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MORPHOLOGICAL DEVELOPMENT IN SORGHUM GRAIN

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Abstract

Immature sorghum grain was harvested at various stages of maturity and its development followed by transmission and scanning electron microscopy. This was done to study the developmental morphology of the sorghum grain. The period immediately following fertilization is a time of rapid development in the sorghum caryopsis. The endosperm expands crushing the nucellar and in the nonbird-resistant sorghums the inner integument is also crushed during this expansion. The cells of the ovary wall expand and elongate to form the pericarp. By the soft dough stage the endosperm has gained most of its storage material and thereafter there is a considerable loss of moisture. During the early stages of development the endosperm cell walls were extensively pitted which could allow for translocation. However, once the period of translocation was over the cell walls became intact.

Introduction

Sorghum (Sorghum bicolor (L.) Moench) is the major cereal grain consumed in the semi-arid tropics. In Africa, sorghum is germinated and used as malt in the brewing of a traditional beer. This brewing is now carried out on an industrial scale. To improve the final product it is important to understand the initial ingredients. Sorghum differs somewhat from barley so it is important to study the structural changes which occur during sorghum malting. Because of this and other uses of sorghum for food, an understanding of the whole grain structure is important. To further aid in an understanding of this structure, this study of sorghum's developmental morphology was undertaken.

To describe the morphology of the sorghum kernel, a standard terminology is now widely recognized and will be used here (Doggett, 1970; Morral et al., 1981; Earp and Rooney, 1982). The main parts of the caryopsis are the embryo with its scutellum which together make up the germ, the endosperm which is a storage tissue and the pericarp which encases the kernel.

Most of the studies reported so far on sorghum grain structure have been concerned with the grain as regards its utilization (Hoseney et al., 1974; Sullins and Rooney, 1975). Insufficient information is available in the literature on the development of immature sorghum to be meaningful. Although limited work has been reported (Subramanyam et al., 1980 a & b) this study was undertaken to show the origin and development of some of the more important structures as relates to changes in the kernel during malting. As the embryology of sorghum grain has already been covered (Artschwager and McGuire, 1949) the present study does not cover embryo development.

KEY WORDS: Sorghum bicolor, grain developmental morphology, endosperm development, pericarp development, cell wall.
Materials and Methods

A non-bird-resistant sorghum variety, NK 283, was grown by the Plant and Seed Control Division, Roodplaat Experimental Farm, South African Department of Agriculture. The different stages of maturity agreed with those described by Vanderlip and Reeves (1972) and samples were collected at the intervals listed in Table 1.

Table 1. Stages of development and times of harvest of immature NK 283 sorghum grain (1982/83 crop)

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Days post-anthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-fertilization</td>
<td>7</td>
</tr>
<tr>
<td>Milk stage</td>
<td>14</td>
</tr>
<tr>
<td>Soft dough</td>
<td>21</td>
</tr>
<tr>
<td>Hard dough</td>
<td>28</td>
</tr>
<tr>
<td>Physiological ripe</td>
<td>35</td>
</tr>
<tr>
<td>Time of harvest</td>
<td>63</td>
</tr>
</tbody>
</table>

The panicles were collected when the kernels in the middle of the panicle had reached the correct stage of maturity. Kernels were taken from the middle of these panicles and fixed within an hour of harvest.

Specimens were prepared for scanning electron microscopy by cutting or fracturing through the middle of the kernel and then fixing in 5% glutaraldehyde buffered to pH 7.4 at 4°C. The samples were then dehydrated through an ethanol series and critical-point dried from amyl acetate-CO₂. Samples were mounted on metal stubs with double-sided Cello-tape and sputter coated with gold-palladium. They were then examined with a JEOL JSM 135 Scanning Electron Microscope with an accelerating voltage of 20 kV. Material for transmission electron microscopy was prepared as previously described (Glennie et al., 1983). Approximately six to eight separate samples were examined for each developmental stage.

Total polyphenols were determined using a modified Jerumanis method (Daiber, 1975). Starch was determined by enzymic hydrolysis and measured as glucose by the glucose oxidase method (Fleming and Pegler, 1963).

Results and Discussion

Post-anthesis in sorghum was found to be a period of rapid development.

Developmental stages will be used when discussing the age of the caryopsis. The age in days is presented in Table 1 but age and developmental stage do not always coincide, depending on season and geographic location.

Post-anthesis was the first stage where the endosperm could be observed expanding into the nucellus (Figure 1a). At this stage the immature sorghum endosperm had a more rigid cellular structure than the coenysic endosperm of the developing wheat caryopsis (Bechtel et al., 1982). The collapse of the sorghum nucellus was probably caused by sample preparation for scanning electron microscopy. When similar samples were prepared for transmission electron microscopy by embedding, this collapse was not observed. It is probable that the nucellus cells had insufficient contents and insufficiently strong cell walls to withstand preparation for EM without embedding. In contrast to Figure 1a, grain at the hard dough stage appeared reasonably mature (Figure 1b). The glumes no longer remained attached, the ovary wall had developed into a pericarp, the endosperm had completely crushed the nucellus and the germ was well developed.

The ovary wall of the immature kernel developed into the pericarp. This development took the form of cell enlargement rather than cell division (Sanders, 1955). Seven days after anthesis the ovary wall was composed of fairly regular almost rectangular cells (Figure 2a). The cell walls of these cells appeared to be regularly pitted. Since these cells develop into a pericarp by expansion it is possible that these pits could be areas of cell wall expansion. A common feature of these cells was plastids containing small, presumably immature, starch granules (Figure 2b). In this respect, sorghum is similar to maize.

As the endosperm expanded, the cells in the pericarp came under pressure which caused them to elongate. Fourteen days after anthesis, at the milk stage, the pericarp cells had both elongated and filled with starch (Figure 3). Although the starch granules in Figure 3 were much larger than those in Figure 2b, those starch granules found in the pericarp were smaller than those found in the endosperm. The starch granules in the pericarp are probably fully developed at the milk stage as the plastolysm of the pericarp cells starts to degenerate about 14 days after anthesis.

There appears to be some confusion in the literature about what is the testa in sorghum grain. Definitions vary from the seed coat which adheres to the outer edges of the inner integument (Hoseney et al., 1981) to that layer whose presence is controlled by the B₁ and B₂ genes (Rooney et al., 1979). In the work described here, reference will be made to the polyphenol containing layer which can confer bird-resistance on the grain and is developed from the inner integument (Morralle et al., 1981). The polyphenol containing layer is usually absent in non-bird-resistant sorghums, the inner integument simply being crushed by the expanding endosperm. The micrographs in Figure 4 show the sequential collapse of the inner integument in a non-bird-resistant sorghum.

The inner integument, which at the time of anthesis surrounded the nucellus, was two cells thick (Figure 4a). The cells were full of cytoplasm and there appeared to be no lateral stretching of the cells. The inner integument was separated from the nucellus by a layer of cutin. Sanders (1955) referred to a cutin layer covering the inner integument but in this study as well as another (Morralle et al., 1981), a cutin layer was found between the integument and
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Fig. 2. Sorghum pericarp at different stages of development.
a) Scanning electron micrography of ovary wall at anthesis. Bar = 100 μm.
b) Transmission electron micrograph of plastid in mesocarp cell at post-fertilization. SG = starch granule; arrow = thylakoid. Bar = 0.5 μm.

Fig. 1. Scanning electron micrographs of sorghum grain at different stages of development.
a) Developing endosperm (arrow) in ovary at post-fertilization. Bar = 100 μm.
b) Grain at hard dough stage. Bar = 1,000 μm. E = endosperm; Em = embryo; FE = floury endosperm; HE = horny endosperm; N = nucellus; OW = ovary wall; P = pericarp; S = scutellum.

Fig. 3. Transmission electron micrograph of the mesocarp at milk stage. Starch granules (SG) are present. Bar = 2.0 μm.
enucleus. At the milk stage it was located between the residual inner integument and the aleurone layer of the endosperm (Figure 4b).

At the milk stage the endosperm had completely crushed the nucellus and was still expanding thus causing the cells of the pericarp to elongate. The cell contents of the inner integument disappeared during this period and by the soft dough stage the endosperm had expanded sufficiently to stretch the inner integument into a narrow band (Figure 4c). The inner integument was completely crushed at the hard dough stage (Figure 4d). The next distal layer of cells (tube cells) now appeared to contain small amount of electron dense material which could be polyphenol as well as some granules which resembled starch granules.

The polyphenol content of the grain at different stages of development is listed in Table 2. Kernels at the milk stage contained no detectable polyphenol but from this stage onward the polyphenol content increased until at the time of harvest it contained 1.50 mg/100 kernels.

Table 2. Starch and polyphenol content in sorghum grain at different stages of development reported on dry weight basis

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Starch g/100 kernels (%)</th>
<th>Polyphenol mg/100 kernels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk stage</td>
<td>0.56 (33.07)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Soft dough</td>
<td>1.38 (48.19)</td>
<td>1.02 (0.02)</td>
</tr>
<tr>
<td>Hard dough</td>
<td>1.42 (51.77)</td>
<td>1.06 (0.03)</td>
</tr>
<tr>
<td>Physiological ripe</td>
<td>2.24 (59.42)</td>
<td>2.13 (0.05)</td>
</tr>
<tr>
<td>Time of harvest</td>
<td>2.18 (70.75)</td>
<td>1.50 (0.04)</td>
</tr>
</tbody>
</table>

Even at the milk stage, a bird-resistant cultivar was found to contain considerable amounts of polyphenol (Glennie et al., 1981). Although the polyphenol content of the nonbird-resistant cultivar used in this study is low when compared to that of the bird-resistant sorghums, it could account for the electron dense areas in Figure 4d.

The development of the endosperm is one of the most important areas of grain development. The kernel gains most of its dry matter during the early stages of development while the later stages are marked mainly by a large reduction in moisture (Figure 5). As shown in Figure 5 the kernels have an early period of very rapid dry weight gain. This gain can be accounted for mainly by the deposition of starch (Table 2).

The subaleurone cells of the endosperm contain both starch granules and protein bodies. Even at the soft dough stage, starch and protein are beginning to be close-packed (Figure 6a). At the hard dough stage the starch granules are more closely packed and will soon become indented by the protein bodies (Figure 6b). At this stage the starch granules contain sufficient water to be susceptible to enzyme attack (note pits). This would probably mean that they are sufficiently plastic to be easily indented by the protein bodies.

At the hard dough stage, starch granules in the central or floury endosperm were still round and relatively soft (Figure 7a). At the physiological ripe stage, the starch granules were packed closely together and were forced to take up polygonal shapes (Figure 7b). Subramanyam et al., (1980a) proposed that the round and relatively soft starch granules are forced into polygonal shapes as water is lost from the kernel. The present study supports this as there is sufficient loss of moisture during this period to

Fig. 4. Transmission electron micrographs of inner integument. The inner integument is the two cell thick area between the two arrows. a) Post-fertilization - cells (between arrows) are beginning to elongate but still retain their contents. Thick arrow - nucleus. Bar = 3.5 µm. b) Milk stage - cells (between thick arrows) are elongating and contents are decreasing. Cutin layer (thin arrow) is visible between inner integument and the aleurone layer of the endosperm. Bar = 2.0 µm. c) Soft dough - only a residual inner integument (between arrows) remains between the aleurone layer and the mesocarp. Bar = 1.0 µm. d) Physiological ripe - residual inner integument (between arrows) crushed between endosperm and mesocarp with traces of polyphenol-like material (P). Bar = 1.0 µm. Al = aleurone cell; C = cutin layer; II = inner integument; P = polyphenol; SG = starch granule.

Fig. 5. Dry weight and moisture content during grain development of sorghum grain. Day 0: anthesis; day 7: post-fertilization; day 14: milk stage; day 21: soft dough; day 28: hard dough; day 35: physiological ripe; day 63: time of harvest.

Fig. 6. Scanning electron micrographs of horny endosperm subaleurone area. a) Soft dough. Bar = 10.0 µm. b) Hard dough. Bar = 2.0 µm. PB = protein bodies; SG = starch granules.
cause the starch granules to pack closely (Figure 5). Also, starch is still being deposited (Table 2) to reinforce this close packing.

Just as the immature panicle contains grains of different degrees of maturity the endosperm of a single immature kernel contains cells of varying physiological ages. It appears that there is a gradient in maturity from the peripheral area near the aleurone layer to the central floury endosperm. The peripheral cells were found to be the more mature. This supports the observation made by Sanders (1955) that the outermost layer of the endosperm was the first area to mature. A similar pattern was reported for immature Zea mays L. kernels where a variability in the starch content of the different cells was reported (Shannon, 1974). During germination, the reverse pattern was found. Endosperm modification began in the central floury endosperm adjacent to the scutellum (Glennie et al., 1983).

The cell walls of the endosperm enclose the storage products deposited there. Yet, during the developmental stages, cell walls must allow for the translocation of the precursors. During the milk stage the endosperm was in an active state of filling and the cell walls contained large numbers of pits (Figure 8a). Although translocation can easily occur through cell walls, the large number of pits in Figure 8a would also allow for rapid translocation. Also, the pits could represent areas of cell wall synthesis as the cell walls expanded with the expanding endosperm.

By the hard dough stage the filling of the endosperm was virtually complete and the cell walls contained only a few random pits as shown in Figure 8b. The cell walls appeared intact at the physiological ripe stage with no visible pitting (Figure 8c). At this stage the kernel had reached its maximum starch content (Table 2) and translocation had probably ceased. It appears that the more starch that was deposited in the endosperm, the fewer pits that appeared in the endosperm cell walls.

During germination it was found that the reverse pattern of cell wall pitting occurred (Glennie, 1984). The entire cell walls that were present in the mature grain were found to be extensively pitted after six days germination. Besides translocation through the cell walls, the pits would allow for translocation of storage materials out of the endosperm. Unfortunately, the micrographs from both of these studies were unable to demonstrate that the pits formed during germination were in the same location as the pits which were present during development.

Conclusions

In the immature sorghum caryopsis a period of rapid development occurred immediately following fertilization. The expanding endosperm crushed the inner integument of the nonbird-resistant sorghum. At the same time, the ovary wall developed into a pericarp. The grain had acquired most of its storage products by the soft dough stage and from this stage onwards development was marked by a considerable loss in moisture. Up to the hard dough stage the endosperm cell walls exhibited extensive pitting but when this period of translocation ceased the pits disappeared and the cell walls became intact.

References

Earp CF, Rooney LW. (1982). Scanning electron microscopy of the pericarp and testa of several sorghum varieties. Food Microstructure 1, 125-134.
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Fig. 7. Scanning electron micrographs of the central floury endosperm.
(a) Hard dough - starch granules are very loosely packed and debris is probably cell contents involved in starch deposition. Bar = 10.0 μm.
(b) Physiological ripe - starch granules are more closely packed with very little debris. Bar = 2.0 μm. CW = cell wall; SG = starch granule.

Fig. 8. Scanning electron micrographs of endosperm cell walls.
(a) Milk stage with many pits (P). Bar = 1.1 μm.
(b) Hard dough with few pits. Bar = 1.1 μm.
(c) Physiological ripe with no visible pits. There is much debris as well as the protein matrix (PM) which surrounds the starch granules in the horny endosperm. Bar = 1.1 μm.


Discussion with Reviewers

L.W. Rooney: What conditions existed in the field during sorghum growth and development? Moulds can and do affect kernels during development. Could they account for the "holes" in the cell walls?

Authors: Sorghum is normally grown in Africa under semi-arid conditions with the grain being left in the field to dry naturally under the sun before harvest. Moulds are rarely a problem. The sorghum for this experiment was grown in 1982/83 in the middle of a severe drought. These conditions are hardly favourable for mould growth. Many sections of grain were examined by both SEM and TEM and not a trace of fungi was found.

L.W. Rooney: What physiological studies exist that support your contention that holes are present in the cell walls? On the basis of a single sample grown in a single season, it is very dangerous to draw the conclusions you have made. What other data support your conclusions?

Authors: We prefer the term "pits" to "holes". Over the last few years we have been isolating the cell walls of sorghum grain as well as sorghum malt. The cell walls of sorghum become extensively pitted during malting but they do not breakdown completely as they do during barley malting. Since pits are usually associated with translocation (e.g. in tracheids in the xylem) and they appeared in the sorghum endosperm cell walls at a time when large amounts of reserve material was being mobilized, we assumed that they could act as areas of translocation.

L.W. Rooney: The objective of your work was to study the sorghum kernel during its development. Why were photos not presented for each of the various sampling periods after anthesis? Why are not photos presented for zero days (at anthesis)?

Authors: Photomicrographs of the caryopsis at fertilization as well as other photomicrographs at some stages of development have already been published (Sanders, 1955; Subramanyam et al., 1980 a and b).

L.W. Rooney: Sorghum kernels within a panicle vary in maturity from top to bottom by as many as 5-7 days. Did you account for this in your sampling procedures?

Authors: Samples were collected by experienced horticulturists and every effort was made to obtain material which fitted the correct stage. Only material from the middle of the panicle was taken in an effort to avoid variation in maturity from top to bottom. The samples were fixed in 5% glutaraldehyde within an hour of collection.

C.F. Earp: When you state differences in starch granule size - what range do you see in pericarp and parts of endosperm?

Authors: Although there is wide variation in starch granule size within any area we find that those in the pericarp are approximately 2-4 µm in diameter while those in the floury endosperm are approximately 10-15 µm (those in the horny endosperm appear to be slightly smaller). For comparison, see Discussion with Reviewers in Earp and Rooney, 1982.

E. Varriano-Marston: Do you think that the debris on the cell walls in Fig. 7a and Fig. 8 is possibly caused by the electron microscopic preparation method (fixation, critical point drying)?

Authors: It is quite possible that this debris is precipitated by fixation and critical point drying. However, what is important is that when the different stages of development are treated in a similar fashion some stages of development produce more debris than others (e.g. Figs. 8a, b and c).

E. Varriano-Marston: Why weren't the samples freeze-dried rather than solution fixing and critical point drying? If the grains were fairly low moisture content then solution fixing undoubtedly caused dislocation of some of the cellular components and large voids in some of the cells, e.g. Fig. 7a.

Authors: It was found that critical point drying preserved more of the structural detail than did freeze drying. However, freeze drying was used to prepare grain samples for energy dispersive X-ray analysis. (Glennie et al., 1981).

Additional reference