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FREE AMINO ACIDS AND PROTEIN CONTENTS OF HEALTHY AND MALFORMED MANGO SHOOTS AND INFLORESCENCES

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ABSTRACT

Fifteen different amino acids were found in the mango leafy shoots and twelve in the inflorescence in both healthy and malformed specimens at different stages of growth. The number of amino acids increased with increase in the age of inflorescence both in healthy and diseased samples. Out of the fifteen amino acids, threonine was absent in healthy shoots whereas the diseased material lacked in cystine, valine, isoleucine and histidine. Likewise, serine and tyrosine were also absent in the diseased, while present in the healthy inflorescence. The quantitative estimation showed higher amount of total free amino acids in healthy than in the diseased material. The healthy inflorescence contained higher amounts of total free amino acids as compared with malformed ones. The level of free amino acids showed a continuous rise from early to full grown stage of inflorescence in both healthy and diseased material. Higher levels of total proteins in the healthy shoots and panicles compared with diseased ones were also observed.

INTRODUCTION

Malformation of mango inflorescence was first reported in Indo-Pakistan sub-continent by Burns (1910). The surveys carried out by Khan and Khan (1960) and Ali and Malik (1980) showed that this disorder was present in almost all orchards of the Punjab Province. Tripathi (1954) found that the malformation of inflorescence and vegetative shoots of mango (bunchy top) were highly correlated with each other.

Pathological observations made by Mohy-ud-Din *et al.* (1972) indicated that *Fusarium oxysporum* (Scheecht) was responsible for causing the bunchy top

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disease. The electron-microscopic studies carried out by them again in 1973 showed virus like particles (Mycoplasma) in malformed inflorescence and bunchy tops, and proved tha t bunchy tops and malformed inflorescence were the manifestations of the same disease. Verma *et al.* (1974) reported that *Fusarium moniliforme* was isolated from the samples of malformed mango inflorescence and vegetative shoots collected from different parts of India. Ali and Malik (1980) also reported the presence of Fusarium in both healthy and malfomed tissues to various degrees.

It is well known that nitrogen is essential for growth in plants and soluble nitrogen, largely as free amino acids, is actively involved in the protein metabolism within the plant. Khan (1960) showed relatively higher amount of carbohydrates and low protein contents in shoots bearing malformed inflorescences as compared with the normal ones. The investigations on these lines in India made by Chadha (1975) brought out that nitrogen was more in healthy panicles compared to the diseased ones. It has also been reported by Sadhu (1975) in India that the leaves on malformed shoots contained large amounts of free amino acids and less bound amino acids than leaves on healthy shoots, whereas amino acid content of the stem showed the opposite trend. Pandey *et al* (1977) reported that hydrolysable polysaccharides and total carbohydrates remained at higher levels in leaves, stems and panicles of malformed than healthy shoots of mango but total nitrogen was little affected.

The aim of the present investigation was to study the qualitative and quantitative characters of the amino acids in the malformed versus healthy shoots and inflorescences.

MATERIALS AND METHODS

The malformed and healthy inflorescences and leafy shoots of Dusehri mango were taken from the Fruit Plant Nursery, Department of Horticulture, University of Agriculture, Faisalabad, Pakistan. The samples were procured from four trees at three different stages of development of the inflorescence, i.e. early, mid, and full grown (Ist, 2nd, and 3rd stages).

From each tree, 12 samples were collected comprising two samples of

inflorescence (diseased and healthy) and two samples of shoots carrying these inflorescences at all the three stages of development. The samples were washed with water and dried in an electric oven at 65°C for 48 hours and were then ground with the laboratory mill (Arthur H. Thomas Company) using 40 mesh sieve. The finely pulverized samples were then stored in paper bags properly labelled for their identification.

The powdered samples were extracted following the procedure as given by Pant and Tulsiani (1968) and the filtrates were collected separately for the quantitative and qualitative analyses of amino acids.

The purified extracts were used for the qualitative determination of free amino acids using paper chromatography technique as explained by Lawrence *et al.* (1961). The identificantion of individual amino acids was made by spraying the paper chromatograms with 0.5 per cent Ninhydrin according to the technique of Clayton and Strong (1954).

The quantities of total free amino acids in the individual samples were estimated by the method given by Kamal and Muhmmad (1967). The total nitrogen was estimated by the Micro-Kjeldahl method. Total protein contents were then calculated by multiplying the amount of total nitrogen by 6.25 (A.O.A.C., 1970).

A two factor factorial experiment was conducted in randomized complete block design with two replications. The data collected were subjected to the analysis of variance. Fisher's L.S.D. test was used for planned comprison of means (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Amino acid status of the mango leafy shoots

The qualitative analysis of the leafy shoots carrying all three stages of developing healthy inflorescence showed that there were eight amino acids in the early, seven in the mid and twelve in the fully grown stage. Five amino acids (glutamine, leucine, proline, phenylalanine and tryptophan) were present in all the three stages while serine, alanine, cystine, valine and isoleucine were detected only in the full grown stage. The amino acids which varied were

histidine, tyrosine, lysine and asparagine. Histidine was present only in the first and third stages, and asparagine in the second and third stages (Table 1).

Table 1. *Occurrence of various amino acids in leafy shoots carrying three stages of developing healthy aud diseased inflorescences*

S. No.	Amino acids	Leafy shoots with healthy inflorescences			Leafy shoots with diseased inflorescences		
		Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
1.	Glutamine	+(2)	+(2)	+(4)	+(1)	+(4)	+(3)
2.	Leucine	+(4)	+(4)	+(4)	+(1)	+(2)	+(1)
3.	Proline	+(1)	+(2)	+(4)	+(1)	+(1)	+(2)
4.	Phenylalanine	+(2)	+(3)	+(3)	+(3)	+(3)	+(2)
5.	Tryptophan	+(2)	+(1)	+(3)	+(1)	+(2)	+(3)
6.	Lysine	+(3)	+(4)	—	+(4)	+(3)	+(2)
7.	Serine	—	—	+(3)	—	—	+(1)
8.	Alanine	—	—	+(2)	—	—	+(3)
9.	Cystine	—	—	+(1)	—	—	—
10.	Valine	—	—	+(1)	—	—	—
11.	Isoleucine	—	—	+(1)	—	—	—
12.	Histidine	+(2)	—	—	—	—	—
13.	Threonine	—	—	—	—	+(1)	+(1)
14.	Asparagine	—	+(2)	+(2)	—	—	+(4)
15.	Tyrosine	+(1)	—	+(1)	+(2)	—	+(1)
	Total amino acids in each stage	8	7	12	7	7	11

The leafy shoots with healthy inflorescence contained fourteen amino acids at the three development stages as compared with eleven amino acids found in sboots with diseased panicles. Of fifteen amino acids given in Table 1, only threonine was absent in the healthy shoots, whereas cystine, valine, isoleucine and histidine were absent in the diseased tissues.

The quantitative analysis revealed that the amount of total free amino acids was more in shoots having healthy panicles (25.5 to 39.2 mmol/100 gm) as compared with those having malformed panicles (22.7 to 37.0 mmol/100 gm). Furthermore, this amount showed a successive rise in leafy shoots with advancement in the growth stages both of healthy as well as diseased inflorescences (Table 3). Similar studies have been made by Sadhu (1975), who showed that the leaves on malformed shoots contained large amount of free amino acids and less bound amino acids than the leaves of healthy shoots. The trend of amino acid composition observed by him was contrary to the results of this study which might be due to certain pathophysiological changes as affected by climatic variation of different localities.

Amino acid status of healthy and diseased mango inflorescences

There were six amino acids in the early stage and ten each in the mid and fully grown stages of healthy inflorescences. The four amino acids which were common in all stages of development were glutamine, leucine, lysine and proline. Phenylalanine was present in the first and third stages, while alanine, tryptophan, serine and asparagine were found in the second and third stages of inflorescence (Table 2).

Results clearly indicate that tyrosine was found in the two stages of healthy inflorescence and was absent in all the three stages in case of diseased inflorescence. Similarly, serine was present in second and third stages of healthy panicles, but absent in all stages of development of diseased ones, which indicates some role of inducing disease resistance ability in the healthy inflorescence.

Total amount of free amino acids in the healthy inflorescence (47.75 to 62.75 mmol/100 gm) was higher than the diseased ones (35.75 to 43.75 mmol/100 gm) and the difference was statistically highly significant (Table 3). The successive rise in the amount of free amino acids was also observed at all stages of deve- lopment as was observed before with regard to the number of amino acids. Furthermore, at all the stages of development the panicles contained higher amounts of free amino acids than the leafy shoot on which they were born.

The qualitative and quantitative analyses of amino acids carried out

Table 2. *Occurrence of various amino acids in three stages of developing healthy and malformed inflorescences*

S- No.	Amino acids	Healthy inflorescences			Malformed Inflorescences		
		Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
1.	Glutamine	+(4)	+(2)	+(2)	+(3)	+(3)	+(4)
2.	Leucine	+(3)	+(3)	+(1)	—	+(2)	+(2)
3.	Proline	+(2)	+(1)	+(1)	—	+(2)	+(2)
4.	Phenylalanine	+(4)	—	+(3)	+(1)	+(1)	+(2)
5.	Tryptophan	—	+(1)	+(3)	—	+(1)	+(2)
6.	Lysine	+(3)	+(1)	+(1)	+(1)	+(3)	+(1)
7.	Serine	—	+(1)	+(1)	—	—	—
8.	Alanine	—	+(1)	+(3)	—	—	+(4)
9.	Cystine	—	+(4)	—	—	+(2)	—
10.	Valine	—	—	—	+(2)	—	+(2)
11.	Asparagine	—	+(1)	+(3)	+(4)	+(1)	+(2)
12.	Tyrosine	+(3)	+(1)	—	—	—	—
	Total amino acids in each stage	6	10	10	4	8	9

Note : The figures in parentheses alongwith plus signs show the number of trees in which specific amino acids were found.

Table 3. *Total free amino acids in leafy shoots with developing healthy and malformed inflorescences (mmol 100 gm) figures for healthy and malformed inflorescences are given in parentheses)*

Tree No.	Leafy shoots with healthy inflorescences			Leafy shoots with malformed inflorescences		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
1	24(49)	35(58)	40(63)	23(36)	26(36)	37(44)
2	25(48)	34(57)	39(62)	22(35)	26(39)	37(43)
3	25(47)	34(59)	38(63)	23(36)	26(40)	37(44)
4	26(47)	35(59)	40(63)	23(36)	26(40)	37(44)

during these studies have demonstrated difference in the nature and amount of amino acids between the healthy and malformed influences as well as in the leafy shoots carrying these panicles. It was pointed out by Goodman *et al.* (1967) that imbalance of amino acids in the host may predispose the host to infection. According to the available evidence *Fusarium* has been implicated in the disease of floral malformation (Mohy-ud-Din *et al.*, 1972; Verma *et al.*, 1974). Thus the present investigations on amino acids led us to believe that there was some role of the imbalance of these important metabolic compounds in the induction of resistance or susceptibility in the host to the disease of malformation. Since the results showed greater quantity of amino acids in the healthy than in the diseased inflorescence as well as in the shoots on which they were borne. It thus seems that these may or may not render the host shoots susceptible to the infection with *Fusarium*, etc.

Total protein in the mango leafy shoots

Total protein contents were significantly higher in shoots with healthy panicles (5.26 to 8.23%) compared with shoots with diseased ones (4.80 to 7.79%) at all the three developing stages of inflorescence (Table 4).

Table 4. *Total protein contents of leafy shoots with developing healthy aud malformed inflorescences (percentage dry matter) (figures for healthy and malformed inflorescence are given in parentheses*

Tree No.	Shoots with healthy inflorescences			Shoots with malformed inflorescences		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
1.	5.32(10.20)	7.20(12 10)	8.31(13.21)	4.80(7.40)	5.32(8.20)	7.79(9.10)
2.	5.21(10.18)	7.10(12.07)	8.20(13.17)	4.79(7.30)	5.31(8.10)	7.78(9.00)
3.	5.21(10.35)	7.10(12.24)	8.11(10.25)	4.81(7.50)	5.33(8.30)	7.80(9.20)
4.	5.31(10.36)	7.20(12.25)	5.30(13 25)	4.80(7.45)	5.32(8.25)	7.79(9.15)

Furthermore there was continuous increase in the protein content with the advancement in the developmental stages in both healthy and diseased

samples. The rise in protein content from early stage of growth to its full grown size was quite natural because as the growth proceeded, synthesis of new enzymes and storage of proteins was likely to increase.

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