'HELMINTHOSPORIOSE' OF RICE IN RELATION TO THE NITROGEN METABOLISM OF THE HOST

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Introduction

Among many metabolic events in a plant, the course and intensity of the nitrogen metabolism of the host have frequently been the most investigated in the problems of the physiology of parasitism by fungi. Reports on the correlation between disease incidence and nitogen content of the host tissue, particularly the soluble fraction, are many (Wingard, 1941; Hashiyoka *et el.*, 1956; Otani, 1953; Samborski *et al.*, 1958).

It has also been demonstrated that the soluble nitrogen fraction is essentially made up of free amino-acids. Hence the 'Helminthosporiose' of rice in relation to the nitrogen metabolism of the host was studied both in the resistant (T 141, Co 20) and susceptible T 1145, B 76) varieties of rice plants and also in the healthy and infected rice plants.

Material and Methods

Leaf samples were collected at different age levels from quadruplicates pots to minimise sampling errors. Fresh material was weighed cut into an inch pieces and exhaustively extracted with 80% ethanol. The extracts were combined and evaporated in an air stream with moderate heat. The residue was taken up in water, centrifuged and used for determination of "Soluble Nitrogen". Dried samples of the alcohol-extracted tissue were used for determination of "Insoluble Nitrogen" (Samborski *et al.*, 1958). Nitrogen determinations were made in duplicate by use of a 'Semi-micro' method as described by Humphries (1956). Rice plants were grown in Knop's culture solution.

For qualitative chromatographic foliar analysis of the free amino-acids, the unidimensional ascending method of Williams and Kirby (1948) was used. For quantitative assay, 0·5 ml. of the extract was spotted and the concentrations of the amino-acids in the test solution were determined by comparing the size and the intensity of the spots with standards, based on the fact that the size and intensity of the amino-acids spots produced after reaction with Ninhydrin is largely a function of the concentration of the amino-acid (Dent, 1948).

Observations

Experiment 1.—Nitrogen status of the host leaves—soluble and insoluble nitrogen in healthy and Helminthosporium oryzae infected leaves of rice varieties at different age levels

Soluble and insoluble nitrogen.—Table I shows the nitrogen levels of healthy and *Helminthosporium oryzae* infected leaves of susceptible

TABLE I

Nitrogen levels of healthy and H. orynae infected rice leaves of different varieties at various age levels*

Variety	Age in days							
	5		10		15		20	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
T 1145								
Healthy ..	0·31	3·6	0·38	3·0	0·38	1·3	0·40	3·3
Infected† ..	0·64	4·1	0·88	5·6	1·05	4·2	0·70	6·3
B 76								
Healthy ..	0·38	5·7	0·56	3·3	0·33	1·3	0·64	5·1
Infected† ..	0·62	3·5	1·18	3·3	1·26	4·8	0·90	3·6
T 141								
Healthy ..	0·20	3·4	0·24	3·8	0·08	3·9	0·14	8·2
Infected† ..	0·47	3·7	0·32	3·4	0·32	3·5	0·20	7·7
Co 20								
Healthy ..	0·37	4·4	0·30	4·1	0·20	1·7	0·12	9·2
Infected† ..	0·61	5·1	0·48	3·7	0·70	3·9	0·14	9·9

* Alcohol and water soluble nitrogen and alcohol-insoluble nitrogen expressed as mg/g fresh weight of leaves.

† Samples collected 24 hrs. after inoculation.

(T 1145, B 76) and resistant (T 141, Co 20) varieties of rice at different age levels. It could be seen from the results that the susceptible varieties contained more soluble nitrogen, especially at the age levels of 15 and 20 days than the resistant plants at the corresponding age. Further, soluble nitrogen appeared to accumulate in the susceptible varieties with increasing age whereas the trend was a decrease in resistant ones. Comparing the healthy leaves with the infected one, it is evident that infection resulted in an increase of soluble nitrogen in all the varieties tested. Insoluble nitrogen was almost at the same level in 5 and 20-day old susceptible plants. But 10 and 15-day old plants registered lower figures.

In resistant varieties 20-day old plants showed considerably higher insoluble nitrogen than 5-day old plants. In general, there seems to be little change in insoluble nitrogen with increasing age in the case of suceptible varieties. On the contrary, in resistant varieties insoluble nitrogen tended to increase with advancing age.

Insoluble nitrogen as a percentage of total nitrogen.—Figure 1 shows the changes in insoluble nitrogen levels in healthy leaves of resistant and susceptible rice varieties at different age levels. The insoluble nitrogen is expressed as a percentage of 'Total' nitrogen, *i.e.*, the sum of the soluble and insoluble fraction. Further it is clear from Fig. 1 that in the resistant plants (T 141, Co 20) insoluble

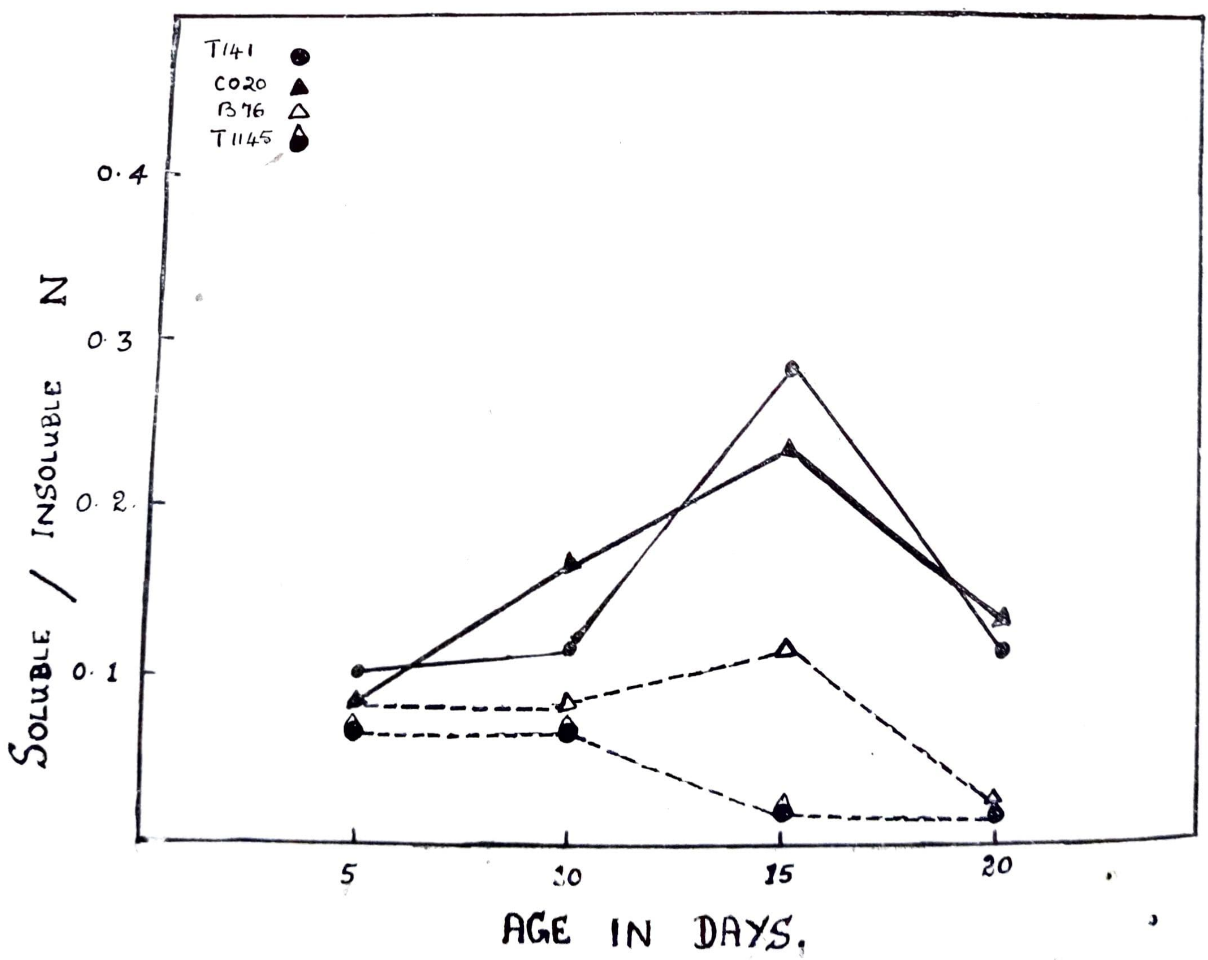

FIG. 1

nitrogen levels were maintained almost steadily with increasing age. In susceptible plants (T 1145, B 76) however a downward drift in the insoluble nitrogen was seen from 5 to 15 days and a rise thereafter. Nevertheless, it might be noted that the percentage of insoluble nitrogen was comparatively lower in the susceptible types than the resistant at all age levels except 5 days. In 5-day old plants these differences were not marked.

Ratio of soluble nitrogen to insoluble nitrogen.—Figure 2 illustrates the accumulation of soluble nitrogen in the resistant and susceptible varieties with reference to age, and presents a contrasting picture to that of insoluble nitrogen. It is evident from curves that proportionately more soluble nitrogen accumulated in the susceptible varieties than the resistant ones.

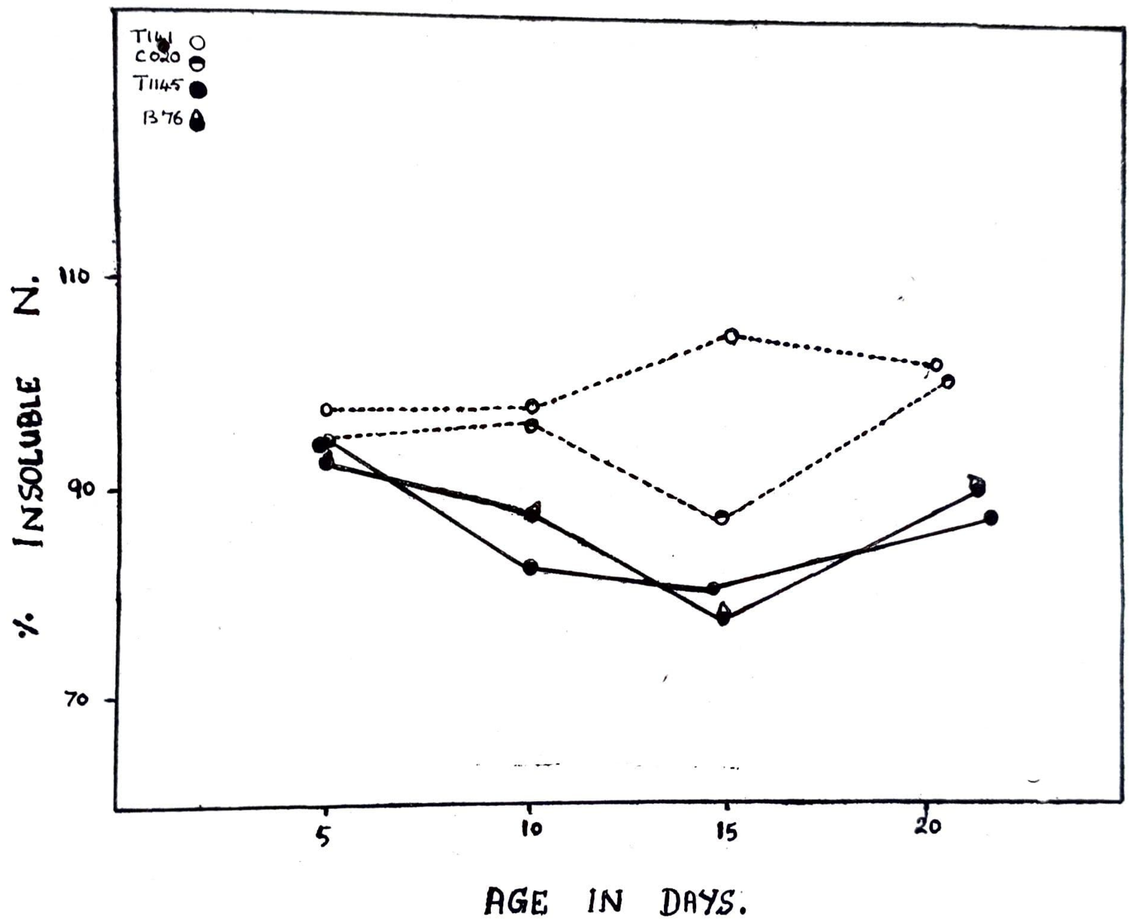

FIG. 2

Experiment 2. Phenylalanine, Tyrosine and glutamic acid contents of leaves of rice varieties resistant and susceptible to H. oryzae at different age levels

Table II clearly indicates that in the susceptible varieties (T 1145, B 76) the concentration of phenylalanine decreased to nearly half the initial amount with increase in age, whereas in the resistant varieties (T 141, Co 20) there was a marked increase in the concentration of phenylalanine at the 20-day age level as compared to 5-day old plants. This increase amounted to nearly 8 times in the T 141 and 3 times in the case of Co 20. An increase in the quantity of glutamic acid was observed in the susceptible varieties at the 20-day age level. Compared to the younger seedlings, and the two resistant varieties had only one-third the quantity of glumatic acid recorded in the susceptible T 1145 and B 76 at the 20-day age level. Significant quantitative changes were not, however, evident with the amino-acid tyrosine.

TABLE II

Amounts of phenylalanine, tyrosine and glutamic acid (mg/g fresh weight) in rice leaves of H. oryzae-resistant and susceptible varieties

Rice variety	Age in days											
	Phenylalanine				Tyrosine				Glutamic acid			
	5	10	15	20	5	10	15	20	5	10	15	20
T 1145 (Sus.) ..	10	2	2	5	5	2	2	5	5	5	3	15
B 76 (Sus.) ..	8	2	3	5	5	2	3	5	2	2	10	15
T 141 (Res.) ..	2	2	1	15	2	1	1	5	5	1	2	5
Co 20 (Res.) ..	5	1	5	15	5	1	2	5	5	1	3	5

Experiment 3. *Effect of externally added L. glutamic acid and DL–β phenylalanine on the incidence of H. oryzae leaf spots in the susceptible variety T* 1145

Since the previous experiment indicated the opposing tendencies with regard to the accumulation of phenylalanine and glutamic acid in the resistant and the susceptible varieties the 10-day old seedlings raised in Knop's solution were fed with various concentrations of glutamic acid and phenylalanine singly and in combination and were inoculated with spore-*cum*-mycelial suspension of *H. oryzae*.

Table III clearly indicates that the mean number of leaf spots per leaf was considerably reduced with 200 μg and above of glutamic acid or phenylalanine added singly or in combination. Plants fed with phenylalanine tended to show a greater reduction in leaf-spot incidence than plants fed with glutamic acid at the concentration of 50 μg each. A mixture of phenylalanine and glutamic acid brought down leaf-spot incidence to a greater degree than the individual amino-acids. The exogenous supply of glutamic acid resulted in the production of bigger infection spots than the control. The increase in area of the spots was directly proportional to the concentration of the glutamic acid supplied. This is evident at the 200 μg concentration level. Thus with 1,000 μg of glutamic acid, the mean area of the infection spots increased to 24·6 sq. mm at the end of 72 hours after inoculation whereas this was only 5936 sq. ft, on the untreated control plants.

TABLE III

Mean number of leaf-spots due to H. oryzae in the susceptible variety T1145 pre-treated to various concentrations of glutamic acid and phenylalanine

Amount in μg (in 30 ml)	1st leaf			2nd leaf		
	Glutamic acid	Phenyl-alanine	Glutamic acid and phenylalanine (1 : 1)	Glutamic acid	Phenyl-alanine	Glutamic acid and phenylalanine (1 : 1)
0	27·4	27·4	27·4	18·8	18·8	18·8
50	17·5	19·5	11·5	17·7	16·2	11·9
200	10·1	8·9	8·8	9·6	8·4	5·5
500	11·5	7·9	6·2	10·3	5·9	6·9
1,000	9·8	6·0	8·8	8·4	6·1	8·4

Table III also indicated the inhibitory effect of externally supplied phenylalanine on the enlargement of the infection spot. The area of the spots on treated plants was considerably smaller at all concentrations of the amino-acid at the end of 72 hours after inoculation. The greatest reduction was noticed with 500 μg of phenylalanine. Addition of equal amounts of amino-acids at (50–200, 500 and 1,000) μg levels increase the rate of the enlargement of the spots during first 48 hour, as compared to the controls. This increase in area was proportional to the concentration of the amino-acids added. Further, it also indicates the inhibitory effect of phenylalanine on the development of the pathogen *in vivo*, since the spots on the untreated control were bigger than those on the treated plants.

Experiment 4. Effect of externally added L glutamic acid on the incidence of H. Oryzae leaf-spot in the resistant variety T. 141

Table IV shows very clearly the beneficial effect of added glutamic acid on increased disease incidence. The number of the spots and the area of the lesion increased with increasing concentration of the amino-acid.

TABLE IV

Percentage increase in lesion area over untreated control (615·6 sq. μ) in T 141 (Res.) with glutamic acid treatment 72 hrs. after inoculation

Glutamic acid (in μg)	Lesion area (in sq. μ)	Percentage increase over control
50	1027·1	66·5
200	1944·6	215·4
500	3568·0	478·6
1,000	9405·0	1425·0

DISCUSSION

Otani (1953) showed an increase in the soluble organic nitrogenous components to be connected with susceptibility of rice plants to the blast disease caused by *Piricularia oryzae* Cav. Indeed very similar results have been obtained in the present work with reference to *Helminthosporium oryzae* infection of rice varieties. The results may be interpreted to mean that the leaf proteins are synthesized at a faster rate in the resistant than in the susceptible. These results are similar to those obtained by Samborski *et al.* (1958) for wheat rust [*Puccinia graminis* (Pers.) of Sp. tritici Erikss and Henn]. From the viewpoint of the host, the stability of the proteins in the resistant varieties would indicate the stability of oxidative enzymes like polyphenol-oxidases which are usually associated with defence mechanism of plants against diseases. It, therefore, seems logical that size of *H. oryzae* lesions on the different rice varieties is the function of the relative availability of soluble nitrogen on the one hand and the relative stability of the protein on the other. This could be the possible reason for the enlargement of the spots on the susceptible varieties (T 1145, B 76) containing higher soluble nitrogen and less stable leaf protein than the resistant Co 20, T 141. It was also observed that the ratio of phenylalanine to glutamic acid was considerably high in the resistant varieties (T 141, Co 20) than in the susceptible variety at the 20 days age level. Further, the rice plants treated with an external supply of L. glutamic acid and DLβ phenylalanine individually or in combination tended to decrease the mean number of leaf-spots in the susceptible variety T 1145 and was also found to be directly proportional to the concentrations of the amino-acids tried. Addition of glutamic acid to the susceptible variety (T 1145) resulted in the rapid expansion of leaf-spots, whereas the addition of phenylalanine to the same variety reduced the area of the leaf-spot considerably. Akai *et al.* (1958) have observed a similar reaction of the leaf-spot of *H. oryzae* on rice plants when fed with L. glutamic acid, whereas in the present study, the deleterious effect of DLβ phenylalanine on *H. oryzae* lesion is a new finding and this confirms the findings of KUC *et al.* (1957) for apple scab with the same amino-acid.

Further, the behaviour of the two amino-acids to *H. oryzae* lesion expansion is one of the building up of an effective concentration of the respective amino-acids in the leaf. Therefore, it seems logical to consider phenylalanine as a potential precursor for synthesis of phenolic substrates as chlorogenic acid. Since it is well known that polyphenolic host factors play an important role in the physiology of the disease (Kirkham, 1959), Schwarze (1958) considered that phenylalanine is converted into chlorogenic acid by *Fagopyrum tataricum* and *Nicotiana tabacum*. Besides, Tamari (1955) has reported chlorogenic acid as the major polyphenolic compound in rice leaves. Hence, it could be inferred that the resistant reaction of T 141 and Co 20 varieties of rice plants to *H. oryzae* is the effect of interplay between the availability of the potential precursor of phenolic substrate on one hand and the relative stability of necessary enzymes on the other, whereas the accumulation of soluble nitrogen, low content of phenylalanine and unstable proteins might explain the reason for rapid enlargement of *H. oryzae* lesions on the susceptible varieties.

In conclusion, the phenotypic expression of resistant/susceptibility of rice varieties to *H. Oryzae* infection could be interpreted in future in terms of "Optimal balance mechanism between polyphenolic and nitrogenous metabolites". A concept that has already been advanced by Keitt *et al.* (1959) in relation to their work on apple scab.

SUMMARY

The reaction of the resistant and susceptible varieties of rice to *H. oryzae* infection was examined in relation to nitrogen metabolism of the host. The resistant varieties were characterised by low soluble nitrogen, high stability of proteins and a high ratio of phenylalanine, whereas the susceptible varieties recorded high soluble nitrogen, low stability of leaf proteins and a high ratio of glutamic acid. Further an exogenous supply of L. glutamic acid and DLβ phenylalanine singly and in combination to rice plants indicated a positive role for the two amino-acids in pathogenesis.

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