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Temperature Effects on Embryonic Abscisic Acid Levels During Development of Wheat Grain Dormancy

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Abstract. Abscisic acid (ABA) levels were determined in both the embryo and remaining grain remnant during development of wheat caryopses under temperature conditions which produced either high or low levels of dormancy in mature grain. Higher levels of grain dormancy were produced in grain from plants grown at 15°C as compared to 25°C. In grain grown at 15°C, embryonic ABA levels steadily increased during development, reaching a maximum at stage IV, just before grain desiccation. At 25°C, ABA levels were very high at the earliest stages of embryonic development, but dropped rapidly during maturation. Only small cultivar differences in ABA levels were observed during development at either temperature. In general, higher levels of dormancy in mature grain correlated with prolonged elevation of ABA levels during grain maturation.

Genetic evidence indicates that abscisic acid (ABA), particularly in the embryo, is required for the induction of seed dormancy. *Arabidopsis* mutants which are ABA deficient or insensitive yield seeds with reduced dormancy (Karssen et al. 1983; Karssen et al. 1987). In such mutants induction of dormancy correlates with embryonic ABA, but not with maternal ABA (Karssen et al. 1983). Developing grains of the viviparous mutants of maize which germinate precociously are deficient or insensitive to ABA (Robichaud et al. 1980). Reduction of ABA levels by the application of fluridone to immature embryos also causes vivipary in developing maize (Fong et al. 1983). In wheat, ABA levels in the whole grain (King 1976) and embryo (Walker-Simmons 1987) peak at the developmental time when maximum fresh weight is attained (stage IV), and dormancy is acquired (King 1982). If ABA is removed from the medium of cultured immature wheat embryos (stages II/III), the embryos precociously germinate. When immature wheat embryos at stages II/III are cultured in ABA, a new set of proteins, characteristic of late seed development (stages III and IV), are induced (Quatrano et al. 1983). Other ABA-inducible proteins which accumulate in the maturing embryo of rice (Gomez et al. 1988; Mundy and Chua, 1988) and barley (Chandler et al. 1988) have recently been reported. Whether any of these ABA-inducible proteins are in turn associated with the induction of dormancy is not yet known.

In wheat the level of dormancy in mature grain is markedly affected by the temperature during seed development. Cereal grains developed under cool temperatures (10–20°C) acquire high levels of dormancy, while development at warmer temperatures (20–30°C) results in low levels of dormancy (Black et al. 1987; Buraas and Skinnes 1985; Reddy et al. 1985; Walker-Simmons 1989).

Since dormancy acquisition can be altered by changing the developmental temperature, this has prompted us to determine how embryonic ABA levels are affected under these same temperature conditions. ABA levels were determined at each stage of caryopsis development in wheat plants grown under the cool or warm temperature conditions which produce large differences in grain dormancy. Two cultivars were compared, one having a high poten-

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tial, and the other low, for producing seeds with grain dormancy.

Materials and Methods

Plant Material

Triticum aesitivum L. cultivars, Brevor and Greer, were grown in a greenhouse at 22°C daytime/15°C nighttime temperatures and a supplemented photoperiod of 18 h. Wheat spikes were tagged at anthesis, and the plants were transferred to growth chambers kept at a constant temperature of 25°C or 15°C with an 18 h photoperiod (400 $\mu E/m^{-2}/s^{-1}$). Plants were maintained under these temperature conditions throughout grain development and watered daily as needed.

Developing grains for ABA analysis and germination assays were sampled from only the primary and secondary florets of the center spikelet of spikes of the main culm. Developmental stages for the wheat caryopsis were assigned according to the classification of Rogers and Quatrano (1983) with the endosperm characteristics of stages II (milky dough), III (soft dough), IV (hard-dough), and V (hard). Percentage dry weight of the seeds was calculated from the fresh weight and the weight of lyophilized grains.

Germination Assay

Ten dissected embryos or grains were placed on blotting paper in a Petri dish (100 × 15 mm). Six milliliters of distilled H_2O with or without 5 μM (±) ABA (Sigma Chemical Co.) were added to each plate. Plates were incubated at 15°C in the dark, and the number of embryos or grains that germinated was counted daily for 9 days. Germination was defined as pericarp rupture over the embryo. A weighted germination index (GI) was calculated which gave maximum weight to seeds or embryos that germinated first and less weight to those that germinated subsequently:

$$GI = \frac{(9 \times n_1 + 8 \times n_2 + \ldots + 1 \times n_9)}{\text{Total days} \times \text{total embryos}}$$

where n_1, n_2 . . . n_9 are the number of embryos (or grains) that germinated on the first, second, and subsequent days until the ninth day, respectively; 9, 8, . . . , are the weights given to the number germinated on the first, second, and subsequent days, respectively. The maximum GI is 1.0 and the minimum is 0.

ABA Analysis

Methanol extracts of dissected embryos or grain remnants were prepared for ABA analysis as previously described (Walker-Simmons 1987). (+)ABA content of the extracts along with appropriate ABA control samples was measured by indirect ELISA (enzyme-linked immunosorbent assay) as described by Walker-Simmons (1987) utilizing a monoclonal antibody specific for (+)ABA. The ABA-4'-bovine serum albumin conjugate used in the immunoassay was prepared according to Weiler (1980).

Results

Effect of Temperature on Induction of Grain Dormancy

The effect of temperature on the development of grain dormancy in the two wheat cultivars, Brevor and Greer, was demonstrated by growing plants of each cultivar from anthesis to maturity at 15° or 25°C (Figs. 1 and 2). Brevor has high potential for producing grains with dormancy (Reddy et al. 1983), while Greer has a low potential (Walker-Simmons 1987). Growth of Brevor at 15°C resulted in low-grain germinability throughout grain development (Fig. 1, upper panel). Grains from Brevor grown at 25°C acquired more germination capacity during the later stages of development. For Greer, growth at the cooler temperature (Fig. 2, upper panel) also reduced germinability of the immature grains. However, seed germinability in Greer was less affected by the two developmental temperatures and stage V grains developed at either temperature had high germinability.

Germinability of the embryos from these grains was measured by dissecting the embryos from the grains and incubating them in water. From stage III on, isolated embryos had high levels of germinability which increased even more with grain maturation (Figs. 1 and 2, lower panels). Responsiveness of the isolated embryos to ABA was measured by the capability of ABA to block embryonic germination. The addition of 5 μM ABA to the incubation solution reduced the germination of the isolated embryos (Figs. 1 and 2, middle panel). Germination indexes for embryos in ABA were similar to the germination indexes of the whole grains in water. ABA severely blocked embryonic germination of Brevor embryos from 15°C grown grain, but ABA had less of an effect on embryos from the 25°C grown grain (Fig. 1). Greer embryos from 15°C grown grain showed a similar enhanced responsiveness to ABA at stages III and IV compared to embryos from 25°C grown grain (Fig. 2).

Grains of both cultivars matured slowly at the cooler temperatures. Time from anthesis to maximum percentage dry weight was 83 days for Brevor and 84 days for Greer at 15°C, and 25 days for Brevor and 31 days for Greer at 25°C. The final dry weight attained for grains from both cultivars at 15°C was approximately three times larger than for grains developed at 25°C.

ABA Levels

The status of embryonic ABA levels during development at the warm or cooler temperatures was

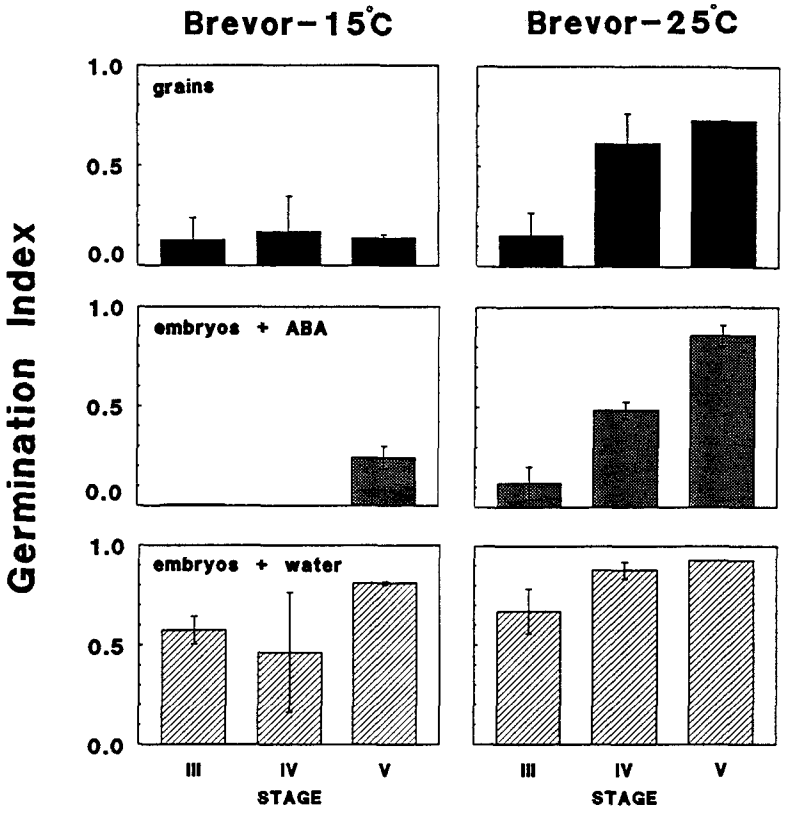

Fig. 1. Effect of development at 15° or 25°C on the germination index of Brevor grains in water, or embryos in water ± 5 μM ABA. Bars indicate mean ± SD.

evaluated by measuring ABA levels in embryos from grains of the same plants as utilized for Figs. 1 and 2. Embryos were dissected throughout development, and ABA measured by immunoassay (Fig. 3). At 15°C ABA levels in the embryos of both the Brevor and Greer cultivars increased through stage III and peaked during stage IV as maximum fresh weight was achieved. Then as the grain entered the desiccation phase during stage V, ABA levels decreased and fell to low levels. This pattern of ABA accumulation with a peak in ABA levels at stage IV is representative of previously reported ABA levels for developing wheat and barley embryos and whole grains grown under 15–20°C controlled temperature conditions (King 1976; Goldbach and Michael 1976; Walker-Simmons 1987).

Development at 25°C resulted in an entirely different ABA accumulation pattern as compared to 15°C. Embryonic ABA levels of 25°C grown grain were very high in the earliest stages of caryopsis development. At stages II–III embryonic ABA levels for each cultivar were over five times higher in the 25°C grown grains compared to those grown at 15°C. During further grain maturation the initial high embryonic ABA levels of both cultivars fell sharply as the grains matured through stages III–V. By the end of stage V, when the grains reached final percentage dry weight, ABA levels in the embryos and grain remnants of both cultivars had dropped to similar low levels. In fact by late stage V, ABA levels in all the embryo and grain remnant samples from both 15° and 25°C grown plants had fallen to similar low levels (Fig. 3), even though stage V Brevor grains grown at 15°C had considerably lower germinability than the other types of grains (Figs. 1 and 2).

The importance of ABA can also be assessed by examining the length of time that ABA levels remained elevated during grain maturation. When results of the ABA embryonic measurements (pg/mg-fresh wt) in Fig. 3 are evaluated as the level of ABA (pg/mg-dry wt) versus days post anthesis as in Fig. 4, differences in the duration of high ABA levels are apparent. Results of the ABA measurements from the earliest possible isolation of intact embryos to the time that grains reached maximum dry weight upon development at 15° or 25°C are presented (Fig. 4). At 25°C embryonic ABA levels were initially far higher than in 15°C grown grain, but ABA levels dropped precipitously with the rapid maturation at the warmer temperature. Very high ABA levels were measured during stages II–III, but those stages only lasted 5 days for both cultivars, compared to a time interval of 24 and 28

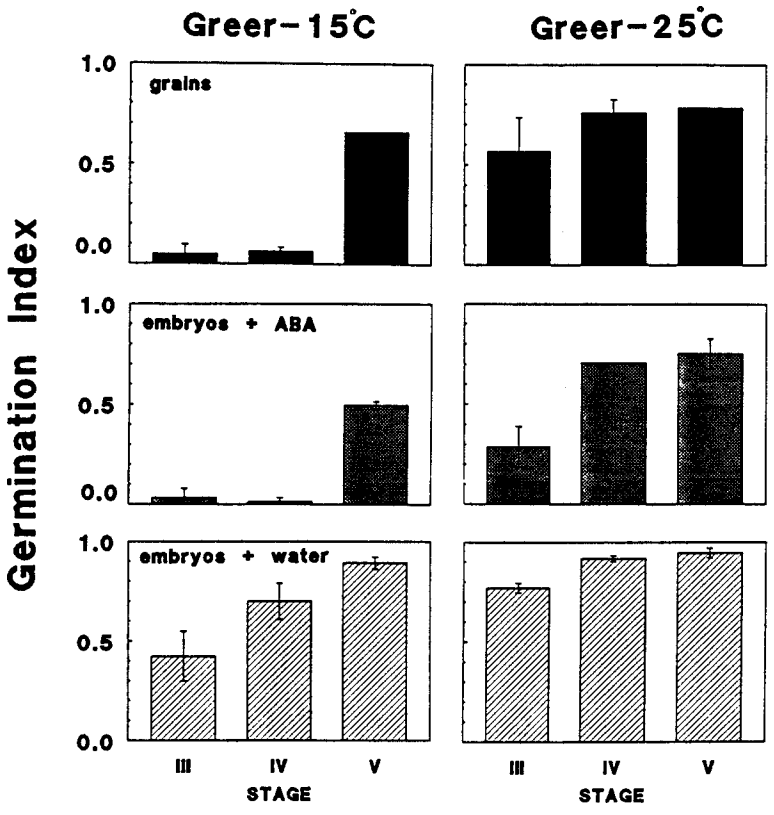

Fig. 2. Same as Fig. 1, except Greer cultivar.

days for stages II–III for Brevor and Greer, respectively, at 15°C. At the cooler temperature, grains matured slowly and elevated embryonic ABA levels were prolonged. ABA levels were maintained at over 350 pg/mg dry wt for over 30 days during growth at 15°C, while at 25°C ABA fell below that level within 6 days. More dormancy was induced in the mature Brevor grain than in the Greer. In general, ABA levels were higher in Brevor embryos than in Greer, although these differences were within the standard deviation. During growth at 15°C ABA levels of the Brevor embryos were often, though not always, 20–40% higher than in Greer embryos.

Discussion

In this study the highest ABA levels measured (Fig. 4) did not result in the largest amounts of grain dormancy. Even though initial embryonic ABA levels were very high during grain development at the warmer temperature, only a low level of dormancy developed in the mature grain. Increased levels of dormancy were produced when ABA levels were elevated for many days during slow grain development at the cooler temperature.

The effects of temperature on ABA levels determined in this study are consistent with previously reported results for cereals grown at cool or warmer temperatures. In a comparison of Brevor and Greer grown 2 years under field conditions, higher ABA levels were measured in the year that was warmer and dryer (Wiedenhoeft et al. 1988). Radley (1976) found that whole grain ABA levels peaked earlier, and ABA levels were two times higher in grain grown at 25°C compared to 15°C. As observed in our study, Quarrie et al. (1988) reported that ABA levels of wheat grain developed at a warm temperature (21–23°C) exhibited one peak, while two peaks in ABA levels were observed during development at a cooler temperature (16°C). In barley, ABA levels peaked earlier in 26°C grown barley compared to 18°C grown grain (Goldbach and Michael 1976), and the cultivar with less dormancy had a lower ABA level during development.

The warmer developmental temperature used in this study may have enhanced grain germinability by causing rapid desiccation of the maturing grains; it has been shown that premature drying of immature seeds of castor bean stops seed development and irreversibly promotes seed germination on seed rehydration (Misra et al. 1984). Also, rapid drying of immature wheat grains at 20°C has been shown to

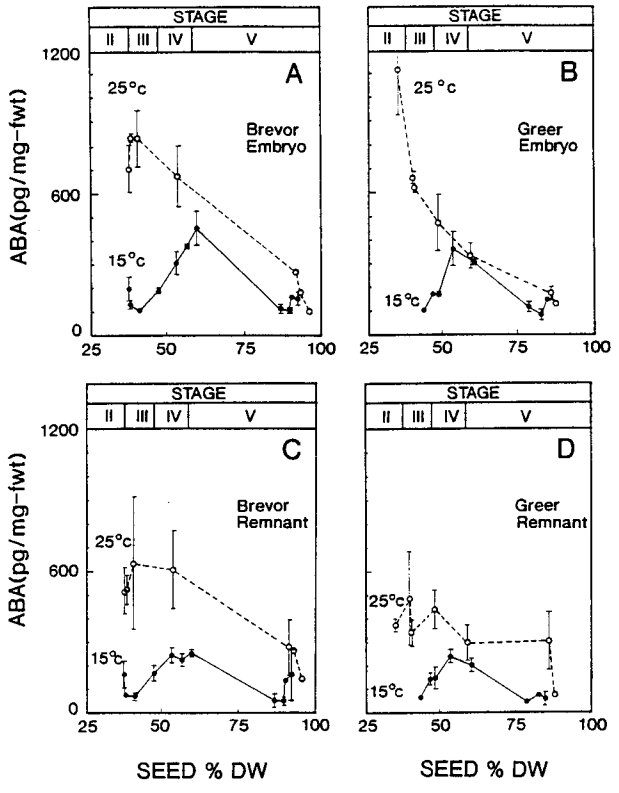

Fig. 3. (+)ABA levels (pg/mg-fresh wt) in embryos and the remaining grain remnant during grain development at 15° or 25°C. ABA levels were measured by immunoassay as described in Materials and Methods. Bars indicate mean ± SD.

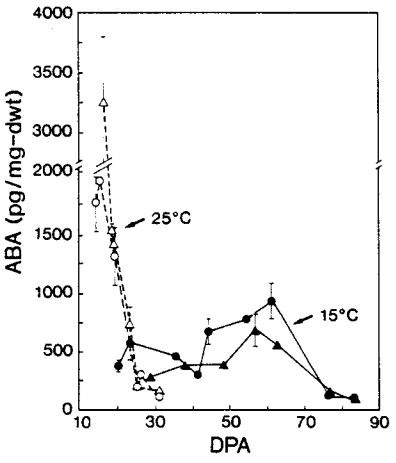

Fig. 4. Embryonic (+)ABA levels (pg/mg-dry wt) of 25°C grown grain, Brevor (○– – –○) and Greer (△– – –△), and 15°C grown grain, Brevor (●——●) and Greer (▲——▲) with days postanthesis (DPA). Sampling ended at harvest ripeness, when the final percentage dry weight was achieved. Bars indicate mean ± SD.

enhance the capacity of GA_3 to induce alpha-amylase accumulation (King and Gale 1980) and to reduce ABA levels in whole grains (King 1982).

It can be concluded from our study that embryos from dormant grains are more responsive to ABA than embryos from nondormant grain. The environmental condition (cooler developmental temperature) and the genotype (Brevor) that yielded grains with the higher level of dormancy (Figs. 1 and 2) also produced embryos that were more responsive to ABA. Other studies have also shown that embryonic responsiveness to ABA in wheat embryos is developmentally regulated and proportional to the degree of grain dormancy (Morris et al. 1989; Walker-Simmons 1987; Walker-Simmons 1988). These observations imply that ABA acts differently in embryos from dormant compared to nondormant grain. Possible differences in ABA action may include the synthesis and maintenance of ABA-inducible mRNAs and proteins.

More dormancy may have developed at the cooler temperature because synthesis and accumulation of ABA-inducible mRNAs and proteins were enhanced by the prolonged elevation of ABA levels during stages III and IV. ABA-inducible proteins are primarily synthesized during stages III and IV in wheat (Quatrano et al. 1983). In our study, embryonic ABA levels of the 25°C grown grain were high at stage III, but dropped rapidly. Embryonic ABA levels in the 15°C grown grain steadily increased during stages III and IV, and ABA levels above 350 pg/mg-dry wt were maintained in both cultivars for over 30 days (Figs. 3–4).

Temperature has been reported to have the largest effect on dormancy induction at the soft dough stage of caryopsis development (stage III, 35–50% dry weight) which suggests that the factors causing dormancy are developed at this time. Sublethal heat stresses at this stage have been shown to reduce dormancy (Belderok 1961; Khan and Laude 1969; Takahashi 1980).

In order to further elucidate the relationship between ABA and dormancy, work is in progress in this laboratory to identify ABA-inducible mRNAs and proteins associated with the initiation and maintenance of dormancy. Evaluation of the effects of varying ABA concentrations on gene expression in immature and mature embryos can now be compared with the endogenous ABA measurements reported in this study made during the development of grain dormancy.

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