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High Performance Liquid Chromatography for the Estimation and Detection of Amino Acids in Root Rot Disease in Sesame

[1]*B. Gokulakumar and* [2]*R. Narayanaswamy*

[*]Department of Physics, Annamalai University, Annamalainagar - 608002, India
[2]Deparment of Engineering Physics, Annamalai University, Annamalainagar - 608002, India

Abstract: The quantity of individual amino acids present in root rot disease roots of sesame are found by high performance liquid chromatography. It is a well developed technique for the separation of amino acids in biological samples. This study has been undertaken to analyse and quantify all the amino acids which are present at different levels in the samples. Certain amino acids show remarkable variations while other show minimum variation. A possible correlation between the variation in the values of the amino acids found in different samples and the diseased conditions of sesame has been attempted. The possible impact of increased levels of free amino acids and resistance to disease are discussed.

Key word: Root rot · Amino acids and Sesame

INTRODUCTION

Sesame (sesamum indicum) is recognized as one of the oldest crops in the world. Archeological records indicate that it has been used in India for more than 5000 years [1,2]. Although it has been cultivated for a long time, the significant increase in productivity is yet to be achieved. It is well recognized that diseases constitute a major constraint in increasing the yield level of sesame crop. Root rot caused by macrophomina phaseolina (Tassi.) Goid. is the most devastating disease limiting the production in almost all the sesame growing areas of Tamilnadu, India [3]. About 5-100 per cent loss in grain yield has been reported by this disease [4]. The disease causing fungi is present in soil causing infection resulting in mortality of the plant. The common symptom of the disease is sudden wilting of the plants throughout its crop period, but mainly after flowering phase. As the fungus survives in soil, effective control of the disease by chemical means is not possible [5]. Under these circumstances, selection of resistant varieties would pave the way in overcoming problem. Several researchers have attempted to screen the possible input of increased levels of free amino acids on resistance to disease or diseased conditions, although some free amino acids are probably related to the observed protection against the disease [6]. In the present study it is aimed to quantify the free amino acids in root rot disease roots of sesame using high performance liquid chromatography (HPLC). HPLC is a well-developed technique for the separation of amino acids in biological samples [7]. The requirement for amino acids in essential quantities is well known as a means to increase yield and over all quality of crops. The application of amino acids for folier use is based on its requirement by plants in general and at critical stages of growth in particular.

Amino acids are estimated by various chromatographic methods, like paper, thin layer, liquid and gas chromatography. But good separation as well as estimation can be achieved only through High Performance Liquid Chromatography (HPLC). HPLC is the most common method for extracting amino acids [8,9]. Organic matter inputs, from plant residues and compost organic wastes, have the potential to significantly reduce the severity of root diseases caused by plant pathogens in natural systems [10]. During the past two decades, major advances have been made in the understanding and predictive commercial use of organic manure mediated suppression of root rot disease [11]. Our aim is therefore to determine whether the competitive ability of plant roots to capture specific free amino acids from organic treated soil so that they can control the disease incidence.

MATERIALS AND METHODS

Field Cultivation: Five different sesame varities namely co-1, vri-1, vri-2, tmv-3 and tmv-4 are obtained from Tamilnadu Regional Research Station, Virithachalam,

Corresponding Author: B. Gokulakumar, Department of Physics, Annamalai University, Annamalainagar - 608002, India

India. All the varities are grown in kharif season in sandy loam soil. In the fields three different manure treatments have been given, viz., as T_1 (control), T_2 (chemical fertilizer) and T_3 (organic manure with neem cake).

Sample Collection: All the diseased roots are collected from the three different manure treated fields at the flowering stage.

Sample Preparation: The oven dried roots are ground well into a fine powder by agate mortar. The powdered sample is weighed (100 mg) and dissolved in boiled and filtered distilled water and kept overnight at room temp, for extraction of free amino acids. The volume of the extraction is kept as 1ml. This is centrifuged and the obtained superarent is filtered and stored and used for free amino acid analysis.

RESULT AND DISCUSSIONS

Increased amounts of some amino acids (glutamine and aspargine) have also been reported in several plants species following infection by fungi and bacteria [12] and circumstantial evidence has been reported linking susceptibility or resistance of the plants to free amino acid levels [13]. HPLC has established its prime position among all chromatographic techniques in the past decade. An added advantage is that many detectors used in HPLC are non-destructive, thus facilitating sample recovery and provide the opportunity for subsequent specific analytical studies [14,15]. Individual amino acids are separated and quantified using an HPLC system as described previously [16] In particular, the direct root uptake of free amino acids from soil may be a mechanism of capturing amino acids released from soil organic matter, effectively short circuiting the reliance of the microbial community to create inorganic N [17].

The quantity of free amino acids present in both root rot and resistant roots are found by injecting the extract into HPLC and the various peak area is integrated. Amino acids play a vital role in the growth procedure of sesame crops. Fifteen amino acids viz., aspartic acid, Glumatic, Serine, Histidine, Glysine, Theronine, Alanine, Arginine, Tyrosine, Valine, Nutinonine, Phenylanine, Isoleucine, Leucine and Lysine are found at different levels in both root rot disease and resistant roots by HPLC. Hanne volpin et al have found from HPLC chromatograms of amino acid extracts from inoculated roots, a consistant increase in certain amino acids during infection of the roots by fungi compared with control roots [18]. In the fifteen free amino acids Aspartic acid, Glumatic acid, Histidine, Glysine, Alanine, tyrosine, Valine, Methionine, Isoleucine and Lysine are at higher levels in diseased roots compared to resistant roots and also those amino acids are found to be higher in T_1 and T_2 treatment fields compared to T_3 treatment. The results presented in Table 1 to Table 5.

Table 1: Free amino acid concentration in nm/ml of root rot disease and resistant roots (Co-1)

	T_1		T_2		T_3	
Amino acide	Disease	Resistant	Disease	Resistant	Disease	Resistant
Aspartic acide	13.55±0.31	7.45±0.21	10.24±0.18	6.82±0.20	8.61±0.18	6.80±0.19
Glutamic acide	12.25±0.24	7.26±0.20	9.89±0.14	7.08±0.19	9.1±0.17	7.06±0.14
Serine	2.2±0.18	5.11±0.20	2.52±0.20	5.77±0.14	4.14±0.18	6.34±0.15
Histidine	10.77±0.20	3.64±0.20	7.81±0.18	4.81±0.18	6.27±0.15	4.69±0.14
Glycine	6.20±0.19	1.35±0.18	4.23±0.20	1.24±0.14	2.37±0.17	1.20±19
Threonine	1.18±0.16	2.28±0.15	1.59±0.11	2.25±0.22	1.63±0.17	2.58±0.20
Alanine	12.53±0.19	7.45±0.15	10.60±0.15	7.23±0.20	9.28±0.12	6.62±0.19
Arginine	0.34±0.2	3.62±0.20	0.44±0.34	3.79±0.18	0.47±0.44	4.59±0.17
Tyrosine	1.20±0.19	0.35±0.19	1.18±0.18	0.26±0.11	0.61±0.21	0.18±0.10
Valine	2.22±0.14	0.39±0.15	1.66±0.18	39±0.15	1.42±0.22	0.31±0.12
Methionine	1.42±0.19	0.64±0.56	1.34±0.14	0.62±0.56	1.17±0.15	0.52±0.10
Phenylalanine	0.11±0.17	1.65±0.12	0.19±0.09	2.08±0.11	0.36±0.18	2.17±0.16
Isleucine	1.78±0.14	0.36±0.19	1.34±6.50	0.35±0.20	1.27±0.15	0.30±0.11
Lecuine	0.12±0.20	1.36±0.21	0.28±0.08	2.42±0.19	0.52±0.21	2.43±0.18
Lysine	12.35±0.15	9.35±0.17	10.33±0.09	8.34±0.16	9.36±0.14	7.36±0.10

Each valur is the mean ± S.E. of four indiviual observations ($P < 0.05$)

Table 2: Free amino acid concentration in nm/ml of root rot disease and resistant roots (VRI-1)

	T_1		T_2		T_3	
Amino acide	Disease	Resistant	Disease	Resistant	Disease	Resistant
Aspartic acid	14.22±0.21	7.36±0.14	11.54±0.32	6.87±0.14	9.60±0.19	6.47±0.17
Glutamic acid	12.43±0.22	7.71±0.20	10.12±0.12	7.13±0.06	8.27±0.10	6.27±0.11
Serine	3.22±0.10	5.33±0.15	4.43±0.15	5.36±0.14	4.78±0.13	6.29±0.14
Histidine	11.46±0.15	5.27±0.12	9.09±0.13	4.35±0.17	7.76±0.13	3.62±0.20
Glycine	6.50±0.21	1.62±0.19	4.51±0.20	1.20±0.10	2.16±0.08	1.08±0.13
Threonine	1.35±0.17	2.64±0.15	1.42±0.19	2.72±0.29	1.72±0.19	3.61±0.25
Alanine	12.25±0.11	7.62±0.16	9.28±0.12	7.35±0.18	9.23±0.13	7.16±0.14
Arginine	0.47±0.10	2.62±0.19	0.61±0.18	3.40±0.20	1.62±0.19	3.58±0.18
Tyrosine	1.14±0.14	0.48±0.12	0.88±0.14	0.32±0.20	0.53±0.20	0.20±0.13
Valine	2.24±0.16	0.43±0.18	1.05±0.15	0.30±0.17	0.61±0.21	0.23±0.17
Methionine	1.81±0.14	0.10±0.10	0.73±0.08	0.09±0.09	0.34±0.16	0.04±0.04
Phenylalanine	0.04±0.04	0.87±0.15	0.05±0.04	1.08±0.10	0.08±0.08	1.21±0.19
Isoleucine	2.05±0.13	0.63±0.18	1.81±0.16	0.23±0.18	1.36±0.14	0.09±0.09
Leucine	0.86±0.1	1.34±0.15	0.09±0.08	2.21±0.20	0.16±0.12	2.30±0.15
lysine	12.24±0.13	9.29±0.13	10.2±0.15	8.24±0.13	9.52±0.19	7.77±0.11

Each value is the mean ± S.E. of four individual observations ($P < 0.05$)

Table 3: Free amino acid concentration in nm/ml of root rot disease and resistant roots (VRI-2)

	T_1		T_2		T_3	
Amino acide	Disease	Resistant	Disease	Resistant	Disease	Resistant
Aspartic acid	14.08±0.13	7.42±0.19	10.71±0.20	7.37±0.13	9.37±0.14	7.16±0.17
Glutamic acid	13.61±0.20	6.72±0.19	9.21±0.14	6.44±0.10	9.74±0.09	6.41±0.20
Serine	3.29±0.20	5.31±0.15	3.35±0.18	5.78±0.15	3.74±0.22	5.80±0.17
Histidine	9.84±0.12	5.27±0.14	9.74±0.18	4.57±0.13	7.6±0.21	3.85±0.12
Glycine	7.33±0.16	1.96±0.09	4.75±0.16	1.66±0.16	3.92±0.10	1.26±0.14
Threonine	1.14±0.14	2.53±0.17	1.53±0.17	2.75±0.64	1.64±0.16	2.88±0.15
Alanine	11.91±0.10	7.09±0.10	10.64±0.16	7.55±0.15	8.63±0.13	7.80±0.14
Arginine	0.32±0.20	3.24±0.17	0.72±0.20	3.35±0.11	1.60±0.12	3.81±0.12
Tyrosine	1.64±0.18	0.44±0.19	0.92±0.08	0.33±0.19	0.56±0.18	0.28±0.11
Valine	2.15±0.12	0.52±0.16	0.89±0.11	0.36±0.15	0.62±0.18	0.35±0.14
Methionine	1.64±0.17	0.08±0.09	0.48±0.21	0.08±0.07	0.21±	0.06±0.07
Phenylalanine	0.15±0.16	1.23±0.18	0.17±0.16	1.24±0.17	0.24±0.18	1.27±0.19
Isoleucine	1.63±0.15	0.50±0.20	1.44±0.14	0.21±0.15	1.19±0.18	0.07±0.10
Leucine	1.26±0.14	1.14±0.18	0.51±0.16	2.26±0.2	0.22±0.18	2.45±0.15
lysine	12.65±0.17	9.13±0.16	10.62±0.16	8.17±0.14	9.64±0.19	7.64±0.18

Each value is the mean ± S.E. of four individual observations ($P < 0.05$)

Table 4: Free amino acid concentration in nm/ml of root rot disease and resistant roots (VRI-3)

	T_1		T_2		T_3	
Amino acide	Disease	Resistant	Disease	Resistant	Disease	Resistant
Aspartic acid	14.25±0.19	7.65±0.16	10.66±0.22	6.70±0.18	9.34±0.18	6.62±0.16
Glutamic acid	13.18±0.11	6.83±0.16	11.29±0.18	6.36±0.16	9.34±0.16	6.35±0.16
Serine	2.21±0.16	4.73±0.19	3.81±0.15	5.55±0.13	4.15±0.08	5.83±0.141
Histidine	11.26±0.20	5.62±0.09	9.84±0.19	4.47±0.14	7.42±0.11	3.9±0.05
Glycine	8.17±0.10	1.85±0.09	5.90±0.06	1.64±0.13	4.83±0.08	1.36±0.09
Threonine	1.35±0.17	2.24±0.14	1.56±0.12	3.44±0.14	1.71±0.125	3.56±0.13

Table 4: Continued

Alanine	12.3±0.36	7.66±0.13	10.62±0.11	7.27±0.09	9.25±0.14	6.55±0.13
Arginine	0.23±0.12	3.43±0.10	0.35±0.12	3.54±0.09	0.78±0.11	3.85±0.08
Tyrosine	1.84±0.13	0.53±0.13	1.05±0.03	0.32±0.08	0.88±0.10	0.27±0.10
Valine	3.83±0.28	0.42±0.12	1.54±0.13	0.36±0.11	1.44±0.13	0.35±0.14
Methionine	1.11±0.07	ND	0.32±0.12	0.12±0.04	0.24±0.11	0.05±0.02
Phenylalanine	0.19±0.05	1.24±0.10	0.28±0.10	1.27±0.10	0.48±0.11	1.35±0.14
Isoleucine	1.62±0.105	0.62±0.16	1.30±0.16	0.15±0.04	1.14±0.12	ND
Leucine	0.37±0.09	2.07±0.07	0.43±0.10	2.32±0.08	0.84±0.14	2.36±0.14
Lysine	12.38±0.13	9.12±0.11	10.41±0.08	8.05±0.03	9.64±0.09	7.65±0.14

Each value is the mean ± S.E. of four individual observations ($P < 0.05$)

Table 5: Free amino acid concentration in nm/ml of root rot disease and resistant roots (VRI-3)

	T_1		T_2		T_3	
Amino acide	Disease	Resistant	Disease	Resistant	Disease	Resistant
Aspartic acid	14.24±0.15	7.26±0.14	10.83±0.10	7.21±0.10	9.65±2.79	6.56±0.14
Glutamic acid	12.46±0.14	7.72±0.12	11.12±0.11	7.45±0.13	9.72±0.10	7.24±0.14
Serine	3.35±0.14	5.12±0.07	4.29±0.19	5.41±0.12	4.37±1.58	6.22±0.10
Histidine	11.43±0.12	5.63±0.15	9.64±0.14	4.77±0.08	7.65±0.13	4.21±0.12
Glycine	6.54±0.11	1.82±0.07	3.62±0.08	1.45±0.13	2.05±0.03	1.25±0.13
Threonine	1.42±0.11	2.35±0.16	1.43±0.13	2.56±0.09	1.65±0.08	2.75±0.07
Alanine	12.21±0.09	7.65±0.13	10.26±0.13	7.55±0.7	8.46±0.14	7.21±0.12
Arginine	1.32±0.10	2.64±0.10	1.60±0.18	3.07±0.02	1.64±0.14	3.27±0.07
Tyrosine	2.05±0.04	0.46±0.16	0.83±0.10	0.32±0.09	0.54±0.15	0.11±0.09
Valine	2.2±0.11	0.55±0.06	1.83±0.15	0.35±0.11	0.75±0.13	0.31±0.09
Methionine	1.46±0.09	0.09±0.12	0.54±0.12	ND	0.24±0.06	0.11±0.07
Phenylalanine	0.06±0.03	0.86±0.09	0.09±0.05	1.28±0.10	0.10±0.07	1.75±0.10
Isoleucine	1.74±0.02	ND	1.55±0.13	0.65±0.14	1.25±0.13	ND
Leucine	0.10±0.07	2.26±0.12	0.29±0.10	2.26±0.09	0.86±0.06	2.34±0.09
lysine	12.21±0.09	9.24±0.07	10.10±0.04	8.17±0.08	9.86±0.11	7.35±0.09

Each value is the mean ± S.E. of four individual observations ($P < 0.05$)

Aspartic acid is found to vary from 14.25 to 8.61 nm/ml in diseased roots and 7.45 to 6.6.56 nm/ml in healthy roots in all treatments although, aspartic acid is not an essential amino acid in all plants [19]. Despite this, it is difficult to form clear causes regarding the significance of amino acid increase with respect to the disease. The effect of fungi exposure and the increased free amino acid levels depends on plant species and the disease organism. Salt stress did enhance the total amino acid concentration in nodulas and roots [20,21]. The amount of Glutamic acid varies from 13.61 to 8.27 nm/ml in root rot disease roots and 7.61 to 6.41nm/ml in healthy roots. Histidine varies from 11.46 to 6.27 nm/ml and 5.63 to 3.64 nm/ml in root rot disease and resistant roots respectively. Similarly Alanine varies from12.53 to 9.23nm/ml in disease roots and 6.62 to 7.64 nm/ml in resistant roots .The Lysine varies from 12.65 to 9.34 nm/ml in disease roots and 8.63 to 7.36 nm/ml in resistant roots. Remaining Glycine, Tyrosine, Valine, Methionine and Isoleucine are slightly high in disease roots compare to resistant roots. Total free amino acid of exudation from resistant roots in the present work is low compared to the disease roots [22]. The fact that only few of the amino acids serine, glutamic acid, aspartic acid and alanine are found in the exudates supports the observation by TA. Et al Ta *et al*. [23]. Glutamic acid, Glycine and Aspartic are the main amino acids translocated from roots [25].

The most abundant amino acids are Glutamine, Asparine, Glumatic acid, Alaine and Tyrosine. SOME amino acids rather rare in plants and its role in plant metabolism is not quite clear [25]. However the high level of glumatic acid again demonstrates the important role of this amino acid in plant metabolism [26]. Serine and thereonine are definitely high in resistant compare to disease roots. In general, toxicity to the host cells of free amino acids showed a positive correlation with susceptibility to root rot, presence of free amino acids in

fungus exudates has been concluded to be a factor in overcoming resistance of the plant disease and facilitating invasion of host tissues [27]. The significance of amino acid exudation by plants is still unclear. It has been shown that they have a limited role in nutrient mobilization [28]. In resistant roots differential responses between T_1, T_2 and T_3 treatments tend there fore to be maximized with such amino acids Serine, Threonine, Arginine, Phenylanlanine and Leucine because of low toxicities [27]. One of the amino acid that might be expected to increase following resistance induction is Phenoline, which has been closely linked to phenol biosynthesis, Phytoalexin accumulation and signification. This is also found by tomato disease and resistant roots by Alvin [6]. The objective of this study is to identify the organic manure treatment (T_3) that could be used for disease control.

CONCLUSION

All the observations strongly indicate certain specific amino acids like, aspartic acid, glumatic acid, histidine, glysine, alanine, tyrosine, valine, nutinonine, isoleucine and lysine are providing the conducive atmosphere for the disease to multiply vigorously. If by some mechanism the amount of the above needed amino acids are decreased or eliminated, then the chance of root rot infection may reduce considerably. Serine, Threonine, Arginnine, Phenylalanine and Leucine are found to be higher in resistant roots and lower in disease roots. This indicates that by using the above amino acids the severety of the disease will be very much reduced. Further work in this direction has to be taken up in order to infer more about root rot infection from other parameters like different soil conditions, different seasons, as well as physical parameters of irrigated water and soil.

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