

Quantitative Estimation of Some Metabolites and Enzymes in Insect induced Leaf Galls of *Mangifera indica*



Kamal Singh Marmit and Suman Lata Sharma
Plant Pathology, Tissue Culture and Biotechnology Laboratory,
Department of Botany,
University of Rajasthan,
Jaipur-302004. (Raj.); India

Abstract : This paper reports the quantitative estimation of some metabolites and enzymes in insect induced leaf galls of *Mangifera indica*. The parameters assayed were total soluble sugar, reducing sugar, starch, α -amylase activity and invertase activity compared to normal tissues. Galls showed significantly higher contents of total sugar, starch, α -amylase and invertase enzymes activity and lower content of reducing sugar.

Key words : *Mangifera indica*, Soluble sugar, Reducing sugar, Starch, α -amylase, Invertase, Enzymes.

Introduction

The mango is the apple or peach of the tropics and one of the most commonly eaten fruit in tropical countries around the world. Mango suffers from several diseases during its life. All the parts of the plant, namely trunk, branch, twig, leaf, petiole, flower and fruit are attacked by a number of pathogens including fungi, bacteria and insects.

Specific interactions between insects and plants are very common in nature. Among these interactions those of galling insects and their host plants are believed to be the most intimate (Fernandes, 1990). Most gall forming insects are highly host and organ specific that they induce galls on only one species or a closely related group of host species (Dreger-Jauffref and Shorthouse, 1992). Carbohydrate amounts are higher in gall tissues than normal tissues (Motta *et al.*, 2005). Present study was thus undertaken on biochemical changes in leaf galls of Mango due to infection of *Amradiplosis allahabadensis*.

Materials and Methods

Normal and heavily galled *Mangifera indica* leaves of equal size were collected from Sodala region of Jaipur, Rajasthan and their biochemical study was done.

(1) Estimation of total soluble sugar:-
The amount of total soluble sugars was estimated by Phenol sulphuric acid reagent method (Dubois *et al.*, 1951).

500 mg each of fresh normal and galled plant material was homogenized with 10 ml of 80% ethanol. Each sample was centrifuged at 2000 rpm for 20 min. The supernatant were collected separately to 1.0 ml of alcoholic extract, 1.0 ml of 5% phenol solution was added and mixed. Then 5.0 ml of 96% sulphuric acid was added rapidly. Each tube was gently agitated during the addition of the acid and then allowed to stand in a water bath at 26-30°C for 20 minutes. The OD of the characteristic yellow orange colour thus developed was measured at 490 nm in a spectrophotometer after setting for 100% transmission against the

* **Corresponding author :** Kamal Singh Marmit, Plant Pathology, Tissue Culture and Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302004. (Raj.); India.

blank standard curve was prepared by using known concentration of glucose. The quantity of sugar was expressed as mg/g fresh weight of tissue.

(2) Estimation of starch:- Estimation of starch was carried out by the method of McCready *et al.* (1950). The residual mass obtained after the extraction of soluble sugar of normal and gall plant material was suspended in 5.0 ml of water and subsequently 6.5 ml of 52% perchloric acid was added to the residue after stirring of the mixture, the contents were centrifuged for 20 minutes at 2000 rpm. The supernatant was decanted and collected and the whole procedure was repeated thrice. Supernatant of each step were then poured and the total volume was made up to 100 ml with distilled water. The mixture was then filtered through whatman filterpaper (No.42) 1.0 ml of aliquot of this filtrate was analyzed for starch content following the same procedure as that of total soluble sugar. Quantity of starch was calculated in terms of glucose equivalent and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed in terms of mg/g fresh wt. of tissue.

(3) Estimation of reducing sugar:- Estimation of reducing sugar was done by the method of Miller (1972). 500mg plant material was treated with 10.0 ml of 80% ethyl alcohol. In 3.0 ml of alcoholic extract, 3.0 ml of DNSA (3, 5-dinitro-salicylic acid) reagent was added. The mixture was heated for 5 minutes in a boiling water bath. After the colour had developed 1.0 ml of 40% Rochelle salt was added when the contents of the tubes were still warm. The tubes were cooled under running tap water. Absorbance was recorded using spectrophotometer at 515 nm. The amount of reducing sugar was calculated using standard curve prepared from glucose. The quantity of reducing sugar was expressed as mg/g fresh weight of tissue.

(4) Determination of alpha-amylase activity:- Alpha-amylase activity was determined by measuring the production of maltose and other reducing sugars from amylopectin of amylase using 3,5-dinitro-salicylic acid (DNSA) colorimetric procedure of Bernfeld (1955). 500mg fresh weight of each tissue sample of normal and gall was crushed with 4.0 ml of 0.02 M phosphate buffer at pH 6.0. The homogenate was centrifuged at 2500 rpm for 20 minutes. The supernatant was used to determine the enzyme activity. The reaction mixture consisted of 1.0 ml of enzyme extract and 1.0 ml of substrate solution (1.0g soluble starch dissolved in 100 ml of 0.02 M phosphate buffer, pH 6.9 containing 0.0067 M NaCl). The reaction mixture was incubated at 30°C for 45 minutes and the reaction was stopped by adding 10.0 ml of DNSA reagent. The tubes were heated in boiling water bath for 15 minutes and then cooled under running tap water. 20.0 ml of cooled water was added to this mixture. Optical density of the yellow colored developed was read at 560 nm against a zero min. blank. The activity was expressed in terms of starch hydrolysed/minutes mg fresh weight of tissue.

(5) Estimation of Invertase activity:- A modified method of Harris and Jaffcoat (1974) was used for estimation of invertase activity. 500 mg normal and galled plant material was crushed in 5.0 ml of 0.2 M acetate buffer (pH 4.8). In a test tube 0.4 ml of 0.4 M sucrose was added. To this reaction mixture 0.4 M enzyme extract was added to make the volume 1.0 ml control was prepared by adding sucrose solution to the tube in which enzyme was inactivated by boiling for about 5 minutes. After incubation at 30°C for 30 minutes, 1.0 ml of 3, 5-dinitrosalicylic acid reagent was added. Tubes were boiled in water bath for 10 minutes and then diluted to 10.0 ml by adding distilled water. Optical density was taken at 560 nm. Invertase activity was expressed as mg sucrose hydrolysed/minutes/mg fresh weight of tissue.

Results

The results are presented in Fig.A-E. In general high amount of total soluble sugars were present in the gall tissue (young, mature and old) as compared to normal. Mature gall showed slightly higher amount of total soluble sugar as compared to young and old galls. High starch content was found in the gall tissue (young, mature and old) as compared to normal tissue. Mature gall showed highest amount of starch as compared to young and old galls.

Higher amount of reducing sugars was present in normal tissue as compared to gall tissue (young, mature and old). Mature gall showed lowest amount of reducing sugar as compared to young and old gall. Alpha-amylase activity was recorded to be more in gall tissue

as compared to normal tissue. Highest alpha-amylase activity was observed in mature gall as compared to young and old gall. Invertase activity was recorded to be more in gall as compared to normal tissues. Maximum invertase activity was observed in mature gall as compared to other young and old gall.

Discussions

The quantity of total soluble sugar was considerably high in gall tissue as compared to normal tissue. According to Mehrotra and Agarwal (2003), sugar has large numbers of stereo-isomer, because they contain several asymmetric carbon atoms (Lindhrost and Thisbe, 2003). Galls have often been described as physiological sinks. Increase in sugar content might be due to accumulation of these

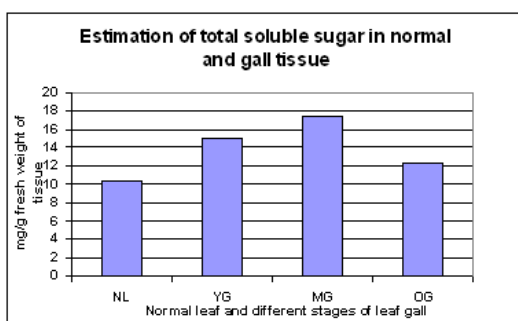


Fig. A

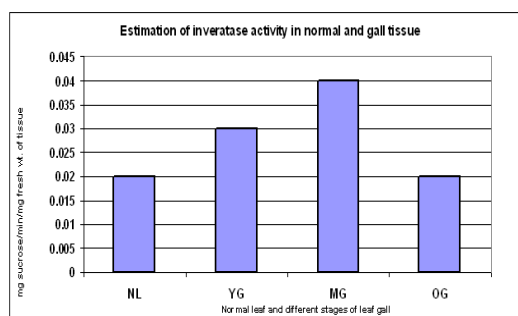


Fig. C

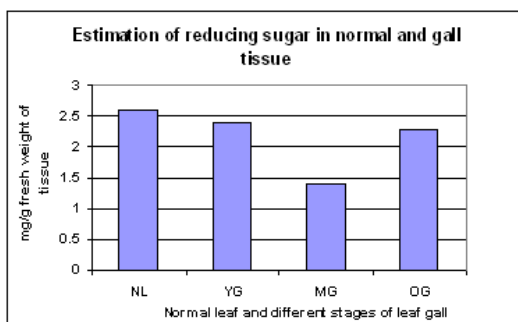


Fig. B

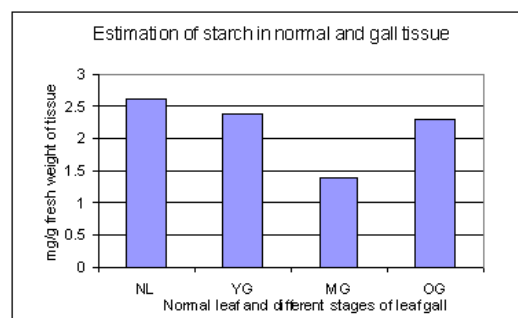


Fig. D

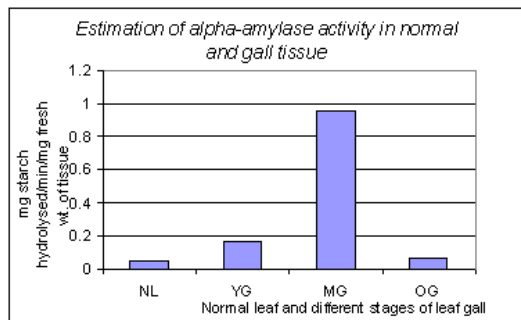


Fig. E

Abbreviations

NL = Normal Leaf; YG = Young Gall

MG = Mature Gall; OG = Old Gall

substances. This accumulation may involve the translocation of soluble sugars from the neighboring healthy tissues to the physiological sinks. This view is supported by the finding of Shaw and Samborski (1956). High sugar contents in young and mature galls may be due to increased metabolic activity under stress which may in turn be responsible for additional synthesis of sugar.

Similarly carbohydrates may also accumulate by depletion of starch due to the activated alpha-amylase activity and other enzymes (Garg and Mandhar, 1975; Shekhawat, 1980 and Rao, 1989). Shekhawat (1980) Purohit (1980) also reported increased activity of alpha-amylase along with increased sugar contents.

References

Bernfeld P. (1955): α and β amylases. *Methods in Enzymol.* **1**, 149-158.

Dreger-Jauffret F. and Shothouse J.D. (1992): Diversity of gall-inducing insects and their galls. In : *Biology of insect-induced galls*, J.D. Shorthouse and O. Rohfritsch (Eds), Oxford University Press, New York. pp. 8-33.

Dubios M.K., Gilles J.K., Robers P.A. and Smith F. (1951): Calorimetric determination of sugar and related substance. *Analyt. Chem.* **26**, 351-356.

Fernandes G.W. (1990): Hypersensitivity : A neglected plant resistance mechanism against insect herbivores. *Environ. Entomol.* **19**, 1173-1182.

Garg I.D. and Mandhar C.L. (1975): Effect of downy mildew on respiration, photosynthesis and carbohydrate synthesis in pearl millet leaves. *Indian Phytopathl.* **28**, 565-566.

Harris G.P. and Jaffcoat B. (1974): Effects of temperature on the distribution C¹⁴-labelled assimilates in the flowering shoot of Carnation. *Ann. Bot.* **38**, 77-83.

Lindhorst T.K. and Thisbe K. (2003). *Essentials of carbohydrate chemistry and biochemistry*. New Delhi. Wiley Eastern Ltd.

McCready R.M., Guggolz J., Silviera V. and Owens H.S. (1950): Determination of starch and amylase in vegetables. *Anal. Chem.* **22**, 1156-1158.

Mehrotra R.S. and Agarwal A. (2003). *Plant Pathology*. TataMchraw-Hill publishing Co. Ltd. New Delhi.

Miller G.L. (1972): Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.* **31**, 426-428.

Motta B., Lucimar, Jane E. Kraus, Antonio S. and Maria L.F.S. (2005): Distribution of metabolites in galled and non-galled foliar tissues of *Tibouchina pulchra*. *Biochemical Systematics and Ecology.* **33(10)**, 971-981.

Purohit S.D. (1980): Deranged physiology of *Sesamum* phyllody induced by mycoplasma like organism (MLO). Ph.D. Thesis, Jodhpur University, Jodhpur, India.

Rao D.V. (1989): *In vivo* and *in vitro* studies of green ear disease of pearl millet (Bajra) caused by *Sclerospora graminicola* (Sacc.) Schroet with special reference to host parasite interaction, biochemistry and control. Ph.D. Thesis, University of Rajasthan, Jaipur, India.

Shaw M. and Samborski D.L. (1956): The physiology of host-parasite relations. The accumulation of radio active substances at infection of facultative and obligate parasites including TMV. *Canad. J. Bot.* **34**, 389-405.

Shekhawat N.S. (1980). Studies on the nature of abnormal growth in plants during pathogenesis *in vitro* and *in vivo* state with special reference to green ear in pearl millet. Ph.D. Thesis, Jodhpur University, Jodhpur, India.