Entomology

Quantitative and qualitative estimation of amino acids accumulation in healthy and insect galls on leaf of *Ficus glomerata* Roxb.

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Abstract

Pauropsylla depressa Crawford (Homoptera: Psyllidae) infests *Ficus glomerata* Roxburgi and produces galls on its leaves, causing extensive damage to the foliage of the plant, (Mani, 1973). Galls are abnormal growth not only foreign organism, but also mechanical irritation, wounds and certain chemicals like mutagenic agents, various amino acids and excessive IAA and other plant growth harmones commonly induce the formation of gall. The gall formation by *Pauropsylla depressa* causing the changes in the histomorphology, ecology and biochemical components of the host plant. The galls are Green and pubescent at the beginning of bud expansion, but mature rapidly and soon after the emergence of the insects become dry, and turned brown. Hence, changes occur in FAA. The data clearly depict those healthy leaves process nine amino acids against clearly depict against 12 presents in galls.

In this way in healthy leaves only Alanine, Cystine, Glutamate, Glycine, Histidine, Lysine, Methionine, Phenylalanine, Proline, Threonine, Tryptophan was present. The gall tissues also exhibited a market increase in the quantities of Cystine Lysine, Phenylalanine as compared to those of healthy tissues, in case of *F. glomerata* gall, the quantitative changes in amino acids spectrum in both healthy and infected leaves as shown in the result clearly indicated that healthy least process 9 amino acids as against 12 presents in galls. The additional ones are Alanine, Threonine and Tyrosine. According to results, gall tissues also exhibited a marked increase in the quantities of Cystine, Lysine and phenylalanine as compared to those of healthy tissues.

Keywords: *Pauropsylla depressa*, *Ficus glomerata*, amino acids, quantitative changes, galls

1. Introduction

Pauropsylla depressa Crawford (Homoptera: Psyllidae) (Dhiman and Vinay, (1983) [3], infests *Ficus glomerata* Roxb. and produces galls on its leaves, causing extensive damage to the foliage of the plant, (Mani, 1973) [9]. Galls are abnormal growth on the plant leaves which provide hinderances in photosynthetic activities of the plant (Fig. 1). In heavy infestations, the leaves turn entirely glomerated with numerous galls. *Pauropsylla depressa* form galls on different parts of host plants. Galls are abnormal growth not only foreign organism, but also mechanical irritation, wounds and certain chemicals like mutagenic agents, various amino acids and excessive indole acetic acid and other plant growth harmones commonly induce the formation of gall. The gall formation by *Pauropsylla depressa* is causing the changes in the histomorphology, ecology and biochemical components of the host plant. The physiology of gall formation is still obscure and it is generally believed that a secretion from nymph stimulates gall formation. When insect emerges out of the gall and moults into adult.

2. Materials and Methods

For biochemical studies include young, mature and old gall, healthy and young (3.0-3.5 cm) and old (9.0-10.0 cm) leaves of almost equal size were collected from *F. glomerata* plant. The leaves were carefully washed with running water and dried in 4 layers of filter paper, leaf and gall tissues of similar size (1-2 mm and 4-5 mm 300 mg each) were carefully removed by scalpel and homogenized in chilled phosphate buffer (10 ml pH 6.1). Homogenate was centrifuged at 5000 rpm. The supernatant was used for the determination of total phenols and some supernatant (kept at 0 degree) was used for enzyme activity. Fresh as well dried healthy and infested leaves (gall tissue of young, mature and old gall) were used for various biochemical estimations.

2.1 Estimation of free amino acid

The amino acids were extracted and estimated by the procedures given by Porter, *et al.*, (1974) [12] and Block, (1963) [1]. The samples were run in n-butanol: acetic acid: water (74: 1: 19: 2) in the other dimension. Chromatograms were developed at 100 ^{0}C after spraying with 0.5% ninhydrin in acetone. All the chromatograms were run in duplicate and for each set of unknown; a standard mixture of known amino acids was also run. The ninhydrin positive spots were eluted in 4 ml of 75% ethanol containing 0.05% $CuSo_4$ $5H_2O$ and read in colorimeter at 540 nm. The concentration of different amino acids was expressed as glycine equivalent.

3. Results

Galls typically are resulting from the interaction between a chemical stimulus produced by the pest organism and the plant's hormones. The resulting gall is usually structurally strong and rich in amino acids in addition to a battery of different hydrolysing enzymes.

Quantitative estimation of free amino acids (FAA) was carried in healthy and galled or infected leaves of *F. glomerata*. These biochemical components such as FAA analysed in infected (galled) leaf showed either increase or decrease levels as compared to healthy leaves. The galls are green and pubescent at the beginning of bud expansion, but mature rapidly and soon

after the emergence of the insects become dry, and turned brown. Hence, changes occur in FAA. The quantitative and qualitative changes in amino acids spectrum in both healthy and gall infected leaf are shown in Table 1 and Table 2. The data of this table clearly depict those healthy leaves process 9 amino acids against clearly depict against 12 presents in galls. In galled tissues, amino acids are Alanine, Cystine, Glutamate, Glycine, Histidine, Lysine, Methionine, Phenylalanine, Proline, Threonine, Tryptophan and Tyrosine found in the infected leaves. The additional once are Alanine, Threonine and Tyrosine. In this way in healthy leaves only Cystine, Glutamate, Glycine, Histidine, Lysine, Methionine, Phenylalanine, Proline, Tryptophan was present. The gall tissues also exhibited a market increase in the quantities of Cystine Lysine, Phenylalanine as compared to those of healthy tissues, (Table 2).

In *F. glomerata* healthy leaf a total of 9 amino acids were identified by paper chromatogram. The amino acids of gall tissues had Alanine, Cystine, Glutamate, Glycine, Histidine, Lysine, Methionine, Phenylalanine, Proline, Threonine, Tryptophan and Tyrosine (Table 1). But, in infected leaf drastic depletion in most of the free amino acid Glycine, Histidine, Methionine, Proline and Tryptophan was recorded. On the other hand, increased quantity of FAA Alanine, Cystine, Glutamate, Lysine, Phenylalanine, Proline, Threonine, and Tyrosine were recorded. The levels of total FAA in gall tissues were altered or depleted due to secretion by *P. depressa*. Quantitative results showed that in healthy leaf average total FAA were 259.9 mg/100 mg dry wt. While in gall tissues average total FAA occurred a gradual decrease, i.e., 254.4 mg/100 mg dry wt (Table 1). Thus, FAA level of gall tissue is greatly affected by the insect *P. depressa.*

Table 1: Quantitative estimation of amino acid spectrum of healthy leaves and gall tissue of *Ficus glomerata* Roxb. Leaves

S. No.	Amino acids (mg/100mg dry wt.)	Healthy leaves	Gall tissues
1.	Alanine	-	301
2.	Cystine	150	302
3.	Glutamate	202	215
4.	Glycine	255	150
5.	Histidine	308	120
6.	Lysine	122	350
7.	Methionine	615	320
8.	Phenylalanine	111	301
9.	Proline	821	250
10.	Threonine	-	408
11.	Tryptophan	535	221
12.	Tyrosine	-	115

Average quantity of Amino acids in Healthy leaves = 259.91 mg/100mg dry wt, Average quantity of Amino acids in Gall tissue = 254.41 mg/100mg dry wt

Table 2: Qualitative estimation of amino acid spectrum of healthy leaves and gall tissue of *Ficus glomerata* Roxb. leaves

S. No.	Amino acids (mg/100 mg dry wt.)	Healthy leaves	Gall tissues
1.	Alanine	-	+++
2.	Cystine	+	+++
3.	Glutamate	+	++
4.	Glycine	++	+
5.	Histidine	++	+
6.	Lysine	+	++
7.	Methionine	+++	++
8.	Phenylalanine	+	++
9.	Proline	+++	+
10.	Threonine	-	++
11.	Tryptophan	+++	++
12.	Tyrosine	-	+

Abbreviation: + = Present, - = Nil, + = Low, ++ = Moderate, +++ = High, ++++ = Very high

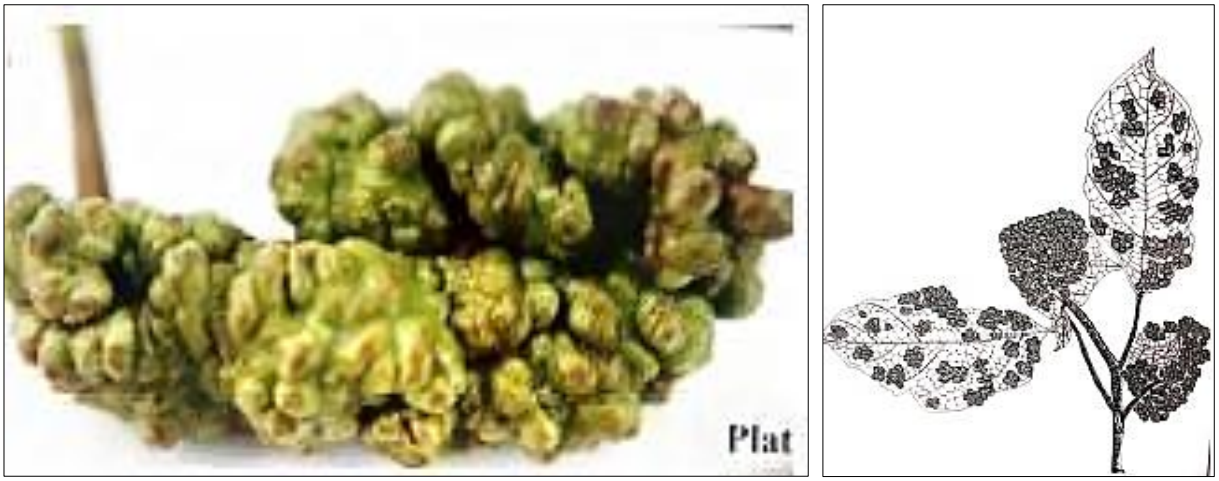
4. Discussion

Substances such as amino acids, proteins, sugars, phenols, enzymes and digestive enzymes of the insect saliva and guts, reports on the biochemical alteration in plant galls are rare. Plant galls induced by *P. depressa* on *F. glomerata* are remarkably close association between the insect and host plant, in which the plant produces an abnormal growth of tissue in response to a specific stimulus from the invading organism. Thus, gall former has the ability to manipulate the growth and development of plant tissues. Manipulation of the host plant by the gall former may extend to control over the chemical composition of gall tissue. Insect galls are usually induced by chemicals injected by the larvae or the adults of the insects into the plants and possibly mechanical damage. After the galls are formed the larvae develop inside until fully grown. Insect secretion as amino acids, phenolic compounds and phenol oxidases pectinases and proteases may be involved in carcinogenesis.

In case of *F. glomerata* gall, the quantitative changes in amino acids spectrum in both healthy and infected leaves as shown in the result clearly indicated that healthy least process 9 amino acids as against 12 presents in galls. The additional ones are Alanine, Threonine and Tyrosine. According to results, gall tissues also exhibited a marked increase in the quantities of Cystine, Lysine and Phenylalanine as compared to those of healthy tissues. Gall tissue is generally, though, to be relatively high in nutrients and low on secondary compounds, compared to healthy plant tissue. Gall tissue represents a higher quality food source than healthy plant tissue.

According to Miles and Lloyd (1967) [11] increase in the number as well as the quantity of these amino acids in the gall tissues may be due to break down of proteins into utilizable units by the enzyme protease, secreted by the salivary glands of the midge. The decrease in the quantity of proline can be explained on the basis of that it is a result of response of plant parts for physiological stress (Levitt, 1972) [8]. On the other

hand, it is shown decrease under pathological stress by Singh *et.* al., (1981). According to Hartley (1998) [4] several studies have found the gall tissue is high in nutrients and low in secondary compounds. On the other hand, according to Showler (2001), the higher quantities and more diverse accumulation of FAA in pigweed leaves occur. Koyama and Akimoto, (2004) [7] observed that the aphid galls accumulate high concentration of amino acids. Khattab and Ibrahim (2005) [5] observed that the decrease in Ca, Mg pigments levels amino acids, lignin, total soluble protein were obtained in the diseased leaves compared with the healthy leaves. Meon *et.* al., (1978) [10] noted the concentration of free Proline was highest in egg and egg sacs and in the galls as compared to uninfected portions of roots of infected tomato plants. Ayman (2009) mentioned that the role of amino acids in plant disease may be due to the correlation between these acids and plant health. Amino acids are used both for production of new cell biomass and produce energy.



(a) (b)

Fig 1: (a) galled leaf, (b) a twig with gall leaf

5. Conclusions

On become gallinaceous leaves become unfit for this activity. Thus, so far study on biochemistry of leaf galls of Ficus glomerata Roxb. Induced by *Pauropsylla depressa* Crawford hence looking to the significance of insect P. deppressa, present investigation has been taken which will arm the economic botanist or entomologists to device a suitable control measure of the insect.

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