SCANNING ELECTRON MICROSCOPY OF HIGH QUALITY AND LOW QUALITY SORGHUM SEEDS

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ABSTRACT

Comparison of the anatomical structures of high density seeds with low density seeds and early germinating with late germinating seeds of sorghum [Sorghum bicolor (L.)] were made using scanning electron microscopy. The differences in anatomical structures of high and low density seeds and early and late germinating seeds were primarily due to differences in maturity.

High density seed had a thinner pericarp, were better filled in the endosperm and had a better developed embryonic axis than the low density seed. Differences in early germinating and late germinating seed between 1.17 and 1.30 specific gravity were not as pronounced as in the low density seeds and late vs. early germinating seed from the ungraded sample. There were no obvious differences in pericarp thickness, celeoptile and plumule development. Differences were evident in the radicle area where structures were not as well organized.

Additional Index Words: Seed quality, Seed density, Seed morphology.

INTRODUCTION

A close and consistent relationship between seed density and/or specific gravity, seed size, and seed quality in sorghum, cotton, rice, has been established (Cortes 1987, Gregg 1969, Sung & Delouche 1962). Weathered seeds tend to be lower in density, germination and vigor than non-weathered seeds, while immature seeds tend to be smaller in size, and lower in density, germination and vigor than mature seeds.

Cortes (1987) found that sorghum seeds from the medium and large size classes were significantly higher in percent germination than those from the small size and unsized classes. Germination increased as specific gravity increased. The three highest specific gravity classes, i.e. 1.26, 1.30, and 1.34 had the highest germination values. Germination decreased sharply as specific gravity declined below 1.26.

Maranville and Clegg (1977) found that sorghum seedling emergence, final stand, and grain yield were not a function of seed size or density when the same number of viable seeds were planted in the field.

Glueck et al. (1977) used electron microscopy to study associations between anatomical properties of sorghum resistance to field grain deterioration (weathering). They concluded that the outer layers of the kernel appeared

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to be at least partially responsible for resistance to field deterioration of grain in some sorghum lines. Glueck and Rooney (1976) characterized 25 sorghum lines for resistance to grain deterioration based on physical and chemical characteristics. They concluded that hybrids developed from more resistant lines have improved grain quality. Earp and Rooney (1982) concluded that microscopic techniques can be used to select for pericarp and testa thickness.

Woodstock et al. (1985) examined the relationship between weathering deterioration and germination, respiratory metabolism, and mineral leaching from cotton (*Gossypium hirsutum* (L.)) seeds. Deterioration of membranes due to weathering was confirmed by electron microscopy of cotyledonary lipid and protein bodies.

The objective of this study was to compare the anatomical structures of high quality sorghum seeds with low quality sorghum seeds by using scanning electron microscopy.

**MATERIALS AND METHODS**

**Study 1**

Sorghum seeds from a high vigor and low vigor lot, as determined by soil cold test emergence, were density separated using sugar solutions (Cortes 1987). Seeds from a high vigor lot with a specific gravity >1.30 and seeds from a low vigor lot with a specific gravity <1.17 were cryofractured in liquid nitrogen with a razor blade (modified from Humphreys et al., 1974). After cryofracture, halved seeds were thawed in absolute alcohol and then rinsed three times in fresh absolute alcohol. The total time in absolute alcohol was 1.5 hours. After the alcohol rinse, half seeds were critical-point dried in carbon dioxide with a Polaron E 3000 critical-point drying apparatus. Two halved seeds per stub were attached with silver paste. When the silver paste was fully dried, the specimens were sputter coated with gold-palladium in a Polaron E 5100 unit. The seeds were examined and photographed in a Hitachi HHS-2R scanning electron microscope with an accelerating voltage of 20 kV.

**Study 2**

Sorghum seeds, from a commercial lot that had not been specific gravity separated, were planted in moistened seed germination towels and placed in a plastic crisper at room temperature (approximately 25°C) in the dark. Seeds which had germinated (radicle penetrating the pericarp) were selected daily and subsequently placed in half-strength Karnovsky’s fixative (2% formaldehyde, 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer). The first group of 12 germinated seed selected was at 28 h after planting. At 76 h after planting, 12 additional germinated seeds were removed and given the same treatment as the seeds removed at 28 h after planting. After 2 d seeds from both groups were taken out of the refrigerator and brought to room temperature. Seeds were then rinsed in 0.1 M phosphate buffer (pH 7.3) for 40 m (4 changes of buffer). The germinated seeds were cut through the radicle with a razor blade while submerged in the buffer. The cut seeds were further fixed for 1 h in fresh half-strength Karnovsky’s fixative. The halved seeds were then rinsed for 3 h in phosphate buffer.
(6 changes), followed by osmication (overnight) in buffered 2% osmium tetroxide. The halved seeds were rinsed twice in 0.1 M phosphate buffer and dehydrated in a graded series of ethanol. The dehydrated cut seeds were critical-point dried in carbon dioxide. The remainder of the procedures were the same as in the first study.

**Study 3**

A third study was conducted following the same selection procedures as in study two except only seeds with a specific gravity between 1.17 and 1.30 were compared.

**RESULTS AND DISCUSSION**

The anatomical differences between high density, high vigor seeds and low density, low vigor seeds are presented in Figures 1 and 2. High vigor, high density (>1.30 specific gravity) seeds were well filled in the endosperm area and had well developed embryonic axes (Figure 1A). Pericarp thickness was 0.30 mm. The pericarp was well organized with a thin mesocarp area with few starch cells (Figure 2A).

Low vigor, low density (<1.17 specific gravity) seeds had a cavity in the scutellar area, and the embryonic axis was not well developed (Figure 1B). The presence of fungi could also be seen in the cavity in the scutellar region. Pericarp thickness was 0.70 mm. The pericarp was less organized, with a thick mesocarp area with numerous starch cells (Figure 2B). The differences in pericarp thickness, organization of the pericarp, and development of the embryonic axis indicated that the differences between high density, high vigor and low density, low vigor seeds were due to differences in stages of maturity.

A second study compared seeds from an ungraded commercial lot that germinated 28 h after planting with seeds that germinated 76 h after planting in moist rolled germination towels. Representative photomicrographs of each group are presented in Figures 3, 4, 5, 6 and 7. Seeds germinating after 28 h had a well developed coleoptile and plumule (Figure 3A and 3B). The radicle and root cap were well developed with a penetrating coleorhiza (Figure 6A). There were starch cells at the area of attachment between the embryonic axis and the scutellum (Figure 7A). The pericarp thickness was 0.30 mm with a thin mesocarp area (Figure 4A). Very few starch cells were found in the mesocarp area.

Seeds germinating at 76 h were less densely filled and had more cavities in the endosperm area (Figure 3B). The coleoptile and plumule were less developed (Figure 5B). The radicle cells collapsed and little differentiation could be seen (Figure 6B). Fewer starch cells were present at the area of attachment between the embryonic axis and the scutellum (Figure 7B). Starch cells were more organized in the scutellar area. The pericarp thickness was 0.65 mm, with a thicker mesocarp area and numerous starch cells (Figure 4B). The differences between early germinating seeds and late germinating seeds also appeared to be due to stages of maturity.
Figure 1. High vigor, high density seed (A) compared to low vigor, low density seed (B). Pericarp (P), endosperm (E), scutellum (S) and embryonic axis (EA). (20X).
Figure 2. Pericarp of high vigor, high density seed (A) compared to low vigor, low density seed (B). Pericarp (P) and mesocarp (M). (500X).
Figure 3. Early germinating seed (A) compared to late germinating seed (B). Ungraded. (20X).
Figure 4. Pericarp of early germinating seed (A) compared to late germinating seed (B). (Ungraded). (500X).
Figure 5. Plumule of early germinating seed (A) compared to late germinating seed (B). (Ungraded). Coleoptile (C) and plumule (P). (100X).
Figure 6. Radicle of early germinating seed (A) compared to late germinating seed (B). (Ungraded). Radicle (R), root cap (RC) and coleorhiza (Ca). (100X).
Figure 1. Area of attachment of scutellum and embryonic axis of early germinating seed (A) compared to late germinating seed (B). (Ungraded). Embryonic axis (EA) and scutellum (S). (60X).
A third study was conducted comparing seeds that germinated at 28 h with seeds that germinated at 76 h with a specific gravity between 1.17 and 1.30. Differences between the seeds germinating within 28 h and the seeds germinating within 76 h could be detected but were not as pronounced nor did they appear to be the same as in the first two studies (Figures 8, 9, 10 and 11). There were no obvious differences in the pericarp development of the seeds (Figure 8). The differences between early and late germinating seeds were more pronounced in the radicle area (Figure 10) as compared to the Coleoptile and plumule area (Figure 9) as in the previous studies. Structures in the late germinating seeds did not appear to be as well developed and well organized as in the early germinating seed (Figures 8, 9, 10 and 11). These differences could be due to stages of maturity or they could be differences due to deterioration. The differences here need to be confirmed through further study and testing.
Figure 8. Early germinating seed (A) compared to late germinating seed (B). Specific gravity 1.17 - 1.30. (20X).
Figure 9. Plumule of early germinating seed (A) compared to late germinating seed (B). Specific gravity 1.17 - 1.30. (90X).
Figure 10. Radicle of early germinating seed (A) compared to late germinating seed (B). Specific gravity 1.17 - 1.30. (90X).
Figure 11. Area of attachment of scutellum and embryonic axis of early germinating seed (A) compared to late germinating seed (B). Specific gravity 1.17 - 1.30. (60X).
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