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Innovation The Research Concept

Changes in Total Amino Acids in Normal and Galled Stem of Coriandrum Sativum L. Caused by Protomyces Macrosporus in Vivo and in Vitro

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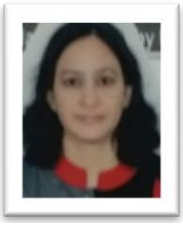

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Abstract

Coraindrum sativum L. (Dhania) belonging to the family umbelliferae (Apiaceae) is an important condiment and occupies a significant place among the non food crops of India. It is grown in fields for green leaves and dry fruits. Unfortunately,this cash crop of great economic and medicinal value suffers severely from stem gall disease caused by the fungus Protomyces macrosporus. When plant tissue is infected by a pathogen, deranged metabolic changes are brought about in the infected tissues.

Present study deals with the changes in total amino acid of healthy and diseased tissues of coriander both in vivo and in vitro.Estimation of amino acids was carried out by the method of Lee and Takahashi (1966). Galled tissues of coriander showed high amino acid contents as compared to their normal counterparts both in vivo and in vitro.

Keywords: Coriandrum sativum, Protomyces macrosporus, in vivo, in vitro

Introduction

Plant galls have attracted the attention of naturalist from early times. They are unique examples of complex inter-specific interaction and mutual adaptation between the plants and tumour inducing agents. In recent years, the study of galls has gained considerable significance in investigations on the etiology of animal and human cancers.

Coriander (Coriandrum sativum L.) belonging to family Umbelliferae is an important condiment and occupies a significant place among the non food crops grown in India. Unfortunately this cash crop of great economic and medicinal value suffers from severe stem gall disease caused by the fungus Protomyces macrosporus.

Several aspects like epidemiology, histopathology, biochemistry and control of coriander gall caused by P.macrosporus have been studied by many scientists viz.(Tayal et al.1981 ,Goyal et.al.1983,Sharma and sharma 2004 and Singhania et al. 2006)[1-4] also worked on occurrence of the disease and its effect on seed production. Jain et al.(1994)[5] recorded an increase in total soluble sugars and alpha amylase activity in galled tissues of coriander both in vivo and in vitro. Mishra et al. (2017)[6] worked on biochemical changes in coriander due to stem gall disease. Jain Smita 2018 reported high protein content in galled tissues of Coriandrum sativum both in vivo and in vitro.

Proteins are the principal constituents of plants and animal life. Amino acids are known to play a key role in the metabolism of plants as they are the building blocks of proteins and are involved in several metabolic activities.

Pathogen induces a spurt in cellular activity in the infected tissue .Due to host pathogen interaction metabolism is shifted in favour of the pathogen. Protein and amino acid composition of various plants under pathogenic state has been worked out by a number of workers viz.(Prasad et al.1976 , Tandon 1985, Rao and Sridevi 1987, Tavernieret al.2007, Ahmed et al.2013 ,Jain Smita 2018,Kumar S.et al.2019.)[7-13]

Infection of plants with pathogenic fungi is found to be associated with accumulation of certain nitrogenous material.(Allen 1954)[14]. The protein content increased in the diseased plants at the early stages of

infection viz. cabbage infected by Plasmodiophora brassicae (Bhattacharya and Williams 1971)[15], Soyabean infected by Phytophthora megasperma (Lazarovitz and Ward 1982)[16].

Chaffei et al. (2004)[17] studied the effect of cadmium toxicity in nitrogen management of Lycopersicon esculentum.Dulermo et al. (2009) [18] studied amino acid changes in sunflower infected by necrotrophic fungi . Solomon and Oliver(2001)[19]] noted an increase in nitrogen level of tomato leaf epoplast during infection by Cladosporium fulvum. Gupta and Naquvi (1979)[20]] studied the changes in amino acid contents of coriander plants infected with Protomyces macrosporus. Kumar Sandeep et.al 2019 studied biochemical changes in coriander plants infected with stem gall disease

The increased protein content at the early stages of infection may be attributed to increased catabolic reactions or decrease in proteolytic degradation or both . The pathogen itself may also contribute the proteolytic enzymes. (Kosuge and Gilchrist 1976, Solomon et al.2003 and Berger et al.2007).[21-23]

The present study provides a precise account of the total amino acid contents of healthy and diseased tissues of Coriandrum sativum infected by Protomyces macrosporus in vivo and in vitro conditions.

Aim of Study

To carry out differences in biochemical estimations of normal and galled tissues of Coriandrum sativum caused by Protomyces macrosporus macrosporus both in vivo and in vitro.

Materials and Methods

Extraction of free amino acids

2.0gm(dry weight) of each sample was homogenized with 100ml of 90% ethanol.The homogenized tissue was stirred separately,kept overnight at room temperature, centrifuged at 2500 rpm for 30 min.,and three times with 90% ethanol. The supernatant was taken. The supernatant of each of the sample was shaken vigorously with chloroform(1.3v/v) The upper aqueous layer from each of the sample was removed and concentrated in vacuo(Awapara 1948)[24]]. The dried residue thus obtained was dissolved separately in 10% isopropanol and stored at 4*C. The isopropanol fractions were dried separately and finally dissolved in 50% ethanol for amino acid analysis. Three such replicates of each of the samples were exmined.

Extraction of Protein bound Amino Acids

The left over residual tissues from both normal and gall samples were treated separately with 6N hydrochloric acid(100mg/g tissues) for 24 hours (Block et al.1958, Khanna and Jain1973, Khanna and Nag 1973)[25-27]. The mixtures from each of the samples were centrifuged (2500 rpm for 30 min.) and supernatant removed. Hydrochloric acid in each case was removed by evaporation of the solution to dryness (25*C) in vacuo , each of the residue thus obtained, was redissolved in 50% ethanol(1.0ml) before using it for analysis of bound amino acids. Isopropanol was used as preservative in order to check esterification of amino acids (Block and Bolling1951 [28]]

Estimation of Amino Acids

Estimation of amino acids was carried out by the method of Lee and Takahashi 1966.[29]

Preparation of citrate buffer- 0.2M citrate buffer, pH 5.0 was prepared by dissolving 10.5g of citric acid in 100ml of 1N NaOH and the final volume was made up to 250 ml with glass distilled water. Ninhydrin reagent was prepared by dissolving following constituents, A,B and Cin the ratio of 5:12:2.

A : 1% ninhydrin in 0.2M citrate buffer(pH5.0)

B : Pure Glycerol

C : 0.2M citrate buffer(pH5.0)

Procedure

0.1ml of test extract was taken in a test tube. 5.0ml of ninhydrin reagent was added to it. The mixture was shaken vigorously and it was heated in boiling water bath for 12 minutes. The test tubes were cooled under running tap water to room temperature and absorbance was recorded at 570nm against blank in a spectrophotometer (Carl Zeiss VSU-2P). Blank was prepared by adding 0.1ml (80%) ethanol in place of extract. The amount of amino acid was calculated using standard curve prepared from glycine and results were expressed in terms of mg amino acid per gram tissue fresh weight.

Qualitative and Quantitative Estimation Of Amino Acids

Each of the extracts of various samples of normal and diseased tissue was applied separately (0.01ml), 2-3cm above the edge of whatman No.1 chromatographic paper strip along with 24 standard, chromatographically homogenous amino acids (BDH chemical Ltd. England). The paper strip was developed in an organic solvent mixture achromatography. The developed strips were dried at room temperature and sprayed with 0.25% ninhydrin dissolved in acetone (Toennies and Kolb 1951[30]].The dried strips were kept in an oven at 100*C for 5-10 minutes. The ninhydrin positive spots coinciding with that of their respective reference to standard amino acids were marked and O.D. was recoded on Toshniwal densitometer.

Results and Discussions

High free amino acid contents were observed in gall tissues as compared to normal tissues both in vivo and in vitro conditions. In vivo state among the three stages of gall (young, mature and old) , maximum amount of free amino acid was found in marure gall followed by young galls. In vitro state dual culture showed highest amino acid content followed by gall callus. However, in all the samples studied maximum amount of free amino acids was observed in galled fruits. Similar results were obtained for bound amino acids but quantity of bound amino acid was less as compared to free amino acids. (fig.1and2)

Qualitative and Quantitative Amino Acid Composition

The normal and gall tissues of coriander showed some qualitative similarities and certain differences in their amino acid composition. (table 1and 2) Seventeen free amino acids and eleven

bound amino acids were identified in both the tissues. Gall tissues showed higher amount of amino acids when measured in terms of optical density as compared to normal tissues. Higher amount of aromatic amino acids namely , phenylalanine, tryptophan and tyrosine were found in all the diseased samples. Alanine and glycine was found only in gall tissues whereas it was absent in normal tissues, while leucine was confined only to the normal tissues. Other amino acids like argenine, aspargine, aspartic acid, cysteine, glutamine, glutanic acid, histidine, lysine, valine, proline and serine were in higher amount in gall tissues both in vivo and in vitro conditions as compared to nomal tissues.

Biochemical studies of diseased and healthy tissues in vivo and in vitro state of various stages revealed metabolic modifications of the host plant which led to abnormal growth. The metabolic regulation in the host tissue is disturbed and the degree of derangement caused is usually found to vary with the nature of parasite and the extent of host parasite interaction. Sakhare and Thite (1986)[31]suggested that the fungal infection induces the oxidative and hydrolytic reactions along with hormonal imbalances in the host tissues affecting the normal metabolism.

There are several reports indicating a net increase in total nitrogen content of various plants infected with fungi. (Rangaswamy and Natrajan 1966, Shekhawat and kothari 1971, Garg and Mandhar 1975 and Jain 1978)[32-35]. Increased protein levels were found to be associated with the diseased tissues as compared to normal ones was reported in various cases.(Novak and Galston 1955, Shaw and Colotelo 1961, Shekhawat 1980, Begnami 2010, Asgarpanha 2012 Bhat et al.2014 and Jain Smita 2018)[36-41]]

Arya and Tiwari (1967)[42] and Jain (1978) found increased level of amino acid in S.graminicola infected bajra plants. Findings of the present experimentation were in complete hormony with the earlier work. The presence of aspartic acid, cysteine, glutamic acid, glycine, proline, tryptophan and tyrosine in abnormally high concentrations in diseased plants may be due to altered regulation of amino acid metabolism under the influence o pathogen.

Higher activities of peroxidase, catalase, glucose-6-phosphate dehydrogenase and alpha amylase were resultant of more protein synthesis. In addition ,isozyme formation in the diseased tissues in case of peroxidase supported this view. Stahamann and Demorst (1972)[43] reported appearance of new isoperoxidase on account of de novo protein synthesis in diseased tissues. Shaw(1963)[44] concluded that final outcome of host parasite interaction, particularly in case of obligate parasites, might depend on the ability of host to synthesize new proteins.

In the present study high amino acid contents were observed in the diseased tissues as compared to the normal counterparts, similar observations were made by Shaw and Colotelo(1961), Rohringer and Somoborski(1967)[45] Arya et al.(1981)[46]. Kiraly and Farkas (1959)[47] reported higher proteolytic activity in the diseased tissues resulting in increased free amino acid levels. Similarly Jain(1978) and Shekhawat(1980) also reported higher levels of protein and bound amino acids in the diseased tissues of bajra

Findings in several studies demonstrated that higher plants possess the Shikimic acid pathway for synthesis of aromatic amino acids Farkas and Kiraly (1962)[48], Hoover et al.(1977)[49]. In general the diseased tissues shoe a higher level of aromatic amino acids i.e. phenylalanine, tryptophan and tyrosine which are precursors for synthesis of auxins and phenolics.

The second explanation may be the synthesis of amino acids along with protein during pathogenesis. The higher level of auxin of diseased tissue possibly enhances the amino acid and protein synthesis. Role of auxin in protein synthesis is a known fact (Galston and Davies, 1969)[50]. Similar results were reported in rust infected wheat leaves where increased protein and amino acid synthesis were reported Pegg and Sequeira(1968)[51].

Conclusion

Total amino acid contents were found to be more in galled tissues as compared to normal both in vivo and in vitro conditions**.** High free amino acid contents were observed in gall tissues as compared to normal tissues both in vivo and in vitro conditions. In vivo state among the three stages of gall (young, mature and old) , maximum amount of free amino acid was found in marure gall followed by young galls. In vitro state dual culture showed highest amino acid content followed by gall callus . However , in all the samples studied maximum amount of free amino acids was observed in galled fruits. Similar results were obtained for bound amino acids but quantity of bound amino acid was less as compared to free amino acids.

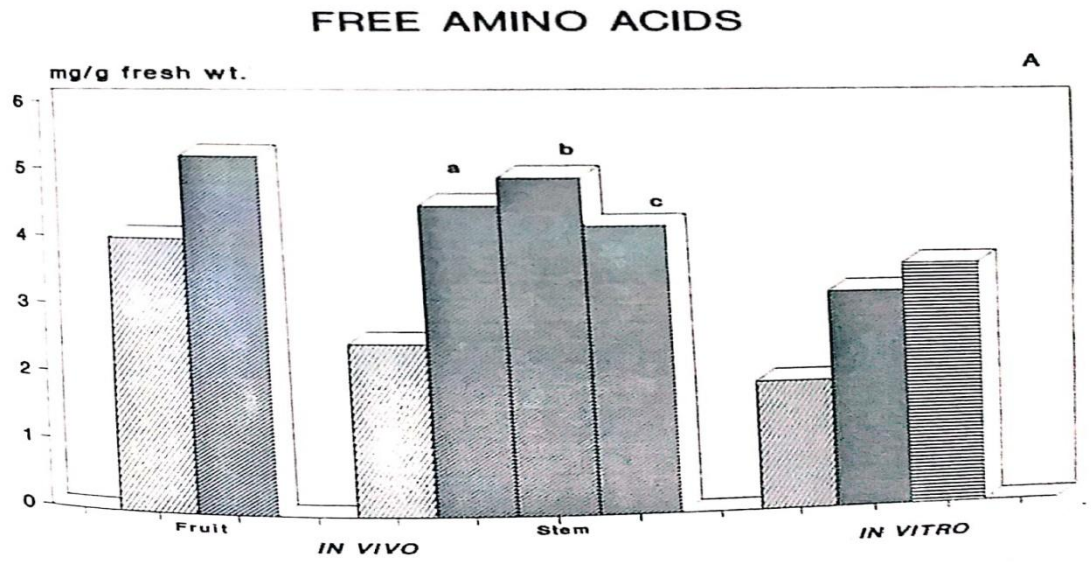

FIG 1 : Free amino acid contents of normal and gall tissues of Coriandrum sativum in vivo and in vitro.

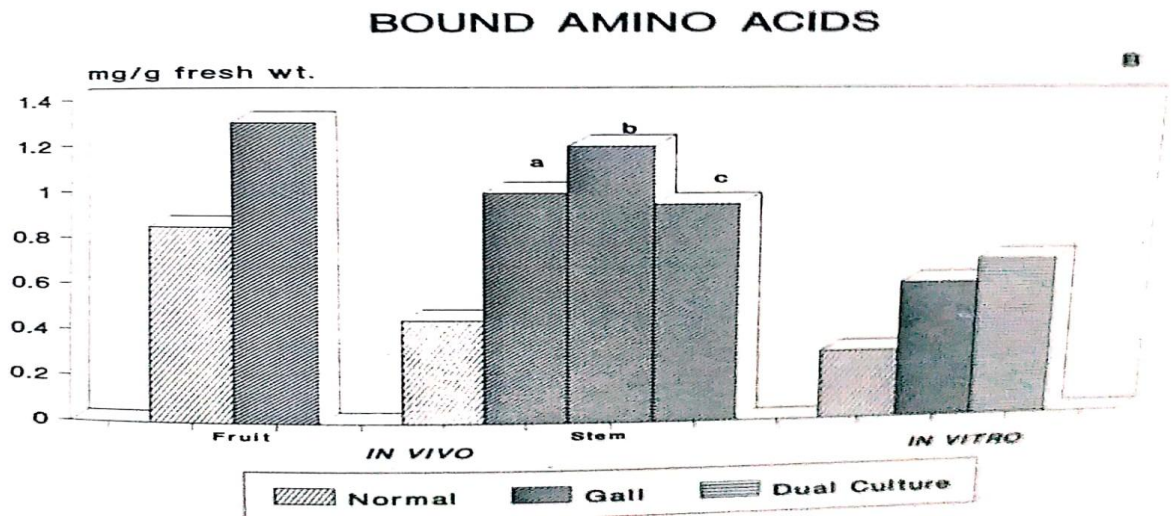

FIG 2 : Bound amino acid contents of normal and gall tissues of Coriandrum sativum in vivo and in vitro.

a = Young Stem gall
b = Mature Stem gall
c = Old Stem gall

Table 1 and 2

Table 6 : **Free amino acid composition of normal and gall tissues of *Coriandrum sativum* L.**

S.No.	Amino acid	*IN VIVO* (O.D.)					*IN VITRO* (O.D.)		
		Standard Rfx100	Normal stem	Galled stem	Normal fruit	Galled fruit	Normal callus	Gall callus	Dual Culture
1.	Alanine	42	0	.202	0	.180	0	.210	.200
2.	Arginine	16	.170	.195	.160	.190	.140	.195	.193
3.	Aspargine	28	.085	.096	.070	.098	.062	.078	.092
4.	Aspartic acid	17	.090	.110	.100	.120	.110	.130	.135
5.	Cysteine	7	.125	.145	.130	.170	.110	.120	.125
6.	Glutamine	26	.030	.035	.040	.048	.023	.030	.045
7.	Glutamic acid	40	.215	.235	.201	.230	.205	.235	.240
8.	Glycine	19	0	.160	0	.175	0	.150	.165
9.	Histidine	13	.085	.095	.070	.095	.060	.095	.123
10.	Leucine	90	.094	0	.090	0	.080	0	0
11.	Lysine	6	.102	.111	.105	.110	.100	.115	.116
12.	Valine	75	.092	.115	.105	.125	.115	.135	.145
13.	Phenylalanine	92	.160	.200	.170	.230	.110	.145	.180
14.	Proline	44	.130	.142	.125	.146	.116	.129	.135
15.	Serine	38	.032	.064	.040	.070	.020	.055	.062
16.	Tryptophan	64	.140	.180	.160	.220	.120	.135	.160
17.	Tyrosine	62	.135	.160	.140	.180	.105	.130	.150

Table 7. **Bound amino acid composition of normal and gall tissues of *Coriandrum sativum* L.**

S.No.	Amino acid	*IN VIVO* (O.D.)					*IN VITRO* (O.D.)		
		Standard Rfx100	Normal stem	Galled stem	Normal fruit	Galled fruit	Normal Callus	Gall callus	Dual Culture
1.	Alanine	42	0	.090	0	.095	0	.091	.092
2.	Arginine	16	.071	.075	.065	.078	.052	.068	.073
3.	Aspartic acid	17	.030	.036	.032	.042	.025	.032	.033
4.	Cysteine	7	.025	.030	.028	.032	.021	.028	.030
5.	Glutamic acid	40	.081	.093	.080	.092	.070	.080	.089
6.	Glycine	19	0	.060	0	.062	0	.052	.058
7.	Lysine	6	.042	.048	.035	.050	.032	.040	.045
8.	Methionine	55	.021	.024	.023	.024	.018	.022	.028
9.	Phenylalanine	92	.064	.070	.062	.072	.065	.072	.075
10.	Proline	44	.034	.036	.028	.038	.025	.033	.040
11.	Threonine	30	.018	.025	.019	.028	.017	.022	.024

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