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High performance liquid chromatography for the detection and estimation of amino acids in relation to Fusarium wilt (panama disease) in banana

Felcy Navajothy. A[1*], Narayanaswamy. R[2], Danis Ponniah[3], Irudayaraj. V[4]

[1]*Department of Physics, St. John's College, Palayamkottai, India-627 002.*
[2]*Engineering Physics Section, Annamalai University, Annamalainagar, India-608 002.*
[3]*Department of Physics, St. Xavier's College (Autonomous), Palayamkottai, India-627 002.*
[4]*Department of Plant Biology & Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India-627 002*

Abstract

Panama disease of banana is an obnoxious disease caused by the fungal pathogen *Fusarium oxysporum*. The variation in the degree of susceptibility and resistivity depends upon several macronutrients, micronutrients, polyphenols and primary metabolites-carbohydrates, protein and lipids and free amino acids in the host. Studies on such relationships between free amino acids and host-pathogen interaction are limited in agricultural crops particularly on materials based on the traditionally cultivated land are scarce. In order to understand the role of free amino acids of the host-banana in relation to Fusarium-wilt disease, HPLC study was carried out in five susceptible (Healthy/Infected) and five resistant varieties of banana from Tirunvelveli district. The results show the quantitative difference in amino acids of susceptible-healthy, susceptible-infected and resistant varieties. Inter-varietal difference in quantity of various amino acids in each group of samples has also been observed. Except two amino acids, tyrosine and lysine, all the other 13 amino acids are present in maximum amount in the infected samples of Monthan and Nadu in which the healthy samples are with very low amount of amino acids. Lysine and tyrosine are present in maximum amount in healthy samples of Rasthali and Rasakathali respectively. Major change in the quantity of arginine is related with the production of Nitric Oxide, an important chemical related to disease resistance. Occurrence of enormous quantity of glutamic acid, an important cellular osmolyte, in infected sample has been attributed to the role in controlling water loss by controlling the stomatal movement and thus ultimately save the infected plant from wilting.

INTRODUCTION

The basic component of living cells is Proteins, with building block materials, amino acids. Proteins are formed by sequence of amino Acids. Plants synthesize amino acids from the primary elements, the Carbon and Oxygen obtained from air, Hydrogen from water in the soil, forming Carbohydrate by means of photosynthesis and combining it with the Nitrogen which the plants obtain from the soil, leading to synthesis of amino acids, by collateral metabolic pathways. About 20 important amino acids are involved in the process of each function. Studies have proved that amino acids can directly or indirectly influence the physiological activities of the plant and they play an important role to overcome various biotic and abiotic stresses of the plants. As in several cases of crop diseases, Integrated Pest Management (IPM) is the best and sustainable method to control the problematic Panama disease in banana, because of the involvement of numerous biotic and abiotic factors involved both on the pathogen (*Fusarium oxysporum*) side for its successful pathogenesis and on the host (banana) side to resist the growth of the pathogen. In order to understand this complicated disease, it is necessary to study the role of mineral nutrients in soil and also the basic units (amino acids) of protein which are structurally and functionally important in every organism, particularly during pathogenesis. The pathogen obtains every nutrient from the host and they synthesize several new compounds for their growth and for the destruction of the host tissues. In contrast, the host cells immediately detect the pathogen by chemical signal and several new compounds are synthesized to inhibit the growth of the pathogen. Thus, the metabolism of both the pathogen and the host change drastically during infection and results in major changes in the composition and concentration of each and every amino acids in both the pathogen and the host. In this connection, there are only limited studies [1] [2] [3] [4] [5] & [6] which show the increase or decrease of some amino acids in healthy and infected plants. But the trend of increase and decrease of particular amino acid varies with pathogen and the host along with several other factors. With such varying results of the qualitative and quantitative changes in the composition of amino acids in plant tissue during infection, it is difficult to draw conclusion as to a general trend. According to the level of amino acids fractions increased in the most susceptible cultivar (Williams) of banana inoculated with *Fusarium moniliforme* and these levels of increase depend on fungal species and its virulence. In contrast, a clear reduction in amino acid fractions was noticed in less susceptible cv. *Paradaica* inoculated with *F. moniliforme*. The present study has been aimed to know the quantitative change in fifteen amino acids in the host-banana in relation to *Fusarium* wilt disease in banana. In order to understand the role of amino acids in disease susceptibility and disease resistance, comparative study on amino acids, through High-performance liquid chromotographic technique, has been made in five selected resistant varieties and five selected susceptible (healthy and infected) varieties of banana from banana-cultivated fields in

*Corresponding Author,
Email: felcyabraham@gmail.com

Tirunelveli district, Tamil Nadu.

MATERIALS AND METHODS

In the present study, five susceptible varieties (Rasthali, Rasakathali, Monthan, Nadu, Karpooravalli) and five resistant varieties (Kathali, Red Banana, Chakai, Robusta, Nendran) of banana are collected from various localities of Tirunelveli district, Tamil Nadu. The samples are collected during the months of November and December, 2009. The plant samples (Rhizome) are about 6 months old. The samples are dried at 60 ^{o}C for 24 hours. The samples are powdered and used for preparation of sample for HPLC analysis. The quantitative study of amino acids present in both susceptible (infected and healthy) and resistant plant samples are done using HP1100 series model HPLC available in the Department of Biochemistry at Sankara Nethralaya, Chennai.

The centrifuged deposits are dissolved in 6 ml of 6N HCl and are subjected to hydrolysis in boiling water bath for a period of 24 hrs. The tubes are cyclo-mixed for every 1 hr for proper hydrolysis to take place. After 24 hrs of hydrolysis, the tubes are centrifuged at 3500 rpm for 15 mts. The supernatant is filtered and is neutralized with 1N NaOH. Then the filtered solution is diluted to 1:100 of the volume (1 ml diluted to 100 ml) with milli-Q water and is proceeded for estimation of amino acids in HPLC.

RESULTS AND DISCUSSION

In order to know the role of amino acids in relation to Panama (*Fusarium*-wilt disease) in banana, the present HPLC study has been carried out to know the qualitative and quantitative differences in free amino in the corm (rhizome) of five different susceptible (healthy and infected) and five different resistant varieties of banana. The HPLC chromatogram for all the fifteen samples has been given in figure 1. From the chromatogram it is seen that the diversity of dominant amino acids is somewhat less in resistant varieties. In the case of susceptible varieties the diversity of dominant amino acids in healthy plants is more in Rasthali and Rasakathali in contrast to Monthan and Nadu, in which the infected samples show more diverse dominant amino acids. In Karpooravalli, both healthy and infected sample show more or less same diversity of dominant amino acids. Thus the HPLC-chromatogram amino acids itself shows the difference between susceptible (healthy/infected) and resistant varieties.

In the highly resistant variety Kathali, there about eight dominant amino acids in contrast to least resistant varieties with only six dominant amino acids. In the case of least susceptible variety, Monthan, only four amino acids are present, each below10 nmoles/ml, in healthy sample, in contrast to infected sample with seven dominant amino acids each with above n moles/ml. In the highly susceptible variety Karpooravalli, the diversity of dominant amino acid is higher in infected sample but the amount is little less than the healthy sample. The comparison of HPLC-amino acids chromatograms of all the samples, show the different kinds of variation among different varieties in relation to the *Fusarium*-wilt disease of banana.

The amount of individual amino acids present in different plant samples has been given in tables 1 and 2. Except two amino acids, tyrosine and lysine, all the other 13 amino acids are present in maximum amount in the infected samples of Monthan (11) and Nadu (2) in which the healthy samples of are with very low amount of above amino acids (Table 3). Lysine and tyrosine are present in maximum amount in healthy samples of Rasthali and Rasakathali respectively (Fig.2). Thus the least susceptible variety Monthan shows high response for the pathogen *Fusarium oxysporum* with the increase of most of the amino acids in the infected sample. Higher level of quantitative variation is seen in glutamic acid, alanine, tyrosine, leucine and isoleucine between different varieties and between healthy / infected samples. Mild variation is seen in quantity of lysine among different samples. Generally, in all the samples two amino acids, histidine and glutamic acids are present in larger quantity when compared to other amino acids.

Histograms in figure 2A, B show the proportion of all the fifteen amino acids in each plant sample. This will give idea about the change in amino acid composition in individual sample. In each sample, there are few (3-6) amino acids present in higher proportion when compared to other amino acids in the same sample. But qualitatively they differ from sample to sample. Thus each and every sample has its own proportion of the presently studied 15 amino acids. Histidine and glutamic acid are present generally in higher proportion in all the samples followed by serine, lysine and alanine. Lysine is present in very small proportion in the infected samples of Monthan and Nadu. Aspartic acid show high variation among different samples. Interestingly, it is present in considerable proportion in all the infected samples of susceptible varieties except, Rasakathali in which it is totally absent. In the healthy plants of susceptible varieties and in samples of resistant varieties aspartic acid is either totally absent (healthy samples of Nadu and Karpooravalli and resistant varieties – Red banana and Chakkai) or present in very small proportion. Although serine is present generally in small proportion in each sample, there is higher degree of variation among different samples. In resistant varieties it is present more or less in uniform amount, from 7.1 nmoles/ml in Robusta to 14.3 nmoles/ml in Chakkai. In the case of susceptible varieties, lot of variation is seen between healthy and infected samples depend upon the variety. In Monthan and Nadu, both healthy and infected samples contain more or less equal proportion of serine. But in other susceptible varieties, there is much of variation of in proportion of serine in healthy and infected samples. Thus it is in higher proportion in healthy sample of Rasthali. In contrast, when compared to healthy sample, serine is present in comparatively low proportion in infected samples of Rasakathali and Karpooravalli. Amino acids like arginine, tyrosine, valine, methionine, phenylanine and isoleucine are present comparatively in smaller proportion in each sample. They are present in very small proportion in healthy sample of susceptible variety Nadu.

In resistant varieties more or less uniform trend is seen in relation to quantity of each amino acid. Thus, in all the resistant varieties, the fifteen amino acids are present in the following order from histidine (maximum), glutamic acid, alanine, lysine, serine, glycine, leucine, threonine, arginine, tyrosine, methionine, phenylanine, valine isoleucine and aspartic acid (minimum). As far as the susceptible varieties are concerned, the proportionate quantity of different amino acid in each sample is more or less with similar trend with little variation. The variation among different sample is comparatively more in glutamic acid, serine, histidine, alanine and glycine with maximum level of variation in histidine followed by alanine (Table 4). It is peculiar to note that the infected plant of the variety Rasakathali stands first in the presence of almost all the amino acids in more quantity followed by Nadu-infected. Comparatively the infected plants of Karpooravalli more or less all the amino acids are present in low quantity. Among the five susceptible varieties, the least susceptible variety Monthan show maximum difference in quantity of each and every amino acids in

healthy and infected samples.

Table 1. Amino Acid Content (nmoles/ml) in infected and healthy plant samples of susceptible varieties as estimated by HPLC

	Rasthali		Rasakathali		Monthan		Nadu		Karpooravalli	
Amino acids	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
Aspartic acid	23.9	6	0	6.7	318.9	1.6	130.5	0	6.2	0
Glutamic acid	111.9	62.6	37.1	131.2	204.7	52.3	126.6	41	19.9	39.6
Serine	13	47.3	19.9	10	219	18	87.7	9.9	35.1	4.5
Histidine	91.3	116.6	36.8	167.2	602.8	68.8	271.2	19.1	35.1	74.3
Glycine	7.8	16.9	7.9	19.1	78.2	6.5	32.6	0.8	4.2	4.2
Threonine	4.7	6.7	5.1	17.6	11	6	61.3	1.9	0.6	0
Alanine	45.8	50.5	13	51.9	278.1	18.8	103.9	42.6	17.8	32.4
Arginine	5.7	6.1	57.4	13.3	60.4	1.6	23.2	0	1.9	4
Tyrosine	10.7	5.5	2.1	12.2	6.1	8	8.5	3.6	5.2	6.7
Valine	12	1.7	3.7	9.5	11.5	1.3	18.4	0	6.2	2.1
Methionine	7.7	5.3	10	4.8	62.9	1.4	17.7	1	2.2	0
Phenylalanine	14.7	11	3.4	9.9	59.5	1.8	17.8	0.8	2.3	6.1
Isoleucine	5.7	5.2	3.1	3.7	17.2	0.7	5.7	1.1	2.2	3.2
Leucine	19.7	20.9	8.6	17.4	80.1	6.1	33.1	1.4	5.8	13.5
Lysine	19.8	23.2	19.6	19.9	16.7	21	18.1	20.6	19.3	15.1

Table 2. Amino Acid Content (nmoles/ml) in plant samples of resistant varieties as estimated by HPLC

Aminoacids	Kathali	Red Banana	Robusta	Chakkai	Nendran
Aspartic acid	2.1	0	1.2	0	0.6
Glutamic acid	39.4	42.6	45.1	50.6	54.2
Serine	12	8.7	14.3	7.1	11.6
Histidine	60.9	49.3	64.6	58.5	63.9
Glycine	10.1	9.7	4.9	6.4	2.8
Threonine	7.2	3.8	5.2	2.9	6.4
Alanine	12.3	18.4	23.7	16.1	30.6
Arginine	7.4	2.9	0.8	4.1	3.3
Tyrosine	6.2	4.6	2.7	5.9	4.5
Valine	2.2	1.6	0.7	3.1	1.4
Methionine	3.7	1.2	2.5	1.6	0.4
Phenylalanine	2.3	3.7	1.8	2.1	4.1
Isoleucine	1.6	2.1	0.5	1.3	1.4
Leucine	7.8	4.2	5.6	7.1	6.1
Lysine	18.2	17.6	14.3	18.4	16.7

Table 3. Minimum and maximum values of Amino acids of plant samples of susceptible (healthy/infected) and resistant varieties of banana (nmoles/ml)

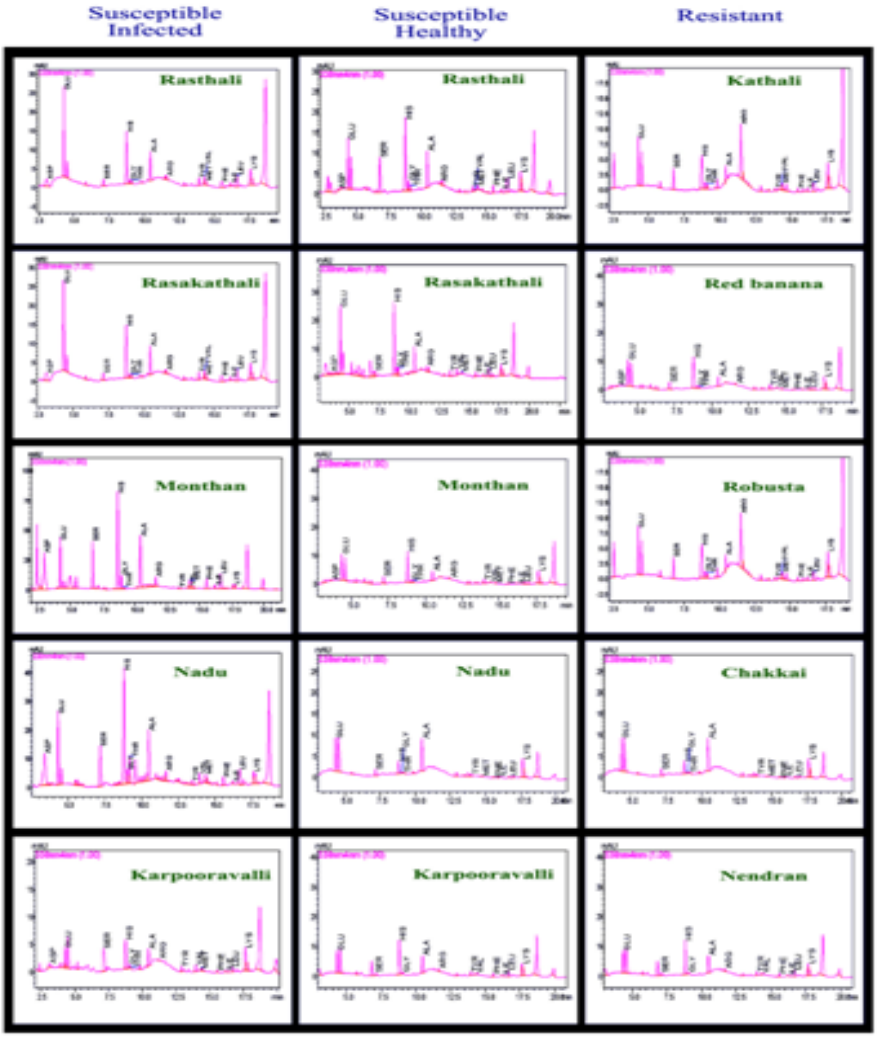
Aminoacids	Susceptible varieties		Resistant varieties
	Healthy	Infected	
Aspartic acid	0.0 - 06.7 Karpooravalli/ Rasakathali	0.0- 318.9 Rasakathali/Monthan	0.0 – 2.1 Red banana/Kathali
Glutamic acid	39.6- 131.2 Karpooravalli/ Rasakathali	19.9- 204.7 Karpooravalli/ Monthan	39.4- 54.2 Kathali/Nendran
Serine	4.5 -47.3 Karpooravali/Rasthali	13.0-219.0 Rasthali/ Monthan	7.1 -14.3 Chakkai/Robusta
Histidine	19.1- 167.2 Nadu/Rasakathali	35.1- 602.8 Karpooravali/ Monthan	49.3 -64.6 Red banana/Robusta
Glycine	0.8 -19.1 Nadu/Rasakathali	4.2 -78.2 Karpooravalli/ Monthan	2.8 -10.1 Nendran/Kathali
Threonine	0.0 -17.6 Karpooravalli/ Rasakathali	0.6 -61.3 Karpooravali/Nadu	2.9 -7.2 Chakai/Kathali
Alanine	18.8 - 51.9 Monthan/Rasakathali	13.0- 278.1 Rasakathali/Monthan	12.3 -30.6 Kathali/Nendran
Arginine	0.0 -13.3 Nadu/Rasakathali	1.9 -60.4 Karpooravalli/ Monthan	0.8 -7.4 Robusta/Kathali
Tyrosine	3.6 -12.2 Nadu/Rasakathali	2.1 -10.7 Rasakathali/Rasthali	2.7 – 6.2 Robusta/Kathali
Valine	0.0 -09.5 Nadu/Rasakathali	3.7 -18.4 Rasakathali/Nadu	0.7 – 3.1 Robusta/chakai
Methionine	0.0 -05.3 Karpooravalli/Rasthali	2.2 -62.9 Karpooravalli/ Monthan	0.4 – 3.7 Nendran/Kathali
Phenylalanine	0.8 -11.0 Nadu/Rasthali	2.3 -59.5 Karpooravalli/ Monthan	1.8 -4.1 Robusta/Nendran
Isoleucine	0.7 -05.2 Monthan/Rasthali	2.2 -17.2 Karpooravali/ Monthan	0.5 – 2.1 Robusta/Red banana
Leucine	1.4 -20.9 Nadu/Rasthali	5.8 -80.1 Karpooravalli/ Monthan	4.2 -7.8 Red banana/Kathali
Lysine	15.1 -23.2 Karpooravali/Rasthal	16.7-19.8 Monthan/Rasthali	14.3 -18.4 Robusta/Chakai

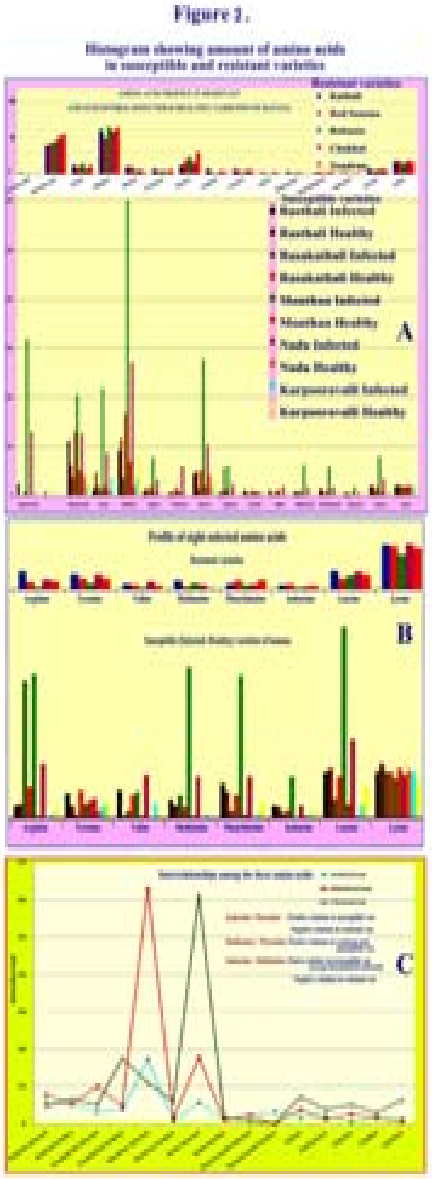
Table 4. Amino acids in Plant samples of highly / least resistant and least / highly susceptible varieties

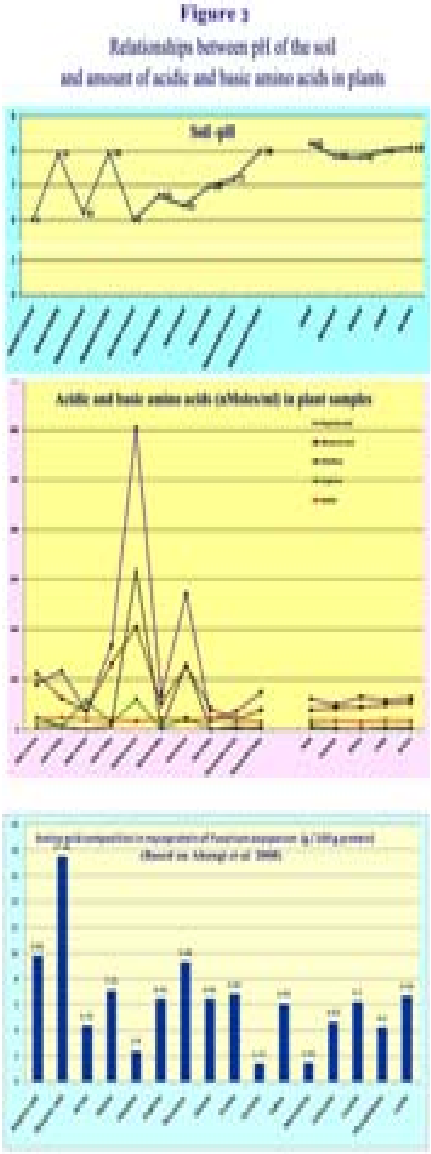
Aminoacids	I Highly Resistant variety: Kathali	II Least Resistant variety: Nendran	III Least susceptible – Healthy Monthan	IV Least susceptible –Infected Monthan	V Highly susceptible – Healthy Rasakathali	VI Highly susceptible – Infected Rasakathali
Aspartic acid	2.1	0.6	1.6	318.9	6.7	0
Glutamic acid	39.4	54.2	52.3	204.7	131.2	37.1
Serine	12	11.6	18	219	10	19.9
Histidine	60.9	63.9	68.8	602.8	167.2	36.8
Glycine	10.1	2.8	6.5	78.2	19.1	7.9
Threonine	7.2	6.4	6	11	17.6	5.1
Alanine	12.3	30.6	18.8	278.1	51.9	13
Arginine	7.4	3.3	1.6	60.4	13.3	57.4
Tyrosine	6.2	4.5	8	6.1	12.2	2.1
Valine	2.2	1.4	1.3	11.5	9.5	3.7
Methionine	3.7	0.4	1.4	62.9	4.8	10
Phenylalanine	2.3	4.1	1.8	59.5	9.9	3.4
Isoleucine	1.6	1.4	0.7	17.2	3.7	3.1
Leucine	7.8	6.1	6.1	80.1	17.4	8.6
Lysine	18.2	16.7	21	16.7	19.9	19.6

Figure 1.

HPLC Chromatogram for amino acids in plant samples of different varieties of banana







From the present HPLC study on amino acids composition and quantity, in resistant and susceptible (healthy and infected) varieties of banana in relation to Fusarium-wilt disease show difference between resistant and susceptible varieties and between healthy and infected plants of susceptible varieties. Due to the infection, major change takes place in the composition of free amino acids in the host. Major change is seen in aspartic acid, glutamic acid, histidine, alanine etc. In general all the amino acids are present in more quantity in susceptible varieties. In susceptible varieties different trend is seen in healthy and infected sample depend upon the variety. Major internal shift in quantity of amino acids is seen between serine and histidine in highly susceptible variety Rasakathali with more amount of serine and less amount of histidine in infected sample with opposite trend in healthy sample (less amount of serine and more amount of histidine). In the case of least susceptible variety Monthan major internal shift is seen between aspartic acid and glutamic acid with the presence of less amount of aspartic acid and more amount of glutamic acid in healthy sample and more amount of aspartic acid and less amount of glutamic acid in infected sample. Such kind of major shift between two amino acids is not seen in the case of highly resistant (Kathali) and least resistant (Nendran) varieties (Table 4). From this observation, it is concluded that the susceptible varieties or more sensitive to the pathogen when compared to the resistant varieties.

The decrease of amino acids in the infected tissues may be due to their utilization by the pathogen or due to their utilization in the synthesis of proteins during host-parasite interactions [7]. The increase in amino acids and or the synthesis of new amino acids may be due to an interference of host metabolism by the pathogen or due to host pathogen interaction as suggested by [8]. Quantitative increase in certain amino acids in the host tissues by infection may either be due to *de novo* synthesis by the host [9] or proteolysis of certain host tissue proteins, since diseased tissues often showed higher proteolytic activity than healthy tissues [10] ; [11]. Thus the increase or decrease of particular amino acid after infection by the pathogen may be due to different reasons connected with either the

pathogen or with the host. Since microbes are rich in protein, amino acids and vitamins, the infected sample may contain more amount of particular amino acid which origin may be from pathogen. Out of 16 amino acids (Fig.3) in the mycoprotein from the pathogen of Panama disease, *Fusarium oxysporum,* glutamic acid (17.48%), aspartic acid (9.81) and threonine (9.28%) are present in higher amount. In the present study, the susceptible variety Monthan is with surprisingly higher amount of almost all the amino acids, including aspartic acid and glutamic acid in the infected plants when compared to the healthy ones. Threonine is more in the susceptible infected plant sample of Nadu. Previous studies on amino acid composition in the host in relation to pathogenesis, show different kinds of trends. The level of amino acids fractions increased in the most susceptible cultivar (Williams) of banana inoculated with *Fusarium moniliforme* and these levels of increase depend on fungal species and its virulence. In contrast, a clear reduction in amino acid fractions was noticed in less susceptible cv. *Paradaica* inoculated with *F. monilifo*. In the present study, the highly susceptible variety Rasakathali, shows the decrease in amount of 12 amino acids. Thus different genotype of the host may respond differently to the different strains of pathogen.

The increase of certain amino acids may be due to the synthesis of such amino acids by the host for particular purpose. For example, arginine induces disease resistance via its effects on Nitric Oxide biosynthesis and defensive enzyme activity [6]. Nitric Oxide plays important role during various biotic and abiotic stresses in plants [12]. It has also been reported that treating cut flowers and other plants with nitric oxide has been shown to lengthen the time before wilting [13]. It is important to note that the Nitric Oxide which keep the flowers and plants without wilting, may definitely keep the banana as fresh during *Fusarium*-wilt disease by increasing the production of NO through the amino acid arginine. In the present study the presence of more amount of arginine in infected plants of susceptible varieties when compared to the healthy plants may be due to the above reason for defense mechanism through the formation of Nitric Oxide from arginine. Exogenous NO can also protect cells against oxidative stress. Thus, the data suggest an emerging model of stress responses in which ABA has several ameliorative functions. These include the rapid induction of stomatal closure to reduce transpirational water loss and the activation of antioxidant defences to combat oxidative stress [14]. In the case of *Fusarium* wilt of banana, the production of higher amount of arginine in the infected plant of highly susceptible variety Rasakathali may also be useful to close the stomata by NO production from arginine and thus preventing the plant from wilting.

Since *Fusarium-wilt* disease causes major problem with water economy of the plant, the regulation of stomas (by closing) to reduce the transpiration is an important step in the survival of the plant infected with *Fusarium.* So there will be a change in the chemicals like abcisic acid, glutamic acid, which control the stomatal movement(http.//www.multibloom.com). This is true in the present study. In the least susceptible variety, Monthan glutamic acid is present in maximum amount (204.7 nmoles/ml) in infected plants in contrast to very low amount in healthy plant (52.3 nmoles/ml). By the presence of more glutamic acid, the stomata may be controlled in a proper way to save the water and thus this variety survive more even after infection. In contrast in the highly susceptible variety, the glutamic acid is low in infected plant (37.1 moles/ml) and high (131.2 nmoles / ml) in healthy plant. So in the infected plant there is no proper control of transpiration by controlling the function of stomata. So this variety is less survivable after infection.

Although there are, several beneficial roles played by several amino acids, there are some amino acids which are toxic to plants plants depends upon the susceptibility and resistivity. Serine, threonine, and valine are definitely more toxic to tobacco varieties which are susceptible to root rot disease. In general, toxicity to the host cells of free amino acids showed a positive correlation with susceptibility to black root rot. Presence of free amino acids in fungous exudates has been concluded to be a factor in overcoming resistance of the plant to disease and facilitating invasion of host tissues[15].

Aspartic acid and glutamic acid are acidic amino acids; arginine, histidine and lysine are basic amino acids. So the difference in amount of different acidic and basic amino acids may be important to maintain the pH of the cellular environment. In the present study, it has been observed that the in resistant varieties which grow in soils with pH in the narrow range 7.5-8.5 show the presence of less amount of acidic and basic amino acids. In contrast, the susceptible varieties (healthy/infected) growing in acidic soils (pH 6-7.98) show great variation in the quantity of both acidic and basic amino acids. The infected plants of Monthan variety growing in acidic soil (pH 6) contains more amount of the basic amino acid histidine, medium amount of acidic amino acid aspartic acid and glutamic acid and low amount of basic amino acids arginine and lysine. Thus the medium amount of aspartic acid and glutamic acid is balanced by high amount of histidine and low amount of arginine and lysine.

Based on the present study along with previous studies, it is concluded that free amino acids in plants play important role in biotic and abiotic stresses, including disease resistance, through several ways. It is true by the change in proportion of several amino acids during pathogenesis. The present HPLC study on amino acids, based on the samples collected from the agricultural fields show the different response of different varieties in relation to *Fusarium* wilt disease and amino acid composition of the plant. By understanding the nutritional aspects during the interaction between the pathogen and the host more knowledge about the favourable and unfavourable biochemical environment of the cell may be acquired and by altering the cellular environment in the host from favourable to unfavourable condition to the pathogen, the entry and growth of the pathogen may be arrested

SUMMARY AND CONCLUSION

Panama disease in banana, caused by the fungal pathogen *Fusarium oxysporum,* is a major problem of banana cultivation throughout the world. Several precautionary measures, soil management practices, cultivation of resistant varieties etc are followed to overcome the Panama disease in banana. But still now, as in other diseases of crop plants, the disease is controlled to certain extent only. It is mainly due to the lack of basic knowledge about the disease in relation to pathogen-host interaction, on which only any precautionary or control measures can be adopted. The pathogen destroys the host and the host resists the growth of the pathogen by using numerous existing or synthesized chemicals. Among these, amino acids of the host are important to resist the pathogen and to recover from the damage caused by the pathogen. Studies on the role of amino acids in host-pathogen interaction are available for only few crops. By comparing the amino acid profile of the host, the nature of susceptibility, resistance and the level of severity of disease etc can be understood thoroughly. The present study on the amino acid composition in healthy / diseased plants of

susceptible varieties along with resistant varieties of banana in relation to *Fusarium*-wilt disease shows the presence of several amino acids in more quantity after infection. The inter-varietal difference in amino acid composition is more in the case of susceptible varieties when compared to the resistant varieties. It clearly indicates that the resistant varieties have various level of inbuilt mechanism of resistance in contrast to susceptible varieties which have the induced resistance mechanism at various levels in different varieties. Thus maximum number of amino acids in maximum quantity has been recorded in the least susceptible variety Monthan in contrast to the highly susceptible variety Rasakathali, in which out of fifteen amino acids, twelve amino acids have been found to be decreased and only three amino acids have been found to be increased in quantity in infected plants. Among the fifteen samples tested, the infected plant of Rasakathali has maximum amount of the amino acid tyrosine. Among the susceptible varieties, maximum level quantitative variation has been observed in one basic amino acid histidine and one acidic amino acid glutamic acid. Major difference in quantity between highly resistant (Kathali) and least resistant (Nendran) varieties is seen in the amino acid alanine. The major difference between the healthy and infected plants of the highly susceptible variety Rasakathali is seen in arginine, histidine, glutamic acid and serine.

In cotton, eight amino acids, isoleucine, valine, proline, phenylalanine, lysine, histidine, tyrosine, and cysteine are accounted for 100% variation in resistance for *Fusarium*-wilt [16]. In the present study, based on the quantitative difference between the healthy and infected plants of susceptible variety in comparison with the resistant varieties, it has been concluded that eight amino acids (aspartic acid, phenylalanine, threonine, methionie, arginine, histidine, glutamic acid and serine) play an important role in the resistance in banana against *Fusarium*-wilt disease.

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