Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; Sp 10(1): 298-300
Received: 20-11-2020
Accepted: 23-12-2020

Basabdatta Das
Division of Plant Biology, Bose Institute, Main Campus, 93/1APC Road, Kolkata, West Bengal, India

Samik Sengupta
Department of Horticulture, Fruit and Fruit Technology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Tapas Kumar Ghose
Division of Plant Biology, Bose Institute, Main Campus, 93/1APC Road, Kolkata, West Bengal, India

Corresponding Author:
Samik Sengupta
Department of Horticulture, Fruit and Fruit Technology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Detection of glycogen in two rice landraces of Assam

Basabdatta Das, Samik Sengupta and Tapas Kumar Ghose

Abstract
Glycogen is a multi-branched polysaccharide and is the primary form carbohydrate storage in humans, animals and fungi. It is obtained in the diet from red and white meat and liver of animals and is also made from ingested carbohydrates via glycogenesis. Glycogen can be rapidly mobilized and can be utilized as a fuel substrate in skeletal muscle and the brain. Since the source of glycogen is of animal origin, it is problematic for vegetarians to obtain an adequate amount of glycogen from their diet. There are reports of Japanese rice cultivars with glycogen. In this study we have detected and extracted glycogen from two rice landraces from the North East Indian state of Assam.

Keywords: Rice landraces, glycogen, extraction protocol

Introduction
Glycogen is a multi-branched polysaccharide of about 120,000 glucose residues (C-6, H-10, O- 5)n; and is the primary form carbohydrate storage in humans, animals and fungi. The monomer alpha-D- glucose units in the polymer chains are connected by an alpha acetyl linkage. All the alpha acetyl links connect the "first" Carbon atom of one glucose molecule to the "fourth" carbon atom of the next glucose molecule, with branches occurring approximately every 8-12 residues. One end of the molecule contains a free first carbon atom of a glucose unit and is called a reducing end. The other ends are all called non- reducing ends (Ball *et al*. 1996) [1].

Glycogen is obtained in the diet from red and white meat and liver of animals. It is also made from ingested carbohydrates via glycogenesis within the brain and stomach (Powel *et al*. 2014, Powel *et al*. 2015) [4, 3]. In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, and functions as the secondary long-term energy storage (Powel *et al*. 2014, Powel *et al*. 2015) [3]. Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. The uterus also stores glycogen during pregnancy to nourish the embryo. It is also essential for athletes, pregnant women and nursing mothers to meet their energy requirements (Powel *et al*. 2014, Powel *et al*. 2015) [3].

Glycogen can be rapidly mobilized in skeletal muscle and can be utilized a fuel substrate in the absence of oxygen but fat oxidation requires energy input. Glycogen can maintain blood glucose levels to be used by certain tissues such as the brain contrarily the carbon atoms of fat cannot be used by any metabolic pathway of the body. Moreover the glycogen stores are more compact than the bulky adipose tissue containing triglycerides (lipids) (Powel *et al*. 2014, Powel *et al*. 2015) [3].

Since the source of glycogen is of animal origin, it is problematic for vegetarians to obtain an adequate amount of glycogen from their diet. This problem is rather prevalent in India as a considerable part of the population is vegetarian. The most popularly known source of plant glycogen in India is obtained primarily from two species of cyanobacteria: *Arthrospira platensis*, popularly known as Spirulina and is consumed in the form of tablets. Certain rice cultivars of Japanese origin (*Oryza sativa* var japonica) like Ohuu No. 344 and Himenomochi have a glycogen in them but these cultivars are not fit for cultivation in India (Nakamura *et al*. 1996, Wong *et al*. 2003) [2, 5].

In our laboratory, we have identified two different accessions from two different landraces of rice from the North East Indian state of Assam which have a considerable amount of glycogen in them. We have also designed a protocol to isolate the glycogen from the rice kernels.

Materials and Methods
The first landraces, Kala Boro dhan was collected from farmer's field at Khoirabari village in Darrang district of Assam. The other landrace, Lal Binni was a collection from a farmer's field at Kachar Hills. Both these two landraces were planted in the experimental plots.

They were cultivated for the purpose of conservation according to regular rice cultivation procedures. For both the landraces, the fittest mature plants were selected and their panicles were harvested separately from the rest. The matured panicles were taken to the laboratory and further quantitative and qualitative experimentation.

The step-by-step procedure for glycogen extraction is as follows

- Rice was dehusked and made into fine powder by thorough grinding.
- The rice powder was then mixed with 10 times the volume of di-ethyl ether and stirred thoroughly, in order to dissolve the fats present in the rice powder.
- The mixture was filtered to separate the ether with dissolved fat from the rice powder. The precipitate (rice powder) was then air dried in front of laminar air flow so that the residual ether evaporates from the rice.
- To the defatted rice powder 10 times the volume of water at was added and vortexed and gently shaken in a shaker. The vortexing should never be less than 1 minute and the shaking should never be less than 5 minutes. The temperature of the added water and the ensuing solution was maintained between 10ºC to 39ºC. Extraction at a temperature of 40° C or more is not preferable because gelatinization of amylose or amylopectin takes place and efficiency of extraction is decreased. Temperatures less than 10ºC will be too cold for the process.
- The mixture was then centrifuged at a speed of about 9000 rpm, preferably at 8500 rpm; for not less than 15 minutes and the supernatant which contains glycogen and proteins, was collected separately. The major constituent of the protein is rice albumin. The precipitate which is mainly rice starch was discarded.
- To separate the rice albumin the obtained supernatant was heated at a temperature range of 85ºC to 100ºC, but never less than 80 ºC; till flocculates appear and the solution becomes cloudy. This flocculates the albumin which then settles at the bottom.
- The mixture was cooled to room temperature and centrifuged at a speed of about 5000rpm, but never less than 4500rpm; until the flocculates separate from the supernatant. The centrifugation time should at least be about 5 minutes.
- The volume of the supernatant was measured and sodium chloride was added to it so as to make the concentration of sodium chloride 0.02 molar. The solution was vortexed and kept undisturbed for 1 to 2 hours and centrifuged at a speed of about 11,000rpm, but never less than 10,000rpm to separate the precipitate from the supernatant. The centrifugation time should at least be about 20 minutes. The supernatant was collected separately and the precipitate containing globulin, the second highest protein fraction, was discarded.
- The weight of the collected supernatant was measured and to it trichloroacetic acid (TCA) is added so that the concentration of the TCA solution becomes 5%.
- The solution was kept undisturbed until the precipitate separates at the bottom and then centrifuged at a speed of about 4500rpm, preferably at 4000rpm but never less than 3500rpm until the all the precipitate separate from the supernatant. The centrifugation time should at least be about 5 minutes.
- The volume of obtained supernatant was measured and mixed with 3 times the volume of methanol. The solution was kept undisturbed for 10 to 20 minutes and then centrifuged at a speed of about 5000rpm for 5 to 10 minutes. The supernatant was discarded and precipitate was retained.

Results and Discussion

The obtained white precipitate is air dried to obtain a white powder. This powder is the extracted glycogen. The procedure was repeated thrice to obtain a statistically valid result. The procedure of glycogen extraction as improvised in this study is fast and easy. It used di-ethyl ether for defatting rice, separated proteins by flocculation and removed the globulin fraction by addition of sodium chloride. Further detailed experiments are now required to elucidate the chemical structure of this glycogen from rice.

Acknowledgement

The authors wish to thank Bose Institute for providing the facilities for conducting this research.

Conflict of Interest

The authors declare that there is no conflict of interest

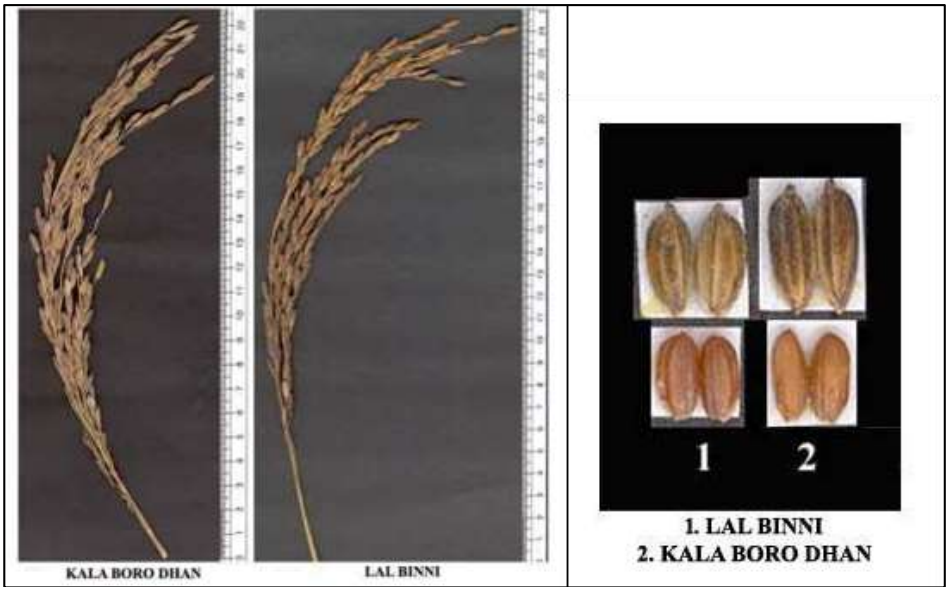

Fig 1: Panicles of the germplasm used in this experiment

References

1. Ball S, Guan HP, James M, Myers A, Keeling P, Mouille G, *et al*. From glycogen to amylopectin: A model for the biogenesis of the plant starch granule. Cell 1996;86(3):349-352.
2. Nakamura Y, Umemoto T, Takahata Y, Komae K, Amano E, Satoh H, *et al*. Changes in structure of starch and enzyme activities affected by sugary mutations in developing rice endosperm. Possible role of starch debranching enzyme (R-enzyme) in amylopectin biosynthesis. Physiologia plantarum 1996;97:491-498.
3. Powell PO, Sullivan MA, Sheehy JJ, Schulz BL, Warren FJ, Gilbert RG, *et al*. Acid hydrolysis and molecular density of Phyto glycogen and liver glycogen helps understand the bonding in glycogenα (composite) particles. PLoS One 2015;10(3):e0121337. doi:10.1371/journal.pone. 0121337.
4. Powell PO, Sullivan MA, Sweedman MC, Stapleton DI, Hasjim I, Robert GGM, *et al*. Extraction, isolation and characterisation of phytoglycogen from su-1 maize leaves and grain. Carbohydrate Polymers 2014;101:423-31.
5. Wong KS, Kubo A, Harada JJ, Satoh KH, Nakamura Y. Structures and properties of amylopectin and phytoglycogen in the endosperm of sugary-1 mutants of rice. Journal of Cereal Science 2003;37(2):139-149.