SOME BIOCHEMICAL ALTERATIONS IN INSECT INDUCED LEAF GALLS OF *BOERHAAVIA DIFFUSA* L.

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Received June 20, 1983

Introduction

The midge *Punaravomyia boerhaaviaefolie* Mani (Order: Diptera) induces leaf galls in *Boerhaavia diffusa*.[11] The leaves at the shoot apex become irregularly enlarged and lack tissue differentiation. The present investigation deals with some biochemical alterations induced by the midge in the leaf galls.

Material and Methods

Healthy and infested leaves of *B. diffusa* were collected from R. B. S. College, campus, Agra and chlorophylls were determined by the methods of BROUGHAM[5] and ARNON[2]. For estimation of total proteins, phenolics and sugars, the samples were dried in oven at 45°C to constant weight. Total proteins were estimated by the procedure of LOWRY *et al.*[10] using Bovin serum albumin as the standard. Total phenolics were estimated by employing Folin Denis reagent as described by BHATIA *et al.*[3] and were calculated from a standard curve earlier prepared using tannic acid. The extraction of sugars was made in 80% acetone by the method of HIGHKIN and FRANKEL.[8] The assay for sugars was carried out by the method of NELSON[14] as modified by SOMOGYI[22] using earlier prepared glucose standard curve. The total sugars were estimated by the procedure of SCOTT.[19] The amino acids were extracted and estimated by the procedures given by PORTER *et al.*,[15] SHAW and COLOTELO[20] and BLOCK *et al.*[4] The samples were run in *n*-butanol: acetic acid: water (4 : 1 : 5) in one dimenstion and phenol: n-butanol: acetic acid: water (74 : 1 : 19 : 2) in the other dimension. Chromatograms were developed at 100°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% $CuSO_4 \cdot 5H_2O$ and read in Spekol 10 colorimeter at 540 nm. The concentrations of different amino acids were expressed as glycine equivalents.

IAA and IAA-oxidase activity in fresh samples was determined by the procedures of SCHNEIDER *et al.*[18] and RABIN and KLEIN[17] as described by WITHAM *et al.*[24]

Results and Discussion

Table 1 shows the quanties of water, chlorophylls, proteins, phenolics and sugars in healthy and gall tissues. It is apparent from the data presented that there was a marked decline in the quantities of total chlorophyll a, reducing sugars, total phenolics and total proteins in galls. On the other hand, the amount of water, total sugars, non-reducing sugars in galls increased significantly. However, the quantity of chlorophyll b was more or less equal in both helathy and infested leaves. Degradation of chlorophylls, phenolic compounds in aphid induced galls have earlier been reported by MILES,[12] URITANI[23] and PUROHIT *et al.*[16] The loss of phenolics in infested plant parts, according to these authors is brought about by the attack of the aphids making the host susceptable. According to MILES[12] and URITANI,[23] the decline in the quantity of proteins in the infested tissues is largely due

TABLE 1. Quantity of water, chlorophylls, total proteins, total phenolics and total sugars in healthy leaves and gall tissues. (Mean value of 10 replications)

	Healthy leaves	Galls
Water content (% fresh wt)	67.4	75.5
Chlorophylls (mg/g fresh wt)		
Chlorophyll a	0.178	0.104
Chlorophyll b	0.241	0.246
Total	0.419	0.350
Total proteins (% dry wt)	4.15	2.75
Total phenolics (μg/g dry wt)	270	195
Sugars (% day wt)		
Reducing	1.95	1.62
Non-reducing	1.02	1.53
Total	2.97	3.15

to their hydrolysis by the enzyme protease produced by the salivary glands of the aphid. The increase in the quantity of total sugars especially the non-reducing ones in a large number of infested plants has been attributed to the conversion of polysaccharides into sugars by MILES.[12]

TABLE 2. Amino acid spectrum of healthy and gall tissues of *B. diffusa* leaves.

Amino acids (μg/100 mg dry wt)	Healthy leaves	Gall tissues
Alanine	—	310
Cystine	165	315
Glutamate	218	121
Glycine	265	198
Histidine	318	127
Lysine	126	371
Methionine	620	355
Phenylalanine	113	322
Proline	830	270
Threonine	—	421
Tryptophan	570	225
Tyrosine	—	124

The quantitative changes in amino acid spectrum in both healthy and infested leaves as shown in the Table 2 clearly indicate that healthy leaves possess nine amino acids as against 12 present in galls. The additional ones are alanine, threonine and tyrosine. The gall tissues also exhibited a marked increase in the quantities of cystine, lysine and phenylalanine as compared to those of healthy tissues. According to MILES and LLOYD[13] and MILES[12], the 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 increase in the number and quantity of certain amino acids may also be due to their transfer from aphid's saliva which is known to contain 4-18 amino acids. On the other hand, the quantity of glutamate, glycine, histidine, methionine, proline and tryptophan was considerably low in galled leaves as compared to healthy ones. According to ANDERS,[1] the amino acids disappearing from the gall tissues act as cecidogenic agents of insects. He has demonstrated the formation of galls in roots of rape seedlings by growing them in the cultures containing amino acids especially tryptophan, histidine and glutamate either singly or in different combinations. In the light of

these facts, it can be concluded that deficiency of these amino acids in the infested tissues may be due to their utilization in gall formation. The decrease in the quantity of proline can be explained on the basis that it is a result of response of plant parts for physiological stress (see LEVITT).[9] On the other hand, it is shown to decrease under pathological stress (SINGH *et al.*[21] and DHINGRA *et al.*[6]) The present material shows an increase in the quantity of water (see Table 1) and pathological state as well. This in the opinion of the present authors may be the cause of decrease in the quantity of free proline in galled tissue.

TABLE 3. IAA and IAA-oxidase levels in the healthy and galls. (Mean value of 10 replications)

	Healthy leaves	Galls
IAA (μg/g fresh wt)	10±1.13	70±6.73
IAA-oxidase (mg IAA destroyed/g fresh wt/hr)	2.44±0.52	3.9±0.61

The levels of IAA and IAA-oxidase in the gall tissues increased as compared to those in the healthy leaves (Table 3). MILES and LLOYD[13] have reported the presence of high amounts of IAA in the infested tissues. The decline in the quantity of tryptophan in the presently studied material may also be held responsible for an increase in IAA levels as earlier suggested by EL-WAZIRI *et al.*[7] This according to him, increases the permeability of the cells which in turn enhance water quantity in the gall tissues as is also presently observed. However, it is surprising to observe the increase in the levels of IAA-oxidase in galls. There is no plausible explanation to this except that this may be due to the presence of higher substrate levels to act upon.

Acknowledgements

Sincere thanks are due to Dr. S. N. CHATURVEDI, Professor & Head, Department of Botany for encouragement, to Dr. Anup GOYAL, S. R. A., Department of Agriculture Chemistry for helping in various ways and to Dr. ROSHAN Singh, Principal, R. B. S. College, Agra for facilities.

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