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Development of Cystine- and Cysteine-rich Aleurone Grains in Bean Seeds

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ABSTRACT

Histochemical techniques were used to study aleurone grains in developing cotyledons of four cultivars of Phaseolus vulgaris L. The ninhydrin-Schiff's reaction and the 2,2'-dihydroxy-6,6'-dinitrophenyl-disulfide (DDD) method were used to identify total protein and protein-bound sulfhydryl and disulfide groups respectively. Aleurone grains of the subepidermal and adjacent mesophyll cells showed a more intense staining reaction to DDD 28 days after flower opening than the rest of the cotyledon, and the staining differentiation increased until the seed matured. The greatest concentrations of these deeply staining aleurone grains were in two areas, at the point where the embryo axis attaches to the cotyledon and adjacent to the hilum. A varietal difference was not demonstrated by these techniques.

Additional index words: Phaseolus vulgaris, Histochemical sulfhydryl groups, Protein bodies, Seed proteins.

THE major portion of the protein of mature seeds is made up of reserve materials located in cotyledons or endosperms. Originally considered single entities, storage proteins have now been demonstrated to be mixtures and are subject to the same controls of protein synthesis as are other proteins (8,9). Altschul et al. (1) point out that a wide variety of plant and animal cells accumulate storage proteins in discrete bodies which they suggest "rightfully should be called 'aleurone grains'" when these sub-cellular bodies are located in seeds. Jennings and Morton (5) reported 'aleurone grains' " when these sub-cellular bodies are located in seeds. Jennings and Morton (5) reported 'aleurone grains' " when these sub-cellular bodies are located in seeds. Jennings and Morton (5) reported 'aleurone grains' " when these sub-cellular bodies are located in seeds.

Dieckert et al. (5) reported two types of aleurone grains in peanuts. The proteins in both types appeared to be the same when judged by zone electrophoresis and chromatography. Kloz et al. (7), in observations using immunofluorescence techniques, demonstrated these new proteins to be synthesized from precursors. Carter et al. (3) reported two types of aleurone grains in developing cotyledons of four cultivars of Phaseolus vulgaris L. Some aleurone grains in immature cells had neither globulin, a very few had only vicilin but most grains contained both globulins. Immature aleurone grains were found in most stages of seed development.

Opik (10) found that aleurone grains arose from the subdivision of vacuoles in bean cotyledons and found no evidence for an alternate protein-forming system. In the same study it was suggested that cells remain unchanged right up to dehydration, or as changes in the protein synthesizing system of the endoplasmic reticulum and ribosomes were concerned.

The object of the experiments reported here was to determine if proteins rich in cystine and cysteine were differentially distributed in the developing cotyledons of beans.

RESULTS AND DISCUSSION

Fifteen-day cotyledons did not contain visible aleurone grains or starch grains. A large nucleus surround-
Table 1. Responses of the granular material in the cotyledonary cells of beans to different staining procedures.

<table>
<thead>
<tr>
<th>Staining procedure</th>
<th>Aleuron grains</th>
<th>Starch grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ninhydrin-Schiff's</td>
<td>Magenta</td>
<td>Negative</td>
</tr>
<tr>
<td>Dcamination + ninhydrin-Schiff's</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Acetylation + ninhydrin-Schiff's</td>
<td>Blue or red</td>
<td>Negative</td>
</tr>
<tr>
<td>DDD N-ethylmaleimide + DDD lodoacetate + DDD</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

See materials and methods section for details.

Subcellular bodies were considered aleurone grains when they were shown to be protein by the ninhydrin-Schiff's test (Table 1). Aleurone grains of 20-day cotyledons were slightly larger (3-6μ) than at later stages of maturity (2-1.5μ) as also noted by Opik (10). The number of aleurone grains per cell increased regularly at each sampling period from a low of 40 at 20 days to a high of 120 at 44 days. The intensity of staining of aleurone grains with ninhydrin-Schiff’s reaction increased during development and reached a maximum at maturity (Fig. 1). Staining was uniform through all areas of the cotyledons indicating a uniform density of protein throughout.

The aleurone grains of bean cotyledons stained blue or red with varying intensity when localized by the DDD reaction. Barnett and Seligman (2) reported that when diazo blue B was used as the dye with the DDD reaction, a difference in color depended upon whether monocoupling (red color) or dicoupling (blue color) occurs. Widely separated sulfhydryl groups showed red or pink coloration, whereas blue coloration indicated a greater concentration of sulfhydryl groups. Aleurone grains stained uniformly red with increasing intensity until 28 days after flowering. Some of the aleurone grains in the subepidermal and adjacent mesophyll cells of 28-day cotyledons stained a deep red with DDD in contrast to the lighter red color of adjacent grains and of grains in the central mesophyll cells. Thirty-two-day cotyledons had blue aleurone grains in the subepidermal and adjacent mesophyll cells. The number of blue-staining aleurone grains increased in these cells until maturity (Fig. 2). These staining behavior responses for protein-bound disulfide and sulfhydryl groups indicated that the primary structure of the proteins being synthesized and stored in the aleurone grains were rich in cystine and cysteine. These aleurone grains were most numerous in the area where the embryo axis attaches to the cotyledons and in the area adjacent to the hilum. Also, the numbers of blue-staining aleurone grains were found to be most numerous in the surface cells with progressively fewer occurring in the interior of the cotyledons and only red-staining ones were found in the center of the cotyledons. Although previous studies have indicated a differentiation of aleurone grains as a function of the time of development, these data describe aleurone grains with specific proteins associated with particular cells. No differences in varieties were observed.

Fig. 1. Cells of a mature bean cotyledon stained with the ninhydrin-Schiff's reaction. The cell contents have shrunk away from the cell wall. The aleurone grains show as small, round to ellipsoidal particles surrounding the large unstained starch grains, ca x 2250.

Fig. 2. Cells of a mature bean cotyledon stained with the DDD reaction. Light and dark staining aleurone grains can be seen in each cell. Sub-epidermal cells are the small cells along the right margin, ca x 2250.