EFFECT OF ACID SCARIFICATION ON LUPINE SEED IMPERMEABILITY

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Varying proportions of hard seed occur among strains of blue lupine (*Lupinus angustifolius*). These hard seed may be rendered permeable by scarification with acid. Since the basic action of this treatment in altering permeability has not been investigated, this study was designed to determine the effect of acid scarification on the path of liquid entry into blue lupine seed. Research on other legumes has shown that liquids enter the seed by different pathways in different species (1, 6, 7, 9).

In this study a hard-seeded strain of sweet blue lupine was used. When seeds of the above strain were soaked in dye for periods ranging up to 2 weeks, 50 to 75% of the seeds were found to be permeable. They absorbed dye through the seed coat as a whole, the entry not being limited to any particular region. In the seeds which remained in the dye solution for 2 weeks without swelling (termed hard seed), the cuticle was stained and in 17% of these seeds some dye had penetrated to the tracheid bar underlying the hilum region (fig 1).

The fact that the dye penetrated the cuticle but did not penetrate the seed coat of the "hard" seeds agrees with the conclusion of most workers that the impermeable layer of the testa is at the outer edge of the palisade epidermis. This is the location of the "light line," an optical phenomena much discussed in the literature (1, 3, 6, 8). The entry through the strophiole reported for other legume seeds (2, 7) did not occur in this strain.

To study the effect of sulfuric acid treatment on the liquid entry, hard seeds were selected by soaking the seeds in water for a week or more and saving the unswollen seeds for study. These seeds were placed in concentrated sulfuric acid for 3 hours. This treatment removed the cuticle of all the seeds and eroded pits through the testa of a small percentage of them.

Seeds treated as above were placed in blue dye and removed at intervals for observation. In all cases in which the seeds were removed before the entire seed was stained, dye entry had occurred through the hilar fissure into the tracheid or through pits eroded through the testa.

Observations of cross sections of the hilum region after scarification revealed that the overlying parenchyma was severely hydrolyzed and that the counter palisades were partially hydrolyzed. The palisade epidermis showed hydrolysis only where pits were formed.

Hyde (4) found that the counter palisades and the palisades of the hilum region control moisture uptake from the air. When the internal moisture of the seed exceeds the external moisture the hilum opens, permitting drying; with reverse conditions the hilum closes, preventing moisture uptake.

To determine the extent to which the hydrolysis of the counter palisades region negated this process, both scarified and unscarified seeds were placed in chambers in which atmospheric moisture was controlled with either a moist blotter or calcium chloride. Observations with a wide field microscope revealed that in the sound seed the hilar fissure was open in the dry chamber and closed in the moist chamber. In the scarified seeds the hilar fissure was open under both conditions.

Since the hilar fissure is merely closed by pressure (fig 1) it was thought that a surfactant might be effective in permitting penetration of this region. Neither this nor a fat solvent was effective in rendering the seed permeable.

To determine the effect of swelling and redrying on the permeability of the hard seeds, the testas were pierced with a needle and the seeds were permitted to swell in water. They were then dried rapidly at

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Fig. 1. Cross section of the hilum region of lupine seed. A, palisade epidermis; B, counter palisades; C, hilum fissure; D, tracheid bar.

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43° C and the hole was sealed with beeswax. When placed in the dye solution 90% of these seeds took up liquid through the strophiole. In the remaining 10% the liquid entered through the seed coat generally. In no case was it primarily through the hilum. Examination of these seeds after the preliminary soaking revealed cracks in the strophiole of most of the seeds. This observation is in accord with that of Kuhn (5). Of the thousands of seeds observed, only 1 seed not treated as above had liquid entry through the strophiole.

**Summary**

Examination of the path of liquid entry into impermeable seeds of *Lupinus angustifolius* (a hard-seeded sweet blue strain) after they were scarified in sulfuric acid indicates that entry occurs through either the hilar fissure or pits eroded through the testa. The impermeable layer of the testa is located at the outer edge of the palisade epidermis. Entry was permitted through the hilar fissure due to partial hydrolysis of the counter palisades which normally cause the hilum to close when the moisture outside the seed exceeds that on the inside.

The only time that the strophiole is important in uptake of liquid is after the seeds have taken up water and been redried. This process causes a cleft to be formed in the strophiole.

**Literature Cited**


**SOME FACTORS WHICH AFFECT THE SYNTHESIS OF CHLOROGENIC ACID IN DISKS OF POTATO TUBER**

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The work of Neish and his colleagues (9, 10) on the synthesis of aromatic compounds in buckwheat indicates that shikimic acid is a precursor to phenolic constituents such as caffeic acid. Chlorogenic acid, a phenol present in a wide variety of plants, is an ester of caffeic acid and quinic acid, the latter being quite similar to shikimic acid in structure. Unfortunately little is known of the mechanism of biosynthesis of this interesting phenol. The recent observations of Johnson and Schaal (3, 4) and of Kuc, et al (6) have shown that, in contrast to the outer periderm, the pulp of the potato tuber is almost devoid of chlorogenic acid. Kuc (5) has further shown that disks of pulp tissue, nevertheless, are capable of producing this compound. Consequently, tissue from the inner core of the potato tuber appears to offer a relatively simple system for study of the pathways by which chlorogenic acid is synthesized.

The purpose of the work reported in this paper, then, is to investigate the effects of environmental factors on the synthesis of chlorogenic acid. Data are also given which offer an explanation for the difference in distribution of this substance between periderm and pulp.

**Materials and Methods**

Chlorogenic acid synthesis was studied in disks, 12.5 mm in diameter and 1 mm thick, sliced with a hand microtome from cylindrical plugs cut out of Kennebec potato tubers. The tubers were kept at 7° C until used. Disks were sliced from the inner part of the tuber only, the outer 5 to 8 mm of tissue and skin being discarded. Each experimental treatment consisted of a sample of 10 disks (weighing about 1.5 g) which were withdrawn at random from the washed slices, blotted lightly, and placed in 5 ml of the appropriate solution contained in a 9 cm Petri dish. The covered dish was held at room temperature (23 to 25° C), in the dark, until the disks were ready for assay. All culture solutions contained 25 ppm of Neomycin sulfate to control bacterial contamination. This addition had no effect on synthesis of chlorogenic acid. The data are presented as the total chlorogenic acid content of each sample. Treatments were run in...