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# Seed Dormancy and Responses of Caryopses, Embryos, and Calli to Abscisic Acid in Wheat[1]

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## ABSTRACT

**Preharvest sprouting of wheat (*Triticum aestivum* L.) is associated with inadequate seed dormancy. Although abscisic acid (ABA) has often been suggested to play a central role in developing seed, its involvement in dormancy of mature seed lacks firm experimental evidence and endogenous ABA levels are not well correlated with germinability. We examined genotypic and temporal variation in wheat seed and embryo germination responses to ABA and determined whether differential sensitivity of embryos to ABA extended to growth of embryo-derived calli. Germination of Parker 76 caryopses, which have little dormancy at maturity, was only slightly inhibited by ABA, whereas germination of Clark's Cream, a highly dormant genotype, was greatly inhibited. Responsiveness of caryopses to ABA and dormancy of seeds decreased concurrently during afterripening. Germination of embryos excised from dormant and nondormant seeds was more responsive to ABA but otherwise was similar to that of caryopses, indicating that differential response to ABA occurs in the embryo. Growth of calli derived from immature embryos of two sprouting-susceptible wheat genotypes exceeded growth of calli from Clark's Cream, but no distinct differences in response to ABA among the genotypes were apparent. We concluded that the action of ABA is similar in developing and mature seeds, that genotypic and temporal variation in embryo responsiveness to endogenous ABA may be involved in dormancy, and that ABA probably acts in concert with other endogenous constituents.**

Embryo maturation is regulated and precocious germination is prevented by ABA in many species, including wheat, during seed development (2, 5, 17). Involvement of ABA in dormancy after maturation and desiccation of seeds is uncertain, however (2, 11, 15, 19, 20), and the physiological basis of dormancy remains unclear. The problem is of considerable economic importance because dormancy is often inadequate to prevent preharvest sprouting of mature seeds during adverse weather (1, 7). Preharvest sprouting induces synthesis of germinative enzymes, notably $\alpha$-amylase, which reduces the value of wheat and other grains (7).

Dormancy of wheat seeds is mediated by factors that are internal and external to the embryo. The embryo lacks true dormancy and germinates readily when excised from the caryopsis (8, 11). ABA levels increase with seed mass during maturation and then decrease dramatically during desiccation (5, 12, 17, 18). Seed dormancy differs markedly among genotypes at maturity but is temporal, and a variable period of afterripening or stratification is necessary for seeds to become fully germinable (1). As in other species, the level of endogenous ABA is poorly correlated with dormancy of mature seeds (5, 12, 17).

Germination responses of mature wheat caryopses and embryos to exogenous compounds are better correlated with seed dormancy than is the level of endogenous ABA (8, 9, 11, 15–18). Genotypic differences in dormancy parallel inhibition of excised embryos by catechin tannin and ABA (15) and by a ubiquitous endogenous compound (8) that is likely tryptophan (9). The effect of the latter is reinforced by exogenous ABA and countered by gibberellic acid (11, 16). Loss of dormancy and diminished responsiveness of excised embryos to exogenous inhibitors coincide during afterripening (8, 9, 11, 16). Thus, any role of ABA in dormancy may depend more on changes in perception of the inhibitor by the embryo than on content of the inhibitor in the caryopsis.

The present study investigated differential response as an alternative mechanism by which ABA might regulate dormancy of mature wheat seed. Seed lots with extreme ranges in dormancy were used to ascertain whether intact caryopses, excised embryos, and calli from embryo explants varied in responsiveness to ABA and to determine if the effect persisted during afterripening to explain genotypic and temporal differences.

## MATERIALS AND METHODS

### Plant Materials

Seeds (caryopses) of 'Parker 76' and 'Clark's Cream' hard winter wheat (*Triticum aestivum* L.) cultivars were harvested from field plots at the North Agronomy Farm, Manhattan, KS, on June 26, 1986. These seedlots were used to examine germination response to ABA during a 4-week afterripening period. Parker 76 has little dormancy at maturity, which causes it to be susceptible to preharvest sprouting, whereas Clark's Cream has high dormancy and excellent sprouting resistance (7). Seed moisture content was reduced from approximately 180 mg $H_2O$/g dry weight at harvest to 120 mg $H_2O$/g dry weight in a forced-air oven (3 d at 40°C). A gravity air-flow table (Forsbergs Inc., Thief River Falls, MN) cleaned the grain and eliminated small light seeds, and subsequent storage at −20°C arrested afterripening (6). Fully afterripened seeds of Clark's Cream were obtained by storing another seed lot at room temperature (about 22°C) for 14 months.

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### Seed Germination

Seeds were removed from the freezer and allowed to afterripen uniformly at room temperature for 0, 1, 2, or 4 weeks before germination assays were made. Seeds of Parker 76 and Clark's Cream were placed crease side down on two layers of Whatman No. 2 filter paper in 100 × 15-mm Petri plates (40 seeds per plate). The plates were irrigated with 4.5 mL of test solution containing 0, 5, 10, 12.5, 25, or 50 $\mu$M ABA, taped shut to prevent water loss, and incubated at 22°C under dim fluorescent lights (5 $\mu E\ m^{-2}\ s^{-1}$). Germination counts were made periodically; seeds with radicle or coleoptile growth in excess of 1 mm were considered germinated and removed from the plate. Each treatment was replicated three times, and cumulative germination (summation of germinated seeds *versus* observational time in d) was calculated for each plate and averaged over replications.

### Embryo Germination

Response of excised embryos to ABA was measured to determine whether differences in seed germination were from differential embryo responsiveness or from extraembryonic effects. Embryonic axes (hereafter termed embryos) from nondormant Parker 76 seeds (afterripened 4 weeks) and dormant Clark's Cream seeds (harvest ripeness) were obtained by a modification of the procedure of Johnston and Stern (4).

Excised embryos were germinated scutellum side down in 96-well tissue culture plates (No. 3596, Costar, Cambridge, MA) containing agar-test solution at a final concentration of 20 g/L sucrose, 6.8 g/L agar, and 0, 0.25, 0.5, or 2.5 $\mu$M ABA. Each well contained 0.2 mL agar-test solution and one embryo. Twelve embryos comprised a replication. Plates were taped shut, incubated at 22°C in darkness, and examined periodically for germination. Embryos were considered germinated if radicle, lateral root, or coleoptile growth exceeded 1 mm. Cumulative germination was calculated as above for each of two replications and averaged.

### Assay of ABA in Embryos

ABA analyses were provided by M. Walker-Simmons, Washington State University. Approximately 50 mg of isolated embryonic axes (about 100) each of Parker 76 (afterripened 4 weeks) and Clark's Cream (harvest ripeness) were divided into three replications, and ABA was assayed by an indirect ELISA technique (17).

### Callus Culture

Growth responses of wheat callus to ABA were examined to determine whether genotypic differences in germination response to ABA were specific to embryos or also occurred in other tissues. 'KS75216,' a sprouting-susceptible hard white winter wheat genotype; Clark's Cream; and 'ND7532,' a red winter wheat genotype that performs well in callus culture (14), were grown in a glasshouse under a 16-h photoperiod. Temperatures were maintained at about 22°C/18°C day/night during vegetative growth and 25°C/22°C day/night during grain development. Immature embryos (10–15 d postanthesis) were used as the explant source. Immature seeds were surface sterilized in 0.8% NaOCl (diluted commercial bleach) for 5 min and thoroughly rinsed in sterile water. Embryos were excised and placed on initiation medium salts (10) plus 20 g/L sucrose, 6.8 g/L agar, 150 mg/L asparagine, 0.5 mg/L thiamine-HCl, and 1 mg/L 2,4-D (pH 5.8), following the method of Sears and Deckard (14). The 2,4-D concentration was used throughout to maintain calli in a largely undifferentiated state.

Calli were cultured at 27°C under 1.5 to 3.0 $\mu E\ m^{-2}\ s^{-1}$ from 20-W cool-white fluorescent lights. About 40 d after initial plating (time 0), healthy calli of various sizes were aseptically weighed and transferred to initiation media containing 0 or 5 $\mu$M ABA. ABA solution was sterilized through 0.2-$\mu$m filters and added to the autoclaved agar after cooling to 60 to 70°C. Plates contained 30 mL of medium and three calli each of Clark's Cream and KS75216 or six calli of ND7532. The total number of calli was 36 for Clark's Cream and KS75216 and 24 for ND7532. Calli were weighed at 10-d intervals for 30 d; after each aseptic weighing, they were transferred to new media.

A second group of immature embryos of KS75216 and Clark's Cream was collected and cultured on initiation media as described above. Calli were transferred to new media at 30-d intervals. After 120 d (time 0), 48 healthy calli of each genotype were aseptically weighed and transferred to initiation media containing 0, 50, or 500 $\mu$M ABA. Four calli of a single genotype were cultured per plate. Calli were weighed on d 15, transferred to new media, and weighed again on d 50.

### Estimates of Experimental Error

Standard deviations of seed germination data were calculated for observation times when cumulative germination was nearest to 0.5. The SD was determined for each ABA concentration (three replications) and averaged over the six ABA concentrations in the trial. This procedure was repeated for each genotype at each afterripening period. Standard deviations for embryo germination were similarly obtained. For Clark's Cream, however, only data from 0, 0.25, and 0.5 $\mu$M ABA treatments, where germination >0, were used. Standard deviations for tissue culture data were obtained by including all data from a single observational time in the calculations.

### Reagents

Two stock solutions of (±) *cis-trans* ABA (Sigma Chemical Co.) were prepared, 40 $\mu$mol/mL ABA (No. A7383) in 0.1 M $NaHCO_3$:ethanol (3:2 v/v) and 10 $\mu$mol/mL ABA (No. A2784) in 0.1 M $NaHCO_3$. All germination assays of Clark's Cream and Parker 76 seeds used the first solution, and embryo germination assays and callus culture experiments used the second solution. The following were also used: purified agar (Difco Laboratories), Murashige and Skoog salt mixture (Gibco Laboratories, No. 500–1117), and 2,4-D (Eastman Kodak Co.). All other chemicals were reagent grade or better.

## RESULTS

### Seed Germination Response

Seed (caryopsis) germination response to exogenous ABA, evaluated during a 4-week afterripening period, differed between the two wheat cultivars, Parker 76 and Clark's Cream (Fig. 1). Seed of Parker 76 had a low level of dormancy at harvest ripeness, and germination was only slightly inhibited by ABA (Fig. 1A). The low level of dormancy at harvest ripeness decreased rapidly, as did response to ABA (Fig. 1, B–D).

Seed of Clark's Cream, in contrast to Parker 76, exhibited considerable dormancy at harvest ripeness, and germination was inhibited by as little as 5 $\mu$M ABA (Fig. 1E). As Clark's Cream seed afterripened, dormancy dissipated slowly and response to ABA decreased concomitantly (Fig. 1, F–H). After 4 weeks of afterripening, most seeds of Clark's Cream germinated rapidly in water, and only traces of dormancy remained (Fig. 1H). Some response to ABA remained, however, and germination was similar to Parker 76 at harvest ripeness (Fig. 1, A and H).

A seed lot of Clark's Cream that had been afterripened for 14 months had no dormancy and germination was not inhibited by 50 $\mu$M ABA (Fig. 2). Its response to ABA resembled that of Parker 76 seed that had been afterripened for 4 weeks.

### Embryo Germination Response

Effects of extraembryonic seed tissues on the differential response of whole-seed germination to ABA were eliminated by using excised embryonic axes (embryos). Embryos from nondormant Parker 76 and dormant Clark's Cream seed germinated more rapidly than caryopses and were more responsive to ABA (Fig. 3). ABA at 2.5 $\mu$M inhibited germination of Parker 76 embryos, whereas 0.25 and 0.5 $\mu$M ABA had little effect (Fig. 3A). Embryos of Clark's Cream were more responsive to ABA; 2.5 $\mu$M completely inhibited germination and the two lower concentrations greatly reduced germination (Fig. 3B). The cumulative germination curves for Parker 76 embryos at 2.5 $\mu$M ABA and Clark's Cream embryos at 0.25 $\mu$M ABA were nearly identical.

### Embryonic ABA Concentrations

The ABA levels in embryos of the two cultivars that represented extreme differences in dormancy were very similar. Mean ABA concentrations (±SD) were 789 ± 79 pg $mg^{-1}$ in embryonic axes from Parker 76 seed that was afterripened 4

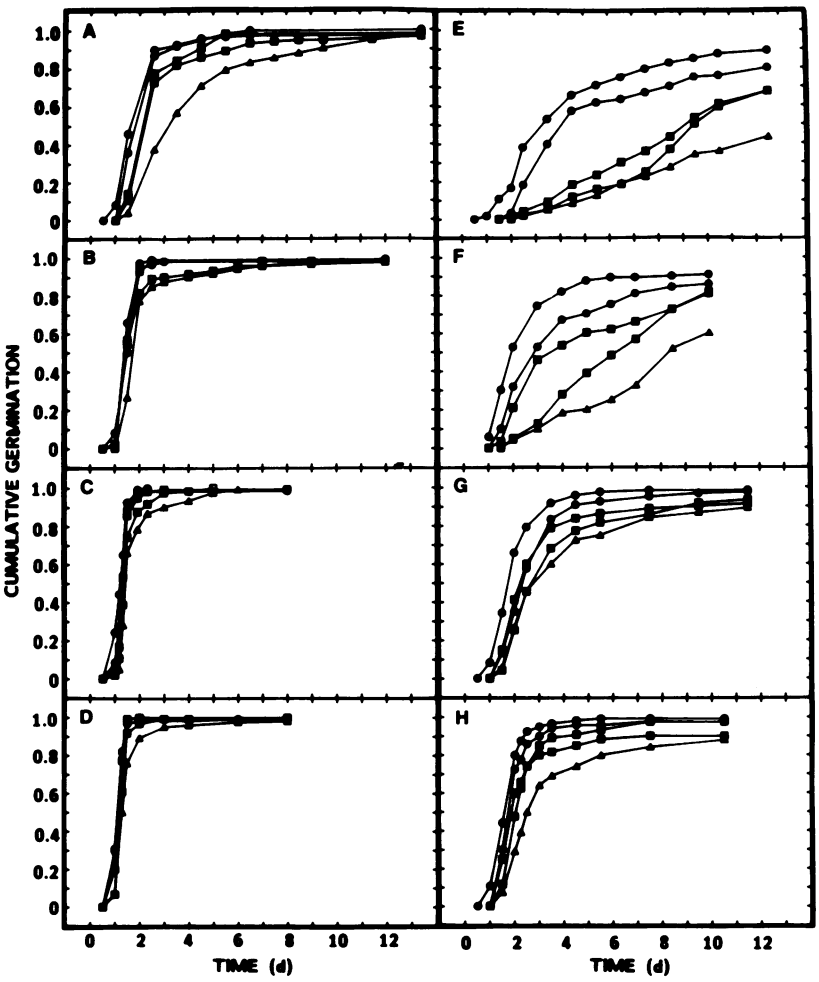


**Figure 1.** Germination response (cumulative curves) of Parker 76 (A–D) and Clark's Cream (E–H) wheat caryopses to ABA during afterripening. Panels A and E, 0 weeks (harvest ripeness); B and F, 1 week; C and G, 2 weeks; and D and H, 4 weeks afterripening at room temperature, respectively. Inhibitor concentrations are 0 (●), 5 (○), 10 (■), 25 (□), and 50 (▲) $\mu$M ABA. The mean SD at each ABA concentration is 0.059, 0.078, 0.134, 0.146, 0.083, 0.108, 0.108, and 0.098, respectively, in panels A–H.

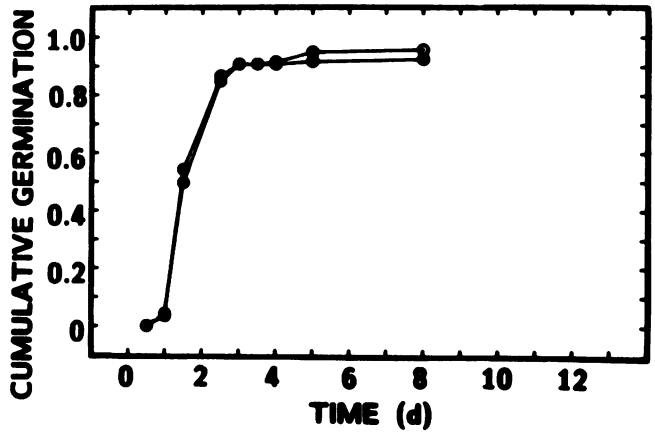


**Figure 2.** Germination response (cumulative curves) of Clark's Cream wheat seeds to 0 (●) and 50 (○) μM ABA after 14 months of afterripening. The mean SD at the two ABA concentrations is 0.075.

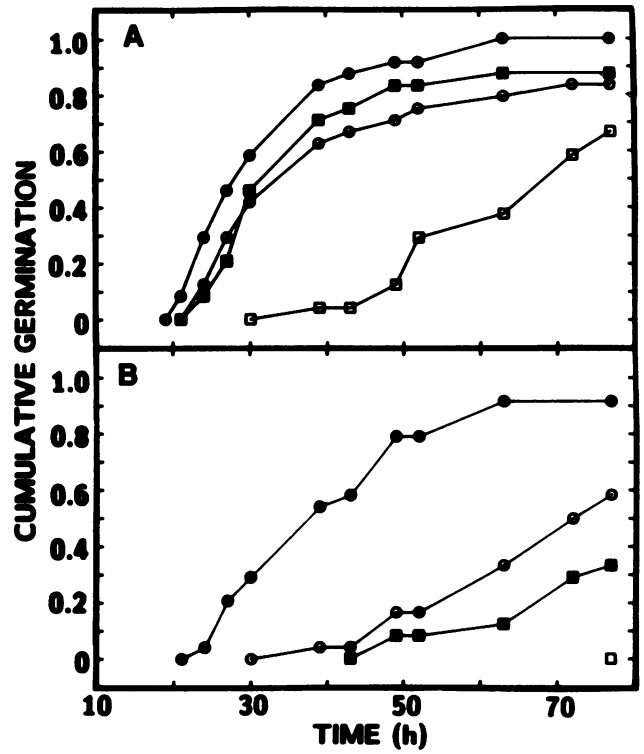


**Figure 3.** Germination response (cumulative curves) of embryonic axes of Parker 76 (panel A) and Clark's Cream (panel B) wheat to 0 (●), 0.25 (○), 0.5 (■), and 2.5 (□) μM ABA. Seeds of Parker 76 and Clark's Cream were afterripened 4 and 0 weeks, respectively, before excising embryos. The mean SD at the four ABA concentrations is 0.08 and 0.09, respectively, in panels A and B.

weeks and 820 ± 47 pg $mg^{-1}$ in embryonic axes of Clark's Cream seed that was harvest ripe and highly dormant.

### Callus Culture Growth Response

ABA at 5 μM inhibited the growth of calli derived from immature embryos of all three wheat genotypes (Fig. 4A). Calli mass differed among genotypes, but the relative retardation of growth by ABA was nearly similar in all cases. Clark's Cream, the sprouting-resistant genotype, grew poorly in culture regardless of ABA treatment, and calli were generally light-colored, nonfriable, and watery in appearance. KS75216 and ND7532, which have little seed dormancy at maturity and are sprouting-susceptible, grew well in callus culture. Calli of KS75216 and Clark's Cream cultured on 0, 50, or 500 μM ABA produced similar results (Fig. 4B). At the high level of ABA (500 μM), cessation of growth of individual calli was more frequent and response was more variable, especially for the sprouting-susceptible genotype, KS75216. After 50 d in culture on 500 μM ABA media, individual calli fresh weights ranged from 50 to 402 mg. Some calli appeared to lose responsiveness to ABA, whereas others remained highly responsive.

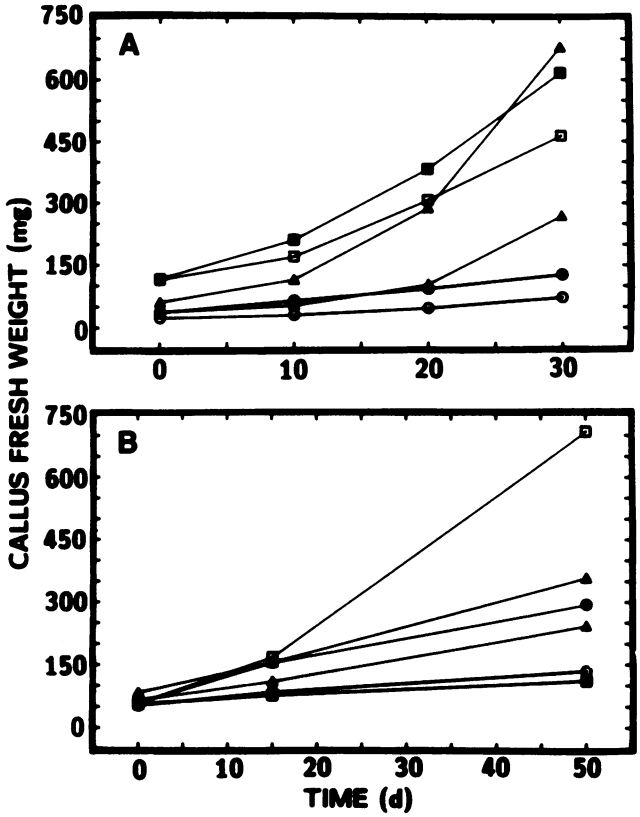


**Figure 4.** Growth response of Clark's Cream (●, ○), KS75216 (■, □), and ND7532 (▲, △) wheat calli to 0 and 5 μM ABA, respectively (panel A), and Clark's Cream (●, ○, ■) and KS75216 (□, ▲, △) calli to 0, 50, and 500 μM ABA, respectively (panel B). The mean SD for sampling dates is 60.3, 105.6, 196.4, and 333.8 for 0, 10, 20, and 30 d, respectively (panel A), and 24.8, 64.7, and 153.3 for 0, 15, and 50 d, respectively (panel B).

## DISCUSSION

Dormancy during seed development in wheat is closely associated with responsiveness of the embryo to ABA (17). Our results show that the phenomenon also occurs in intact caryopses of mature seeds and persists in both them and excised embryos during afterripening. Similar responses of intact caryopses and excised embryos but not of calli indicate

that the effect is localized and does not depend on extraembryonic tissues. While the results may suggest a role for ABA in seed dormancy, the specificity of the inhibition is still in question. Numerous other compounds in wheat caryopses mediate dormancy and germination, including some that react similarly to ABA with excised embryos (8, 9, 11, 15, 16). ABA probably does not act alone to mediate dormancy but, instead, functions in concert with these other endogenous constituents.

The contrasting responsiveness of dormant and nondormant caryopses or embryos excised from them may explain the poor correlation between dormancy and endogenous ABA levels (5, 12, 17). Responsiveness to ABA was nearly qualitatively distinctive in embryos from the most extreme seedlots, dormant Clark's Cream and afterripened Parker 76. The difference was not likely attributable to ABA content: endogenous ABA levels did not differ significantly between the two genotypes prior to imbibition in our studies, and ABA levels did not differ appreciably in embryos of similarly contrasting seedlots during imbibition in other studies (17).

Although there was some question whether ABA is incorporated or inhibits germination of intact caryopses (17), the present results leave little doubt. Extraembryonic tissues undoubtedly slowed movement of ABA during imbibition, but they did not appear to be a barrier to absorption of ABA by the caryopsis or to its action on the embryo. Instead, the response of sensitive Clark's Cream caryopses was directly proportional to the concentration of ABA that was applied to them. Similar responses occur in intact triticale caryopses, in which germination and protein synthesis are strongly inhibited by imbibed ABA (19, 20).

The differential response to ABA exhibited during seed and embryo germination was not evident in callus culture, probably because tissue-specific responses are often lost in 'dedifferentiated' callus (3). The genotypes differed widely in their general response to callus culture, however, a phenomemon that has been noted elsewhere (14). A general inverse relationship existed between level of seed dormancy at maturity and growth in tissue culture in our studies, with explants from nondormant wheat genotypes growing well and explants from dormant wheat genotypes growing poorly.

The present studies demonstrate the strong caryopsis-embryo-inhibitor interaction in wheat seed dormancy, but the nature of the interaction remains to be elucidated. The specificity and other characteristics of the embryo-ABA interaction are typical of those between receptors and some growth regulators (13). It is apparent, in any case, that embryonic responsiveness to ABA may be a factor in regulating both genotypic and temporal differences in wheat seed dormancy.

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