Evidence for the role of abscisic acid in the genetic and environmental control of dormancy in wheat (*Triticum aestivum* L.)

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Abstract

Grain production of two wheat cultivars (*Triticum aestivum* L. cv Recital and Scipion), known for their different germination behaviour, was studied at two different temperatures. The study of dormancy onset during grain development showed that in both cultivars, dormancy developed on the mother plant. In Recital grain, after a period of high germinability, dormancy developed for a transient period. However, full germination was obtained on medium supplemented with fluridone, showing that dormancy was associated with abscisic acid (ABA) biosynthesis inside the grain. Dormancy progressively disappeared during natural drying, at a slower rate for grains developing at 15°C than for grains developing at 25°C. However, at both temperatures, the release from dormancy was complete at maturity. In Scipion grain, dormancy was almost total throughout grain development irrespective of the temperature. However, grains could germinate in the presence of fluridone: changes in sensitivity to fluridone were observed during grain development, showing an increase in dormancy during the first half of development, followed by a progressive decrease during the second half. This decrease occurred later for grains developing at 15°C than for those developing at 25°C. In Scipion grain, unlike the cultivar Recital, release from dormancy was not completed before the end of development on the mother plant. An additional dry storage period was necessary which was shorter for grains developed at 25°C than for grains produced at 15°C. A comparison of embryo ABA levels after a 24-h culture in the presence or absence of fluridone, allowed the ABA synthesis to be estimated. It appears that the depth of dormancy was related to the estimated ABA synthesis capacity of the embryos.

Keywords: abscisic acid biosynthesis, fluridone, seed dormancy, temperature effect, *Triticum aestivum*

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Introduction

Over the past fifteen years, work with hormone-deficient and hormone-insensitive mutants of *Arabidopsis thaliana* and *Lycopersicon esculentum* (reviewed in Hilhorst and Karssen, 1992) has provided a considerable body of evidence for the involvement of abscisic acid (ABA) in inducing primary dormancy during seed development. The role of ABA in the dormancy of cereal grains is not yet well understood. During development of the cereal grain, whether the mature grain is dormant or not, an accumulation of free ABA was observed until maximum grain dry weight was attained (McWha, 1975; King, 1976). Thereafter, in mature grains, free ABA content was generally low or even undetectable. The pattern of change in embryonic ABA content during development was found to be similar in cultivars whose grains were dormant or non-dormant at maturity (Walker-Simmons, 1987). Recently, three wheat mutants that lacked dormancy at maturity were isolated from an ethylmethane sulphonate-treated population of a dormant line (Kawakami *et al.*, 1997). The profile of embryo ABA content during seed development was similar in mutants and in the dormant line, except that ABA peaked earlier in the dormant line. The authors suggested that this early increase in ABA content during development can affect the dormancy mechanism, as already proposed by Goldbach and Michael (1976) for barley and King (1993) for wheat.

Large differences in the response to applied ABA were often reported. Mature dormant grains were much more sensitive to ABA than non-dormant grains (Stoy and Sundin, 1976). Embryo responsiveness to ABA decreased with loss of grain dormancy during after-ripening in wheat (Walker-Simmons, 1987) and sorghum (Steinbach *et al.*, 1995). Similarly, embryos of non-dormant mutants grains rapidly lost sensitivity to ABA during the latter half of seed maturation while embryos of the dormant line maintained their sensitivity even after maturity (Kawakami *et al.*, 1997).

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A new perspective on the mechanism of seed dormancy has been developed, focused on the role of ABA biosynthesis. Studies based on genetic approaches using hormone-deficient mutants (Karssen *et al*., 1983) and inhibitors of ABA synthesis, such as fluridone (Hole *et al*., 1989; Le Page-Degivry and Garello, 1992; Wang *et al*., 1995) and norflurazon (Jullien and Bouinot, 1997), show that only ABA synthesized inside the embryo is responsible for the induction of dormancy while maternal ABA only transiently inhibits precocious germination. In wheat, the application of fluridone during culture of detached spikes, from anthesis to maturity, prevents both ABA accumulation and dormancy induction (Rasmussen *et al*., 1997). Ried and Walker-Simmons (1990) suggested that dormant grain embryonic axes may be distinguished from non-dormant ones by their capacity to produce ABA during the first hours after imbibition.

The aim of this work was to investigate the role of ABA synthesis in wheat grain dormancy. As the intensity of grain dormancy is a cultivar character with wide genetic variations, two cultivars showing largely different germination behaviour were compared. The cultivar Recital produces non-dormant grains while those of Scipion are deeply dormant (Beaux and Ladonne, 1990). Depth of grain dormancy is also strongly influenced by environmental factors during grain development, especially by the temperature (Black *et al*., 1987; Walker-Simmons and Sesing, 1990). Grains of both cultivars were therefore produced at low temperature (15°C) to promote the production of highly dormant grains or at high temperature (25°C), which results in only slightly dormant grains. Changes in the germination capacity during development and dry storage of the grains were studied in controled conditions and in the presence of fluridone, which inhibits ABA synthesis. The results demonstrate the role of ABA biosynthesis both in the onset and the maintenance of grain dormancy.

Material and Methods

Plant material

Caryopses of two wheat (*Triticum aestivum* L.) cultivars, Recital and Scipion, were germinated on wetted filter paper in a Petri dish at 23°C. Germinated seedlings were selected, transferred into rock wool blocks and allowed to vernalize at 8–10°C for 2 months under continuous fluorescent light (Sylvania Lifeline Blanc Industrie, 10 $W.m^{-2}$). Thereafter, they were grown in sand, ten per 20 cm pot, in a growth room at 25° ± 1°C, with a 16 h photoperiod of white, fluorescent light (Sylvania Lifeline Blanc Industrie) supplemented to 370 $W.m^{-2}$ with high intensity mercury vapour lamps (Sylvania HSL-BW), at a relative humidity of 65–75%. Nutrient solution (macro and microelements) was applied in the morning (10.00 h) and in the evening (22.00 h). Spikes were tagged on the first day of anthesis. From anthesis to maturity, the temperature of the growth room was maintained at 25° ± 1°C, or 15° ± 1°C.

Spikes were harvested at different developmental stages. To compare caryopses developed at 25°C and 15°C, spikes were harvested at intervals defined by the sum of the mean daily temperature, based on the equation: temp.°C (base temperature 0°C) × number of days; i.e. respectively 4 d or 7 d to give a sum of 100°C for growth at 25 and 15°C, respectively. The sum of daily temperature is often used as a reference by agronomists studying preharvest sprouting in cereals (Masse, 1981). Grains were harvested from the first and second florets of the six middle spikelets.

Germination of caryopses

Caryopses were aseptically isolated and cultured immediately following harvest on 15 ml of 6% agar at 23°C, under fluorescent light (Sylvania Lifeline Blanc Industrie, 45 W. m^{-2}, 16 h day^{-1}). These conditions of relatively high temperature allowed a strong expression of dormancy, as shown by Black *et al*. (1987). Moreover, the presence of agar slowed the eventual leaching of inhibitory substances from the grain (Barthe and Bulard, 1982). These cultures remained sterile and could be observed over a long period (two months). Experiments were performed two or three times using 24 caryopses for each experimental batch. A grain was considered to have germinated when elongation of the radicle reached 1 mm.

Fluridone (1-methyl-3 phenyl-5-[3-(trifluoromethyl) phenyl]-4-(1H)-pyridinone), generously provided by Dow Elanco, was resuspended in 10% (v/v) acetone-water to give a 10 mM stock solution and filter-sterilized through a Millipore filter (HA 0.45 µm) and introduced into the agar medium at 40°C at a final concentration of 100 µM.

ABA extraction and quantification

ABA was quantified by radioimmunoassay (RIA) (Le Page-Degivry *et al*., 1984). Polyclonal antibodies were shown to be highly specific towards the + (S)-ABA molecule. However, because they were prepared with an immunogen obtained by coupling ABA to human serum albumin through the carboxyl group, cross-reactivity was observed whether the acid function was free, esterified or linked by an amide linkage. The conversion of the ABA carboxyl group into an amide

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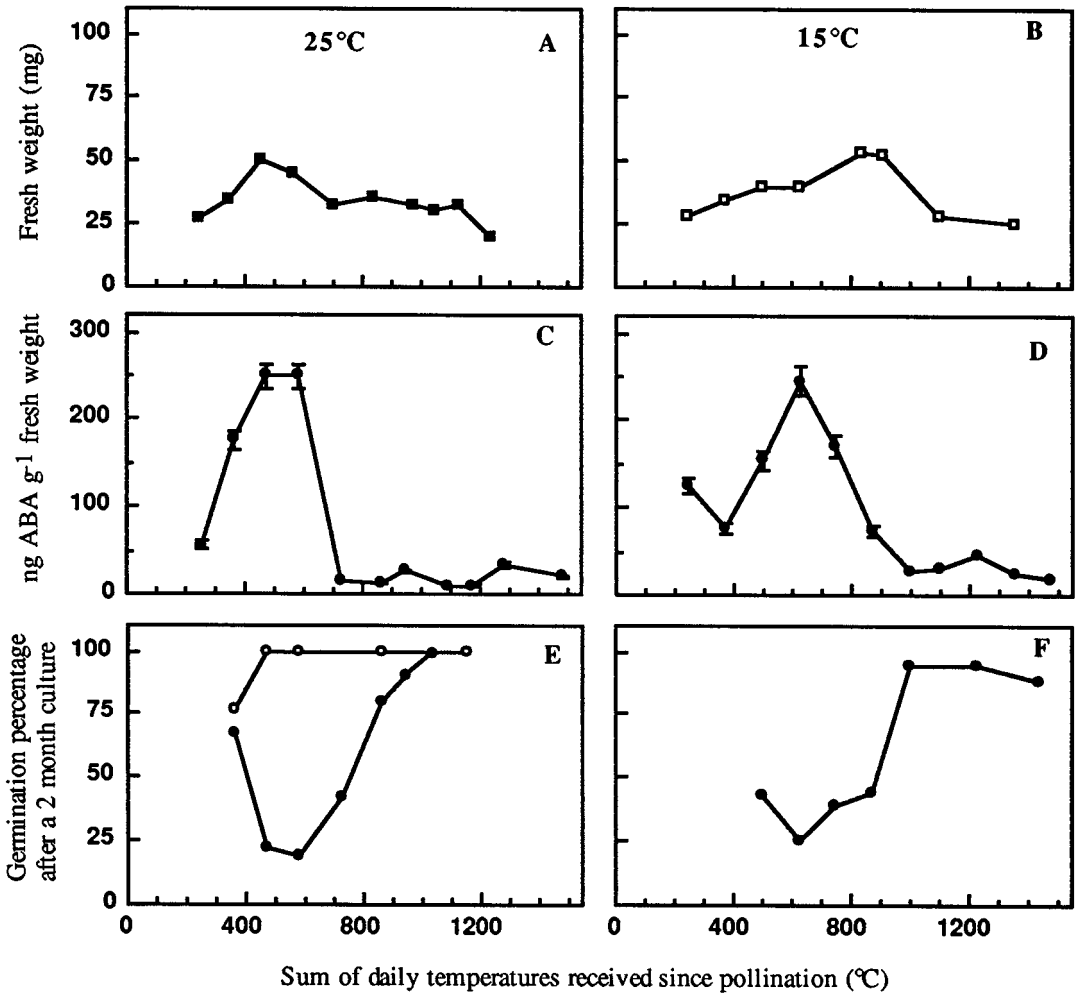

Figure 1. Changes in characteristics of grains (*Triticum aestivum* cv Recital) developed at 25°C (left : A, C and E) or 15°C (right : B, D and F). A and B : Fresh weight; C and D : ABA content (data are means ± SE vertical bars, n=4); E and F : Germination percentages of grains cultured on water-agar (●) or on water-agar supplemented with fluridone at 100 µM (○) for 2 months.

induced an increase in sensitivity of the free (+) ABA estimation and allowed ABA values to be calculated by differential measurement before and after amidation (Le Page-Degivry and Garello, 1992; Bianco-Trinchant *et al.*, 1993).

Results

Plants of the cultivar Recital (produces non-dormant grains) and Scipion (produces dormant grains) were grown at 15 and 25°C from anthesis to grain maturity (Figs. 1 and 2A and 2B). In order to observe the effect of temperature on grain development, changes in grain fresh weight were measured. For both varieties, development was slower at 15°C than at 25°C. For example, the maximum fresh weight of Recital grain was obtained with a cumulated temperature (= c.t.) of 950°C at 15°C and of 500°C c.t. at 25°C.

Changes in ABA content during grain development

Changes in grain ABA content throughout grain development are shown in Fig. 1 for Recital and in Fig. 2 for Scipion. In Recital grains grown at 25°C (Fig. 1C), the ABA content was low at 300°C c.t., increased to a maximum of 250 ng/g at 600°C c.t. and then sharply decreased to a low value which remained steady until maturity. In the Recital caryopsis developing at 15°C (Fig. 1D), the ABA content was at a minimum at 400°C c.t. It reached a maximum of 250 ng fresh g^{-1} weight at 600°C c.t. Thereafter, the ABA content slowly decreased, reached a minimum at 1000°C c.t. and remained steady until maturity.

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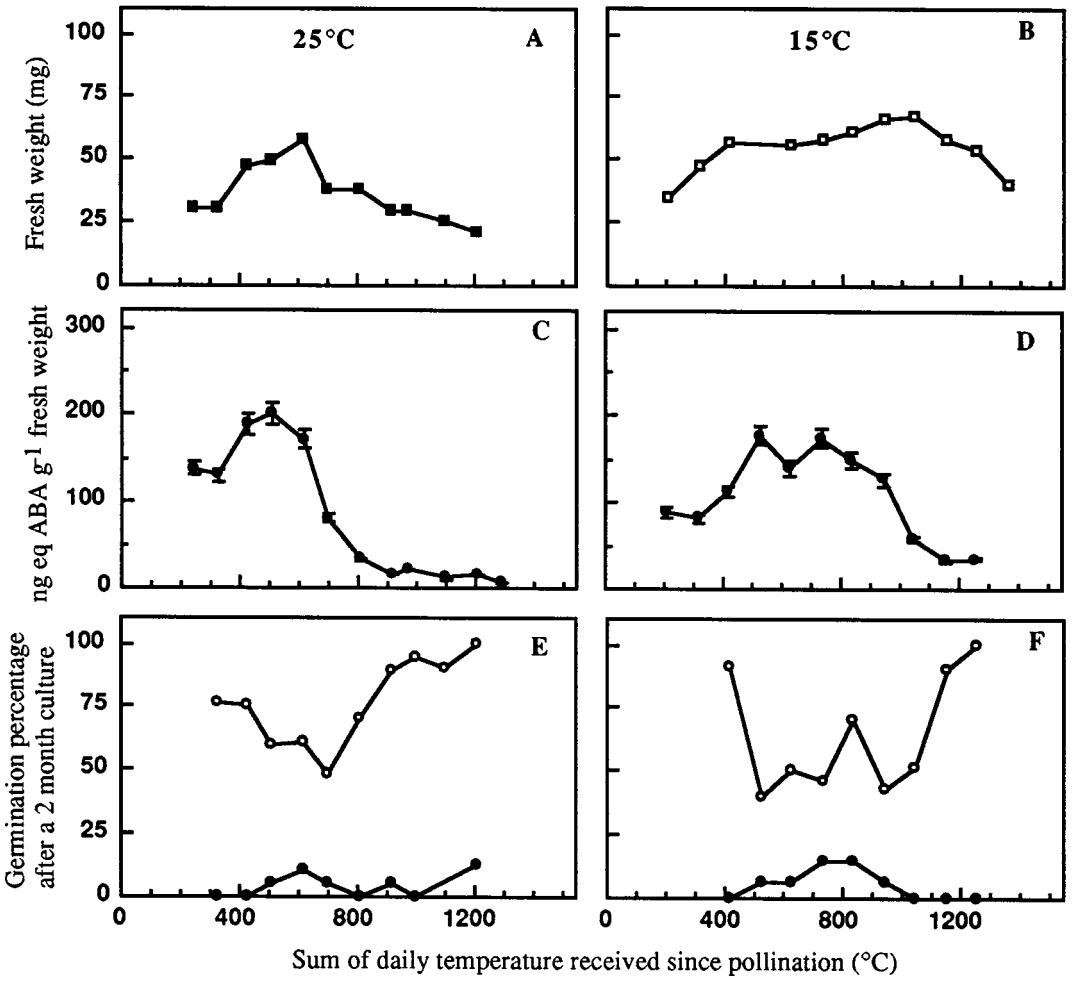

Figure 2. Changes in characteristics of grains (*Triticum aestivum* cv Scipion) developed at 25°C (left : A, C and E) or 15°C (right : B, D and F). A and B : Fresh weight; C and D : ABA content (data are means ± SE vertical bars, n=4); E and F : Germination percentages of grains cultured on water-agar (●) or on water-agar supplemented with fluridone at 100 µM (○) for 2 months.

In Scipion grains grown at 25°C (Fig. 2C), the ABA content was already high at 250°C c.t. It increased slightly until 500°C c.t. and then decreased slowly, reaching a minimum at 800°C c.t. In Scipion grains developing at 15°C (Fig. 2D), the ABA content increased later (420°C c.t.). It peaked at 510°C c.t. and remained high for a long period (from 510° to 810°C c.t.). Thereafter, it slowly decreased and reached a minimum at more than 1100°C c.t.

Therefore, for both cultivars, a lowering of the temperature during grain development led to a longer duration of ABA accumulation. The amplitude of the maximum was not modified. However, whatever the temperature, ABA accumulation began slightly earlier and lasted longer in the Scipion cultivar compared to the Recital cultivar.

Changes in germination capacity during grain development

When young (300°C c.t.), green caryopses of the Recital cultivar that still had liquid endosperm, were obtained from plants developing at 25°C (Fig. 1E); their germination efficiency was high (75%). However, within the two-month culture, root growth was delayed and poor and the plantlets remained dwarfed. Therefore, at this stage, embryo differentiation was not achieved, and grain exhibited a precocious germination (Raghavan, 1976). Thereafter, during the period extending from 450 to 600°C c.t., a low germination percentage (20–25%) was observed. Later on, Recital grains germinated more rapidly and at a higher percentage as maturation progressed. From

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900°C c.t., all the caryopses were able to germinate in our culture conditions.

When Recital caryopsis development was examined at 15°C (Fig. 1F), the percentage of grain germination from 450°C c.t. to 900°C c.t. was low (25–40%), with a minimum (25%) at 600°C c.t. Thereafter, all the caryopses were able to germinate.

Scipion grains germinated poorly on water agar (0 to 15%) whatever their developmental stage and whatever the temperature applied during their development (Fig. 2E, and F).

Changes in sensitivity to fluridone during grain development

The effect of fluridone treatment on grain germination was studied during grain development of both cultivars. Fluridone, initially dissolved in 10% acetone-water was introduced at 100 µM in the culture medium of developing caryopses. A control experiment showed that the residual acetone had no effect on grain germination. In Recital grains developed at 25°C (Fig. 1E), the behaviour of young caryopses (300°C c.t.) was identical to that on water-agar, showing about 75% germination. For grains harvested at higher cumulated developmental temperatures, fluridone treatment provoked germination in all grains whatever their developmental stage. However, plantlets were white owing to inhibition of carotenoid biosynthesis and the subsequent destruction of chlorophyll.

In Scipion grains developed at 25°C (Fig. 2E) fluridone strongly stimulated germination of young caryopses: 75% of caryopses harvested at 300 and 400°C c.t. were able to germinate. Thereafter, the germination percentage decreased until a minimum (45%) at 700°C c.t. and then it progressively increased to 100% from 900°C c.t. to maturity.

Scipion grains produced at 15°C (Fig. 2F) showed a similar behaviour: a high germination percentage (85%) at 400°C c.t., which decreased to 40–45% thereafter. This low germinability was observed for the period from 500 to 900°C c.t. but then, it progressively increased, reaching 100% at maturity. However, for each stage, germination occurred more slowly and in lower percentage than for grains developed at 25°C.

Manipulation of ABA biosynthesis by fluridone treatment of mature seeds

During dry storage, the germinability of mature grains of Scipion progressively increased. After a two-month dry storage period, (Fig. 3A) 70% of the grains produced at 25°C germinated within a one-month culture while germination of grains produced at 15°C remained poor (10–15%). After a 4–6-month dry storage period, grains germinated to a high percentage (95–100% germination for grains produced at 25°C and 90% germination for grains produced at 15°C).

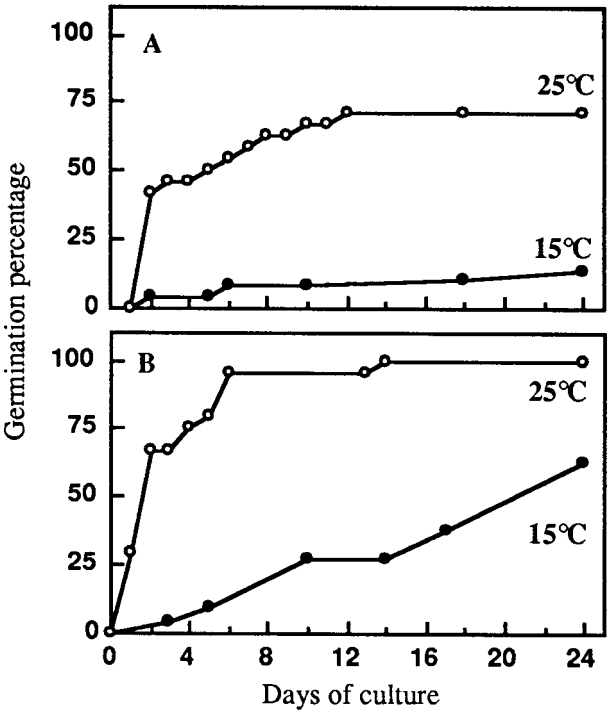

Figure 3. Changes in germination percentage of grains (*Triticum aestivum* cv Scipion) developed at 25°C (○) or 15°C (●), harvested at maturity and then stored dry for 2 months before: A; culture on water-agar B; culture on water-agar supplemented with fluridone at 100 µM.

The effect of fluridone treatment on grain germination was studied in the more dormant cultivar, Scipion. Fluridone was introduced in the germination culture medium (100 µM) of Scipion caryopses previously harvested at maturity and stored in dry conditions for two months (Fig. 3B). There was a high percentage of germination of caryopses produced at 25°C within two days of culture while germination of caryopses produced at 15°C was delayed. Because the two types of caryopses behaved very differently and the response of caryopses produced at 25°C was rapid and homogenous, changes in ABA content were compared both in endosperms and in embryos after a 24-hour culture in the presence and in the absence of fluridone. Whether or not the culture was performed in the presence of fluridone, endosperm ABA content was similar; in contrast, the presence of fluridone led to a lowering of ABA content in the embryos (Table 1). The embryo ABA synthesis capacity was estimated by measuring the difference in ABA content in the absence and in

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Table 1. Effect of fluridone treatment on endosperm and embryo ABA contents in cv. Scipion. Grains were allowed to develop at 15°C and 25°C until maturity, then stored for 2 months. ABA was estimated after a 24 h culture of the grains in the absence (control) or in the presence of 100 µM fluridone. Data are means ± SE (n=4).

	Grains produced at 15°C		Grains produced at 25°C	
	ng/g FW	pg/embryo	ng/g FW	pg/embryo
Endosperm				
Control	4.6 ± 0.5	280 ± 28	3.3 ± 0.3	167 ± 17
Fluridone-treated	5.3 ± 0.5	322 ± 30	4.5 ± 0.4	189 ± 19
Embryo				
Control	53.5 ± 5	212 ± 20	35.9 ± 4	147 ± 15
Fluridone-treated	16.3 ± 2	68 ± 7	21.9 ± 2	79 ± 8
Estimated ABA synthesis	37.2	144	14	68

the presence of fluridone. Whether expressed in ng per gram of fresh weight or in pg per embryo, estimated ABA synthesis was higher for grains produced at 15°C than for those produced at 25°C.

Discussion

The strong difference in seed germination behaviour between the two cultivars and the influence of temperature during seed development on the depth of dormancy was confirmed by comparing the germination of grains harvested at maturity and cultured in controlled conditions. At harvest, Recital grains were able to germinate while Scipion grains were dormant. Moreover, grains developed under cool temperatures (15°C) acquired a high level of dormancy while development at warmer temperatures (25°C) resulted in low levels of dormancy as shown previously by Black *et al.* (1987) and Walker-Simmons and Sesing (1990).

Germination of dormant grains could be induced by fluridone treatment. Fluridone was shown to be inactive on ^{3}H-(+)-ABA metabolism by rose buds (Le Bris, personal communication). Therefore the difference between the steady state level of ABA in grains cultured in the absence or in the presence of fluridone can be considered to be mainly caused by the ABA synthesized during the culture. The intensity of grain dormancy was associated with the ABA synthesis capacity of the embryos. Indeed, embryos isolated during culture of partly after-ripened Scipion grains exhibited high ABA content when maturation took place at 15°C. ABA content was lower for Scipion grains matured at 25°C. Such a relationship between the depth of dormancy and the ABA synthesis capacity was previously reported in sunflower (Le Page-Degivry *et al.*, 1996), barley (Wang *et al.*, 1995) and beech (Le Page-Degivry *et al.*, 1997). It should be noted that the same fluridone concentration induced a higher germination percentage of grains matured at 25°C compared to grains matured at 15°C. Thus, the sensitivity of grains to fluridone appeared inversely related to the intensity of ABA synthesis and the degree of their dormancy.

Since ABA induces dormancy during seed development (reviewed in Hilhorst and Karssen, 1992), the timing of dormancy onset and the involvement of ABA during grain development in wheat were investigated. When developing at a relatively high temperature (25°C), grains of the non-dormant cultivar Recital exhibited a period of high germinability followed by a period of strongly reduced germination. Germination capacity was inversely related to ABA contents. At a lower (15°C) maturation temperature, the initial period of high germinability was absent and the grains had an extended period of low germinability associated with an extended period of high ABA contents. Such behaviour agrees with observations reported for other species that show no dormancy at maturity. For example, in embryos of *Phaseolus vulgaris* (Prévost and Le Page-Degivry, 1985), the duration of the lag-phase that precedes germination was directly correlated with embryo ABA content. It was suggested that it correlated with the catabolism of ABA accumulated inside the embryo during its development on the mother plant. However, for Recital grain, the germination percentage was severely restricted even after a two-month culture. Moreover, culturing in the presence of fluridone promoted high germination values throughout grain maturation at 25°C. This observation emphasized that ABA was synthesized inside the grain during its early development for a transient period. These findings also show that dormancy develops during grain development but can disappear later during the course of maturation, as already suggested by King (1976) and Black *et al.* (1987).

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In the second cultivar (Scipion), developing grains were dormant throughout their development on the mother plant. Germinability was very low throughout development although ABA levels were comparable or even lower to those in the Recital cultivar. When no correlation could be observed between ABA content and germinability, differences in sensitivity to ABA have been invoked (Walker-Simmons, 1987; Kawakani et al., 1997). However, our results show that germination can be induced by culture in the presence of fluridone. We observed that the response to fluridone changed with time after pollination. These changes in sensitivity to fluridone reflected possible changes in the intensity of ABA synthesis and in dormancy level.

In conclusion, the ABA synthesis capacity inside the grain appears to play a key role in induction and maintenance of dormancy in wheat, as already demonstrated in *Helianthus* (Le Page-Degivry and Garello, 1992) and beech (Le Page-Degivry *et al.*, 1997). Moreover, both genetic and temperature-induced variations in the intensity of grain dormancy were associated with changes in intensity and duration of grain ABA synthesis.

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