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Abscisic Acid Levels in Soybean Reproductive Structures during Development[1]

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ABSTRACT

Abscisic acid (ABA) concentrations and growth rates of developing soybean (*Glycine max* [L.] Merr. cv. Wye) seeds and pod walls were determined from anthesis to maturation using high pressure liquid chromatographic techniques. Developing soybean seeds contain up to 12,200 ng/g fresh weight of ABA compared to 330 ng/g fresh weight for pod walls. In the developing seeds ABA levels correlated with growth rates, being the highest during the most active growth period of seed enlargement, and then decreasing to less than 10 ng/g fresh weight at maturity. Higher levels of ABA were found to occur in the cotyledons and seed coats than the root-shoot axes at 21 days postanthesis. The time required for excised root-shoot axes to initiate growth in liquid culture decreased as seed development progressed and ABA levels of the seeds declined.

Abscisic acid (ABA) is recognized as a naturally occurring plant hormone of major importance in the regulation of plant growth and development. It has been implicated in such plant growth processes as senescence; abscission of leaves, flowers, and fruits; rest and dormancy of seeds, buds, and tubers; and inhibition of vegetative growth (1, 10–12, 16). In mature seeds, relatively high levels have been found in some species and its occurrence implicated in dormancy and the inhibition of germination (1, 4, 10–12, 15, 16, 18, 19, 21). In developing seeds, little is known of the concentration changes and the physiological role of ABA. In this investigation, we examined the ABA content and growth rates of the developing seeds and pod walls and the growth of root-shoot axes excised from the developing seed at different developmental stages. A preliminary report of this work has been presented (14).

MATERIALS AND METHODS

Plant Material. Soybeans (*Glycine max* [L.] Merr. cv. Wye) were grown to maturity in a controlled environment growth room (6,000 ft-c, 12-hr photoperiod, 75% relative humidity, 24 C day, 18 C night) in 18-cm plastic pots containing a Jiffy-Mix (Jiffy Products of America, West Chicago, Ill.) pea gravel mixture (1:1, v/v). Nutrients were supplied daily using a modified Hoagland solution (6). Samples of 20 pods were randomly selected from a population at regular intervals after full bloom (35 days postplanting) until maturation (60 days postanthesis). The developing seeds and pods were immediately dissected in a 4 C cold room, weighed and frozen in liquid N_2, and stored at −40 C until extraction for ABA analysis.

Germination of Root-Shoot Axes. Root-shoot axes from developing seeds were removed aseptically at 21, 28, 35, 45, and 60 days after anthesis. Freshly harvested pods were surface-sterilized for 8 min in a 5% sodium hypochlorite solution, and the developing seeds removed from the pod and rinsed in sterile distilled H_2O. Twenty root-shoot axes per treatment were incubated in two 125-Erlenmeyer culture flasks with 25 ml of sterile White basal medium (20) containing 10% sucrose (w/v) at pH 6. The flasks were rotated at 1 rpm under continuous fluorescent light (100 ft-c) at 25 C, and the medium was changed every 10 days. The number of root-shoot axes which showed visual signs of growth and elongation of the radicle in each treatment was recorded daily during the incubation period.

Preparation of Plant Extracts. All solvents used in these studies were ACS grade. The over-all scheme for the cleanup of plant tissue extracts and extraction of free ABA was the same as outlined by Sweetser and Vatvars (17). One to 10 g of plant tissue were removed from the freezer and extracted in a blender containing 75 ml of cold 80% methyl alcohol to which was added a known aliquot of ^{14}C-ABA (1.66 mCi/mmol, California Bionuclear Corporation, ^{14}C in carboxyl group). Partial cleanup of the extracts was made with conventional ether extractions at pH 8 and 2.8. Further cleanup was achieved by passing the sample through a column of Sephadex G-25 (0.7 × 30 cm) as described previously (17).

ABA Analysis by HPLC.[2] The ABA analysis of the soybean pod walls and seeds (see Figs. 3 and 4) was carried out with the HPLC procedure outlined by Sweetser and Vatvars (17). Briefly, this procedure uses a Du Pont model 820 liquid chromatograph with a strong cation exchange column, Zipax-SCX (3 m × 2.1 mm i.d.). The mobile phase was distilled H_2O adjusted to pH 1.7 with HNO_3. The column was operated at 50 C, 500 to 900 p.s.i. and a flow of ~1 ml/min.

The ABA levels in soybean seed parts (Table I) were determined by a modified HPLC procedure in which the ABA separations were made using a Du Pont model 840 liquid chromatograph with a Whatman Partisil-10-SCX column (25 cm × 4.6 mm i.d.). As shown (Figs. 1 and 2), with this column-mobile phase system, it is possible to make ABA separations at room temperatures in less than 6 min. The ABA of the samples was calculated from a standard curve constructed from peak height measurements obtained from known amounts of ABA (Polyscience Inc.). The per cent ABA recovery was based on the per cent of the total radioactivity recovered in the ABA peak from ^{14}C-ABA spike added prior to blender extraction. The recoveries of the ^{14}C-ABA were quite consistent, generally varying between 40 and 50% with a standard deviation of 5 to 7%. The authenticity of ABA was confirmed by mass spectrographic analysis of collected ABA fractions.

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[2] Abbreviation: HPLC: high performance liquid chromatograph.

Table I. *Distribution of ABA in Developing Soybean Seeds Sampled at 21 Days Postanthesis*

Seed Parts	ABA Content	
	ng/g	ng/seed
Seed Coats	10,108	398
Cotyledons	11,909	955
Root-shoot axes	6,139	59
Intact seeds	14,890	1441

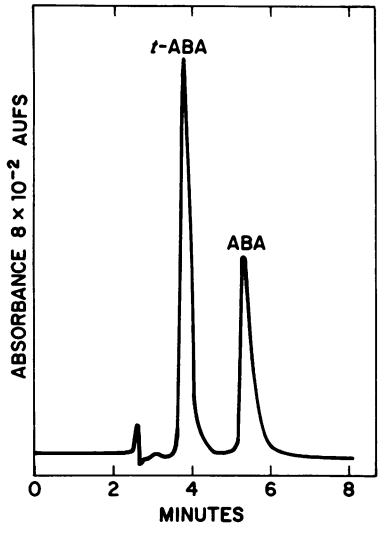

FIG. 1. HPLC separation of a 300-ng mixture of ABA and *t*-ABA. Chromatographic conditions were: Partisil-10-SCX column 25 cm × 4.6 mm with distilled H_2O (pH 3.5 with .05 M KH_2PO_4) mobile phase; ambient °C, 600 p.s.i., and a flow of 1.2 ml/min.

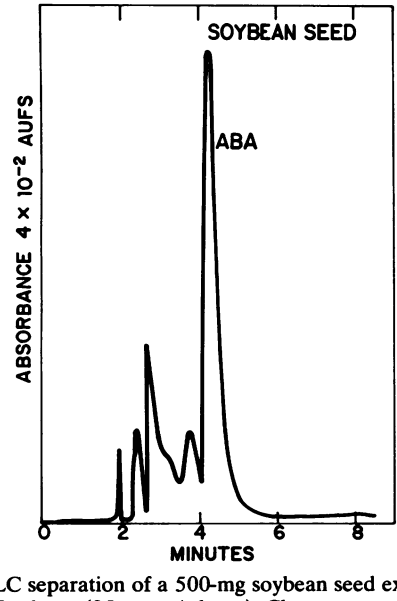

FIG. 2. HPLC separation of a 500-mg soybean seed extract with the Partisil-10-SCX column (25 cm × 4.6 mm). Chromatographic conditions were: mobile phase distilled H_2O at pH 1.7 with HNO_3; ambient °C, 800 p.s.i., and a flow of 1.6 ml/min.

RESULTS AND DISCUSSION

Fresh and dry weight changes and ABA concentrations of pod walls and developing seeds of soybean determined from anthesis to maturation are presented in Figures 3 and 4, respectively. The changes observed in fresh and dry weights of developing soybean seeds and pod walls (Figs. 3B and 4B) are similar to those recently reported (5, 13). ABA could be detected in all seed and pod wall extracts sampled throughout the course of seed development. In developing soybean seeds (Fig. 4A), the ABA concentration is unexpectedly high with maximum levels up to 12,200 ng/g fresh weight. These values are the highest reported in the literature for seeds or any other plant tissue using similar analytical procedures (11, 12, 17). The ABA concentration in the developing seed on a ng/g fresh weight basis increases shortly after full bloom (0 days postanthesis), reaching a maximum level during the active growth stage of pod filling (15 days postanthesis), and then it decreases steadily to less than 10 ng/g fresh weight at maturity and plant senescence (60 days postanthesis). Compared with the developing seed, the pod wall in general contains smaller amounts of ABA (Fig. 3A). The ABA concentration on a ng/g fresh weight basis in the pod wall decreases as the wall increases to its maximum fresh weight. The ABA levels then increase just prior to pod senescence to a maximum concentration of 332 ng/g fresh weight, which is similar to the concentration changes reported for developing and senescing leaves (9). In contrast to the developing seed, the maximum ABA concentration in the pod wall is not associated with rapid growth rates but rather with ABA concentration changes in the seed and plant senescence. Similar ABA concentration changes over the entire growth cycle of the developing pod wall and seed were obtained in three separate growth analyses with maximum ABA

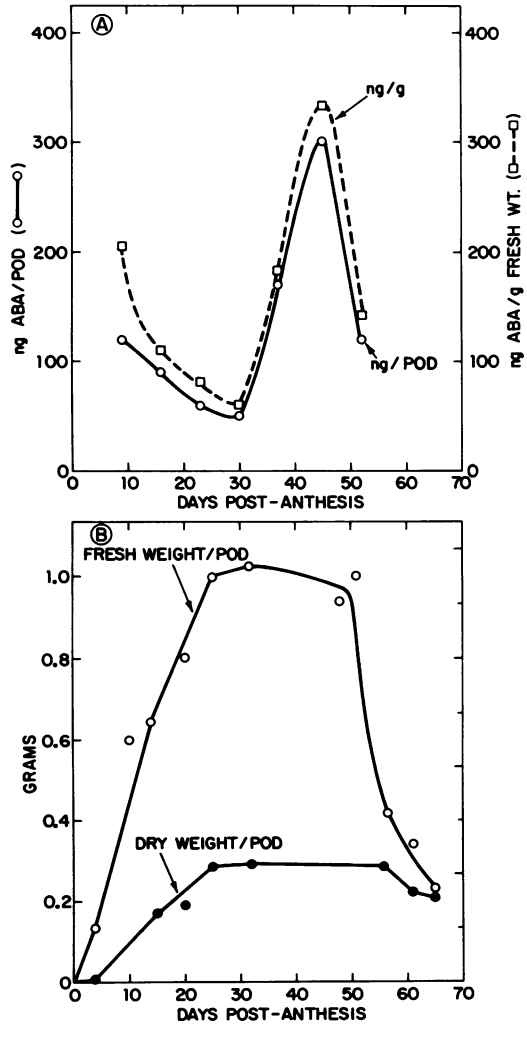

FIG. 3. Changes in fresh and dry weight (A) and ABA concentrations of the developing soybean pod wall (B), sampled at regular intervals from anthesis to seed maturation.

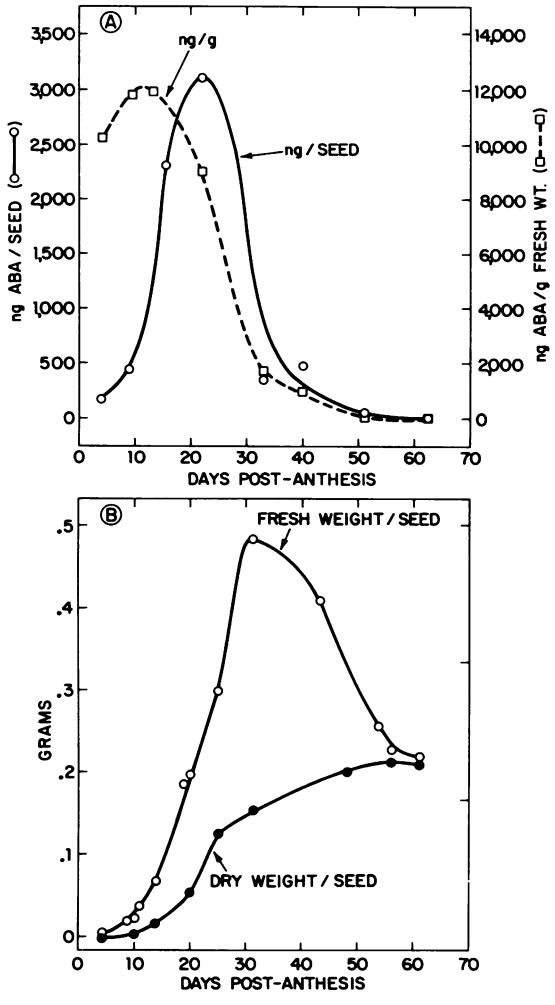

FIG. 4. Changes in fresh and dry weight (A), and ABA concentrations of the developing soybean seed (B), sample at regular intervals from anthesis to seed maturation.

levels in the seed ranging from 6,500 to 14,000 ng/g fresh weight.

Maximum ABA accumulates in the developing seed during the most active growth period of seed enlargement. Rapid increases in the rate of fresh and dry weights of the seed from 15 to 32 days postanthesis (Fig. 4) appear to be correlated with a rapid rise to maximum ABA concentration in the developing seed. A comparison of the rate of dry weight accumulation and the ABA profiles suggests that the onset and rate of increase in growth and in ABA content are correlated. A decrease in the rate of dry weight accumulation of the seed is paralleled by a sharp decrease from high ABA concentration to less than 10 ng/g fresh weight. The ABA concentration changes over the entire growth cycle of the developing soybean seeds reported here are the first for any major legume crop. The ABA levels and concentration changes for developing soybean seeds and pod walls differ from those reported for other crop species. For example, in developing cotton fruits (3), a rapid rise in ABA levels is correlated with the period of young fruit abscission and a second peak at fruit maturation during the period of fruit wall senescence. In grape berries (2), maximum ABA accumulates at fruit maturation during fruit ripening. In wheat (9, 14), maximum ABA accumulates in developing seeds during the most active growth period, similar to soybeans but the maximum level is approximately 30-fold less. It appears that the maximum ABA concentration changes may vary with each species and its regulatory role in reproductive development may differ for each crop species. In addition, the data presented for developing soybeans support the view that ABA need not be regarded solely as a general inhibitor of plant growth or a substance involved only in dormancy, senescence, abscission, and water stress, but also seed growth and development. The question of whether endogenous ABA stimulates seed growth and development is difficult to answer because there is no reported effective means of regulating endogenous ABA levels in developing seeds. Furthermore, ABA could either be synthesized within the seed or translocated from other parts of the plant to the seed.

To explore the ABA distribution within the developing seed, a sample of seeds harvested at 21 days postanthesis was dissected and carefully separated into seed coats, cotyledons, and root-shoot axes and extracted for ABA analysis. Higher ABA concentrations were found in the cotyledons than the seed coats and root-shoot axes (Table I). The root-shoot axes contained the lowest ABA concentrations. These results indicate that ABA is not equally distributed within the seed, suggesting either different rates of biosynthesis, metabolism, or accumulation.

To determine the possible significance of the endogenous ABA levels in the developing seed, we determined the ability of the root-shoot axes to grow in liquid culture when excised from the developing seeds at different developmental stages. As shown in Figure 5, all root-shoot axes at all stages of development were found to grow, but the lag between excision and growth decreased with increasing development and declining ABA concentration of the intact seed. Root-shoot axes excised from mature seeds containing less than 10 ng/g fresh weight of ABA grew within 2 days whereas those excised at 21 days postanthesis and (stage 1) containing high ABA levels (10,000 ng/g fresh weight), required nearly 50 days. Intermediate lag times for root-shoot growth were observed at 28 (stage 2), 35 (stage 3), and 45 (stage 4) days, corresponding to increases in development and a decline in the ABA concentration of the intact seed. A marked increase in germination capacity of the root-shoot axes from 21 days to 60 days postanthesis and its association with a decline in ABA content are consistent with the hypothesis (4, 7, 8) that ABA plays a regulatory role in suppressing germination, allowing normal development of the embryo by inhibiting precocious germination. For example, in developing cotton seeds, the data of Ihle and Dure (7, 8) suggest that the ABA in the embryo is absorbed from the tissues surrounding the embryo where it is produced, and regulates precocious germination. Although in their studies, no measurements of endogenous ABA in the developing seed were presented, our results of the relationship of the endogenous ABA levels and

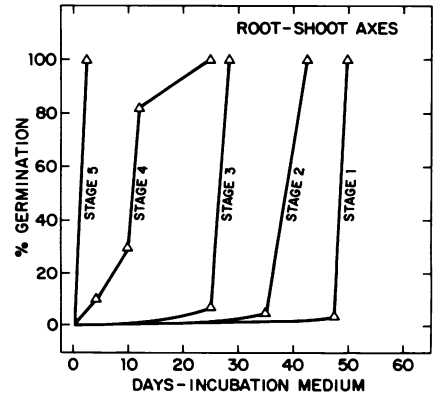

FIG. 5. Germination velocity of soybean root-shoot axes. Lag time from excision to growth of root-shoot axes excised from developing seeds at various stages after anthesis: stage 1 (21 days), stage 2 (28 days), stage 3 (35 days), stage 4 (45 days), stage 5 (60 days to mature). Each treatment represents 20 root-shoot axes incubated in culture flasks containing White's basal medium and 10% sucrose.

germination velocity of excised root-shoot axes from developing soybean seeds would tend to support this hypothesis.

The results from the present study indicate that the ABA content of developing soybean seeds is unexpectedly high during the initial stages of seed growth and development compared to vegetative plant parts. Conclusions regarding an ABA regulatory role in seed development appear premature at this time because of our limited knowledge of the mode of action of ABA in developing seeds at the biochemical level. Elucidation of its regulatory roles in reproductive structures may provide information useful in increasing seed yields.

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