THE HILAR LAYER OF WHITE CORN[1]

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ABSTRACT

The structure, color, and absorbance of the hilar layers of several hybrids and inbred lines of white corn were investigated. In many kernels the hilar layer appeared as a single band, but in others it showed a distinctly double nature. Observed *in situ,* after tip caps were broken off, hilar layers were light tan, brown, or almost black. Hilar layers were isolated by dissecting away other tissues after the kernel had been boiled for 65 minutes in a 0.5% aqueous solution of KOH. These layers varied in absorbance from 0.10 to 1.00. The absorbance was presumably affected by the concentration of pigments and by the thickness of the isolated hilar layer.

Possible significance of the variability of the hilar layer to the corn dry-milling industry is pointed out.

The hilar layer (closing tissue) of the corn kernel has much biological significance because it is part of a protective covering about the seed. It is formed in the placento-chalazal region – the region of the attachment of the ovule to the ovary. As in other grains, the ovule develops into the seed and the ovary becomes the pericarp which is fused with the seed. Vascular tissues, which conduct water and nutrients from the corn plant to the kernel, traverse the tip cap but do not extend across the placento-chalazal region into the growing seed. Transfer of materials from the vascular tissues to the seed is made through parenchymatous cells (largely unspecialized cells).

Origin and development of the hilar layer in a yellow dent corn has been studied by Kiesselbach and Walker (2). Their observations are outlined briefly here. By 20 days after pollination a plate of tissue, several cells thick, is differentiated across the placento-chalazal region. This differentiated tissue, which is the beginning of the hilar layer, remains active in conduction for another 2 weeks, but then its cells begin to shrink and to lose their contents. During a subsequent 2-week period the cells become compressed into a nearly structureless dark-brown plate – the hilar layer. Cell walls in the inner part of the hilar layer become suberized, and this tissue forms a semipermeable closing layer which separates the seed from the parent plant. The margin of the hilar layer is united with another semipermeable membrane, the seed coat (3). The seed is enclosed by this combination of

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hilar layer and seed coat except for a small area opposite the primary root.

The covering, composed of hilar layer and seed coat, undoubtedly constitutes a partial barrier to the passage of moisture and to the entrance of fungal hyphae. However, the seed can still lose and absorb moisture through it (2). Johann (1) found no anatomical variations in the closing layer sufficient in themselves to account for the marked differences in resistance or susceptibility of various strains of yellow dent corn to infection by *Diplodia zeae.*

The mature hilar layer of corn is commonly described as a dark-brown layer, but few details have been published concerning its structure. Its cellular nature has been recognized (2,3), and Johann (1) observed variations in the thickness and compactness of the layer and in the completeness of its union with the suberized membrane of the seed coat.

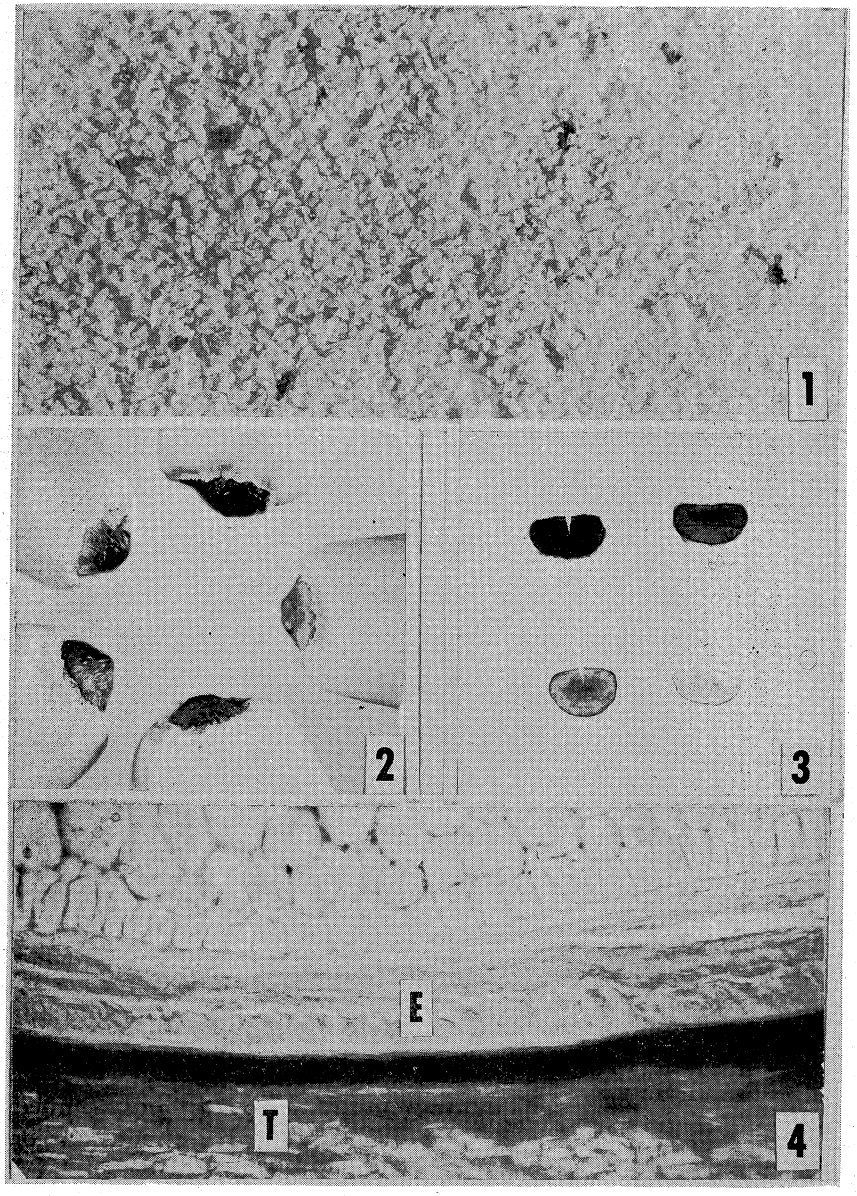
Fragments of the hilar layer are often present in corn grits—a food product of the corn dry-milling industry (Fig. 1). Because some customers object to these dark specks, a strain of corn with small light-colored hilar layers might be of value to corn dry-millers, provided such hilar layers retained their essential protective function. An investigation was therefore begun to determine the variability of the hilar layer of white corn with respect to its color and size. The present report concerns the method used in isolating hilar layers and also some general observations on the structure and pigmentation of the hilar layer. Data on the variation in color and size of the hilar layer in inbred lines will be presented in a later paper.

Materials and Methods

Samples of hybrids and of inbred lines of white corn were provided through the kindness of M. S. Zuber, Crops Research Division, U.S. Department of Agriculture, stationed at the University of Missouri. The principal ones are noted in legends under the figures.

Structure of the hilar layer was studied from longisections cut at 30 μ on a freezing microtome through the proximal end of kernels that had been softened somewhat in water. The sections were mounted, unstained, in glycerol so that appreciable differences in pigmentation of the hilar layers and adjacent tissues would be apparent. Eighty-two kernels were sectioned; they were from five hybrids and from five inbred lines.

For observation of the hilar layer *in situ* (Fig. 2), the tip cap was broken off and any tissue still adhering to the hilar layer was carefully removed under a dissecting microscope.



Figs. 1–4. Fig. 1. White corn grits showing fragments of dark hilar layers (3×).

Fig. 2. Exposed hilar layers selected to show the range in intensity of color (1.5×).

Fig. 3. Isolated hilar layers selected from kernels of hybrid U.S. 523W to show the range in absorbance (2×).

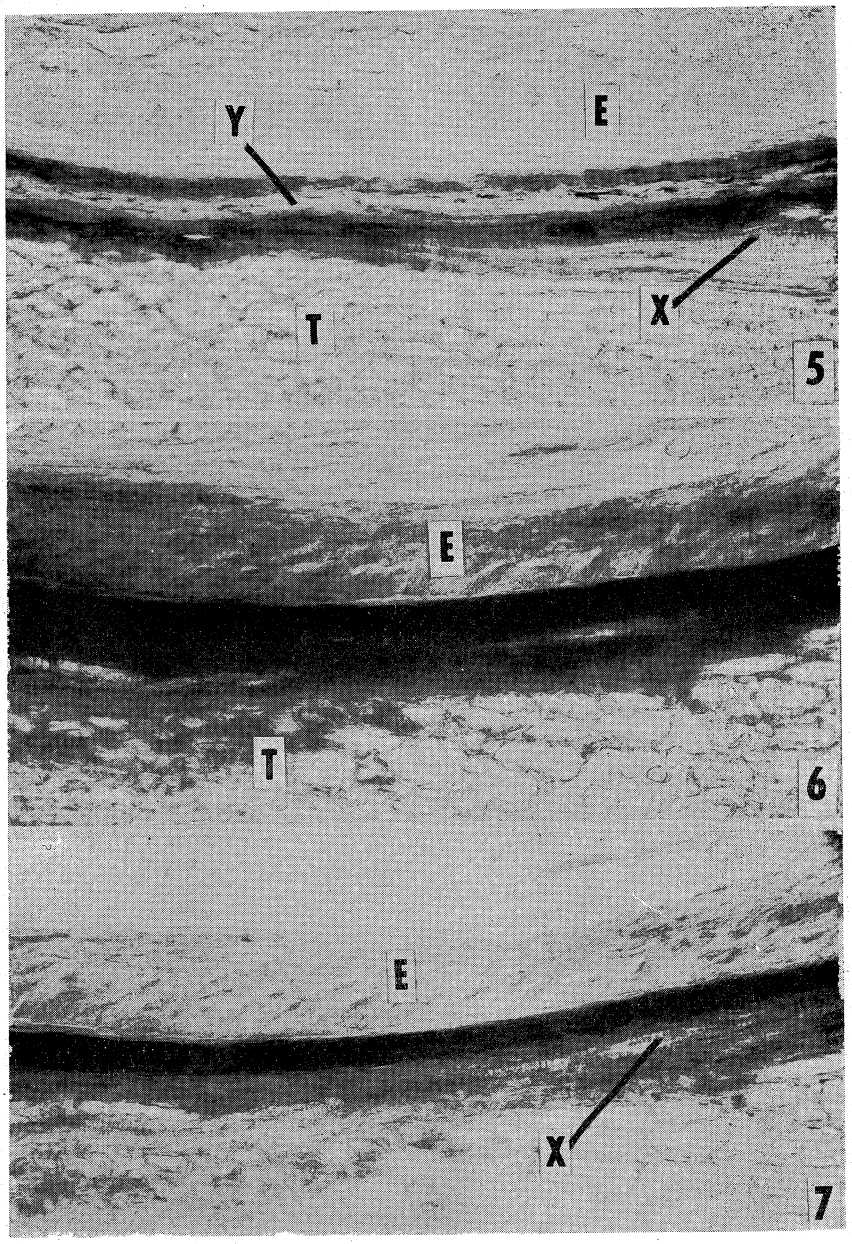
Fig. 4. Unstained, 30-μ section through the hilar layer of a white corn hybrid, Dixie 33 × U.S. 523W (200×): E, endosperm; T, tip cap.

The following method was used to isolate hilar layers so that their absorbances could be determined. The kernels were placed in a flask containing 0.5% aqueous solution of potassium hydroxide and refluxed over an electric heater for 65 minutes, after allowing 5 minutes to bring the solution to a boil. The flask was left on the heater for another 10 minutes after the current was turned off. After the kernels were washed in hot tapwater two or three times, the tip caps and adhering hilar layers were removed. Occasionally, tip caps came off the kernels in the potassium hydroxide solution. Under a dissecting microscope, the tip cap and the inner lining of endosperm were removed from each hilar layer. Because an isolated hilar layer is not flat but rises to a peak in the portion that lies over the lowermost tip of the embryo (Fig. 2), a slit was made in the elevated portion so that the layer could be flattened when a cover glass was added (Fig. 3). The specimens were mounted in glycerol.

Transmission of the isolated hilar layers was determined in a darkened room by projecting their images with a low-power microscope onto a ground-glass plate at the top of a camera bellows. Lenses used were a 2.8× objective and a 6.4× ocular, and the final magnification was 25×. A Weston photronic cell in series with a galvanometer was used to measure the transmission. Intensity of the light source was adjusted to give a reading of 100 on the galvanometer in a blank area adjacent to the image of the hilar layer before the light transmission was measured through the hilar layer. The readings of both blank and hilar layer were made through a circular opening 25 mm. in diameter. If a hilar layer had light and dark areas, an attempt was made to include in the circular opening light and dark parts in the same proportion as they occurred in the whole hilar layer. Absorbance of the hilar layers was calculated from their percentage of transmission.

Characteristics of the Hilar Layer

Structure. The hilar layer, seen in section, is a pigmented band of tissue that is usually sharply defined on both the inner and the outer face (Fig. 4). It is separated from the endosperm by a few layers of crushed parenchyma cells of the placento-chalazal tissue. Occasionally, there is another definite layer of pigmented cells to the outside of the primary one but separated from it by colorless tissue (Fig. 5). A double pigmented layer was found most commonly in the distal part of the hilar layer, but it was also seen in the proximal, or peaked, portion. The kernels of any one sample tend to be similar with respect to the single or double nature of the hilar layer. There may be pigmented



Figs. 5–7. Unstained, 30-μ sections through hilar layers of white corn (200×): E, endosperm; T, tip cap; Y, colorless tissue; X, xylem elements of a vascular strand. Fig. 5, inbred line T13. Fig. 6, inbred line H28. Fig. 7, the same as Fig. 6, but a different filter and exposure time were used to give more detail.

cells in the tip cap just outside of the hilar layer (Figs. 6,7).

Wolf *et al.* (3) stated that the hilar layer of yellow dent corn is frequently "split into an upper and a lower layer separated by a large air space in the dry kernel." A somewhat similar condition was found in the white corn kernels sectioned. However, in about half of the 14 samples sectioned, the air space in the tip cap was small or entirely lacking. When the air space was moderate or large in size, the tip cap tissue bordering on it was practically colorless as often as it was pigmented.

Color. Hilar layers *in situ* vary in color from light tan through yellowish brown and reddish brown to almost black. Only the range in color intensity can be shown in a black and white photograph. It is apparent from Fig. 2 that this range is considerable.

Evidence that hilar layers differ in intensity of color was also obtained from sections. The photomicrographs for Figs. 5 and 6 were taken under the same conditions of lighting and exposure. Developing and printing of the films were also uniform. Both the hilar layer and the endosperm in Fig. 6 are darker than the same tissues in Fig. 5. A different filter in photographing the dark hilar layer brought out more detail (Fig. 7), but the photograph still shows how dark the hilar layer is in comparison with that of Fig. 5.

Absorbance. Some isolated hilar layers selected to show the approximate range in absorbance are pictured in Fig. 3. The absorbance readings obtained for these four hilar layers are: Upper row, 0.82 and 0.52; lower row, 0.38 and 0.12. The highest reading made was 1.00 and the lowest, 0.10. Preliminary observations indicate that the absorbance of the hilar layers is more uniform within individual inbred lines than in hybrids of white corn.

Discussion and Conclusion

The absorbance of an isolated hilar layer is presumably affected by both the thickness of the layer and the concentration of pigment in it. Consequently, if comparative studies are to be made, it is important to know whether the hilar layer can be isolated with accuracy. The endosperm that lines the inner surface of the hilar layer often slips out easily after treatment with potassium hyroxide. Sometimes it adheres rather firmly and is removed only with difficulty. This adherence seems to be noticeable especially when the hilar layer is dark and the endosperm also contains much pigment. In either case, after the endosperm is removed, there are only a few layers of crushed parenchyma cells remaining on the inner surface of a sharply defined hilar layer. The separation of the tip cap from a hilar layer is seldom as accurate

and clean-cut as the separation of the endosperm from the hilar layer. This difference is perhaps inevitable. The tip cap is not a morphological entity; it is merely part of the pericarp.

After treatment with potassium hydroxide there is, at times, a rather easy separation between the hilar layer and the tissue traversed by the vascular strands that spread out fanwise from the base of the tip cap and that lie close to the hilar layer. Sometimes the vascular strands adhere closely to the hilar layer. If they are removed, some pigmented tissue is taken away. This loss would be expected if considerable pigment is present outside the hilar layer, as in Figs. 6,7. It is concluded that the potassium hydroxide method for isolation of hilar layers makes possible a fairly accurate separation of them from adjacent tissue, although it does not give a perfect separation. The large differences in absorbance which were found are considered to be the result of true differences between the hilar layers.

Further work on a greater range of hybrids and inbred lines is required to establish the potential value of this variability to the corn processor. Extension of the work to yellow dent corn may also be justified, because corn dry-millers have a similar black-speck problem in the processing of yellow dent corn.

Acknowledgments

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