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Abscisic Acid Levels during Early Seed Development in *Sechium edule* Sw.

Paolo Vernieri*, Pierdomenico Perata, Roberto Lorenzi, and Nello Ceccarelli

Dipartimento di Biologia delle Piante Agrarie, Università degli Studi di Pisa, Viale delle Piagge No. 23, 56124 Pisa, Italy

ABSTRACT

The time-course growth of single tissues in pollinated and unpollinated ovules of *Sechium edule* Sw. is described in relation to the endogenous levels of abscisic acid. Quantitation of abscisic acid (ABA) in the minute amounts of material obtained after ovule dissection has been performed by using a highly specific and sensitive solid-phase radioimmunoassay based on a monoclonal antibody raised against free (S)-ABA. While the absolute amount of ABA rises in both types of ovules, only in unpollinated ones does this leads to an increase in the hormone concentration. Infact in pollinated ovules the rapid growth following pollination prevents, through a dilution effect, the increase in ABA concentration. Growth patterns and endogenous ABA levels are similar for integuments and nucellus tissues either in pollinated or unpollinated ovules. It is suggested that the growth inhibition induced by the increase in ABA concentration after anthesis could be counteracted by the pollination triggered fast ovule growth.

Accumulation of ABA during seed development has been reported in many species (10, 14, 17), and it is generally considered to be implicated in the regulation of seed dormancy and germination (19, 20). While the role of ABA as a unique factor in inducing seed dormancy has been questioned in recent years (20, 22), evidence has been reported in support of a specific role of ABA in the prevention of "*in vitro*" and "*in vivo*" precocious germination of seeds through specific genome regulation (1, 2, 9, 16). Embryo culture experiments in a number of species have shown that ABA inhibits the synthesis of a set of germination specific proteins while allowing the accumulation of storage proteins and their respective mRNAs (3, 8, 13). However, most studies on ABA in seeds concern the levels and the possible role of this hormone in relation to embryo development and germination while very little information is available on the very early stages of ovule development immediately after pollination.

Seeds of *Sechium edule*, a viviparous species, contain high levels of gibberellins and cytokinins throughout seed growth and development (11, 12). Endogenous levels of ABA in the same seeds have been also investigated (4), and absence of hormone accumulation in coincidence with the maximal seed growth, as observed in other species (1, 10), has been noticed.

We report here on the quantification of ABA in integuments and nucellus of pollinated and unpollinated *Sechium* ovules. The very small amount of tissues available for each stage of ovule development has raised the need for a very sensitive and accurate methodology. This was accomplished by using a recently developed solid-phase RIA[1] based on a monoclonal antibody against free (S)-ABA (18). This monoclonal antibody has affinity and specificity high enough to allow the precise quantitation of very low levels of ABA in aqueous plant crude extracts.

MATERIALS AND METHODS

Plant Material

Plants of *Sechium edule* Sw. (Fam. Cucurbitaceae, tribe: Sicyoideae, common name: "chayote") were grown in the field during June to October 1988 and flowers were singularly tagged at anthesis. Ovaries were dissected and single tissues measured and weighed throughout seed development in order to characterize the growth patterns.

The material for the experiments on ABA content in pollinated and unpollinated ovules was obtained as follows. During September a sufficient amount of female flowers was selected on the basis of synchronous flower opening and divided in two groups. The first group was hand pollinated, whereas flowers of the second group were capped with cheesecloth in order to prevent pollination. Ovaries harvested at anthesis in coincidence with hand pollination and flower capping were referred to as time 0. Ovaries from the two groups were collected and dissected every 2 d and processed as follows.

Extraction

Fruits were opened, ovules were dissected on ice under a microscope, and the material was weighed and immediately frozen in liquid nitrogen. Isolated ovule tissues (nucellus and integuments) were then separately extracted in distilled water (v/w = 20:1) for 16 h at 4°C in the dark. Extracts were centrifuged (13,000*g*, 10 min) and the supernatants, opportunely diluted, used for RIA analysis. Water extraction efficiency for ABA in *Sechium* tissues was previously checked (18) and found to be more than 95%.

ABA Determination

Quantitation of ABA in the tissues was accomplished employing a solid-phase RIA (18) based on the use of a monoclonal antibody (DBPA 1) raised against free (S)-ABA.

[1] Abbreviation: RIA, radioimmunoassay.

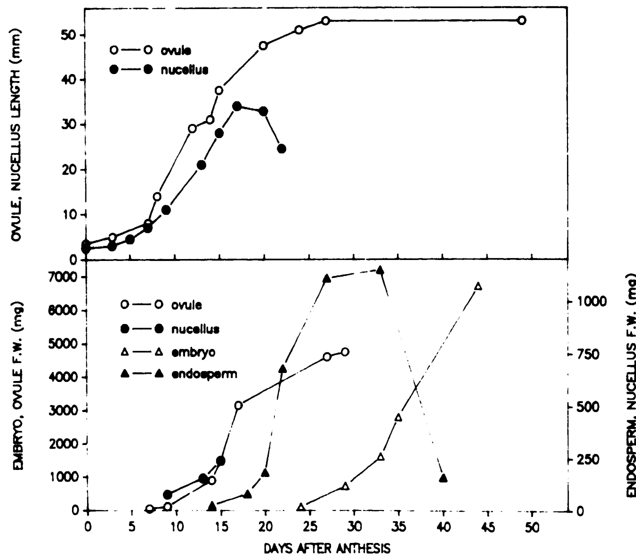

Figure 1. Time-course growth of single seed tissues after anthesis. Fifteen days after anthesis nucellus become undetachable from integuments.

Validation of RIA Results

The presence of competitive interferences was investigated after HPLC fractionation of the crude aqueous extracts of both nucellus and integuments. An HPLC instrument (LDC) equipped with a UV absorbance detector operating at 254 nm was used. The column (15 cm × ¼ inch o.d., packed with LiChrosorb RP18, 10 μm) was eluted at a flow rate of 1 mL/min using different proportions of methanol and water (added with 0.05 M acetic acid): 30% methanol for 6 min; a linear gradient 30 to 50% methanol for 20 min; 50% methanol for 6 min; a linear gradient 50 to 100% methanol for 15 min. Two mL fractions were collected, dried under vacuum, and resuspended in 75 mM PBS (pH 7). Each fraction was assayed in triplicate by RIA.

Noncompetitive interferences were evaluated by internal standardization experiments. In brief, aliquots of crude aqueous extracts were added to standard curves, and the recovered ABA quantities were plotted against the amounts of ABA added, in order to check the parallelism of the lines obtained.

RESULTS

The growth pattern of single components of the *Sechium* seed after pollination is shown in Figure 1. Following pollination a rapid growth of both integuments (see ovule length, Fig. 1) and nucellus takes place, while the endosperm and the embryo are in a state of arrested development which lasts for about 10 and 20 d, respectively. The phase of rapid embryo growth begins when the integuments and the endosperm are reaching their maximal size.

As shown in Figure 1, the growth period of the endosperm is relatively short and characterized by a very high growth rate. Concomitantly with the maximal growth rate of the endosperm the degeneration of nucellus begins. Before d 12 after pollination the nucellus is the only tissue, beside integuments, which shows appreciable growth. On the whole,

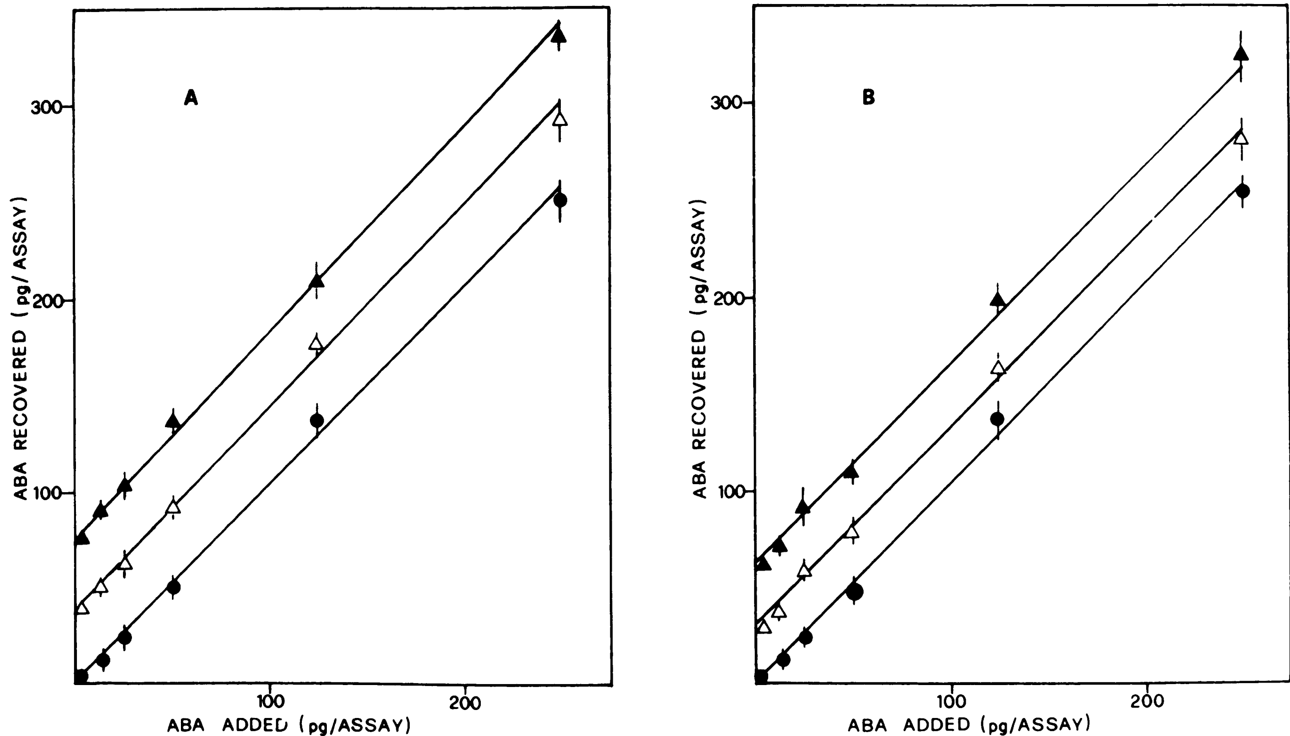

Figure 2. Internal standardization with integuments (A) and nucellus (B) crude aqueous extracts: ●; standard curve Δ: standard curve plus 10 μL extract ▲: standard curve plus 20 μL extract. Bars = SE (n = 3).

therefore, seed growth in *Sechium* during the 25 d after pollination is predominantly ascribable to integuments, nucellus, and endosperm while embryo growth is quite slow.

Preliminary experiments showed the presence of ABA in the whole ovules a few days after pollination. Although the size of the ovule in *Sechium* is quite large, the weight of the single tissues isolated is in the order of milligrams, therefore hampering the analysis of endogenous ABA by physicochemical methods. We therefore decided to investigate the concentration of ABA in the isolated integuments and nucellus by RIA. While the characteristics of the antibody used have been described (18), the reliability of the results obtained analyzing these tissues was checked as described in "Materials and Methods." The parallelism of the lines (shown in Fig. 2) obtained by plotting the recovered against added ABA in the presence of constant aliquots of aqueous plant extract demonstrates the absence of interfering substances in nucellus and integuments extracts. Moreover the absence of cross-reacting substances, other than ABA, in the same extracts was ascertained by assaying the single fractions after HPLC separation of the crude aqueous extracts. As shown in Figure 3 only insignificant cross-reactivity was observed in the extracts.

Endogenous levels of ABA in integuments and nucellus from pollinated and unpollinated ovules are presented in Figure 4. While the analysis of pollinated ovules was extended for 20 d after pollination, data for unpollinated ovules are limited to 10 d after full bloom when abscission of these ovaries occurred.

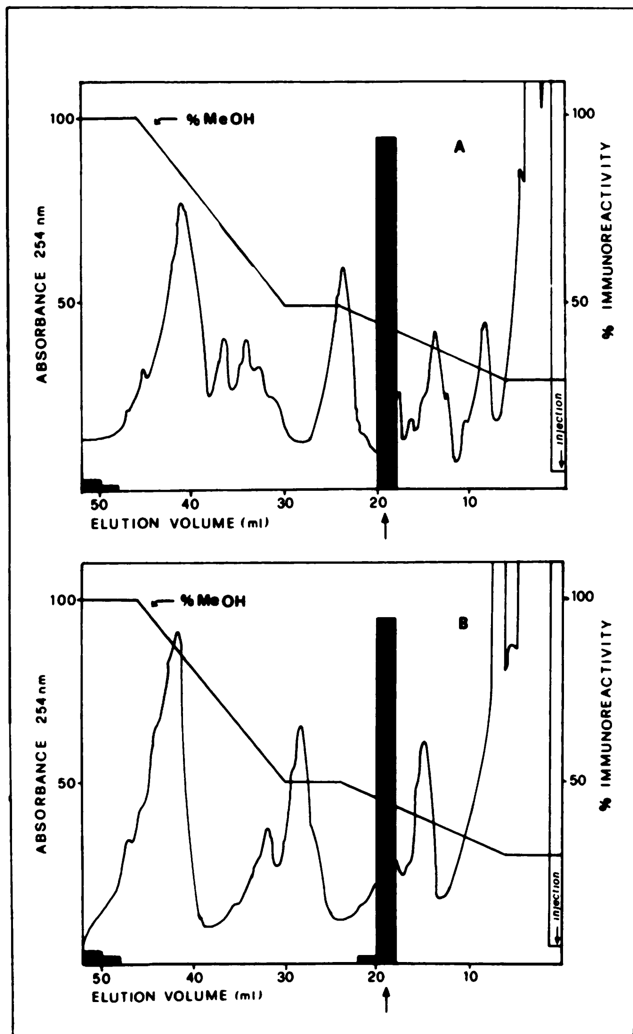

Figure 3. Distribution of immunoreactivity on HPLC fractionated crude aqueous extracts of integuments (A) and nucellus (B). The arrow indicates ABA migration.

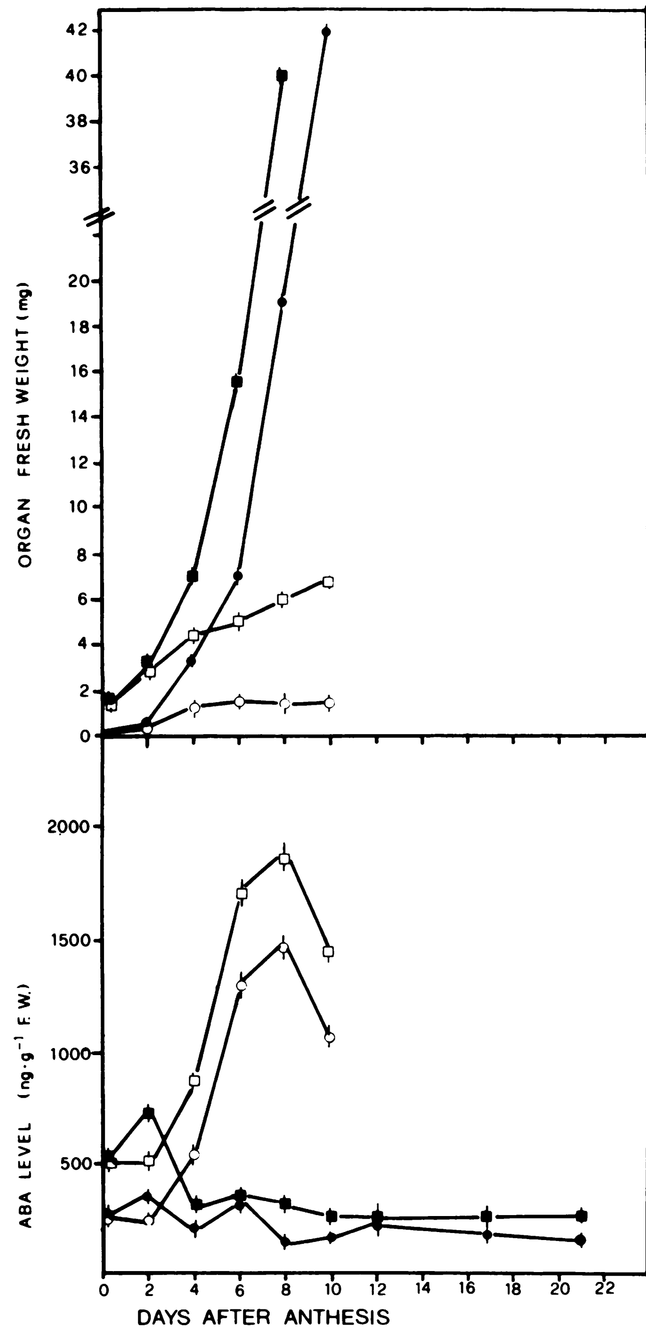

Figure 4. Pattern of endogenous ABA concentration and growth rate in pollinated (■, ●) and unpollinated (□, ○) ovules after anthesis. Nucellus (●, ○); integuments (■, ●). Bars = SE (n = 6).

In pollinated ovules the concentration of ABA does not show noticeable changes during the 20 d after pollination, although, starting at d 3, a dramatic increase in the fresh weight of integuments and nucellus is observed. Unpollinated ovules, while experiencing only a modest increment in fresh weight during the 10 d following full bloom show a considerable increase in the concentration of ABA which rises three to four times above the initial level. It is interesting to note that the increase of ABA concentration in unpollinated ovules coincides with the phase of rapid weight increment in pollinated ovules. No significant differences in the pattern of ABA concentration were observed between nucellus and teguments either in pollinated or unpollinated ovules.

DISCUSSION

In *Sechium edule* the feasibility of morphological separation and the easiness to obtain discrete amounts of material allow the characterization of the time-course growth of single ovule tissues. These characteristics, together with the availability of a very sensitive and specific immunoassay allow the investigation of the hormonal content of single ovule tissues in the first stage of development after pollination and the comparison between pollinated and unpollinated ovules.

Almost all information concerning ABA during seed development is restricted to the stages coincident with embryo growth, while most lacking are data on the levels and function of this hormone in seed tissues immediately after pollination. The large body of data concerning ABA in later stages of seed development suggests a role for this hormone in the physiology of seed maturation and germination (3, 9, 13). However, the significance, if any, of ABA in the very early stages of ovule development following pollination, as evidenced by the few reports available (5, 6, 14), is completely unknown. Moreover, information on endogenous levels of ABA in single seed tissues at this early stage of development is lacking.

The results obtained in the present study suggest that, besides the role in the prevention of precocious germination, ABA could also be involved in the very early ovule growth in relation to the pollination event, before the growth of embryo begins. In *Sechium* the endogenous concentration of ABA does not rise after pollination while the ovule is experiencing a very rapid increase in fresh weight. This picture is quite different from the one often observed in later stages of seed development when an increase in ABA concentration is usually associated with the most rapid phase of embryo growth (7, 10, 15, 20, 21).

In fact the concentration of ABA in pollinated ovules remains almost constant at around 200 ng/g. fresh weight, at least until d 21 after pollination when the ovule weighs more than 3 g. Nevertheless, the total amount of ABA per ovule from pollination to d 21 rises from 0.66 to 886 ng (Table I). This suggests active synthesis in the ovule or import of ABA from other maternal tissues. However, ABA concentrations do not increase owing to a dilution effect due to the rapid growth of the ovule following pollination.

In the absence of pollination the ovules undergo some growth, although at a much lower rate than pollinated ones. At the same time, starting at d 4, ABA concentration rises from the initial 765 (d 0) to 3400 ng/g fresh weight at d 8 after full bloom. As clearly evident in Figure 4, the increase in ABA concentration in unpollinated ovule starts (d 4) together with the beginning of the very rapid growth of pollinated ovules.

Table I. *Total Amount of ABA in Integuments and Nucellus of Pollinated Ovules during the 3 Weeks following Pollination*

Values ± SE, $n = 6$.

Days after Pollination	ABA content	
	Teguments	Nucellus
	ng/organ	
0	0.62 ± 0.2	0.04 ± 0.01
2	1.69 ± 0.4	0.14 ± 0.01
4	2.58 ± 0.5	0.72 ± 0.2
6	6.70 ± 0.7	1.60 ± 0.3
8	28.58 ± 5	5.53 ± 1
10	67.37 ± 6	12.85 ± 0.5
12	102 ± 10	21.48 ± 4
13	206 ± 17	51.71 ± 5
17	520 ± 25	60.0 ± 4
21	846 ± 32	40.00 ± 3

The observed differences in ABA levels between pollinated and unpollinated ovules suggest, as a working hypothesis, that following anthesis the ovules are subjected to an accumulation of ABA, either as a result of "*in loco*" synthesis or import, which, in turn may contribute to the ovule degeneration in absence of pollination. The pollination triggers ovule growth, thus compensating for the increase of ABA and keeping the level constantly low. Therefore, the different ABA concentrations in pollinated and unpollinated ovules do not seem to be the result of a variation of hormone import rate or metabolism. The lower ABA concentration in pollinated ovules is likely due to the dilution arising from the fast organ growth rate stimulated by pollination.

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