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Molecular Cloning and Expression of Abscisic Acid-Responsive Genes in Embryos of Dormant Wheat Seeds[1]

Craig F. Morris, Robert J. Anderberg, Peter J. Goldmark[2], and Mary K. Walker-Simmons*

U.S. Department of Agriculture/Agricultural Research Service, Wheat Genetics, Quality, Physiology and Disease Research (C.F.G, R.J.A., M.W.-S.), Department of Agronomy and Soils (P.J.G.), Washington State University, Pullman, Washington 99164-6420

ABSTRACT

Hydrated dormant cereal seeds do not germinate even when environmental conditions are favorable for germination. By using cDNA cloning and differential screening, we have identified mRNAs from five gene families that are abundant in the embryos of imbibed, but developmentally arrested wheat (*Triticum aestivum* L.) seeds. Gene transcript levels of these mRNAs are maintained and even increase in embryos of imbibed dormant seeds for as long as the seeds remain dormant. In contrast, transcript levels decline in nondormant seeds after imbibition and disappear as germination occurs. All the identified genes are ABA responsive. Based on these data we conclude that wheat seeds in the hydrated dormant state exhibit prolonged expression of ABA-responsive genes.

Upon hydration, most mature seeds germinate readily over a range of environmental conditions. By contrast, dormant seeds do not germinate under conditions favorable for germination and can remain viable in a hydrated state for prolonged periods without cell elongation until a signal is recognized by the seed embryo which initiates germination. The mechanism of seed dormancy poses an intriguing problem in plant molecular biology and has major agricultural importance. For the first few hours after imbibition dormant and nondormant seeds exhibit similar physiological responses, including the same rates of water uptake and new protein synthesis (6). In dormant seeds, however, there appear to be molecular and biochemical restrictions which prevent cell expansion and germination. The intent of this research is to contribute toward the identification of the restrictions which regulate seed dormancy in mature seeds.

In some plant species seed dormancy is released by prolonged storage of dry seeds (afterripening), while other species require hydration at low temperatures (6, 20). Proposed dormancy mechanisms in mature seeds have focused on regulation by inhibitors acting alone or in combination. Such mechanisms have included those attributed to the seed coat such as chemical inhibitors and oxygen restriction. Proposed embryo-linked inhibitors of dormancy have included phenolics, ABA, coumarins, and tryptophan (6, 20, 27, 29). However, correlation between inhibitor concentrations and dormancy has proven elusive.

Hormones such as GA[3] can promote germination of dormant seed. GA-responsive genes identified to date are generally associated with secondary events of germination, rather than initiators of germination. Because ABA inhibits the transcription of many GA-responsive genes, the relative levels of ABA and GA could govern dormancy in mature seeds. However, evidence obtained with ABA-deficient and GA-deficient mutants disputes this hypothesis (19). The induction and the release of dormancy is not affected by GA deficiency. Seeds of GA-deficient mutants produce dormant seeds, and just as in the wild-type seeds, dormancy release requires a prolonged storage period or chilling of hydrated seeds (19).

ABA is absolutely required for the induction of seed dormancy. Arabidopsis mutants either deficient in embryonic ABA or nonresponsive to ABA during seed development produce seeds lacking dormancy (18, 19). Precocious germination is observed in cultured immature wheat embryos when ABA is removed from the media (30). Other evidence indicating that embryonic ABA must be present for dormancy induction is provided by the application of fluridone during seed development, which blocks the accumulation of ABA in the embryo and results in the inhibition of dormancy induction in developing maize embryos (17).

The role of ABA in maintaining dormancy in mature seeds is less certain. Applied ABA can inhibit the germination of isolated embryos from mature wheat seeds, especially in embryos from dormant seeds. A 10- to 100-fold greater concentration of ABA is required to block germination of embryos from nondormant compared to dormant seeds (26, 38). However, it does not appear that high ABA levels in mature seeds are correlated with seed dormancy. Many studies have shown that ABA levels are similar in mature dormant and nondormant seeds (20, 38). Even though initial ABA levels are similar we find that dormant seed embryos are more ABA responsive. When dormant grain axes are imbibed there is prolonged and active synthesis of ABA-responsive proteins (32). The prolonged synthesis of ABA-responsive proteins is not observed in germinating embryos. These results have led us to examine the hypothesis that dormancy is under positive control result-

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[2] Visiting Scientist; permanent address: DJR Research, Inc., Star Route 69, Okanogan, WA 98840.

[3] Abbreviation: GA, gibberellic acid.

ing in the continued expression of specific genes, and to determine if at least some of these genes are ABA responsive.

To identify such genes we have differentially screened a cDNA library from ABA-maintained dormant embryonic axes with cDNA prepared from dormant and nondormant axes. The embryonic axis provides a unique model experimental system. Dormancy in wheat does not result from true embryo dormancy, but is instead associated with the caryopsis and responsiveness of the embryo (28). When separated from the caryopsis, the embryonic axes of dormant seeds germinate readily while retaining responsiveness to external germination inhibitors. Dormancy can be restored by incubating the axes in low ABA concentrations and there is good correlation between embryonic responsiveness to ABA and whole seed dormancy (26, 38). Use of isolated embryonic axes whose dormancy has been maintained with ABA has enabled us to screen for transcripts preferentially expressed in this organ, and then to extend the results to embryos within intact hydrated seeds.

Even though preferential expression of specific genes among different developmental states is well documented (15), genes expressed preferentially in hydrated dormant tissue have not been previously reported. In this paper our results show the enhanced and prolonged expression of specific gene transcripts in imbibed dormant wheat grains. In all cases the level of these transcripts was increased by ABA.

MATERIALS AND METHODS

Plant Material

The soft white winter wheat (*Triticum aestivum* L.) cv Brevor was vernalized and then grown under controlled glasshouse conditions using common cultural practices. Spikes were collected during a 5-d period from plants whose peduncles had turned yellow the previous 1 to 2 d. Spikes were dried 2 d at 37 to 40°C in a forced-air oven and threshed, and the grain was stored at −20°C to arrest afterripening. This combination of genotype, growing environment and postharvest treatment provided a supply of highly dormant seed. Nondormant Brevor seed was obtained by dry afterripening at room temperature 3 to 4 months and then stored at −20°C.

Embryonic axes were prepared as previously described (27). Isolated axes were stored several days at −20°C until used. Germination assays were conducted at 20°C in the dark. Axes were plated scutellum side down on agar (6.8 g/L Noble agar, Difco[4]) containing 20 g/L sucrose with or without 5 μM (±) ABA. Whole seeds were placed crease side down in Petri dishes on water-saturated blotters. Axes or seeds were considered germinated when the coleorhiza was greater than 1 mm in length or the radicle appeared.

Hosts, Clones and Protocols

Escherichia coli XL1-Blue and BB4 host strains, and R408 interference-resistant helper phage were obtained from Stratagene (La Jolla, CA). p1015 was obtained from Dr. Ralph S. Quatrano, University of North Carolina, Chapel Hill, NC (22). pHVA39 was obtained from Dr. Peter M. Chandler, CSIRO, Division of Plant Industry, Canberra, ACT, Australia (10). JR050 was obtained from Dr. John C. Rogers, Washington University School of Medicine, St. Louis, MO (33). pNVR1 was supplied by Dr. Natasha V. Raikhel, Michigan State University, East Lansing, MI (31). Unless otherwise indicated, protocols, buffers, media, and so forth are as described by Maniatis *et al.* (23).

cDNA Library Construction

Wheat axes (200, *ca.* 0.1 g) were plated as described for germination assays with 5 μM (±) ABA and incubated for 12 h. Total RNA was isolated by modifying the methods of Glisin *et al.* (14); Ullrich *et al.* (37) and Chirgwin *et al.* (9). Total RNA yield was 1.1 mg; A_{260}/A_{280} ratios were 1.7 to 1.8. Poly(A)$^+$ RNA isolation followed the method of Aviv and Leder (2) as modified by Kingston (21). Poly(A)$^+$ RNA was selected by two rounds of oligo(dT)-cellulose (type VII, Pharmacia LKB) affinity chromatography; mRNA yield was 8.2 μg.

cDNA library construction was conducted by Stratagene using oligo(dT) priming, size fractionation (>500 bp), and the *Eco*RI cloning site of Lambda Zap. The library was amplified in XL1-Blue.

Isolation of Differential Clones

Approximately 7000 clones (plaque-forming units) of the primary amplified library were screened for ABA-responsive and/or dormancy-associated clones by the "+/−" (differential) screening strategy of Sargent (34). Replicate plaque lifts were made according to the manufacturer's instructions (Colony/Plaque Screen, DuPont New England Nuclear) (4).

cDNA probe was prepared using mRNA from dormant wheat seed axes incubated as described for germination assays with 5 μM (±) ABA for 12 h and nondormant (afterripened) wheat seed axes incubated in similar fashion, but without ABA. Axes were homogenized (1, 16) and total RNA was precipitated with LiCl concentration (7). Poly(A)$^+$ RNA was isolated using an oligo(dT)-cellulose batch method (3).

The cDNA probe synthesis reaction began by drying 10 μL of [α-^{32}P]dCTP (3000 Ci/mmol, 10.0 mCi/mL, DuPont New England Nuclear) in a 1.5 mL microfuge tube. In a second tube, 1 to 3 μL water, 0.5 μg mRNA (1–3 μL), and 0.25 μg oligo(dT)$_{15}$ (0.25 μg/μL, Pharmacia LKB) were heated to 70°C for 5 min and cooled to room temperature. To this tube was added 1 μL of 10 × reaction buffer (500 mM Tris-Cl [pH 8.3], 750 mM KCl, 100 mM $MgCl_2$, 5 mM spermidine), 1 μL DTT (100 mM), 1 μL dATP, dCTP, dGTP, and dTTP (10 mM each), 0.5 μL RNasin (32 units/μL, Promega), and 0.5 μL sodium pyrophosphate (80 mM). These components were mixed, transferred to the first tube and 1 μL AMV reverse transcriptase was added (22 units/μL, Promega). The reaction was incubated at 42°C for 45 min, an additional 1 μL of AMV reverse transcriptase was added and the reaction was incubated an additional 45 min at 42°C. The volume was brought to 100 μL with STE (24) (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 8.0) and applied to a spun column. Total incorporation equaled 3 to 4 × 10^6 cpm.

[4] Mention of a specific product name by the U.S. Department of Agriculture does not constitute an endorsement and does not imply a recommendation over other suitable products.

Plaque lifts were placed in heat-sealable bags (Dazey Corp.) and prehybridized at 70°C for 30 to 60 min in hybridization buffer (10 g/L BSA, 1 mM EDTA, 0.5 M Na_2HPO_4 [pH 7.2], 10 g/L SDS, 1 g/L sodium pyrophosphate). Probes (equal cpm) were heat denatured and added to the bag with extensive mixing. Lifts were then hybridized at 65°C for 40 h. Lifts were removed from the bags and washed twice (15 and then 90 min) with 5 g/L BSA, 1 mM EDTA, 40 mM Na_2HPO_4 [pH 7.2], 5 g/L SDS, and then once (90 min) with 1 mM EDTA, 40 mM Na_2HPO_4 [pH 7.2], 1 g/L SDS, all at 65°C. Fluorography was conducted with X-OMAT AR film (Kodak) and intensifying screens (Lightning Plus or Quanta III, DuPont Cronex) at −80°C.

Differential clones were identified and rescreened using the "large plaque assay" of Meeks-Wagner *et al.* (25). Lifts were taken, probed (12 h), washed, and fluorographed as above. Clones were replated at low titer, rescreened, and one well-isolated plaque from each was plugged into SM (23) medium containing 5.8 g NaCl, 2 g $MgSO_4$ $7H_20$, 50 mL of 1 M Tris, and 5 mL of 2% (w/v) gelatin per L) for excision/rescue.

The clones were excised and rescued according to the protocol provided by Stratagene using XL1-Blue host cells, Lambda ZAP phage stock, and R408 helper phage. Clones now existed as pBluescript SK plasmids and the resulting Ampicillin-resistant colonies were used to obtain DNA following the mini-prep method of Del Sal *et al.* (11). Insert size was estimated by co-electrophoresing *Hin*dIII/*Eco*RI-restricted lambda DNA as mol wt markers. On the basis of recovery from excision/rescue and restriction/gel analysis, 14 clones were selected for further analysis.

Isolation of Dehydrin-Like Clones

Dehydrin-like clones were isolated from the cDNA library by probing plaque lifts with probe prepared using insert from pHVA39 following the random hexanucleotide method of Feinberg and Vogelstein (13). Approximately 5600 clones from the primary amplified library were screened. Seven plaques which produced positive signals were plugged, replated, and rescreened. After excision/rescue and restriction analysis, two clones were selected and identified as pMA80 and pMA94b.

Identification of Gene Families

Preliminary screening for classification of the clones into gene families was conducted with Southern dot blots. Classifications were confirmed with Southern gel blots conducted under high stringency conditions. DNA was alkaline blotted onto nylon membrane (Zeta Probe, Bio-Rad) using a dot blot manifold (Minifold I, Schleicher and Schuell) or capillary transfer following the supplied protocols. The blots were hybridized at 65°C in 0.5 M phosphate buffer (pH 7.2) containing 1 mM EDTA, 1% (w/v) BSA, and 7% (w/v) SDS.

Northern Analysis of Transcripts

For Northern blots, embryonic axes were incubated on agar for 2, 6, or 12 h at 20°C in the dark. Poly(A)$^+$ RNA was isolated following the method of Badley *et al.* (3). Approximately 0.5 to 1.0 μg of mRNA was denatured and electrophoresed in 1.2% agarose gels containing Mops buffer and 0.37 M formaldehyde (35). Gels were soaked in 50 mM NaOH for 30 min on a rotary shaker and then capillary blotted onto Zeta Probe following the supplied protocol except that transfer was conducted using 10 mM NaOH overnight at 4°C. Blots were then rinsed for 10 min in 2 × SSC (23), air-dried, and vacuum baked for 2 h at 80°C or UV-cross-linked (Stratalinker, Stratagene). Probe prepared from gel-isolated cDNA inserts, hybridization, washing, and fluorography were as described above. Selected blots were quantified using a radioanalytic imaging system (Ambis, San Diego, CA).

ABA Analysis

ABA was extracted and assayed with a monoclonal antibody for (+) ABA as previously described (38).

RESULTS

Differential Screening of the cDNA Library

Embryonic axes, consisting of the root and shoot apices, were utilized for differentially screening mRNAs prevalent in dormant and/or ABA-responsive tissue. Embryonic axes were isolated from mature grains of both dormant and nondormant seedlots of the same genotype. The rates of germination for whole seeds and isolated axes from dormant and nondormant seedlots are shown in Figure 1. Removal of the axes from dormant grains removed most of the constraints of whole seed dormancy, which were restored by the addition of 5 μM ABA (Fig. 1).

A cDNA library was prepared using poly(A)$^+$ RNA from the ABA-treated axes from dormant grain at 12 h postimbibition. This time point was chosen for poly(A)$^+$ RNA isolation and differential screening, because by 12 h postimbibition, new protein synthesis reaches a maximum rate (32) and the nondormant axes are poised to germinate. The cDNA library was differentially screened with ^{32}P-labeled cDNA prepared from poly(A)$^+$ RNA from nondormant axes and ABA-maintained dormant axes. Approximately 7000 clones were screened.

Isolation and Southern Analyses of cDNA Clones Prevalent in Imbibed Dormant Axes

Fourteen cDNA clones were identified for mRNA transcripts abundant in dormant, but rare in nondormant axes at 12 h postimbibition and are listed in Table I. Two additional clones (pMA80 and pMA94b) were selected by hybridization to an ABA-responsive clone (pHVA39) from barley which encodes a dehydration stress protein, dehydrin (10). These two clones were also included in this study (Table I).

The 16 clones were classified into five major gene families (DORM 1–5) based on DNA hybridization homology. Sequence homology among the various clones was examined by first conducting a preliminary screening with Southern dot blots and then putative gene family groupings were confirmed with Southern gel blots of restricted clones conducted under high stringency conditions. The two clones pMA80 and pMA94b selected by homology to the barley dehydrin clone

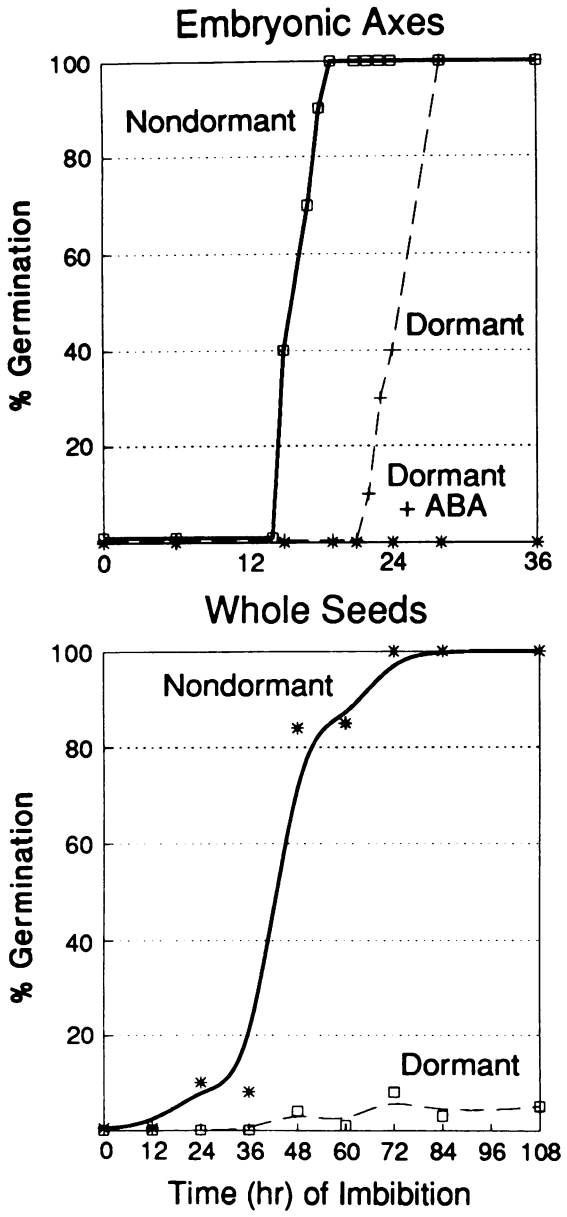

Figure 1. Cumulative germination of isolated embryonic axes (top) and whole seeds (bottom) Isolated axes from nondormant seeds imbibed in water and isolated axes from dormant seeds imbibed in water or 5 μM (±) ABA.

(pHVA39) were classified in the DORM-1 group with clone pMA1906 selected by differential screening. Two of the groups (DORM-2 and DORM-3) contained five or more independent clones, while DORM-4 and DORM-5 contained a single cDNA clone, as classified by Southern analyses.

Southern hybridization analyses of all the clones were conducted with cDNA clones for some known ABA-responsive genes including barley dehydrin (10), Em (22) and wheat germ agglutinin (31) (data not shown). As expected clones pMA80 and pMA94b, as well as clone pMA1906, in the DORM-1 group all hybridized with the dehydrin cDNA clone. All the clones in the DORM-2 group showed homology with the Em cDNA clone. The wheat germ agglutinin clone showed no homology with any of the clones. Partial sequence data indicate that all three of the DORM-1 clones, at both their 5′ and 3′ ends, have homology with barley dehydrin (9). When compared to *dhn*18, the 5′ end of pMA94b showed 96% homology over a stretch of 140 bases within the open reading frame while at the 3′ noncoding end there was 80% homology over a stretch of 119 bases. For clone pMA80, sequencing extended 45 bases into the 5′ end of the open reading frame and showed 89% homology for those bases while at the 3′ end sequencing extended into the last 30 bases of the open reading frame and showed 80% homology for those bases. Clone pMA1906 showed 100% homology to clone pMA80 at the 3′ end for the 135 bases sequenced. At the 5′ end of pMA1906 there was 64% homology to *dhn*18 over a stretch of 199 bases within the open reading frame. All the clones in the DORM-2 family have sequence homology with Em (J Curry, M Walker-Simmons, unpublished results).

Temporal Expression of the Dormancy-Associated Genes in Imbibed Embryonic Axes

Transcript levels for the selected clones were compared in dormant and nondormant grain axes before imbibition and during the first 12 h after wetting. No exogenous ABA was added to the axes in this experiment. A cDNA clone from each of the five gene families was utilized as a hybridization probe. As shown in Figure 2, isolated axes from mature dry dormant and nondormant grains have relatively high levels of all the transcripts. Upon imbibition gene expression was maintained and even increased for all the clones in the dormant grain axes. Transcript levels rose earlier and declined sooner for the Em homolog, pMA1959, compared to the other transcripts. In the hydrated dormant axes transcript levels for pMA1959 peaked by 2 h and declined thereafter. The other transcripts were highest at 6 h in the hydrated

Table I. *Characteristics of Selected cDNA Clones(p-2708)*

Group (Known Homology)	Clones	Estimated Size of cDNA
		kilobases
DORM-1 (Dehydrin, RAB)	pMA1906	1.0
	pMA80	1.5
	pMA94b	0.8
DORM-2 (Em)	pMA1910	0.65
	pMA1950	0.84
	pMA1952	0.83
	pMA1958	0.25
	pMA1959	0.78
	pMA2004	0.65
DORM-3	pMA1931	0.98
	pMA2001	1.6
	pMA2002	0.75
	pMA2005	0.98
	pMA2110	0.40
DORM-4	pMA1949	1.0
DORM-5	pMA1951	0.82

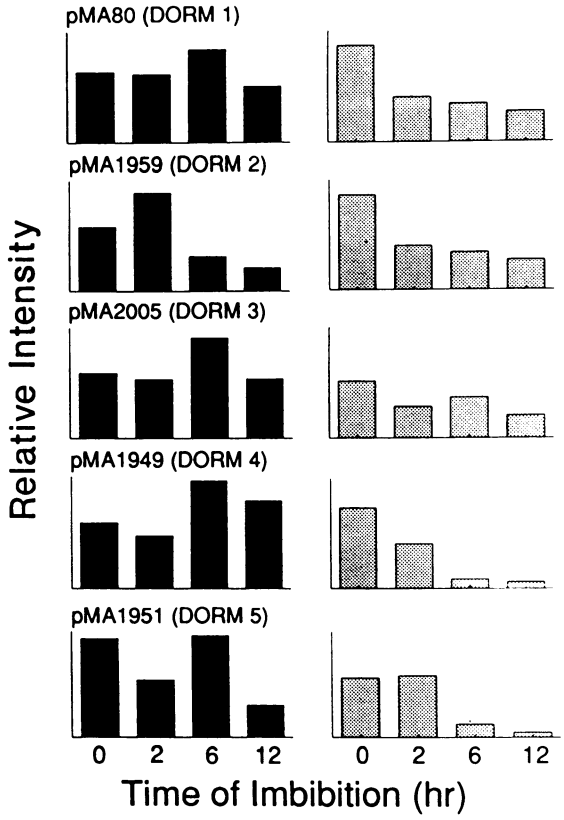

Figure 2. Transcript levels in imbibed axes. Northern blot analysis was conducted with each cDNA clone. The bands were quantified by radioimaging and the relative intensities of the bands within each blot are presented. Dormant (■) and nondormant (□) grain axes.

dormant grain axes. In the nondormant axes, transcript levels declined following imbibition.

Gene Expression in Embryos Isolated from Imbibed Seeds

To assess embryonic gene expression in intact seeds, transcript levels for selected clones were measured in embryos from whole imbibed seeds. Intact seeds from the dormant and nondormant seedlot were imbibed and their germination rate is presented in Figure 1. At the times indicated in Figure 3, embryos were dissected from the whole seeds, poly(A)$^+$ RNA isolated and Northern gel blot hybridization analyses performed. Temporal patterns of gene expression for transcripts corresponding to pMA80, pMA94b, pMA1959, pMA2005, and pMA1951 were measured. Transcript levels for those clones were similar in the mature dry seed from the dormant and nondormant seedlots. As with the axes, prolonged maintenance of transcript levels for the selected clones was positively correlated with imbibed dormant tissue. As shown in Figure 3, embryonic transcript levels were maintained in the dormant imbibed whole grains for 2 d, while levels declined steadily in the germinating, nondormant seeds. Transcript levels corresponding to all the clones continued to be high in the dormant grain embryos at 3 d postimbibition (data not shown).

When clone pMA80 was used as a hybridization probe, three mRNA bands were detected with sizes 1900, 1400, and 900 bases (Fig. 3). This result indicates that there is a family of transcripts related to pMA80. That result would be expected because clone pMA80 has hybridization homology to the barley *dhn* and rice *rab* genes, and each of these consists of a small, multigene family encoding several homologous proteins (10, 28, 36). In this report all three mRNA bands were quantified together by radioanalytic imaging, even though the individual bands did not always vary precisely in parallel. Another member of the same gene family as clone pMA80, clone pMA94b, hybridized to only a smaller mRNA band of approximately 900 bases.

Correlation of gene expression for the selected clones with dormant seed embryos contrasted with expression of α-amylase, an enzyme associated with germinating tissue. A cDNA

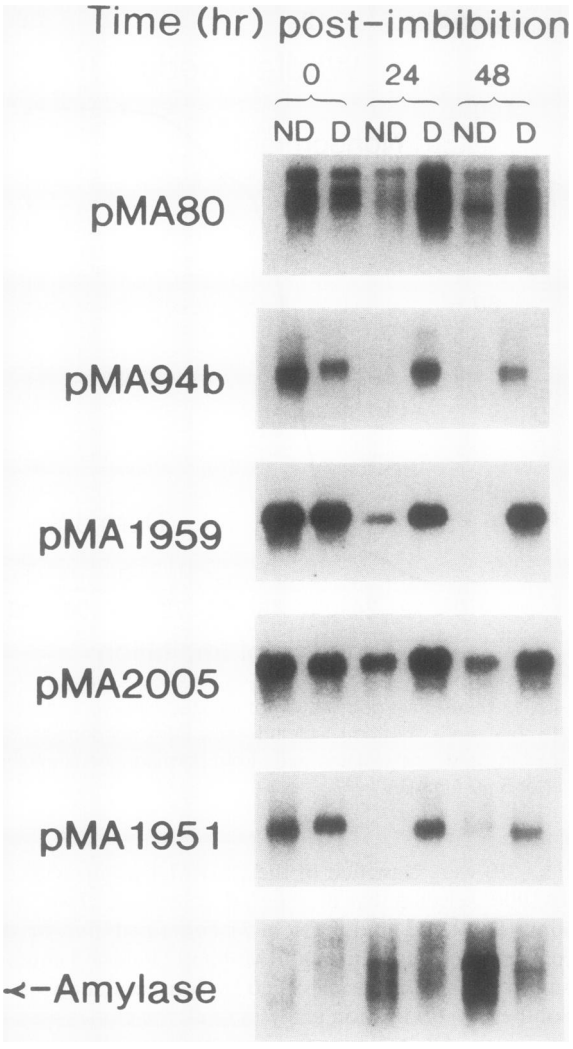

Figure 3. Transcript levels in embryos from imbibed intact dormant and nondormant seeds. Seeds were imbibed at time 0. Before imbibition and at the times indicated embryos were dissected from the seed, poly(A)$^+$ RNA extracted and Northern blot analyses performed. cDNA clone JR050 was used to measure α-amylase.

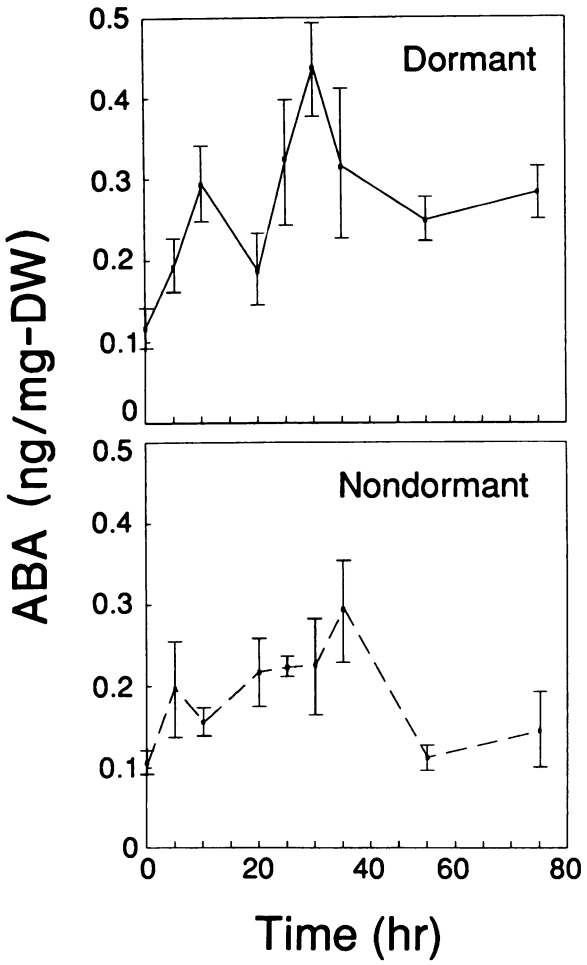

Figure 4. Comparison of endogenous ABA in embryos from imbibed dormant and nondormant grain. Seeds were imbibed in water at 20°C. At the times indicated embryos were dissected, ABA was extracted and measured by immunoassay. The mean of three separate samples is shown and the error bars represent one SD above and below the mean.

clone (JR050) for low isoelectric point α-amylase (33) was used to measure α-amylase transcript levels. As shown in Figure 3, α-amylase transcripts were not detected in the mature dry seed embryos. Amylase transcript levels were detectible by 24 h postimbibition in the germinating seeds only and increased to higher levels by 48 h.

ABA in the embryos was measured to compare endogenous hormonal concentration with gene expression in the imbibed dormant and nondormant seeds and is shown in Figure 4. ABA concentrations were similar in the embryos of the dormant and nondormant seeds before imbibition. Upon hydration an increase in ABA levels occurred in the dormant seed embryos followed by a gradual decline. In nondormant seeds embryonic ABA increased slightly after imbibition and then declined steadily. Based on total tissue water content, the endogenous ABA concentration in the dormant grain embryos was estimated to be 1.1 μM at 20 h, dropping to 0.5 μM by 72 h. ABA concentration in the nondormant embryos was lower and was calculated to be 0.8 μM at 20 h and 0.1 μM by 72 h.

Effect of ABA on Gene Expression and Differences in ABA Responsiveness

The effect of ABA on gene expression of the selected transcripts was assessed by incubating axes in water or 0.05 to 50 μM (±) ABA and measuring transcript levels after 24 h and are presented in Figure 5. By 24 h postimbibition in water, transcript levels have declined from the time 0 levels even in dormant grain axes. Transcript levels increased in the axes incubated for 24 h in ABA. Transcript levels of clones pMA80, pMA1959, and pMA2005 all increased with axes imbibed in higher ABA concentrations. Transcript levels of all the clones examined to date are enhanced by ABA. Eight different clones representing all the five DORM families have been examined so far (data not shown).

The results presented in Figure 5 show that dormant as compared to nondormant grain axes, are far more responsive to ABA as measured by ABA-enhanced increases in the selected transcripts. Incubation of the axes in 0.05 to 0.5 μM ABA resulted in considerably higher transcript levels in the dormant grain axes compared to the nondormant. These differences in embryonic axis responsiveness to ABA correlated positively with the effects of ABA on axis germination. In this, as in our previously reported studies (26, 38), ABA

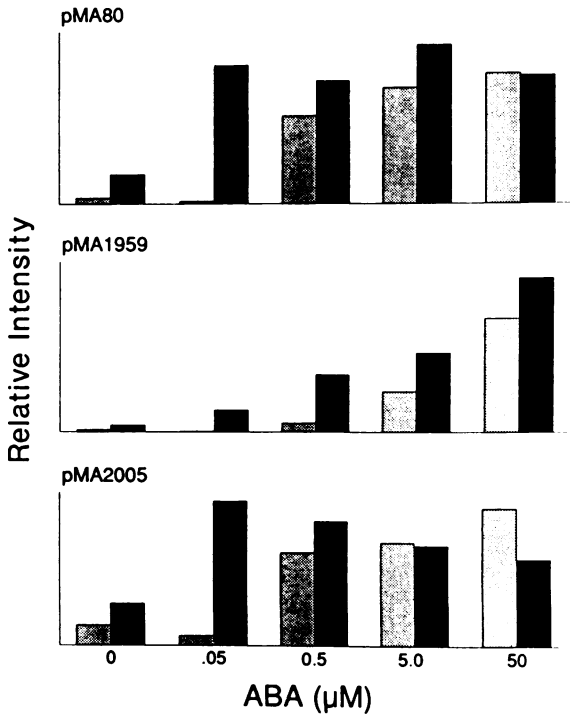

Figure 5. Transcript levels of embryonic axes incubated for 24 h at 20°C in water or varying levels of (±) ABA (0.05, 0.5, 5.0, 50 μM). At 24 h axes were harvested, poly(A)$^+$ RNA extracted, and Northern blot analyses performed. Bands were quantified by radioimaging and the relative intensities of the bands within each blot are presented. Dormant (■) and nondormant (□) grain axes.

concentrations of 0.05 to 0.5 μM blocked germination of the dormant grain axes, while higher ABA concentrations (5–50 μM) are required with the nondormant axes (data not shown).

DISCUSSION

Gene transcripts maintained at elevated levels in isolated dormant grain axes have been identified (Fig. 2). These transcripts are also maintained within embryos of intact hydrated dormant seeds (Fig. 3). Clones from five different gene families (Table I) were identified by differential screening for gene expression in dormant embryonic tissue. Our experiments suggest that dormancy in hydrated seeds is an active process and may be under positive molecular control requiring the continuous expression of certain genes. These results are consistent with our previous finding that there is active and prolonged synthesis of specific ABA-responsive proteins in imbibed, but dormant wheat grain axes (32). Our results in wheat are also consistent with our findings in dormant grass weed seeds. Such weed seeds possess far higher levels of dormancy than wheat. We have determined that a set of specific gene transcripts are also maintained at elevated levels in hydrated dormant seeds of several grass weed seed species (P Goldmark, M Walker-Simmons, manuscript in preparation).

Prolonged maintenance of these selected transcripts was observed only in imbibed dormant tissue. When nondormant seeds were imbibed, ABA-responsive transcript levels declined, α-amylase mRNA increased and germination occurred. Transcripts representative of the five gene families all exhibited similar temporal regulation except for cDNA clone pMA1959, which has homology to Em (30). In comparison to the other transcripts, Em expression occurred earlier after imbibition and also declined earlier (Figs. 2 and 3).

ABA is required for the induction of dormancy (17) during seed development and our results suggest that ABA has a role in the maintenance of dormancy as well. Previous physiological experiments have shown that ABA application (0.05 to 5 μM) can restore dormancy to embryos removed from mature dormant seeds (26, 38). In this report we have demonstrated that incubation of isolated axes in this range of ABA concentrations enhances expression of the selected transcripts (Fig. 5). These concentrations were within the range of calculated endogenous ABA concentration measured in embryos from imbibed seeds (Fig. 4). Dormant, compared to nondormant, grain axes are far more responsive to ABA as defined by enhancement of transcript expression in response to lower ABA levels (Fig. 5). This enhanced response of dormant grain embryos to ABA provides a possible mechanism for dormancy. Such a dormancy mechanism could include factors such as the *vp1* gene product (24) associated with the ability of embryonic tissue to respond to ABA.

Whether expression of these selected gene transcripts actually restricts germination or is only associated with an ABA-regulated process cannot be answered until it is possible to block the production of the gene products or introduce them into nondormant axes. The function of these gene products in imbibed dormant seeds is also not known. Two of the identified gene products, dehydrin or RAB proteins (36) and Em (30) have been characterized as late embryogenic abundant (LEA) proteins (12), and each protein is known to increase as developing seeds enter the desiccation stage. Transcript levels for these two genes increase in leaves in response to osmotic stress (5, 8, 28), which is characteristic of most ABA-responsive genes (36). This raises the question of whether embryos of imbibed dormant seeds are under a self-imposed osmotic stress, which causes the continued expression of this group of ABA-responsive genes. Both Em and dehydrin are very hydrophilic proteins (10, 12) and under water-limiting conditions, enhanced levels of these hydrophilic proteins in dormant seeds could sequester water and restrict germination. In conclusion our work demonstrates that, even though there is no visible growth in imbibed dormant seeds, there is prolonged expression of specific ABA-responsive genes. The presence of these transcripts is associated with the dormant state. Identification of these genes should be useful in advancing our understanding of the factors that determine whether a cereal seed upon hydration will remain dormant for weeks or will germinate readily.

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