LOCALISATION AND DISTRIBUTION OF METABOLITES DURING BULBIL DEVELOPMENT IN *Dioscorea alata* L. (TRUE YAMS)

N. DIKSHIT

*National Bureau of Plant Genetic Resources, Base Centre, Cuttack-753 006, Orissa*

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The distribution of various metabolites like total polysaccharides, proteins, starch and lipid content were studied in the bulbils of *Dioscorea alata* L. during different stages of development. It revealed that polysaccharides in the form of starch granules mostly accumulated in the cells of ground tissue compared to some deposition in cell wall. Total proteins and lipids are located in the phellogen cells and periderm region respectively. The role of these metabolites as a nutritional source in developing bulbil is discussed.

Key Words: *Dioscorea alata* bulbil, localisation, distribution, metabolites.

* Dioscorea alata* L. (true Yams) is one of the main source of carbohydrate food in Asian, Caribbean and South American countries (Okonkwo, 1985). It is indispensable during periods of scarcity and famine. It mainly perennates through rhizome or through bulbil, the aerial perennating structure. A good amount of work have been done on *Dioscorea* species on taxonomy and vegetative anatomy [Burkill (1960); Ayensu, (1965, 70 & 72)] the floral anatomy [Murty & Purnima (1983)] Embryology [Rao (1951)] and ontogeny [Sharma (1974, 1975)]. Further the knowledge regarding histochemical changes during bulbil development of *Dioscorea alata* is insufficient. Therefore, in the present paper the role of important metabolites like polysaccharides, total proteins, starch and lipid on bulbil development is discussed.

MATERIALS AND METHODS

Bulbils of different size of *D. alata* were collected from the State Bank Colony, Meerut and were fixed in Formalin Acetic Acid alcohol (FAA) and Acetic alcohol (1:3). Dehydration of the material was done in ethyl-alcohol-xylene series. After dehydration and infiltration the material was embedded in paraffin wax (M.P. 60°C). The wax embedded blocks were sectioned at 6-10 microns using rotatory microtome. Both serial transverse and longitudinal sections were cut. Uniformity in the thickness of sections was maintained in order to avoid error in observing the staining intensity for a particular reactions. Haupts adhesive was used for affixing the section on slides. Periodic Acid Schiff's (PAS) reagent, Mercury-bromophenol Blue, Starch IKI and Sudan dyes were used for localising total polysaccharides, proteins, starch and lipid content. The qualitative difference in the intensity of colour reaction of metabolites thus localized was taken as indication of its quantity comparatively (Fig. 1).

RESULTS AND DISCUSSION

In *D. alata* the mature bulbils are globose to elongated in shape. The length varied from 0.2-1.4 cms. Bulbils of different breadth i.e. 0.3, 0.6, 0.9 and 1.2 cms are considered as stage A, B, C and d respectively. The anatomy, development and distribution of metabolites of *Dioscorea alata*, bulbils are as follows:

**Anatomy**

The mature bulbil in a transverse section appears to be smooth in outline or irregular due to the presence of tubercles. The outer most layer is epidermis, which appears thick walled (Phellem). Following epidermis, there is a region of four to five layered parenchymatous cells described as cortical region by Ayensu (1972). The cells of this region are thick-walled and suberized. Below this region there is a zone of three or four layers of suberized cells resembling cork cambium (Phellogen). Ground tissue composed of parenchymatous cells are filled up with starch grain and other cell inclusions. Vascular bundles are collateral and are found scattered in the ground tissue.

**Development**

Bulbils develop in the axil of a leaf on the abaxial side of an organised apex. After the formation
of accessory buds, the cells below it become active showing their meristematic nature. Due to the activity of these cells hump like mass is formed which can be distinguished as a bulbil primordum. This further enlarges in its size due to the activity of isolated meristem. Simultaneously, the apical growth of the accessory buds is arrested. Due to the divisions and enlargement of the cells, bulbil gradually increase in
Metabolites during bulbil development in *Dioscorea alata* L. (True Yams)

Table 1. Distribution pattern of Polysaccharides, Proteins, Starch and lipids in different stages of bulbil development in *Dioscorea alata* L.

<table>
<thead>
<tr>
<th>Stage</th>
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<th>Polysaccharides</th>
<th>Proteins</th>
<th>Starch</th>
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Tissues refers to PH-Phellem, PG- Phellogen, GT-Ground Tissue, MC-Mucilage. 
1, 2, 3, 4 and (-) indicates degrees of intensity as less, moderate, high, more intense and absent respectively for metabolites studied.

Stages refers to breadth of bulbil A = 0.3, B = 0.6, C = 0.9 and D = 1.2 cm respectively.

its size. There is no definite arrangement of vascular bundles in a bulbil. At a later stage when the bulbil becomes quite mature the differentiation of root primordium takes place from the tissue of the bulbil. After cell division the enlargement of parenchymatous tissue in the bulbil takes place and various types of cell inclusion develop in these cells.

**Localisation of insoluble polysaccharides**

Localisation of insoluble polysaccharides in the different sizes of bulbils reveals that PAS granules are absent in the periderm and present in the cambium which again varies during development. In the bulbils of 0.3 cm size there is a light stain of PAS but in the 0.6 cm size there is moderate stain and in the 1.2 cm size there is dark stain. The ground cells also shows affinity for PAS. In the smaller bulbils there is less stain in the periphery which gradually increases up to moderate stain in the mature bulbils. Starch grains in the smaller bulbils takes very light stain and during the development process it increases in colour to dense pink in the mature bulbils. These starch grains occupy the whole of the ground cells. In the small bulbils they are very smaller in size but in mature bulbils they are bigger. The grains exhibit a variety of size and form. Most grains measures between 15 to 80 µm in length and are 10 to 45 µm in breadth. In outline the hilum is either oval or elliptical and is usually near the narrow end and it is seldom encountered in the centre of the grain. Hilum takes no stain. They are black. Mucilage cells distributed in the ground tissue also shows affinity for PAS. The developing small bulbils take light stain and the intensity increases to dark pink stain in the mature bulbils.

**Localisation of total protein**

Bulbils shows a heterogeneity of colour towards mercury bromophenol reaction. Low intensity of protein was observed in mature bulbils of 1.2 cm size breadth whereas cambium shows moderate to high intensity. In the small bulbils, phellogen takes light stain which again increases to moderate and dense in the mature bulbil. Ground tissue in the small bulbils takes little or no stain whereas the colour increases to moderate in the mature bulbils. In the small bulbils protein like bodies are present in the ground tissue. Mucilage takes blue stain in the mature bulbils.

**Localisation of Starch**

The most important cellular content of the bulbil is starch grains which are present only in the ground tissue. In all the stages, the periderm and phellogen takes no stain whereas the ground tissue shows a high intensity to starch. The mature bulbil shows dense colour whereas the young bulbil shows moderate stain which again shows the quantitative amount of starch in the mature bulbil.

**Localisation of lipids**

Localisation of lipid shows that they are mainly present in the periderm region but not in the ground tissue region. The intensity of colour varied from light to dark from young to mature bulbils. Mucilage takes light stain in the mature bulbils.

The evaluation of qualitative localisation of polysaccharides, proteins, starch and lipid in the developing bulbils revealed that there is a gradual accumulation of these metabolites in the developing bulbil. Polysaccharides are accumulated more in the ground tissue and the intensity increased in stage C & D whereas Periderm and phellogen takes moderate
to high stain in the stage D compared to moderate in the stage A, B & C. Phellem takes no PAS stain in all the stages. Total proteins are accumulated moderate to high in the phellem and phellogen compared to absence of proteins in the ground tissue. The accumulation of starch showed a steady increase from moderate to more intense in the ground tissue from stage A to stage D compared to absence in the phellem, phellogen and periderm. Lipids are distributed only in the phellem and ground tissue region. These results indicated that the metabolites are accumulated in the bulbils according to the need which are utilised during germination.

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