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RESEARCH PAPER

H₂O₂ mediates the regulation of ABA catabolism and GA biosynthesis in *Arabidopsis* seed dormancy and germination

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Abstract

 H_2O_2 is known as a signal molecule in plant cells, but its role in the regulation of aqbscisic acid (ABA) and gibberellic acid (GA) metabolism and hormonal balance is not yet clear. In this study it was found that H_2O_2 affected the regulation of ABA catabolism and GA biosynthesis during seed imbibition and thus exerted control over seed dormancy and germination. As seen by quantitative RT-PCR (QRT-PCR), H_2O_2 up-regulated ABA catabolism genes (e.g. *CYP707A* genes), resulting in a decreased ABA content during imbibition. This action required the participation of nitric oxide (NO), another signal molecule. At the same time, H_2O_2 also up-regulated GA biosynthesis, as shown by QRT-PCR. When an ABA catabolism mutant, *cyp707a2*, and an overexpressing plant, *CYP707A2-OE*, were tested, ABA content was negatively correlated with GA biosynthesis. Exogenously applied GA was able to over-ride the inhibition of germination at low concentrations of ABA, but had no obvious effect when ABA concentrations were high. It is concluded that H_2O_2 mediates the up-regulation of ABA catabolism, probably through an NO signal, and also promotes GA biosynthesis. High concentrations of ABA inhibit GA biosynthesis but a balance of these two hormones can jointly control the dormancy and germination of *Arabidopsis* seeds.

Key words: ABA, ABA catabolism, Arabidopsis, GA, GA biosynthesis, hydrogen peroxide (H₂O₂), nitric oxide (NO), seed dormancy.

Introduction

Seed germination is a complex process. Germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994; Holdsworth et al., 2008). Seeds of most angiosperms are dormant at maturity, and the dormancy must be lost before germination can occur (Bewley, 1997). Seed dormancy has been defined by Finch-Savage and Leubner-Metzger as the incapacity of a viable seed to germinate under favourable conditions (Finch-Savage and Leubner-Metzger, 2006). Many factors are involved in seed dormancy regulation, including some plant hormones, such as abscisic acid (ABA), gibberellic acid (GA), and ethylene (Bewley, 1997; Zhou et al., 1998; Ghassemian et al., 2000; Nakajima et al., 2006; Carrera et al., 2008; Holdsworth et al., 2008), some environmental factors, such as light intensity and low

temperatures (Holdsworth *et al.*, 2008), and several signalling molecules, such as nitric oxide (NO) and some reactive oxygen species (ROS) (Batak *et al.*, 2002; Bethke *et al.*, 2004, 2006; Sarath *et al.*, 2007). However, the mechanisms of dormancy holding and breaking remain unclear because it is unknown how these factors are inter-related. The mechanisms of ABA catabolism and GA biosynthesis regulation are of particular interest.

 H_2O_2 acts as a signalling molecule, participating in a series of processes including plant development, stress responses, and programmed cell death (Pei *et al.*, 2000; Bethke and Jones, 2001; Apel and Hirt, 2004; Foyer and Noctor, 2005). In plants, H_2O_2 is generated in chloroplasts, mitochondria, and peroxisomes (Mittler *et al.*, 2004). Plasma membrane NAD(P)H oxidase is reported to be the pivotal enzyme involved in H_2O_2 generation (Kauss and Jeblick, 1995,

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