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STANDARDIZATION OF DESCRIPTIVE TERMINOLOGY OF STARCH GRANULES WITH REFERENCE TO IDENTIFICATION OF RAW DRUGS

A.B.D. Selvam[*1]

[1]Pharmacognosy Section, Botanical Survey of India, Howrah – 711 103, India.

ABSTRACT
Starch is produced by plants and stored in the form of small granules (starch granules/grains) in their various parts, such as roots, rhizomes, tubers and seeds. The stored starch granules when viewed with polarised light under light microscope show certain basic structural features useful in the identification of plant species in general and drug plants/raw drugs in particular, although some of the starch granules may be of less diagnostic importance than others. The current botanical knowledge about how and where these starch granules are produced and stored in plants, their morphological features, physical, chemical and optical properties that may be useful in the identification of plants/drug plants/raw drugs are summarized in this paper. The size and shape, surface characteristics, presence of the hilum, reaction with iodine, birefringence properties and extinction cross are some of the important characteristic features to be observed carefully while describing the starch granules, which can be used as a diagnostic aid for the identification of drug plants/raw drugs.

Keywords:- *Starch grains, Starch granules, Identification, Standardization, Description, Terminology*

Correspondence Author

A.B.D. Selvam

Pharmacognosy Section, Botanical Survey of India, Howrah – 711 103, India.

Email: abd_selvam@yahoo.co.in

INTRODUCTION

Starch constitutes the principal form of carbohydrate reserve in the green plants and is found especially in seeds and underground parts. Starch granules are synthesised in different parts of plants mainly in leaves that contain chloroplasts and are stored in different parts of plants in the form of storage energy. When the plant needs energy, the synthesised starch grains are converted into sugar and transferred to different parts of plants where it is required. When the rate of photosynthesis is high during the day, small starch granules of intermediate shape, about 1 µm in diameter may be formed within the chloroplasts. These are called temporary, transitory or transient starch granules. During the night, the transient starch granules are moved to different locations in the plants and are used as energy or transformed into storage starch.

The starch granules are stored in the plants both in the underground storage parts (roots, rhizomes and tubers) and also in the aerial parts (stems, leaves, flowers, pollens, fruits and seeds) as an energy reserve. The stems of some of the species of Palms and Cycads contain soft starchy pith, known as 'sago' starch that are important food sources in many parts of the world. Some of the algae and other microorganisms are also known to produce small quantities of starch granules in different forms[1].

In general, cereal grains such as rice, maize, wheat, millets (pearl millet, foxtail millet and finger millet), soybeans, sorghum, barley, oats and rye, mostly contain starch granules. In addition to nutrient properties as a food, starch granules have wide range of utilities mainly in drug industries. Most of the drug plants possess starch granules, which can be utilized as a diagnostic aid for the identification of plant species in their crude form[2].

The morphology of starch granules is largely depending on the genetic composition of the plant, but size and shape can be modified to certain extent by both the internal and external environment of the plant. The size and shape of the starch granule as well as a number of additional properties discussed below are often considered as characteristics of plant species. However, not all starch granules found in plants are diagnostic. Some of the granules are highly characteristic/diagnostic, useful for identifying the plant species, whereas other granules are having less diagnostic value.

There are two basic forms of starch have been recognized, native starch (comprising of raw, unmodified starch derived directly from plants) and modified starch (processed by pounding, abrasion and heating).

Starch granules are also used for understanding the culture of people who lived in the past. It is learned that ancient people used starch for edible and non-edible purposes. Therefore, it also plays role in archaeological and paleo-environmental research on ancient starch retrieved from archival sites[1].

DESCRIPTION AND IDENTIFICATION OF STARCH GRANULES

Starch occurs in the form of granules (starch grains), the shape and size of which are characteristic of the species as is also the ratio of the content of the principal constituents, amylose and amylopectin. A number of starches are recognized for pharmaceutical use. Starch occurs in irregular, angular masses or as a white powder. It is insoluble in cold water, but forms a colloidal solution on boiling with 15 times its weight of water, the solution forming a translucent jelly on cooling[3].

There are two basic methods for identifying starch granules. The first one involves diagnostic tests of chemical and physical properties of starch granules, whereas the second one uses the optical properties of the starch granules.

Starch grains found in plants have characteristic morphological features, physical, chemical and optical properties, which can be best-observed under Scanning Electron Microscope (SEM) as well as under light microscope using polarising filters. Surface ornamentation of starch granules varies from plant to plant, which may be used as the diagnostic characters to identify and distinguish the plant species from its closely allied and related species. In precise, starch grains are characteristic and species-specific[4].

Microscopical characters

Powdered plant parts containing starch grains should be mounted in water or Smith's starch reagent (equal parts of Water, Glycerine and 50% Acetic acid) and observed under microscope. Some of the most important microscopic characters of the plant starches, which may be useful in the detailed description and identification of plants at species level, are listed in **Table – 1**. Morphological features of starch grains are depicted in **Plate – 1** using photomicrographs and Scanning Electron Micrographs taken from different species of *Aconitum* (Aconites).

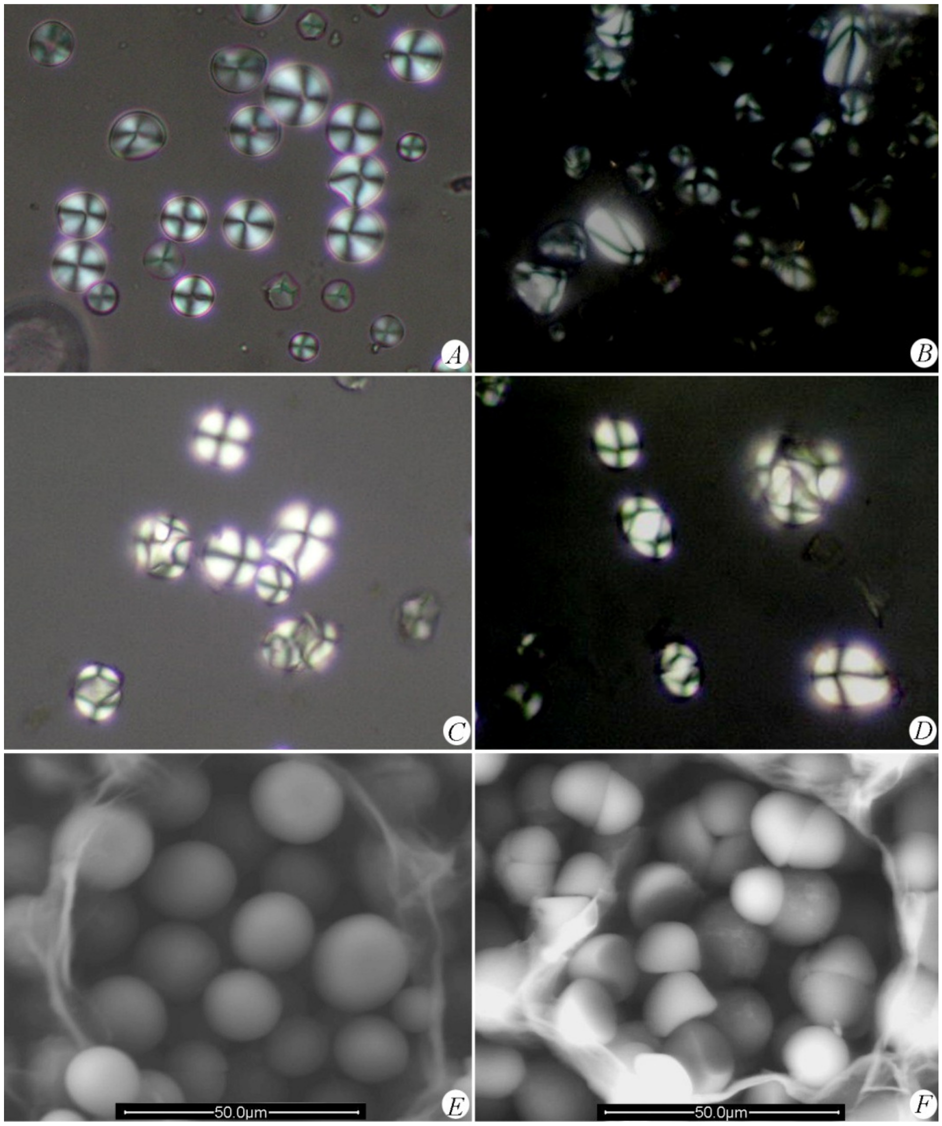

Plate – 1. Photomicrographs (*A* to *D*) and Scanning Electron micrographs (*E* & *F*) of the tuberous roots of *Aconitum* species illustrating differernt structural features of starch granules.
A – Simple starch grains showing **+** or X-shaped extinction cross with concentric hilum;
B – Simple starch grains showing Scissor-shaped polari mark with eccentric hilum;
C & *D* – Simple and compound starch granules with 2 to 3 units;
E - Simple starch grains (Oval-shaped); *F* – Simple and compound starch grains with 2 to 3 units

Table-1: Descriptive terminology of Starch granules used for identification of raw drugs

Characteristic features of Starch granules	Descriptive terms
Basic type of starch grains	Transitory or storage
Location of storage starch grains	Roots, rhizomes, tubers, stems, leaves, pollen grains, seeds, etc.
Occurrence of tissues/cells	Mainly in ground tissues (pith and cortex)
Basic forms of starch	Native starch or modified starch
Distribution of starch grains	Rare, common, abundance, occasional, frequent
Grain/granule type	Simple, compound or semi-compound, if compound, number of components 2 – 5

Size (diameter of starch grains)	Ranges from 1 – 100 µm (rarely up to 150 µm, exceptionally up to 175 µm)
Shape of starch granules (in outline)	Circular/spherical, sub-spherical, ovoid, elliptical, oblongoid, cylindrical, triangular, papillate, polyhedral, angular, irregular etc.
Position of hilum	Distinct or indistinct, if distinct, whether centric/concentric (hilum situated near the middle of the granule) or eccentric (hilum located towards one end of the granule)
Number of hilum in a grain	Single, or more than one
Striations	Well-marked, faintly marked, may be absent (no striations)
Extinction cross or polari mark pattern or dark lines seen against white background of starch granules	Plus(+)-shaped, X-shaped (winged), Y-shaped, Cross-shaped, Scissor-shaped
Visibility of starch grains under polarised light	Surface ornamentation clearly seen, hazy or not seen
Overall view (in appearance)	Freshwater mussel-shaped, egg-shaped, Muller-shaped, etc.

Granule types

Starch granules are classified as simple, compound or semi-compound depending on how they are formed in the amyloplast. Simple granules have only one component in the amyloplast, but in contrast, compound granules are comprised of several separate parts, known as subgranules or granula, which have formed simultaneously within a single amyloplast, but each separate granula of a compound granule still exhibits a polarising cross. Some starch-bearing plants produce semi-compound granules as in the case of the bulb of *Ledebouria ovatifolia* (Baker) Jessop. (= *Scilla ovatifoia* Baker) and the edible seeds of *Amaranthus retroflexus* L. (American pigweed). These begin as compound granules, but the separate subgranules become fused together by a surrounding layer of amorphous starch. The resulting semi-compound granule has one exterior surface but two or more hila, using the term 'pseudo-compound granules' for single granules that appear compound but have several fissures, for example, as in wrinkled peas[1].

Size and shape

The size, shape and structure of the starch granules of every plant species only vary within definite limits, so that it is possible to distinguish between the starches derived from different species. Starch granules may be simple or compound and the description of a starch granule as 2-, 3-, 4- or 5-compound refers to the number of component granules present in the compound granule, formed by the aggregation of a large number of simple granules, e.g. rice[3].

Starch granules exhibit a wide range of shapes, and are helpful in identification of species. Shapes such as, disc, sphere, oval, elongated, rounded, kidney-shaped, fresh-water mussel-shaped, polyhedral and irregular forms are common[1,4]. Cereals such as, wheat, rye and barley display two distinct granule forms, viz., large lenticular or disc-shaped and smaller spherical granules.

Structurally, starch consists of colourless and highly refractive granules (refractive index about 1.5), which are microscopical in size. The size of starch granules generally varies from 1 to 100 µm, not exceeding 150 µm in maximum dimension, and possess definite morphological characters which are evident under microscope[5]. However, those in the rhizome of *Canna* plant can reach up to 175 µm exceptionally. Size is positively correlated with the amount of water in the granule[1].

Hilum

The starting point of formation of the granule in the amyloplast is marked by the hilum, which may be central/concentric or marginal/eccentric in position. Most commonly it is situated near the middle of the granule (concentric), but it can be eccentric, i.e., towards one end of the granule[1,3]. The hilum, which appears as darker or lighter lines/points according to the focussing adjustment of the microscope, is varied. Both the hilum and the striations are more clearly seen in commercial starches than in granules observed *in situ* in the plant cells[5].

Striations

The starch granule is built up by deposition of successive layers around the hilum, and concentric rings or striations are often clearly visible in larger granules, e.g., potato[3]. The striations or concentric rings, the fine lines surrounding the hilum or in the case of granules showing a marked eccentricity, forming parallel arcs, transversing the granules more or less transversely; they result from the stratified structure of the majority of the starch granules[5].

Birefringence

Viewed with polarising filters under light microscope, starch granules show *birefringence*, which means that they appear bright white against a black background. This pattern occurs because the semi-crystalline arrangement of starch molecules causes the polarised light to travel at different velocities through the granules. Starch granules are thus anisotropic. Birefringence of starch granules has been attributed to their semi-crystalline nature, but is due to a highly ordered molecular structure. Chemical extraction and drying of granules can result in reduced birefringence, less-marked lamellae and centrally developed cracks at the hilum. This has implications for both reference collections and identification of ancient starch granules.

One of the most important methods for starch identification is the use of polarising microscope to detect the extinction cross within the bright of the granule. An important property of the cross is that it rotates when the polariser is turned. The relative length of the arms of the cross and the angle at which they meet at the hilum vary widely between different plant species.

Iodine staining

Starch reacts to several stains in particular ways, which is useful for identification. For example, pure amylose gives a deep-blue colour when stained with iodine-potassium-iodide, while amylopectin gives a reddish colour. The relative proportions of these components, which vary from species to species, determine the ultimate colour of stained starch granules.

Gelatinisation

Starch granules are insoluble but are permeable to water. In contact with water at low temperatures, they swell. This process is reversible. Native starch can hold up to 30 percent of its dry weight in moisture. Hydration increases the birefringence of the granules[1].

CONCLUSION

Starch grains are produced by plants with the help of cell organelle, known as chloroplast, and are stored mainly in roots, rhizomes, tubers and seeds in the form of small granules. The size and shape of starch granules are characteristic of the plant species in which they occur, although some granule forms may be of less diagnostic than others.

The characteristic features of starch grains, such as size, shape, surface characteristics, presence of hilum, reaction with iodine and appearance of characteristic birefringence and extinction cross when observed under light microscope using polarising filters are useful in identification plant species. The shape (3D view) and size of the starch granules can be clearly studied using Scanning Electron Microscope (SEM). The obtained features are useful in delimiting the plant species from its closely allied or related species.

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