a seed orchard located in Amberley (New Zealand).
- O6—*P. radiata* var. *radiata* (GF 17): provided by Proseed and collected from control-pollinated trees located in Kaingaroa (New Zealand).

2.1.2. Growth conditions

Prior to sowing, seeds were subjected to cold stratification, placing them into a cold chamber at 4 °C in dark for three weeks. Afterwards, seeds were placed in sterilized water for two days at the same conditions to induce germination. Finally, seeds were sown in pots of 17 cm Ø filled with peat:perlite (7:3, v/v). Plants were grown in a greenhouse under controlled conditions ($T = 23 \pm 1$ °C and RH = 70 ± 5%) for two years.

2.1.3. Experimental design

Two-year-old saplings were analyzed during the summer time (from July to September). Ten plants per breed were used, half were randomly selected for water stress treatment (D) by withholding water and the remainder were kept well-watered [Control plants (W)]. For four weeks, water was withheld until all breeds showed more than half of the plants with external symptoms such as needle epinasty and apical curvature (De Diego et al., 2012). Immediately after, plants were watered for one week and subjected to a second drought cycle. Due to external symptoms of needle epinasty and apical curvature in water stressed *P. radiata* plants being directly correlated to plant water status (De Diego et al., 2012, 2013a), in this second drought cycle breeds were evaluated when half of the plants from each breed presented these symptoms (C2.*i*; where *i* represented each breed from O1–O6).

2.2. Biometric, growth and water balance parameters

Total aerial height (cm) and root collar diameter (mm) of each plant were measured at C2.*i*. Relative aerial height (RdGR, cm day^{-1}) and diameter (RdGR, mm day^{-1}) growth ratio was also calculated and estimated as described by Sánchez-Gómez et al. (2010) according to the following mathematical equation:

$$\mathrm{RGR} = \frac{\ln G_i - \ln G_j}{t_i - t_j}, \qquad (1)$$

where G represents the height and root collar diameter in time i and j (t_i and t_j respectively, with $i > j$).

2.2.1. Water potential

Plant water potential was determined at C2.*i* before (Ψpre, MPa) and after dawn (Ψh, MPa) using a Scholander chamber (Skye SKPM 1400) and the pressure-equilibration technique (Scholander et al., 1965).