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Fluctuating temperatures terminate dormancy in *Cynara cardunculus* seeds by turning off ABA synthesis and reducing ABA signalling, but not stimulating GA synthesis or signalling

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Abstract

Fluctuating temperatures terminate seed dormancy in many species, including *Cynara cardunculus* (L.) var. *sylvestris*. Termination of physiological dormancy requires low ratios of abscisic acid (ABA)/gibberellins (GA). In a previous paper we have shown that physiological responses to fluctuating temperatures comprise a reduction of abscisic acid (ABA) content and sensitivity. However, a possible stimulation of GA synthesis was also suggested as part of the mechanism. That possible stimulation, as well as the identification of potential regulatory sites for ABA and GA metabolism and signalling involved in the termination of dormancy by fluctuating temperatures, are yet to be determined. In this study, we measured GA content and sensitivity in seeds incubated under constant and fluctuating temperatures. We also assessed the expression of several genes involved in ABA and GA metabolism and signalling. Our results show that fluctuating temperatures reduce ABA/GA ratios through a reduction in ABA accumulation during incubation but without altering GA synthesis as compared to that observed under constant temperatures. On the other hand, fluctuating temperatures did not increase sensitivity to GA. Fluctuating temperatures reduced the expression of *CycaNCED* and *CycaABI5* (ABA synthesis and signalling genes) with a temporal pattern that coincides with the interruption of ABA accumulation that precedes germination of seeds incubated under fluctuating temperatures. However, fluctuating temperatures did not modify the expression of *CycaCYP707A2* (ABA inactivation) as compared to that observed under constant temperatures. Consistent with our determinations of GA content and sensitivity, fluctuating temperatures did not modify the expression of GA synthesis (*CycaGA3ox*) and signalling genes (*CycaRGL2* and *CycaGAI*) in relation to that observed at constant temperatures. These results show that fluctuating temperatures terminate dormancy in *Cynara cardunculus* seeds through an interruption in ABA accumulation and a reduction in ABA signalling exerted at the level of *CycaNCED* and *CycaABI5* expression.

Keywords: abscisic acid, dormancy, fluctuating temperatures, gene expression, gibberellins

Introduction

Dormancy could be defined as the failure of an intact viable seed to complete germination in a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination (Bewley, 1997; Baskin and Baskin, 2007). Dormancy is a common trait in non-domesticated plants which increases the ability of a species to avoid competition between individuals of the same species and prevents germination out of place or season (Finkelstein *et al.*, 2008). Seeds are dormant at the time of dispersal from the mother plant (Hilhorst, 1995) and dormancy is progressively lost as a consequence of seed interaction with environmental signals such as

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