Anatomy and Histochemistry of Taro Seed

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ABSTRACT

This study reports findings on seed anatomy and histochemistry in taro (Colocasia esculenta). Perhaps because taro is propagated vegetatively, little information is available regarding taro sexual propagules (seeds). Major significance of this study is the first time observation of internal seed structure in taro and the identification of an aleurone layer.

Seeds were fixed in 5% glutaraldehyde and post-fixed in 2% osmium tetroxide and subsequently embedded in epoxy resin. One micrometer thick sections were cut on an ultramicrotome and were subjected to various histochemical stains.

A uniseriate aleurone layer which is rich in protein bodies is clearly differentiated as the outermost layer of the endosperm. A starchy endosperm underlies the aleurone layer. In addition to protein bodies, the aleurone layer contains starch granules and lipid bodies. The cotyledon is rich in lipid, but also contains numerous starch granules.

Introduction

In contrast to abundant information on seed anatomy and histochemistry of numerous plant families, especially the Poaceae (Buttrose, 1963; Evers, 1970; Jones, 1971), few studies have been of seed structure in the Araceae, especially the important tuber crop taro, Colocasia esculenta. One reason may be difficulty of preparative techniques in embedding and sectioning of the small seed, especially if paraffin embedding is employed. Another reason may be that taro is generally propagated by asexual means, thus bypassing the sexual propagules (seeds). Development of seed handling techniques and establishment of breeding programs for taro (Jackson and Pelomo, 1980; Strauss et al., 1979 and Shaw 1975, 1982), however, necessitates an understanding of seed structure and physiology.

This study reports findings on seed anatomy and histochemistry in taro, with special reference to the previously undescribed aleurone layer.

Materials and Methods

Dry, ungerminated seed of taro, Colocasia esculenta were obtained from Dr. G.V.H. Jackson, Ministry of Agriculture and Home Affairs, Solomon Islands, and fixed in 5% glutaraldehyde in a 0.05 M cacodylate buffer, pH 6.8, for 6 hours,

followed by post-fixation overnight at 4 C in 2% OsO4. Prior to fixation an incision was made in the testa to aid in infiltration of fixative solutions. Following dehydration in an acetone series, seeds were infiltrated and embedded in Spurr's low viscosity epoxy resin (Spurr, 1969). For light microscopy, thick sections, 1-2 um, were cut with glass knives on an LKB Ultratome III, mounted on glass slides, and stained by the following standard histochemical procedures.

1. Lipids and starch: Sudan black B (Bronner, 1975).
2. Water insoluble polysaccharides: Periodic acid-Schiff's (PAS) reaction (Jensen, 1962).
3. Proteins: Bromophenol blue (Chapman, 1975).

Results and Discussion

The embryo is small in comparison to size of the seed, and consists of a hypocotyl-root axis and a single bilobed cotyledon (Figure 1) which is epigeal. It is rich in lipid and starch as determined by the PAS reaction and Sudan black B staining. Following emergence from the seed coat, the cotyledon becomes green and photosynthetic. The embryo comprises approximately one-third the volume of the seed and the endosperm approximately two-thirds. It is situated at the micropylar end and is surrounded by starch-filled endosperm except at the root tip. The testa or seed coat is a multilayered structure which contains numerous pockets of calcium oxylate crystals (Figure 2).

The uniseriate aleurone layer is the outermost layer of the endosperm and consists of large cuboidal aleurone cells which have thin cell walls (Figures 2, 3). The thin walls appear to be in contrast to the thick aleurone walls in wheat (Morrison, Kuo and O'Brien, 1975) and barley (Taiz and Jones, 1973). Wheat aleurone cell walls exhibit autofluorescence because of presence of ferulic acid in the walls. Taro aleurone cell walls, however, were not autofluorescent. The aleurone layer is in direct contact with the embryo only at the lower portion of the hypocotyl-root axis where it extends along the lateral edge of the hypocotyl-root axis. A second type of aleurone cell, or modified aleurone, is continuous over the root axis of the embryo (Figure 1). This modified aleurone consists of rectangularly shaped cells. In Poaceae, aleurone transfer cells have been described in this region (Esau, 1977). An electron microscopic study is in progress which will clarify if this modified aleurone is indeed a transfer cell layer. As determined with the periodic acid-Schiff's reaction (Figure 3), the aleurone cells contain starch granules. The sub-aleurone endosperm is filled with large starch granules (Figure 3). Protein bodies, which stain with bromophenol blue, are dispersed throughout the cytoplasm of the aleurone cells (Figure 4).

Taro seed provides an opportunity to investigate the structure and physiology of seed germination in a monocotyledonous, but non-grass family (Araceae). Particular interest is in answering several questions. Do the protein-storing aleurone cells of taro synthesize and release amylases as well as other hydrolases in response to gibberellic acid? Is the primary function of the tissue to mobilize the total food reserves of the endosperm for the growing embryo during germination? Answers to these questions are currently being explored along with an ultrastructural analysis of the aleurone and other seed tissues.

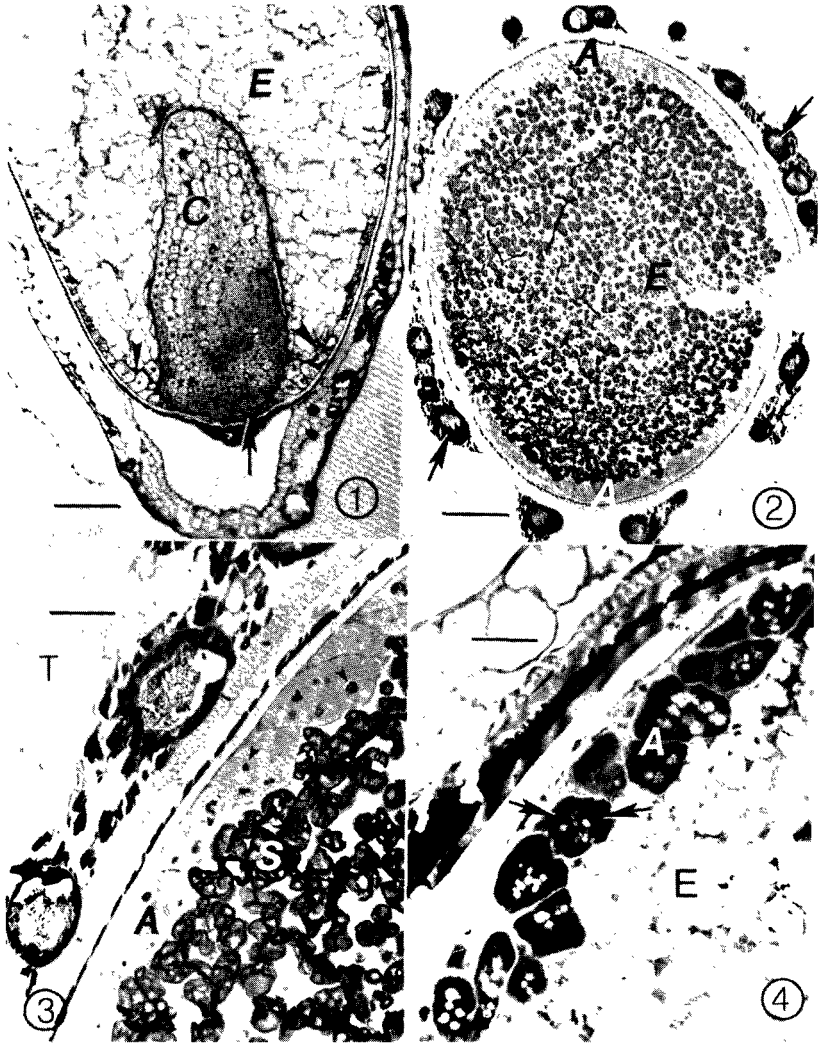

Figure 1. Longitudinal section through taro seed. Smaller arrow indicates aleurone layer adjacent to the hypocotyl-root axis. Longer arrow indicates modified aleurone layer. Toluidine blue staining. C = cotyledon; E = endosperm. Scale bar = 100 um. x 125.

Figure 2. Transverse section through taro seed illustrating differentiation of endosperm into an outer uniseriate layer, the aleurone layer (A), and the underlying starchy endosperm (E). Starch granules appear red (dark in this micrograph) after reaction with the periodic acid-Schiff's reagent. Pockets of crystals are evident (arrows) in the seed coat. PAS staining. Scale bar = 100 um. x 125.

Figure 3. PAS reaction of aleurone (A) and starchy endosperm. Starch granules (small arrows) are evident in the aleurone layer. Larger starch granules (S) fill the starchy endosperm. T = testa. Scale bar = 25 um. X 500.

Figure 4. Bromophenol blue staining of aleurone (A). The cytoplasm contains dark staining protein bodies (arrows). Starch granules appear clear against the dark background. Starchy endosperm (E) contains no protein bodies. Scale bar = 25 um. X 500.

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