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AN ALTERNATIVE METHOD TO LOCALISE STARCH GRAINS AND INULIN CRYSTALS IN PLANT TISSUE SECTIONS

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ABSTRACT

Starch and inulin are two different storage polysaccharides of plant cells of diverse taxa. Starch has nutritive and medicinal value as well as is a source of simple sugars in industry. Inulin also has value in food industry as a source of fructose and in preparation of diabetic bread. Techniques to identify their presence and relative quantities in plant would be useful for commercial exploitation. In the past, histochemical localization procedures have been developed using Lugol's iodine and periodicacid - Schiff's reaction (PAS reaction) for starch and phloxine and thymol-sulphuric acid method for inulin. In this paper alternative and fairly stable histochemical localization procedures using Dragendorff reagent, (that is conventionally used for detecting alkaloids) for localizing these two polysaccharides are suggested.

Keywords: Dragendorff reagent, inulin crystals, lugol's iodine, starch grains

Starch constitutes the principal form of carbohydrates in plants (Evans, 1989). It is intracellular and occurs as granules in membrane-bound cytoplasmic organelles, especially plastids. Starch storage occurs widely in the plant body in the parenchyma cells of the cortex, pith and vascular tissues of roots and stems, parenchyma cells of rhizomes, tubers, corms, endosperm of seeds and guard cells of stomata. Starch is composed of two basic molecules: amylose and amylopectin (Krishnamurthy 1988).The amylose chain is unbranched and amylopectin is a branched chain of molecules (Evert 2006). Starch grains or granules are varied in shape and size, commonly layering around a point, the hilum, which may be the centre of the grain (concentric) or to one side (eccentric).

The layering of starch grains is attributed to an alternation of the above mentioned two polysaccharide molecules (Evert 2006). The position of hilum and the presence or absence of well-defined striations are of importance in characterization of starch grains (Evans 1989).as well as in distinguishing them from other storage polysaccharides such as inulin. Starch has varied uses; it is nutritive, demulcent and absorbent. It is used as antidote in iodine poisioning, and as a disintegrating agent in pills and tablets. Starch is also a starting material for the commercial manufacture of liquid glucose, dextrose and dextrin (Kokate *et al* 2005).

Inulin derives its name from *Inula helenium* from which it was first isolated (Evans 1989). Inulin is found widely distributed among numerous angiosperm families. This carbohydrate occurs in solution in the cell sap of some species, in others it accumulates as an amorphous material, and in some plants inulin occurs as crystals deposited on the inside of cell walls (Blaydes 1943). Chemically, inulin consists of a chain of 35-50 1, 2-linked fructofuranose units terminated by one glucose unit. Inulin is not metabolized by the human body and is excreted unchanged making it a useful reagent for the testing of renal efficiency. Inulin also has a potential use in the food industry as a source of fructose (Evans, 1989). Inulin finds use in preparation of culture media as fermentative identifying agent for certain bacteria. It is an ingredient of diabetic bread (Kokate *et al*, 2005).

The two most commonly used methods for the histochemical detection of starch are Lugol's iodine (iodine-potassium iodide) and periodicacid- Schiff's reaction (PAS reaction) (Yoder and Mahlberg, 1976).

This article suggests an alternative method for the detection of starch grains in a plant tissue. This method was found out while carrying out the histochemical study for alkaloids. The general histochmeical method used for detection of alkaloids in plants is the employment of Dragendorff reagent (Yoder and Mahlberg, 1976; Joger, 1998). This reagent is prepared as per the following procedure:

Solution 1: Dissolve 0.85 g basic bismuth nitrate in 10 ml glacial acetic acid and 40 ml water under heating.

Solution 2: Dissolve 8 g potassium iodide in 30 ml water.

Mix solutions 1&2 in the ratio of 1:1. Add 1 ml of this mixed stock solution to 2ml glacial acetic acid and 10 ml water (Wagner and Bladt, 2001).

Dragendorff reagent gives an orange colouration with alkaloids, when the plant sections are left overnight in the reagent (as per our observation). It was found by us that Dragendorff reagent gives a positive reaction not only with alkaloids but also with starch grains

which stains to blue-black immediately, similar to the reaction obtained when the starch grains are stained with Lugol's iodine. This reaction was tested over a wide range of plants and was found to be consistent and effective in the localization of starch in plant tissues. Hence this method can be used as an alternative histochemical method for localization of starch in plant tissues.

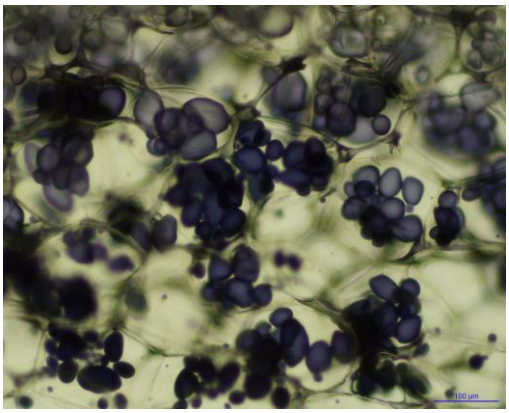

Fig.1- A section of potato, showing starch grains stained with Lugol's iodine

Fig.2- A section of potato, showing starch grains stained with Dragendorff reagent

There are already two methods for the histochemical testing of inulin. One method involves the use of thymol and sulphuric acid, but this method does not seem to be good for the study as the inulin produces a red colour with the reagent but it disappears quickly, sometimes even before taking the material for examination under the microscope. The other method is staining with phloxine which gives a reddish pink colour to inulin (Kokate *et al*, 2005). In this article we have come up with two other methods for the localization of inulin one using Lugol's iodine and the other using Dragendorff reagent. Sections of *Inula racemosa* were taken and stained with Lugol's iodine and Dragendorff reagent. In the sections treated with Lugol's iodine, the inulin grains stained blue-black immediately, while in those sections stained with Dragendorff reagent the blue-black colour did not appear immediately. So, the sections were left in Dragendorff reagent for 2 hours and it was found that the inulin crystals were stained blue-black. The reaction/colour produced in case of both the above reagents was persistent for a longer time as compared to the previously followed other methods. It is to be further mentioned that the staining of starch and inulin is very specific and that no other structure in the cell gets stained even if kept for a very long time. Stained starch and inulin can be distinguished from each other by their location specificity, starch is always present in the protoplast and inulin is always attached to the cell wall. Also, starch will show the alternate

amylase and amylopectin layers, while inulin is highly crystalline. Moreover starch and inulin are not found together in any plant in the same cell.

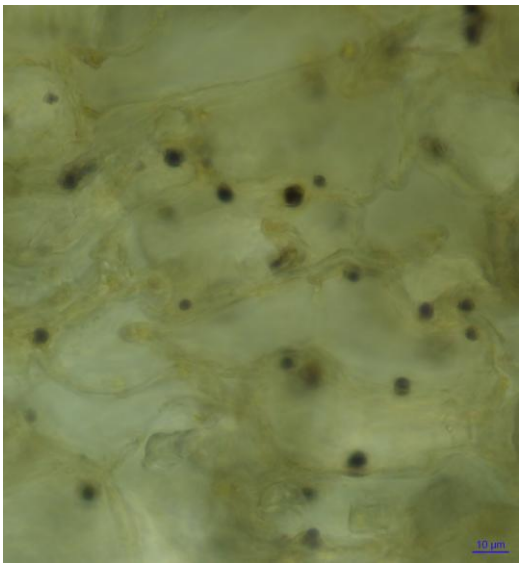

Fig. 3- Section of *Inula* stained with Lugol's iodine for inulin

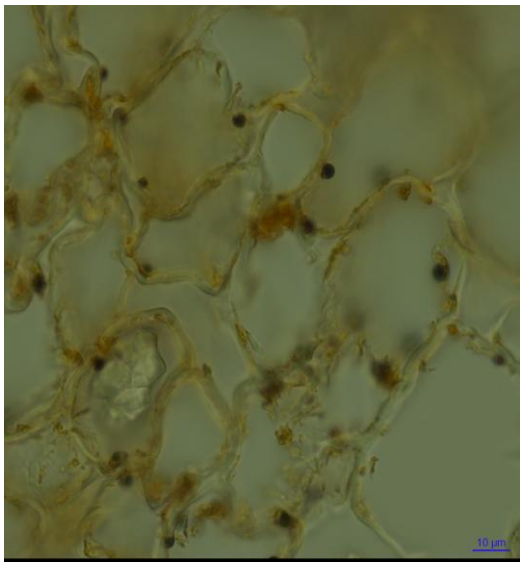

Fig. 4- Section of *Inula* stained with Dragendorff reagent for inulin

The rationale of starch staining through Lugol's iodine is as follows: Starch grain is composed of amorphous and crystalline regions, whose chains are held together by hydrogen bonds (Evert, 2006). The amylose chain can exist in three main forms: an amorphous conformation and two differential helical forms or in a free form where it can bind with another hydrophobic guest molecule such as Iodine, fatty acid or an aromatic compound. This is known as the 'V' form and this is how amylopectin binds with amylose to form starch. It has been reported by several authors that the amylopectin is relatively stable as compared to amylose and the colour given with iodine is purple but the iodine-binding is low (Evans, 1989). Amylose produces the blue colouration with iodine and the binding is strong. The left-handed amylose helix contains a 5Å wide central channel in which iodine (as iodide ions) is embedded (Saenger, 1984) after staining. There are other authors who believe that the reaction appears to be accumulation of iodine in the centre of the helical starch molecule. Shorter starch molecules give a red colour while longer molecules give a blue colour (Krishnamurthy, 1988). In the method using Dragendorff reagent also it appears that the iodine ion is responsible for the blue-black colouration. The presence of bismuth salt in Dragendorff solution enhances the intensity of starch colouration by iodide ions, but the mechanism of enhancement is not clear.

The intensity of staining of starch with Lugol's iodine fades slowly after sometime, but staining with Dragendorff reagent does not fade.

The principle behind staining of inulin by Lugol's iodine method and Dragendorff method can be attributed to the fact that the iodine molecules get lodged in the fructofuranose molecule in the same way as iodine (as iodide ions) gets embedded in the wide central channel in the Lugol's iodine method. It is emphasised here that Dragendorff method is more useful as this one reagent can be used to stain not only alkaloids but also starch and inulin, in a long-lasting way.

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