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Comparison of Non - Wrinkled and Wrinkled Soybean Seedcoats by Scanning Electron Microscopy

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COMPARISON OF NON-WRINKLED AND WRINKLED SOYBEAN SEEDCOATS BY SCANNING ELECTRON MICROSCOPY

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Abstract

Cross-sectioned soybean seed of the 'Bragg' and 'Davis' cultivars with either wrinkled or non-wrinkled seedcoats were examined by scanning electron microscopy for their seedcoat characteristics. The typical soybean seedcoat structure observed for both cultivars consisted of an outer cuticle with internal palisade, hourglass, and parenchyma cell layers. However, the layer of hourglass cells in both wrinkled and nonwrinkled seedcoats of both cultivars decreased gradually in thickness until it disappeared completely in the area opposite the hilum. In addition, hourglass cells in the area of seedcoat wrinkling in both cultivars appeared twisted, compressed, and distorted. The appearance of these cells may explain the wrinkled seedcoat condition which usually occurs opposite the hilum when seeds are exposed to alternate wetting-drying cycles. The role of the hourglass cells as a supporting, "cushion-like" mechanism is suggested.

Additional index words: Glycine max (L.) Merr., soybean seedcoat, cuticle, hourglass cells, palisade cells, parenchyma cells.

Exposure of soybean seed to alternate wet and dry weather while awaiting harvest after maturity causes seedcoat wrinkling which is referred to by Moore (6, 7) and Wolf et al. (10) as water damage. Moore (6, 7) pointed out that unequal expansion of the seedcoat during rehydration and dehydration leads to unequal stresses which causes wrinkling. The outer surface of the cotyledons just beneath the area of seedcoat wrinkling becomes damaged and may result in either abnormal seedling or complete absence of germination. Pereira (8) observed reduced seedling emergence for soybean seeds with wrinkled seedcoats compared to those with non-wrinkled seedcoats. Pereira (8) also stained seeds with both wrinkled and non-wrinkled seedcoats with 2, 3, 5 triphenyl tetrazolium chloride and observed discolored "bands" on the cotyledons corresponding to the same patterns of the visible wrinkled on the external surface of the seedcoat. These "bands" were atypical in their staining pattern, either intensely stained or almost white, and were invariably prominent on the cotyledonary area opposite the hilum (6, 7, 8).

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Recently, the scanning electron microscope (SEM) has been used in studying the structural features of soybean seedcoats (1, 9, 10) as well as assessing water absorption and disease infection in seeds (1, 4). Seedcoats of soybeans and legumes possess characteristic layers -the cuticle, and layers of palisade, hourglass and parenchyma cells (1, 2, 3, 4, 9, 10). It is not known, however, what happens to these layers when they are subjected to alternate rehydration-dehydration cycles. The objective of this study was to use SEM to examine modifications of the seedcoat structure that might be implicated with the wrinkling process.

Materials and methods

Soybean seeds of the 'Bragg' and 'Davis' cultivars were produced in 1981 at Mississippi State, MS. Seeds of both cultivars were sorted by hand into wrinkled and non-wrinkled categories according to the external appearance of their seedcoat.

Seeds were cross-sectioned transversely through the hilum with a single-edge razor blade and were mounted with the cut surface upwards on aluminum stubs with epoxy glue (Ross Chemical Co., Detroit, MI 48209). The specimens were coated with gold/palladium (60:40) in a Polaron E 5100 Series II Cool sputter coater (Palaron Equipment Ltd., Watford, England) and examined in a Hitachi HHS - 2 R Scanning Electron Microscope (Hitachi Electronics, Ltd., Tokyo, Japan) at an accelerating voltage of 20KV. Photomicrographs were taken with Polaroid type 55 P/N 4 x 5 Land film (Polaroid Corp., Cambridge, MA 02139) at a working distance of 15 mm.

Results and Discussion

The SEM micrographs revealed seedcoat patterns for both cultivars which were quite similar. The cuticle and palisade, hourglass, and parenchyma cell layers were highly developed at the subhilar region (Fig. 1 and 4), but the size of the hourglass cells gradually decrease and disappeared in the region opposite the hilum (Fig. 2 and 5). McEwen et al. (5) showed the existance of hourglass cells in faba beans (Vicia Faba L.) on the side directly opposite the hilum but made no reference to their size when compared to those in other regions of the seedcoat. During our work, however, we did not observe any hourglass cells in the area of the seedcoat directly opposite or distal to the hilum. In addition, a comparison of wrinkled and non-wrinkled seedcoats of both Davis and Bragg showed that the hourglass cells of the wrinkled and non-wrinkled seedcoats in the area where seedcoat wrinkling began to occur appeared compressed and twisted (Fig. 3 and 6). However, hourglass cells were not altered in non-wrinkled seedcoats.

The conspicuous hourglass cells of the subhilar region act like a "cushion" preventing the occurrence of wrinkles which have been identified by Moore (6, 7) as the consequence of successive cycles of rehydration and dehydration. Since there are no hourglass cells present in the region opposite the hilum, the expansion and contraction of the seedcoat cannot be "cushioned", and it is forced to wrinkle. Seeds with moisture contents below 18% are more susceptible to rapid rehydration-dehydration cycles which promote seedcoat wrinkling (Fig. 10), while seeds with moisture contents above 18% are less affected and exhibit little, if any, seedcoat wrinkling (Fig. 7). This suggests that seedcoat wrinkling does not occur in those seeds which are not yet sufficiently dry (mature) when rehydration-dehydration cycles occur during the critical post-maturation, pre-harvest interval just prior to harvest. This cyclic phenomenon exerts its greatest influence on those seeds which have dried down (matured) to field maturity, approximately 13-15% moisture. The cotyledonary cells underlying these wrinkles are subject to pressure which causes them to bruise or even die (Fig. 11 and 12). Thus, deterioration is initiated which eventually spreads throughout the entire seed, decreasing its quality and reducing field emergence (6, 7, 8). On the other hand, seeds not yet dry enough to be stressed by variable moisture do not exhibit seedcoat wrinkling and cotyledonary deterioration (Fig. 8 and 9).

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References

Calero, E., S. H. West, and K. Hinson. 1981. Water absorption of soybean seeds and associated causal factors. Crop. Sci. 21:926-933.

Carlson, J. B. 1973. Morphology, p. 17-95. In Caldwell, B. E. (ed.), Soybeans: Improvement, Production and Uses. Agronomy series #16, Amer. Soc. of Agronomy, Madison, WI.

Esau, K. 1977. Anatomy of seed plants. 2nd edition, John Wiley and Sons. New York, NY. 550p.

Hill, H. J. and S. H. West. 1982. Fungal penetration of soybean seed through pores. Crop Sci. 22:601-605.

McEwen, T. J., B. L. Dronzek, and w. Bushuk. 1974. A scanning electron microscope study of faba bean seed. Cereal Chem. 51:750-757.

Moore, R. P. 1972. Effects of mechanical injuries on viability, p. 94-113. In Roberts, E. H . (ed.), Viability of Seeds. Syracuse Univ. Press. Syracuse, NY.

Pereira, L. A. G. 1974. Comparisons of selected vigor tests for evaluating soybean seed quality. M.S. Thesis, Miss. State Univ., Miss. State, MS.

Wolf, W. J. and F. L. Baker. 1972. Scanning electron microscopy of soybeans. Cereal Sci. Today 17:125-130.

Wolf, W. J., F. L. Baker, and R. L. Bernard. 1981. Soybean seedcoat structural features: pits, deposits and cracks. Scanning Electron Microsc. III. 531-544.

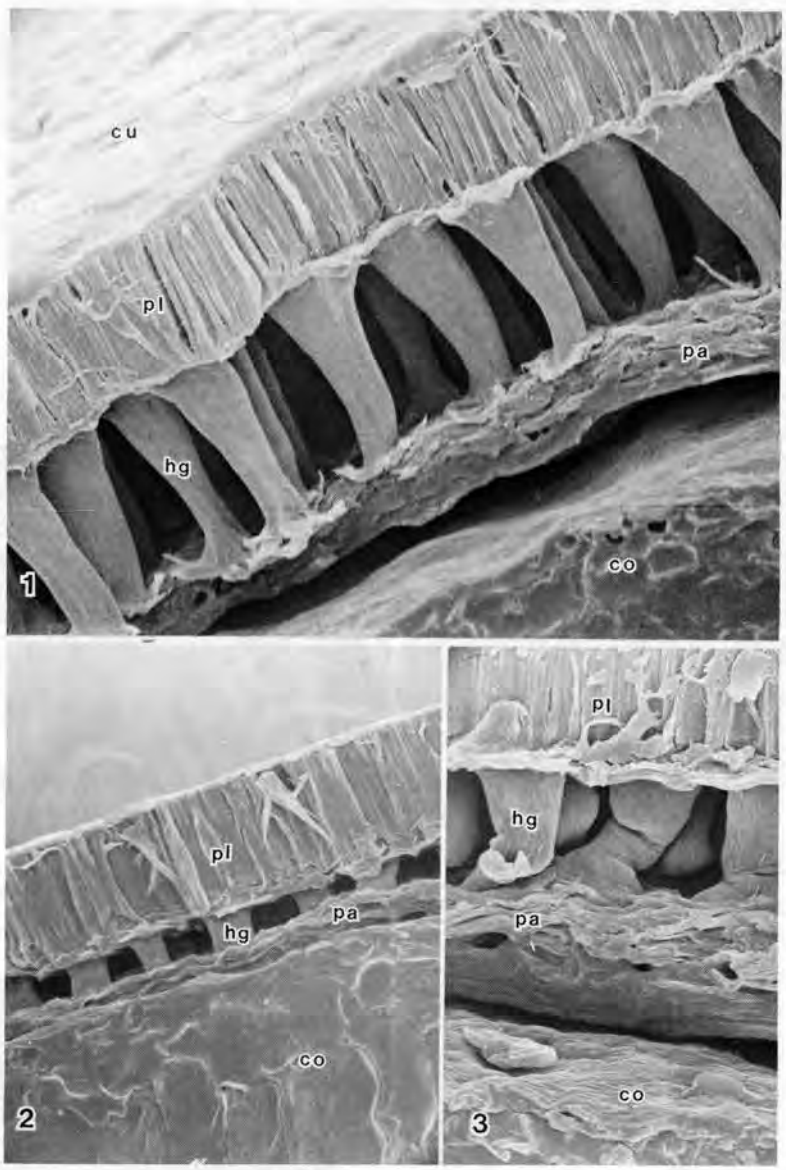

Fig. 1-3. SEM micrographs of seedcoat structure, cv. Davis. Fig. 1 at the subhilar region; X 250. Fig. 2 at an intermediary point between the hilum and its distal region; note that hourglass cells are visibly smaller; X 500. Fig. 3 wrinkled seedcoat near the subhilar region; note the compressed twisted hourglass cells; X 800. cu = cuticle; pl = palisade cells; hg = hourglass cells; pa = parenchyma cells; co = cotyledon.

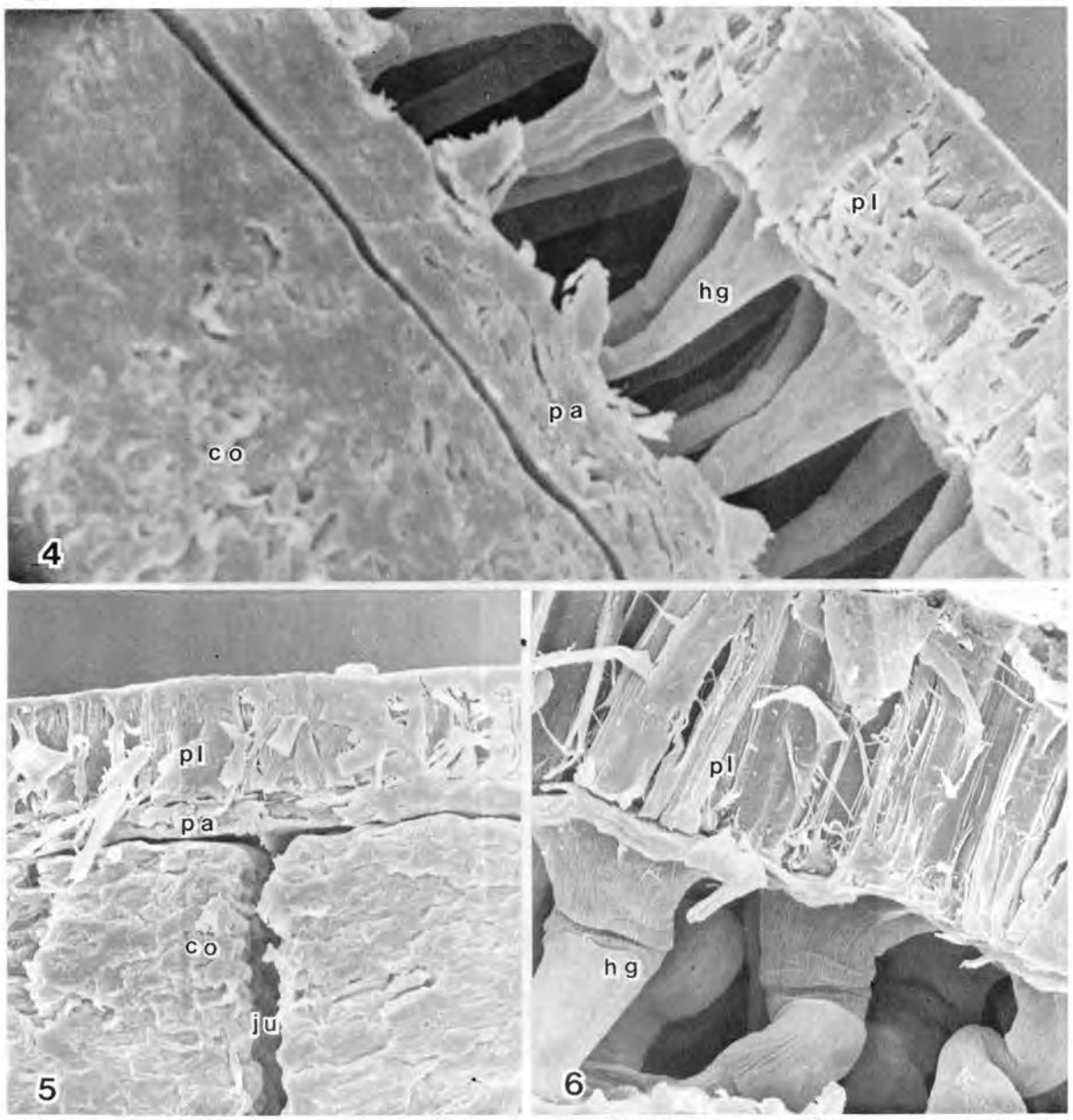

Fig. 4-6. SEM micrographs of seedcoat structure, cv. Bragg. Fig. 4 at the subhilar region; X 350. Fig. 5 at the region directly opposite the hilum; note the absence of hourglass cells; X 400. Fig. 6 wrinkled seedcoat near the subhilar region; note the compressed, twisted hourglass cells; X 850. pl = palisade cells; hg = hourglass cells; pa = parenchyma cells; co = cotyledon; ju = juncture between cotyledons.

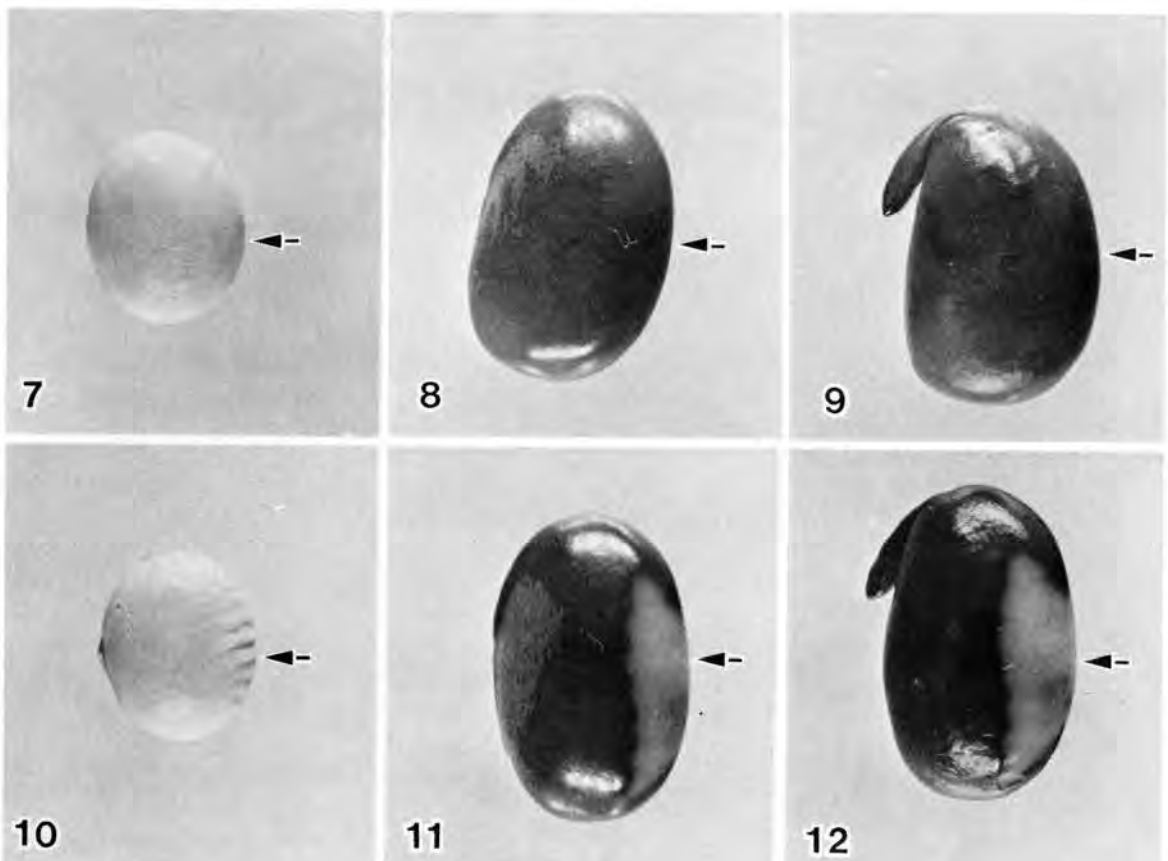

Fig. 7-9. Non-wrinkled seed of Davis showing smooth seedcoat (7), TZ stained seed with seedcoat intact (8), and TZ stained seed with seedcoat removed (9). Arrows point out absence of wrinkled seedcoat and areas of deterioration in TZ stained seed.

Fig. 10-12. Wrinkled seed of Davis showing wrinkled seedcoat (10), TZ stained seed with intact seedcoat (11), and TZ stained seed with seedcoat removed (12). Arrows point out wrinkled seedcoat and areas of deterioration just beneath wrinkled in TZ stained seed.