

CHANGES IN NITROGENOUS COMPOUNDS IN *NARENGA PORPHYROCOMA* DURING INFECTION WITH *USTILAGO CONSIMILIS*

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The common reed *Narenga Porphyrocoma* is often infected with the fungus *Ustilago consimilis*. During the infection to the formation of hypertrophy a lot of biochemical changes more particularly of nitrogenous compounds takes place in the host plant. The level of amino acids increases from 2.9 mg/g to 4.5 mg/g during initial infection, however, the amount decreases to 2.1 mg/g during severe infection. The highest level of amino acids is recorded in the teleutospores (6 mg/g). The protein content increases from healthy (3.36 mg/g) to severely infected tissues (4.28 mg/g). The highest accumulation of protein is recorded in the teleutospore (9.6 mg/g). There is increase in individual amino acids with the severity of infection.

Keywords : Amino acids; *Narenga porphyrocoma*; Nitrogen; Protein; *Ustilago consimilis*.

Introduction

Narenga porphyrocoma belonging to the family Gramineae and sub-family Pamicoideae and the tribe Andropogoneae, is a tall reed growing wild everywhere in Manipur. It is an important reed used by the people of Manipur for thatching their houses. The plant is often infected by the fungus *Ustilago consimilis* Sydow causing hypertrophy, at the apical portion of the plant. It is recorded that the *U. consimilis* is parasiting on the inflorescence axis of the host plant *N. porphyrocoma*. Complete degeneration of the hypertrophy accompanied with the liberation of the black spores is seen during the severe infection. The hypertrophy is a rich protein food for the people of Manipur. The inner soft mass of the hypertrophy and the teleutospores are eaten as raw or after roasting.

The aim and object of the present work have been to find out the changes that take place in the host plant in respect of the nitrogenous compounds during the infection with the pathogen.

Materials and Methods

The mature hypertrophy, in other words, the fully infected host plant and the teleutospores, were collected for the biochemical analysis. The methods used for the determination of amino acids was the Ninhydrin Method of Moore and Stein¹. In the case of protein analysis the method prescribed by Lowry *et al*². was used. Nitrogen estimation was made following Micro Kjeldahl process of digestion, distillation and titration. The estimation of nitrites and nitrates was conducted following the process adopted by Paech and Treacy³.

Nitrite was determined by reacting the extract with water and mixture of solutions 1 (sulphuric acid + glacial acetic acid) and solution 2 (α - naphthyl amine + H₂O + glacial acetic acid). After resting half an hour, colours were compared with the standard using sodium nitrite in Bausch and Lomb colorimeter.

Result and Discussion

It is evident from the results that due to the infection of host plant *Narenga porphyrocoma* with *Ustilago consimilis* there is fluctuation of the nitrogenous compounds in the infected

tissues. The free amino acid contents were found significantly higher in the infected culm at the initial stage of infection, however, the amount decreased when the infection was severe. In all cases, on the contrary, the teleutospore of the fungus showed the highest amount of amino acids (Table 1).

In all the three samples, viz., healthy tissues, infected tissues and teleutospores, ten ninhydrin positive spots were detected, however, the concentrations of different individual amino acids vary. Interestingly, the proline was detected in the teleutospores. Although no qualitative difference was observed among the individual amino acid spectrum of the infected and healthy culms, but their differences could be observed in

relative concentrations of certain individuals (Table 2). In the infected culm, cysteic acid and glutamic acid were found decreased however, there was increase in the levels of glycine, histidine, tyrosine, valine and arginine. In the teleutospores of the fungus levels of individual amino acids, like, cysteic acid, aspartic acid, glutamic acid, cystine, histidine and valine were found higher when compared with that of the host culms. Among the individual amino acids of teleutospores, histidine retained the highest intensity.

The results show that there is variation in the protein levels before and after the infection (Table 3). At the initial infection stage there is accompanied with the increase of protein concentration (3.364 to 4.882 mg/g), however, when the infection is severe

Table 1. Changes of free amino acids content in *Narenga porphyrocoma* infected with *Ustilago consimilis*.

Samples	Amino acids mg/g fresh wt.	% decrease (-) or increase (+) over healthy
Healthy	2.902	-
Initial Infection	4.485	(+) 54.54
Severe infection	2.111	(-) 27.25
Teleutospores	6.015	(+) 107.27

Table 2. Changes in contents of free amino acids in *Narenga porphyrocoma* during the pathogenesis with *Ustilago consimilis*.

Name of the free amino acids	Free amino acids ug/g fresh wt.		
	Healthy	Infected (IIS)	Teleutospores
Cysteic acid	149.86	93.75	262.50
Aspartic acid	149.86	156.25	262.50
Glutamic acid	524.44	312.50	1162.50
Cysteine	37.46	62.50	75.00
Arginine	74.92	156.25	112.50
Glycine	112.38	625.00	225.00
Histidine	337.14	843.75	1387.00
Unidentified	299.68	187.50	Nil
Valine	37.46	93.75	112.50
Tyrosine	37.46	125.08	37.50
Proline	Nil	Nil	112.50

IIS - Initial infection stage.

Table 3. Changes in Protein content of *Narenga porphyrocoma* infected with *Ustilago consimilis*.

Particulars	Protein mg/g
Healthy	3.364
Infected culm (IIS)	4.882
Infected culm (SIS)	4.279
Teleutospores	9.599

IIS - Initial infection stage; SIS - Severe infection stage.

the protein concentration is lowered (4.279 mg/g). In teleutospore the protein content is highest (9.599 mg/g). There is significantly high nitrogen content in the teleutospore (3.599 mg/g), however, the concentration of nitrite is very low (0.100 mg/g). The nitrate content showed considerably higher (1.185 mg/g) compared to the nitrite. With severity of infection the levels of nitrate and nitrite decreased, however, the levels of these two compounds were found highest in teleutospores (Table 4).

Several reports are available regarding the decrease or depletion of amino acids during the severe infection⁴⁻⁵. Increase of amino acids both in quantity and quality during pathogenesis is also reported by many workers⁶⁻⁷. Presence of high levels of amino acids in the teleutospores and increase in the infected culm (initial infection stage) might be due to the synthesis of these compounds by the fungus, or it is likely that the parasitism

stimulates the amino acid synthesis of the host during the course of pathogenesis. Synthesis of amino acids by the growing fungus would also cause an increase in the amino acid content of the invaded cell.⁸ Amino acid might have accumulated in the infected tissues due to blockage of protein synthesis⁹ or due to enhanced protease activity in the diseased tissues¹⁰. On the other hand protein amino acids are sometimes found to increase simultaneously with the free amino acids¹¹, in which case amino acid must be translocated from other parts of the host to the infected tissues or amino acids as well as protein synthesis might be increased in the infected tissues.

Decrease of free amino acid content in the severe stage of infection might be either due to utilization of the host amino acids by the fungus in the process of sporulation and accumulated in the spores or decreases metabolic activities of the host plant. The changes taking place in the amino spectrum allow us to conclude that the amino acid metabolism of the host tissues significantly changes as a result of parasitism of *Ustilago consimilis*.

Changes in protein content due to infection was reported by earlier workers⁶⁻¹². The present finding is in conformity with the above. Increase in the levels of protein content in the infected culms of *N. porphyrocoma*

Table 4. Changes in Total Nitrogen, Nitrate and Nitrite in the culms of *Narenga porphyrocoma* during pathogenesis with *Ustilago consimilis*.

Samples	Total nitrogen (%)	mg/100g dry wt.	
		Nitrate	Nitrite
Healthy culm	0.599	37.04	7.15
Infected culm (IIS)	0.980	33.34	6.43
Infected culm (SIS)	0.784	29.63	5.05
Teleutospores	3.599	118.54	10.01

IIS - Initial infection stage; SIS - Severe infection stage.

might be due to the increased synthesis of protein by the pathogen or by the host plant in the course of pathogenesis. During the course of investigation it was also observed that the activities of Nitrate and Nitrite reductases were increased during the pathogenesis. The explanation of which may be made from the findings in which there is higher detection of Nitrate than the Nitrite. The increase in the Nitrate and Nitrite reductases in the infected culms might have helped in increasing the levels of protein.

Maximum content of protein in the spores of the fungus could be due to the storage of protein synthesized by the fungus or translocation of the same from the host plant in the course of pathogenesis. The increase metabolic activity in stress may cause the synthesis of additional protein¹³.

The decrease levels of nitrate and nitrite in the infected culms than the healthy ones might be due to the various metabolic activities of the fungus, which stimulate increase in the uptake of nitrate nitrogen from the host plant. The highest levels of nitrate and nitrite in the teleutospores might be due to the accumulation of these substances in the spores during the pathogenesis.

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