Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [4] April 2018 : 07-10

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Histochemical localization of starch in stem and root of *Jatropha curcas* (Euphorbiaceae)

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ABSTRACT
Histochemical methods are used in the identification, density of accumulation and distribution of chemical compounds within cells and tissues under microscope using the color-stain reaction technique. This includes the preparation of fixed variably stained specimens and then the examination under the microscopic devices. In the current research work, carbohydrate storage in the form of starch grains has been examined in stems and roots of Jatropha curcas. The predominant starch-storing tissues were identified, and the cellular localization of the starch grains within these tissues was determined. In stem sections, starch was seen predominantly in parenchymatous cortex, medullary rays, pith while in the root sections, starch was seen highly concentrated only in cortical tissues and to some extent as brownish black patches in the medullary rays. We conclude that starch research is not yet a mature subject and that novel experimental and theoretical approaches will be important to advance the field.
Key words: *Histochemical, plant, tissues, cortex, medullary rays, pith, starch*

Received 12.02.2018 Revised 02.03.2018 Accepted 16.04.2018

INTRODUCTION

Histochemistry is devoted to study the identification and distribution of chemical compounds within and between biological cells, using stains, indicators and light and electron microscopy [1]. To understand the definitions of histochemistry, it may be helpful to recall the definitions of histology as the microscopic study of the structure of biological cells and tissues, whereas, chemistry is the science of matter and the changes that occur between and via different chemical reactions. Thus, leading to the changes occur in molecules and cell components. It is a powerful technique for localization of trace quantities of substances present in biological tissues [2, 3]. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as protein, lipid, starch, phytin and minerals such as calcium, potassium and iron in rice grains [4, 5].

Plants use photosynthesis to assimilate carbon and fuel their metabolism and growth. Atmospheric carbon dioxide is converted into organic compounds, utilizing the energy provided by sunlight. During the night, when photosynthesis is not possible, plants must rely on stored reserves of carbohydrates built up during the previous day. Carbohydrates are important group of carbon compounds which are essential to sustain life. They are large biological molecule, consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen: oxygen atom ratio of 2:1 (as in water). Numerous polysaccharides purified from Chinese medicinal herbs are bioactive and possesses anti-tumour and antibacterial activities.

Starch is an insoluble, non-structural carbohydrate composed of α-glucose polymers. It is composed of 2 α-D-Glucose homopolymers: amylose and amylopectin. The ratios of amylose and amylopectin vary between plant species [6]. In native wild type plants, the major polysaccharide, amylopectin accounts for 70-80% of the starch granule. Amylopectin is a large and highly branched polymer. The remaining 20-30% of the starch granule is composed of amylose, which is a small and relatively unbranched polymer. It is synthesized by plants and algae to store energy in a dense, osmotically inert form. It is also use in the production of paper and board of biodegradable plastics and packaging material amongst others [7, 8, 9].

J. curcas is a perennial lactiferous Euphorbiaceae, native to Mexico in Central America, where high genetic diversity was reported [10]. It was spread from Central America to Africa and Asia by Portuguese traders as valuable hedge plant [11]. It has now become naturalized in many tropical and subtropical countries, including India, Indonesia, Philippines' besides, Africa and North America. The plant is well-adapted to semiarid climate, although more humid environmental conditions result in better crop performance [12, 13]. The plants are deciduous with juicy stem and fast growth. The species has great economic potential, either in landscaping as hedge, pharmacology, and especially in the production of biodiesel oil. The potential use of Jatropha oil as biodiesel feedstock has been well studied [14, 15, 16]. The *J. curcas* plant develops in several kinds of soils, including sandy, stony, saline, alkaline and rocky ones, which, from the physical nutritional point of view, are restrictive to full root development. The roles of the various starches located throughout the plant confirm that starch participates in roles fundamental to the plants growth, development and survival. Therefore present study is focus on localization of starch in stem and root of *Jatropha curcas.*

MATERIALS AND METHODS

The study was carried out at Department of Botany, University of Rajasthan, Jaipur, Rajasthan (India) in the year 2015. The stems and roots of the plants taken for the study were collected from Rajasthan University campus and identified in the Rajasthan University herbarium. Only healthy parts of the plants were collected.

Collected plant parts were fixed in Formalin Acetic Alcohol (FAA) fixative for 24 hrs. The FAA fixative was prepared following method as given in Table 1. Fixed plant parts were dehydrated in tertiary butyl alcohol (TBA) series [17]. TBA dehydration series is given in Table 2.

Dehydrated materials were infiltrated in an oven maintained at $62^0 \pm 2^0$ C, using paraffin wax of melting point 58-60^0 C. Transverse sections of the paraffin embedded material were cut on a rotary microtome (Waswox) between the range of 12-15µ. The sections in paraffin ribbon were spread out on the chemically cleaned microslides and fastened with the help of Haupt's adhesive (Table 3) [18] and were dried at least for 24 hrs before staining.

Method of staining for starch localization

IKI solution was prepared by dissolving 2 gm of KI in 100 ml of distilled water. To this 0.2 gm of iodine was added which was followed by stirring until iodine crystals were dissolved.

Transverse sections of stem and roots of *Jatropha curcas* are stained separately in IKI solution for 10 min and mounted in glycerin. The observations were recorded and photo micrograph taken.

RESULTS AND DISCUSSION

In the stem and root sections, the older starch grains appeared blue black while the newly formed as purple. In stem section of *Jatropha curcas,* starch was seen predominantly in parenchymatous cortex, medullary rays, pith (Fig. A and B). The starch grains stained bluish black with iodine–potassium iodide solutions, and were abundant in the cortex and pith. The starch grains occurred not only inside the cortical and pith cells but also in the intercellular spaces.

In the root sections of the plant, starch was seen highly concentrated only in cortical tissues and to some extent as brownish black patches in the medullary rays (Fig. C, D). Starches from different botanical sources vary in terms of their functional properties (e.g., gelatinization onset temperature, final viscosity of paste, formation of two-phase pastes or paste stickiness) and thus in their end-uses. This variation stems from differences in the structure of starch, such as the size of starch granules, their composition, and molecular architecture of the constituent polymers [19]. Starch structure also influences its digestibility in the gut. Those with reduced digestibility (resistant starch), such as high-amylose starches, are increasingly valued due to their health-promoting effects, potentially serving as a preventive measure against conditions such as colorectal cancer and diabetes [20] . Understanding starch biosynthesis and its relationships to structure and functionality is of enormous interest as it represents a prerequisite for the targeted improvement of starch crops.

Starch is the most important reserve food material of the higher plants and is found in cereals, legumes and vegetables. It is usually present inside the plant cells as compact insoluble granules which may be spherical, ovoid or compact crystals and which have a distinctly layered structure. The shape and size of the starch granules varies between 2-175 microns depending upon botanical source and tissue type [21]. In the current research work, carbohydrate storage in the form of starch grains has been shows the variation in size.

Table 1: Preparation of FAA fixative

S. No.	Chemicals	Quantity (ml)
1.	90% Ethanol	50ml
2.	Glacial Acetic Acid	5ml
3.	Formalin	5ml
4.	Distilled Water	40ml
5.	Total	100ml

Table 2: Preparation of TBA dehydration series

S. No.	% of TBA	Distilled water (ml)	TBA(ml)	Ethyl Alcohol 95% (ml)	Ethyl Alcohol 100%	Time of treatment (hours)
1	10%	50	10	40	-	2
2	20%	30	20	50	-	12
3	35%	15	35	50	-	1
4	55%	-	55	45	-	1
5	75%	-	75	-	25	1
6	100%	-	100	-	-	1

Table 3: Preparation of Haupt's adhesive.

S. No.	Chemicals	Quantity
1.	Gelatin	1gm
2.	Distilled warm water (90^0 C) (mixture cooled to 30^0 C)	100ml
3.	Glycerin	5ml
4.	Phenol crystals	2gm

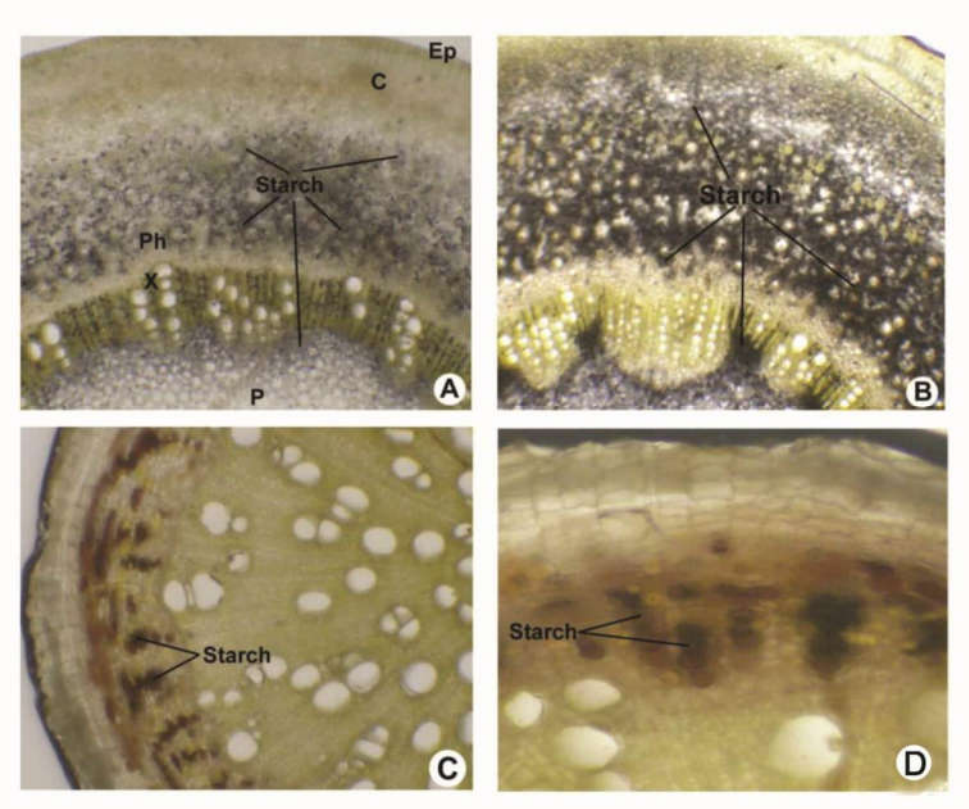

Figure1: Histochemical localization of starch
A- B. T.S. of *Jatropha curcas* stem showing starch localization (×40)
C. T.S. of *Jatropha curcas* root showing starch distribution (×40)
D. An enlarged portion of *Jatropha curcas* root T.S. showing starch localization (×100)
Abbreviations:
Ep: Epidermis, C: Cortex, Ph: Phloem, X: Xylem,P: Pith

CONCLUSION

Here, we have presented recent research findings in the context of histochemical localization of starch. Furthermore in the present study, it is however concluded that such methods are proved as good tool to be rapidly and efficiently employed in different vital aspects of biological research.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Botany, University of Rajasthan, Jaipur for providing necessary facilities to carry out the study. I am not getting any fellowship or financial aid. Grateful acknowledgement is to the supervisor for their sincere suggestions and moral support during research work.

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CITATION OF THE ARTICLE

V Kumari, S Gupta, N Kumari, A Shrivastav and K K Verma. Histochemical localization of starch in stem and root of *Jatropha curcas* (Euphorbiaceae). Bull. Env. Pharmacol. Life Sci., Vol 7 [5] April 2018 : 07-10