ABA Levels and Sensitivity in Developing Wheat Embryos of Sprouting Resistant and Susceptible Cultivars

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ABSTRACT

A sprouting-resistant and a sprouting-susceptible wheat cultivar were utilized to examine the role of ABA levels and sensitivity responses in wheat embryonic germination. Endogenous embryonic ABA levels were measured in both cultivars throughout grain maturation utilizing a new and sensitive ABA immunoassay. Embryonic ABA levels of each cultivar were similar with the sprouting-susceptible cultivar having about a 25% lower ABA level than that of the sprouting-resistant cultivar. Larger differences between the cultivars were noted in sensitivity to ABA, as measured by capability of ABA to block embryonic germination. ABA inhibited embryonic germination much more effectively in the sprouting-resistant cultivar.

ABA has been demonstrated to have an important role in regulating seed embryonic maturation and germination (13, 22). Exogenously applied ABA prevents embryo germination, blocks the expression of germination-specific enzymes, and promotes embryonic development. ABA is an effective inhibitor of embryonic germination in immature wheat grain (23) and in seeds of other species including rape (5) and soybean (4). Precocious germination has been observed in cultured immature wheat embryos when ABA was removed (23) and in wheat (13) and soybean embryos (1, 2) when ABA levels were reduced by drying. ABA deficient and ABA insensitive mutants of Arabidopsis are susceptible to precocious germination and have reduced seed dormancy (11, 14). Viviparous mutants in corn that are ABA deficient or insensitive precociously germinate (26). ABA prevents the expression of germination-specific proteins in isolated embryos of cotton, soybean, rice, and wheat (22). Application of ABA to immature embryos maintains or causes an increase in mRNA and proteins characteristic of later stages of embryonic development in wheat (23), rape (5), soybean (4), rice (29), and cotton (7).

Involvement of ABA in regulating embryonic germination and the activity of germination enzymes is of particular importance in wheat. Germination of wheat before harvest can severely reduce the quality of the grain for food products resulting in large economic loss. This preharvest sprouting damage is caused by hydrolysis of starch by α-amylase accompanied by proteolytic damage of grain proteins. Both α-amylase activity and synthesis in cereals have been shown to be regulated by ABA through inhibition of GA3-induction of α-amylase synthesis (3, 10, 32). Not only is the grain embryo considered to be the site of ABA action, but the earliest significant sprouting damage caused by α-amylase has recently been shown to occur in the grain embryo (16-18). It has been previously difficult to measure actual embryonic ABA levels due to the small amount of tissue in developing embryos.

Wheat cultivars vary in susceptibility to preharvest sprouting and in the duration of seed dormancy (25). Grains removed from the maturing plant of a sprouting-susceptible cultivar and placed in water will easily germinate. In contrast, grains removed from a maturing sprouting-resistant cultivar will not sprout easily. Similar cultivar differences in preharvest sprouting susceptibility occur in field-grown grains still within the wheat spike under rainy weather conditions. We have been utilizing a sprouting-susceptible and a sprouting-resistant cultivar to examine the role of embryonic ABA in regulating embryonic germination in wheat. Under field conditions, 50 μM ABA more effectively blocked embryonic germination in a sprouting-resistant than in a sprouting-susceptible cultivar (9). However, the amount of ABA sensitivity and endogenous levels of ABA varied widely in response to different environmental conditions (9).

The objectives of this research were to determine, under controlled growth conditions, if embryonic ABA levels in sprouting susceptible and resistant cultivars account for sprouting differences, and to determine if embryonic sensitivities to ABA correspond to whole seed germination capabilities of the cultivars. A sensitive immunoassay utilizing a monoclonal antibody to (+)ABA was developed to measure ABA in small amounts of embryonic tissue. Embryonic ABA sensitivity in the two cultivars was compared by measuring the effectiveness of a broad range of ABA concentrations to inhibit embryonic germination. Finally, it was demonstrated that the embryonic ABA sensitivity responses reflect the capacity of whole seeds to germinate during maturation.

MATERIALS AND METHODS

Plant Material. Triticum aestivum L. cultivars Brevor and Greer were grown in a greenhouse with 22°C day/15°C night temperatures and a supplemented photoperiod of 16 h. Wheat spikes were tagged at anthesis. To minimize variation, grains were selected only from the primary and secondary florets of the main culm. Embryos from the early stages of development were dissected from the grain using a sterile forceps. In the later grain maturation stages embryos were dissected using a sterile razor blade. These embryos had small amounts of adhering endosperm.

Germination Assay and Index. Ten dissected embryos or whole seeds were placed on blotting paper in a Petri dish (100 × 15 mm) containing 6 ml of distilled H2O with or without (+)ABA (Sigma Chemical Co.). The Petri dishes were incubated at 15°C for 7 days at 15°C.
in the dark. Embryos or seeds that germinated were counted daily for 12 d. A germination index (25) was calculated with maximum weight given to the embryos or seeds that germinated first and less weight to those that germinated later.

Germination Index (GI) =

\[ 12 \times n_1 + 11 \times n_2 + \ldots + 1 \times n_{12} \]

where \( n_1, n_2, \ldots, n_{12} \) are the number of embryos or seeds germinated on the first, second, and subsequent days until the 12th d, respectively; 12, 11, \ldots, and 1 are the weights given to the number germinated on the first, second, and subsequent days, respectively.

**ABA Extraction.** Dissected embryos or the grain remnants were frozen immediately in liquid N\(_2\), lyophilized, and powdered. The powdered samples were suspended in extracting methanol (methanol containing 100 mg/L butylated hydroxytoluene and 0.5 g/L citric acid monohydrate) at a ratio of 0.017 g dry tissue:1.0 ml extracting methanol. \(^{14}\)CABA (0.14 nCi; 97 \( \mu \)Ci/mg; Amer sham, Arlington Heights, IL) was added to each of the samples as an internal standard. Extracts were stirred overnight, then spun at 2000g and the supernatants dried in a Speed Vac Concentrator \(^{2}\) (Savant, Farmingdale, NY). This procedure gave optimum extraction of ABA as measured by immunoassay and 96.5% recovery (se: 3.6%) of the \(^{14}\)C counts. For ELISA\(^{3}\) assay the dried samples were resuspended in 100 \( \mu \)l methanol and 900 \( \mu \)l water. Before immunoassay the resuspended samples were spun at 2000g, if necessary, to remove insoluble material. Several serial dilutions in TBS of each sample extract were assayed.

**ELISA Assay Materials.** This procedure is a modification of our previously reported immunoassay method (33).

Buffers. TBS: 6.05 g Tris, 0.20 mg MgCl\(_2\), 8.8 g NaCl per liter (pH 7.8); washing buffer: TBS containing 0.05% (v/v) Tween 20, and 0.1% (w/v) BSA (ELISA grade, Sigma).

**ABA-4'-BSA Conjugate.** The conjugate was prepared according to Weiler (34) and lyophilized. Conjugate was suspended in 0.05 M NaHCO\(_3\) (pH 9.6), at a concentration of 7 mg/ml and stock aliquots of 60 \( \mu \)l were kept frozen at \(-20^\circ\)C. Before ELISA assay a 60 \( \mu \)l aliquot was thawed and diluted with 0.05 M NaHCO\(_3\) (pH 9.6), to a final volume of 16 ml which is sufficient to coat the assay wells of one microtitration plate.

Monoclonal Antibody. MAb to free cis, trans (+)-ABA (19) was purchased from Idetect, Inc., 1057 Sneath Lane, San Bruno, CA 94066. Two mg MAb was mixed into 135 ml TBS, containing 0.2% (w/v) BSA. Aliquots of 2.25 ml of the MAb solution were stored at \(-20^\circ\)C. Before ELISA assay a 2.25 ml aliquot was thawed and diluted in TBS to a final volume of 11.25 ml, which is sufficient for the assay wells of one microtitration plate.

Second Antibody. Rabbit antinouse alkaline phosphatase conjugate (Sigma Chemical Co.) was diluted 1:2000 in TBS.

Alkaline Phosphatase Substrate. The substrate, \( p \)-nitrophenyl phosphate was prepared at a concentration of 1 mg/ml in 0.05 M NaHCO\(_3\) (pH 9.6).

**ABA Standards.** (+)-cis-trans ABA purchased from Sigma Chemical Co. was dissolved in TBS in concentrations ranging from 5 to 250 pg/100 \( \mu \)l.

Microtitration Plates. Immulon 2 flat bottom, 96-well microtitration plates, Dynatech Laboratories, Inc., Alexandria, VA were utilized. Better binding of the ABA-4'-BSA conjugate was obtained with this type of plate than with other types tested. The upper and lower row of wells of the plate were not used.

ELISA Assay Procedure. All samples and solutions were kept in the dark during incubations.

1. **Coating of Wells with ABA-4'-BSA Conjugate.** A 200 \( \mu \)l aliquot of the conjugate was added to each well of the microtitration plate, excluding the upper and lower rows. Plates were incubated at 4°C overnight.

2. **Incubation of ABA Samples with MAb.** A 350 \( \mu \)l aliquot of the ABA sample was pipetted into a test tube. Then 350 \( \mu \)l of MAb solution was added and mixed. The solutions were incubated overnight at 4°C. This is enough material for three replicate well assays.

3. **Addition of ABA Samples Incubated with MAb.** Plate wells coated with conjugate were washed three times with washing buffer. For this step only, the final washing solution was left in the plates for 10 min and then discarded. Then, 200 \( \mu \)l aliquots of the samples incubated with MAb was added to three replicate wells. Plates were incubated for 2.5 h. This incubation and all the following steps were performed at room temperature.

4. **Addition of the Second Antibody.** Wells were washed three times with washing buffer. Rabbit antinouse alkaline phosphatase conjugate (200 \( \mu \)l) was added to each well. Plates were incubated for 2 h.

5. **Measurement of Alkaline Phosphatase.** Wells were washed three times with washing buffer. \( p \)-Nitrophenyl phosphate substrate (200 \( \mu \)l) was added to each well. Plates were incubated for about 1 h until the \( A \) at 405 nm of control samples containing no ABA, was approximately 1.0. The incubation was stopped by adding 50 \( \mu \)l of 5 N KOH, and the sample absorbance measured at 405 nm with a Bio-Rad Enzyme Immunoassay Reader. The absorbance of the samples is inversely proportional to the amount of ABA in the original sample incubated with MAb.

Replicate ABA standards were assayed for each plate. A linear regression analysis was computed using the ABA standards, and the amount of ABA in the plant extract samples calculated based on the coefficient of the ABA standard curve for each plate. All sample results were the average of three serial dilutions within the linear range of the ABA standard curve.

**Partition Procedure.** Tissue samples extracted in methanol as described above for the ELISA assay were partitioned against ethyl acetate before HPLC analysis. The dried methanol extracts were resuspended in 4 ml water and the pH of the solution was lowered to less than pH 3 using acetic acid. The aqueous samples were partitioned three times against ethyl acetate, and the pooled ethyl acetate fractions were dried. Samples were resuspended in 2 ml methanol (HPLC grade), filtered through a 0.2 \( \mu \)m pore size nylon-66 membrane filter (Rainin), and dried.

**HPLC.** Samples were resuspended in 450 \( \mu \)l of 0.2 n acetic acid containing 10% (v/v) ethanol, and 350 \( \mu \)l was injected on to a reverse phase uBondpak \( \mathrm{C}_{18} \) (3.9 mm x 30 cm) HPLC column (Millipore-Waters, Milford, MA) (9). Samples were injected with a Sample Processor 710P (Millipore-Waters) directed with a Programmable System Controller (Millipore-Waters). An initial 3.5 min linear solvent gradient from 10 to 35% (v/v) ethanol in 0.2 n acetic acid was followed by constant 35% ethanol in 0.2 n acetic acid at a flowrate of 1 ml/min. The fractions containing ABA were collected based on retention time of ABA standard and the fractions were dried. These semipurified samples were methylated (9) and then dissolved in 500 \( \mu \)l chloroform. Samples were injected onto a silica Porasil HPLC column (3.9 x 30 cm, Millipore-Waters Associates). Development was isocratic with chloroform:hexane (80:20, v/v) at a flow rate of 2 ml/min (B Heimbigner, MG Hagemann, DR Gealy, unpublished procedure). The methylated peak was measured at 270 nm using a UV monitor (model 440, Waters Associates) and integrated using a Hewlett Packard model 3392A Integrator. Identification of the methylated peak was confirmed by HPLC chromatography of a.

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\(^{2}\) Mention of a specific product name by the United States Department of Agriculture does not constitute an endorsement and does not imply a recommendation over other suitable products.

\(^{3}\) Abbreviations: ELISA, enzyme-linked immunosorbent assay; ABA-4'-BSA, ABA-bovine serum albumin-conjugate; DPA, days postanthesis; MAb, monoclonal antibody; TBS, Tris-buffered saline.
methyllated ABA standard (Sigma Chemical Co.) and of methylated \[^{14}C\]ABA prepared in the same manner as the samples. Efficiency of each step of the purification process was monitored by counting \(^{14}C\) from the \[^{14}C\]ABA internal standard added to the original samples, and the final ABA values were calculated based on the recovery of \(^{14}C\).

**RESULTS**

**Development of an Immunoassay for Embryonic ABA.** A sensitive ABA immunoassay utilizing a monoclonal antibody for (+)ABA was developed for analysis of the small developing embryos (33). The sensitivity of the assay to as low as 5 pg, and a simple extraction method, enabled measurement of ABA in as few as one to two embryos. This quantitative ABA immunoassay is an indirect ELISA. The assay is linear from 5 to 250 pg (+)ABA (33). A major advantage of this indirect ELISA assay is that only small amounts (0.3 μg/assay well) of a commercially available MAb to (+)ABA are required.

A comparison of ABA levels in initial crude grain extracts determined by immunoassay or by HPLC analysis of methylated samples after further purification by ethyl acetate partitioning and HPLC separation on a C18 column, gave equivalent results (Table I). ABA immunoassay results utilizing the ABA-4'-BSA conjugate in an indirect ELISA assay have been verified by GC-MS by Norman et al. (21; personal communication from S Norman).

Addition of wheat extracts to standard amounts of ABA did not interfere with the linearity of the immunoassay. Aliquots of crude grain extract containing 30 or 60 pg of (+)ABA were added to (+)ABA standards (5–83.3 pg) (Fig. 1). The slopes of the lines with added plant extract were comparable to that of (+)ABA standards alone. Standard ABA recovery checks were routinely made with embryonic and grain remnant extracts at all stages of development. Known quantities of ABA were added to appropriately diluted tissue extracts and the added ABA was accurately detected with the immunoassay. ABA recoveries of 95% (SD, 5%), as measured by ELISA, were obtained for ABA added to embryonic extracts.

**Measurement and Comparison of Embryonic ABA.** ABA was measured in embryos dissected from the sprouting-resistant wheat cultivar (Brevor) and the sprouting-susceptible cultivar (Greer) during grain development and maturation (Fig. 2). Grains from the two cultivars had the same rate of maturation reaching maximum fresh weight near 40 DPA. Embryonic ABA in both cultivars peaked as the grain reached maximum fresh weight. After that time the grains dried rapidly and embryonic ABA in both cultivars declined to a low level. Embryonic ABA levels of the two cultivars throughout grain development have been measured in three separate experiments. In all the experiments, embryonic ABA levels in the sprouting-susceptible Greer grains were about 20 to 40% lower than in the sprouting-resistant Brevor grains from 20 to 40 DPA, although all values were within the range of 1 sd. The embryonic ABA levels of Greer averaged 74.6% (SD, 9.1%) of the levels of Brevor at the time of maximum fresh weight in the three experiments.

Embryonic ABA concentrations from the experiment presented in Figure 2 were calculated utilizing the percentage moisture content of the embryos and the results are presented in Table II for each stage of embryo development (27). The concentration calculations are useful for relating actual endogenous ABA concentrations to published reports of the effects of ABA concentration on gene expression and protein induction in *in vitro* experiments. The concentrations were similar for the two cultivars, although the ABA concentrations in Brevor were higher at Stage V. ABA levels in the remaining part of the grain after embryo removal were also measured in Brevor and Greer (Fig. 2). The calculated ABA concentrations utilizing grain percentage moisture are presented in Table II. Generally, ABA levels and concentration in the remaining part of the grain were lower than for the embryos in both cultivars. Brevor and Greer concentrations

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Table I. **ABA Measurement by ELISA or HPLC of Crude and Purified Sample Extracts**

<table>
<thead>
<tr>
<th>Sample Purification</th>
<th>Assay Method</th>
<th>(+)ABA (%) of initial extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial crude extract</td>
<td>ELISA</td>
<td>100.0 ± 5.1</td>
</tr>
<tr>
<td>Ethyl acetate partitioned</td>
<td>ELISA</td>
<td>100.9 ± 4.4</td>
</tr>
<tr>
<td>HPLC chromatography (C18 column)</td>
<td>ELISA</td>
<td>107.3 ± 15.4</td>
</tr>
<tr>
<td>HPLC (silica) of methylated samples</td>
<td>HPLC</td>
<td>104.5 ± 9.9</td>
</tr>
</tbody>
</table>

**Fig. 1.** Effect of added grain extracts on the ABA standard curve. Diluted grain extracts (1/10 and 1/20 dilution) were added to (+)ABA standards ranging from 5 to 83 pg. The amount of ABA was measured by indirect ELISA in the samples containing only ABA standards (+), ABA standards + 1/10 diluted grain extract (O), and ABA standards + 1/20 diluted grain extract (●). Data are the means of three sample dilutions.

**Fig. 2.** ABA levels in isolated embryos (●—●) and the remaining part of the grain (O—O) of developing Brevor (upper) and Greer (lower). Arrows indicate time of maximum fresh weight. Data are the means ± SD of three replicate extractions of 10 embryos or grains each. The SD was within the data point if not shown.
Embryos from both cultivars were more sensitive to ABA during the earlier stages of development. Embryonic sensitivity to ABA was first measured at about 25 DPA when isolated embryos can germinate (31). At that time a 10 times higher concentration of ABA (5 \textmu M compared to 0.5 \textmu M) was required to reduce the germination index equivalently in Greer embryos compared to Brevor. Similarly large cultivar differences in sensitivity to ABA continued from 25 to 38 DPA as the grains steadily increased in fresh weight. Near 38 DPA grain (as indicated by the arrow) each cultivar reached maximum fresh weight, and then the desiccation stage of grain maturation began. The grains rapidly dried after 38 DPA and embryonic sensitivity to ABA declined. After 40 DPA Greer embryos lost sensitivity to 5 and even 50 \textmu M ABA. After the grain reached the maximum percentage dry weight at 43 to 44 DPA, sensitivity to ABA was lost, and Greer embryonic germination was no longer blocked by ABA.

Embryos from the sprouting resistant cultivar (Brevor) maintained sensitivity to low levels of ABA longer than Greer. Between 25 to 38 DPA, 0.5 \textmu M ABA effectively reduced the germination index of Brevor embryos. Once the maximum fresh weight had been reached there was a decline in sensitivity to the lower ABA concentrations. However, the higher concentration of 50 \textmu M ABA still reduced the Brevor embryonic germination index by 50\% even 10 d after the final 90\% dry weight was achieved at 43 DPA. Maximum inhibition of embryonic germination in each cultivar was obtained with 50 \textmu M ABA. Increasing the concentration to 500 \textmu M ABA in this study gave very similar results to that obtained with 50 \textmu M for each cultivar (data not shown). The sensitivity experiment has been repeated in two other separate experiments with a similar pattern each time. Brevor embryos were more sensitive to ABA than Greer.

Comparison of Cultivar Seed Germination Index with Embryonic ABA Sensitivity. To relate the embryonic sensitivity results to the germination capacity of whole seeds, the germination indexes for whole seeds from the two cultivars were compared during grain maturation (Fig. 4). Brevor seeds had a much lower germination index throughout grain maturation which is consistent with previous reports that Brevor is more resistant to

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Table II. Concentration of ABA (\textmu M) in Embryos and Seed Remnants

ABA (pg/mg fresh weight) was measured by immunoassay. Values were converted to \textmu M based on the assumption that all water in the tissue was available to ABA. Data are the means of three replicate extractions and immunoassays of 10 embryos or grain remnants each.

<table>
<thead>
<tr>
<th>DPA (Stage*)</th>
<th>Seed Dry Wt</th>
<th>Embryo ABA</th>
<th>Grain Remnant ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B\textsuperscript{a}</td>
<td>Gr\textsuperscript{b}</td>
<td>Bv</td>
</tr>
<tr>
<td>22-23 (III)</td>
<td>38.0</td>
<td>42.7</td>
<td>1.20</td>
</tr>
<tr>
<td>30-31 (III/IV)</td>
<td>44.8</td>
<td>45.9</td>
<td>1.52</td>
</tr>
<tr>
<td>33-39 (IV)</td>
<td>46.8</td>
<td>46.1</td>
<td>1.54</td>
</tr>
<tr>
<td>43-63 (V)</td>
<td>86.5</td>
<td>72.3</td>
<td>4.12</td>
</tr>
</tbody>
</table>

\(\textsuperscript{a}\) Stages of wheat caryopsis development (27). \(\textsuperscript{b}\) Brevor. \(\textsuperscript{c}\) Greer.

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FIG. 3. Brevor (upper) and Greer (lower) embryonic sensitivity to ABA. Embryos were isolated from grains at the times presented in DPA. Germination index was measured for 10 embryos treated in water (●—●), 0.5 \textmu M ABA (O—O), 5.0 \textmu M ABA (+——+), or in 50 \textmu M ABA (▲—▲). Germination assay procedure is described in "Materials and Methods." Arrow indicates time of maximum fresh weight.

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FIG. 4. Comparison of the germination index for whole seed (●—●) and for isolated embryos incubated in 5 \textmu M (△—△) or 50 \textmu M ABA (○——○), (upper) Brevor; (lower) Greer. Arrows indicate time of maximum fresh weight. Each data point is for 10 grains or embryos.
preharvest sprouting (25). (Applied ABA had no effect on the germination index of these seeds and [14C]ABA was not incorporated, so the ABA sensitivity experiments could not be conducted with whole grain in this study.)

Cultivar embryonic sensitivity to ABA was similar to the germination response of whole seeds in water. Embryos from these grains were incubated in water or 5 or 50 μM ABA, and the effect on the germination index measured (Fig. 4). As in the experiments presented in Figure 3, embryos from both cultivars germinated well in water (data not shown). In this particular experiment, perhaps due to minor temperature fluctuations, Greer embryos were more sensitive to 5 μM ABA than in the experiment presented in Figure 3, but still less sensitive than Brevor embryos. As with the whole seeds, the germination index for Greer embryos in ABA increased as the seed reached maximum fresh weight near 40 DPA. Brevor embryos treated with ABA (Fig. 4), like seeds of Brevor, had a reduced germination capacity throughout maturation.

**DISCUSSION**

ABA levels of both cultivars were consistently 2 to 3 times higher in the embryo than in the remaining part of the grain until late maturation. The highest embryonic ABA levels occurred as the grains reached maximum fresh weight, and then ABA decreased rapidly as the grains began to desiccate and decrease in fresh weight. The pattern of embryonic ABA accumulation is similar to that reported by King (12) in wheat for maturing whole grains. Recently Raikhel et al. (24) reported wheat embryonic ABA levels for the cultivar Marshall at 10, 25, and 40 DPA as measured by a direct immunoassay. The ABA levels reported were similar to the embryonic levels found in my study.

It was necessary to develop a new ABA immunoassay for this study because so little embryonic tissue was available. At the same time as this immunoassay for wheat embryos was being developed, a similar indirect ELISA assay for ABA was developed for citrus leaves (21). ABA immunoassay results utilizing the commercial monoclonal antibody in the indirect ELISA assay with the ABA-4′-BSA conjugate have been verified by GC-MS by Norman et al. (21). These indirect ELISA assays offer the advantage of requiring considerably less monoclonal antibody than direct ELISA assays.

Results of this study indicate that there is a small difference between the endogenous embryonic levels of ABA in the two cultivars. Embryonic ABA levels in the cultivar Greer with less sprouting resistance averaged 75% that of the sprouting resistant cultivar Brevor at the maximum ABA level for three separate experiments, but the differences were within 1 sp of the replicate samples. Goldbach and Michael (8) compared whole grain ABA levels in two barley varieties with different sprouting resistance and obtained results similar to this embryonic study. They observed that the sprouting susceptible barley cultivar had a lower ABA content throughout maturation, and the ABA level in that cultivar was around 60% of the sprouting resistant cultivar at the maximum ABA level.

The calculated concentration of ABA from my measurements of the developing embryos was in the 1 to 5 μM range with the concentrations increasing as water was lost from the grain even though the ABA level decreased. The concentration values can only be considered an estimation because the calculations are based on the assumption that endogenous ABA is uniformly distributed throughout the available water in the tissue. A concentration range of 1 to 100 μM ABA has been shown to have a significant effect on embryo-specific gene expression in wheat (22, 23). Lakhani and Sachar (15) reported that 10 μM ABA blocked the stimulatory effect of GA3 on poly(A) polymerase activity in wheat embryos. In barley aleurone 25 μM ABA largely reduced the accumulation of α-amylase mRNA caused by GA3 (3) and 50 μM induced the accumulation or synthesis of an α-amylase inhibitor (20). In this study, similar embryonic ABA concentrations were measured in the sprouting-resistant and sprouting-susceptible cultivar. The small variations in embryonic ABA concentration do not appear sufficient to fully explain the differences in sprouting capability between the two cultivars.

A large difference between the two cultivars in sensitivity to ABA, as measured by ABA inhibition of embryonic germination was found. The germination response of isolated embryos from both cultivars to ABA closely resembled the characteristic germination response of whole seeds from each cultivar in water. Large ABA embryonic sensitivity differences that varied in response to changing environmental conditions in sprouting resistant and susceptible cultivars grown under field conditions have been previously observed (9). Recently we have expanded the studies to more cultivars varying in degree of resistance to sprouting, and have consistently found that the cultivars with more resistance to sprouting have more embryonic sensitivity to ABA (Y Hu, RE Allan, M Walker-Simmons, unpublished results). Embryonic sensitivity differences to 50 μM ABA were also found by Stoy and Sundin (30) in four wheat cultivars measured at one developmental time in mature grain.

In this report the sprouting susceptible cultivar lost sensitivity to ABA as the grain entered the desiccation stage. Loss of embryonic sensitivity to ABA upon grain maturation or desiccation has been reported in rape (5) and in soybean (4). In this study wheat embryos from the sprouting resistant cultivar showed a similar diminishing response to ABA upon grain desiccation. However, embryos from the sprouting-resistant cultivar continued to show sensitivity to ABA even upon grain desiccation. My preliminary experiments indicate that the decrease in embryonic sensitivity is not due to a decrease in ABA uptake capability.

Since the concentration range that was measured for endogenous embryonic levels has been shown to be effective in regulating embryonic germination enzymes (23) we must look further to explain why the wheat cultivars can have such different sprouting capabilities during grain maturation. We are now actively investigating the following possible causes for this ABA sensitivity difference. The sprouting susceptible cultivar may have more ability to take up or degrade exogenously applied ABA, and to turnover endogenous ABA. Embryonic water uptake may be regulated differently in the two cultivars and ABA may reduce water uptake in the embryos of the sprouting resistant cultivar. Schopfer and Plachy (28) have reported that ABA inhibits embryonic water uptake. In rape, Finkelstein and Crouch (6) have found that placing embryos in high osmoticum blocked water uptake and prevented germination. The high osmoticum was an effective germination blocker in immature embryos but not in mature (desiccation stage) embryos. Additionally, the ABA sensitivity differences observed in this study may result from different amounts of gene expression caused by ABA in the two cultivars. A comparison of ABA inducible proteins between the two cultivars will determine if embryonic proteins are affected by these differences in ABA sensitivity. Investigation of the causes of the sensitivity differences observed in this study should yield new information on the role of ABA in regulating embryonic germination.

Acknowledgments— I thank Joan Sesing for excellent technical assistance. A sample of MAB to (+)ABA was kindly provided by E. W. Weiler.

**LITERATURE CITED**

6. Finkelstein RR, ML Crouch 1986 Rapeseed embryo development in culture on high osmoticum is similar to that in seeds. Plant Physiol 81: 907–912
34. Weiler EW 1979 Radioimmunoassay for the determination of free and conjugated abscisic acid. Planta 144: 255–263