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BIOCHEMICAL CHANGES OF VARIOUS METABOLITES IN LEAF GALL OF *FICUS ROXBURGHII*

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ABSTRACT

Biochemistry encompasses the study of the chemistry inherent to life and its processes. Any infection symptom caused by a pathological organism in plants is invariably linked to biochemical changes in the tissues. Disease onset in plants stems from a sequence of physiological transformations within host tissues triggered by the pathogen at various stages of disease progression. The pathogen elicits cellular responses by releasing secretions containing auxins, enzymes, and toxins, which modify the metabolism of the host cell. As the disease advances, a complex series of biochemical reactions unfolds in a coordinated and integrated manner. During the localized infection phase, a metabolic equilibrium is established between the host and the parasite (Sharma, 2004).

Keywords: *Biochemistry, Metabolites, Plants Stems, Physiological Transformations, Biochemical Reactions.*

Introduction

The sourced of primary metabolites from higher plants for commercial purposes are high-volume, low-value chemicals. They play crucial roles in energy regulation, tissue growth, and overall organismal development, serving as the foundational components of life. Primary metabolic pathways produce essential primary metabolites that are ubiquitous in nature and vital for all living lifeforms. These compounds, such as proteins, carbohydrates, fats, and nucleic acids, serve fundamental roles in life creation and maintenance. With the exception of fats, these compounds are typically large, polymeric molecules. Generally, In on going investigations, biochemical changes in various cellular metabolites and enzymes have been observed in leaf gall of *Ficus roxburghii*.

Carbohydrates are widely distributed compounds found throughout the plant and animal kingdoms. They are primarily produced through photosynthesis, which is fundamental to metabolic processes like photosynthesis. Carbohydrates serve multiple roles such as storage of energy, structural components of cell walls, and as raw materials for various synthetic processes. Alongside plant growth substances, the quality and quantity of carbohydrates significantly influence cellular growth and metabolic events (Maretzki et al., 1974). Glucose, fructose and other six carbon compounds are the most abundant biomolecules in nature. They are also known as saccharides (from the Greek word Sakcharon, that's meaning sugar or sweetness).

Starch is composed of D-glucopyranose units linked by α-1-4 glycosidic bonds, consisting predominantly of a mixture of amylose and amylopectin in a 1:4 ratio. Both amylose and amylopectin are high molecular weight compounds. Chemically, carbohydrates are derivatives of aldehydes or ketones of polyhydroxy alcohols. Each molecule of carbohydrate contains either an aldehyde or ketone group and is categorized accordingly as an aldose or a ketose. They are typically classified into monosaccharides, derived monosaccharides, oligosaccharides, and polysaccharides. Hexoses, for example, are sugars with six carbon atoms, and starch serves as the primary reserve substance in plant cells.

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Researchers have investigated the carbohydrate compositions of various medicinal plant species, including Morus alba and Psoralea corylifolia (Singh, 2004), Bacopa monnieri (Mohapatra and Rath, 2005).Starch stands out as the primary storage carbohydrate in plant cells. It consists of two polysaccharides, amylose and amylopectin. Starch in leaves undergoes transitory storage, accumulating during the day and relocating to other plant parts during the night. Numerous studies have been conducted on starch, as documented by Dinges et al. (2001) and Critchley et al. (2001). Alpha-amylase is a widely distributed in plants responsible for the hydrolysis of starch. Known formally as endo-1,4-alpha-D-glucan glucohydrolase (EC 3.2.1.1), it acts extracellularly by cleaving internal bonds within polysaccharides, specifically breaking alpha-1,4 linkages in a random fashion to produce a mixture of glucose and maltose. This enzyme plays a crucial role in starch breakdown within chloroplasts (Beck, 1985), making it essential for mobilizing carbohydrate reserves in plants

Medicinal use, the alpha-amylase inhibitory activity of plants like Phyllanthus amarus from Malaysia has been explored for treating diabetes (Hasenah et al., 2006). Numerous studies have investigated alpha-amylase activity in both in vivo and in vitro settings involving medicinal plants. Examples include Cordia gharaf and Jatropha curcas (Rajore, 2002), barley (Tull et al., 2003), Morus alba and Psoralea corulifolia (Singh, 2004), and Lantana camara (Nagamani et al., 2007).

Materials and Methods

Total Soluble Sugar

Amount of total soluble sugars was determined using the phenol-sulfuric acid reagent method developed by Dubois et al. (1951).

Reagents

- 80% ethanol
- 96% sulphuric acid
- 5% phenol solution
- A standard glucose solution was prepared by dissolving 50 mg of glucose in 50 ml of distilled water.
- **Working Standard:** A working standard solution was prepared by taking 10 ml of the standard glucose solution prepared earlier and diluting it to 100 ml with distilled water. This resulted in a solution with a known concentration of glucose.

To create a series of calibration standards, various volumes (ranging from 0.1 ml to 1.0 ml) of this working standard were measured and diluted to 10 ml with distilled water. These volumes corresponded to concentrations ranging from 10 μg/ml to 100 μg/ml of glucose in each standard solution.

The same procedure used for preparing and analyzing the sample was followed for estimating the glucose concentration in these calibration standards. This process ensured accurate measurement and calibration of glucose levels across the specified concentration range.

Procedure

- **Extraction of total soluble sugar**
- To extract total soluble sugars from both normal and gall tissues, 500 mg of each tissue sample was homogenized with 10.0 ml of 80% ethanol. Each homogenized sample was then centrifuged at 2000 rpm for 20 minutes. After centrifugation, the supernatants from each sample were carefully collected separately. These supernatants were subsequently used for the estimation of total soluble sugars.
- **Total soluble sugar**

1.0 ml of each alcoholic extract was mixed with 1.0 ml of 5% phenol. Following this, 5.0 ml of 96% sulfuric acid was added quickly while gently agitating the mixture. The solution was then allowed to stand in a water bath at 26-30°C for 20 minutes. After incubation, the optical density of the resulting yellow-orange color was measured at 490 nm using a spectrophotometer. A standard curve was established using known concentrations of glucose. The total sugar content was quantified and expressed as mg per gram of fresh tissue weight.

Reducing Sugar

Reducing sugars were estimated using the dinitrosalicylic acid (DNSA) method developed by Miller in 1972.

Materials and Methods

Reagents

- 40 persentageRochelle salt (Potassium-sodium tartarate)
- 0.1% DNSA (Dinitro salicylic acid)
- Ethanol eighty persentage
- Standard glucose solution

Procedure

- **Extraction of Reducing Sugar**

500 mg of both normal and gall tissues were homogenized with 10.0 ml of 80% ethanol. Each homogenized sample was centrifuged at 2000 rpm for 20 minutes. The supernatant from each sample was collected and analyzed for reducing sugars.

- **Estimation of Reducing Soluble Sugar**

1.0 ml of extract from each sample was separately collected into a test tube. To each tube, 1.0 ml of DNSA (dinitrosalicylic acid) reagent was added. The mixture was then heated for 5 minutes in a boiling water bath to develop color. After color development, 1.0 ml of 40% sodium potassium tartrate (Rochelle salt) was added to each tube while it was still warm. The tubes were cooled in running tap water, and the absorbance of the resulting solution was measured at 575 nm using a spectrophotometer. The absorbance values were compared against a standard curve prepared with known concentrations of glucose. The quantity of reducing sugars present in each sample was calculated and expressed as mg per gram of fresh tissue weight.

Starch

The McCready et al. in 1950 was used to estimate the starch content.

Reagents

- 80% ethanol
- 52% perchloric acid
- Standard glucose solution

Procedure

- **Extraction of Starch**

500 mg each of normal and gall tissues were homogenized with 10.0 ml of 80% ethanol. Each homogenized sample was then centrifuged at 2000 rpm for 20 minutes. After centrifugation, the supernatant from each sample was discarded, and the residue (pellet) was collected.

- **Estimation of Starch**

After extracting total soluble sugars from normal and gall tissues, the residual mass was suspended in 5.0 ml of distilled water. To this suspension, 6.5 ml of 52% perchloric acid was added and stirred for 15 minutes. The mixture was then centrifuged at 2000 rpm for 20 minutes, and the supernatant was carefully decanted and collected. This process was repeated three times. The supernatants from each extraction step were combined (pooled) and the total volume was adjusted to 100.0 ml with distilled water. The mixture was then filtered through Whatman No. 42 filter paper to obtain a clear filtrate. From this filtrate, a 1.0 ml aliquot was taken and analyzed for starch content using the same procedure as for estimating total soluble sugars. The quantity of starch was calculated in terms of glucose equivalent, and a conversion factor of 0.9 was applied to convert the glucose value to starch. The amount of starch was expressed as mg per gram of fresh tissue weight.

Alpha Amylase

Alpha-amylase activity was assessed by measuring the production of maltose and other reducing sugars from amylopectin using the 3,5-dinitrosalicylic acid (DNSA) colorimetric method developed by Bernfeld in 1955.

Reagents

- A phosphate buffer of pH 6.9 was prepared using 45 ml of 0.02 M KH2PO4 and 55 ml of 0.02 M K2HPO4.

- DNSA (3,5-dinitrosalicylic acid) reagent.
- The substrate solution consisted of 1.0 g of soluble starch dissolved in 100 ml of 0.02 M phosphate buffer, pH 6.9, containing 0.0067 M NaCl.

Procedure

- **Extraction of Enzyme**

200 mg of fresh weight from both normal and gall tissue samples were crushed in 4.0 ml of 0.02 M phosphate buffer (pH 6.9). The homogenate was then centrifuged at 2500 rpm for 20 minutes, and the resulting supernatant was used to determine enzyme activity.

- **Determination of Alpha Amylase Activity**

The enzyme activity assay was conducted by preparing a reaction mixture consisting of 1.0 ml of enzyme extract and 1.0 ml of substrate solution containing 1.0 g of soluble starch dissolved in 100 ml of 0.02 M phosphate buffer (pH 6.9) with 0.0067 M NaCl. Following incubation at 30°C for 45 minutes, the reaction was halted by adding 1.0 ml of DNSA (3,5-dinitrosalicylic acid) reagent. Subsequently, the reaction tubes were subjected to a 15-minute boiling water bath followed by rapid cooling under tap water. After cooling, 20 ml of distilled water was added to each tube to dilute the contents. The yellow color developed due to unhydrolyzed starch was then measured for optical density at 560 nm using a spectrophotometer against a zero-hour blank. Enzyme activity was expressed as mg of starch hydrolyzed per hour per gram of fresh tissue weight, determined from absorbance readings and calibrated against known starch hydrolysis standards.

Results

- **Reducing Sugar**

he results shown in Plate 1, Fig. A indicate that the reducing sugar content was higher in normal plant tissue compared to galls in both plant species. Within the gall tissue of *Ficus roxburghii,* the highest levels of reducing sugars were observed in mature leaf galls, with lower amounts in young and old galls. Additionally, the reducing sugar content decreased gradually as the galls aged.

- **Starch**

The results depicted in Plate 1, Fig. B show that the total starch content was higher in young leaf galls of *Ficus roxburghii* compared to their normal counterparts. However, the starch content decreased in mature and old galls.

- **Total Soluble Sugar**

The results depicted in Plate 2, Fig. A show that the total soluble sugar contents were higher in leaf galls (young, mature, and old) compared to the leaf galls of *Ficus roxburghii* and their normal counterparts. Specifically, mature gall tissues exhibited slightly higher amounts of total soluble sugars compared to young and old galls.

- **Alpha Amylase**

The results shown in Plate 2, Fig. B indicate that alpha-amylase activity decreased in all gall tissues compared to their normal counterparts in *Ficus roxburghii*. Among the gall tissues, the highest alpha-amylase activity was observed in young galls, which then decreased gradually with the age of the galls.

Conclusion

- Gall tissues of *Ficus roxburghii* exhibited higher total soluble sugar contents compared to normal leaves, whereas re*ducing sugar content was lower in gall tissues.*
- *Young gall tissues in Ficus roxburghii* showed higher starch contents compared to their normal counterparts.
- Alpha-amylase activity was lower in tissues of *Ficus roxburghii* gall compared to their normal counterparts.
- The high starch contents observed in gall tissues may be correlated with the low alpha-amylase activity in these tissues.

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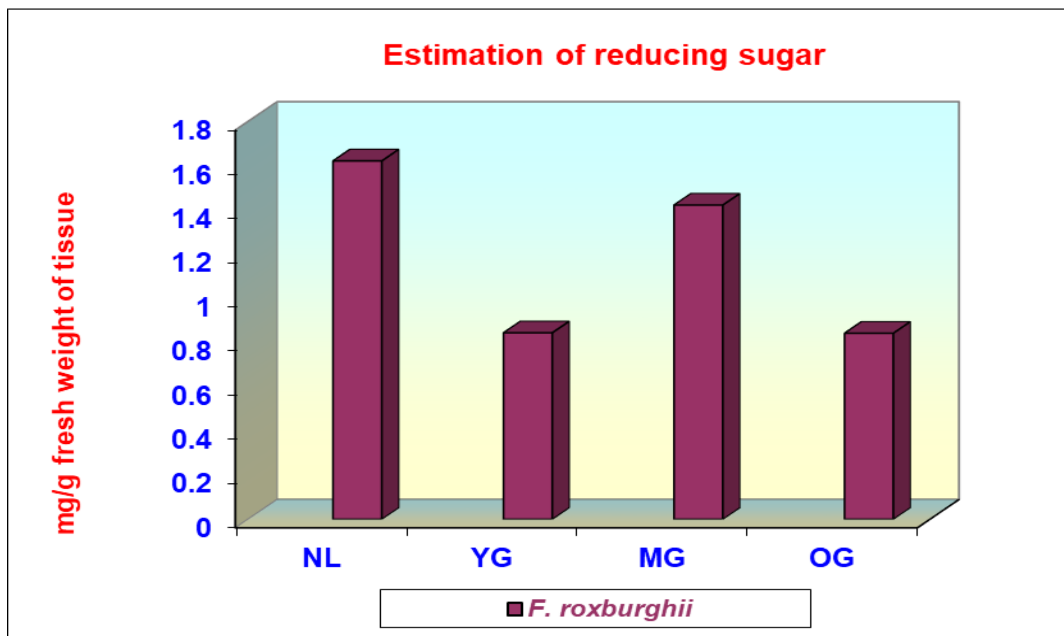

Fig. A

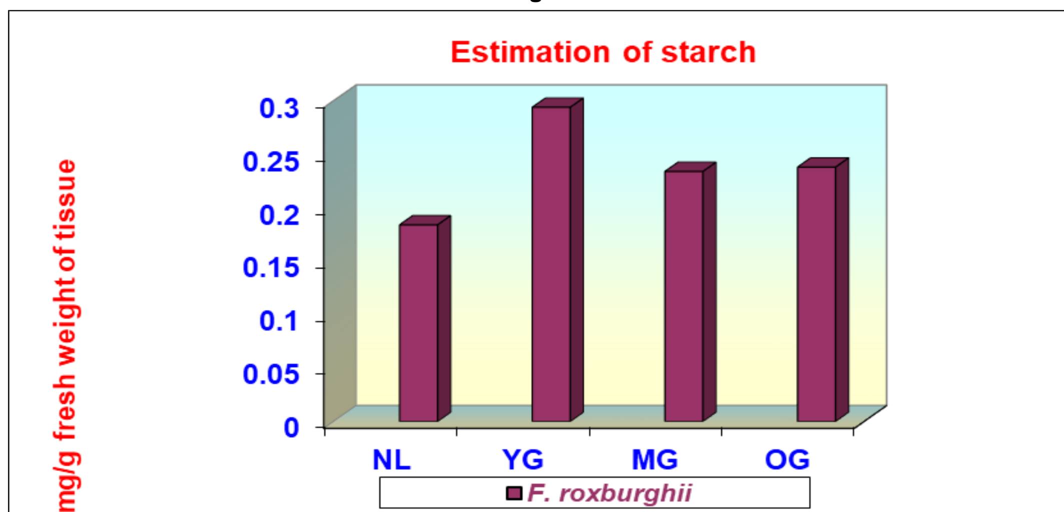

Fig. B

Plate 1

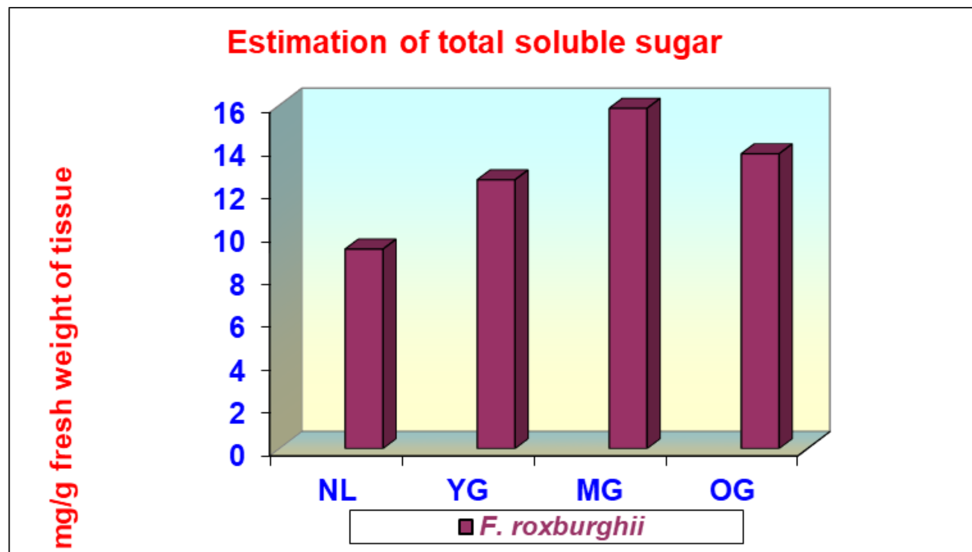

Fig. A

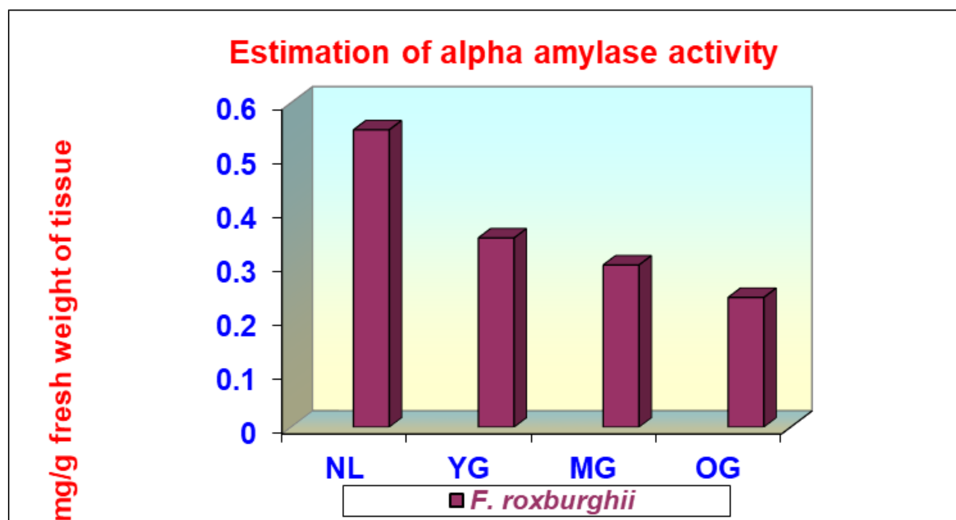

Fig. B

Plate 2