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Foliar Biochemical Changes During Gall Formation in *Mitragyna parvifolia*

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ABSTRACT

Mitragyna parvifolia is a medicinally important plant which is native to India and Shrilanka. This plant is widely used by tribal people of Rajasthan and other Ayurvedic practitioners. Normal leaf and leaf gall of *Mitragyna parvifolia* were collected and categorized in normal (healthy leaf) and galled leaf. In the present investigation an attempt was made to study the changes of some metabolites during the gall formation on leaf of *Mitragyna parvifolia* attacked by gall insect. Total soluble sugar, total phenol and peroxidase activities were found high in gall leaves as compared to healthy leaves but chlorophyll contents and polyphenol oxidase activities were recorded high in normal leave and protein contents were slightly high in normal leaves as compared to infected or gall leaves.

Figure : 00 References : 26 Table : 01
KEY WORDS : Biochemical changes, Healthy leaf, Leaf gall, *Mitragyna parvifolia*

Introduction

Mitragyna parvifolia belongs to family Rubiaceae. This plant is also known as Kaim[24], is an ethano-medicinally important plant which is widely used by tribal people of Rajasthan and other Ayurvedic practitioners.The fruit juice of this plant is used to increase breast milk in lactating mothers. Leaves of plant are used to dress wounds and ulcers to alleviate pain, swelling and is better for healing[18,19,21]. Leaf extract of this plant is to be both analgic and has antimicrobial potential[10] and fruits have anthelmintic activity[13]. With the development of disease, a complex series of biochemical reactions proceed in an orderly and highly integrated manner. Initially metabolic activities of parasites are much less than host metabolic activities because of relatively simple body structure but after or during the infection, equilibrium is formed between host and parasite suggesting their metabolic equality[26]. Metabolic activities of host decrease and parasite activities increase simultaneously which lead to gall formation. Hence an attempt has been made to study the changes in the biochemical profile of healthy and infected leaves (gall) of *Mitragyna parvifolia*.

Materials and Methods

Healthy and infected leaf samples of *Mitragyna parvifolia* were collected from Kadamdungri adjoining areas of Jaipur. Visual scorning method was adopted for the observation of gall development in plants. Infected leaves were categorized into young, old and mature and healthy leaves were used for the comparative analysis.

Analysis of total chlorophyll content

Total chlorophyll content and healthy and galled leaves were estimated[20]**.** One gram quantity of fresh leaves of each sample of healthy and galled leaf homogenized with 10 ml acetone and centrifuged homogenate at 3000rpm for 10 minutes. Supernatant was collected in separate tubes and repeated acetone extraction of tissues until extract was free from pigment and finally optical density was measured at 645 and 883 nm and calculated total chlorophyll content.

Total soluble sugars

Quantitative analysis of total sugars was done by the phenol sulphuric acid reagent method[2]. The optical density at 490 nm was measured and then calculated total sugars and standard curve was prepared by using known concentrations of glucose. The quantity of total sugar was expressed as mg/g fresh weight of tissue.

Total protein

Normal and gall fresh tissues extracted with 5.0 ml of 5% trichloroacetic acid (TCA)[11]. 5.0 ml of alkaline copper reagent was added to the dissolved residue and allowed to stand for 10 minutes and samples were calculated with a standard curve prepared from bovine serum albumin.

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Total phenol

Total phenolic contents were estimated[1]. Ethanolic extract was used for estimation of total phenols to 1.0 ml of alcoholic extract, 1.0 ml of folin-ciocalteu reagent (diluted with equal volume of distilled water before use) followed by 2.0 ml of 20% sodium carbonate were added. The samples were placed in a boiling water bath for exactly one minute and then cooled under running tap water. Each of the reaction mixture was diluted to 20.0 ml with distilled water. Total phenols were calculated from standard curve prepared from different concentrations of caffeic acid.

Peroxidase and poly phenol oxidase activity assay

The polyphenol oxidase activity was assayed[17]. Took reaction mixture and 3.0 ml of 5 mM solution of DL-DOPA in 0.033 M potassium phosphate buffer pH (7.0). The increase in optical density at 470 nm in spectrophotometer was observed after mixing enzymes and substrate, recorded at15 seconds interval upto 3 minutes. An enzyme activity was expressed in terms of units (change in O.D.)/ sec/g fresh weight of tissue**.**Peroxidase activity was determined[26]. 7 ml of 0.2 M phosphate buffer (pH = 6.1), 0.1 ml of 1mM H_2O_2, 0.5 ml of enzyme solution as added in a cuvette. The absorbance was calibrated to zero. To the mixture 0.1 ml of 2.0 mM o-dianisidine was added and mixed quickly. The absorbance was recorded at 460 nm at 15 second intervals for 10 min. The enzyme activity was expressed in units (change in O.D.)/sec/mg fresh weight of tissue.

Statistical analysis

It was carried out after the collection of data and their mean values were calculated. After that one way ANNOVA was used to find out the f and r values.

Results

Gall induction on *Mitragyna parvifolia* leaves gave significant result for chlorophyll content estimations and biochemical changes. The observation in healthy leaves sample and infected leaves samples are in Table-1.

Chlorophyll contents

Total chlorophyll content in healthy leaves was estimated high 0.395mg/g fresh weight of tissues while in different stages of gall formations, total chlorophyll content was 0.297mg/g fresh weight of tissues in young gall (YG), 0.217mg/g fresh weight of tissues in old gall (OG) and 0.228mg/g of fresh weight of tissues in mature gall (MG).

Total soluble sugar

Similar to chlorophyll content, soluble sugar was decreased in healthy leaf (0.95±0.020) mg/g while in three stage of infected leaf such as young gall (1.21±0.010) mg/g, old gall(1.19±0.080) mg/g and (1.265±0.120) mg/g fresh weight of tissue recorded respectively.

Total protein

Total protein in healthy leaf was higher than galled or infected leaf. In healthy leaf total protein was (0.298±0.022) mg/g was recorded while in young gall, old gall and mature leaf the total protein content were recorded(0.243±0.019)mg/g,(0.285±.013) mg/g and 0.223±0.017) mg/g fresh weight of tissues respectively.

Total phenol

High phenol was recorded in infected leaves and healthy leaf has low amount of total phenol (0.285±0.013)mg/ g fresh weight of tissues(Table-1).

Polyphenol oxidase and peroxidase activity

Healthy leaf showed higher polyphenol oxidase activity (1.35±0.060) unit /sec/mg fresh weight of tissue as compared to gall leaf in all the three stages (young, old and mature) tested.Higher peroxidase activity was recorded in gall leaf as compared to the healthy leaf (0.03±0.001)unit /sec/mg fresh weight of tissue (Table-1).

Discussion

The gall induced in *Mitragyna parvifolia* by Hemiptera (Aleyrodidae).This insect not caused gall in the plant only, causing extensive damage to agriculture foliage plant and as well as economically forest plants.

Total Chlorophyll was recorded low in gall tissue because due to the gall formation chlorosis and necrosis were observed. Similar observations were recorded in rice varieties[3], in Urd bean[12] and Ash gourd infected with three viruses[16]. Low chlorophyll contents *in terminalia* plant species during insect induced gall was because loss of palisade tissues, chloroplast disappearances and modified spong tissues[15] and decreasement of chlorophyll a, chlorophyll b and total chlorophyll contents in infected leaf of *Capsicum annuum*[14].

Increase in sugar contents in galls might be due to accumulation of these substances. During the gall formation cell differentiations and growth of cells formed of nutritive substance between host plants to neighboring parts of the gall[4]. Some other scientist correlate high amount of sugar content due to the increase activity of alpha amylase[22]. Total soluble sugar was recorded high in gemini virus infected leaf of *capscicum annuum*[14].

The proteins are nitrogenous organic compounds with high molecular weight. They are important elements of living organisms. Proteins are made from amino acid chain. In leaf gall samples of *Mitragyna parvifolia* high protein level was recorded as compared to healthy leaf.

TABLE-1 : Changes in biochemical profile of *Mitragyna parvifolia* infected with insect

Sample	Total chlorophyll contents mg/g	Total soluble sugars mg/g	Total protein mg/g	Total phenol mg/g	Poly phenol oxidase Unit / sec/mg	Peroxidase Unit / sec/mg
Normal leaf	0.395±0.014	0.95±0.012	0.298±0.022	0.285±0.013	1.35±0.060	0.30±0.001
Young gall	0.297±0.010	1.21±0.010	0.243±0.019	0.623±0.019	0.98±0.020	0.49±0.006
Old gall	0.217±0.020	1.19±0.080	0.285±0.013	0.310±0.017	1.01±0.032	0.60±0.007
Mature gall	0.228±0.018	1.26±0.120	0.223±0.017	0.298±0.022	1.25±0.023	0.50±0.012
F value	0.2859	0.0039	6.8041	0.0930	0.2747	1.889
R value	0.6121	0.9528	0.3014	0.7706	0.61897	0.2185

Similar results have been reported in various cases[8,14]. IAA is essential for gall formation which is synthesized *via* the quinines and tryptophan reactions and hyrolyzation of host plant protein. This aspect supports the higher amount of phenol in infected leaf of *Mitragyna parvifolia* due to the acceleration of phenol synthesizing pathway with the insect attack.

Phenols are defensive chemicals which are involved in protecting the invading organism[23] and activate defense enzymes[5]. Defense responses are induced by insect herbivores upon leaf[6]. Peroxidase, proline and phenol are actively involved in defense mechanism and provide resistance of plants.In the present investigation high peroxidase and low polyphenol oxidase activity was observed in gall tissues as compared to its normal counterparts in all the plant parts tested. High peroxidase activity in infected leaf is due to increased concentration of phenol which influences resistance in host plant. However, lower polyphenol oxidase activity of the galled tissue might be another factor for hyperphenolicity levels. It is believed that the level of phenolic compounds is regulated by polyphenol oxidase[25]. Workers[9] observed involvement of defense enzymes and phenols in resistance of wheat crop towards aphid. Similar results such as increased level of total phenol contents, high peroxidase activity and low level of polyphenol oxidase activity were estimated in *salvadora persica*[7].

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