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Determination of Total Chlorophyll, Carbohydrate and Hydrolyzing Enzymes Contents in *Mitragyna Parvifolia* Leaves

Om Prakash Meena

Government College Rajgarh, Alwar, India
op21meena@gmail.com

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Abstract

Histochemical studies have shown that gall-inducing agents can alter the cell and tissue metabolism on the host plant part. Morphological changes in the host tissues are ultimately linked with physiological changes. Histochemistry enables the localization of various metabolites present in the tissue. To understand the morphological changes in terms of physiology certain histochemical studies have been carried out. An attempt has been made to interpret the biochemical data in terms of cells, tissues and tissue systems. Fresh hand-cut sections were used to study the changes brought about in the distribution of various metabolites and enzymes of the normal and gall tissues. The metabolites studied during the present investigation were chlorophyll, totally insoluble polysaccharides and enzymes.

Keywords

Histochemistry, metabolites, galls

1. Introduction

Chlorophyll is the green pigment of plants and omnipresent in all green plant parts that are necessary for the synthesis of carbohydrates as a result of photosynthesis. Chlorophyll a and b are the most important pigments and are present in almost

all green plants. Chlorophyll absorbs light for the process of photosynthesis which is the basis of all food for plants and animals. It is the reason that chlorophyll is regarded as the most important biochemical substance in nature. Chlorophyll efficiently absorbs visible light, and the light energy is converted into chemical energy by the pigments.

The chlorophyll content of plant leaves is an indicator of photosynthetic capacity, plant productivity, and plant vigor. Total chlorophyll contents in leaves are affected by plant pathogens, leafage, nutritional and environmental factors. Gall-inducing insects and other pathogens reduced chlorophyll contents and photosynthetic rate in leaves (Prade et al., 2016). Gall-inducing infections affect nutritional composition by lowering the concentration of chlorophyll and carotenoids in galled tissues (Huang et al., 2014). The quantity and qualitative yield of photosynthetic products could be compromised due to the feeding activity of galling organisms (Gonzales et al., 2005). Macioszek et al., (2020) studied the effect of Alternariabrassicicola development on the photosynthesis of Brassica juncea. Chlorophyll a and chlorophyll b concentration decreased significantly, followed by chloroplast breakdown.

In the present investigation, total chlorophyll pigments were analyzed in leaf gall (young, mature, and old) and normal tissues.

2. Material and Methods

Fresh healthy and diseased leaves were collected from the field and the surface cleaned. Take 1 gram sample of each and rapidly crush and homogenize with a few drops of 80 percent acetone in a pestle and mortar. Centrifugation of the homogenate was done. The procedure was repeated till the chlorophyll was totally removed from the residue, and the obtained extract was diluted to 50 ml. The optical density of the extracted pigments was recorded against the wavelength ranging from 400-700 nm at an interval of 20 nm and the chlorophyll content was estimated by taking optical density at 645 nm and 663 nm with the help of spectropolarimeter 'SPEKOL' Carl Zeiss Jena with cuvette of 20 ml capacity and 1.0 cm light path by using 80% acetone as blank and calculated the equation given by Arnon (1949).

3. Results

3.1. Chlorophyll Contents

The results are represented in Plate1, Fig. 1. and Table 1. Total chlorophyll contents were found lowest in old, galled tissue (0.217±0.020 mg/g fresh weight of tissue) followed mature and young galled tissue compared to normal leaf (0.395±0.014 mg/g fresh weight of tissue).

Plate 1

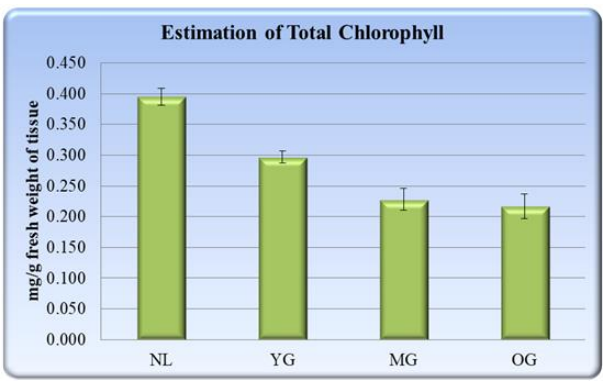

Figure 1. Estimation of Total Chlorophyll in Normal, Young, Mature and Old Gall Tissue

(NL=Normal leaf, YG=Young gall, MG=Mature gall, OG=Old gall)

3.2. Estimation of Total Carbohydrate Contents and Hydrolyzing Enzymes

Carbohydrates have a crucial part in a number of metabolic activities of plants and are indispensable for tissues grown in culture. These are formed by the storage of energy trapped from sunlight in the process of photosynthesis. Secondly, carbohydrates are the main constituents of the supporting tissues of plants. These are the supreme lavish grade of biomolecules in the environment. These are widely distributed molecules and are found in the repository units of plants and hepatic and adipose tissues of animals as the bank of nourishment.

The knowledge of the carbohydrate contents of both normal and gall tissues will aid in understanding the metabolism of the diseased and normal tissues. Hence, total soluble sugar contents, reducing sugar contents, starch contents and activity of enzymes viz. alpha-amylase and invertase of leaf galls of Mitragyna parvifolia and their normal counterparts were analyzed.

3.3. Total Soluble Sugar Contents

The concentration of total soluble sugar was calculated using Dubois et al. phenol's sulphuric acid reagent technique (1951).

3.4. Estimation of total soluble sugar

One ml of five percent phenol was added to 1 ml of each ethanolic extract and thoroughly mixed. 5.0 mL of 96 % H_2SO_4 was promptly added to the aforesaid mixture. Allow to stand in the water bath for 20 minutes at 26-300C stirring slowly while adding H_2SO_4. A distinctive yellow-orange color was obtained, and the optical density (OD) at 490 nm was measured in a spectrophotometer. The standard graph was generated using previously determined glucose dilutions. The overall sugar content was measured in milligrams per gramme of fresh tissue mass.

3.5. Reducing sugars contents

Miller's dinitrosalicylic acid (DNSA reagent) method was used to calculate reducing sugar (1972).

3.6. Estimation of total soluble sugar

Every sample was decorated in 1.0 mL and placed in a test tube separately. The extract was treated with a 1.0 mL DNSA reagent. This solution was also warmed in a boiling water bath for 5 minutes. Following the color development, 1.0 ml of 40 percent sodium-potassium tartrate was added in while the mixture was still warm. The intensity was measured at 575 nm against a standard glucose solution after the tubes were cooled in running tap water. In terms of amount, the reducing sugars were measured in milligrams per gramme of fresh tissue mass.

3.7. Total soluble sugar contents

The results are shown in Plate 2, Fig. A. and Table 1

The amounts of total soluble sugar were estimated highest in mature leaf gall tissues (1.26±0.120 mg/g fresh weight of tissue) followed by young and old galled tissue in comparison to healthy normal leaf tissue (0.95±0.012 mg/g fresh weight of tissue).

3.8. Reducing sugar content

The results are shown in Plate 2, Fig. B. and Table 1. Reducing sugar, were lowest in young gall tissues (0.699±0.18 mg/g fresh weight of tissue) followed by old galled and mature galled tissue compared to normal healthy tissues (1.201±0.03 mg/g fresh weight of tissue).

PLATE-2

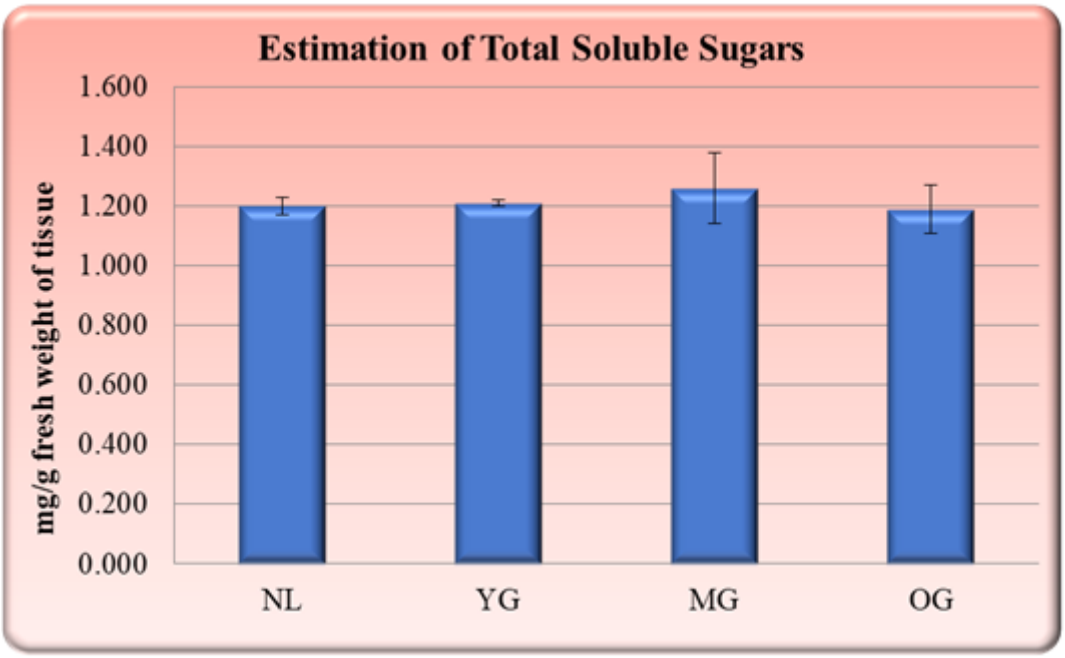

Figure 2. Estimation of Total Soluble Sugars in Normal, Young, Mature and Old Gall Tissue (NL=Normal leaf, MG=Mature gall,YG=Young gall,OG=Oldgall)

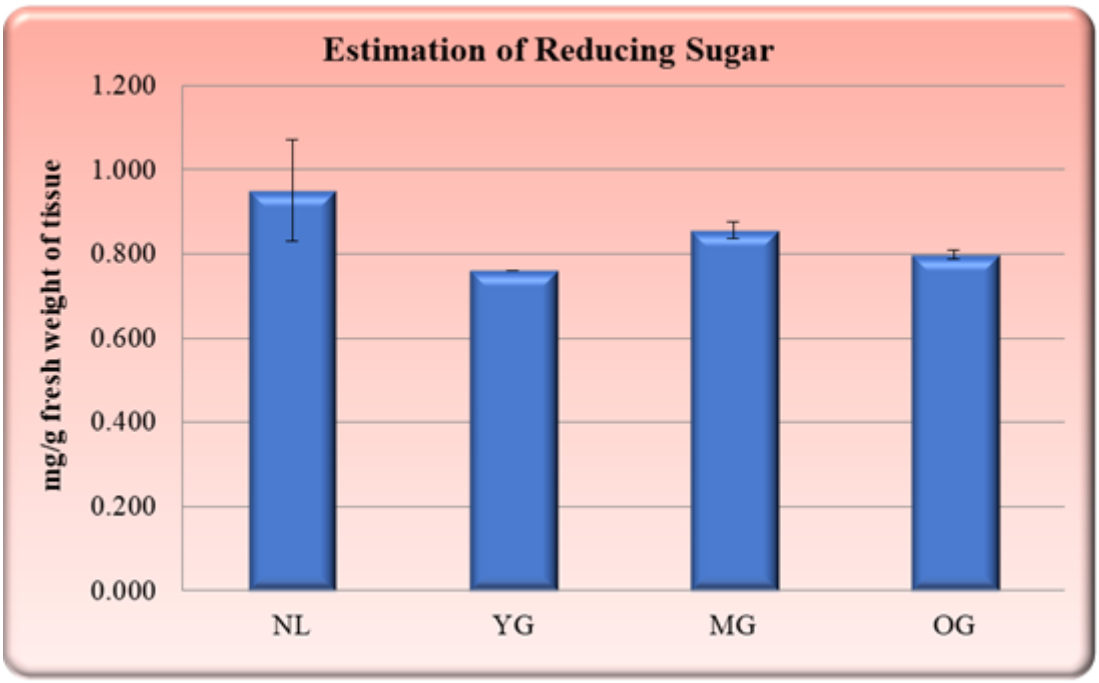

Figure 3. Estimation of Reducing Sugar in Normal, Young, Mature and Old Gall Tissue (NL=Normal leaf, MG=Mature gall, YG=Young gall, OG=Old gall)

Table 1. Changes in biochemical profile of Mitragyna parviflora infected galls induced by insects

Sample	Total Chlorophyll mg/g fresh weight of tissue	Total soluble suger mg/g fresh weight of tissue	Redusing sugermg/g fresh weight of tissue
Normal leaf	0.395+0.014	1.201+0.03	0.95+0.012
Young leaf	0.297+0.010	1.21+0.010	0.760+0.001
Mature leaf	0.228+0.018	1.26+0.120	0.857+0.020
Old leaf	0.217+0.020	1.19+0.080	0.799+0.010

4. Conclusions

4.1 The normal leaves showed high total chlorophyll content, but in leaf galls, total chlorophyll contents were low in young, old and mature gall. Marginal difference in old and mature gall but in young gall, it was recorded comparatively higher than other two stages.

4.2 The total soluble sugar content of the young, old, and mature leaf galls differed just slightly. The total soluble sugar content of normal leaves was lower than galled leaves.

4.3 The reducing sugar was more in normal leaves in comparison within various stages of leaf galled, such as young, mature and old, the content was noticeably low.

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