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EFFECT OF LEAF RUST INFECTION ON AMINO ACID AND AMIDE CONTENT OF WHEAT (*TRITICUM AESTIVUM* L.) Var. Pak-70

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Studies were made on amino acid and amide content of wheat (*Triticum aestivum* L.) Var. Pak-70 infected with leaf rust fungus (*Puccinia recondita*). Healthy plants were taken as control for comparative studies. 19 ninhydrin positive compounds were detected from the leaf and 15 from the stem. In the leaves glutamate, glutamine, histidine, arginine and tryptophan were present only in diseased samples whereas cystine and tyrosine were present only in healthy controls. Glycine, leucine, alanine, phenylalanine asparatate and asparagine were found both in healthy and diseased samples. In the case of the stem alanine appeared only in diseased samples whereas lysine was present only in healthy samples. Aspartic acid, arginine, glycine, phenylalanine, serine and leucine were found both in healthy and diseased stem samples.

INTRODUCTION

Several investigators [17, 5, 11, 13, 14, 10, 15, 1, 9, 18, and 4] have studied changes in amino acid content and amides but their observations are contradictory to each other in most of the cases. For instance Patel and Walker [10] reported an increase in methionine content of bean leaves and finger millet infected by pathogenic fungi, whereas Ashok and Singh [2] showed a decrease in methionine content of sorghum leaves infected by *Colletotrichum graminicolum*. Many workers showed an accumulation of certain amino acids, i.e. alanine, aspartic acid, glutamic acid and their amides asparagine and glutamine in infected plants have been demonstrated by many workers [3, 12, 15].

The present studies were carried out to determine the changes in amino acid and amide contents of one of the major cereal crop of this region, i.e. wheat infected by leaf rust fungi and are a part of the research work being conducted on the physiology of diseased plants with special reference to *Puccinia: Triticum* host parasite system.

MATERIAL AND METHODS

Healthy seeds of wheat (*Triticum aestivum* L.) var. Pak-70 were grown under normal field conditions after surface sterilization with 0.1% mercuric chloride. The seedling were allowed to grow for one month at a temperature ranging between 11 – 24° and average humidity of 37%. The seedlings were then inoculated with the uredospores of *Puccinia recondita* Rob. ex. Desm. (approximately 250 spores per drop) by hypodermic and foliar inoculations. The inoculum was about a year old and showed 80% viability when tested in moist chamber at 20°. Control plants were grown at a distance from the infected plants to minimize dissemination of spores from the infected plants. Healthy and diseased stem and leaf samples were analysed weekly for changes in amino acid content and amides.

Amino acids were determined qualitatively by paper chromatography. They were extracted in 80% ethanol and evaporated to a final volume of 0.5 ml. The samples were loaded as a single spot for chromatography on Whatman No. 3 chromatographic paper and developed in *n*-butanol: acetic acid: water (4:1:5 v/v). Amino acids were than located by spraying the chromatogram with 0.2% ninhydrin solution in acetone.

RESULTS

The results obtained are illustrated in Table 1 and Table 2. 19 ninhydrine positive compounds were detected in healthy and diseased leaf samples (Table 1) an 15 in healthy and diseased stem samples (Table 2). Among these, 18 in the case of the leaf and 14 in the case of the stem were identified as amino acids and amides. In leaf samples neutral amino acid like glycine, leucine, alanine, phenylalanine and acidic amino acids and its amide i.e. asparatic acid and asparagine were present both in healthy and diseased samples. Methionine was present in appreciable amount in healthy leaves but its amount decreased in diseased leaves.

Cystine and tyrosine were absent in the case of diseased leaves but were observed in healthy samples. A characteristic feature was the presence of both the acidic amino acids i.e. aspartic acid and glutamic acid and their amides asparagine and glutamine in the diseased leaves of wheat, although aspartic acid was also present in healthy leaves, yet the presence of glutamic acid just before earing seems signficant (Table 1). Besides the acidic amino acids and their amides, arginine, histidine and tryptophan were also present in diseased samples. These compound were absent in healthy samples.

In the case of the stem (Table 2) aspartic acid, arginine, glycine, phenylalanine, serine and leucine were present both in healthy and diseased samples but the amount of glycine and arginine was much higher in diseased samples than in the healthy ones (Table 2). Lysine was absent in

Table 1. Changes in free amino acids and other ninhydrin positive compounds in healthy and rusted leaves of Wheat

Days after inoculation	Plant samples	AMINO ACIDS**																		
		Alanine	Arginine	Asparagine	Aspartic acid	Cystine	Glutamic acid	Glutamine	Glycine	Histidine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Threonine	Tryptophan	Tyrosine	Valine	Unidentified
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0	Healthy								++											
	Diseased																			
7	Healthy				++	+												+		
	Diseased																			
14	Healthy								+++		++									
	Diseased		++						++		+									
21	Healthy												+++							
	Diseased	++																		
28	Healthy	++											++						+	
	Diseased						+++													
35	Healthy	++							+							+				
	Diseased	++		+					++											
42	Healthy			+++	+++				+++		+	++			+++				++	
	Diseased			+++				+++	++	+++				++		++	++		++	
49	Healthy								+++		++	++		++				+++		
	diseased	+++		+++	+++				+++		+++		+++							
56	Healthy										+	++							++	
	Diseased										++									
63	Healthy	+++		+	+++				+++		++		++	+++						
	Diseased										+								+	

* Healthy plants = Plants grown under ordinary field condition.
Diseased plants = Plants inoclated with *P. recondita*

** The number of + signs indicate the relative amount of each amino acids as estimated by visual examination of paper chromatograms.
\+ = Low concentration
++ = Medium concentration
+++ = High concentration.

'Table 2. Changes in free amino acids and other ninhydrin positive compounds in healthy and rusted stem of Wheat

Days after inoculation	Plant samples	AMINO ACIDS														
		Alanine	Arginine	Asparagine	Aspartic acid	Glutamine	Glycine	Leucine	Lysine	Phenylalanine	Proline	Serine	Tryptophan	Tyrosine	Valine	Unidentified
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
28	Healthy			++				+++								
	Diseased	++								++				++		
35	Healthy				+											
	Diseased				+											
42	Healthy															
	Diseased		++				+++									
49	Healthy		+++		+++		+++		++	+++		+++				
	Diseased		++			+++	+++	++				++				
56	Healthy													++		+
	Diseased							++		+++			++			+
63	Healthy		++	+	+		++	+								
	Diseased		++				++	+		++					++	

diseased samples whereas alanine was absent in healthy samples.

DISCUSSION

Very little changes have been observed in amino acid content of wheat plant after infection with rust fungi (*Puccinia recondita*) except for a few amino acids which appeared as a result of infection and were absent in healthy control.

These observations are in agreement with the work of Patel and Walker [10] on bean infected by *Pseudomonas phaseolicola;* Khatri and Chenulu [5] on cowpea infected by mosaic virus and Sekhawat and Kothuri [14] on opium poppy infected by downy midlew.

Activation of enzymes involved in amino acids and amides synthesis in rust affected plants have been observed in certain cases resulting in increased levels of various amino acids and amides [7]. A parallel protein synthesis as well as proteolysis can occur in rust infected plants. The accumulation of alanine might be the result of protein hydrolysis which might enter the tricarboxylic acid cycle either through pyruvate by di-amination or by transamination with α-ketoglutaric acid or oxaloacetic acid resulting in the formation of glutamic acid or aspartic acid, respectively. The operation of tricarboxylic acid in rust infected tissues has been proved by Shaw and Colotelo [15].

In the rusted wheat leaves the methionine content was found to be significantly lower. This observation is in agreement with the observation of Ashok and Singh [2] on sorghum infected with *Colletotrichum graminicolum.* The decrease in the methionine content of diseased leaves may be due to the result of transamination of methionine with *alpha*-ketoglutaric acid. Increased concentration of methionine is known to inhibit the growth of pathogen as well as the toxins and enzyme producing ability of the pathogen [18].

Accumulation of glutamic acid in rusted leaves may be either due to enhanced activity of glutamic dehydrogenase which catalyzes the formation of glutamate from *alpha-*

ketoglutarate or due to the transmination of alanine with *alpha*-ketoglutaric acid. An increased synthesis of glutamic dehydrogenase has been reported by Smith [16] in sunflower cotyledons infected by *Puccinia helianthii*. An early accumulation of glutamic acid and then its disappeance in the later stages with the corresponding appearance of the amide glutamine positively indicates the increased activity of enzyme glutamine synthetase. In most of the higher plants under normal conditions glutamine serves as the nitrogen reserve. The increased activity of glutamine synthetase may thus be correlated with the storage of glutamine to prevent the accumulation of ammonia in toxic levels, as observed in oat plants infected with crown rust [8].

Similarly a decrease in aspartic acid content after an initial increase and corresponding appearance of asparagine suggests an increased activity of asparagine synthetase which converts aspartic acid into asparagine.

Though the increase in certain other amino acids, i.e. histidine, cystine, glycine, lysine and tryptophan was also observed yet it showed no definite pattern. It may be assumed that they are intermediate products of nitrogen metabolism involved in the synthesis of other nitrogenous compounds.

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