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Determining the Structure of the Barley Kernel by Scanning Electron Microscopy¹

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(Refer to pages 7-9 for Figures 1-7)

ABSTRACT

The barley kernel consists of the husks (that include the lemma and the palea), the caryopsis, and the rachilla. The caryopsis is composed of the pericarp, seed coat, endosperm, and germ. The detailed structure of husks and awns, ridges and stomata-like cells on the husks, the rachilla, the pericarp, and the endosperm are illustrated by scanning electron microscopy. Detailed pictures and description of aleurone cells and their contents, subaleurone layer, and starchy endosperm and its contents in barley are given.

Few accounts of either gross or detailed structure of barley are available in the literature, although structures of other cereals are well documented. Some of the general accounts of barley-kernel structure were given by Winton and Winton (1), Mann and Harlan (2), and Bawtree and Gordon (3). Structure of the barley kernel is important in relation to kernel function and to utilization in malting and brewing. A clear understanding of the structural details is therefore of interest to cereal chemists, plant physiologists, plant breeders, plant pathologists, and barley processors.

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The present study considers the gross and detailed structures (other than germ) of the immature and mature barley kernel. The detailed structure was determined by scanning electron microscopy. Application of the technique to cereal specimens has been discussed in recent publications (4,5,6). Its most attractive feature is the possibility of obtaining three-dimensional images of sample surfaces with minimal preparation.

MATERIALS AND METHODS

The whole grain was six-row malting barley (*Hordeum vulgare* L.) of Dickson variety from the 1969 crop grown in Madison, Wis. Grain was harvested 7 or 35 days after heading, and freeze-dried. In addition, mature grain was steeped in water for 30 hr. at 16°C. and freeze-dried. Sections were prepared by slicing the grain with a razor blade. Samples were mounted on circular (9-mm. diam.) specimen holders with an adhesive, coated with graphite, covered with a 200 to 300 Å gold layer, and examined with a Cambridge Stereoscan electron microscope at 20 KV.

SCANNING ELECTRON MICROSCOPY OF BARLEY TISSUES

Before giving structural details, we will describe briefly the main characteristics of the Dickson barley used. The kernels are broad at the center and plump on the crease side; they taper sharply to both ends, and lateral kernels are slightly to moderately "twisted". The crease is V-shaped: open in the upper half of the kernel and tight at the base. The point of attachment has a depression; the glumes are covered with short hairs. The hairs on the rachilla are short; the awn is rough. The aleurone layer is white.

The Awn

An example of the rough appearance of the awn in Dickson barley is given in Fig. 1, which shows several barbs with broken tips. The barbs are about 30 μ in diameter and are about 150 μ long. The rough surface of the awn is accentuated by ridges and numerous small, blunted protrusions.

The Rachilla

The ventral view of the kernel showing the attachment end, the crease, and the rachilla in mature barley can be seen in Fig. 2, a; a higher magnification in Fig. 2, b shows the rachilla hairs in more detail. The hairs in Dickson barley are at the base about 10 μ thick and up to 200 μ long.

Husk, Pericarp, and Seed Coat

A cross-section through the lemma and palea is shown in Fig. 3, a. An oblique view of the husks (Fig. 3, b) shows the four main lemma tissues described by Bawtree and Gordon (3) as well as the hairs on the lemma surface. The differences in appearance of the top of a cross-section and of the side of the lemma (with several protruding tubes) and of the pericarp are shown in Fig. 3, c. Higher magnification (top view) of the lemma shows (Fig. 3, d) the fibro-vascular cells surrounded by secondary walls. Details of the pericarp layers, seed coat, and adjacent aleurone layer can be seen in Fig. 3, e.

Close to the point at which the lemma is drawn out into the awn, the surface in both mature and immature barley is covered with an amorphous, silica-like scale.

Ridges and stomata-like cells are seen on the surface of palea in immature (Fig. 4, a) and mature barley (Fig. 4, b). The presence of stomata-like cells would not be surprising, since husks are remnants of the protective envelope of the flower and are part of the leaf system of the plant. The larger diameter (about 12μ) of the oval-shaped cells is within the range of stomatal cells of cereal leaves. The presence of stoma in barley bran chaff was reported by Winton and Winton (1). However, the cells in Fig. 4, a and 4, b show none of the interior details normally seen in electron micrographs of viable stomata. The round, stomata-like cells showed no openings such as were observed by us in oats. According to Bawtree and Gordon (3), no true stomata are present on the outer epiderm of barley hulls. A fivefold magnification (not reproduced here) of one of the cells in Fig. 4, a indicated the presence of at least a two-layered structure. The ridges in immature barley are regular and relatively deep (Fig. 4, a); they are more variable in mature barley, however (Fig. 4, b).

Endosperm

A transverse section taken through the pericarp, seed coat, multilayered aleurone, and endosperm is shown in Fig. 5. The average length of the aleurone cell (in a transverse section at the germ end) was 28μ and its average width about 15μ . The whole wall between adjacent cells was about 3μ thick. Several compound middle lamellae can be seen between the cell-walls of two aleurone cells in Fig. 5. The section shows the multilayered structure of the pericarp, the two-cell-deep aleurone layer, and the subaleurone layers. The aleurone layers are in part open and show the spherical aleurone grains (diameter of about 1.5μ), and are in part covered by a wrinkled cell-wall material. The section through the aleurone cell-wall seems to indicate in several places a multilayered fibrous structure. Some of the aleurone grains have a rugged surface, indicating the possible presence of spherosomes, structures which can be seen by transmission electron microscopy (7,8). According to Jones (7), the aleurone grains of water-imbibed cells are roughly spherical in shape, with diameters ranging from 2 to 4μ . The size of the aleurone grains determined in this study on dry cells is substantially smaller, indicating considerable swelling from water imbibition. The spherosomes of fixed tissue are also spherical; they are found in close association with the aleurone grains and show a distinct pattern of arrangement around the periphery of the grain. In the subaleurone layer one can see (Fig. 5) several lentil-shaped (6 to 8μ long) starch granules, apparently embedded in a relatively thick protein matrix and forming cells separated by endosperm cell-wall material.

One of the aleurone cells is shown under high magnification in Fig. 6. Clearly seen at the inside bottom and upper left of the aleurone cell is the spotted appearance (presumably from pits in the surface of the cell-wall) which was observed in many of the sections studied.

A longitudinal section through the pericarp, aleurone, and starchy endosperm of immature barley showed no clear cellular differentiation of the aleurone layer. A higher magnification of material in the central endosperm of immature barley (Fig. 7, a) showed only isolated and small (diameter about 4μ) starch granules. The pitted appearance of the starch granules in Fig. 7, b from the longitudinal section of mature barley is an artifact that resulted from prolonged exposure to the electron beam in the scanning microscope. The high concentration of starch granules in the

central endosperm of the mature endosperm is in sharp contrast to the picture in Fig. 7, a. This contrast would be expected, since the ratio of protein to starch may be as much as 50 times higher in the earliest stages of growth than in the mature barley grain (9). According to Harris (10), the starch granules of barley are first seen as small spheres in the cells of the endosperm a few days after the beginning of seed development. These spheres later develop into the bean-shaped and lenticular forms characteristic of mature starch. The diameters of the large starch granules in Fig. 7, b are as much as 16μ , though many small-diameter granules (about 1.5μ) can be seen. The large granules seem to be relatively free; the smaller ones are embedded in a protein matrix. Some material, apparently proteinaceous, adheres to several of the larger starch granules. Starch granules are formed in amyloplasts, which probably accounts for adherent proteinaceous material. The presence of cell-wall material is indicated in Fig. 7, b. In addition to the kidney-bean-shaped starch granules, granules with surface indentations are present. The indentations indicate areas in which starch granules pressed against each other. As Buttrose (11) observed, the surface of the starch granules seems to be spongy, although the effect of surface modification by the electron beam cannot be excluded.

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