PHYSIOLOGY OF PARASITISM

3. Nitrogen mobilization in mung bean seedling infected with *Rhizoctonia solani**

LUNG-CHI WU**

(Received June 13, 1969)

Introduction

In the previous report (Wu, 1967), it was found that the infection of mung bean by *Rhizoctonia solani* caused a rise in amino acid fraction during the early stage of disease development. The ninhydrin positive materials in the infected plants rose to 1.4 times over that in the control plants followed by an appearance of characteristic symptoms one day later (Wu, 1967). An increase in the contents of certain amino acids is often accompanied by a decrease of number of others (van Andel, 1966).

The effect of fungal infection on the free amino acid and amide contents has been studied (Andal and Subba Rao, 1956; Burton and de Zeeuw, 1961; Farkas and Király, 1961; Hrushovetz, 1954; Jones, 1963 McCombs and Winstead, 1961, 1964; Rohringer, 1957; Rohringer *et al.*, 1958; Stretch and Cappellini, 1965). However, there is little light on the exact role played by these amino acids in the establishment of infection. Nevertheless, in some cases, susceptibility or resistance of the host plants seems to correlate with the content of one of a few amino acids (van Andel, 1966). Changes in amino acid content may be due to pathological aberrations in the fate of host proteins.

Proteins, peptides, and amino acids are the most importannt constituents of living matter. Studies of protein metabolism in both healthy and diseased plants indicate that the metabolic relationship between proteins and free amino acids is hardly separated. Protein and enzyme changes in infected plants have been studied with obligate parasites (Andreev and Shaw, 1965; Barret and McLaughlin, 1954; Johnson *et al.*, 1966; Staples and Stahmann, 1964) as well as facultative parasites (Akazawa *et al.*, 1957; Heitefuss *et al.*, 1960;

*The research was partly supported by the National Science Council. The author is indebted to Dr. J.-Y. Lin for his assistance in automatic analyses of amino acids.

**Professor, Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan, China. The author is concurrently a Reasearch Fellow of the Institute of Botany, Academia Sinica.

Kawashima *et al.*, 1964; Shishiyama *et al.*, 1968; Uritani and Stahmann, 1961; Weber and Stahmann, 1964). Stahmann (1963) suggests that the patterns for many proteins are similar to abnormal hemoglobins which have been shown to differ in their amino acids in their polypeptide chains. Perhaps similar changes occur in the proteins and enzymes of infected plant tissues.

To ascertain the effect of Rhizoctonia infection on the nitrogen metabolism in mung bean seedling, the compositions of free amino acids and soluble proteins are studied by means of chromatography and electrophoresis in the present paper. An abstract of part of the work has been published previously (Wu, 1968).

Materials and Methods

An isolate of *Rhizoctonia solani* (ReCS5) and mung bean (*Phaseolus aureus*) of large grain variety were used throughout the experiments. Briefly, mung bean seeds were disinfected, placed on 1% water agar, and allowed to germinate and grow for a given period of time at 25°C in a Freas photosynthesis incubator (Model 806) as previously described (Wu, 1967). Plant inoculation was made by pouring onto seeds a fragmented mycelial suspension of *R. solani* grown on potato-dextrose agar for 2 days at 25°C.

Seeds or seedlings of the same size were harvested at intervals of 24 hours weighed, and dissected either into cotyledon and axial part or into cotyledon, hypocotyl and epicotyl prior to storage in a freezer at −15°C. Essentially, initial seed samples were dissected into embryo and cotyledon with seed coat. In order to obtain an adequate amounts of individual tissues for extraction and analysis, 50 seeds were selected for each initial seed sample of which fresh weight ranged from 4 to 5 gm. The seedlings of different age consisted of 50, 30, 25, or 20 seedlings weighing within the range of 7 to 15 gm of fresh weights for each sample.

Samples of seed or seedling were fractionated in the following manner as already mentioned previously (Wu, 1967). The frozen samples were blended with chilled 80% (v/v) ethanol solution (100 ml) for 1 minute to extract free amino acids. The extract combined with 20 ml washing from a blender jar was placed on a hot plate to boil for 10 minutes, cooled, and centrifuged at 10,000×g for 10 minutes. The supernatant was collected by decantation and the residue was suspended in 20 ml of 80% ethanol and centrifuged. The supernatants were combined and diluted to 100 ml with 80% ethanol. An aliquot portion of the alcohol extract was taken for amino acid analysis and 80 ml of alcohol extract were concentrated for further analyses. The residue after the alcohol extraction were suspended in 100 ml of 0.2% sodium hydroxide, heated for 10 minutes at 90°C with occasional stirring, and centrifuged at 10,000×g for 10 minutes.

The supernatant was saved for the analysis of protein. Free amino acids were determined by the colorimetric method of Rosen (1957) with 3% ninhydrin solution and proteins were determined by the Folin-Ciocalteu method (Lowry *et al.*, 1951) as already described (Wu, 1967).

Alcohol extracts were concentrated with aid of a rotating vacuum evaporator at 45°C. The concentrated extract and washing of the evaporator flask were combined, usually 10 to 15 ml. Then, they were transferred in a hematocrit tube which was sustained in a test tube with side arm to evaporate the extracts less than 5 ml under partial vacuum and heating at 65°C. All the samples were brought to 5 ml and centrifuged at 25,000×g for 20 minutes to obtain clear extracts. The supernatants were saved in screw tubes and stored at −15°C until used for paper chromatography. For the automatic method, 1 ml concentrated extract was diluted to 2 ml, added 10 ml 1% picric acid, and centrifuged to clarify the extract. The supernatant was passed through Dowex 2-×8 column, washed with 0.02 N HCl for 3 times, each time 5 ml, and all eluents were collected. The eluent was concentrated to 1 ml under vacuum and then adjusted pH value to 2.2 with 1 N NaOH.

A method for the quantitative determination of amino acids and amides described by Potor *et al.* (1957) was applied with a slight modification for paper chromatography. One-dimensional descending chromatography was carried out on 28.0×46.0 cm Whatman No. 1 paper using aqueous phenol. Two-dimensional descending chromatography was also on 46.0×46.0 cm Whatman No. 1 paper using aqueous phenol in the first direction and n-butanol: acetic acid: water in the second direction. All chromatograms were run in a cabinet at 26°C. The solvents employed were Mallinkrodt liquefied phenol with deionized water in 4:1 (v/v), adjusted to an pH of 5.5 to 5.8 with N NaOH and a Beckman Zeromatic II pH meter, and n-butanol: acetic acid: water (90:10:29, v/v/v) which were made up fresh before use. For the detection of the amino acids, the paper were sprayed with 1% ninhydrin (Sigma Chemical Co.) in absolute ethanol containing 0.1% 8-hydroxyquinoline (Wako Pure Chemical Ind. Ltd.) and kept in an ethanol-saturated atmosphere approximately 60°C for 30 minutes. The Technicon automatic analyzer was used for automatic recording chromatography and the method of Spackman *et al.* (1958) was adopted.

Preparation of sample for electrophoresis was done by the method of Steward and Barber (1964). The frozen tissues were homogenized with a minimum amount (approximately 1 gm tissue to 1 ml 0.1 M tris-glycine buffer, pH 8.3) and centrifuged for 30 minutes in a MSE 'Highspeed 18' refrigerator centrifuge at 25,000×g. The clear pale yellow extract was used immediately for the electrophoresis. Disc electrophoresis was carried out at 26°C using simplified procedure described by Clarke (1964). The equal amounts of buffer-

soluble proteins were applied in 2% sucrose solution without use of sample gel. Gel tubes containing protein extracts from healthy and diseased tissues were run simultaneously in the direction of the anode. Bromophenol blue (British Drug Houses Ltd.) was added as a front marker to obtain a uniform running distance of proteins in the gels. The current was initially 2 m Amp/column and increased to 5 m Amp/column upon the appearance of the dye front reached 1 cm below the gel surface. Proteins were stained in a solution of 0.7 gm of amidoschwarz 10 B (Schmid & Co.) in 100 ml of 7.0% acetic acid and destained the gel background in 7.0% acetic acid.

Results

Dry weight changes.—Temperature effect on the growth of mung bean was studied (Wu, 1965). However, changes in the dry weight of individual tissues in the mung bean associated with *R. solani* at various periods of ontogenesis were not known. Healthy and diseased mung beans grown on water agar were harvested at 0, 1, 2, 3, 4, and 5 days after inoculation. The harvested samples were dissected into cotyledons and root-shoot axes. Dry weights were determined on comparable material oven-dried at 105°C for 20 hours.

As shown in Fig. 1, there was slight change in dry weight of cotyledons

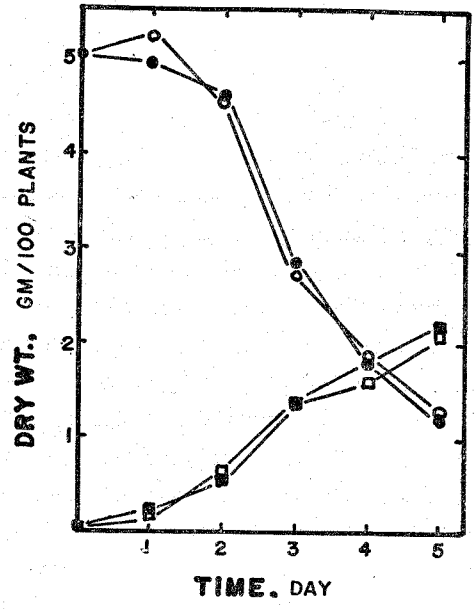

Fig. 1. Dry weight of cotyledons and axial parts of healthy (H) and diseased (D) mung bean seedlings determined at various intervals following inoculation. —●—●—H-cotyledons,—■—■—H-axial parts,—○—○—D-cotyledons,—□—□—D-axial parts. Each point represents 20 to 50 seedlings.

between day 0 and day 2, and an extremely rapid decline occurred between day 2 and day 5. By day 3 the dry weight of the cotyledons accounted for nearly half of the initial dry weight. On the other hand, the dry weights of the axial parts gradually increased through day 5, i. e. from 0.07 gm to 2.22 gm/100 plants. The patterns of the dry weight changes in healthy and diseased plants grown on water agar were essentially the same through day 5, though there was a very slight fluctuation between them.

Similar experiments were also carried out with the mung bean plants dissected into cotyledons, hypocotyls, and epicotyls. The same trend of dry weight patterns of different individual tissues was obtained, namely from 5.27 gm at day 0 to 0.76 gm at day 5 in healthy cotyledons; from 0.07 gm at day 1 to 2.09 gm at day 5 in healthy hypocotyls; from 0.27 gm at day 3 to 0.53 gm at day 5 in healthy epicotyls. No difference between healthy and diseased plants was observed. Although the dry weight increase was observed in both hypocotyls and epicotyls during the lapse of time, but the rate of increase was much higher in hypocotyls than that in epicotyls (Table 1).

Table 1. *Dry weight of cotyledons, hypocotyls, and epicotyls of healthy and diseased mung bean seedlings (gm/100 plants).*

Time (Day)	Healthy			Diseased		
	Cotyledon	Hypocotyl	Epicotyl	Cotyledon	Hypocotyl	Epicotyl
0	5.274	0.065	—	—	—	—
1	5.278	0.240	—	4.898	0.228	—
2	4.545	0.764	—	4.024	0.886	—
3	2.110	1.450	0.268	2.580	1.320	0.268
4	1.546	2.052	0.420	1.282	1.929	0.434
5	0.763	2.088	0.526	0.549	2.051	0.672

Calculation of moisture content indicated that it was increased rapidly between day 0 and day 1 and the rate of increase was much less thereafter. The range of moisture contents was from 38% to 95% and there was no difference in moisture contents between healthy and diseased mung bean grown on water agar.

Protein changes.—Pronounced decrease was observed when the protein contents of healthy and diseased cotyledons were compared at day 1. (Fig. 2). However, this apparent difference was simply due to the increased amount of cotyledon protein in healthy plant since amount of protein in healthy cotyledon was increased from 705 mg at day 0 to 916 mg/100 plants at day 1 whereas the protein content of diseased cotyledon was more or less the same between day 0 and day 1.

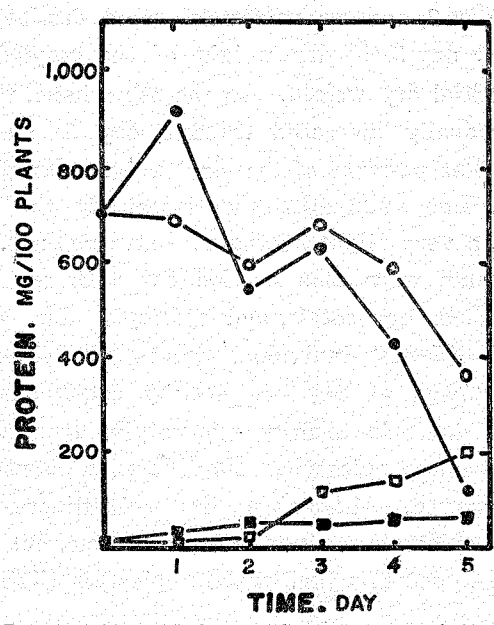

Fig. 2. Proteins in cotyledons and axial parts of healthy (H) and diseased (D) mung bean seedlings determined at various intervals following inoculation. —●—●— H-cotyledons, —■—■— H-axial parts, —○—○— D-cotyledons,—□—□—D-axial parts. Each point represent 20 to 50 seedlings.

It is worthy to note that the protein content of the axial parts from diseased plants started to increase at day 3 when the typical lesions were observed on hypocotyls (Fig. 2). The protein content in the axial parts from healthy plants was essentially constant from day 2 to day 5. Furthermore, the protein content in diseased cotyledons was found to be higher than that in healthy cotyledons from day 2 and thereafter. However, the patterns of protein changes in these comparable tissues were modified when the protein

Table 2. *Proteins in cotyledons, hypocotyls, and epicotyls of healthy and diseased mung bean seedlings (mg/100 plants).*

Time (Day)	Healthy			Diseased		
	Cotyledon	Hypocotyl	Epicotyl	Cotyledon	Hypocotyl	Epicotyl
0	680	—	—	—	—	—
1	600	38	—	620	52	—
2	520	84	—	553	120	—
3	517	158	94	468	175	133
4	356	180	169	264	227	133
5	223	200	228	187	221	231

contents in cotyledons, hypocotyls, and epicotyls from both healthy and diseased plants were compared (Table 2). Nevertheless, the Rhizoctonia infection seemed to induce an increase of the protein contents in both hypocotyls and epicotyls obtained from diseased mung bean plants.

Amino acid analysis.—Changes in free amino acid content and distribution were very striking during the course of germination (Fig. 3). In the initial seed samples, free amino acids accounted for very low level in both cotyledons and axial parts and only slight increase was observed in healthy and diseased mung bean at day 1. However, the free amino acid contents in the axial parts began to increase between day 1 and day 2 and then a striking increase was observed through day 5. By day 2, a day before the appearance of typical lesions on hypocotyls, the free amino acid content in the axial parts from the diseased mung bean was 1.6 times over that in the control plants, but the rate of increase in free amino acids was much less than that of the axial parts from healthy plants after day 3. Free amino acid contents in cotyledons was only slightly increased regardless of the Rhizoctonia infection. The similar trend of the amino acid changes in the axial parts was also observed in the further experiments of which tested plants were dissected into cotyledons, hypocotyls, and epicotyls (Table 3).

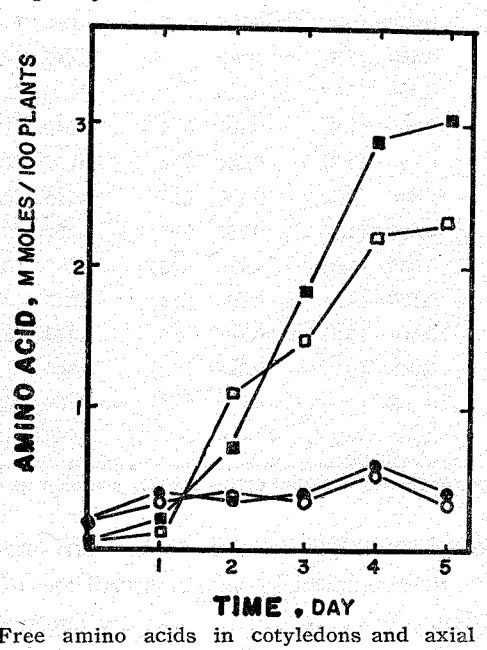

Fig. 3. Free amino acids in cotyledons and axial parts of healthy (H) and diseased (D) mung bean seedlings determined at various intervals following inoculation. —●—●—H-cotyledons,—■—■—H-axial parts,—○—○—D-cotyledons,—□—□—D-axial parts. Each point represents 20 to 50 seedlings.

Table 3. *Free amino acids in cotyledons, hypocotyls, and epicotyls of healthy and diseased mung bean seedlings (μ mole/100 plants).*

Time (Day)	Healthy			Diseased		
	Cotyledon	Hypocotyl	Epicotyl	Cotyledon	Hypocotyl	Epicotyl
0	178	—	—	—	—	—
1	417	56	—	483	487	—
2	361	293	—	383	374	—
3	201	597	17	601	467	39
4	140	706	32	125	850	50
5	107	1,083	62	125	873	108

Table 4. *Alcohol extractable amino acids of healthy and diseased mung bean seedlings grown on water agar for 2 days in the dark (μ mole/ml extract).**

Amino acid	Healthy		Diseased	
	Cotyledon	Axial part	Cotyledon	Axial part
Lysine	0.122 (1.8)	0.462 (11.3)	0.240 (5.0)	1.380 (17.0)
Histidine	0.414 (6.2)	0.324 (8.0)	0.447 (9.3)	0.864 (10.7)
Arginine	0.660 (9.9)	0.312 (7.7)	0.061 (1.3)	0.984 (12.1)
Aspartic acid	0.480 (7.2)	1.082 (26.6)	0.447 (9.3)	1.098 (13.5)
Glutamic acid	1.300 (19.4)	0.210 (5.2)	0.508 (10.6)	0.276 (3.4)
Proline	0.508 (7.6)	0.702 (17.2)	0.649 (13.5)	1.162 (14.3)
Glycine	0.080 (1.2)	0.024 (0.6)	0.797 (16.6)	0.072 (0.9)
Alanine	2.664 (39.8)	0.150 (3.7)	0.155 (3.2)	0.276 (3.4)
Valine	0.068 (1.0)	0.234 (5.7)	0.127 (2.7)	0.420 (5.2)
Methionine	0.064 (1.0)	0.036 (0.9)	0.033 (0.7)	0.264 (3.3)
Isoleucine	0.120 (1.8)	0.168 (4.1)	0.045 (0.9)	0.444 (5.5)
Leucine	0.064 (1.0)	0.186 (4.6)	0.113 (2.4)	—
Tyrosine	0.048 (0.7)	0.048 (1.2)	0.066 (1.4)	0.120 (1.5)
Phenylalanine	0.108 (1.6)	0.132 (3.2)	1.108 (23.1)	0.744 (9.2)
Total	6.700 (100.0)	4.070 (100.0)	4.796 (100.0)	8.104 (100.0)

* The figures in the parentheses are per cent of total μ moles of listed amino acids.

Paper chromatographic analysis of the alcohol extracts indicated that no qualitative differences were induced in the compositions of free amino acids by Rhizoctonia infection. However, leucine was not detected with an automatic recording apparatus in the axial parts from the diseased mung been (Table 4).

When the alcohol extractable amino acids of healthy and diseased mung bean were compared, a flow of the amino acid from cotyledons to the axial parts seemed to be affected by Rhizoctonia infection, i. e. histidine, arginine,

alanine, and methionine were stimulated while leucine and phenylalanine were lessened (Table 4). The similar trend was only found in arginine and methionine by comparing the relative amounts of amino acids.

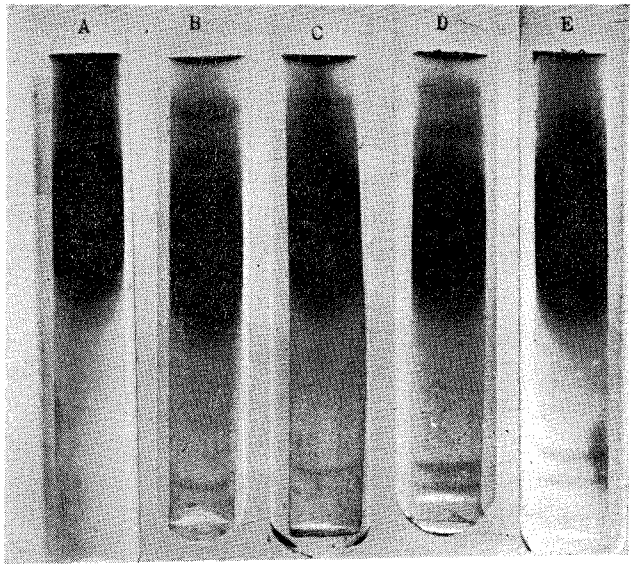

Fig. 4. Electrophoretic patterns of soluble cotyledon proteins on acrylamide gels. Extracts from healthy seedlings at 0 time (A), 1-day (B), 2-day (C) and diseased seedlings at 1-day (D), 2-day (E).

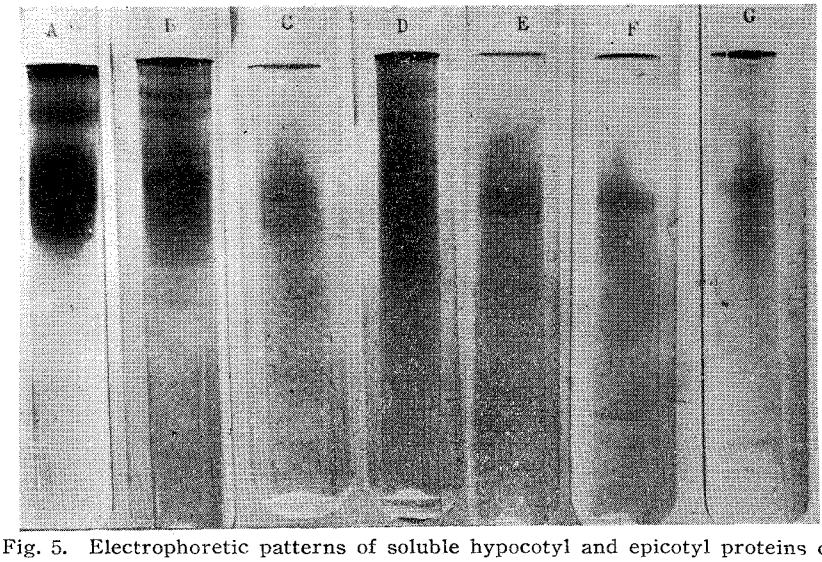

Fig. 5. Electrophoretic patterns of soluble hypocotyl and epicotyl proteins on acrylamide gels. Extracts from healthy hypocotyls at 0 time (A), 1-day (B), 3-day (C), and epicotyls at 3-day (F); diseased hypocotyls at 1-day (D), 3-day (E), and epicotyls at 3-day (G).

Fourteen amino acids were detected in the present experiments. They were lysine, histidine, arginine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine (Table 4). Among 14 amino acids listed, lysine, histidine, glycine, tyrosine, and phenylalanine were increased in both cotyledons and axial parts of the diseased plants whereas glutamic acid and alanine were decreased by Rhizoctonia infection when relative amounts of amino acids in the comparable individual tissues of healthy and diseased plants were compared.

Disc electrophoresis.—The electrophoretic patterns of response to the Rhizoctonia infection were different in the comparable individual tissues during the process of seed germination (Fig. 4 and Fig. 5). And the difference in electrophoretic patterns between the healthy and diseased plants was found considerably significant in the buffer-soluble proteins from hypocotyls (Fig. 5).

The effect of the fungal infection might reflect an interference with the early development of young seedlings. Before symptoms were evident in the inoculated plants, a new band appeared in the slow moving protein profiles of the cotyledons from inoculated mung beans at day 1 whereas 2 bands were disappeared in the soluble protein fraction from the inoculated plant at day 2. Brown colored substances were also found to move downward to the direction of anode soon. These two colored bands were always darker in the gels with infected tissues (Fig. 4).

On the other hand, a slow moving protein band disappeared and two fast mobilizing bands were found to be intensified in the soluble hypocotyl proteins from inoculated plants at day 1. In the later stages of disease development the deleterious effect of infection on the comparable individual tissues was no longer discernible under the experimental conditions (Fig. 5).

Discussion

Nitrogen metabolism plays an important part in host-parasite interaction (van Andel, 1966). Changes in free amino acids, amides, and proteins of diseased plants are known in many diseases (Wu, 1967, 1968). There is, however, little light on the exact role played by these nitrogen compounds in establishment of Rhizoctonia infection. The studies on the nitrogen mobilization in mung bean infected with *R. solani* are attempted in the present investigation.

Fourteen amino acids are detected in the present experiments. Among 14 amino acids, the amounts of lysine, histidine, glycine, tyrosine, and phenylalanine are increased in diseased mung bean whereas those of glutamic acid and alanine are decreased. Apparently, an increase in the contents of certain amino acids is in company with a decrease in a number of others (van Andel,

1966). McCombs and Winstead (1961) report an increase in the glutamic acid and a decrease of glutamine and citrulline contents in the watermelons infected with anthracnose fungus. Glutamine and asparagine are also found to be reduced in scab-infected cucumber foliage (Burton and DeZeeuw, 1961) whereas wheat leaves infected by stem rust exhibit an increase in ammonia, glutamine, and asparagine (Farkas and Király, 1961). Accumulation of alanine is prominent in blueberry fruits infected with *Glomerella cingulata* (Stretch and Cappelini, 1965), cucumber fruits infected with *Pythium aphanidermatum* (McCombs and Winstead, 1964), and wheat root infected by *Helminthosporium sativum* (Hrushovetz, 1954).

A flow of the amino acid from cotyledons to axial parts are affected by Rhizoctonia infection. The flow of histidine, arginine, alanine, and methionine are enhanced by infection while leucine and phenylalanine are lessened and the mobilization of arginine and methionine are significant. Sempio and Raggi (1966) also find a strong flow of amino acids freed by hydrolysis in rust-infected broad bean from uninfected to infected tissue. These amino acids and amides may be transported through xylem since, in most cases, glutamine and aspargine are predominated in plant exudates (Bollard, 1960). However, the transformation of amino acids should not be overlooked (Kretovich, 1965). Contamination of amino acid due to fungal origin (Ogura *et al.*, 1961) may affect the flow of the amino acids in diseased plants since one amino acid in high concentration interferes uptake of other amino acids (Jones, 1963).

The Rhizoctonia infection seemingly affect the buffer-soluble proteins in diseased mung bean, particularly the germinating seeds, since the amidoschwarz protein profiles on polyacrylamide gels manifest the host response to the Rhizoctonia infection. This is not surprising in asmuch as de novo synthesis of several enzymes is evidenced in germinating seeds (Altschul *et al.*, 1966). Since nitrogen metabolism is very active in germinating seeds, which is characterized by an intense turnover of protein (Koller *et al.*, 1962), the studies on the detailed picture of the changes in nitrogen pool of the infected seedlings will be very promising in study of the infection mechanism.

Summary

Dry weights of healthy and diseased mung beans grown on water agar were determined. There was a slight change in dry weight of cotyledons between day 0 and day 2, and an abrupt decline occurred between days 2 and 5. On the other hand, the dry weight of axial parts gradually increased up to day 5. Moisture contents of whole plants were increased rapidly between day 0 and day 1 and the rate of increase was slower thereafter. Changes in the dry weight and moisture content of healthy and diseased plants were

essentially the same. A marked decrease in cotyledon proteins was observed in early stage of pathogenesis whereas the protein content of the axial parts started to increase as soon as the typical lesions appeared. The difference in electrophoretic patterns between the healthy and diseased plants was found to be greater in the buffer-soluble proteins from hypocotyls.

Changes in free amino acid content and distribution indicated that the amino acid pool was considerably affected in the early stage of disease development. Fourteen amino acids were identified in the alcohol extracts of healthy and diseased seedlings by an amino acid autoanalyzer. They were lysine, histidine, arginine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine. Among 14 amino acids, the amounts of lysine, histidine, glycine, tyrosine, and phenylalanine were increased in diseased mung bean whereas those of glutamic acid and alanine were decreased. Apparently, an increase in the contents of certain amino acids is in company with a decrease in a number of others. A flow of the amino acids from cotyledons to axial parts was affected by Rhizoctonia infection. The flows of leucine and phenylalanine were lessened while those of histidine, arginine, alanine, and methionine were enhanced by infection. The mobilization of arginine and methionine was particularly remarkable. No qualitative differences, however, were observed in the compositions of free amino acids between healthy and diseased plants.

寄 生 生 理

3. 罹病綠豆苗體內氮化合物之變化

吳 龍 溪

健全綠豆苗與罹病苗之子葉乾重量，發芽後逐漸減少，但胚莖却增加。其含水量之變化不受苗立枯病之影響而改變，子葉可溶性蛋白質在罹病初期顯然減少，而胚莖在病徵出現後驟然增多。水溶性蛋白質之電氣泳動結果顯示綠豆苗罹病後各種蛋白質之變化顯著。

綠豆苗乙醇抽出液含有十四種氨基酸：離氨基酸，纖氨酸，鮭卵酸，天門冬酸，麩氨酸，吡咯氨酸。氨基乙酸，氨基丙酸，甲型氨基異戊酸，甲硫氨酸，異白氨基酸，白氨基酸，酥氨基酸，以及苯氨基丙酸。在罹病植物幼苗中離氨基酸，纖氨酸，氨基乙酸，酥氨基酸，以及苯氨基丙酸之含量增加，但麩氨酸和氨基丙酸含量却減少。綠豆苗氨基酸之轉移受苗立枯病菌感染之影響，如罹病苗之纖氨酸，鮭卵酸，氨基丙酸以及甲硫氨酸被促進，但白氨基酸及苯氨基丙酸却被抑制。

Literature Cited

AKAZAWA, T., Y. UMEMURA, and I. URITANI, 1957. Electrophoretic study of protein metabolism in sweet potato infected with *Ceratostomella fimbriata*. Bull. Agr. Chem. Soc. Japan, **21**:192-196.

ALTSCHUL, A. M., L. Y. YATSU, R. L. ORY, and M. ENGLEMAN. 1966. Seed proteins. Ann. Rev. Plant Physiol. **17**:113-136.

ANDAL, R. and N. S. SUBBA RAO. 1956. Amino acid changes in the healthy and foot-rot infected rice. J. Indian Botan. Soc. **35**:414-416.

ANDREEV, L. N. and M. SHAW. 1965. A note on the effect of rust infection on peroxidase isozymes in flax. Can. J. Bot. **43**:1479-1483.

BARRET, R. E. and J. H. MCLAUGHLIN. 1954. Disease resistance factors in wheat, electrophoretic and chromatographic analysis of protein extracts of wheat seedlings. J. Agr. Food Chem. **2**:1026-1029.

BOLLARD, E. G. 1960. Transport in the xylem. Ann. Rev. Plant Physiol. **11**:141-166.

BURTON, C. L. and D. J. DEZEEUW. 1961. Free amino acid constitution of healthy and scab-infected cucumber foliage. Phytopathology **51**:776-777.

CLARKE, J. T. 1964. Simplified "Disc" (polyacrylamide gel) electrophoresis. Ann. New York Acad. Sci. **121**: 428-436.

FARKAS, G. L and Z. KIRÁLY. 1961. Amide metabolism in wheat leaves infected with stem rust. Physiol. Plant. **14**:344-353.

HEITEFUSS, R., D. J. BUCHANAN-DAVIDSON, M. A. STAHMANN, and J. C. WALKER. 1960. Electrophoretic and immunochemical studies of proteins in cabbage infected with *Fusarium oxysporum* f. *conglutinans*. Phytopathology **50**:198-205.

HRUSHOVETZ, S. B. 1954. The effect of infection by *Helminthosporium sativum* on the amino content of wheat roots. Can. J. Bot. **32**:571-575.

JOHNSON, L. B., B. L. BRANNAMAN, and F. P. ZSCHELLE, Jr. 1966. Protein and enzyme changes in barley leaves infected with *Erysiphe graminis* f. sp. *hordei*. Phytopathology **56**:1405-1410.

JONES, O. T. G. 1963. The accumulation of amino acids by fungi, with particular reference to the plant parasitic fungus *Botrytis fabae*. J. Exptl. Botany **14**:399-411.

KAWASHIMA, N., N. HYODO, and I. URITANI. 1964. Investigations of antigenic components produced by sweet potato roots in response to black rot infection. Phytopathology **54**:1086-1092.

KOLLER, D., A. M. MAYER, A. POLJAKOFF-MAYER, and S. KLEIN. 1962. Seed germination. Ann. Rev. Plant Physiol. **13**:437-464.

KRETOVICH, W. L. 1965. Some problems of amino acids and amide biosynthesis in plants. Ann. Rev. Plant Physiol. **16**:141-154.

LOWRY, D. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. **193**: 265-275.

MCCOMBS, C. L. and N. N. WINSTEAD. 1961. The amino acids and sugar compositions of healthy and anthracnose-infected watermelons. Phytopathology **51**:644. (Abstr.)

MCCOMBS, C. L. and N. N. WINSTEAD. 1964. Changes in sugars and amino acids of cucumber fruits infected with *Pythium aphanidermatum*. Phytopathology **54**:233-234.

OGURA, H., S. AKAI, and T. SATO. 1961. Studies on *Pellicularia filamentosa* (Pat.) Rogors. II. On the formation of free amino acids and sugars in *P. filamentosa*, Ann. Phytopathol. Soc. Japan **26**:31-36.

PORTER, C. A., D. MARGOLIS, and P. SHARP. 1957. Quantitative determination of amino acids by paper chromatography. Contrib. Boyce Thompson Inst. **18**:465-476.

ROHRINGER, R. 1957. Untersuchungen zur Biochemie van Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici*. Phytopathol. Z. **29**:45-64.

ROHRINGER, R., M. A. STAHMANN, and J. C. WALKER, 1958. Effect of *Fusarium oxysporum* f. *lycopersici* and its metabolites on leaf constituents of susceptible and resistant tomatoes. Agr. Food Chem. **6**:838-843.

ROSEN, H. 1957. A modified ninhydrin colorimeter analysis for amino acids. Arch. Biochem. Biophys. **97**:10-15.

SEMPIO, C. and V. RAGGI. 1966. Amminoacidi liberi e combinati in piante di Fova (Vicia faba major) infecttate con Uromyces fabae. (Free and bound amino acids in broad bean plants infected with *U. vicia-fabae*) Phytopath. Z. **55**:117-171. (Abstract in Rev. Appl. Mycol. 45, 2666,1966).

SHISHIYAMA, J., T. OGUCHI, and S. AKAI. 1968. The change of protein fractions in diseased leaves of rice plants in the early stage of infection by *Helminthosporium oryzae*. Ann. Phytopath. Soc. Japan **34**:16-22.

SPACKMAN, D. H., W. H. STEIN, and S. MOORE. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. **30**:1190-1206.

STAHMANN, M. A. 1963. Plant proteins. Ann. Rev. Plant Physiol. **14**:137-158.

STAPLES, R. C. and M. A. STAHMANN. 1964. Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. Phytopathology **54**: 760-764.

STEWARD, F. C. and J. T. BARBER, 1964. The use of acrylamide gel electrophoresis in the investigation of the soluble proteins of plants. Ann. New York Acad. Sci. **121**: 525-531.

STRETCH, A. W. and R. A. CAPPELLINI. 1965. Changes in free amino acids and reducing sugars in Highbush blueberry fruit infected with *Glomerella cingulata*. Phytopathology **55**:302-303.

URITANI, I. and M. A. STAHMANN. 1961. Changes in nitrogen metabolism in sweet potato with black rot. Plant Physiol. **36**:770-782.

VAN ANDEL., O. M. 1966. Amino acids and plant disease. Ann. Rev. Phytopath. **4**:349-368.

WEBER, D. J. and M. A. STAHMANN. 1964. Ceratocystis infection in sweet potato: its effect on proteins, isozymes and acquired immunity. Science **146**:929-931.

WU, L.-C. 1965. Physiology of parasitism. 1. Growth, pathogenicity, and toxin production of *Rhizoctonia solani* Kuehn. Bot. Bull. Acad. Sinica **6**:144-152.

WU, L.-C. 1967. Physiology of parasitism. 2. Biochemical changes in the mung bean seedling infected with *Rhizoctonia solani* Kühn. Bot. Bull. Acad. Sinica. 8:271-283.

WU, L-C. 1968. Biochemical changes in the mung bean seedling infected with *Rhizoctonia solani*. p. 321-334. In T. Hirai, Z. Hidaka, and I. Uritani (Ed.) Biochemical regulation in diseased plants or Injury. The Phytopathological Society of Japan, Tokyo.

WU, L.-C. 1968. Nitrogen mobilization in mung bean seedling infected with *Rhizoctonia solani*. Plant Physiol. 43 (Suppl): S-9.