Journal of Experimental Botany, Vol. 57, No. 6, pp. 1423–1430, 2006
doi:10.1093/jxb/erj122 Advance Access publication 17 March, 2006

Journal of Experimental Botany

RESEARCH PAPER

Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant barley grains

Roberto L. Benech-Arnold[1,*], Nicolas Gualano[1], Juliette Leymarie[2], Daniel Côme[2] and Françoise Corbineau[2]

[1] *IFEVA-Cátedra de Cerealicultura Facultad de Agronomia, Universidad de Buenos Aires/CONICET, Av. San Martin 4453, 1417 Buenos Aires, Argentina*

[2] *Université Pierre et Marie Curie-Paris 6, Physiologie Végétale Appliquée, EA 2388 Physiologie des semences, Site d'Ivry, Boîte 152, 4 Place Jussieu, F-75252 Paris cedex 05, France*

Received 30 September 2005; Accepted 13 January 2006

Abstract

Two mechanisms have been suggested as being responsible for dormancy in barley grain: (i) ABA in the embryo, and (ii) limitation of oxygen supply to the embryo by oxygen fixation as a result of the oxidation of phenolic compounds in the glumellae. The aim of the present work was to investigate whether hypoxia imposed by the glumellae interferes with ABA metabolism in the embryo, thus resulting in dormancy. In dormant and non-dormant grains incubated at 20 °C and in non-dormant grains incubated at 30 °C (i.e. when dormancy is not expressed), ABA content in the embryo decreased dramatically during the first 5 h of incubation before germination was detected. By contrast, germination of dormant grains was less than 2% within 48 h at 30 °C and embryo ABA content increased during the first hours of incubation and then remained 2–4 times higher than in embryos from grains in which dormancy was not expressed. Removal of the glumellae allowed germination of dormant grains at 30 °C and the embryos did not display the initial increase in ABA content. Incubation of de-hulled grains under 5% oxygen to mimic the effect of glumellae, restored the initial increase ABA in content and completely inhibited germination. Incubation of embryos isolated from dormant grains, in the presence of a wide range of ABA concentrations and under various oxygen tensions, revealed that hypoxia increased embryo sensitivity to ABA by 2-fold. This effect was more pronounced at 30 °C than at 20 °C. Furthermore, when embryos from dormant grains were incubated at 30 °C in the presence of 10 μM ABA, their endogenous ABA content remained constant after 48 h of incubation under air, while it increased dramatically in embryos incubated under hypoxia, indicating that the apparent increase in embryo ABA responsiveness induced by hypoxia was, in part, mediated by an inability of the embryo to inactivate ABA. Taken together these results suggest that hypoxia, either imposed artificially or by the glumellae, increases embryo sensitivity to ABA and interferes with ABA metabolism.

Key words: Abscisic acid, barley, glumellae, hypoxia, seed dormancy.

Introduction

Barley is one of the most important grain crops in the world. The barley grain is mostly used for malting, an industrial process which requires grain germination. For that reason, the presence of dormancy in the barley grain is a major constraint for its industrialization. Barley cultivars with low dormancy at harvest are usually welcomed by the industry because the grain can be malted soon after harvest, thus avoiding costs and deterioration derived from long-term storage until dormancy is terminated (Corbineau and Côme, 1996; Benech-Arnold, 2004). On the other hand, barley cultivars that lose dormancy prior to harvest are prone to sprout. Therefore, the possibility of adjusting the timing of exit from dormancy to a narrow and precise time window (i.e. neither too early so as to subject the crop to the risk of sprouting, nor too late so as to have to store the grain until the germination capacity has been

* To whom correspondence should be addressed. E-mail: benech@ifeva.edu.ar

acquired) requires a thorough knowledge of the physiological mechanisms involved in dormancy imposition and maintenance.

Dormancy of the barley grain is typically imposed by the seed-covering structures (lemma and palea, pericarp plus seed coat). Indeed, embryos can germinate well from the very early stages of development if they are isolated from the rest of the grain and incubated in water (Benech-Arnold *et al*., 1999). Limitation of oxygen supply to the embryo by oxygen fixation as a result of oxidation of phenolic compounds in the lemma and palea (hereafter referred to as the glumellae or the hull) has been suggested to be responsible for the dormancy of dressed caryopses of cereals such as barley (Lenoir *et al*., 1986) and oat (Corbineau *et al*., 1986). In dormant grains of barley, for example, whole intact caryopses germinated with difficulty, even in the air, while de-hulled caryopses were all able to germinate under oxygen tensions of at least 10%, suggesting that oxygen concentration under the covering structures might be less than 10% (Lenoir *et al*., 1986). Calculations taking into account the diffusion coefficient of oxygen through the seed coats of various seeds, the thickness of the latter and the respiratory activity of the embryo have shown that the embryos enclosed in their seed coats are subjected to hypoxia (Edwards, 1973). Dormancy of barley and other cereals from temperate climates can be regarded as a relative phenomenon, the expression of which depends on the incubation temperature. It is usually not expressed at relatively low temperatures (10–20 °C) while its expression increases as the temperature rises (Lenoir *et al*., 1983; Corbineau and Côme, 1996; Benech-Arnold, 2004).

The phytohormone abscisic acid (ABA) has largely been implicated in both the imposition and the maintenance of dormancy (Fong *et al*., 1983; Karssen *et al*., 1983; Walker-Simmons, 1987; Bewley, 1997; Steinbach *et al*., 1997). The role of ABA in dormancy imposition is clear from the low dormancy displayed by freshly harvested seeds of ABA-deficient or -insensitive mutants of *Arabidopsis* and other species whose seeds normally present primary dormancy. Moreover, application of the ABA-synthesis inhibitor, fluridone, has been shown to anticipate the release from dormancy in developing seeds of a number of species (Fong *et al*., 1983; Le Page-Degivry and Garello, 1992; Steinbach *et al*., 1997). The role of ABA in dormancy maintenance is a bit more controversial, although some reports have shown that dormancy expression in seeds of some species is associated with ABA *de novo* synthesis and/or a lower rate of ABA inactivation upon imbibition, which does not occur in seeds that do not express dormancy (Le Page-Degivry and Garello, 1992; Bianco *et al*., 1997; Le Page-Degivry *et al*., 1997; Yoshioka *et al*., 1998; Grappin *et al*., 2000). Dormancy of the barley grain also appears to be under ABA control: termination of glumellae-imposed dormancy during grain development was shown to be correlated with a sharp decline both in ABA embryonic content and sensitivity (Benech-Arnold *et al*., 1999). A role for ABA in dormancy maintenance of the barley grain has also been suggested: ABA embryonic content declines during the first hours of incubation of non-dormant seeds, whereas it remains at high levels in embryos of dormant grains (Wang *et al*., 1998). However, most functional aspects of this role of ABA in dormancy maintenance in barley have not been explored so far. Although it is known that incubation temperature can affect embryo responsiveness to ABA (Walker-Simmons, 1988; Poljakoff-Mayber *et al*., 1990; Corbineau and Côme, 2000), it is not known whether the differential expression of dormancy with temperature is also mediated by a different rate of ABA inactivation (i.e. catabolism/conjugation). The way in which oxygen influences germination of dormant seeds is largely unknown, but it has been proposed that oxygen concentration might determine the rate with which germination inhibitors or ABA are catabolized (Neill and Horgan, 1987; Barthe *et al*., 2000). If indeed ABA is implicated in dormancy maintenance, it could be hypothesized that, under conditions that allow dormancy expression (i.e. incubation at high temperatures) the hypoxia condition imposed by the glumellae is instrumental for such expression by interfering with ABA inactivation and, perhaps, by enhancing embryo sensitivity to the hormone.

In this paper these possibilities are explored. A correlation was determined between dormancy expression (i.e. through incubation at different temperatures) and ABA content evolution pattern during grain incubation in hulled and de-hulled grains. In addition, through the incubation of de-hulled grains under hypoxia, the effect of the glumellae was mimicked both in the expression of dormancy and in the evolution of ABA content, thus suggesting that glumellae interfere with ABA inactivation through oxygen deprivation. It was also tested whether the hypoxic condition imposed on the embryo can modify its responsiveness to ABA.

Materials and methods

Plant material

Barley (*Hordeum vulgare* L., cv. Pewter) grains harvested in 2002 and received from the 'Coopérative Agricole de Toury' (western France) were used throughout this study. Experiments were carried out with dormant grains, which were maintained at −20 °C from harvest until the experiments began in order to maintain their initial dormancy (Lenoir *et al*., 1983), and non-dormant grains, which were stored dry in the open air for at least 3 months at 25 °C in order to break their dormancy (Corbineau and Côme, 1996).

Germination assays

Germination assays were carried out using whole grains, de-hulled grains (seeds without glumellae), and isolated embryos which had been separated from the endosperm of dry seeds by a sharp scalpel blade, with as little endosperm as possible. Both types of grains, and embryos were germinated in 9 cm Petri dishes (50 individuals per

dish, two replicates) on a layer of cotton wool imbibed with deionized water. To test for embryo sensitivity to ABA, isolated embryos were incubated in aqueous solutions of abscisic acid (mixed isomers, Sigma) at the concentrations indicated in the text, ranging from 0 mM (water) to 1 mM. Assays were performed in darkness at 20 °C, a temperature at which dormant and non-dormant grains germinated, and 30 °C, a temperature at which only non-dormant grains were able to germinate.

Germination in atmospheres with different controlled oxygen tensions was performed at both temperatures in darkness, using the procedure of Côme and Tissaoui (1968). Gas mixtures containing 5–21% oxygen were obtained through capillary tubes from compressed air and nitrogen. The gaseous atmospheres were passed continuously through germination chambers at a constant flow rate (4.0 l h^{-1}). The oxygen tensions were measured daily using a Servomex analyser (Servomex, type 570A).

A grain or an isolated embryo was regarded as germinated when the radicle had pierced the coats in de-hulled or whole grains, or had reached 2–3 mm in length in isolated embryos. Germination counts were made every day during 7 d. The results presented are the means of the germination percentages obtained after various durations in two replicates ±arithmetical spread.

ABA quantitation

In parallel with all the germination assays carried out, batches of whole seeds, de-hulled grains and embryos were incubated and sampled at different times after incubation. Thirty embryos were dissected from the rest of the seed structure (except when isolated embryos were incubated) and immediately frozen in liquid N_2. The embryos were then lyophilized, powdered, weighed, and stored at −30 °C until assayed for ABA content with a radioimmunoassay as described elsewhere (Steinbach *et al*., 1995). The results presented are the means of three measurements carried out in duplicate ±standard deviation.

Results

Germination and embryo ABA content of dormant and non-dormant whole grains incubated at 20 °C and 30 °C

Figure 1a shows the germination, as a function of time, of dormant and non-dormant whole grains at 20 °C and 30 °C. Both dormant and non-dormant grains germinated easily at 20 °C, although dormant grains reached a somewhat lower germination percentage (76%) than non-dormant ones (100%) and also displayed a slower germination rate. Maximum germination percentage was reached in less than 24 h by non-dormant grains while it took 48 h in the case of dormant ones. By contrast, more than 80% of non-dormant grains were able to germinate when incubated at 30 °C, while almost no dormant grain germinated at this temperature. Embryo ABA content throughout the incubation period also depended on grain dormancy level and the incubation temperature (Fig. 1b). While embryo ABA content declined sharply a few hours after incubation in embryos from grains that germinated (i.e. non-dormant grains incubated at 20 °C and 30 °C, and dormant grains incubated at 20 °C), it increased during the first hours of incubation at 30 °C in dormant grains and decreased smoothly afterwards. Twenty-four hours after incubation, ABA content in embryos from dormant grains incubated at 30 °C was between 2 and 4 times higher than that measured in embryos from dormant and non-dormant grains incubated at 20 °C and non-dormant grains incubated at 30 °C. ABA depletion was evident 6 h after the initiation of incubation, well before any visible germination was detected. The close association found between germination behaviour and the changes in embryo ABA content during incubation, suggests a role for ABA in dormancy maintenance of the barley grain.

Germination and embryo ABA content of de-hulled dormant grains incubated at 30 °C in the air

Since incubation at 30 °C allowed the expression of dormancy in dormant grains, and this expression appeared to be mediated by changes in ABA content that are substantially different from that determined in embryos from grains in which dormancy is not expressed, it was

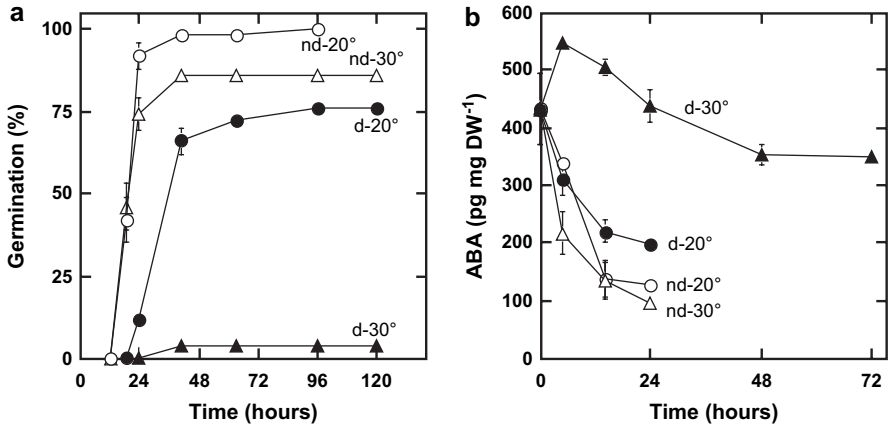

Fig. 1. Germination (a) and embryo ABA content evolution (b) during incubation at 20 °C (open circles, filled circles) and 30 °C (open triangles, filled triangles) of dormant (filled circles, filled triangles) and non-dormant (open circles, open triangles) grains. Freshly harvested (dormant, d) grains and grains stored dry for at least three months at 25 °C (non-dormant, nd). Means of two measurements ±arithmetical spread (germination) and of three measurements ±SD (ABA content).

investigated whether the glumellae interfered with germination through ABA metabolism. Therefore, intact and de-hulled dormant grains were incubated at 30 °C and embryo ABA content was followed throughout incubation.

Removal of the glumellae strongly reduced the inhibition of germination imposed by incubation at 30 °C; de-hulled grains reached almost 100% germination after about 5–6 d of incubation (Fig. 2a), whereas intact grains almost did not germinate throughout the experimental period (Fig. 1a). Changes in ABA content during incubation were also different between de-hulled and intact grains: although ABA embryo content did not decrease as sharply as observed in dormant and non-dormant whole grains incubated at 20 °C and in non-dormant ones incubated at 30 °C (Fig. 1b), an initial increase in ABA content was not detected in de-hulled dormant grains (Fig. 2b) as observed in intact grains (Fig. 1b). After 24 h of incubation, ABA content in embryos from intact dormant grains (Fig. 1b) remained similar (around 350 pg mg^{-1} DW) to that measured in embryos from de-hulled grains (Fig. 2b). These results strongly suggest that the glumellae, which are involved in the expression of dormancy of dormant grains incubated at 30 °C, interfere with ABA metabolism.

Germination and embryo ABA content of de-hulled dormant grains incubated at 30 °C under hypoxia

To determine whether the interference of the glumellae with grain germination and ABA inactivation was through the hypoxia condition imposed on the embryo, de-hulled dormant grains were incubated under 5% oxygen and both germination and ABA content were followed throughout incubation.

Incubation of de-hulled dormant grains under 5% oxygen suppressed germination at 30 °C (Fig. 2a) resembling the behaviour of intact dormant grains incubated in air at this temperature (Fig. 1a). Whereas the ABA content in embryos from de-hulled grains decreased steadily throughout incubation in the air (Fig. 2b), changes in ABA content in embryos from de-hulled grains incubated under 5% oxygen was similar to that of embryos from intact dormant grains incubated at 30 °C in air as described in Fig. 1b: ABA content increased immediately after incubation, stayed high until 16 h and then decreased, reaching values similar to those observed in de-hulled grains incubated under 21% oxygen (Fig. 2b).

These results show that incubation under hypoxia of de-hulled dormant grains can mimic the presence of the glumellae in terms of both germination of the grains and changes in ABA content. Thus, it seems likely that interference with grain germination and ABA metabolism exerted by the presence of the glumellae is through the hypoxia condition imposed on the embryo by this structure.

Effects of oxygen supply on embryo responsiveness to ABA

The possibility was explored that hypoxia, in addition to interfering with ABA inactivation, can modify embryo responsiveness to ABA. With this aim, embryos isolated from dormant and non-dormant grains were incubated under a range of ABA concentrations, at 20 °C and 30 °C and under different oxygen tensions.

Embryos from dormant grains were able to germinate under a wide range of ABA concentrations if they were incubated at 20 °C and under 21% oxygen; only when ABA concentration was as high as 1 mM did the hormone produce some degree of inhibition (Fig. 3a). Reducing the oxygen concentration to 10% increased embryo sensitivity 2-fold; an ABA concentration of 0.1 mM was sufficient to suppress germination almost completely (Fig. 3a).

Incubation at 30 °C markedly increased embryo sensitivity to ABA and the effect of hypoxia was even more evident than at 20 °C. When incubation was performed under 21% oxygen, embryo germination was suppressed with 1 mM ABA and highly inhibited with 0.1 mM ABA

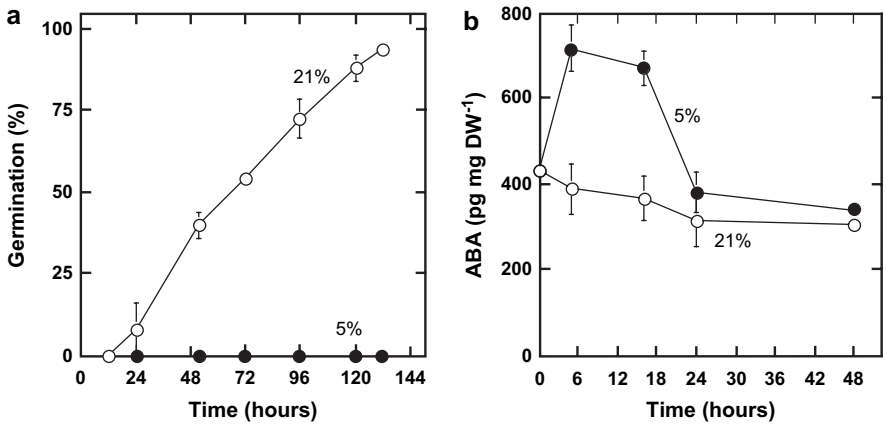

Fig. 2. Germination (a) and embryo ABA content evolution (b) during incubation at 30 °C of dormant de-hulled grains placed in 5% (filled circles) and 21% oxygen (open circles). Means of two measurements ±arithmetical spread (germination) and of three measurements ±SD (ABA content).

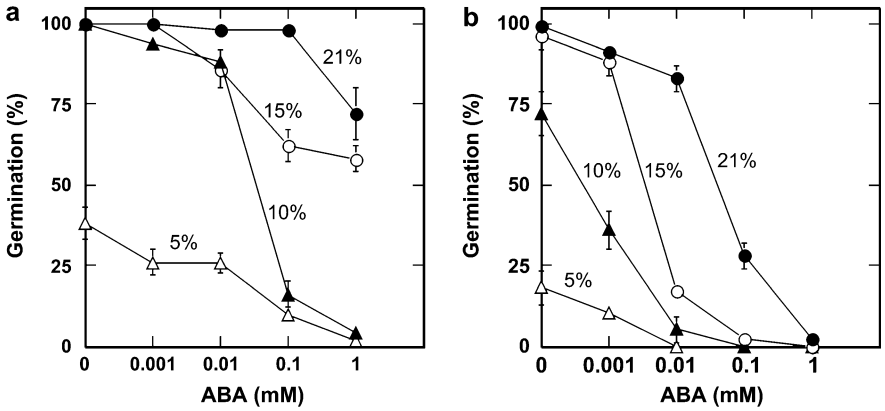

Fig. 3. Effects of ABA concentration on the germination percentages obtained after 7 d with embryos isolated from dormant grains and placed at 20 °C (a) and 30 °C (b) in 21% (filled circles), 15% (open circles), 10% (filled triangles), and 5% (open triangles) oxygen. Means of two measurements ±arithmetical spread.

(Fig. 3b). However, if incubation was carried out under 15% and 10% oxygen, embryo responsiveness to ABA increased more than 1- and 2-fold, respectively (Fig. 3b). For example, with embryos incubated under 10% oxygen, an ABA concentration as low as 0.01 mM (10 μM) was nearly sufficient to suppress germination and it was severely inhibited with 0.001 mM ABA (Fig. 3b). As in de-hulled dormant grains (Fig. 2a) the germination of isolated embryos was strongly inhibited under 5% oxygen (Fig. 3b) regardless of ABA concentration.

Embryos from non-dormant grains were tested at 30 °C only. Overall, embryo responsiveness to ABA was lower than in embryos coming from dormant grains and the effect of hypoxia was also to a lesser extent than observed in those embryos. Embryo germination was maximum with ABA concentrations up to 0.1 mM and germination was only partially reduced at 1 mM ABA if incubation was performed under 21% oxygen (Fig. 4). When embryos were incubated under 10% and 5% oxygen, germination was partially inhibited (more than 50% embryos germinated) at ABA concentrations of 0.1 and 0.01 mM, respectively (Fig. 4).

These results demonstrate that hypoxic conditions markedly increased embryo responsiveness to ABA and that this effect is partially overcome by afterripening.

To investigate the extent to which this increase in embryo responsiveness to ABA caused by hypoxia was in fact the result of hypoxia interference with ABA inactivation, embryos isolated from dormant grains were incubated in the presence of 0.01 mM ABA, at 20 °C and 30 °C and under 21% or 5% oxygen, and embryo ABA content was determined at different times after incubation. Embryos incubated at 20 °C displayed an initial increase in ABA content (i.e. until 6 h of incubation) which was evident both under 21% and 5% oxygen (Fig. 5a). After this increase, and irrespective of oxygen concentration of the atmosphere, ABA content stabilized around 1000 pg ABA mg^{-1} dry weight until the end of the experiment

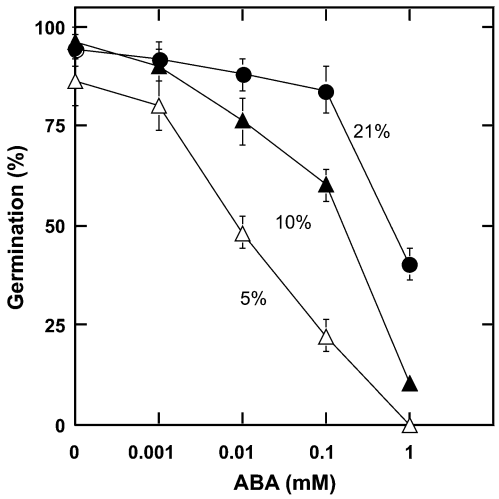

Fig. 4. Effects of ABA concentration on the germination percentages obtained after 7 d with embryos isolated from non-dormant grains and placed at 30 °C in 21% (filled circles), 10% (filled triangles), and 5% (open triangles) oxygen. Means of two measurements ±arithmetical spread.

(i.e. 48 h of incubation under 5% oxygen and 24 h under 21% oxygen since embryos germinated at that time in this condition) (Fig. 5a). By contrast, changes in ABA content in embryos incubated at 30 °C depended on the oxygen concentration of the atmosphere: while in embryos incubated under 21% oxygen this pattern was similar to that observed in embryos incubated at 20 °C (i.e. an initial increase followed by stabilization around 1000 pg ABA mg^{-1} dry weight), embryos incubated under 5% oxygen appeared to have lost their ability to inactivate ABA beyond 24 h of incubation (Fig. 5b). Indeed, when measured after 48 h of incubation, ABA content had increased almost five times with respect to the value measured after 24 h of incubation.

Taken together, these results suggest that part of the enhanced responsiveness to ABA observed in embryos

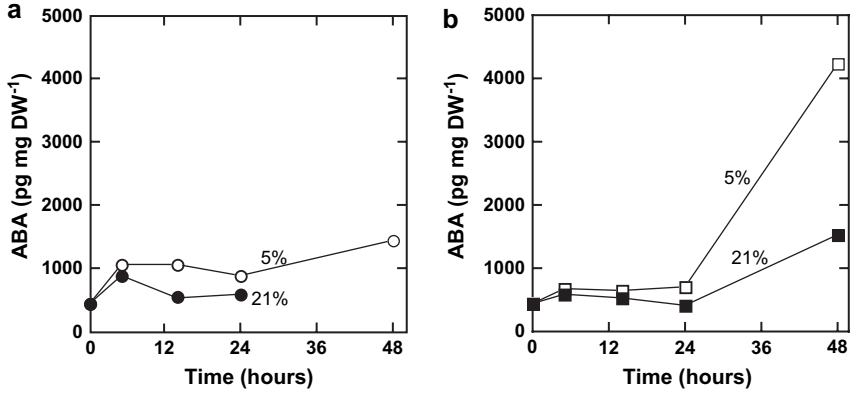

Fig. 5. Change in ABA content of embryos isolated from dormant grains and incubated at 20 °C (a) and 30 °C (b) in the presence of 10 μM ABA under 21% (filled circles, filled squares) and 5% (open circles, open squares) oxygen. Means of three measurements ±SD. The spread of SD is less than the size of the symbols.

from dormant grains, incubated at 30 °C and under hypoxia, might be due to an interference of hypoxia in the capacity of the embryo to inactivate ABA. By contrast, the increase in sensitivity to ABA displayed by embryos incubated under hypoxia but at 20 °C, would not include this interference on ABA inactivation.

Discussion

Barley grains exhibited a dormancy, which was expressed at high temperature (30 °C), but hardly at all at 20 °C (Fig. 1a). As in other cereal seeds, this dormancy expression mainly resulted from an inhibitory action of the glumellae, since de-hulled grains germinated at 30 °C (Fig. 2a). It was suggested by Lenoir *et al.* (1983) that the covering structures (glumellae adhering to the grain and pericarp+ seed coat) prevent germination by placing the embryo under hypoxia, because they fixed oxygen through polyphenol oxidase-mediated oxidation of phenolic compounds present in high amounts (Lenoir *et al*., 1986). Even though incubation under high oxygen tensions (i.e. >21%) did not overcome the inhibition to germination imposed by the glumellae, this failure was attributed to a concomitant increase in polyphenol oxidase activity in the hull with oxygen concentrations higher than 21% (Lenoir *et al*., 1986). Only incubation in the presence of a strong oxidant like hydrogen peroxide can overcome hull-imposed dormancy (Wang *et al*., 1998). This barrier to oxygen supply to the embryo might play a role in the metabolic regulation of the embryo (Lecat *et al*., 1992; Corbineau and Côme, 1996, 2003) or hormonal regulation by preventing ABA leaching into the medium (Bianco *et al*., 1997; Wang *et al*., 1998) or by interfering with ABA inactivation (Neill and Horgan, 1987; Barthe *et al*., 2000). However, the inability of dormant seeds placed at 30 °C to germinate did not result from an inability of the embryo to synthesize ATP, since the energy charge is similar in dormant and non-dormant grains until germination occurred (Lecat *et al*., 1992; Corbineau and Côme, 2003).

Although the embryo ABA content was similar in dry dormant and non-dormant grains, these results clearly demonstrated that a relationship exists between the dormancy level of the barley grain and the evolution of embryo ABA content during incubation (Fig. 1b). The ABA level of the embryo largely decreased during the first hours of incubation and prior to any visible germination in both dormant and non-dormant grains at 20 °C, the temperature at which dormancy is not expressed, and in non-dormant grains incubated at 30 °C, suggesting that ABA catabolism and/or conjugation exceeds ABA biosynthesis. By contrast, at 30 °C dormancy expression was associated with a maintenance of ABA at high levels: after an initial increase, ABA content decreased very smoothly and was always between 2- and 4-fold higher than in embryos from grains in which dormancy was not expressed (Fig. 1b). It appears, then, that a high dormancy level, together with incubation at high temperatures (i.e. 30 °C), promotes ABA biosynthesis and prevents ABA inactivation through metabolism and/or conjugation. The possibility of *de novo* ABA synthesis upon incubation of dormant grains at 30 °C is supported by the observation that germination at this temperature was improved in the presence of fluridone, an ABA synthesis inhibitor (data not shown). On the other hand, the difference with respect to ABA content in embryos from grains that did not strongly express dormancy, might result from a reduction of ABA inactivation, either through catabolism or conjugation. In most plants, inactivation of ABA occurs via the oxidative pathway, the key step being the hydroxylation of the 8′ methyl group of ABA through the action of 8′-hydroxylase (Saito *et al*., 2004). For example, in *Arabidopsis*, this enzyme is known to play a major role in the decline of ABA content during seed imbibition (Kushiro *et al*., 2004).

Both the removal of the glumellae and the incubation of de-hulled grains under low oxygen concentrations modified grain germination behaviour and ABA content evolution throughout incubation at 30 °C (Fig. 2). Although

the potential multiplicity of factors might contribute to the regulation of germination, these results indicate that the presence of the glumellae is instrumental for dormancy maintenance because it imposes oxygen deprivation to the embryo which, in turn, promotes ABA synthesis and/or inhibits ABA inactivation. Indeed, removal of the glumellae in dormant grains incubated at 30 °C under 21% oxygen (Fig. 2B) suppressed the initial increase in ABA content that was observed in dressed dormant grains incubated at the same temperature (Fig. 2b). By contrast, incubation under hypoxia (5% oxygen) of de-hulled grains restored it (Fig. 2b) and inhibited germination (Fig. 2a). The physiological significance of this initial peak is not known, but its existence seemed to be closely associated with dormancy maintenance. Although evidence exists showing that hypoxia can impair ABA catabolism, the authors are not aware of any previous report showing that hypoxia can promote ABA biosynthesis (Xiong and Zhu, 2003; Nambara and Marion-Poll, 2005). Hypoxia interference with ABA catabolism could be through the regulation of ABA 8′-hydroxylase activity. However, evidence exists showing that the enzyme ABA 8′-hydroxylase works in a wide range of temperatures and oxygen availabilities (Krochko *et al*., 1998). Therefore, it could be that temperature and oxygen concentration regulate the functioning of this enzyme at the level of expression of the gene that codifies it. The authors current efforts are devoted to clone this gene in barley and to study its expression under different temperatures and oxygen supplies as related to grain dormancy. The biochemical and molecular bases of afterripening are largely unknown, but it seems from these results that breaking of dormancy results in a loss of sensitivity of ABA metabolism to the temperature of incubation and to oxygen deprivation.

Involvement of embryo responsiveness to ABA in the regulation of seed dormancy has been reported for various cereals, such as wheat (Walker-Simmons, 1987; Corbineau *et al*., 2000), barley (Wang *et al*., 1995; Benech-Arnold *et al*., 1999; Corbineau and Côme, 2000), oat (Corbineau *et al*., 1991), and sorghum (Steinbach *et al*., 1995) and other species (Groot and Karssen, 1992; Le Page-Degivry *et al*., 1996; Corbineau *et al*., 2002). It is also known to be modulated by incubation temperature as reported by other authors (Poljakoff-Mayber *et al*., 1990; Corbineau *et al*., 1993) and our results (Fig. 3). In this paper it is clearly demonstrated that embryo sensitivity to ABA can also be affected by oxygen availability (Figs 3, 4). Incubation under low oxygen tensions enhanced the inhibitory effect of ABA on embryo germination by several degrees of magnitude. To the best of our knowledge, this is the first time that such an effect is reported. These results suggest that, in addition to interference with ABA metabolism, the presence of the glumellae increases embryo responsiveness to the phytohormone. It is also demonstrated that, in embryos coming from dormant grains and incubated at 30 °C, this apparent increase in embryo sensitivity to ABA is, in part, mediated by an inability of the embryo to inactivate ABA. Consistently, with results reported by Grappin *et al*. (2000) in *Nicotiana plumbaginifolia* and by Le Page-Degivry *et al*. (1996) in sunflower, the results seem to indicate that there exists a correlation between ABA responsiveness and capacity for ABA biosynthesis and metabolism.

Overall, the results reported in this paper shed new light on the role of ABA in dormancy maintenance in the barley grain, and on the way its synthesis and catabolism is modulated by the environment imposed to the embryo by the surrounding structures. Since germination was scored as radicle elongation, the possibility cannot be ruled out that hypoxia imposed by the glumellae, even if it did not reduce the embryo energy charge (Corbineau and Côme, 1996, 2003), might affect various biochemical processes or expression of genes involved in cell elongation independently of ABA metabolism. However, hypoxia imposed by the glumellae seems to be of paramount importance for controlling hormone-mediated dormancy maintenance through content and tissue responsiveness. The authors' present work is focused on elucidating the molecular features underlying these mechanisms.

Acknowledgements

The authors want to thank Silvina Enciso for excellent technical assistance with ABA determinations. This work has been financed by Université Pierre et Marie Curie, University of Buenos Aires (G012) and Agencia Nacional de Promocion Cientifica y Tecnica (Argentina) (Project 08-14402).

References

Barthe P, Garello G, Bianco-Trinchant J, Le Page-Degivry MT. 2000. Oxygen availability and ABA metabolism in *Fagus sylvatica* seeds. *Plant Growth Regulation* **30,** 185–191.

Benech-Arnold RL. 2004. Inception, maintenance, and termination of dormancy in grain crops: physiology, genetics, and environmental control. In: Benech-Arnold RL, Sánchez RA, eds. *Handbook of seed physiology. applications to agriculture*. Binghamton, NY: Food Products Press, 169–198.

Benech-Arnold RL, Giallorenzi MC, Frank J, Rodriguez V. 1999. Termination of hull-imposed dormancy in barley is correlated with changes in embryonic ABA content and sensitivity. *Seed Science Research* **9,** 39–47.

Bewley JD. 1997. Seed germination and dormancy. *The Plant Cell* **9,** 1055–1066.

Bianco J, Garello G, Le Page-Degivry MT. 1997. *De novo* ABA synthesis and expression of seed dormancy in a gymnosperm *Pseudotsuga menziesii*. *Plant Growth Regulation* **21,** 115–119.

Côme D, Tissaoui T. 1968. Induction d'une dormance embryonnaire secondaire chez le pommier (*Pyrus malus* L.) par des atmosphères très appauvries en oxygène. *Comptes Rendus de l'Académie des Sciences, Paris, Série III* **266,** 477–479.

Corbineau F, Benamar A, Côme D. 2000. Changes in sensitivity to ABA of the developing and maturing embryo of two wheat

cultivars with different sprouting susceptibility. *Israel Journal of Plant Science* **48,** 189–197.

Corbineau F, Bianco J, Garello G, Côme D. 2002. Breakage of *Pseudotsuga menziesii* seed dormancy by cold treatment as related to changes in seed ABA sensitivity and ABA levels. *Physiologia Plantarum* **114,** 313–319.

Corbineau F, Black M, Côme D. 1993. Induction of thermodormancy in *Avena sativa* seeds. *Seed Science Research* **3,** 111–117.

Corbineau F, Côme D. 1996. Barley seed dormancy. *Bios Boissons Conditionnement* **261,** 113–119.

Corbineau F, Côme D. 2000. Dormancy of cereal seeds as related to embryo sensitivity to ABA and water potential. In: Viemont JD, Crabbé J, eds. *Dormancy in plants: from whole plants behaviour to cellular control*. Oxon: CAB International, 183–194.

Corbineau F, Côme D. 2003. Involvement of energy metabolism and ABA in primary and secondary dormancies in oat (*Avena sativa* L.) seeds: a physiological approach. In: Nicolas G, Bradford KJ, Côme D, Pritchard H, eds. *The biology of seeds: recent research advances*. Oxford: CAB International, 113–120.

Corbineau F, Lecat S, Côme D. 1986. Dormancy of three cultivars of oat seeds (*Avena sativa* L.). *Seed Science and Technology* **14,** 725–735.

Corbineau F, Poljakoff-Mayber A, Côme D. 1991. Responsiveness to abscisic acid of embryos of dormant oat (*Avena sativa*) seeds. Involvement of ABA-inducible proteins. *Physiologia Plantarum* **83,** 1–6.

Edwards MM. 1973. Seed dormancy and seed environment: internal oxygen relationships. In: Heydecker W, ed. *Seed ecology*. London: Butterworths, 169–188.

Fong F, Smith JD, Koehler DE. 1983. Early events in maize seed development. *Plant Physiology* **73,** 899–901.

Grappin P, Bouinot D, Sotta B, Miginiac E, Julien M. 2000. Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* **210,** 279–285.

Groot SPC, Karssen CM. 1992. Dormancy and germination of abscisic acid-deficient tomato seeds: studies with the *sitiens* mutant. *Plant Physiology* **99,** 952–958.

Karssen CM, Brinkhorst-Van der Swan DLC, Breekland AE, Koorneef M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.). *Planta* **157,** 158–165.

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E. 2004. The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO Journal* **23,** 1647–1656.

Krochko JE, Abrams GD, Loewen MK, Abrams SR, Cutler AJ. 1998. (+)-Abscisic acid 8′-hydroxylase is a cytochrome P450 monooxygenase. *Plant Physiology* **118,** 849–860.

Lecat S, Corbineau F, Côme D. 1992. Effects of gibberellic acid on the germination of dormant oat (*Avena sativa* L.) seeds as related to temperature, oxygen and energy metabolism. *Seed Science and Technology* **20,** 421–433.

Lenoir C, Corbineau F, Côme D. 1983. Rôle des glumelles dans la dormance des semences d'orge. *Physiologie Végétale* **21,** 633–643.

Lenoir C, Corbineau F, Côme D. 1986. Barley (*Hordeum vulgare*) seed dormancy as related to glumella characteristics. *Physiologia Plantarum* **68,** 301–307.

Le Page-Degivry MT, Bianco J, Barthe P, Garello G. 1996. Changes in hormone sensitivity in relation to onset and breaking of sunflower embryo dormancy. In: Lang GA, ed. *Plant dormancy. physiology, biochemistry and molecular biology*. Oxford: CAB International, 221–231.

Le Page-Degivry MT, Garello G. 1992. *In situ* abscisic acid synthesis. A requirement for induction of embryo dormancy in *Helianthus annuus*. *Plant Physiology* **98,** 1386–1390.

Le Page-Degivry MT, Garello G, Barthe P. 1997. Changes in abscisic acid biosynthesis and catabolism during dormancy breaking in *Fagus sylvatica* embryo. *Journal of Plant Growth Regulation* **16,** 57–61.

Nambara E, Marion-Poll A. 2005. Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56,** 165–185.

Neill SJ, Horgan R. 1987. Abscisic acid and related compounds. In: Rivier L, Crozier A, eds. *The principles and practice of plant hormone analysis*. London: Academic Press, 111–167.

Poljakoff-Mayber A, Corbineau F, Côme D. 1990. A possible mechanism of high-temperature dormancy regulation in seeds of *Avena sativa* L. (cv. Moyencourt). *Plant Growth Regulation* **9,** 147–156.

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M. 2004. *Arabidopsis* CYP707As encode (+)-abscisic acid 8′-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiology* **134,** 1439–1449.

Steinbach HS, Benech-Arnold RL, Kristof G, Sánchez RA, Marcucci Poltri S. 1995. Physiological basis of pre-harvest sprouting resistance in *Sorghum bicolor* (L.) Moench. ABA levels and sensitivity in developing embryos of sprouting resistant and susceptible varieties. *Journal of Experimental Botany* **45,** 701–709.

Steinbach HS, Benech-Arnold RL, Sánchez RA. 1997. Hormonal regulation of dormancy in developing *Sorghum* seeds. *Plant Physiology* **113,** 149–154.

Walker-Simmons MK. 1987. ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. *Plant Physiology* **84,** 61–66.

Walker-Simmons MK. 1988. Enhancement of ABA responsiveness in wheat embryos by high temperature. *Plant, Cell and Environment* **11,** 769–775.

Wang M, Heimovaara-Dijkstra S, Van Duijn B. 1995. Modulation of germination of embryos isolated from dormant and non-dormant grains by manipulation of endogenous abscisic acid. *Planta* **195,** 586–592.

Wang M, van der Meulen RM, Visser K, Van Schaik H-P, Van Duijn B, de Boer AH. 1998. Effects of dormancy-breaking chemicals on ABA levels in barley grain embryos. *Seed Science Research* **8,** 129–137.

Xiong L, Zhu JK. 2003. Regulation of abscisic acid biosynthesis. *Plant Physiology* **133,** 29–36.

Yoshioka T, Endo T, Satoh S. 1998. Restoration of seed germination at supraoptimal temperature by fluridone, an inhibitor of abscisic acid biosynthesis. *Plant Cell Physiology* **39,** 307–312.