Nature of Hard-Seededness in Lima Beans (*Phaseolus lunatus* L.)

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**Abstract.** We investigated the areas of water penetration and the anatomical structures of hilar regions of permeable and impermeable seed coats of lima beans (*Phaseolus lunatus* L.). Results indicate that water can enter permeable seeds through the hilum, raphe, and micropyle. In impermeable seeds water cannot pass through any of these areas. Anatomical data confirm that there were no structural differences in the testae of permeable and impermeable seeds, but a noticeable difference was apparent in the hilar region. In permeable seeds the palisade layer did not connect evenly in the hilar canal. By contrast, the hilar canals of impermeable seeds had connected palisade layers that were uniformly coated with a cuticular layer. Micropylar openings were clearly visible in permeable seeds, but these openings were occluded and well covered with cuticle in impermeable seeds. Visible differences were evident in the raphe.

Uniform germination is desirable in producing lima beans for fresh market, processing, or seed purposes. However, almost all cultivars of lima beans have hard or impermeable seeds. Cultivars with hard seeds do not germinate uniformly, and reduced stands result.

The terms “hard” and “impermeable” are applied to seeds that do not imbibe water after being placed under normal germination conditions for a specified time (12). The cause of hard-seededness in important leguminous seed has been attributed to heredity (5, 6, 7), storage conditions (6), character and amount of thickening of the cell walls (1, 5, 11), and the regulatory function of the micropyle and hilum (2, 3, 5).

The objective of this study was to determine the route of water penetration and the anatomical structure of the seed coat in the hilar region of lima bean seeds.

**Materials and Methods**

Permeable (lines 628, 631, 958, 959, 997, 1012, 1013, 1014, and 1015) and impermeable (lines 455, 457, 611, 612, 615, 616, and 617) selections of lima beans (*Phaseolus lunatus* L.) from L. H. Pollard’s breeding stock were employed in this study. Seeds were stored at room temperature in the laboratory until used in experiments, at which time they had a moisture content of about 10-12%.

The micropyle, raphe, and hilar areas of lima bean seeds were coated with paraffin wax (M.P. 58° C), petroleum jelly, and adhesive materials. Embedding wax was used for most of this study because of its superior coating quality. Because preliminary observations indicated that little change occurred in the uptake of water during the period from 24 to 48 hr, the period of 0 to 24 hr was used for these studies. The areas of water penetration were studied by 3 methods:

- **Soaking method:** Eight hundred permeable seeds were selected and replicated 4 times in each of the following 8 treatments (9).
  1. Control
  2. All 3 areas coated: hilum, raphe, and micropyle (H + R + M)
  3. 2 areas coated: hilum and raphe (H + R)
  4. 2 areas coated: hilum and micropyle (H + M)
  5. 2 areas coated: raphe and micropyle (R + M)
  6. 1 area coated: hilum (H)
  7. 1 area coated: raphe (R)
  8. 1 area coated: micropyle (M)

Each treatment contained 25 seeds per replication. Seeds that had imbibed water at the end of 24 hr of soaking at room temperature (25° C) were counted. A planned comparison was utilized to analyze the data (8).

- **Diffusion of iodine vapor:** Permeable and impermeable seeds were treated as in “a” above and placed in a desiccator containing a small amount of iodine crystals. After 3 days they were removed from the desiccator, dissected and observed under a stereoscopic binocular microscope.

- **Dye method:** Permeable and impermeable seeds were soaked in 0.5 percent fast-green solution for 30 min. The seeds then were examined as above.

For the anatomical portion of this study, mature seed coats containing the hilar, micropylar and rapheal region were excised. The samples were fixed in FFA, dehydrated in TBA-ETOH series, and embedded in Tissuemat (4). Sections were cut at 10 μ and stained with sodium III and safranin-fast green.

**Results and Discussion**

Results from the soaking treatment indicated that water can pass through the hilum, raphe, or micropyle in permeable seed within 24 hr. The number of seeds imbibing water in each treatment were: control, 100; hilum, raphe, and micropyle covered with wax, 9; hilum and raphe covered, 62; hilum and micropyle covered, 97; raphe and micropyle covered, 99; hilum only covered, 95; raphe only covered, 93; and micropyle only covered, 92. There was no difference in the number of seeds absorbing water through hilum, raphe, raphe + micropyle, hilum + micropyle, hilum + raphe and the control. There was, however, a significant difference in the number of seeds imbibing water when the hilum, raphe and micropyle were all covered with wax and when only the hilum and raphe were covered with wax, and, also, between these 2 treatments and the control and all other treatments. Water entered through the hilum with slightly greater ease than through the raphe. These data on lima beans differ from those of Snyder (10) and Kyle and Randall (5) who observed that the micropyle was the primary site of water entry in ‘Great Northern’ beans.

The diffusion of iodine vapor and dye experiments confirm that water can enter the permeable seed through the hilum, raphe, or micropyle. Vapor of iodine was useful for detecting invisible cracks in any part of the seed coat. The palisade layer of cells in the permeable seeds failed to connect completely in the hilar canal, and the resultant fissures allowed...
water to enter (Fig. 1). In impermeable seed, water did not enter any of the areas within 24 hr. Light micrographs of these seeds indicated that the hilar fissure was sealed or occluded (Fig. 2). The lower palisade layer appeared to be connected and coated with a cuticular layer. Even where the upper palisade layer did not adjoin at the hilar fissure, the lower palisade layer and its cuticular layer effectively prohibited water from passing through.

Fig. 1. Transverse section of mature permeable seed coat showing hilum area. The palisade layer failed to connect completely (x875). CL = Cuticular layer – PL = Palisade layer.

Fig. 2. Transverse section of mature impermeable seed coat showing hilum area. Note uniform development of 2 palisade layers, accompanied by the cuticular layer between these 2 layers (x875).
Anatomically, there was a noticeable difference between the hila of permeable and impermeable seeds (Figs. 3 and 4). The palisade layer of the hilar canal did not connect evenly in permeable seeds. The breakage occurred on the canal's surface. By contrast, mature impermeable seeds had a connected palisade layer at the canal's surface and a closed fissure along the furrow of the hilar canal. In addition, there was a complete cuticle layer covering this area. The concept that the hilum operates as a hygroscopic valve, as reported in some members of Papilionaceae by Hyde (3) and in snap beans by Honma and Denma (2), seems inapplicable to hard-seeded lima beans. Our data agree with those of Watson (12), in that the cuticle as well

Fig. 3. Median section of mature permeable seed coat, showing opened palisade layer (x2188).

Fig. 4. Median section of mature impermeable seed coat, showing closed palisade layer (x2188).
as the palisade layer appears to be blocking the passage of water in the impermeable lines. Moreover, it is doubtful that the impermeable condition of these seeds would be altered by changing the relative humidity of the storage atmosphere. The connected palisade and cuticle layers appear to be genetic traits. Under natural conditions and where uniform germination and emergence are not critical, such as a home garden, these lines would probably do well as the impermeable layers would be altered naturally by micro-organisms. In a commercial breeding program, however, there would be selective pressure against such traits.

Sub-epidermal layers of cells at the raphe ridge of both permeable and impermeable seeds differentiated from a more or less isodiametric form into radially elongated parenchyma-like cells with thin walls. These cells absorbed water readily and seemed spongy-like. The vascular system of both types of seeds occurred beneath the raphe ridge. It was not possible to determine whether this system was involved in the penetration of water through the testa.

The permeable seed did not develop a palisade layer from the raphe ridge and micropylar region to the hilar canal. Most of the impermeable seed, however, had 2 palisade layers into the hilar canal. A cuticular layer was present between these 2 palisade layers. Individual palisade cells appeared to be normal in size and in secondary thickening of the walls.

The testa of both permeable and impermeable seed were structurally similar and impermeable to water. In this respect our data agree with those of Watson (12). Observations show that the secondary thickening of the testa of lima beans was very pronounced. All observed cells within the seed coat were dead. The epidermal cells had developed into a palisade layer with its sclereid, macrosclereid, and Malpighian cells having uneven secondary wall thickening. The individual cells were elongated and rod-like with small lumen, and they had simple pits that were interconnected between cells. One or 2 sub-epidermal layers had differentiated into columnar cells.

These have sometimes been called “hourglass” or “pillar cells”, but usually they are referred to as “osteosclereids” (11). The middle layers of sub-epidermal cells were tangentially enlarged. Parenchyma cells of the lower layers, however, were small. The mature seed coat had 2 major parts: palisade cells that had thick walls and a cuticle, and a sub-epidermal layer of cells. Although secondary thickness appeared in these sub-epidermal cells, they were loosely arranged with some intercellular spaces.

Literature Cited
8. LeClerg, E. L. 1957. Mean separation by the functional analysis of variance and multiple comparisons. ARS 20-3. ARS, USDA.

Effects of Tomato Juice on Seed Germination and Seedling Growth

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Abstract. Tomato seeds were more responsive than wheat or lettuce seeds to the presence of an inhibitor in the juice of tomato fruits. Seed germination and seedling growth decreased with increasing concentrations of juice. Inhibition of seed germination in 20% juice with an osmotic concentration of less than 0.1 M was significantly less than in 0.1 M glucose or mannitol with 0.01 M citric acid at pH 4.4. The inhibitor in tomato juice was thermostable, but the effect decreased with prolonged storage at -20°C. There were cultivar differences in the amount of inhibitor present in ripe tomato fruits.

Many physiological effects of tomato juice on plant growth have been noted (5, 9, 13, 14, 16). Konis (10) reported on the properties and action of an inhibitor in tomato fruits, and Tetjarew (15) considered that the organic acids in the juice were responsible for the growth retarding effect. The inhibitory effect of the juice was considered by Juel (8) to be due to acidity, and, according to Akkerman and Veldstra (2), caffeic and ferulic acids are responsible for inhibition of seed germination in tomato fruits. Nitsch, however, (14) found that the organic acids were slightly stimulatory to growth.

Because of these varied and sometimes confusing reports, and the recent finding of sprouted seed in intact ripe tomato fruits by Yamaguchi et al. (18), a study was made to clarify the action of tomato juice on seed germination and seedling growth.

Materials and Methods

Tomato juice free of insoluble solids was obtained by pulping ripe fruits in a Waring blender and filtering through Whatman No. 2 paper. Streptomycin at 50 ppm was effective in retarding the growth of mold in the more dilute solutions of tomato juice. Since streptomycin inhibits seedling growth (4, 7), other methods for sterilization of the juice were sought. It was not known whether the inhibitor was thermolabile, so heat was not...