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Abscisic Acid Inhibition of Endosperm Cell Division in Cultured Maize Kernels[1]

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ABSTRACT

The response of developing maize (*Zea mays* L.) endosperm to elevated levels of abscisic acid (ABA) was investigated. Maize kernels and subtending cob sections were excised at 5 days after pollination (DAP) and placed in culture with or without 90 micromolar (±)-ABA in the medium. A decreased number of cells per endosperm was observed at 10 DAP (and later sampling times) in kernels cultured in medium containing ABA from 5 DAP, and in kernels transferred at 8 DAP to medium containing ABA, but not in kernels transferred at 11 DAP to medium containing ABA. The number of starch granules per endosperm was decreased in some treatments, but the reduction, when apparent, was comparable to the decreased number of endosperm cells. The effect on endosperm fresh weight was slight, transient, and appeared to be secondary to the effect on cell number. Mature endosperm dry weight was reduced when kernels were cultured continuously in medium containing ABA. Endosperm (+)-ABA content of kernels cultured in 0, 3, 10, 30, 100, or 300 micromolar (±)-ABA was measured at 10 DAP by indirect ELISA using a monoclonal antibody. Content of (+)-ABA in endosperms correlated negatively ($R = -0.92$) with endosperm cell number. On the basis of these studies we propose that during early kernel development, elevated levels of ABA decrease the rate of cell division in maize endosperm which, in turn, could limit the storage capacity of the kernel.

In maize (*Zea mays* L.) and other cereal grains, ABA accumulates late in kernel development and has been associated with the induction and maintenance of embryo dormancy (13). ABA also induces synthesis of embryo-specific polypeptides that characteristically accumulate late in embryogenesis (9). However, ABA accumulates during early kernel development if plants are subjected to water deficit (11, 21). Water deficit during anthesis reduces kernel number in wheat (17, 25) and maize (6, 28), but when water deficit occurs after anthesis, during early kernel development, a reduction in grain mass occurs (6, 28). Whether or not ABA is involved in this reduction of grain mass is unknown.

Maize endosperm cell division occurs from approximately 24 h after pollination until about 12 DAP[2], with peak mitotic index between 8 and 10 DAP (22). A transition from division to expansion occurs in those endosperm cells destined to become storage cells. Following this transition the number and size of starch granules and protein bodies increase, concomitant with nuclear expansion and DNA amplification (22). Both cell division and cell expansion may be important in determining the storage capacity of the kernel. Water or temperature stress during early kernel development decreases the number of endosperm cells and starch granules (5, 14, 18, 19) and also decreases dry matter accumulation (18, 28). Since ABA accumulates in plant tissues subjected to water deficit and other types of stress, it is plausible that the effects of water deficit on kernel development may be due, in part, to elevated levels of ABA in the kernel.

Exogenously applied ABA has been found to inhibit cell division in some plant tissue systems. The rate of cell division in maize root tips was diminished with exogenously applied ABA (3). Cell division was also inhibited in decapitated pea buds after applications of 10 to 100 μM ABA (20). In wheat pollen, exposure to ABA or water stress caused sterility by decreasing the rate of meiosis in pollen mother cells (25).

The effects of increased levels of ABA on expansion growth are varied. In root/shoot studies of etiolated soybean seedlings, ABA at low concentrations inhibited shoot growth, whereas root elongation was slightly stimulated or unaffected (7). Although a stimulation of tissue expansion was also reported in excised pea root tips (10) and bean tissue (1), many have suggested that ABA suppresses cell expansion rates (4, 20, 23).

In the current studies, the effects of ABA on developing maize endosperm were examined *in vitro* by supplying exogenous ABA to whole kernels. The objective was to determine if elevated levels of ABA would affect the processes involved in early endosperm development, including cell division, cell expansion, and starch granule initiation.

MATERIALS AND METHODS

Plant Material

Hybrid maize (*Zea mays* L.) seeds (cultivar 3925, Pioneer Hybrid International, Des Moines, IA) were sown in 12-L pots containing either a peat mix (peat moss/vermiculite/perlite [1:1:1,v/v]) or a soil/peat mix (1:2, v/v). Plants were thinned to 2 per pot and grown in the greenhouse under supplemental lighting (1000-W metal halide lamps, Duraglo, GE Inc., Hendersonville, NC) which provided approximately 500 μmol of photons (400–700 nm) m^{-2} s^{-1} at the top of

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[2] Abbreviation: DAP, days after pollination.

mature plants. Automatic watering with a nutrient solution (1 g L^{-1} of Peter's 15–16–17, W. R. Grace and Co., Fogelsville, PA) every 2 h during the light period delivered about 1 L/d to each pot. Previous study has shown that plants grown under these conditions are well watered and have low levels of maternal ABA (21). The ear shoots were bagged prior to silk emergence and all florets on each ear were synchronously pollinated 4 or 5 d after silk emergence.

In Vitro Culture

At 5 DAP the middle portion of each ear was aseptically dissected into six-kernel cob sections. Five of the six kernels were excised leaving a single kernel per section to place in culture (8). Cob sections were placed on a 15% (w/v) sucrose culture medium (8) modified to contain 1 g of L-glutamine/L as the sole nitrogen source. The medium was adjusted to pH 5.8 with 1 N NaOH before autoclaving. Difco Bacto-agar (0.5%, w/v) was used to solidify the medium. Streptomycin sulfate was added to the medium after autoclaving to a final concentration of 10 mg L^{-1}. ABA ([±]-2-*cis*-4-*trans*-ABA; Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water (pH 8.0, adjusted with 1 N NaOH), the pH was adjusted to pH 5.8 with 1 N HCl. The solution was filter-sterilized into cooling, autoclaved medium. The final concentration of ABA in the medium was 90 μM for all studies except the concentration study, where the range was 3 to 300 μM. Fifty mL of medium was poured into each Petri dish (100 x 25 mm). Cob sections were randomly assigned to treatments and sampled as described below. All cultures were kept in a dark, humidified chamber at 27°C (±1°) for the duration of the sampling time.

In study 1, kernels remained in the same medium, without renewal, for the entire study. To determine the timing of treatment effects, kernels were sampled daily from 2 to 7 d after start of culture (7–12 DAP) for analysis as described below, and at maturity (37 DAP), for dry matter determination. In study 2, kernels were cultured in either control or ABA-containing medium during the period of most rapid cell division (from 5–10 DAP), followed by transfer of both ABA-treated and untreated kernels to fresh control medium at 10 DAP. Kernels were randomly sampled at 5, 8, 12, and 16 d after start of culture (10, 13, 17, and 21 DAP). In study 3, treatment periods were designed to encompass various portions of kernel development so that effects on cell division, expansion growth and other processes would be compared. Kernels were cultured at 5 DAP with or without 90 μM ABA in the medium. Randomly selected control kernels were transferred at 8 and 11 DAP to ABA-containing medium. Kernels were sampled from each treatment at 5, 9, and 12 d after start of culture (10, 14, and 17 DAP). In study 4, the effect of ABA concentration in the medium was determined. Culture began at 5 DAP in medium containing 0, 3, 10, 30, 100, or 300 μM ABA. All kernels were harvested after 5 d in culture (10 DAP) and analyzed for endosperm ABA content and cell number.

Cell and Starch Granule Number Determinations

Kernels were excised from the cob sections and immediately placed in ethanol:glacial acetic acid (3:1, v/v). After at least 24 h the kernels were sequentially equilibrated in 80, 50, 25, and 0% ethanol (v/v) and the endosperms were excised. An endosperm digestion procedure was developed by modifying the method of Reddy and Daynard (24). A pectinase solution for endosperm digestion was prepared by mixing 1 part pectinase (technical grade with inert filler, No. 102588, ICN Biomedicals, Cleveland, OH) to 3 parts citrate-phosphate buffer (8.8 g Na_2HPO_4 + 3.6 g citric acid L^{-1} [pH 4.0], + 0.1% [w/v] NaN_3), then filtering twice through Whatman No. 1 paper to remove inert filler. Endosperms were dissected from the kernels, placed in 1 mL of pectinase solution in a tightly capped tube, and incubated at 40°C until soft. Endosperms less than 10 DAP required approximately 12 to 24 h in the pectinase solution; older endosperms required up to 48 h to soften. Nuclei and starch granules were dispersed by forcing the tissue through a 20 gauge needle with a syringe. An aliquot of the suspension was diluted with an equal portion of aceto-carmine (Carolina Biological Supply Co., Burlington, NC) to stain the nuclei. Another aliquot was removed from the suspension and diluted with an equal volume of an iodine solution (3.3 g I_2 + 6.7 g KI L^{-1}) to stain the starch granules. Stained nuclei or stained starch granules were counted on a hemacytometer (Improved Neubauer Ultra Plane, model 3500, Hausser Scientific, Blue Bell, PA) at ×400 magnification. The counts were multiplied by the appropriate dilution factors to determine cell (nuclei) and starch granule number per endosperm. Nuclear diameter measurements were made using a calibrated ocular micrometer.

ABA Extraction and Analysis

Each endosperm was excised from a frozen kernel, weighed, and homogenized in 0.5 mL of extraction medium (80% methanol, 1% [v/v] glacial acetic acid, 10 mg L^{-1} butylated hydroxytoluene). After 24 h of shaking at 4°C, the homogenate was centrifuged, supernatant was removed, and the pellet reextracted. The combined supernatants (1.0 mL) were evaporated to dryness at 40°C *in vacuo*. The dried extract was redissolved in 50 μL of methanol, then diluted with 950 μL of Tris-buffered saline (50 mM Tris, 1 mM $MgCl_2$, 10 mM NaCl, 0.1% (w/v) NaN_3 [pH 7.5]). Aliquots from this solution were assayed by indirect ELISA (21, 27) using an Idetek (San Bruno, CA) monoclonal antibody that is specific for the (+)-ABA enantiomer. In this method the extract is assayed without further clean-up and it typically has little loss of ABA (27); therefore, an internal tracer of radioactive ABA was not added and the values reported were not corrected for any loss.

[^{3}H]ABA Uptake in Developing Endosperm

To determine the rate of ABA uptake in the developing endosperm of cultured kernels, 1 μCi (37 kBq) of (±)2-*cis*,4-*trans*-[^{3}H]ABA (Amersham, specific activity = 33.2 Ci $mmol^{-1}$) was added to 50 mL of medium containing 90 μM ABA. Kernels with subtending cob sections were excised and placed in culture at 5 DAP, then harvested after 12, 36, and 111 h (5.5, 6.5, and 10 DAP). Kernels were excised from cob sections before endosperms were removed to avoid endosperm contact with the labeled culture medium. ABA was extracted from excised endosperm tissue as described above.

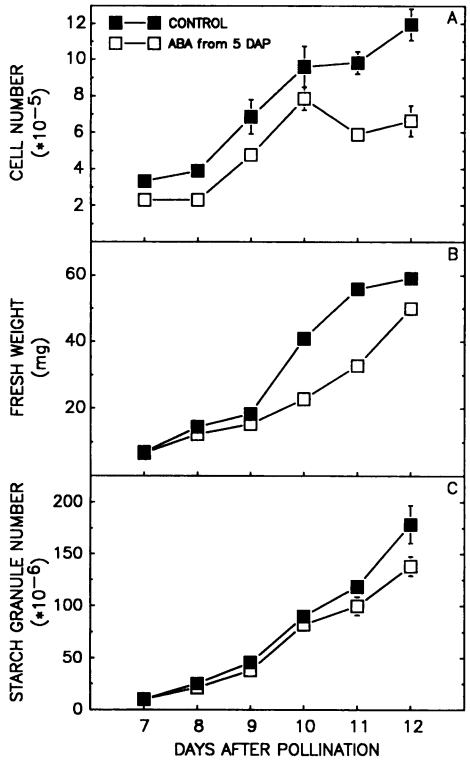

Figure 1. Study 1. Effects of continuous culture of maize kernels on medium containing 90 μM (±)-ABA (□), compared to culture in medium without ABA (■), on endosperm cell number (A), endosperm fresh weight (B), and endosperm starch granule number (C). In (B), a single, pooled estimate of average fresh weight was obtained from six endosperms for each data point. In (A) and (C), means ± SE (where error bars exceed symbol dimensions) of six replicate endosperms are shown.

The total supernatant (1 mL) was added to 10 mL of scintillation cocktail (Liquiscint, National Diagnostics, Manville, NJ) and counted in a Beckman LS 100 liquid scintillation counter. Counts were also obtained from aliquots of the medium at the start of culture. Total [^{3}H] counts per minute minus background were converted to disintegrations per minute using the external standard ratio. The efficiency of counting was approximately 30%.

RESULTS

Endosperm Cell Number

Kernels cultured at 5 DAP in medium containing 90 μM ABA formed fewer endosperm cells than kernels cultured in medium without ABA (Fig. 1A). ABA-treated kernels maintained a rate of cell division comparable with control kernels through 10 DAP. However, from 10 to 12 DAP the number of endosperm cells in ABA-treated kernels did not increase while endosperm cells of control kernels continued dividing. At 12 DAP ABA-treated kernels had about 40% fewer endosperm cells than control kernels.

Kernels in study 2 were cultured with 90 μM ABA from 5 to 10 DAP, then transferred to control medium to allow study of starch granule initiation and further expansion growth during the subsequent period. Endosperms in the ABA treatment had about 40% fewer cells at 10 and at 13 DAP (Fig. 2A). There was no significant ($P \leq 0.05$) increase in cell number in either treated or control kernels from 10 to 13 DAP. Attempts were made to determine the number of endosperm cells at later times of development, but degradation of the nuclei after about 15 DAP resulted in inaccurate counts.

In study 3, treatment periods were designed to encompass various portions of endosperm development so that effects of ABA on cell division could be distinguished from the effects on fresh weight gain and starch granule accumulation. The rate of endosperm cell division was decreased by ABA treatment during a 5-d period (from 5–10 DAP), but not during a 2-d period from 8 to 10 DAP (Fig. 3A). At 14 DAP, kernels in both treatments (ABA from 5 or from 8 DAP) had about 30% fewer endosperm cells than controls. Transferring the kernels to ABA-containing medium at 11 DAP had no effect on the number of endosperm cells observed at 14 DAP, probably due to the near completion of endosperm cell division at the time of transfer.

Total endosperm cell numbers of control kernels at 10 DAP in studies 1, 2, and 3 were significantly different from one

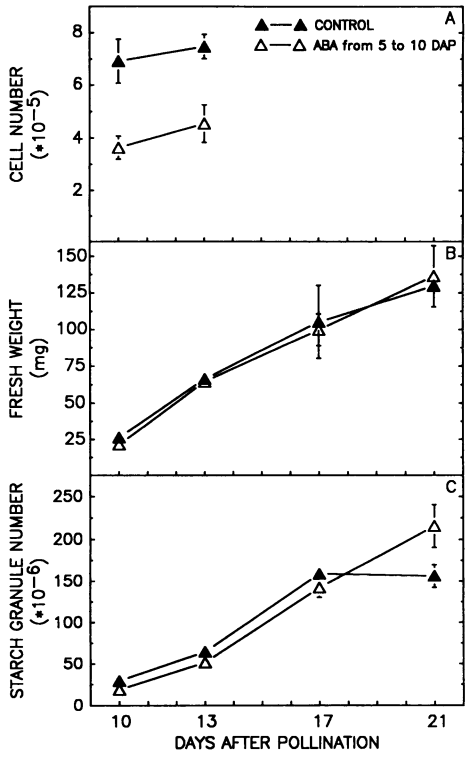

Figure 2. Study 2. Effects of culturing maize kernels from 5 to 10 DAP on medium containing 90 μM (±)-ABA (△) or medium without ABA (▲) from 5 to 10 DAP, followed by culture in medium without ABA from 10 to 21 DAP, on endosperm cell number (A), endosperm fresh weight (B), and endosperm starch granule number (C). The bars (where they exceed symbol dimensions) represent the SE of five replicate endosperms.

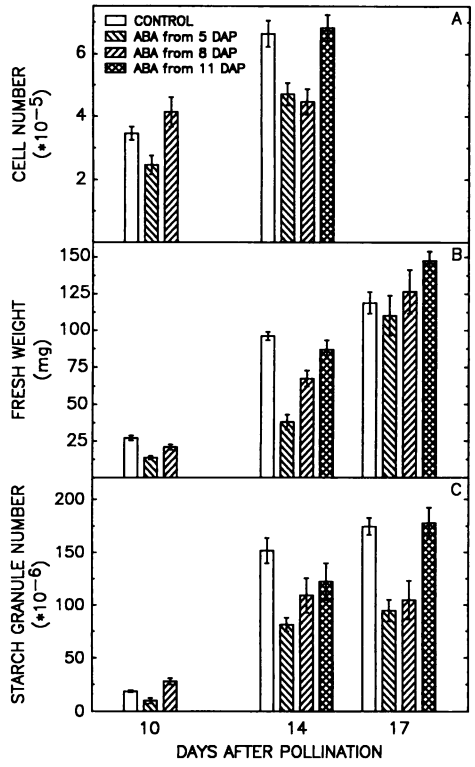

Figure 3. Study 3. Effects of culturing maize kernels on medium containing 90 μM (±)-ABA at three different developmental periods, early cell division (from 5 DAP), late cell division (from 8 DAP), and early starch granule initiation (from 11 DAP), on endosperm cell number (A), endosperm fresh weight (B), and endosperm starch granule number (C). Kernels were cultured at 5 DAP in either control (□) or ABA-containing (▨) medium. Additional transfers from control to ABA-containing medium were made at 8 DAP (▨) or 11 DAP (▩). Bars represent the SE of 3 to 10 replicate endosperms.

another (Figs. 1A, 2A, 3A). It is possible that differences between studies were due to differences in the starting plant material, possibly due to the light environment. Although artificial lighting was supplied for 12 h/d with about 500 μmol of photons (400–700 nm) m^{-2} s^{-1} at the top of mature maize plants, natural radiation differed in the three studies. Total solar radiant energy (300–3000 nm) for the period from silk emergence to 5 d after pollination (time of culture), averaged 15.5, 6.6, and 2.4 MJ/d for studies 1, 2, and 3, respectively. Control kernels from each of these studies at 10 DAP had 960, 602, and 346 thousand endosperm cells, respectively, with a correlation coefficient to solar radiant energy of R = 0.994. In each study, however, ABA-treated kernels had 30 to 40% fewer endosperm cells than controls.

Expansion Growth

Treatment with ABA from 5 DAP appeared to decrease endosperm fresh weight at 10 and 11 DAP relative to kernels cultured in control medium (Fig. 1B). By 12 DAP, however, endosperms in both ABA-treated and control kernels had comparable fresh weight. When treatment was limited to 5 d (from 5–10 DAP), and sampling was extended to 21 DAP, there was no effect of ABA treatment on endosperm fresh weight (Fig. 2B). In the third study involving three different ABA treatment times, a reduction in fresh weight was observed at 14 DAP in endosperms of kernels cultured in medium containing ABA from 5 or from 8 DAP (Fig. 3B). These two treatments encompassed the period of most active cell division and, correspondingly, the period in which ABA treatment decreased cell numbers (Fig. 3A). However, when kernels were cultured in medium containing ABA after cell division was near completion (from 11 DAP), there was no effect on fresh weight (Fig. 3B). At 17 DAP endosperms from all treatments had comparable fresh weight.

Starch Granule Number

Starch granule number increased linearly from 9 to 12 DAP in both controls and ABA-treated kernels (Fig. 1C). During this time interval there was no significant ($P \leq 0.05$) effect of ABA treatment on starch granule number. When sampling was extended to 21 DAP following a brief ABA treatment (5–10 DAP), an apparent failure of the control kernels to increase in starch granule number between 17 and 21 DAP made it appear that the ABA treatment resulted in increased numbers of starch granules (Fig. 2C). However, at 21 DAP, there was no statistically significant ($P \leq 0.05$) difference in the number of starch granules in treated and untreated kernels. In study 3, kernels cultured in medium containing ABA during the period of most active cell division (from 5 or 8 DAP), had fewer starch granules at 14 and 17 DAP (Fig. 3C). When kernels were cultured in medium containing ABA after cell division was near completion (from 11 DAP), there was no significant ($P \leq 0.05$) effect on starch granule number. Differences in total starch granule numbers at 10 DAP between controls in studies 1, 2, and 3 corresponded to the total endosperm cell numbers. Both were possibly affected by the preculture light environment of the plant material.

Nuclear Diameter

Nuclear diameters were measured to determine if culturing with ABA in the medium would affect nuclear size (Table I). In the first study, sampled from 7 to 12 DAP, endosperm cells from control kernels appeared to have consistently larger nuclei throughout sampling than those of ABA-treated kernels, although the differences were slight. In study 2, nuclear diameters were measured on endosperms harvested at 10 and 13 DAP following culture in control medium, or medium containing ABA from 5 to 10 DAP. The trend was similar to that of the first study, but no significant ($P \leq 0.05$) treatment effects were found. In study 3, however, ABA treatment from 5 or 8 DAP significantly ($P \leq 0.05$) decreased nuclear diameters at 10 DAP compared to controls. Also, nuclear enlargement was inhibited to a greater extent in those kernels cultured with ABA from 5 DAP, than from 8 DAP.

Dry Weight of Mature Tissues

To test the long-term effect of culturing kernels with or without ABA, kernels remained in the same culture medium from 5 DAP until near maturity (37 DAP). The endosperms

Table I. *Nuclear Diameters*

Endosperms were digested as described in the text to obtain representative samples of suspended nuclei. Nuclear diameters of 50 to 200 individual nuclei were measured at ×400 using an ocular micrometer. ABA treatments began on the dates indicated and continued until sampling except in study 2, where ABA treatment was from 5 to 10 DAP. Means ± SE of the indicated number (*n*) of nuclei from 5 to 10 replicate kernels are shown.

Study and Sampling Date, DAP	Control	Cultured with ABA in the Medium	
		from 5 DAP	from 8 DAP
		μm	
Study 1[a]			
7	11.9 ± 0.4	10.8 ± 0.4	
8	12.3 ± 0.4	11.6 ± 0.3	
9	14.3 ± 0.5	13.1 ± 0.6	
10	14.8 ± 0.8	14.3 ± 0.7	
11	16.5 ± 0.8	15.6 ± 0.8	
12	17.3 ± 0.7	16.5 ± 0.7	
Study 2[b]			
10	12.0 ± 0.8	11.7 ± 0.9	
13	17.4 ± 1.0	17.0 ± 1.0	
Study 3[c]			
10	19.8 ± 0.5	13.5 ± 0.3	17.0 ± 0.8[d]

[a] $n = 120$. [b] $n = 50$. [c] $n = 200$. [d] Mean ± SE of 62 nuclear measurements from 3 replicate kernels.

of ABA-treated kernels had 34% less dry matter than those of controls (Table II). There was no significant ($P \leq 0.05$) difference, however, between embryo dry weights of kernels cultured in medium containing ABA and kernels cultured in control medium.

ABA Concentration and Analysis

In study 4, kernels were cultured from 5 to 10 DAP in a range of (±)-ABA concentrations from 3 to 300 μM to determine the concentration dependence of the cell division response. At 10 DAP, endosperm cell number from control kernels was approximately the same as from kernels cultured in medium containing 3, 10, or 30 μM (±)-ABA (Fig. 4). Culturing kernels in medium containing 100 or 300 μM (±)-ABA resulted in an equivalent reduction in the rate of cell division, with about 30% fewer endosperm cells formed at 10 DAP compared to controls. Assays indicated the concentrations of (+)-ABA in endosperms were similar throughout the treatment range from 0 to 30 μM, whereas endosperms from kernels cultured with 100 μM (±)-ABA had (+)-ABA concentrations more than twofold higher than controls (Fig. 4). A negative correlation ($R = -0.92$) existed between endosperm cell number and endosperm (+)-ABA content.

The uptake of [^{3}H]ABA into cultured kernels indicated that little or no ABA was transported into the developing endosperm during the first 12 h of culture (data not shown). After 36 and 111 h, sufficient [^{3}H] accumulated in endosperms to allow quantitation. These data and the initial specific radioactivity of ABA in the culture medium (200 μCi mmol^{-1}) were used to calculate the amount of ABA taken up in the endosperm. Such analysis indicated that endosperms took up 107 pmol of (±)-ABA after 36 h, and 522 pmol of (±)-ABA after 111 h of treatment (10 DAP). In contrast, the amount of (+)-ABA in kernels cultured in medium containing 100 or 300 μM (±)-ABA was only 11.0 or 9.6 pmol, respectively, after a similar time in culture (about 120 h, data from study 4). Thus, it appeared that the effective dose of the naturally occurring (+) enantiomer of ABA was substantially decreased, compared to the amount of ABA taken up, presumably through catabolism in the tissue.

DISCUSSION

The rate of cell division in the developing endosperm of cultured maize kernels was adversely affected by the presence of 90 μM (or greater) ABA in the culture medium (Figs. 1A, 2A, 3A, and 4). Endosperm cell numbers were reduced substantially in all studies when kernels were cultured in media containing ABA during the early stage of endosperm development (5–10 DAP). In other plant systems, inhibition of cell division was also observed following exogenous ABA applications. Barlow and Pilet (3) reported a diminished rate of cell division in maize root tips following exogenously applied 50 μM, but not 5 μM ABA. Similarly, Nougarède *et al.* (20) demonstrated that 10 to 1000 μM ABA inhibited cell division in decapitated, meristematic pea buds. Both investigators concluded that ABA reduced the rate of cell division, but even at high concentrations (100–1000 μM), ABA did not completely inhibit cell division.

Perhaps high levels of exogenously applied ABA are necessary to overcome the rapid rate of ABA catabolism within the plant. Uptake of [^{3}H]ABA from a medium with 90 μM ABA indicated that about 522 pmol of (±)-ABA [approximately 260 pmol of the naturally occurring (+)-ABA] was transported into the endosperm over a 5-d period, yet ELISA [which measured only the (+) enantiomer] demonstrated that kernels cultured in 100 μM ABA accumulated only 11 pmol of (+)-ABA per endosperm. A high rate of turnover, either through oxidative degradation or conjugation, within the cob and/or kernel tissues, could explain the relatively low amount of (+)-ABA found in the endosperm after 5 d of culture in 90 μM (±)-ABA. These data suggest that the rate of (+)-ABA catabolism in endosperms limited the extent of ABA accumulation when kernels were treated via ABA-containing medium. Thus, the lack of a more substantial response to high levels of applied ABA (Fig. 4) may have been due to the inability to establish higher (+)-ABA concentrations in endosperm cells.

The biological activity of the unnatural (−) enantiomer of

Table II. *Endosperm and Embryo Dry Weights at 37 DAP*

Kernels were cultured in 90 μM (±)-ABA or control medium from 5 to 37 DAP. Kernels were excised, separated into embryo and endosperm, dried and weighed. Means ± SE of 11 and 8 replicates of control and ABA-treated kernels, respectively, are shown.

Treatment	Endosperm	Embryo
	mg	
Control	95.7 ± 5.9	14.2 ± 1.9
(±)-ABA	61.1 ± 6.7	15.6 ± 3.1

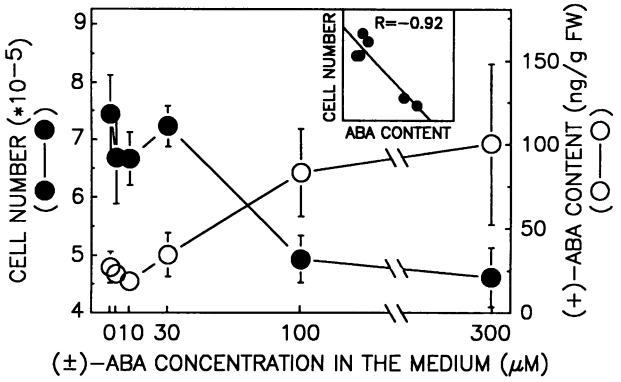

Figure 4. Study 4. Effects of culturing maize kernels from 5 to 10 DAP on a range of (±)-ABA concentration on endosperm cell number (●) and (+)-ABA content (○) of the endosperm. All kernels were sampled at 10 DAP. The bars (where they exceed symbol dimensions) represent the SE of six replicate endosperms. The inset shows the linear regression of endosperm ABA content plotted against endosperm cell number.

ABA is still uncertain. Although it has been suggested that in short-term responses the (−) enantiomer has no effect, in long-term responses it has exhibited comparable effects to the (+) enantiomer (16). In *Vicia faba* L., stomatal closure, a short-term response, occurred after 30 min in the presence of (+)-ABA (12). In contrast, the (−) enantiomer, purified by immunoaffinity chromatography, was so inactive that it was used as a control. Investigations using (+) and (−)-ABA, resolved by acetylcellulose chromatography, indicated that both enantiomers were biologically active in eliciting long-term responses (26). At a given dose, the naturally occurring (+)-ABA was initially a more effective inhibitor of expansion of embryonic bean axes than (−)-ABA, but after 12 h its effectiveness decreased. The decreased effectiveness of (+)-ABA corresponded to its substantial catabolism during this time period, whereas (−)-ABA was not appreciably catabolized. Differences in ABA metabolism were also observed in cell suspension cultures of bromegrass (*Bromus inermis* Leyss) after 21 d with (±)-ABA in the medium (2). These cells catabolized 79% of the (+) and only 7% of the (−) enantiomer, indicating that the (−)-ABA was a poor substrate for catabolic breakdown in that plant system. In the current study, the amount of (+)-ABA detected in the endosperm correlated well with the number of cells in the endosperm (Fig. 4, $R = -0.92$), whereas the amount of (±)-ABA in the medium was not as well correlated with endosperm cell number ($R = -0.84$). Although the concentrations of ABA in the medium that were required to elicit an inhibitory effect on cell division were quite high, the levels of (+)-ABA in the endosperm were comparable to the 100 ng/g fresh weight of (+)-ABA found in water stressed maize kernels at 9 and 11 DAP (21).

Elevated levels of ABA had little effect on endosperm expansion growth. An apparent decrease in fresh weight was observed when ABA was applied early in endosperm development (Figs. 1B and 3B), but this was a transient effect, lasting only a few days. Subsequent rapid fresh weight gain occurred in ABA-treated kernels that resulted in similar endosperm fresh weight in ABA-treated and control kernels. When the start of ABA treatment was delayed until cell division in the endosperm was nearly complete (11 DAP), there was no discernible effect on endosperm growth (Fig. 3B).

Nuclear size in maize endosperm is considered to be directly proportional to cell size (15) and may be a determinant of cell growth capacity. A trend toward smaller nuclei in ABA-treated endosperms was evident in study 1 (Table I), but the differences were slight. In study 3, however, ABA-treated kernels had smaller nuclei whether the treatment was begun at 5 or 8 DAP, with the earlier treatment resulting in the most dramatic decrease. Additional studies are required to determine whether ABA affects cell expansion directly, or whether the effect on cell expansion is a secondary consequence of ABA's effect on cell division and its apparent effect on the DNA synthesis associated with nuclear enlargement.

Starch granule number, an indirect measure of the number of amyloplasts in the endosperm, has correlated with final kernel mass in several cereal grain species, including maize (14), wheat (18, 19), and barley (5). In the present studies, culturing kernels on medium containing ABA had no apparent effect on the number of starch granules per endosperm during the period of most rapid starch granule initiation (ABA treatment from 11 DAP, Fig. 3C). Culturing on ABA-containing medium during the period of cell division, followed by control medium during the period of starch granule initiation was also without effect, although it appeared that the ABA-treated kernels had extended their time of starch granule initiation beyond that of the control kernels (Fig. 2C). However, when kernels were cultured on ABA-containing medium during a period extending throughout cell division and starch granule initiation, starch granule numbers were decreased (ABA from 5 and 8 DAP, Fig. 3C). Therefore, the reduction in starch granule number observed when kernels were cultured continuously in ABA-containing medium was not necessarily related to the presence of ABA in the culture medium during the period of starch granule initiation. Consequently, we suggest that the effects of ABA on expansion growth and starch granule initiation may have been secondary to earlier effects which decreased endosperm cell number.

Cultured kernels are generally 30 to 50% smaller at maturity than field grown material, but even at their reduced size they maintain the ability to germinate. When kernels from study 1 were grown to maturity in culture, ABA-treated kernels had endosperms that were 35% smaller than controls (Table II). In contrast, embryo dry weight was unaffected by ABA treatment. ABA has been shown to induce synthesis of late embryogenesis abundant proteins in many species (9). It is possible that such induction contributes to the maintenance of dry matter accumulation in embryos exposed to elevated levels of ABA. Perhaps maize has evolved a mechanism of differential tissue response such that increases in ABA at early stages of seed development decrease endosperm growth more than embryo growth, thereby ensuring production of viable seed.

In maize kernels of commercial genotypes, the embryo comprises only 10 to 15% of the total grain weight at maturity. Decreases in final kernel mass in response to environmental stresses have correlated well with decreases in endosperm cell

number in maize (14), as well as in other cereal grain species (5, 18, 19). Perhaps in the present case also, the ABA-induced decrease in endosperm cell number limited the maximal storage capacity of the kernel, and in turn, decreased the mature dry mass (Table II), even though fresh weight growth and starch granule initiation were relatively unaffected.

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