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Endogenous Abscisic Acid Levels in Germinating and Nongerminating Lettuce Seed[1, 2]

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ABSTRACT

The concentrations of abscisic acid in Grand Rapids lettuce (*Lactuca sativa L.*) seeds imbibed under conditions which promote or inhibit germination were determined by electron capture-gas chromatography. The concentration of abscisic acid in dry seeds was 12 to 14 nanograms per 100 milligrams. During 24-hour imbibition, the abscisic acid content diminished more rapidly during conditions which allow germination (25 C in light) than in conditions which inhibited germination (35 C in light or darkness at 25 C). A decrease in endogenous levels of abscisic acid was not always correlated with germination.

It is well established that seed germination of the lettuce (*Lactuca sativa* L.) cultivar Grand Rapids is inhibited by darkness at moderate temperatures (1) and by high temperatures (30–40 C) in light (3, 18). Dark germination of Grand Rapids lettuce seeds is promoted by GA_3 (4) and ABA inhibits germination induced by GA_3 or light (5, 13). Increasing the concentration of GA_3 in the presence of ABA does not overcome ABA inhibition unless a cytokinin is also present (5). Kinetin has also been shown to largely overcome thermodormancy in lettuce seeds imposed by high temperature during imbibition whereas GA_3 has little effect (3, 11, 12, 18). Similarities in the effects of high temperature (35 C) and ABA and their interactions with kinetin and GA_3 have been shown recently (7).

Probably the absence of germination in darkness or at high temperatures is controlled at some stage by a high level of inhibitor(s) and/or a low level of promoter(s) (6). Gas chromatographic evidence for the presence of ABA in dry Grand Rapids lettuce seed has recently been reported (19). The present investigation was undertaken to determine the contents of ABA in Grand Rapids lettuce seeds during imbibition under conditions which prevent and allow germination.

MATERIALS AND METHODS

In the light *versus* dark experiment, Grands Rapids lettuce (*Lactuca sativa* L.) seeds were imbibed at 25 C in diffuse white light or in total darkness. In the 25 *versus* 35 C temperature experiments, seeds were imbibed in diffuse white light at these two temperatures.

Seeds (400 mg dry weight) were collected in test tubes and cold 80% ethyl alcohol was added. Seeds from the dark treatment were collected under a dim green safelight. Tubes with samples were then stored at −30 C until homogenized. Seed samples were homogenized in cold ethyl alcohol (80%) in a TenBroeck homogenizer and transferred to flasks, the final volume being 40 ml. The homogenates were left overnight at 5 C on a shaker.

The preparations were then filtered *in vacuo* through a Büchner funnel, the filtrates were collected in round bottomed flasks, and the extract was evaporated to near dryness using a rotary film evaporator under vacuum.

Extract purifications were based on the method described by Seeley (14). After evaporating the ethyl alcohol, the residue was taken up in water, and the pH was adjusted to 8.3 with 10% ammonium hydroxide. The aqueous extract was partitioned three times with equal volumes (30 ml) of dichloromethane which was discarded. The aqueous fraction was adjusted to pH 2.6 with 10% HCl and again partitioned three times with dichloromethane. The dichloromethane phases were pooled and evaporated to dryness. The residue was quantitatively transferred with ethyl acetate and divided equally between two test tubes and the ethyl acetate evaporated with a stream of dry nitrogen gas.

Quantitative determination of ABA in the seed extracts was achieved by adaptation of the electron capture-gas chromatography techniques described by Seeley and Powell (15), Seeley (14), and Braun (2). The present determinations were performed using a Perkin-Elmer 900 gas chromatograph equipped with a 3% OV-1 column and a Nickel-63 electron capture detector operated in the pulsed voltage mode (10,000 pulses/sec).

At the time of injection into the gas chromatograph, the diazomethylated extract was taken up in ethyl acetate. The quantity of methyl-*cis*,*trans*-ABA in the extract was then determined by reference to standard curves of the peak heights.

To substantiate that the chromatograph peaks measured actually represented esterified ABA, similarly prepared extract samples were esterified with N-ethyl-N′-nitro-N-nitrosoguanidine to yield the ethyl esters or with N′-nitro-N-nitroso-N-propylguanidine to yield the propyl esters. The appropriate retention time shift occurred in the sample extracts (2). Extracts subjected to each of the esterifying reagents were also subjected to UV light exposure and the appropriate isomerization from *cis*,*trans* to *trans*,*trans* isomer observed (9).

Abscisic acid data for each experiment were subjected to a one-way analysis of variance for unequal groups. Duncan's multiple range test at the 5% level as modified by Kramer (8) was used to separate group means of unequal replication (two to eight-replications).

RESULTS AND DISCUSSION

The contents of ABA in lettuce seeds before and during imbibition in dark or light (A, 1972 seed) and at 25 or 35 C (B, 1972 seed; C, 1973 seed) are shown in Figure 1. The initial endogenous ABA content of the dry seeds was 12 to 14 ng/100 mg, which is

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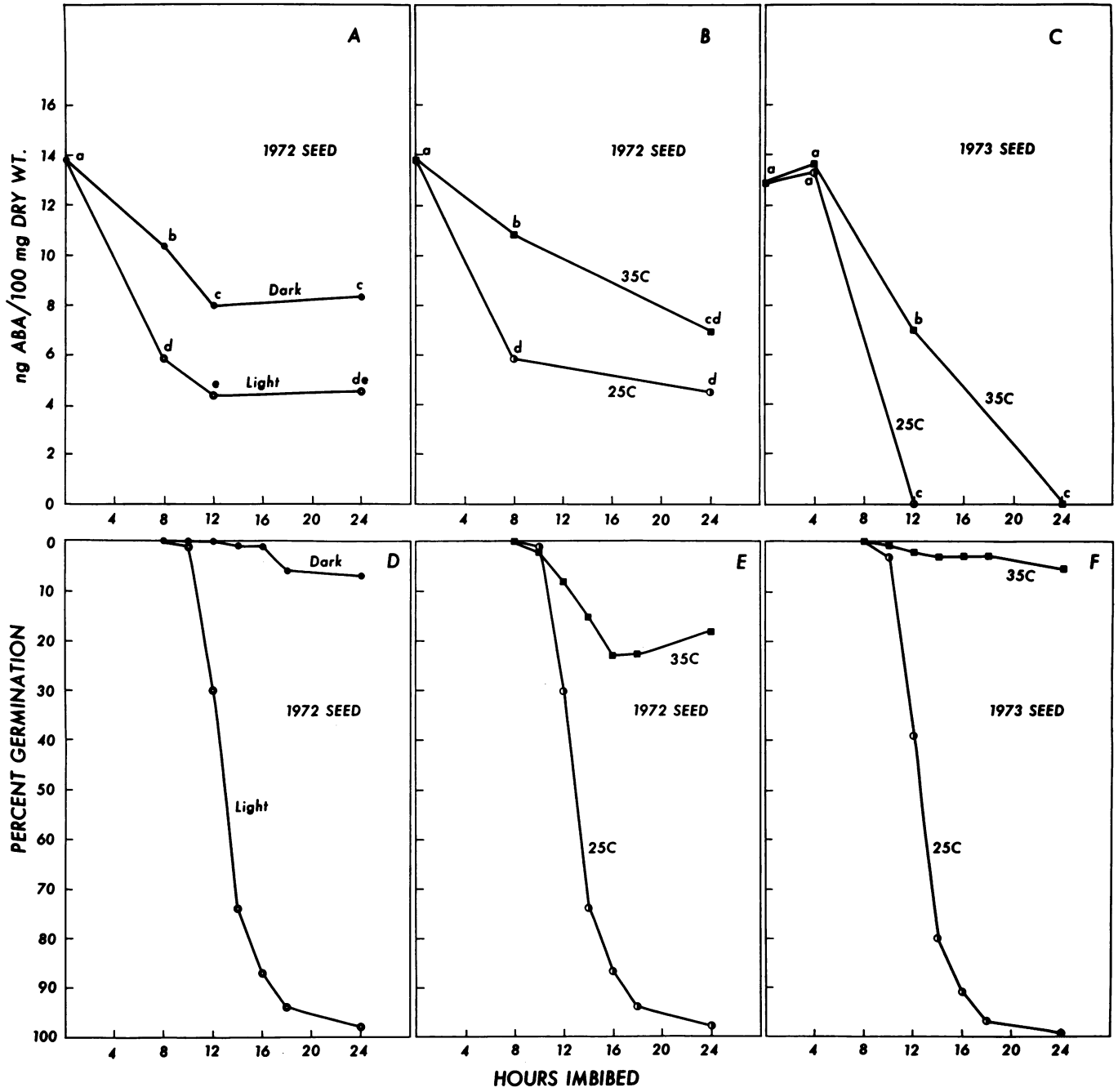

FIG. 1. ABA content and per cent germination of Grand Rapids seed during 24 hr imbibition; A, D: dark *versus* light, at 25 C, 1972 seed; B, E: 25 *versus* 35 C, in light, 1972 seed; C, F: 25 *versus* 35 C, in light, 1973 seed. Mean separation within each experiment is by Duncan's multiple range test as modified by Kramer (8), 5% level.

substantially lower than that reported in dormant seed of *Fraxinus americana* (45 ng/100 mg) (17), in dormant seeds of *Acer saccharum* (82.6 ng/100 mg) (20), and in young expanding strawberry leaves (40 ng/100 mg) (2).

During 24 hr of imbibition the ABA content was found to diminish more rapidly in conditions which allow germination, *i.e.* 25 C in light, than in conditions which inhibit germination, *i.e.* 35 C in light or 25 C in darkness (See Fig. 1, D–F for germination comparisons).

The decrease in ABA content may be due to its metabolism and/or leaching into the medium. McWha and Hillman (10) found that when lettuce seeds were imbibed in an ABA solution and then placed in water the seeds lost substantial ABA by leaching during the first 2 hr after transfer. Metabolism did not become a substantial cause of reduction until after 12 hr of imbibition. Leaching of an endogenous inhibitor, presumably ABA, into water medium has recently been reported (19). Decline of ABA content in *Fraxinus americana* seeds previously permeated with ^{14}C-ABA in dichloromethane was found to result largely from metabolism and only to a small extent from leaching (16).

Even though the ABA levels found in germinating and nongerminating seeds are significantly different at several points during imbibition, on a physiological basis the differences are very small and of doubtful physiological significance (Fig. 1, A–C). Investigation of lettuce seed germination in the presence of exogenous ABA indicates that ABA incorporation into lettuce seeds

in amounts much higher than the endogenous levels found in the present study only delayed germination. McWha and Hillman (10) report that germination of Great Lakes (photoinsensitive) seeds imbibed continuously at 25 C in a 10 μM ^{14}C-ABA solution became evident after 36 hr *versus* 8 to 10 hr for the water control. Calculations performed on their data indicate that at the time germination became evident the content of ^{14}C-ABA in the seeds was approximately 78 ng/100 mg dry weight (assuming 100 seeds/100 mg) and was still accumulating. In the present investigation the ABA content was never higher than 14 ng/100 mg of dry weight of seed. This suggests that germination is probably independent of the endogenous level of ABA. This conclusion is further supported by the observation that no ABA was detected in the 1973 seeds after 24 hr of imbibition at 35 C (Fig. 1C), even though germination was effectively inhibited (Fig. 1F) at this temperature. In the 1972 seeds (Fig. 1B) a significant level of ABA was found after 24 hr imbibition at both 25 and 35 C, but germination was not inhibited at 25 C and was substantially less inhibited at 35 C (Fig. 1E).

A lack of relationship between ABA content and germination has been shown for cold-requiring seeds of *Fraxinus americana*. Sondheimer *et al.* (16) found that these seeds were able to metabolize ABA both under conditions of continued dormancy (25 C) and stratification (5 C). However, only stratified seeds were able to germinate.

In the present investigation the initial content of endogenous ABA was much lower than that which appears to be necessary for inhibition of germination. The level of ABA diminished during imbibition under both germinative and nongerminative conditions. It is not known what amounts of endogenous ABA are present specifically at the active sites or are necessary to effect control on germination.

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