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Distinction between the Responses of Developing Maize Kernels to Fluridone and Desiccation in Relation to Germinability, α-Amylase Activity, and Abscisic Acid Content[1]

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ABSTRACT

Developing kernels of the maize (*Zea mays*) hybrid W64A × W182E germinated precociously following fluridone treatment. Likewise, following premature drying, the kernels germinated upon subsequent rehydration. Tolerance of the aleurone layer to premature desiccation considerably preceded that of the embryo. The increase in α-amylase activity following premature drying was substantial and was equal to, or exceeded, the increase which occurred following normal maturation drying. In contrast, there was only a small increase in enzyme activity, regardless of the concentration of the supplied gibberellic acid, following fluridone treatment. Both fluridone and drying cause a decrease in abscisic acid content within the developing kernels. While this decline in growth regulator may permit kernels to germinate, alone this is not sufficient to permit an increase in α-amylase activity. Thus drying is necessary to sensitize the aleurone layer to gibberellin, and thereby elicit enzyme synthesis. For this tissue to achieve its full potential to produce α-amylase, it must not only be free of the inhibitory effects of abscisic acid, but it must also be competent to respond to gibberellin.

During development, the aleurone layer serves as a storage tissue, whereas following germination, its function is largely hydrolytic, as a source of enzymes for the mobilization of stored reserves (6). While it must be ensured that the catabolic events of postgermination do not occur during development, a switch in metabolism must occur to ultimately trigger reserve mobilization and its associated enzyme synthesis and release.

The nature of this switch remains elusive, but it is possible that drying and the growth regulator ABA, which are known to affect both development and germination of the embryo (reviewed in ref. 13), also play a role in the aleurone layer. Drying, when prematurely imposed on developing grains, enables the aleurone layer of wheat and barley to respond to GA and produce α-amylase (1, 2, 5, 17), a starch-hydrolyzing enzyme which is the predominant protein synthesized by this tissue (6). Drying, it is argued, renders the tissue sensitive to gibberellin (1, 5). Conversely, others have suggested that high levels of ABA prevent the developing aleurone layer from initiating α-amylase biosynthesis, a function that ABA is known to effect in germinated grains (14, 18). These authors propose that drying serves to reduce endogenous ABA, allowing the aleurone layer to produce enzyme. This contention is supported by studies of viviparous (*vp*) mutants of corn; the ABA-deficient and -insensitive aleurone layer of these mutants produces elevated levels of α-amylase late in development while still attached to the cob (24).

There has been no previous attempt to simultaneously assess the relative significance of drying and ABA depletion in the triggering of α-amylase biosynthesis in the aleurone layer or how, if at all, the two are interrelated. Here, we compare the responses of maize kernels to premature drying and application of fluridone, an inhibitor of ABA-precursor synthesis (8); both treatments are known to elicit the precocious germination of maize kernels. If drying serves merely to reduce inhibitory levels of ABA in the developing tissue, then reducing the endogenous levels of ABA with fluridone in developing kernels should allow the aleurone layer to respond and thus result in the precocious production of enzyme in the absence of a drying treatment. It is evident, however, that germination and α-amylase synthesis cannot be attributed only to a modulation in ABA levels.

MATERIALS AND METHODS

Plant Material

Hybrid W64A × W182E kernels of *Zea mays* were planted and maintained outdoors at the University of Guelph. Plants were self-pollinated by hand. Cobs were treated with fluridone as described by Fong et al. (7). Exposed cobs were sprayed liberally with 125 ppm fluridone in 1% acetone at 10 DAP[2], then the husks were replaced and covered with a waxed paper bag. Other cobs, sprayed either with 1% acetone (1987 and 1988) or left untreated (1989), were harvested at 21 DAP (desiccation-intolerant stage), 35 DAP (desiccation-tolerant

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[2] Abbreviations: DAP, days after pollination; HAI, hours after imbibition.

stage), or 77 DAP (maturity) and either frozen in liquid nitrogen and stored at −80°C (fresh), allowed to dry under ambient laboratory conditions (22°C, <20% RH) while suspended by the shank of the cob (dry), or maintained in 100% RH for a duration equal to the drying period, between 10 and 14 d (moist controls). For ABA and α-amylase determinations, kernels from the basal end of the cob were used.

Endosperm Water Content

Immediately after harvest, the pericarp, testa, and embryo were removed from fluridone-treated and nontreated (fresh) kernels and the fresh weight of the remaining endosperm was recorded. The endosperm was dried over silica gel for 4 d and the dry weight was recorded. The water content was calculated as a percent of the fresh weight using the formula (fresh weight-dry weight) × 100%/fresh weight.

Incubation Conditions for Development of α-Amylase Activity

In vivo α-amylase activity was followed using a method modified from Goldstein and Jennings (9). Kernels were sterilized for 10 min in 70% ethanol and for a further 20 min in 20% bleach, followed by repeated rinsing in sterile distilled water. In the case of fluridone-treated, fresh and moist-control kernels, the embryos were removed immediately after sterilization with a sterile scalpel and forceps and the remaining tissue rinsed in sterile distilled water. Dry kernels were hydrated for 6 h at room temperature following sterilization, to ease embryo removal. Four or five deembryonated kernels were placed embryo-side down in a 6-cm Petri dish on autoclaved filter paper moistened with buffer solution (10 mM $CaCl_2$ and 1 mM sodium acetate buffer [pH 4.8]) containing 28.1 μM penicillin, 31.0 μM chloramphenicol, and 171.6 μM streptomycin and, where specified, growth regulators. After allowing the kernels to equilibrate with the buffer solution for 4 to 6 h, they were placed embryo-side up and incubated at 28°C for 4 d. The total incubation time for all kernels from the completion of surface sterilization was 96 h. Incubated kernels were freeze-dried and stored at −20°C.

α-Amylase Assay

Individual freeze-dried kernels were homogenized with 1 mL ice-cold incubation buffer in a chilled mortar and pestle with sand. The homogenate was centrifuged for 5 min at 18,000*g*, the enzyme-containing supernatant was quick-frozen in liquid nitrogen and stored at −20°C. A modified Hejgaard and Gibbons (11) gel method was used for quantifying α-amylase activity. Blue dye-linked substrate was prepared from Phadebas amylase test tablets (Pharmacia Diagnostics, Uppsala, Sweden). The tablets were pulverized, washed free of the manufacturer's buffer with repeated washes in distilled water, and the resulting blue powder freeze-dried. Two-millimeter-thick gels containing 1% agarose and 0.5% Phadebas powder in incubation buffer were poured on horizontal glass plates. Using a 3.5 mm-cork borer, 20 μL wells were punched from the gel. Enzyme standards were prepared fresh daily with barley α-amylase (Sigma) in incubation buffer. Following application of extracts and standards to the wells, the gel was incubated overnight at 27°C, suspended over water in a sealed Tupperware container. The reaction was stopped by gently shaking the gel in 0.05 N NaOH solution, followed by washes in distilled water. The activity of the applied sample was proportional to the diameter of the cleared area around the wells. A minimum of 4, but typically 5, samples were assayed per treatment.

ABA Determinations

Abscisic acid was extracted according to the method of Raikhel *et al.* (19), with modifications. Deembryonated kernels were freeze-dried and ground in a Wiley Mill (Thomas, Philadelphia, PA), yielding 0.5 to 1.0 g powder. The powder was homogenized in a chilled mortar with pestle with 5 mL ice-cold acidic 80% methanol containing 10 mg/L butylated hydroxytoluene. The homogenate was transferred to a 50 mL polypropylene tube, 6400 DPM (0.43 pmol) DL-*cis*, *trans*-[G-^{3}H] ABA (Amersham, Oakville, Canada) was added as an internal recovery standard and the homogenate shaken at 4°C for 30 min. Debris was pelleted from the solution by centrifugation at 48,000*g* for 5 min. The supernatant was kept at 4°C. The pellet was washed twice with ice-cold 80% methanol containing 10 mg/L butylated hydroxytoluene. The pooled supernatants were lyophilized to dryness and the resulting ABA-containing residue dissolved in 1.5 mL Tris-buffered saline (pH 7.5). Samples were stored at −80°C until assayed.

ABA was determined using the Phytodetek (San Bruno, CA) monoclonal antibody ELISA system. The absolute tissue content of ABA was calculated from the extracted (assayed) amount of hormone using the percent recovery of the radioactive [^{3}H]ABA as a conversion factor.

RESULTS

Fluridone and Premature Drying Treatments

The germinability of intact kernels following fluridone treatment and prematurely imposed drying increased only after 20 DAP (Fig. 1). At this age of development, kernels germinated poorly in response to either treatment; kernels were either intolerant of drying or had yet to exhibit viviparous germination in response to fluridone. By 35 DAP, prematurely dried kernels were completely germinable upon rehydration and the leaves developed green pigmentation as seedling growth proceeded. Kernels taken from cobs detached from the plant but not allowed to dry (moist control) were not germinable, demonstrating that drying, rather than mere detachment, elicited the germinative response. When embryos were isolated from the moist-control kernels, they were fully germinable. Fluridone-treated kernels exhibited 85% viviparous germination on the cob at a similar age of development. While fluridone-treated kernels exhibited even greater viviparity beyond 35 DAP, the cobs were increasingly infested with insects and fungus and could not be used. Thus, using the germination of the embryo as an indicator, fluridone-treated or prematurely dried kernels acquired the ability to fully respond by 35 DAP.

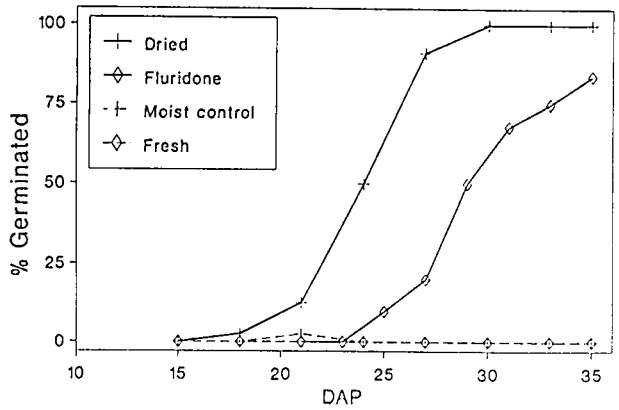

Figure 1. Germination of fluridone-treated and prematurely dried maize kernels. Fresh cobs were harvested during development and allowed to dry (dried) or maintained in a hydrated state (moist control). After the drying period, 50 kernels from the basal portion of three randomly chosen cobs were selected, and allowed to imbibe for 7 d at 28°C to assess germinability. Viviparous germination of fluridone-treated and fresh developing kernels was determined *in situ* immediately upon harvest (without a prior incubation period) of three to four randomly chosen cobs. Germination was considered complete when the radicle (from prematurely dried kernels) or the coleoptile (from fluridone-treated kernels) extended 2 mm beyond the scutellum. Cobs harvested in 1989.

α-Amylase in Deembryonated Fluridone-Treated Kernels

Prior to the onset of viviparous germination of fluridone-treated kernels (*i.e.* at 21 DAP), α-amylase activity was very low (Fig. 2A). Regardless of the growth regulators supplied in the buffer, the already low levels of α-amylase in the tissue at harvest (0 HAI) declined. Exogenous GA_3 limited the extent of enzyme loss, but did not elicit a net increase in enzyme activity (Fig. 2A). Deembryonated kernels not treated with fluridone were similarly unresponsive, although they contained lower initial levels of enzyme (Fig. 2A).

Deembryonated fluridone-treated kernels at 35 DAP, a time of development at which precocious germination occurred (Fig. 1), exhibited a net increase in α-amylase activity (Fig. 2B). While incubation in buffer alone resulted in a net biosynthesis of enzyme (Fig. 2B) this was enhanced fourfold by exogenous GA_3 (Fig. 2B); little activity was detected in the presence of 10 μM ABA. Control kernels (*i.e.* those not sprayed with fluridone) did not exhibit the increase in enzyme activity, regardless of the incubation conditions (Fig. 2B). Fluridone treatment, therefore, enabled developing 35-DAP kernels to respond to growth regulators in a manner typical of germinated wheat and barley grains, without the requirement for normal maturation drying.

Effect of Fluridone Treatment on Water Content

While fluridone treatment elicited a postgerminative response in 35-DAP deembryonated kernels, the possibility existed that the primary effect of this treatment is to cause a reduction in the water content of the developing tissues. Thus, the synthesis of α-amylase could have been a secondary response to premature drying rather than a direct response to fluridone treatment. Measurement of the relative water content of fluridone-treated and untreated fresh tissue showed that this is unlikely; the endosperm from both treatments contained similar relative amounts of water throughout development (Fig. 3).

α-Amylase Activity in Prematurely Dried Tissue

Premature drying, like fluridone treatment, elicited a net increase in α-amylase activity upon incubation (Fig. 4). This postgerminative response of the prematurely dried deembryonated kernels differed from that of the fluridone-treated (nondried) kernels in two ways: the developmental age at which the tissue was able to respond and the extent of enzyme activity. First, unlike fluridone-treated kernels of similar developmental age (Fig. 2A), 21-DAP prematurely dried tissue

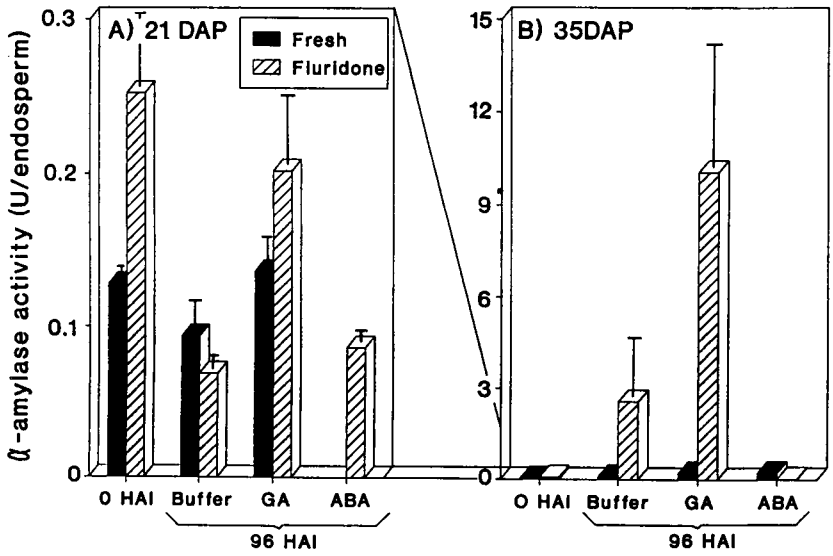

Figure 2. α-Amylase activity in deembryonated, previviparous (21 DAP), and viviparous (35 DAP) fluridone-treated and control maize kernels. Embryos were excised immediately upon harvest and the enzyme was assayed directly (0 HAI) or following a 96-h incubation in the presence of buffer alone, 10 μM GA_3, or 10 μM ABA. Activity in 21-DAP fresh endosperm incubated in ABA was not determined. Note the difference in the scale of the ordinate axis between A and B. Kernels from cobs harvested in 1988.

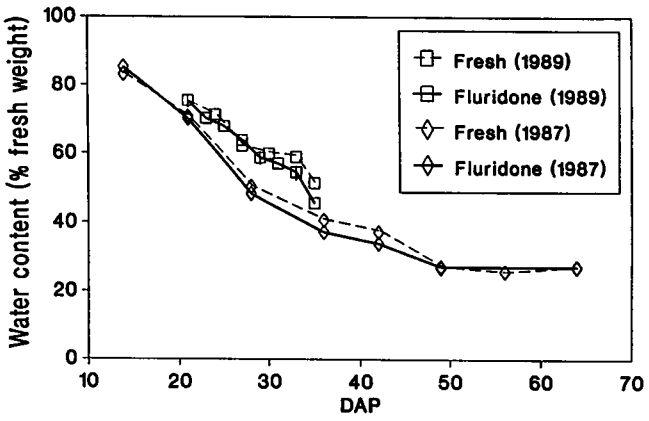

Figure 3. Water content of endosperm tissue from fluridone-treated and fresh (nontreated) kernels throughout the development of two harvests of maize.

exhibited a substantial net increase in enzyme activity in the presence or absence of GA_3 (Fig. 4). Thus, prematurely dried deembryonated kernels evidently acquired the ability to produce α-amylase at a younger developmental age than fluridone-treated kernels. It is also noteworthy that prematurely dried 21-DAP deembryonated kernels (Fig. 4) were able respond to incubation at a younger age of development than the embryos of prematurely dried intact kernels which germinated only poorly (Fig. 1). Second, the qualitative difference in response extended to the 35-DAP treatments, when prematurely dried tissue (Fig. 4) produced 13-times the amount of enzyme of the fluridone-treated tissue (Fig. 2B) in response to GA_3. This difference in response was not due to the toxicity of fluridone since treated kernels, when allowed to dry, also exhibited elevated levels of enzyme following rehydration (data not shown). Both 21- and 35-DAP tissue produced amounts of enzyme comparable to, or greater than, mature aleurone tissues (77 DAP; Fig. 4).

Incubation in GA_3 had no significant effect (Student's t test, 95% confidence interval) on the amount of enzyme activity of either prematurely dried (21-DAP and 35-DAP; see also Fig. 6) and mature (77 DAP) tissue (Fig. 4). Sensitivity to ABA, conversely, was high; its addition to the incubation buffer resulted in a substantial reduction in enzyme activity.

ABA Content of Deembryonated Prematurely Dried and Fluridone-Treated Kernels

The limited ability of the deembryonated fluridone-treated kernels to produce α-amylase, relative to the high levels in response to drying, may have been due to the inability of the inhibitor to completely block ABA biosynthesis. A comparison of the ABA content of embryoless kernels, however, showed that both drying and fluridone treatment result in low levels of this growth regulator, which were almost equal to those of mature, 77-DAP tissue (Fig. 5).

Cob Detachment in the Induction of α-Amylase Activity

To determine whether the precocious increase in α-amylase activity by prematurely dried tissue was elicited by detachment of the cob from the plant rather than the subsequent drying process, cobs were removed from the plant but maintained in a hydrated state for a time equal to the drying treatment. Deembryonated kernels isolated from these moist control kernels contained substantially less enzyme activity than the dried and rehydrated tissue at 21 and 35 DAP (Fig. 6). Although the amount of enzyme produced by the 35-DAP moist controls (Fig. 6) was nearly 100-fold greater than the very low amount produced by freshly harvested kernels (Fig. 2B), it was still 11-fold less than that in the mature or prematurely dried and rehydrated material (Fig. 6), regardless of the growth regulators provided (GA_3 being generally ineffective). Thus detachment alone could not account for the major postgerminative response of the dry tissue.

Optimization of Exogenous GA_3 for α-Amylase Activity Increase

The possibility existed that deembryonated fluridone-treated kernels showed small increases in α-amylase activity because of nonoptimal amounts of supplied GA_3. When GA_3 was supplied to fluridone-treated kernels at 10 μM (the concentration used throughout this study) following embryo excision the greatest increase in α-amylase activity was recorded (Fig. 7). However, the enzyme never increased to the levels achieved by buffer-imbibed, deembryonated kernels following

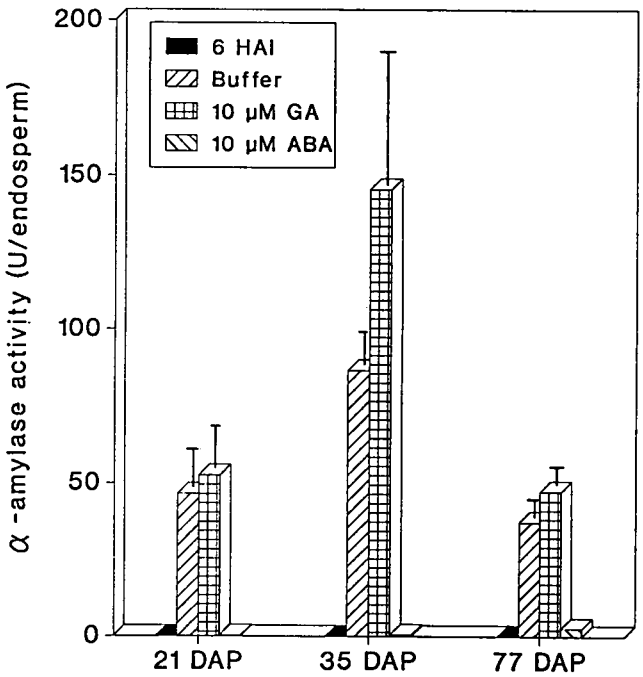

Figure 4. α-Amylase activity in deembryonated, air-dried, desiccation-intolerant (21 DAP), -tolerant (35 DAP), and mature (77 DAP) kernels. Dried kernels were incubated in ABA- or GA_3-containing buffer at 28°C for 6 h before embryo excision. The enzyme activity was then determined immediately (6 HAI) or following a further 90-h incubation in the appropriate incubation buffer. The 77 DAP kernels were mature and had completed maturation drying; they were treated as the 21-DAP and 35-DAP kernels. Kernels from cobs harvested in 1988.

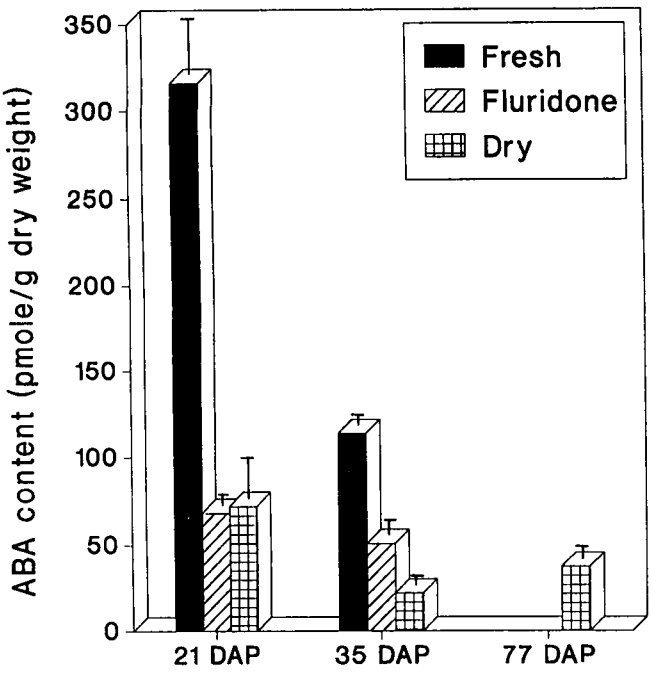

Figure 5. Effect of fluridone treatment (21 and 35 DAP), premature drying (21 and 35 DAP) and maturation drying (77 DAP) on the ABA content of deembryonated kernels. ABA was assayed using material freeze-dried upon harvest (fresh and fluridone) or following the drying treatment (dry).

drying and rehydration (Fig. 7). Since GA_3 applied at optimal levels to fluridone-treated (nondried) kernels failed to induce an increase in α-amylase to the levels of dried and rehydrated tissues, it is likely that the ability of the aleurone layer to respond was a consequence of drying.

DISCUSSION

Developing kernels of the maize hybrid W64A × W182E germinate precociously following fluridone-treatment (Fig. 1), as has been shown in other cultivars of maize (7, 8). Germination of viviparous (*vp*) mutants of maize has been attributed to either ABA-deficiency (3, 16, 23) or ABA-insensitivity (16). The chemically induced viviparous response occurs in both the embryo and the aleurone layer (Figs. 1 and 2) and is coincident with reduced ABA levels in deembryonated kernels (Fig. 5).

The increase in α-amylase activity of prematurely dried and mature tissue (*cf.* Figs. 4, 6, and 7) in response to incubation is substantial. There is only a small increase in enzyme activity in response to the fluridone treatment (Fig. 2B), however, indicating that while ABA-deficiency enables the embryo to germinate, removal of this growth regulator alone does not result in increased activity of the aleurone layer. Drying, on the other hand, stimulates α-amylase to increase to levels equal to, or greater than, those produced by mature tissue (Fig. 4; ref. 18 and A Oaks, personal communication), although both drying and fluridone-treatment similarly reduce ABA content (Fig. 5). The importance of drying is further supported by the finding that fluridone-treated kernels produce elevated levels of enzyme following drying and rehydration. Thus, drying affects more than a change in endogenous ABA levels and appears to elicit the tissues competency to respond to gibberellin upon rehydration.

Dried endosperm tissue is unresponsive to exogenous GA_3, producing similar amounts of enzyme as tissue incubated in buffer alone (Fig. 4). These results, like those of Harvey and Oaks (10) and Goldstein and Jennings (9), are attributed to the presence of near-optimal levels of gibberellin in the tissue, rather than the possibility that the aleurone layer does not need stimulation by this growth regulator. Ingle and Hageman (12) found that certain cultivars of corn are responsive to GA_3. Inbred lines of these cultivars which contain lower endogenous levels of this growth regulator, exhibit a greater response than their relatively gibberellin-rich hybrid progeny (22).

Beyond the quantitative differences in their response to exogenous GA_3, fluridone-treated and dried kernels differ in their sensitivity. While dry aleurone tissue is unaffected by exogenous GA_3, fluridone-treated tissue shows a significant, albeit small, response (Fig. 2B). This graded response to increasing levels of GA_3 suggests that gibberellin was limiting in kernels which have not been dried. Rood *et al.* (21) found that mature seeds contain predominantly glucosyl conjugates

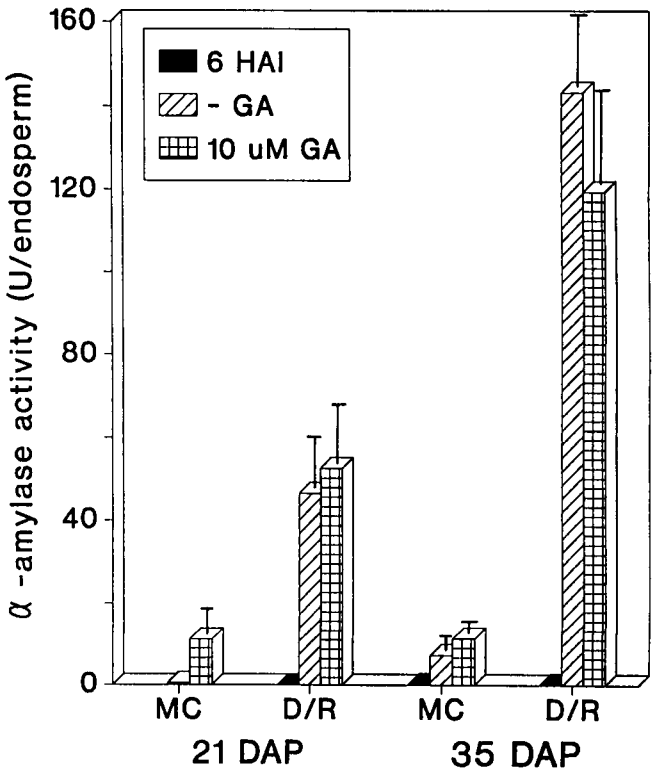

Figure 6. Levels of α-amylase in deembryonated prematurely dried and rehydrated (D/R) and moist control (MC) maize kernels. Kernels were incubated in GA_3-containing buffer at 28°C for 6 h before embryo excision. The enzyme activity was then determined immediately (6 HAI) or following a further 90-h incubation in the appropriate incubation buffer. Levels in 21-DAP moist-control tissue incubated in the presence of 10 μM GA_3 were not determined. Kernels from cobs harvested in 1988 (21 DAP) and 1989 (35 DAP).

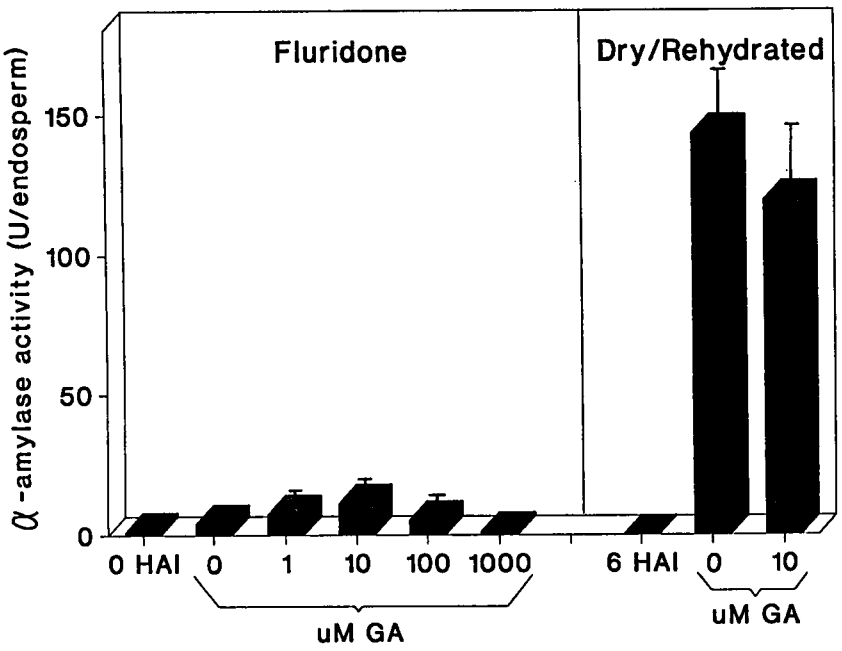

Figure 7. α-Amylase activity in response to GA_3 supplied to deembryonated 35-DAP fluridone-treated maize kernels. Embryos were excised immediately following surface sterilization of fluridone-treated kernels or after a 6-h incubation in buffer for dry kernels. Total incubation time was 96 h in all cases.

of gibberellins which are subsequently mobilized into active forms during germination. Thus, the gibberellin which accumulates during grain development is stored in an inactive form. The possibility exists that drying stimulates the endosperms to reversibly conjugate these molecules, as well as eliciting the ability to synthesize de novo bioactive-forms of gibberellin.

The significance of ABA in the physiology of the developing aleurone tissue only now is being established. Napier *et al.* (15) were unable to identify, by two-dimensional gel electrophoresis, novel ABA-induced proteins synthesized in developing wheat aleurone tissue, although they note that treatments which deplete ABA levels in developing tissue (*i.e.* drying and washing) provoke gibberellin-responsiveness. Thus, the ABA-regulated inhibition of α-amylase synthesis by developing tissue does not appear to be associated with the synthesis of novel ABA-induced proteins. Dooner (4), in contrast, was able to identify a variety of unrelated enzymes which are synthesized in wild-type maize kernels but not in ABA-insensitive *vp1* mutants. Interestingly, the failure of *vp1* kernels to produce these proteins is temporally correlated to the onset of precocious α-amylase synthesis during late development (24). The pleiotropic nature of this mutant leads us to speculate that ABA-insensitive, and perhaps ABA-deficient, mutants fail to produce a protein which serves to maintain α-amylase synthesis at low levels in developing maize grains.

There is an apparent lack of synchrony in the ability of embryo and aleurone layer to respond to drying. While the embryo is fully desiccation tolerant only around 30 DAP (Fig. 1), the dried and rehydrated 21-DAP deembryonated grains are able to produce α-amylase at levels equal to mature tissue (Fig. 4). Thus, this normal postgerminative event will occur in the absence of the kernel being germinable. The difference in the onset of desiccation tolerance may be related to the relative complexity of the two tissues. We might expect that a structure as complex as the embryo, which probably requires the survival of all tissues for germination, will show a more marked response to severe water loss than the aleurone layer, a tissue which will produce a detectable response to drying even if not all cells of the tissue survive. Alternatively, increased levels of embryonic ABA, which are known to occur as a result of desiccation stress in barley (20), may limit germination of the 21-DAP kernels.

Nicholls (17) offers evidence that detachment from the plant, possibly due to an interruption of the flow of photosynthate, is sufficient to trigger α-amylase synthesis by developing wheat aleurone tissue. In this study, we detached cobs from the plant but kept them hydrated for a period equivalent to the drying treatment; there is no resulting responsiveness of the aleurone layer as far as α-amylase activity is concerned. It is arguable that the cob itself contains a substantial reserve of carbohydrate. However, the kernels show only slight differences in dry weight accumulation between the moist control or the dried kernels. The dry weight of 21-DAP moist control kernels increase 8.5% during treatment relative to a 7.4% increase during drying. In contrast, both 35-DAP dried and moist control kernels actually lose dry weight (3.1 and 2.9%, respectively) during the treatment period (data not shown).

Our results suggest that in developing maize kernels, endogenous ABA plays a role in preventing embryo germination and suppressing α-amylase synthesis by the aleurone layer. However, for this tissue to achieve its full potential to produce the enzyme it must not only be free of the inhibitory effects of ABA, but it must also be competent to respond to gibberellin. A drying treatment, either prematurely imposed or as occurs normally during the course of kernel maturation, triggers the transition to complete competence.

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LITERATURE CITED

1. **Armstrong C, Black M, Chapman JM, Norman HE, Angold R** (1982) The induction of sensitivity to gibberellin in aleurone tissue of developing wheat grains. I. The effect of dehydration. Planta **154:** 573–577
2. **Bilderback DE** (1971) Amylases in developing barley seeds. Plant Physiol **48:** 331–334
3. **Brenner ML, Burr B, Burr F** (1977) Correlation of genetic vivipary in corn with abscisic acid concentrations. Plant Physiol **59:** 420
4. **Dooner HK** (1985) Viviparous-1 mutation in maize conditions pleiotropic enzyme deficiencies in the aleurone. Plant Physiol **77:** 486–488
5. **Evans M, Black M, Chapman J** (1975) Induction of hormone sensitivity by dehydration is one positive role for drying in cereal seed. Nature **258:** 144–145
6. **Fincher GB** (1989) Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. Annu Rev Plant Physiol Plant Mol Biol **40:** 305–346
7. **Fong F, Koehler DE, Smith JD** (1983) Fluridone induction of vivipary during seed development. *In* JE Kruger, DE LaBerge, eds, Third International Symposium on Pre-Harvest Sprouting in Cereals. Westview Press. Boulder, CO, pp 188–196
8. **Fong F, Smith JD, Koehler DE** (1983) Early events in maize seed development: 1-methyl-3-phenyl-5-(3-[trifluoromethyl] phenyl)-4-(1H)-pyridinone induction of vivipary. Plant Physiol **73:** 899–901
9. **Goldstein LD, Jennings PH** (1975) The occurrence and development of amylase enzymes in incubated, de-embryonated maize kernels. Plant Physiol **55:** 893–898
10. **Harvey BM, Oaks A** (1974) The role of gibberellic acid in the hydrolysis of endosperm reserves in *Zea mays.* Planta **121:** 67–74
11. **Hejgaard J, Gibbons GC** (1979) Screening for α-amylase in cereals. Improved gel-diffusion assay using a dye-labelled starch substrate. Carlsberg Res Commun **44:** 21–25
12. **Ingle J, Hageman RH** (1965) Metabolic changes associated with the germination of corn III. Effects of gibberellic acid on endosperm metabolism. Plant Physiol **40:** 672–675
13. **Kermode AR, Oishi MY, Bewley JD** (1989) Regulatory roles for desiccation and abscisic acid in seed development: a comparison of the evidence from whole seeds and isolated embryos. *In* PC Stanwood, MB McDonald, eds, Seed Moisture. Crop Sci. Soc. of America (Special Publication No. 14), Madison, WI, pp 23–50
14. **King RW** (1976) Abscisic acid in developing wheat grains and its relationship to grain growth and maturation. Planta **132:** 43–51
15. **Napier JA, Chapman JM, Black M** (1989) Calcium-dependent induction of novel proteins by abscisic acid in wheat aleurone tissue of different developmental stages. Planta **179:** 156–164
16. **Neill SJ, Horgan R, Parry AD** (1986) The carotenoid and abscisic acid content of viviparous kernels and seedlings of *Zea mays* L. Planta **169:** 87–96
17. **Nicholls PB** (1979) Induction of sensitivity to gibberellic acid in developing wheat caryopses: effect of rate of desiccation. Aust J Plant Physiol **6:** 229–240
18. **Oaks A, Winspear MJ, Misra S** (1983) Hydrolysis of endosperm protein in *Zea mays* (W64A × 182E). *In* JE Kruger, DE LaBerge, eds, Third International Symposium on Pre-Harvest Sprouting in Cereals. Westview Press, Boulder, CO, pp 204–212
19. **Raikhel NV, Hughes DW, Galau GA** (1987) An enzyme-immunoassay for quantitative analysis of abscisic acid in wheat. *In* J Fox, M Jacobs, eds, Molecular Biology of Plant Growth Control. Alan R Liss, Inc, New York, pp 197–207
20. **Robertson M, Walker-Simmons M, Munro D, Hill RD** (1990) Induction of α-amylase inhibitor synthesis in barley embryos and young seedlings by abscisic acid and dehydration stress. Plant Physiol **91:** 415–420
21. **Rood SB, Pharis RP, Koshioka M** (1983) Reversible conjugation of gibberellins in situ in maize. Plant Physiol **73:** 340–346
22. **Rood SB, Larsen KM** (1988) Gibberellins, amylase, and the onset of heterosis in maize seedlings. J Exp Bot **39:** 223–233
23. **Smith JD, McDaniel S, Lively S** (1978) Regulation of embryo growth by abscisic acid in vitro. Maize Gen Coop Newslett **52:** 107–108
24. **Wilson GF, Rhodes AM, Dickinson DB** (1973) Some physiological effects of viviparous genes vp_1 and vp_5 on developing maize kernels. Plant Physiol **52:** 350–356