

Permeability of Seed Coats to Water as Related to Drying Conditions and Metabolism of Phenolics¹

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ABSTRACT

The seed coat of *Pisum elatius* is normally impermeable to water. When seeds are dried in the absence of oxygen their coats are totally permeable to water. Structural differences are observed between permeable and impermeable seed coats. In the genus *Pisum*, species with normally impermeable seed coats have a high content of phenolics and of catechol oxidase, while seed coats of *P. sativum* contain very little catechol oxidase and have a very low content of phenolics. Such differences are not noted in the cotyledons. We hypothesized that during dehydration of seeds, oxidation of phenolic compounds in seed coats through catalysis of catechol oxidase in presence of O₂ might render the seed coats impermeable to water.

The permeability of the seed coat of the Leguminosae has been the subject of many studies (1, 3, 7), but little is known about its control. Conditions during seed storage may effect permeability, and mechanical damage usually increased permeability to water and breaks dormancy caused by hard seededness. In *Ononis sicula* a genus of the Papilionaceae seed color appears to be correlated with permeability (4), and structural differences between green-brown and yellow seeds have been reported (5). The phenolic compounds of the seed coat may be involved in permeability changes in *Hedysarum* a genus of the Leguminosae (2).

The seed coat of the wild type peas *Pisum elatius* and *Pisum fulvum* are rather thick and brown in color and are impermeable to water, whereas the seed coat of the cultivated pea *Pisum sativum* is thinner, yellowish green to light brown, and is permeable to water.

We report some preliminary comparisons of seed color, phenolic compounds, and levels of enzymes oxidizing phenolics and the permeability of the seed coat in the genus *Pisum*.

MATERIALS AND METHODS

Pea seeds, *Pisum sativum* var. Alaska were purchased either from Ferry Morse Seed Co. or grown in Jerusalem. Seeds of *P. elatius* and *P. fulvum* were all collected from plants grown in Jerusalem. Seeds of *Cercis siliquastrum* and of *Robinia*

pseudacacia were collected from single trees in the Botanical Gardens of the Hebrew University. Fruit set was started at the same time for all the pods from which the seeds were collected. The seeds were dried over CaCl₂ in desiccators for 3 days under four different conditions—in air, in an atmosphere of pure oxygen, under vacuum, or in an atmosphere of pure nitrogen. For inhibition studies the seeds were put in a beaker and covered with distilled water for 24 hr. The percentage of imbibed seeds was determined. In most cases it was an all or nothing effect. The seeds either imbibed fully or failed to take up water at all, as determined by weighing.

Preparation of Extracts for Enzyme Determination. Seed coats were ground in 0.4 M sucrose 0.1 M phosphate buffer, pH 7.3, and the homogenate was centrifuged at 20,000g for 30 min to give a particulate and a supernatant fraction. The particulate fraction was resuspended in the same buffer used for grinding. In the case of cotyledons, grinding was carried out as above but the homogenate was first centrifuged for 10 min at 1,000g, and the precipitate was retained and resuspended in buffer used for grinding. The supernatant was centrifuged again at 20,000g for 30 min to give a particulate, and a supernatant fraction and the 20,000g precipitate also were resuspended in the buffer used for grinding.

Determination of Catechol Oxidase. Activity was according to Harel and Mayer (6) using 5 mM 4-methyl catechol as substrate. Phosphate or citrate buffer (0.1 M) was added to the reaction mixture to give a final pH of 7.3 or 5.3.

Ascorbic Acid Oxidase Activity was determined by following O₂ uptake with a polarographic oxygen electrode at pH 7.3 in 0.1 M phosphate buffer, using 50 mM Na ascorbate as substrate. The phenolic content of the tissues was measured according to Mapson *et al.* (7) using the sodium molybdate method which determines *o*-diphenols.

RESULTS AND DISCUSSION

The color of the seed coat and its permeability to water might be determined by conditions during the last stages of drying. In order to test this assumption, we collected pods (legumes) of *Pisum elatius* as they began to dry, when the seeds inside were still green. The seeds were dried over CaCl₂ in desiccators at room temperature for 3 days under four different conditions: in air, in an atmosphere of pure oxygen, under vacuum, or in an atmosphere of pure nitrogen. The results in Table I indicate that both the browning of the seed coat and its impermeability depend on the presence of oxygen during the last stages of drying of the seeds.

The green coats of seeds dried under nitrogen or *in vacuo* could not be transformed into brown ones by reimbibing the seeds and then drying them in air. Treatment of green-coated seeds with 1% H₂O₂ for up to 24 hr also failed to change seed color. In both treatments, the seeds also remained permeable to

¹The untimely death of Ernest Sondheimer will be felt by all plant biochemists who are deprived not only of an outstanding contributor but also of a personal friend. This paper is dedicated to his memory and in appreciation of our close contacts and common interests.

Table I. Effect of Different Drying Conditions on the Seed Coat Color and Permeability to Water of *P. elatius*

The experiments were carried out in triplicate.

Drying Conditions	Seed Coat Color	Seeds Imbibed	Permeability of Seed Coat to Water
		%	
Air	Brown	0	Impermeable
Oxygen	Brown	0	Impermeable
Vacuum	Green	100	Completely permeable
Nitrogen	Green	100	Completely permeable

Table II. Effect of the Drying Conditions on the Permeability of the Seed Coat of *Cercis siliquastrum*

The seeds were collected while still green and were dried under varying conditions. Results are based on 300 seeds for each treatment.

Conditions of drying	Imbibed
	%
Air	40
Oxygen	50
Vacuum	87
Nitrogen	82

water. Apparently, once the seed has been dried in the absence of oxygen, subsequent exposure to oxygen or mild oxidizing agents cannot turn the seed coat from green to brown or change its permeability.

It seemed possible that conditions during drying might affect the structure of the seed coat. Scanning electron microscopy was used to study surface structure in *P. elatius* dried in air or in vacuum (Figs 1-6). Clear differences were observed in the outer structure of the seed coat of the two types of seeds. The seed coats of the air-dried seeds have organized and regular structures while the seeds of the vacuum-dried seed showed deformations and structural irregularity. This is probably due to disturbances in cutin deposition due to the treatment.

The question whether the oxygen dependency of the seed coat color and its impermeability to water is a general phenomenon in the Leguminosae was tested on two other species, seeds of *Cercis siliquastrum* and *Robinia pseudacacia*. Both plants normally have brown and impermeable seeds. The effect of the conditions during drying on the permeability of the seed coat of *Cercis* is shown in Table II.

In contrast to the pea species, in *Cercis siliquastrum* green and brown seeds and seeds of intermediate color were found in each treatment. However, the majority of the green seeds were observed in the samples dried *in vacuo* or under N₂. Since there were some brown seeds in this group and some green seeds in the air and O₂ treatments, the permeability was not quite as uniform within a batch as for *Pisum*, but the trend here is quite similar.

Green seeds of *Robinia pseudacacia* were collected once a week for 3 weeks from a single tree and were dried under the above four conditions and checked for permeability to water. Since the pods had set at the same time, the different harvest times represent different stages of development (Table III).

Only the seeds from the second harvest show a consistent relation between conditions of drying and permeability. The seeds from the first and the third harvest were hardly affected by the drying conditions. This may indicate that the presence of oxygen influences permeability of the seed coat only at a

certain stage of its development. Too early drying induces completely permeable seed coats. If the treatment is delayed beyond a certain period, the seeds become almost impermeable, although the results of treatment under N₂ clearly differs from the other treatments.

Some experiments were made to study the possible mechanisms relating seed color, drying, and coat permeability. Catechol oxidases are known to require O₂ and cause browning. Therefore, the activity of catechol oxidase was determined in the seed coat and in the cotyledons of the cultivated pea *Pisum sativum* and the wild species of peas: *Pisum elatius* and *Pisum fulvum* (Tables IV and V).

The seed coats of the wild species of pea have higher activity of catechol oxidase in comparison to the seed coat of the cultivated pea and to the cotyledons of both the wild and cultivated types. Most of the catechol oxidase activity in the seed coats of wild peas is located in the fraction sedimenting at 20,000g, both at pH 5.3 and at pH 7.3. The activity of the supernatant fraction is low in all cases. In contrast catechol oxidase activity in the cotyledons is much lower and similar in magnitude in the three species.

The relative weights of the seed coat in the wild species is three times higher than that of the cultivated species (8.8% of the weight of the whole seed of *P. sativum* in contrast to 22.4% in *P. elatius* and 31.4% in *P. fulvum*). The contribution

Table III. Effect of Drying Conditions on the Permeability of *Robinia pseudacacia* Seeds from Three Successive Harvests

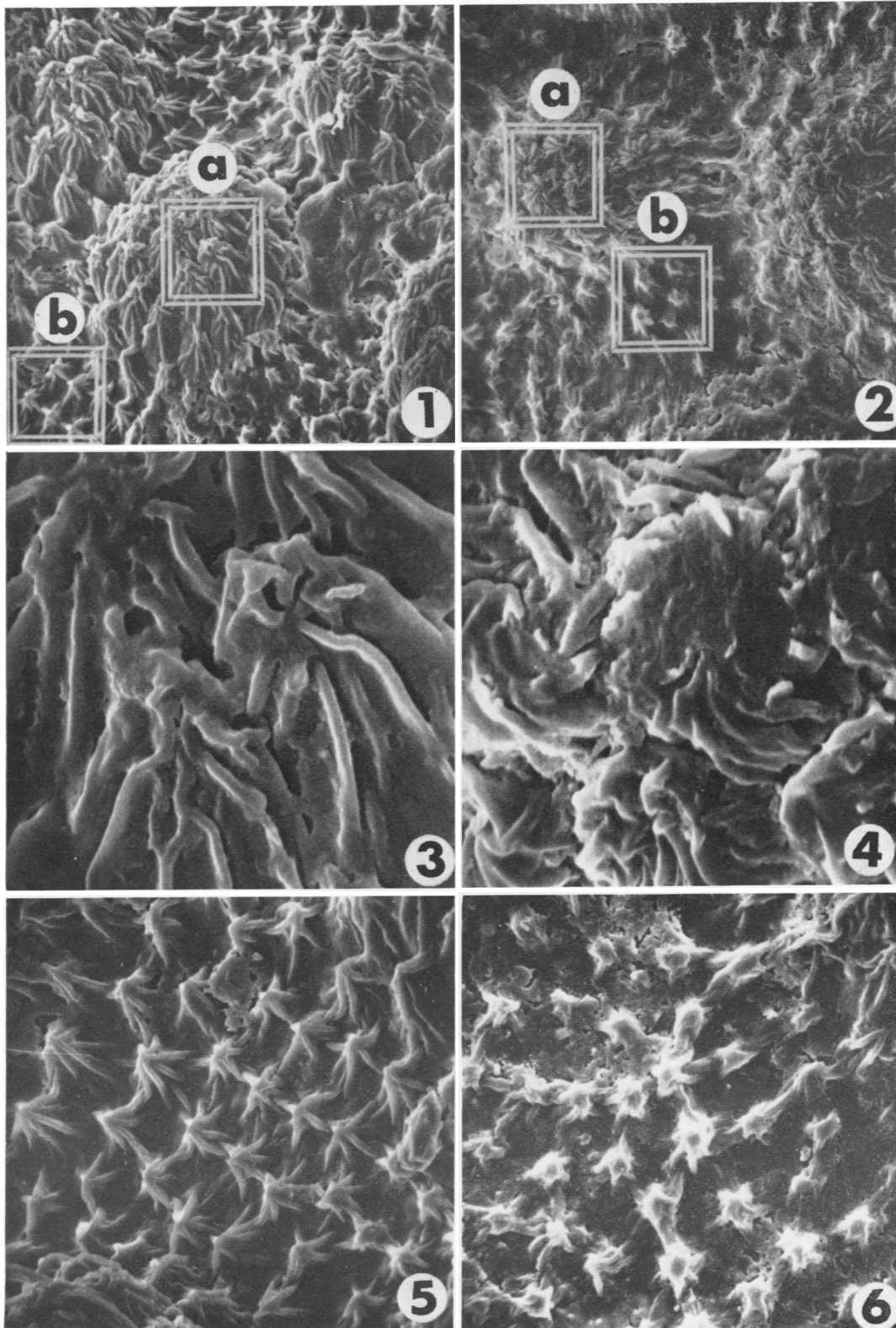
Results were based on 200 seeds for each treatment.

Date of Collecting	Drying Conditions	Imbibed
		%
5 July 1973	Air	100
	Oxygen	100
	Vacuum	100
	Nitrogen	100
13 July 1973	Air	20
	Oxygen	16
	Vacuum	50
	Nitrogen	53
20 July 1973	Air	0
	Oxygen	0
	Vacuum	2
	Nitrogen	15

Table IV. Catechol Oxidase Activity in Seed Coats of Air-dried Seeds

Total activity is expressed in $\mu\text{l O}_2$ uptake min^{-1} g seed coats⁻¹, and specific activity in $\mu\text{l O}_2$ uptake min^{-1} mg protein⁻¹.

Species	20,000g Particulate Fraction				20,000g Supernatant Fraction			
	pH 5.3		pH 7.3		pH 5.3		pH 7.3	
	Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity
<i>Pisum elatius</i> *	6.8	1.0	12.8	2.0	1.2	0.1	1.2	0.1
<i>Pisum fulvum</i>	3.1	0.7	9.1	2.0	0.8	0.1	2.0	0.3
<i>Pisum sativum</i> var. Alaska	0.5	0.2	1.5	0.6	0	0	1.6	0.2



FIGS. 1-6. Scanning electron microscope photographs of section of seed coat of *P. elatius* dried in air or in vacuum. 1: Air-dried seeds ($\times 1100$); 2: vacuum-dried seeds ($\times 1100$); 3: air-dried seeds 5600 magnification of section a in Fig. 1; 4: vacuum-dried seeds 5500 magnification of section a in Fig. 2; 5: air-dried seeds 2000 magnification of section b in Fig. 1; 6: vacuum-dried seeds 2200 magnification of section b in Fig. 2.

of the seed coat to the catechol oxidase activity of the whole seed is therefore very much higher in the seeds of the wild species. We compared catechol oxidase activity with another oxygen-requiring copper enzyme, ascorbic acid oxidase, which

is also present in the seed coat but which is not normally involved in browning reactions. The activity of ascorbic acid oxidase is similar in the three species as shown in Table VI. The differences in catechol oxidase activity do not simply re-

Table V. *Catechol Oxidase Activity in Cotyledons of Air-dried Seeds*Total activity is expressed in $\mu\text{l O}_2$ uptake $\text{min}^{-1} \text{g cotyledon}^{-1}$ and specific activity in $\mu\text{l O}_2$ uptake $\text{min}^{-1} \text{mg protein}^{-1}$.

Species	1,000g Precipitate Fraction				20,000g Precipitate Fraction				Supernatant Fraction			
	pH 5.3		pH 7.3		pH 5.3		pH 7.3		pH 5.3		pH 7.3	
	Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity	Total activity	Specific activity
<i>P. elatius</i>	0.7	0.024	1.6	0.05	0.3	0.02	1.1	0.1	1.2	0.006	0.8	0.004
<i>P. fulvum</i>	0.4	0.02	0.8	0.03	0.6	0.05	1.3	0.12	0.3	0.002	0.7	0.004
<i>P. sativum</i>	0.1	0.01	1.6	0.07	0.5	0.05	1.0	0.1	0.5	0.004	0.9	0.006

Table VI. *Ascorbic Acid Oxidase Activity in Seed Coat*

Species	Total Activity	
	20,000g fraction	Supernatant fraction
	$\mu\text{l O}_2$ uptake $\text{min}^{-1} \text{seed coat}^{-1}$	
<i>P. elatius</i>	4.5	6.0
<i>P. fulvum</i>	4.3	7.0
<i>P. sativum</i> var. Alaska	4.0	6.0

Table VII. *Phenolic Content of Seed Coats and Cotyledons*

Species	Phenolic Compounds	
	Seed coat	Cotyledons
	mg/g	
<i>P. elatius</i>	25.8	1.4
<i>P. fulvum</i>	5.9	1.3
<i>P. sativum</i>	0.4	0.4

fect a general difference in enzyme activity in the coats of the species examined.

The phenolic content of the seed coats and the cotyledons in the three species of *Pisum* is shown in Table VII. From the results it is clear that the *o*-diphenol content of the coats of *P. elatius* and *P. fulvum* is much higher than that of *P. sativum*. The same was also true for the total phenolic content of the seed coats. The highest phenolic content was observed in *P. elatius*, although the color of the seeds of *P. fulvum* was much darker. No such marked differences in *o*-diphenol content is observed in the cotyledons of the different species.

The results obtained here suggest that the permeability of seed coats to water is related to the content of phenolic com-

pounds in the seed coat, and to their level of oxidation. The oxidation of the phenolics may be catalyzed by catechol oxidase present in the seed coat itself and is O_2 -dependent. Moreover, the oxidation process may cause structural changes.

Although these results show only a correlation between the metabolic events and seed coat permeability, it seems likely that a causal relation may exist. The fact that the oxygen content during drying influences seed coat permeability could explain the great variability observed in the water permeability of wild seed populations. It could also account for differences in permeability depending on the position of the seed on the mother plant and on ecological conditions during seed maturation. During all these processes the access of oxygen to the drying seeds could well show considerable variability. Thus a major factor in determining the seed coat permeability, at least in the genus *Pisum*, has been determined. We feel that similar mechanisms may be of much wider occurrence in seeds.

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