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STUDIES ON THE RELATION BETWEEN
THE PRINCIPAL COMPONENTS OF RICE PLANT AND
ITS SUSCEPTIBILITY TO THE BLAST DISEASE
AND ON THE PHYSIOLOGICAL
CHARACTERS OF THE BLAST FUNGUS

By

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Introduction

As an outbreak of the rice blast disease is one of the most horrible disasters for the rice cultivation, studies concerning this disease have been made from various view points by many investigators especially in our country. But it seems that there remain yet some problems to be solved, for example problems concerning the susceptibility or the resistance of the rice plant against the blast disease or problems concerning the virulence of the causal fungus and so on. Among them the studies on the cause of the susceptibility or resistance of the rice plant against the blast fungus have recently come to be the most important as well as the most interesting ones in the field of plant pathology.

Studies on that problem may be carried on from two different angles: the one is studies from the side of the rice plant and the other is studies from the side of the blast fungus. Speaking of the studies from the former side, the first problems to be examined may be the morphological characters of the plant surface through which the fungus enters the host plant. If the hyphae of the causal fungus could only mechanically penetrate the epidermis of the host plant, as described by W. BROWN and C. HARVEY (1927), it is quite natural to suppose that there are some relations between the susceptibility of the plant and such morphological characters of its epidermal cells as the thickness of the cuticular layer, the thickness of the cell wall, the presence or absence of the cork layer and the fibre tissue. It is known from the studies of MATSUURA (1928), SUETA (1928), ITO and KURIBAYASHI (1931), IKATA, MATSUURA and TAGUCHI (1931), and YOSHII (1936) that the hyphae of rice blast fungus can penetrate almost mechanically the epidermal cell of the rice leaf. According to the studies of ITO and SHIMADA (1937), SHIMADA (1937), and TOCHINAI and KOMIYA (1939) those rice plants which were wounded at the leaf are apt to be severely attacked by the disease. SAKAMOTO (1940) gives the name "Kaze-imochi" (wind-blast) to such a case when the penetration of the blast fungus is greatly facilitated by bruises or wounds which were made on the host tissues by the wind. ITO and SHIMADA (1937) stated that the nodule part of the rice plant is apt to be severely attacked by blast fungus because of weak development of the cuticle. NAGAI and IMAMURA (1933) reported that the fungus invasion is facilitated on such a panicle stalk as one that is constructed from too much assimilation tissues compared

with the mechanical tissues. From the same view points, the well known fact, that the rice plants supplied with excessive amounts of nitrogen are apt to be severely attacked by the blast fungus, is often attributed to the decline in the hardness of the leaves which is caused by such excessive supply of nitrogen. H. SUZUKI (1934, '35), working with the rice plants different in their water supply and with many rice varieties different in their blast susceptibility, came to the conclusion that the thickness of epidermal cell walls of the leaves is the most important factor determining resistance of the rice plant against the blast disease. On the other hand the relationships between the silification of rice leaves and the resistance to blast disease have been discussed from some years ago. ONODERA (1917) reported the results of chemical analysis both of the blast diseased and of the healthy leaves of the rice plants and showed that the silica contents are less in the former than in the latter. By the chemical analysis of plants of several rice varieties different in their blast susceptibility, MIYAKE and ADACHI (1922), KAWASHIMA (1927) recognized also the fact that the more resistant plants contain the larger amounts of silica. ITO and HAYASHI (1931), MIYAKE and IKEDA (1932), IKEDA (1932), AKAI (1953) studied the relations between silica manuring and susceptibility. AKIMOTO (1939, a) reported that the blast resistance was correlated with the ability of silica absorption which differs in different rice varieties. IKEDA (1933) studied the influence of nitrogen manuring upon the silica content of the rice plant and ISHIZUKA and TANAKA (1950) studied the effect of three manurial ingredients upon the silica content. According to ISHIZUKA and HAYAKAWA (1950) deficiency of magnesium prevents the absorption of silica by the rice plant, which leads in turn to the high blast susceptibility of the rice plant. Many researchers such as H. SUZUKI (1933-1951), HEMMI and H. SUZUKI (1933), AKAI (1938, 1940), HEMMI (1940), YOSHII (1940, '41), SAWADA (1939), TERA0 (1939), N. R. ADYANTHAYA, and G. RANGASWAMI (1952), studied the relations between the varietal susceptibility of the rice plants to blast disease and the silification of their epidermis. Among them the studies of SUZUKI go into detail; he shows that the more resistant rice plants have the more so called "silicated cells" among epidermal tissues.

While silica found in the graminicolous plants is ordinarily thought to be concerned only with the toughness of the plant, W. ENGEL (1953) found that great part of silica present in the plant is combined with galactose and their ratio of combination is 1 : 2 in the grown up plants

and on the other hand it is 1:1 in the plants in growth. In consideration of these results he suggests the physiological function of silica. Therefore, there may be some significance of silica for determining resistance to blast disease other than only mechanical defence. Apart from such problems, it is indubitable that the morphological characters of the plant surface, as described above, are closely related with the facility or difficulty of causal fungus penetration into the plant. But those morphological characters of the rice surface are concerned only with the penetration of the causal fungus and they do not seem to have any close correlations with the fungus growth in the host plant. Such growth may have rather close correlation with the chemical components of the host plant. There have been many researches which treated the problem of the susceptibility or resistance from such view point (G. GASSNER and K. HASSENBRANK: 1931, C. B. SMITH: 1951, J. C. WALKER, and M. E. GALLEGLY: 1951, H. TORIKATA and Y. KOMAE: 1952, C. T. WEI, J. C. WALKER and R. P. SCHEFFER: 1952, BABA, TAKAHASHI and IWATA: 1951, '52, '53, G. K. LINK, K. WILCOX et al: 1953, AKAI and UYAMA: 1953). According to TAHARA (1937) the blast fungus can penetrate well into the seedlings which look yellowish because of their lack of nitrogen, but the fungus cannot grow favourably in such seedlings owing to the deficiency of nourishment, producing comparatively small lesions only. He made chemical analysis on those rice plants which were made more susceptible by being placed in a dark room or by the application of an excess amount of nitrogen, and got the following conclusion: though the increase of the amount of the total nitrogen in the rice plant is not directly concerned with the higher blast susceptibility, the disturbance of the composition balance in the nitrogenous compounds which accompanied the increase of total nitrogen contents, such as extraordinary increase of the amount of non-protein nitrogen compared with that of protein nitrogen, always favours the fungus growth in those rice plants, resulting in the higher blast susceptibility of those rice plants. TANAKA and KATSUKI (1952) reported the higher blast susceptibility accompanied by the higher content of three basic amino acid and OTANI (1948, '52) reported the enhancement of blast susceptibility in close correlation with the higher content of soluble protein or α -amino acid. On the other hand, the extraordinary accumulation of certain components may be caused by physiological disturbance or may cause the physiological disturbance of the rice plant, and such physiological disturbance, in turn, may enhance

the blast susceptibility. NISHIKADO and MATSUMOTO (1935, '36), MIYAZAKI (1928), AKIMOTO (1939 b) and SAKAMOTO (1949) discussed the problem from such a view point. Among them SAKAMOTO reported that the accumulation of ammonium nitrogen in the rice plant causes the increase of the water permeability in the protoplasm of the rice plants, which makes them more susceptible to the blast disease. According to NISHIKADO and MATSUMOTO the blast susceptibility of the rice plant was enhanced inversely proportional to the decrease of the specific electric conductivity of the cell sap or to the decrease of the ratio between depression degree of freezing point and specific electric conductivity. As shown by FUKUCHI (1940) and TOCHINAI and KOMIYA (1939), the treatment of rice plants with ether vapour greatly enhances their blast susceptibility. This enhancement is thought to be concerned with some changes in the protoplasm caused by that treatment.

Studies treating the problem of blast susceptibility of the rice plant from the side of the parasite are to be briefly described next. As known well, the conidiospores which fall into the water on the rice leaves germinate into germ-tubes on the tips of which appresoria soon appear. At last the infection hyphae which are produced from the appresoria penetrate the epidermis of the rice leaf. Thus the attack of the blast fungus upon rice plants begins with the germination of its spores. There have been many reports concerning the spore germination of the blast fungus (ABE: 1933; HEMMI, IKEYA and INOUE: 1936; AOKI: 1937; HEMMI and IMURA: 1939, etc.). According to AOKI, the spores of the blast fungus can germinate well at pH 5-6 and also at pH 8-9; the addition of glucose and glycerine to the spore suspending water drops favours the spore germination proportional to the concentration of added material up to 1 mol. On the other hand, the want of oxygen or the presence of brown spot fungus spore in mixture with the blast fungus spore hinders the germination of the blast fungus spore. HEMMI, IKEYA and INOUE reported that the addition of the culture filtrate of the brown spot fungus to the spore suspension of the blast fungus hindered the germination of the spores. According to HEMMI and IMURA there is not any correlation between the virulency of the fungus and germination rate of the spores or the growth rate of the germ tubes. ABE (1930, '31, '33), HEMMI and ABE (1931), IMURA (1940), JOHNSON and HALLIN (1952) studied the effects of some environmental conditions upon the penetration of fungus hyphae through the rice epidermis. H. SUZUKI (1943 c) showed that the presence of NH_4

promoted the formation of appresoria and on the contrary the presence of K hindered that formation. Moreover, according to him, the rice plants which contain comparatively large amounts of ammonium nitrogen are the more susceptible and the rice leaves which contain the greater amount of K are the more resistant against the blast fungus. SUZUKI explained this fact by supposing the exosmosis of excess ammonium or K through the rice epidermis. IMURA, examining the effect of intensity of sunlight upon the enlargement of the diseased lesions, found that the rate of the lesion enlargement was favoured by a weak shading for a while after the incubation period but with the further lapse of time its enlargement was hindered by the same shading. IMURA explained these facts as follows; the better lesion enlargement soon after the incubation period was thought to be due to the changes in the host vitality which were caused by the shading and the worse lesion enlargement with the lapse of time after shading was due to the diminution of the assimilation products of host plant which affect the growth of the causal fungus in the plant. However, in order to understand well the fungus growth in the host plant, it may be most convenient to inspect the fungus growth on synthesized culture media. It is SCGA (1918) who first cultured the blast fungus on culture medium; he was followed by HIRAYAMA (1931), ITO and TERUI (1931), YOSHII (1936), ABE (1938), INOUE (1939), TOCHINAI and NAKANO (1940), and TOCHINAI and HARA (1942). Thus some physiological characters of the blast fungus came to be known. Among them the studies of TOCHINAI and NAKANO, culturing the fungus on a synthesized culture medium, yielded experimental results on the nitrogen or carbon sources for the growth of the blast fungus, but in those days some natural substances such as rice straw decoction were usually added to the culture media, as otherwise the fungus growth was quite poor. Therefore it can not be helped that some of those experimental results are unreliable. However it has recently become known through the studies of F. W. LEAVER and al. (1947), TANAKA and KATSUKI (1951, '52, '53) and Y. OTANI (1952) in succession that biotin and vitamin B₁ are indispensable for the growth of the blast fungus as the growth factor. Then the blast fungus came to be easily cultured on the pure synthesized culture medium to which biotin and vitamin B₁ are added. Working with such synthesized medium Y. OTANI showed that some amino acids or amides are a better nitrogen source than inorganic nitrogen for the fungus growth. The same author reported that while some kinds of sugars

such as sucrose, maltose and glucose were most excellent carbon sources for the growth of the blast fungus, some kinds of organic acids as succinic acid or citric acid could be utilized as the carbon source to some extent by the same fungus. NAKAMURA and SHIMOMURA (1953) cultured blast fungus on the sugar-free medium which contained leucine and found that the fungus can utilize the ketonic acid, decomposition product of the leucine, as its carbon source. On one of those cultural studies Y. OTANI (1954, '55) reported the production of protease and amino acid oxidase by the blast fungus.

Some of those studies which treated the problem concerning the blast susceptibility of the rice plant from the side of the rice plant or from the blast fungus may be reviewed as above. Recently there have appeared researchers who pay attention to the observation of the actual struggle between the infected plant and the causal fungus and try to ascertain the cause of the resistance or susceptibility in this struggle itself. Studies from such a viewpoint which is thought to originate from the work of DUFRÉNOY (1936), have been making some worthy findings, aided by the development of the histochemical method. As for the rice blast disease, ONO (1950) examined particularly the diseased lesions which appeared on some rice varieties different in their susceptibility and classified the lesions into several types. According to him the brown-spot type lesions, which usually appear on the high resistant varieties, come into appearance because on those blast resistant varieties the epidermal cells which were penetrated by the infection hyphae turn into brownish immediately after the penetration; such changes of the epidermal cells prevent any farther growth of the hyphae in that rice plant, leaving the lesions in the form of brown spots. According to N. SUZUKI (1952), those brownish materials which appeared in the penetrated cells of the rice plant prevent the formation of the conidiospore of the blast fungus; the same author and co-workers (1953) considered this brownish substance to be chlorogenic acid. There are some other reports which describe the protective action of chlorogenic acid or other related phenol compounds against the fungus growth in the plant tissue and consider the appearance of these compounds as one of the causes of disease tolerance (G. JOHNSON and L. A. SCHAAL: 1952, K. PAECH und H. RUCKENBROD: 1953, H. NIENSTAEDT: 1953). But there seem to be some doubts regarding the opinion that these phenolic compounds themselves prevent the fungus growth. According to N. SUZUKI, stimulation of the fungus

penetration causes some changes such as the increase of respiration of the infected cell, which leads in turn to the formation of the brownish materials in that cell, and the antifungal actions begin to appear in the cells during the formation of the brownish material. However there remain many problems to be solved concerning the antifungal action which may appear during the development of the infection.

TAMARI and KAJI (1954) have recently succeeded in extracting toxic substances from the filtrate of blast fungus culture: according to them the toxic action of these substances relative to the growth of rice plants is diminished by the addition of chlorogenic acid. But it remains to be solved how these toxins act during the development of the blast infection, and how they are related with the susceptibility or resistance of the rice plant.

Besides all these works above mentioned, some breeders have been making great effort to develop a variety of the rice plant which would be highly resistant against the blast disease. It is quite true that the breeding of rice varieties which are highly resistant against the blast infection and at the same time excellent in other chief characters is most desirable for the control of this disease. But as no such excellent rice variety has yet been developed, it is necessary to cultivate rather susceptible varieties because of their excellency in the other chief characters. Therefore it is quite important to establish a way of cultivation by which the tolerance of the rice plant will be made as great as possible against the blast disease. In order to answer this purpose it is needful first to learn why and how the same rice variety varies its susceptibility or resistance against blast disease under different environmental conditions. The problems concerning the fluctuation of resistance or susceptibility of the same variety under different environmental conditions seem to have been discussed in confusion with the problems on the varietal differences of the blast susceptibility. It is rather convenient to-day that the problems on the fluctuation of blast susceptibility in the same rice variety be discussed separately from the problem of the varietal differences in blast susceptibility, although naturally there exist some connections between those phenomena. In the case of the highly resistant rice varieties the swiftness of the appearance of the resistance reaction which appears originally rather quickly after the penetration, may determine the degree of resistance of the rice plant. But in the case of rather susceptible varieties the appearance of this reaction is originally rather slow even

when they were cultivated under the ordinary conditions, so the penetrated hyphae can make their growth previous to the appearance of this resistance reaction. As is well known, such rice varieties come often to suffer from more severe attacks by the blast fungus because of some environmental conditions. Such fluctuation of susceptibility can not be explained only by the resistant reaction; some relations are suggested between the fluctuation of susceptibility and the amount of certain chemical components of the rice plant which are utilized by the fungus as nourishment for their growth. The present writer has tried to examine those relations, employing through all his experiments the variety "Eiko", which is medium in its blast susceptibility. First by the inoculation experiments some instances of the fluctuations of blast susceptibility, which are caused by some environmental conditions or by the differences in the growing stages, were ascertained. Then the amount of the chief chemical components in those rice plants were determined, while examination was made of some morphological characters of their epidermal cell. On the other hand the present writer examined the growth of the blast fungus on the synthesized culture media in which those chemical compounds related to the chemical components of the rice plant itself were applied at different levels. Likewise examination was made of some enzymes which are produced by the fungus and may constitute the aggressive forces against the rice plants. Summarizing these experimental results, he undertakes to discuss the causes of the fluctuation of the blast susceptibility in the same rice variety.

The present work was done in the Botanical Institute, Faculty of Agriculture, Hokkaido University, Sapporo Japan. The writer wishes to express here his sincere gratitude to Dr. Y. TOCHINAI and to Dr. S. ITO for their valuable directions and cordial encouragement and also to Dr. T. FUKUSHI and Dr. T. TAGAWA for their valuable suggestions and encouragement throughout the present experiments. The writer wishes to extend his thanks to Dr. ISHIZUKA and Dr. Y. NAKAMURA, Profs. of Agricultural Chemistry of Hokkaido University, who gave the writer their kind guidance and helpful suggestions concerning the chemical analysis of the plant and water culture of the rice plant, and also to Dr. S. TANAKA, Prof. of Kyoto University, who gave the writer many appreciable suggestions and inspirations on the growth factor of the fungus. The seed of the rice plant was supplied by the Hokkaido

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EXPERIMENTAL

PART A

Relations between the susceptibility of rice plants
to the blast disease and
their principal chemical components.

Chapter I

Differences between the rice seedlings raised
on the hot bed nursery and on the ordinary nursery
in respect to their susceptibility to blast and
to their chemical components.

Hot bed nursery has recently come to be widely employed for rice seedling cultivation in the cold weather districts, Tōhoku and Hokkaido of Japan; the employment of this method gives the various advantages to the rice cultivation in those districts. The hot bed nursery, however, is a kind of land nursery and the temperature and the humidity inside are apt to become too high; moreover the light which is necessary for the growth of the rice seedlings inside the nursery is apt to be scarcely sufficient on account of the presence of the frame cover. The seedlings which are raised under such conditions are supposed to be rather susceptible to the rice blast disease in view of the previous investigations, so the present writer undertook to examine the degree of their susceptibility for comparison with the susceptibility of those rice seedlings raised in the ordinary nursery. Then he examined their principal chemical components and some morphological characters in order to determine the relations between the susceptibility and those characters.

The hot bed nursery used in the following described experiments was made in the usual manner at a sunny place of the Botanical Garden of Hokkaido University. The frame cover was made of linseed oil paper. Manures were given at the rate of the following; ammonium sulfate 91 kg, superphosphate of lime 114 kg, straw ash 46 kg per acre.

The rice seeds were sown at the rate of 0.85 kg per 1/1000 acre. The conditions inside this hot bed nursery were carefully controlled through all the growing period, spraying water in accordance with soil dryness and lifting the frame cover in accordance with the temperature inside. But in spite of these careful controls the temperature inside fluctuated from 6.0°C to 30.5°C; its average was 19.5°C which was about 4.7°C higher than the outside. While the relative humidity inside fluctuated from 53% to 100% all through the seedling growing period with an average 83.2% which was about 5.4% higher than the outside. Though the percentage of light which came through the new linseed oil paper of the cover frame was about 80%, it decreased with the days by reason of the weather exposure and it came to be that the only 55% of the whole light could penetrate into the nursery through the weather-beaten linseed oil paper at the end of the seedling culture period. Experiments were repeated four times in three years. Through all the experiments the rice variety "Eiko" was used, which was supplied by the Hokkaido Agricultural Experiment Station (Same through the all experiments of part A). "Eiko" is a variety which is said to be medium in its blast susceptibility. Most of the lesions developed on the inoculated leaves under the ordinary condition are the chronic-type (according to the ONO's classification), mixing with a few acute-type ones. But from the observations of the process of their enlargement, it is known that they appear first as a small brown spot and then somewhat transparent looking parts develop around these spots taking an appearance somewhat like to the acute-type lesions, but in the meanwhile these transform into the chronic-type enlarging their length and width. Thus the lesions develop on this rice variety under the ordinary condition in the following process; brown spot → acute type → chronic type. But it must be noticed that the period of the acute-type is often prolonged and lesions continue to enlarge in that type for rather a long time, if the rice plant happens to become more susceptible to the blast disease than usual under some environmental conditions. At any rate, however, all the lesions change into the chronic type after a long lapse of time.

1. Inoculation experiments

Exp. 1: The hot bed nursery and ordinary nursery were prepared at the beginning of May and the seeds were sown on 10 May. In the hot bed nursery the germination began on 16 May while it was on

20 May in the ordinary nursery. At first the growth rate of the hot bed nursery seedlings seemed to exceed that of ordinary nursery seedlings, but the latter gradually came up with the former. And the seedlings of the both nursery beds came to the full-growth with four leaves each on 7 of June. At that time the average of leaf length of the ordinary nursery seedlings was clearly longer than that of the hot bed nursery seedlings while the height of the former was a little greater than that of the latter (Table 1). This fact is owing to the greater elongation of internodes of the latter compared with that of the former.

The seedlings were inoculated with the blast fungus which had been collected at ŌNOMURA of OSHIMA district, Hokkaido, and was cultured on sterilized rice-straw. Inoculum was prepared by adding sterilized water so as to make the content 2500 spores per 1 cc, and 300 cc of this spore suspension was sprayed over about 100 seedlings. Some seedlings which were grown on the pot buried in the nursery were dug up with the pot and carried into the inoculation box in which the temperature was regulated at 28°C and the humidity at 100% while the remainder were inoculated in the nursery. The diseased lesions which developed were examined at two weeks after the inoculation. The results are shown in table 1.

TABLE 1. Inoculations of the blast fungus on both kinds of seedlings. (1)

	ordinary nursery	hot bed nursery seedling	
	seedling	I	II*
height	9.9 cm	9.5 cm	9.5 cm
number of leaves per seedling	4	4	4
average leaf length	8.3 cm	5.2 cm	5.2 cm
number of leaves examined	315	146	232
total number of lesions	20	76	188
average number of lesions per leaf	0.06	0.52	0.81
average number of lesions per 100 cm length of leaves	0.7	9.9	16.2
length of lesions	max.	0.5 cm	1.3 cm
	min.	0.1 "	0.3 "
	aver.	0.3 "	0.7 "
type of lesions	brown spot, chronic	chronic, acute	chronic, acute

* inoculated in the inoculation box.

As shown in the table, the lesions were more numerous on the hot bed nursery seedlings than on the ordinary nursery seedlings. While the most of the lesions developed on the latter were the chronic-type mixing with a few brown spot-type, the lesions developed on the former were both the acute-type and the chronic-type without any brown spot ones. As for the largeness of the lesions the former were two or three times as large as the latter. On comparing the two of the hot bed nursery (I and II in the table), it is known that more numerous and somewhat larger lesions develop on those seedlings which are inoculated in the inoculation box than those inoculated in the nursery.

Exp. 2: Two experimental sections were prepared and in both the seedlings were grown in the soil of the pots. In the one the soil was in the muddy condition the same as in the ordinary nursery, and in the other the soil was kept wet only by spraying water in accordance with its dryness. The both were placed in the glass house. The latter may be correctly called a land nursery, but the hot bed nursery and the land nursery resemble each other so far as their soil condition is concerned. Therefore in this experiment the latter was treated as the hot bed nursery. When the seedlings of the both sections reached to about 10 cm height, they were carried into the inoculation box and inoculated with blast disease fungus in the way as in exp. 1.

TABLE 2. Inoculations of the blast fungus on the two groups of seedlings. (2)

	ordinary nursery seedling	hot bed nursery seedling
height	9.6 cm	9.8 cm
number of leaves per seedling	4	4
average leaf length	8.3 cm	6.9 cm
number of leaves examined	168	99
total number of lesions	6	70
average number of lesions per leaf	0.04	0.90
average number of lesions per 100 cm length of leaves	0.3	13.1
length of lesions	max.	0.5 cm
	min.	0.1 "
	aver.	0.2 "
type of lesions	brown spot, acute	acute, chronic

Comparing the growth of both groups of seedlings at the time of inoculation, the hot bed nursery seedlings were inferior to the ordinary nursery seedlings in their average leaf length, while the latter a little surpassed the former in their height. The results of the inoculation experiments are shown in the table 2. More numerous and larger lesions were clearly seen on the hot bed nursery seedlings than on the ordinary nursery seedlings. The lesions developed on the former were acute type or chronic type, while those developed on the latter were chronic or brown spot type.

Exp. 3 and Exp. 4: The nursery was prepared at the end of April in experiment 3 and at the beginning of May in experiment 4. In each experiment the seedlings were inoculated with blast fungus on the 20th day after germination. The inoculated seedlings were somewhat younger than those used in the foregoing experiments, and the hot bed nursery seedlings were a little superior to the ordinary ones in both height and average leaf length. The results of the inoculation experiments are summarized in table 3. As shown in the table, the number of lesions developed on the hot bed nursery seedlings was greater than on the ordinary nursery ones.

TABLE 3. Inoculations of the blast fungus on both groups of seedlings. (3)

	exp. 3		exp. 4		
	ordinary nursery seedling	hot bed nursery seedling	ordinary nursery seedling	hot bed nursery seedling	
height	9.5 cm	9.8 cm	9.3 cm	9.8 cm	
number of leaves per seedling	3.5	3.5	3.5	3.5	
average leaf length	6.5 cm	6.8 cm	6.2 cm	7.0 cm	
number of leaves examined	310	305	290	300	
total number of lesions	68	146	75	136	
average number of lesions per leaf	0.22	0.48	0.26	0.45	
average number of lesions per 100 cm length of leaves	3.4	7.0	4.2	6.5	
length of lesions	max.	0.8 cm	1.5 cm	1.0 cm	1.5 cm
	min.	0.1 "	0.2 "	0.1 "	0.1 "
	aver.	0.4 "	0.8 "	0.5 "	0.9 "
type of lesions	brown spot, chronic	chronic, acute	brown spot, chronic	chronic, acute	

Moreover the lesions on the former were chronic-type or acute-type while the lesions on the latter were brown-spot type or chronic-type. Their size was evidently larger on the former.

Summarizing the four experiments above described, it is clear that the seedlings grown in the hot bed nursery are more susceptible to the blast disease than those in the ordinary bed. This may be due not only to the rather high temperature, rather high humidity and light deficiency but also to the soil condition of lower humidity in the hot bed nursery, in view of the results of experiment 2.

2. Some morphological characters of the epidermis in the two kinds of seedlings.

It is often said that some morphological characters such as the thickness of the outer walls of the leaf epidermal cells or their silification are concerned with the blast susceptibility of the rice plant. In the following some of those morphological characters are examined on the two kinds of seedlings which were shown in the previous experiments to be different in their blast susceptibility. Materials were the same as those used in the previous inoculation experiments. Some of the seedlings were collected previous to the inoculations and their 3rd leaves were fixed in Carnoy's solution. The thickness of the outer walls of the epidermal cells in those leaves was examined on the preparates which were prepared by the paraffin method and were stained with haematoxylin. According to H. SUZUKI⁽⁶⁹⁾ the epidermal cells of the leaf may be classified into the following seven groups; (1) the epidermal cells above the sclerenchymatous tissue on the reverse side of the mid-rib (epidermal cells of mid-rib), (2) the long and short cells on the reverse side of the leaf (epidermal cells on the reverse side), (3) the long and short cells between the stomatal row and row of motor cells (long and short cells I), (4) the long and short cells between the row of the dumb-bell shaped rice cells and the row of long and short cells neighbouring to the row of motor cells (long and short cells II), (5) the motor-cells, (6) the accessory cells on the outside of the leaf (accessory cells on outside), (7) the accessory cells on the reverse side of the leaf (accessory cells on reverse side).

The silification of the epidermis was examined by counting the numbers of the silicated epidermal cells. The preparates were prepared as follows; about 5 cm long pieces that were taken from the three parts of the leaves (upper part, middle part and lower part) were

soaked in phenol for a few minutes and then they were mounted in clove oil on the slide-glass. On the preparates the silicated epidermal cells can be easily identified.

The results are given by calculating their numbers per unit area (1mm^2) of the leaves.

a) The thickness of the outer walls in the leaf epidermal cells: The results of the measurement of about 20-50 cells in 5-8 pieces of the leaves are summarized in table 4.

TABLE 4. Thickness of the outer walls of the leaf epidermal cells on both kinds of seedlings.

exp.	kind of seedlings	kind of cells	upper part		middle part		lower part	
			range	aver.	range	aver.	range	aver.
1	ordinary nursery seedling	epidermal cells of midrib	4.95 μ -8.80	6.44 μ	5.50 μ -8.80	7.83 μ	5.50 μ -8.80	6.60 μ
		epidermal cells on reverse side	3.30 -5.50	4.48	4.40 -5.50	4.95	4.40 -5.50	4.95
		motor cells	3.30 -4.40	3.54	3.30 -4.40	3.71	3.30 -5.50	4.06
		long and short cells I	3.30 -4.40	3.85	3.30 -4.40	4.19	3.30 -3.85	3.92
		long and short cells II	3.30 -5.50	3.16	3.30 -5.50	4.26	3.30 -5.50	4.19
		accessory cells on outside	3.30 -4.40	3.52	3.30 -4.40	3.99	3.30 -4.40	3.44
		accessory cells on reverse side	3.30 -4.40	3.85	3.30 -5.50	4.47	3.30 -4.40	4.19
	hot bed nursery seedling	epidermal cells of midrib	4.40 -6.05	4.90	4.40 -7.70	8.80	5.50 -8.80	6.38
		epidermal cells on reverse side	3.30 -4.40	3.91	3.85 -4.40	4.22	3.30 -4.40	3.67
		motor cells	2.75 -3.85	3.42	2.75 -3.85	3.48	2.75 -5.50	3.67
		long and short cells I	3.30 -4.40	4.00	3.30 -4.40	3.85	3.30 -4.40	3.85
		long and short cells II	2.20 -3.85	3.36	3.85 -4.40	4.03	2.75 -4.40	3.85
		accessory cells on outside	1.65 -3.85	3.12	3.30 -4.40	3.85	3.30 -3.85	3.39
		accessory cells on reverse side	2.20 -4.40	3.30	3.30 -3.85	3.48	3.30 -4.40	3.67

exp.	kind of seedlings	kind of cells	upper part		middle part		lower part	
			range	aver.	range	aver.	range	aver.
2	ordinary nursery seedling	epidermal cells of midrib	4.75 μ -8.20	6.11 μ	5.50 μ -8.20	7.30 μ	5.13 μ -6.95	6.00 μ
		epidermal cells on reverse side	2.95 -5.20	4.31	3.85 -4.40	4.31	2.75 -4.40	3.64
		motor cells	2.20 -4.40	3.73	3.30 -3.85	3.43	2.20 -2.75	2.29
		long and short cells I	3.30 -4.40	3.63	3.30 -4.95	3.63	3.30 -4.40	3.83
		long and short cells II	3.30 -4.40	4.13	2.75 -4.40	3.85	3.30 -4.40	4.03
		accessory cells on outside	2.20 -4.85	3.20	2.65 -4.20	3.11	2.20 -3.85	3.12
		accessory cells on reverse side	2.75 -4.40	3.60	3.30 -4.40	3.51	3.30 -4.40	3.48
	hot bed nursery seedling	epidermal cells of midrib	4.00 -5.60	4.50	4.40 -7.21	5.67	4.50 -6.60	5.83
		epidermal cells on reverse side	3.30 -4.40	3.58	3.30 -4.40	3.56	2.75 -4.40	3.48
		motor cells	2.20 -3.30	2.99	2.75 -3.30	3.02	2.10 -2.75	2.22
		long and short cells I	3.30 -4.40	3.48	3.30 -4.40	3.75	3.30 -4.40	3.71
		long and short cells II	3.30 -4.40	3.77	3.30 -4.40	3.71	3.30 -4.95	4.13
		accessory cells on outside	2.20 -3.85	3.12	2.55 -3.85	3.04	2.20 -3.30	2.88
		accessory cells on reverse side	2.20 -4.20	3.12	2.85 -3.85	3.12	2.85 -4.40	3.12
3	ordinary nursery seedling	epidermal cells of midrib	4.40 -3.50	6.54	4.95 -6.60	5.63	4.40 -6.60	5.13
		epidermal cells on reverse side	2.75 -4.95	4.32	3.30 -4.40	3.53	2.75 -4.40	3.82
		motor cells	3.30 -4.40	3.73	3.30 -3.85	3.43	3.30 -4.40	3.44
		long and short cells I	3.30 -4.40	3.66	3.30 -3.85	3.65	3.30 -4.40	3.82
		long and short cells II	3.30 -4.95	3.63	3.30 -4.40	3.98	3.30 -4.40	3.91
		accessory cells on outside	2.20 -4.95	3.21	3.30 -4.40	3.61	3.30 -4.40	3.43
		accessory cells on reverse side	2.75 -4.40	3.61	2.75 -4.40	3.67	3.30 -4.40	3.51

exp.	kind of seedlings	kind of cells	upper part		middle part		lower part	
			range	aver.	range	aver.	range	aver.
	hot bed nursery seedling	epidermal cells of midrib	4.40 μ -7.70	5.98 μ	4.40 μ -7.70	5.87 μ	5.58 μ -8.80	6.29 μ
		epidermal cells on reverse side	2.75 -4.95	4.23	3.30 -4.95	3.55	2.75 -4.40	3.64
		motor cells	2.50 -3.50	3.11	3.30 -3.85	3.38	2.85 -3.85	3.11
		long and short cells I	2.75 -4.40	3.85	3.30 -3.85	3.56	3.30 -3.85	3.46
		long and short cells II	2.75 -4.95	3.64	3.85 -4.95	4.31	3.30 -4.40	3.71
		accessory cells on outside	2.75 -3.55	2.75	2.75 -3.85	3.30	3.30 -4.40	3.80
		accessory cells on reverse side	2.75 -4.40	3.55	2.75 -3.80	3.55	3.30 -3.85	3.51
4	ordinary nursery seedling	epidermal cells of midrib	4.75 -8.20	6.34	5.50 -8.20	6.99	5.13 -6.95	6.12
		epidermal cells on reverse side	2.95 -5.20	4.21	3.85 -4.40	4.10	2.75 -4.40	3.32
		motor cells	2.20 -4.40	3.76	2.30 -3.85	3.45	2.20 -2.75	2.41
		long and short cells I	3.30 -4.40	3.94	2.75 -4.40	3.64	3.30 -4.40	3.91
		long and short cells II	3.30 -4.40	3.84	2.75 -4.40	3.59	3.30 -4.40	3.93
		accessory cells on outside	2.20 -4.85	3.32	2.65 -4.20	3.31	2.20 -3.85	2.98
		accessory cells on reverse side	2.75 -4.40	3.60	3.30 -4.40	3.85	3.30 -4.40	3.69
	hot bed nursery seedling	epidermal cells of midrib	4.00 -5.60	4.85	4.40 -7.21	5.99	5.13 -6.95	5.88
		epidermal cells on reverse side	2.95 -4.40	3.86	3.85 -4.40	4.10	2.75 -4.40	3.16
		motor cells	2.20 -3.30	2.85	2.75 -3.30	3.11	2.20 -2.75	2.37
		long and short cells I	3.30 -4.40	3.95	2.75 -4.40	3.55	3.30 -4.40	3.88
		long and short cells II	3.30 -4.40	3.87	2.75 -4.40	3.61	3.30 -4.40	3.86
		accessory cells on outside	2.20 -3.85	2.96	2.55 -3.85	3.09	2.20 -3.30	2.76
		accessory cells on reverse side	2.20 -4.20	3.01	2.85 -3.85	3.20	2.85 -4.40	3.12

As will be seen in the table, the outer walls of the epidermal cells of mid-rib and of the reverse side are thickest among all the kinds of epidermal cells irrespectively of the kinds of seedlings. But on comparing their thickness in each same kind of epidermal cells and in the same part of the leaf between the two kinds of seedlings, one sees that the outer wall of the hot bed nursery seedlings are generally thinner than those of the ordinary bed seedlings though there are some fluctuations. Moreover it is noteworthy that such relations are quite certain in the case of the motor cells, through which the fungus enters the rice plant most frequently.

b) Silicated epidermal cells: The number of silicated epidermal cells in a unit area (1 mm^2) of the leaves was calculated from the observations on ten optical fields for each material. The results are summarized in table 5.

As will be seen in the table, regardless of the kind of epidermal cells and the kind of the seedlings the number of silicated cells was greatest on the upper part of the leaf, and diminished in order on the middle part and the lower part. But comparison of the number of silicated motor cells in the same part of the leaf between the two kinds of seedlings, shows that they are clearly fewer in the hot bed nursery seedlings than in the ordinary bed seedlings, while as to the long and short cells the relations are often the reverse.

On summarizing the results of this section, it is concluded at least that the thickness of the outer walls of the motor cells are thinner in the hot bed nursery seedlings, which are rather susceptible to blast disease, than it is in the ordinary nursery seedlings, which are rather resistant to blast disease, and also the silification of the outer walls of the motor cells are fewer in the former than in the latter. It is noteworthy that the blast fungus penetration into the rice plant occurs more often through the motor cells than by any other route.

3. Chemical analysis of the ashes of the two kinds of seedlings.

It is certain that such morphological characters as examined above are concerned with the penetration of the blast fungus into the rice plant, but the proliferation of penetrated fungus inside the rice plant seems rather to be concerned with the chemical components in the rice plants. Therefore in the following, the contents of organic matter, inorganic matter and moisture are determined on both kinds of

TABLE 5. Number of silicated epidermal cells on both kinds of seedlings.

exp.	kind of the seedlings	parts of the leaf	motor cells	long cells	short cells	total
1	ordinary nursery seedling	upper part	9.44	0.69	0.12	10.25
		middle part	2.79	0.23	0.11	3.13
		lower part	0.52	0.08	0	0.60
		total	12.75	1.00	0.23	13.98
	hot bed nursery seedling	upper part	1.65	2.36	0.18	4.19
		middle part	0.19	0.86	0.05	1.10
		lower part	0.02	0.05	0	0.07
		total	1.86	3.27	0.23	5.36
2	ordinary nursery seedling	upper part	8.78	1.55	0.21	10.54
		middle part	2.43	0.41	0.10	2.94
		lower part	0.22	0.08	0	0.30
		total	11.43	2.04	0.31	13.78
	hot bed nursery seedling	upper part	1.84	3.43	0.15	5.47
		middle part	0.23	0.48	0.11	0.82
		lower part	0.11	0.04	0	0.15
		total	2.18	4.00	0.26	6.44
3	ordinary nursery seedling	upper part	14.55	11.52	0.86	26.93
		middle part	9.86	2.05	0.39	12.39
		lower part	8.83	1.08	0.26	10.17
		total	33.24	14.65	1.51	49.40
	hot bed nursery seedling	upper part	6.80	12.98	0.98	20.76
		middle part	2.11	1.37	0.46	3.94
		lower part	1.34	0.95	0.36	2.65
		total	10.25	15.30	1.80	27.35
4	ordinary nursery seedling	upper part	12.45	11.63	1.52	25.60
		middle part	8.87	3.43	1.00	13.30
		lower part	5.31	1.24	0.61	7.16
		total	26.63	16.30	3.12	46.06
	hot bed nursery seedling	upper part	5.22	3.15	0.21	8.58
		middle part	1.54	1.23	0.11	2.88
		lower part	0.95	0.86	0.09	1.90
		total	7.71	5.24	0.41	13.36

seedlings which are raised in the hot bed nursery and in the ordinary nursery. The materials are the same as those used in the preceding experiments. Moisture content was measured by drying the materials in 95°-98°C thermodesiccator, and the dried materials were burned to ash in 5000°C Muffle furnace. The reduction in weight by the burning of the dried material was treated as the organic matter. Then the ash was dissolved in concentrated hydrochloric acid and prepared for the determination of P, K, Ca, Mg, Si by the usual method. Table 6 gives the results on the contents of moisture, organic matter and inorganic matter.

TABLE 6. Content of organic- and inorganic matters and moisture in the two kinds of seedlings.

	hot bed nursery seedling		ordinary nursery seedling	
	percentage of fresh weight	percentage of dry weight	percentage of fresh weight	percentage of dry weight
organic matter	15.1	83.8	15.1	71.8
inorganic matter (ash)	2.9	16.2	5.9	28.2
moisture content	82.0	—	79.0	—

As shown in the table, the moisture content of the hot bed nursery seedlings was higher than that of the ordinary nursery seedlings and the fresh weight percentage of the organic matters were the same in both kinds of seedlings. Therefore the fresh weight percentage of inorganic matters is higher in the ordinary nursery seedlings than in the hot bed nursery seedlings. Thus as clearly known from the results on the dry weight percentage, the hot bed nursery seedlings contain more organic matter and less inorganic matter on comparison with the ordinary nursery seedlings.

The results of the chemical analysis on the ashes of the two kinds of seedlings are given in table 7.

As will be seen in the table, every kind of inorganic matter is smaller in content in the hot bed nursery seedlings than in the ordinary nursery seedlings, but the content of other inorganic matters in the table, which were not determined directly, are somewhat higher in the former than in the latter. These other inorganic matters may be Na, SO₄, Cl etc.. As clearly shown in the percentage composition of the ash, the most part of the ash is occupied by silica in both sorts

TABLE 7. Content of each kind of inorganic matter in the two sorts of the seedlings.

	hot bed nursery seedling		ordinary nursery seedling	
	percentage of dry weight	percentage composition of ash	percentage of dry weight	percentage composition of ash
SiO ₂	7.9	48.3	16.4	58.3
P ₂ O ₅	1.4	8.8	2.1	7.4
CaO	0.9	5.3	2.7	9.5
Mg	0.4	2.6	0.8	2.9
K ₂ O	2.0	12.4	3.2	11.3
other inorganic matter	3.6	22.6	3.0	10.6

of seedlings. And therefore the differences in the silica content between the two kinds of seedlings draw one's attention particularly. It must be noticed that silica is concerned greatly with the hardness of the rice plant tissue

4. The content of various kinds of nitrogen in the both kinds of seedlings.

The contents respectively of the various kinds of nitrogen both in the hot bed nursery seedlings and ordinary nursery seedlings were determined. Samples used in these experiments were the same as those which were used in the inoculation experiments above described. They were collected just previous to the inoculations. About 10 grams of the seedlings were collected from the two experimental sections by cutting the stalk just above the roots. Their fresh weights were accurately determined. The procedures of separation and determination of the various fractions of nitrogen were as follows; the samples were first roughly ground in a mortar, then they were transferred into beaker and reasonable quantities of 3% acetic acid and water were added. The acidified mixture was heated to boiling for about half an hour, and after cooling, the mixture was filtrated by suction funnel. The residues were washed twice with some quantity of hot 3% acetic acid, and were employed for the determination of protein nitrogen by KJELDAHL'S method. Filtrate which was combined with the two washings was employed for the determination of total soluble nitrogen, amino acid nitrogen, amide nitrogen, soluble protein nitrogen, basic

nitrogen, ammonium nitrogen and nitrate nitrogen. For the determination of the total soluble nitrogen in those filtrate the nitrate nitrogen was reduced first by treatment with reduced iron according to PUCHER et al⁽⁶⁰⁾ and then the total nitrogen was determined by KJELDAHL'S method. The sum of the protein nitrogen and the total soluble nitrogen makes the total nitrogen in the samples. Some aliquots of the filtrate were carefully concentrated on the water bath and were employed for the determination of alpha amino nitrogen by the VAN SLYKE'S method after subtracting the ammonium nitrogen. For the determination of ammonium nitrogen some aliquots of the filtrate were weakly alkalized (pH 8.0) with magnesium oxide and they were subjected vacuum distillation at 40°C. For the determination of amide nitrogen, some aliquots of the filtrate were treated with some quantity of 30% sulfuric acid and they were heated over a boiling water bath for 3 hours; then the ammonia produced was determined in the same way as described above. The values which were obtained by subtracting the ammonium nitrogen previously determined from the results just obtained were multiplied. Thus the content of the amide nitrogen was obtained. Nitrate nitrogen was determined by the phenol-disulfonic acid method after subtracting soluble protein from the filtrate according to ROBIN C. BURREL & THOMAS G. PHILLIPS⁽⁶¹⁾. Some aliquots of the filtrate were treated with some quantities of lead acetate (10 g of lead acetate in 90 cc of 1.5% acetic acid) and allowed to stand overnight. The precipitates were separated from the supernatant liquid by centrifugation (8000-10000 r.p.m.), and they were washed with some quantity of 80% alcohol. The supernatant liquid combined with the alcohol washings were then treated with some quantities of mercuric acetate (10 g of mercuric acetate in 100 cc of 2.0% acetic acid). The mixture was allowed to stand overnight, and the precipitate was removed by centrifugation (8000-10000 r.p.m.). The two precipitates were combined and were used for the nitrogen determination by the KJELDAHL process. This fraction was classified as soluble protein nitrogen. After removal of the lead, mercury and also amide by distillation, the supernatant was acidified with H₂SO₄ and treated with phosphotungstic acid. The mixture was allowed to stand overnight and the precipitate was removed by filtration. The precipitate was analyzed for nitrogen by KJELDAHL'S method. This fraction of nitrogen was classified as basic nitrogen.

Indicator which was used for the titration was a mixture of

methyl red and methylene blue. Blank tests were performed sometimes. All the determinations were completed in two days after the sampling, while the determination of the ammonium nitrogen was completed on the day of the sampling.

The total nitrogen contents in the two groups of seedlings is shown in table 8. The figures represent mg of nitrogen in the seedlings of fresh weight per 1 g.

TABLE 8. Total nitrogen in the two kinds of seedlings.

	exp. 1	exp. 2	exp. 3	exp. 4
ordinary seedlings	11.7 mg	10.8 mg	8.97 mg	9.32 mg
hot bed nursery seedlings	15.9 "	13.6 "	10.33 "	10.88 "

As will be seen in the table, the hot bed nursery seedlings contain a greater amount of total nitrogen than the ordinary nursery seedlings. The total nitrogen is divided into two parts, protein nitrogen and total soluble nitrogen. The contents of each nitrogen are recorded in table 9.

TABLE 9. Content of protein- and soluble-nitrogen in the two kinds of seedlings.

exp.	fractions of soluble nitrogen	ordinary nursery seedling		hot bed nursery seedling	
		mg per 1 g fresh weight	percentage composition of total nitrogen	mg per 1 g fresh weight	percentage composition of total nitrogen
1	protein-N	7.10 mg	60.68 %	8.10 mg	50.94 %
	soluble-N	4.60 "	39.32 "	7.80 "	49.06 "
2	protein-N	6.80 "	62.96 "	7.50 "	55.15 "
	soluble-N	4.00 "	37.04 "	6.10 "	44.85 "
3	protein-N	7.18 "	80.04 "	6.52 "	63.12 "
	soluble-N	1.79 "	19.96 "	3.81 "	36.88 "
4	protein-N	7.51 "	80.60 "	6.43 "	59.20 "
	soluble-N	1.81 "	19.40 "	4.45 "	40.80 "

As shown in the table the seedlings raised on the hot bed nursery always contain greater amount of total soluble nitrogen than the seedlings raised on the ordinary nursery; however the percentages of protein nitrogen component in the total nitrogen are always lower in

the former seedlings than in the latter seedlings while the percentage of total soluble nitrogen are always higher at the former than at the latter.

The total soluble nitrogen was divided into fractions and their amounts were determined as in the following. The results are summarized in table 10.

TABLE 10. Content of each kind of soluble nitrogen in the two kinds of seedlings.

exp.	fractions of soluble nitrogen	ordinary nursery seedling		hot bed nursery seedling	
		mg per 1 g fresh weight	percentage composition of total soluble nitrogen	mg per 1 g fresh weight	percentage composition of total soluble nitrogen
1	amide N	0.24 mg	5.22 %	0.60 mg	7.69 %
	ammonium N	0.06 "	1.30 "	0.12 "	1.53 "
	nitrate N	0.05 "	1.09 "	0.44 "	5.63 "
	other soluble N	4.25 "	92.39 "	6.64 "	85.14 "
2	amide N	0.32 "	8.00 "	0.48 "	7.87 "
	ammonium N	0.09 "	2.25 "	0.11 "	1.80 "
	nitrate N	0.06 "	1.50 "	0.39 "	6.38 "
	other soluble N	3.53 "	88.25 "	5.12 "	83.95 "
3	soluble protein N	0.40 "	22.35 "	1.42 "	37.27 "
	α -amino N	0.84 "	46.93 "	1.53 "	40.16 "
	basic N	0.18 "	10.06 "	0.57 "	14.96 "
	amide N	0.23 "	12.85 "	0.18 "	4.72 "
	ammonium N	0.08 "	4.47 "	0.08 "	2.10 "
	nitrate N	0.02 "	1.12 "	0.01 "	0.26 "
4	soluble protein N	0.64 "	35.40 "	1.58 "	35.50 "
	α -amino N	0.71 "	39.20 "	1.55 "	34.80 "
	basic N	0.20 "	11.00 "	0.70 "	15.40 "
	amide N	0.19 "	10.50 "	0.25 "	5.60 "
	ammonium N	0.05 "	2.80 "	0.07 "	1.60 "
	nitrate N	0.04 "	2.20 "	0.05 "	1.10 "

In experiments 1 and 2, amide, ammonium and nitrate nitrogen were determined directly and the values which were given by sub-

tracting these three fractions from the total soluble nitrogen were classified as "other soluble nitrogen". The most part of this "other soluble nitrogen" may be soluble protein, α -amino and basic nitrogen. As will be seen in the table, those three fractions, ("other soluble nitrogen" in exps. 1 and 2) were always greater in amounts in the hot bed nursery seedlings than in the ordinary nursery seedlings. It is noticed that the greater part of the soluble nitrogen is supplied by these three fractions and amide, as clearly shown in the percentage composition of total soluble nitrogen in the table. On the other hand the contents of amide, ammonium and nitrate nitrogen seem to be widely variable even in the same kind of seedlings. In the exp. 3 the content of amide nitrogen and nitrate nitrogen in the hot bed nursery seedlings was rather lower than in the ordinary nursery seedlings and the contents of ammonium nitrogen were equal in the two kinds of seedlings, while in exps. 1 and 2 the relations were clearly the reverse.

On summarizing the results it is safely said that the hot bed nursery seedlings are apt to contain a greater amount of nitrogen in comparison with the ordinary nursery seedlings and moreover the contents of soluble nitrogen such as soluble protein nitrogen, α -amino nitrogen and basic nitrogen are always greater in the former seedlings than in the latter while the content of protein nitrogen is always less in the former.

5. Sugar contents in the two kinds of seedlings.

The differences in the fractions of nitrogen between the two kinds of seedlings may suggest the differences in their sugar contents, as the sugars are utilized for the synthesis of organic nitrogenous matters as their constitutional materials. In the following the sugar contents in the two kinds of seedlings were determined. Samples were the same as those used in the former experiments, and the same kinds of filtrates as used for the determination of total soluble nitrogen were employed for the measurements of the sugar content. The sugar contents in the original filtrate itself which were determined by micro-BERTRAND method were classified as reducing sugar. Then the filtrates were hydrolized with 25% HCl treatment, and the sugar contents in the solution which were determined by micro-BERTRAND method were classified as total sugar content. The differences of the two values give the amount of the non-reducing sugar. The total sugar content

in the two kinds of seedlings are given in the table 11.

TABLE 11. Total sugar in the two kinds of seedlings.

	exp. 1	exp. 2	exp. 3	exp. 4
ordinary nursery seedling	6.9 mg	5.2 mg	5.6 mg	6.3 mg
hot bed nursery seedling	2.0 "	4.6 "	2.8 "	2.1 "

As will be seen in the table, the sugar content in the hot bed nursery seedlings was always less than that in the ordinary nursery seedlings. The sugars found in the plant are considered to be the balance between the sugar amounts produced by the photosynthesis and the sugar amounts used by the plant as respiration or constitutional material. And it is supposed that the amount of sugar used as constitutional materials by the hot bed nursery seedlings are greater than those used by the ordinary nursery seedlings, because much organic nitrogenous matters are synthesized in the former seedlings as known from the previous experiments. On the other hand the sugars produced by the photosynthesis are thought to be greater in the latter seedlings

TABLE 12. Content of reducing- and non-reducing sugar in the two kinds of seedlings.

exp.	fractions of sugar	ordinary nursery seedling		hot bed nursery seedling	
		mg per 1 g fresh weight	percentage composition of total sugar	mg per 1 g fresh weight	percentage composition of total sugar
1	reducing sugar	0.2 mg	2.9 %	1.2 mg	60.0 %
	non-reducing sugar	6.7 "	97.1 "	0.8 "	40.0 "
2	reducing sugar	1.8 "	34.6 "	2.4 "	52.2 "
	non-reducing sugar	3.4 "	65.4 "	2.2 "	47.8 "
3	reducing sugar	0.7 "	12.5 "	2.2 "	78.6 "
	non-reducing sugar	4.9 "	87.5 "	0.6 "	21.4 "
4	reducing sugar	0.9 "	14.3 "	1.6 "	76.2 "
	non-reducing sugar	5.4 "	85.7 "	0.5 "	23.8 "

on comparison with the former, because the hot bed nursery seedlings are covered with cover frame and so are apt to receive comparatively less light. These considerations may explain why the sugar content is rather less in the hot bed nursery seedlings.

Then the sugars were divided into two parts, reducing sugar and non-reducing sugar; the results are summarized in table 12.

As will be seen in the table, reducing sugar supplies the greater part of the total sugar in the hot bed nursery seedlings, while in the ordinary nursery seedlings the greater part of the total sugar is supplied by the non-reducing sugar. Thus the hot bed nursery seedlings contain the greater amount of reducing sugar and the less amount of non-reducing sugar on comparison with the ordinary nursery seedlings. The fact, that in the former seedlings the greater part of the sugar is supplied by the reducing sugar, may suggest the transfer of the sugar into a simple form in preparation for the greater consumption in the hot bed nursery seedlings.

6. Conclusion.

It is true that the temperature and the moisture are largely variable in the hot bed nursery according to the weather or to the way of its management, but they are apt to rise to unnecessarily high points in spite of the most careful management. The light which comes through the linseed oil paper of the cover frame into the hot bed nursery is apt to decrease with the days as the paper becomes weather beaten and the dust becomes attached on it. Moreover the hot bed nursery is a kind of land nursery in its soil conditions and it is considerably different from the ordinary nursery bed in its soil moisture. Seedlings grown in such hot bed nursery are apt to be elongated in their internode compared with the ordinary nursery bed seedlings. The moisture content is higher and the content of inorganic matter, such as SiO_2 , P_2O_5 , CaO , Mg , and K_2O , is lower in the former seedlings than in the latter while there are not so great differences in their content of organic matter. The inoculation experiment of the blast fungus indicates that the seedlings raised in the hot bed nursery are apt to be more severely attacked by the fungus, producing more numerous, larger and often acute type diseased spots.

The cause of such differences of susceptibility to blast fungus disease is partly attributed to the differences of such morphological characters as the thickness of outermost layer and the silification of rice epidermis.

On comparing such morphological characters between the two kinds of seedlings, one finds that in respect to thickness the outer layer of the motor cells at least is clearly less in the hot bed nursery seedlings than in the ordinary nursery seedlings. And as for the silification of the epidermal cells the former seedlings are inferior to the latter, especially in the motor cells, while the silica content in the former is lower than that in the latter as described above. As is well known, the blast fungus penetrates into rice plants most frequently through the motor cells and therefore the results above summarized will explain the fact that more numerous lesions appear in the hot bed nursery seedlings on inoculation experiment. But those results cannot explain the fact that the lesions produced on the hot bed nursery seedlings are larger and also most of them are acute type ones. It can be supposed that the proliferation of blast fungus which has penetrated into the rice plant is related with the contents of chemical components in the rice plant. The hot bed nursery seedlings contain greater amount of total nitrogen than the ordinary nursery seedlings. Dividing these total nitrogen between protein and soluble nitrogen, the difference in the soluble nitrogen content is remarkable while the difference in protein nitrogen is slight. As for the sugar, the content in the hot bed nursery seedlings is lower than that in the ordinary nursery seedlings. This may be owing partly to the presence of the frame cover. On dividing the sugars into the reducing and non-reducing sugar, the content of the former is found to be greater, while the content of the latter is less in the hot bed nursery seedlings than in the ordinary nursery seedlings.

Chapter II

Growing stages of rice plants and their susceptibility to the blast disease.

It is often observed in the fields that the blast susceptibility of the rice plant may vary according to the progress of their growing stages. In order to ascertain such relations and to know the cause of the susceptibility, the following experiments were carried on employing as material rice plants which were grown in the pot, in water-culture solution and in the paddy field. The growing stages were divided into the following six, viz., (1) the seedling stage, (2) the elongation stage (when the transplanted rice plants begin to grow

vigorously, increasing their height and beginning to make tillers), (3) the ear formation stage (when the tillering is most vigorous and the young ears of about 5 mm length are beginning to develop), (4) boot stage (when the ear grows to about 10 cm length and its presence is recognizable from outside, though it does not appear yet), (5) flowering stage, and (6) the ripening stage (about 2 weeks after the flowering).

1. Inoculation experiments.

Exp. 1: Rice seedlings raised in the ordinary nursery were transplanted to soil in WAGNER pots (about 17 cm in diameter). Previous to the transplantation the soil in one pot was mixed with 1.5 g ammonium sulphate, 2.8 g superphosphate of lime and 0.7 g potassium sulphate. Soon after the transplantation of the seedlings the rice plants began to increase in height; their growth was most vigorous in the elongation stage. They began to tiller from elongation stage and the number of tillers continued to increase till the boot stage. The average leaf length continued to increase vigorously till the boot stage and then

TABLE 13. Inoculations of the blast fungus on the rice plants of various growing stages.

	seedling stage	elongation stage	ear formation stage	boot stage	flowering stage	ripening stage	
height	9.5 cm	23.6 cm	40.0 cm	75.0 cm	94.7 cm	101.7 cm	
number of tillers	—	3	9	12	12	12	
average leaf length	6.5 cm	17.5 cm	24.7 cm	27.9 cm	27.5 cm	27.4 cm	
number of leaves examined	310	273	232	270	315	103	
total number of lesions	68	8	43	27	25	7	
average number of lesions per leaf	0.22	0.03	0.19	0.10	0.08	0.07	
average number of lesions per 100 cm length of leaves	3.4	0.2	0.8	0.4	0.3	0.2	
length of lesions	max.	0.8 cm	0.8 cm	2.0 cm	1.5 cm	0.8 cm	0.8 cm
	min.	0.1 "	0.1 "	0.3 "	0.3 "	0.1 "	0.1 "
	aver.	0.4 "	0.5 "	1.4 "	1.1 "	0.4 "	0.5 "
type of lesions	brown spot, chronic	brown spot, chronic	chronic, acute	chronic, acute	chronic	chronic	

ceased to increase. At each growing stage some rice plants were carried into the inoculation box and were inoculated by spraying the spore suspension of the blast fungus after their height, number of tillers, etc. were recorded. The diseased lesions developed were examined at the 10th day after the inoculation. The results are summarized in table 13.

As will be seen in the table the rice plants are attacked most severely by the blast fungus in two growing stages, the ear formation stage and the boot stage. In both growing stages comparatively greater number of lesions are observed and their size is largest. Moreover there are seen some acute type lesions besides the chronic type lesions. And it should be noticed that the acute type lesion is never seen in the other growing stages. On the other hand, the rice plants are most resistant to blast fungus during the elongation stage, producing comparatively fewer and smaller lesions as a result of the inoculation. Furthermore there are to be seen some brown spot lesions, which are never seen in the other growing stages.

Exp. 2: As shown in the previous chapter, the seedlings which are raised in the hot bed nursery are more susceptible to the blast fungus in comparison with the ordinary nursery seedlings. And it is important as well as interesting to know if such characters of the seedlings raised in the hot bed nursery remain long after the transplantation. Therefore seedlings which were raised in the hot bed nursery were transplanted onto soil in pots and inoculated with the blast fungus at each growing stage. The state of the growth of the plants after the transplantation was not so different from what was noted in the case of experiment 1. The results are given in table 14. As will be seen in the table, the rice plant remarkably increases its resistance to the blast disease at elongation stage as compared with seedling stage. The number of lesions becomes less, the size of the lesions becomes smaller and the brown spot type lesions are seen here and there, though the chronic type lesions are dominant. However, on comparing with the rice plant at the same stage in experiment 1 the plants in this experiments seem to be a little higher in their susceptibility to the blast disease.

On the other hand the rice plants become again increasingly blast susceptible at the ear formation stage and also at the boot stage, but this the same tendency as was seen in exp. 1. It is also same as in exp. 1 that the plant leaves decrease again in blast susceptibility during

TABLE 14. Inoculations of the blast fungus on the rice plants of various growing stages.
(hot bed nursery seedling)

	seedling stage	elongation stage	ear formation stage	boot stage	flowering stage	ripening stage	
height	9.8 cm	24.2 cm	38.5 cm	72.1 cm	91.8 cm	99.5 cm	
number of tillers	—	4	9	12	12	12	
average leaf length	6.8 cm	15.6 cm	23.6 cm	27.5 cm	27.8 cm	28.1 cm	
number of leaves examined	305	290	248	288	290	240	
total number of lesions	146	12	45	35	17	12	
average number of lesions per leaf	0.48	0.04	0.18	0.12	0.06	0.05	
average number of lesions per 100 cm length of leaves	7.0	0.27	0.77	0.44	0.21	0.18	
length of lesions	max.	1.5 cm	1.0 cm	2.0 cm	1.5 cm	0.8 cm	1.0 cm
	min.	0.2 "	0.1 "	0.3 "	0.3 "	0.1 "	0.1 "
	aver.	0.8 "	0.6 "	1.4 "	1.0 "	0.5 "	0.6 "
type of lesions	chronic, acute	brown spot, chronic	chronic, acute	chronic, acute	chronic	chronic	

and after the flowering stage. Thus it is certain that high blast susceptibility of the hot bed nursery seedlings does never continue long after their transplantation in the field, although the blast susceptibility of the rice plants in the elongation stage is a little higher on comparison with the blast susceptibility in the same growing stage of exp. 1 in which the ordinary nursery seedlings were used.

Exp. 3: In this experiment the rice plants were cultured in water culture solutions. Seeds were sown on quartz sand which had been thoroughly washed previously, and the seedlings were transplanted in the culture solutions at the time when second leaf began to develop. The employed culture solution was recommended by Dr. ISHIZUKA⁽³⁹⁾; its prescription was as follows: NH_4HO_3 114.3 mg/1ℓ, KH_2PO_4 53.6 mg/1ℓ, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 117.2 mg/1ℓ, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 183.4 mg/1ℓ, KCl 2.3 mg/1ℓ, traces of FeCl_3 , MnSO_4 , and silica sol. The culture solution was changed generally once a week, but it was changed once each five days from the boot stage to the flowering stage as the absorption of the solution

by the plant was most vigorous. In this culture solution the rice plants grew quite favorably through all the growing stages, although their height and the number of tillers were a little inferior to those of the soil cultured rice plants. Inoculations of the blast fungus at each growing stage were carried on in the same way as in the previous experiments. As will be noted in table 15, the diseased lesions developed on the plants both in the ear formation stage and in the boot stage were largest in size, including acute type lesions which were never seen on the plants in the other growing stages. Thus it may be safely said that the rice plants are most susceptible to the blast disease during the ear formation and boot stages. On the other hand lesions developed on the plants in the elongation stage were smallest in size and moreover include considerable numbers of the brown spot type one, which are thought to develop on the blast resistant rice plant. It may, therefore, safely be said that the elongation stage is the most blast resistant stage of all rice growing stages, although the number of the lesions developed was remarkably great in this experiment.

TABLE 15. Inoculations of the blast fungus on the rice plants in various growing stages.
(water culture)

	elonga- tion stage	ear for- mation stage	boot stage	flowering stage	ripening stage	
height	25.0 cm	51.8 cm	60.3 cm	73.5 cm	75.0 cm	
number of tillers	3	8	8	8	8	
average leaf length	9.4 cm	15.7 cm	18.5 cm	18.8 cm	18.8 cm	
number of leaves examined	37	80	89	116	125	
total number of lesions	32	88	44	52	10	
average number of lesions per leaf	0.86	1.10	0.49	0.45	0.08	
average number of lesions per 100 cm length of leaves	9.2	7.0	2.7	2.2	0.5	
length of lesions	max.	0.5 cm	1.2 cm	1.5 cm	1.0 cm	0.7 cm
	min.	0.1 "	0.1 "	0.2 "	0.2 "	0.2 "
	aver.	0.18 "	0.44 "	0.55 "	0.35 "	0.35 "
type of lesions	brown spot, chronic	chronic, acute	chronic, acute	chronic	chronic	

Exp. 4: Rice seedlings raised on the ordinary nursery were transplanted to a 1/125 acre paddy field of the Hokkaido University Farm; the diseased lesions which were developed by the natural infection of the blast fungus were examined at each growing stage. The results of the observations in 1950 are described in the following. The diseased lesions on the rice plants were seen first in the beginning of July when the rice plants were entering to the ear formation stage. In the ear formation stage 35 rice plants selected at random were pulled out and the diseased lesions on their leaves were examined. Then at about five days before reaching each of the other growing stages 30-50 rice plants were marked at random and the leaves on which the diseased lesions were seen were cut off. About 10 days later the newly developed diseased lesions were examined. The results of these investigations are shown as the infections of each stage respectively in table 16.

TABLE 16. Inoculation of the blast fungus on the rice plants in various growing stages.
(Paddy field)

	elonga- tion stage	ear for- mation stage	boot stage	flowering stage	ripening stage
height	26.7 cm	53.8 cm	70.3 cm	83.5 cm	91.1 cm
number of tillers	3	5	6	6	6
average leaf length	18.4 cm	23.7 cm	25.2 cm	25.4 cm	25.5 cm
number of leaves examined	340	295	311	321	280
total number of lesions	0	27	31	13	9
average number of lesions per leaf	0	0.09	0.10	0.04	0.03
average number of lesions per 100 cm length of leaves	0	0.35	0.40	0.15	0.11
length of lesions	max.	1.2 cm	1.5 cm	1.2 cm	1.0 cm
	min.	0.2 "	0.2 "	0.1 "	0.1 "
	aver.	0.64 "	0.77 "	0.43 "	0.39 "
type of lesions	—	chronic, acute	chronic, acute	chronic	chronic

As will be seen in the table, the lesions found both at the ear formation stage and the boot stage were most numerous and also

largest in size, while there were seen some acute type ones besides the chronic type ones. Considering from these facts it is clear as in the previous experiments that the rice plants are most susceptible to blast disease at the two stages above mentioned. No diseased lesions were found in the elongation stage. Referring to the previous experiments, it is certain that the rice plants at the elongation stage are quite resistant to the blast disease, but a part of the reason why no lesions were found at that stage may lie in the scantiness of the dispersing fungus spores in the air because of the unsuitable climate for spore formation in this season in Hokkaido.

Exp. 5: In this experiment the rice plants were grown in the culture solutions in which three times as much nitrogen as in the standard solutions was applied. As in the previous experiments, the inoculation of the blast fungus was carried on at each growing stage. On comparing the growth of the rice plants in this culture solution with the growth in the standard solution (experiment 3), both the height and the average leaf length in each growing stage are seen to be greater in the former but no noticeable differences were seen in the number of tillers (table 17).

Examining the number of the diseased lesions which were developed by the inoculation at each growing stage, one sees that they are rather fewer on the rice plant leaves both in the flowering stage and in the ripening stage. Examining the type of lesions, the acute type ones are generally dominant all through the growing stages, and it must be noticed that all the lesions which are developed on the rice plants from the elongation stage till the boot stage are acute type, while there are seen some chronic type lesions as well as the acute type ones on the plant leaves in both the flowering stage and the ripening stage. Examining the size of the lesions, those which developed on the rice plant both in the ear formation stage and in boot stage are largest and those which developed in the elongation stage are next to them, while those developed on the leaves in the flowering stage and in the ripening stage are smallest.

Thus it may be concluded that the rice plants in the elongation stage lose their characteristics of the higher blast resistance as a result of the supply of the high level nitrogen, while the rice plants are as well most susceptible to the blast infection at the two growing stages of ear formation and boot stage. On the other hand the leaves of the rice plants become rather resistant to the blast disease during

TABLE 17. Inoculation of the blast fungus on the rice plants of various growing stages.
(high level nitrogen supply)

	elonga- tion stage	ear for- mation stage	boot stage	flowering stage	ripening stage	
height	25.7 cm	47.5 cm	82.1 cm	93.5 cm	103.6 cm	
number of tillers	3	8.2	9.0	8.8	9.2	
average leaf length	23.7 cm	20.8 cm	22.8 cm	28.2 cm	33.3 cm	
number of leaves examined	232	240	230	221	220	
total number of lesions	44	45	42	25	22	
average number of lesions per leaf	0.19	0.19	0.18	0.11	0.10	
average number of lesions per 100 cm length of leaves	0.8	0.9	0.8	0.4	0.3	
length of lesions	max.	1.5 cm	2.0 cm	2.0 cm	1.5 cm	1.5 cm
	min.	0.3 "	0.3 "	0.3 "	0.2 "	0.2 "
	aver.	0.93 "	1.30 "	1.20 "	0.70 "	0.68 "
type of lesions	acute	acute	acute	chronic, acute	chronic, acute	

and after the flowering stage, this is the same result as was noted in the experiments with the standard amount nitrogen supply.

Summarizing the five experiments above described, it may be concluded as follows; the rice plants are quite resistant against the blast infection in the elongation stage, but when they grow up to the ear formation stage and the boot stage the rice plants become remarkably susceptible to the disease. In the flowering stage and in the ripening stage the leaves become rather resistant to the disease. It was shown in the previous chapter that the rice seedlings raised on the hot bed nursery are more susceptible to the blast disease on comparison with those raised on the ordinary nursery but it is certain from the experiments described in this chapter that this character at the seedling stage never continues long after their transplantation in the field. On the other hand, if the rice plants were supplied with high level nitrogen, they become rather susceptible to the blast disease in the elongation stage to be almost equally susceptible as the plant in the ear formation stage. In other words the elongation stage loses the characteristic of the most resistant stage of all the growing stages

by the supply of the high level nitrogen, but even in this case the leaves of the rice plant in the flowering stage and in the ripening stage are comparatively resistant to the disease.

2. Comparison of some morphological characters of the rice plants in different growing stages.

In this section the results of the measurement of some morphological characters, such as the thickness of the outer wall and the silification of the epidermal cell of the rice plants in each growing stage are described. On the rice plants before the boot stage 2nd leaves from above and on the rice plants after the flowering stage 1st leaves from above are employed for the measurement. Methods of measurements and classification of the epidermal cells etc., are quite the same as in the previous chapter. The measurements were carried on nearly all the rice plants, which were used in the inoculation experiments from experiment 1 to experiment 4, but the results are almost the same through all these experiments and therefore in the followings the results on the experiment 1 are described as representative. In experiment 5 in which the rice plant supplied with excess amount nitrogen were used, the number of silicated epidermal cells was smaller than in other experiments, but the data are omitted here because more detailed data are shown in the following chapter.

a) The thickness of the outer walls of epidermal cells: The results of the measurement are shown in table 18. In the same growing stage the thickness of the outer wall varies according to the kind of epidermal cells. Epidermal cells of midrib and epidermal cells on reverse side are thickest in its outer wall in all kinds of epidermal cells but it seems that the blast fungus never comes into the rice plant through these epidermal cells. Comparing the outer wall thickness of each kind of epidermal cells between the different growing stages, it is clear that the thickness increases with the progress of the growing stages till the boot stage. That is to say, in the elongation stage, when the rice plants are most resistant against the blast disease through all growing stages, the thickness of the outer wall is less than that of the rice plant in ear formation stage when the plants are most susceptible. Thus it is rather difficult to discover any relations between the fluctuation of blast susceptibility of the rice plant which is shown with the progress of growing stages and the thickness of the outer wall of the plant epidermis.

TABLE 18. Thickness of the outer walls of the epidermal cells on the rice plants in the various growing stages.

growing stages	kinds of epidermal cells	upper part		middle part		lower part	
		range	aver.	range	aver.	range	aver.
seedling stage	epidermal cells of midrib	4.40 -8.80	6.54	4.95 -6.60	5.65	4.40 -6.60	5.13
	epidermal cells on reverse side	2.75 -4.95	4.32	3.30 -4.40	3.53	2.75 -4.40	3.82
	motor cells	3.30 -4.40	3.73	3.30 -3.85	3.43	3.30 -4.40	3.44
	long and short cells I	3.30 -4.40	3.66	3.30 -3.85	3.67	3.30 -4.40	3.83
	long and short cells II	3.30 -4.95	3.63	3.30 -4.40	3.98	3.30 -4.40	3.91
	accessory cells on outside	2.20 -4.95	3.21	3.30 -4.40	3.61	3.30 -4.40	3.43
	accessory cells on reverse side	2.75 -4.40	3.61	2.75 -4.40	3.67	3.30 -4.40	3.51
elongation stage	epidermal cells of midrib	4.40 -8.80	6.61	4.40 -8.80	5.93	4.40 -7.20	5.66
	epidermal cells on reverse side	2.88 -5.04	4.51	3.36 -4.40	3.73	2.88 -4.32	3.58
	motor cells	3.12 -4.55	3.75	3.12 -4.33	3.62	3.12 -4.33	3.55
	long and short cells I	3.36 -4.33	3.66	3.36 -4.33	3.71	3.36 -4.56	3.75
	long and short cells II	3.36 -4.33	3.66	3.36 -4.33	3.59	3.36 -4.33	3.81
	accessory cells on outside	2.16 -4.33	3.18	3.36 -4.33	3.58	3.30 -4.33	3.58
	accessory cells on reverse side	2.64 -4.33	3.56	2.64 -4.33	3.62	2.64 -4.33	3.54
ear formation stage	epidermal cells of midrib	4.55 -8.80	6.85	4.40 -7.20	6.02	4.40 -7.20	5.61
	epidermal cells on reverse side	3.12 -5.05	4.62	3.36 -5.04	3.88	3.12 -4.32	3.80
	motor cells	3.12 -4.55	3.77	3.12 -4.33	3.72	3.12 -4.33	3.63
	long and short cells I	3.12 -4.33	3.66	3.36 -4.33	3.68	3.36 -5.04	3.81
	long and short cells II	3.36 -4.33	3.72	3.36 -5.04	3.69	3.36 -5.04	3.94
	accessory cells on outside	2.16 -4.33	3.32	3.12 -4.33	3.83	3.12 -4.33	3.55
	accessory cells on reverse side	2.64 -4.33	3.71	2.64 -4.33	3.72	2.64 -4.33	3.60

growing stages	kinds of epidermal cells	upper part		middle part		lower part	
		range	aver.	range	aver.	range	aver.
boot stage	epidermal cells of midrib	5.04 -8.80	7.32	5.04 -8.80	6.20	4.40 -7.20	5.30
	epidermal cells on reverse side	3.12 -7.20	5.76	3.36 -4.40	4.02	3.36 -5.04	4.12
	motor cells	3.36 -5.04	4.32	3.12 -5.04	4.12	3.12 -4.33	3.76
	long and short cells I	3.36 -5.04	3.84	3.36 -4.33	3.81	3.36 -4.56	3.88
	long and short cells II	3.36 -5.04	3.98	3.36 -5.04	4.18	3.36 -5.04	4.14
	accessory cells on outside	2.40 -4.80	3.85	3.36 -5.04	4.19	3.30 -4.33	3.52
	accessory cells on reverse side	2.64 -4.80	4.00	3.36 -4.80	4.29	3.36 -4.80	3.65
flowering stage	epidermal cells of midrib	5.04 -8.80	7.32	5.04 -8.80	6.18	4.40 -7.20	5.89
	epidermal cells on reverse side	3.12 -7.20	5.75	3.36 -5.04	4.55	3.36 -5.04	4.22
	motor cells	3.36 -5.04	3.98	3.12 -5.04	3.95	3.12 -4.33	3.24
	long and short cells I	3.36 -5.04	3.91	3.36 -4.33	3.85	3.36 -4.56	3.96
	long and short cells II	3.36 -5.04	4.00	3.36 -5.04	4.20	3.36 -5.04	4.01
	accessory cells on outside	2.40 -4.80	3.71	3.36 -4.33	3.81	3.30 -4.33	3.63
	accessory cells on reverse side	3.21 -4.80	3.92	3.36 -4.80	4.02	3.36 -4.80	3.74
ripening stage	epidermal cells of midrib	5.04 -8.80	6.98	5.04 -8.80	6.30	5.04 -8.80	6.18
	epidermal cells on reverse side	3.12 -7.20	5.77	3.36 -5.04	4.62	3.36 -5.04	4.22
	motor cells	3.36 -5.04	4.00	3.12 -5.04	3.95	2.88 -3.84	3.33
	long and short cells I	3.36 -5.04	3.85	3.36 -4.33	3.85	3.36 -4.56	3.85
	long and short cells II	3.36 -5.04	3.95	3.36 -5.04	3.96	3.36 -5.04	4.00
	accessory cells on outside	2.40 -4.80	3.82	3.36 -4.80	3.81	3.30 -4.33	3.71
	accessory cells on reverse side	3.12 -4.80	3.89	3.36 -4.80	3.95	3.36 -4.80	3.74

b) The silification of the epidermal cells: The number both of each kind of epidermal cells and of the silicated ones in 1 mm² of the leaf surface were examined and the percentage of silicated epidermal cells of each kind were calculated. As the difference in the number of silicated cells between the different parts of one leaf were quite small, the percentage was calculated by summarizing all the number of silicated ones in every part of the leaf. The silicated accessory cells were scarcely found in every growing stages, and therefore their counts are omitted in the table. Table 19 shows the silicated cell percentage of motor cells, long cells and of short cells. As will be seen in the table, the silification percentage is highest in the motor cell, and increases with the progress of the rice plant growing stages. Accordingly there seems not to be any notable relationship between the fluctuation of the blast susceptibility of the rice plants with the progress of the growing stages and the silification of their epidermal cells.

TABLE 19. Percentage of silicated epidermal cells on the rice plants of the various growing stages.

	motor cell	long cell	short cell
elongation stage	3.55 %	1.62 %	0.24 %
ear formation stage	3.71 "	1.68 "	0.28 "
boot stage	3.94 "	1.75 "	0.31 "
flowering stage	4.20 "	1.78 "	0.44 "
ripening stage	4.51 "	1.80 "	0.51 "

3. The results of the ash analysis of the rice plant leaves in each growing stage.

Some of the rice plants in each growing stage were dried in 95-98°C thermodesiccator, and after the measurements of their moisture content they were burnt to ashes in the MUFFLE furnace of 5000°C. Then the content of the inorganic matters in the ashes were measured by the usual methods as in the previous chapter. The measurements were carried on the all rice plants which were used in the inoculation experiments, but the results of these experiments were almost same each other, and therefore in the followings the results of the experiment 1 are mentioned. Table 20 and figure 1 show the content of

moisture, organic matters and inorganic matters of the rice plant in each growing stage. As will be seen in the table and the figure, the contents of the organic matter increase with the progress of the growing stages. While the contents of the inorganic matters decrease gradually from seedling stage to the boot stage and afterward they begin to increase till ripening stage. On the other hand the content

TABLE 20. Content of organic and inorganic matters and of moisture in the rice plants of the various growing stages.

growing stages	items	organic matter	inorganic matter	moisture
seedling stage	fresh weight percentage	15.1 %	5.9 %	79.0 %
	dry weight percentage	71.8 "	28.2 "	—
elongation stage	fresh weight percentage	20.0 "	3.5 "	76.5 "
	dry weight percentage	85.1 "	14.9 "	—
ear formation stage	fresh weight percentage	22.7 "	2.5 "	74.8 "
	dry weight percentage	90.2 "	9.8 "	—
boot stage	fresh weight percentage	26.0 "	2.7 "	71.3 "
	dry weight percentage	90.5 "	9.5 "	—
flowering stage	fresh weight percentage	26.3 "	5.3 "	68.4 "
	dry weight percentage	83.2 "	16.8 "	—
ripening stage	fresh weight percentage	29.6 "	5.2 "	65.2 "
	dry weight percentage	85.0 "	15.0 "	—

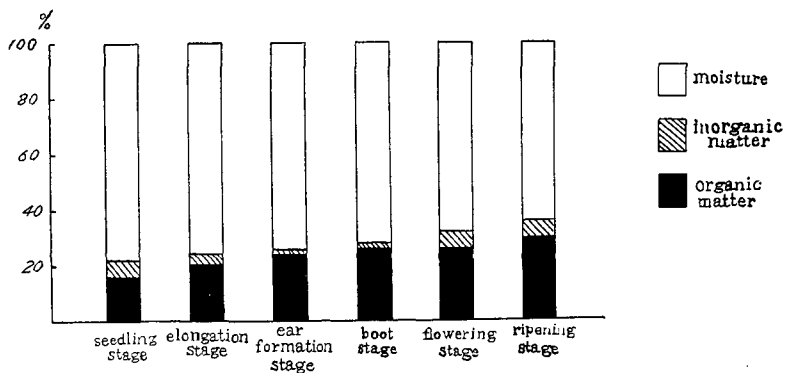


Fig. 1. Percentage of organic- and inorganic matters and moisture in the rice plant of the various growing stages.

of the moisture decrease with the progress of the growing stages. And therefore the dry weight percentage of the organic matter increase from the seedling stage to the boot stage and then after begin to decrease, while the dry weight percentage of inorganic matters decrease from the seedling stage to the boot stage and then after begin to increase. These may suggest some changes in the rice components which are taken place at the neighbour of the boot stage. The results of the analysis of the ashes are shown in table 21.

TABLE 21. Content of the various kinds of inorganic matters in the rice plant of the various growing stages.

growing stages	items	SiO ₂	P ₂ O ₅	CaO	Mg	K ₂ O	the other inorg. matters
		%	%	%	%	%	%
seedling stage	fresh weight percentage	3.44	0.44	0.57	0.17	0.67	0.63
	dry weight percentage	16.4 "	2.1 "	2.7 "	0.8 "	3.2 "	3.0 "
elongation stage	fresh weight percentage	1.97 "	0.26 "	0.26 "	0.09 "	0.49 "	0.42 "
	dry weight percentage	8.4 "	1.1 "	1.1 "	0.4 "	2.1 "	1.8 "
ear formation stage	fresh weight percentage	1.36 "	0.20 "	0.20 "	0.05 "	0.48 "	0.20 "
	dry weight percentage	5.4 "	0.8 "	0.8 "	0.2 "	1.9 "	0.8 "
boot stage	fresh weight percentage	1.69 "	0.14 "	0.14 "	0.06 "	0.49 "	0.17 "
	dry weight percentage	5.9 "	0.5 "	0.5 "	0.2 "	1.7 "	0.6 "
flowering stage	fresh weight percentage	3.63 "	0.22 "	0.22 "	0.10 "	0.79 "	0.35 "
	dry weight percentage	11.5 "	0.7 "	0.7 "	0.3 "	2.5 "	1.1 "
ripening stage	fresh weight percentage	3.80 "	0.17 "	0.17 "	0.07 "	0.63 "	0.35 "
	dry weight percentage	10.9 "	0.5 "	0.5 "	0.2 "	1.8 "	1.0 "

As will be seen in the table, the contents of every kinds of the inorganic matters decrease with the progress of the growing stages till the boot stage and thenceforth on the contrary they begin to increase. This is the same both in the fresh weight percentage and

in the dry weight percentage. This fact seems to be concerned with the facts that the content of amino acid, protein and carbohydrate increase greatly with the progress of the growing stages till the boot stage and thenceforce their increase became quite slow as will be seen in the following section. The percentage of each inorganic matters in the ash are shown in figure 2.

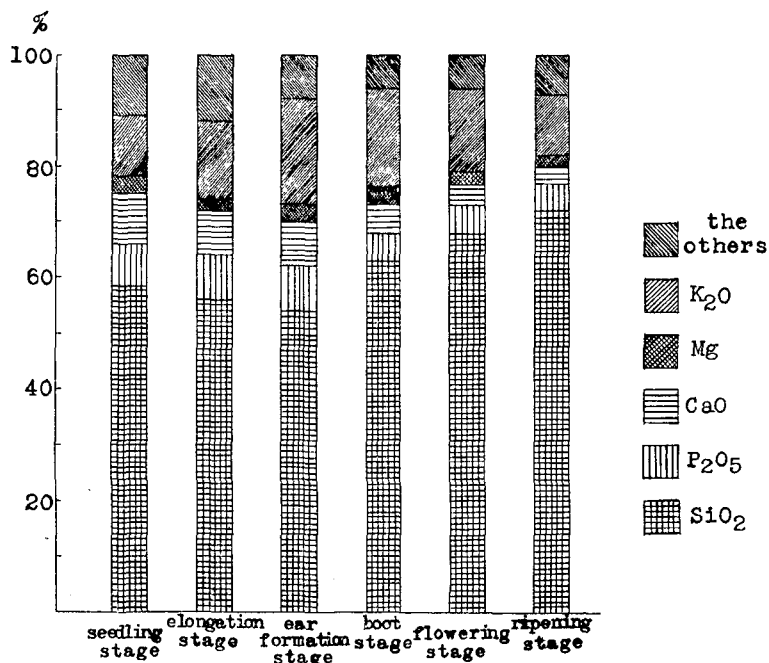


Fig. 2. Percentage of various kinds of inorganic matters in the ash of the rice plants of various growing stages.

As will be seen in the figure, most parts of the ash are occupied by SiO₂ through all the growing stages but its percentage in the ash decreases with the progress of the growing stages till the ear formation stage and then after it begins to increase. The percentage of K₂O are next to SiO₂, and it increases with the progress of the growing stages till the ear formation stage and thenceforce follow the decreasing. The fluctuation of percentage of P₂O₅ are likely to K₂O while the percentage of CaO and Mg decrease with the progress of the growing stages. Thus it is known from the experiments in this section that the content of the organic matters in the rice leaf increase remarkably with the progress of the growing stages till the ear formation stage

or the boot stage and then begin to decrease gradually, while on the contrary the content of the inorganic matter decrease with the progress of growing stages till the above mentioned stage and then begin to increase. Accordingly the rice plant contain the greatest amounts of organic matters and the least amount of inorganic matters in the ear formation stage or the boot stage which is the most susceptible stage to the blast disease all through the growing stages as shown in the previous section. Moreover as the most parts of the inorganic matter are occupied by the SiO_2 , the fluctuation of its content with the progress of the growing stages takes one's attention particularly. And it must be noticed in the present experiments that the less content of the silica tends to go parallel with the higher susceptibility against the blast infection.

4. The contents of the various kinds of nitrogen in the rice plants at each growing stage.

The content of various kinds of nitrogen in each growing stage was determined. Samples used in the determinations were the same as those used in the inoculation experiment above described. Just before the inoculation experiment at each growing stage, about 10g leaves were collected from 5-20 rice plants; they were ground in a mortar, after their fresh weight was accurately determined. The procedures of the separation and the determination of the various kinds of nitrogen were the same as described in the previous chapter. The total nitrogen content in each growing stage is given in the table 22. The figures show mg of nitrogen in the leaves of fresh weight 1g. In experiment 2, in which hot bed nursery seedlings were employed,

TABLE 22. Total nitrogen in the rice plants in the various growing stages.

growing stages	exp. 1	exp. 2	exp. 3	exp. 4	exp. 5
seedling stage	8.79 mg	10.33 mg	—	8.56 mg	—
elongation stage	9.48 "	9.84 "	13.07 mg	10.77 "	14.03 mg
ear formation stage	10.00 "	10.21 "	12.06 "	11.32 "	13.94 "
boot stage	9.71 "	9.89 "	11.31 "	10.98 "	14.20 "
flowering stage	8.28 "	8.66 "	9.87 "	9.76 "	14.03 "
ripening stage	8.53 "	8.46 "	12.26 "	9.54 "	13.84 "

the total content of nitrogen in the seedling stage was greater than that found in the other experiments, and it decreased from seedling stage to the elongation stage, approaching to the content of the other experiments. In experiments 3 and 5 in which the rice plants were grown in the water culture solutions, the total nitrogen content was generally greater comparing with that found in the other experiments; especially in experiment 5 the content was greatest because of the excess amount nitrogen supply. Also, in some experiments the nitrogen content was pretty high in the ripening stage but this was because the moisture content in the leaves becomes remarkably lower at that stage. Thus, although there are some differences in the experiments, it is safely to be said, in general, that the total nitrogen contents increase usually with the progress of the growing stages till the ear formation stage or the boot stage and then decrease gradually. The total nitrogen was divided into the two parts, the protein nitrogen and the total soluble nitrogen. The contents of each nitrogen in every growing stages are summarized in table 23. Between the experiments there are some differences in the content of protein nitrogen and total soluble nitrogen even at the same growing stage, but the tendency

TABLE 23. Contents of the protein nitrogen and soluble nitrogen in the rice plants of various growing stages.

growing stages	fractions of nitrogen	exp. 1	exp. 2	exp. 3	exp. 4	exp. 5
seedling stage	protein-N	7.18 mg	6.25 mg	—	6.85 mg	—
	total soluble-N	1.79 "	3.81 "	—	1.71 "	—
elongation stage	protein-N	7.78 "	7.76 "	11.83 mg	8.62 "	9.04 mg
	total soluble-N	1.70 "	2.08 "	1.24 "	2.15 "	4.63 "
ear formation stage	protein-N	5.91 "	6.15 "	10.37 "	7.26 "	9.06 "
	total soluble-N	4.09 "	4.06 "	1.69 "	4.06 "	4.88 "
boot stage	protein-N	6.75 "	6.81 "	9.32 "	7.67 "	9.94 "
	total soluble-N	2.96 "	3.08 "	1.99 "	3.31 "	4.26 "
flowering stage	protein-N	7.00 "	7.21 "	8.29 "	7.70 "	10.94 "
	total soluble-N	1.28 "	1.45 "	1.58 "	2.06 "	3.09 "
ripening stage	protein-N	6.97 "	6.48 "	10.70 "	7.63 "	11.07 "
	total soluble-N	2.04 "	1.98 "	1.56 "	1.91 "	2.77 "

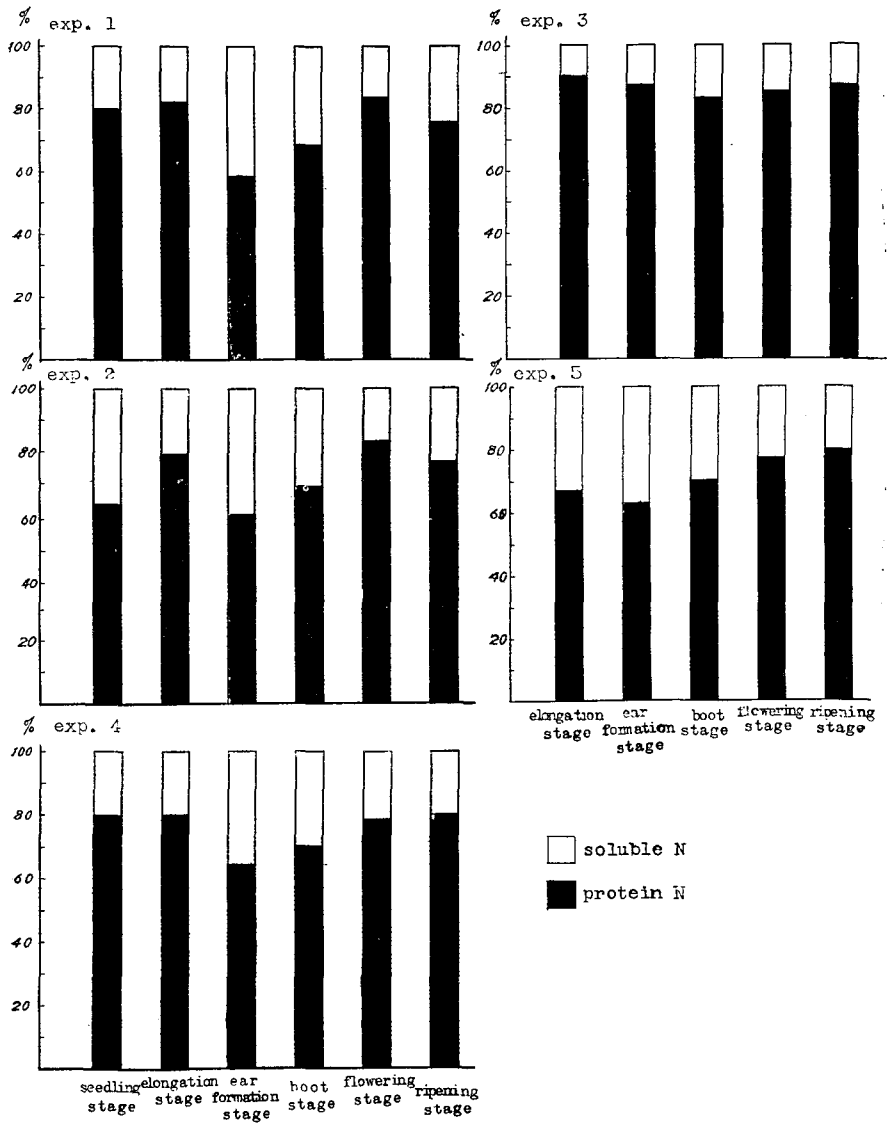


Fig. 3. Percentage of protein and soluble nitrogen in the total nitrogen of the rice plants of various growing stages.

of their fluctuation with the progress of the growing stages seems to be almost the same. Generally speaking, the protein nitrogen content increases from the seedling stage to the elongation stage but it decreases rather remarkably from the elongation stage to the ear formation stage or the boot stage, and then increases again gradually to the ripening stage. On the other hand the content of soluble nitrogen decreases once from the seedling stage to the elongation stage but increases remarkably from the seedling stage to the ear formation stage or the boot stage, and then decreases gradually till the ripening stage. In experiment 2, the content of protein nitrogen was comparatively lower while the content of soluble nitrogen was comparatively higher at the seedling stage because of the employment as material of those seedlings which had been raised on the hot bed nursery; but after the elongation stage the content of those nitrogens comes near to that of the other experiments. Thus it is clear that such characters in the seedling stage do not remain long after the transplantation. In experiment 5, in which the excess amount nitrogen was supplied, the content of soluble nitrogen in the elongation stage was rather higher than that in the ear formation stage of the other experiments, while its content was rather higher in every growing stages comparing with the case of the other experiments. However even in this case, the tendency of the fluctuation of its contents with the progress of the growing stages was the same as in other experiments. At any rate it should be noticed that the content of protein nitrogen is lowest while the content of soluble protein is highest at the ear formation or the boot stage through all the experiments. Figure 3 shows the percentage of the two classes of nitrogen in the total nitrogen. It is clearly shown in the figure that the percentages of protein nitrogen are lowest while the percentages of the soluble nitrogen are highest at the ear formation stage or the boot stage on comparison with those at other growing stages. Next, the fractions of the soluble nitrogen were determined. The results are summarized in table 24. Generally speaking, the content of every kind of soluble nitrogen increases from the elongation stage to the ear formation stage and then it decreases gradually with the continuance of growth. In the ripening stage pretty high content is shown in every kind of soluble nitrogen, but that high value seems to be concerned with the remarkable decrease of the moisture content in the rice leaf. Thus a peak is seen at the ear formation stage and that is most conspicuous in the following three

TABLE 24. Fractions of the soluble nitrogen in the rice plants of various growing stages.

exp.	fractions of soluble nitrogen	seedling stage	elongation stage	ear formation stage	boot stage	flowering stage	ripening stage
1	soluble protein-N	0.40 mg	0.39 mg	0.96 mg	0.59 mg	0.50 mg	0.81 mg
	α -amino-N	0.81 "	0.28 "	0.98 "	0.58 "	0.45 "	0.57 "
	basic-N	0.84 "	0.74 "	1.60 "	1.51 "	0.22 "	0.24 "
	amide-N	0.23 "	0.17 "	0.26 "	0.20 "	0.04 "	0.17 "
	ammonium-N	0.08 "	0.07 "	0.20 "	0.04 "	0.04 "	0.19 "
	nitrate-N	0.02 "	0.02 "	0.04 "	0.03 "	0.02 "	0.05 "
2	soluble protein-N	1.42 "	0.48 "	1.06 "	0.62 "	0.58 "	0.79 "
	α -amino-N	1.53 "	0.35 "	0.81 "	0.58 "	0.49 "	0.55 "
	basic-N	0.57 "	0.94 "	1.42 "	1.54 "	0.27 "	0.24 "
	amide-N	0.81 "	0.21 "	0.30 "	0.22 "	0.05 "	0.15 "
	ammonium-N	0.08 "	0.03 "	0.25 "	0.05 "	0.03 "	0.12 "
	nitrate-N	0.01 "	0.02 "	0.03 "	0.02 "	0.02 "	0.04 "
3	soluble protein-N	—	0.53 "	0.58 "	0.52 "	0.38 "	0.84 "
	α -amino-N	—	0.33 "	0.53 "	0.79 "	0.23 "	0.47 "
	basic-N	—	0.19 "	0.44 "	0.53 "	0.23 "	0.15 "
	amide-N	—	0.15 "	0.02 "	0.02 "	0.61 "	0.06 "
	ammonium-N	—	0.02 "	0.10 "	0.12 "	0.13 "	0.07 "
	nitrate-N	—	0.01 "	0.02 "	0.01 "	0.01 "	0.01 "
4	soluble protein-N	0.43 "	0.52 "	0.91 "	0.70 "	0.83 "	0.77 "
	α -amino-N	0.22 "	0.33 "	1.02 "	0.82 "	0.70 "	0.55 "
	basic-N	0.73 "	0.85 "	1.60 "	1.49 "	0.33 "	0.27 "
	amide-N	0.25 "	0.20 "	0.33 "	0.27 "	0.08 "	0.13 "
	ammonium-N	0.05 "	0.09 "	0.14 "	0.02 "	0.04 "	0.08 "
	nitrate-N	0.02 "	0.07 "	0.06 "	0.01 "	0.05 "	0.06 "
5	soluble protein-N	—	1.16 "	1.22 "	0.85 "	0.99 "	0.89 "
	α -amino-N	—	1.71 "	1.80 "	1.66 "	0.83 "	0.69 "
	basic-N	—	0.56 "	0.49 "	0.34 "	0.49 "	0.39 "
	amide-N	—	1.06 "	1.21 "	1.07 "	0.56 "	0.56 "
	ammonium-N	—	0.14 "	0.15 "	0.13 "	0.09 "	0.08 "
	nitrate-N	—	0.12 "	0.12 "	0.12 "	0.10 "	0.08 "

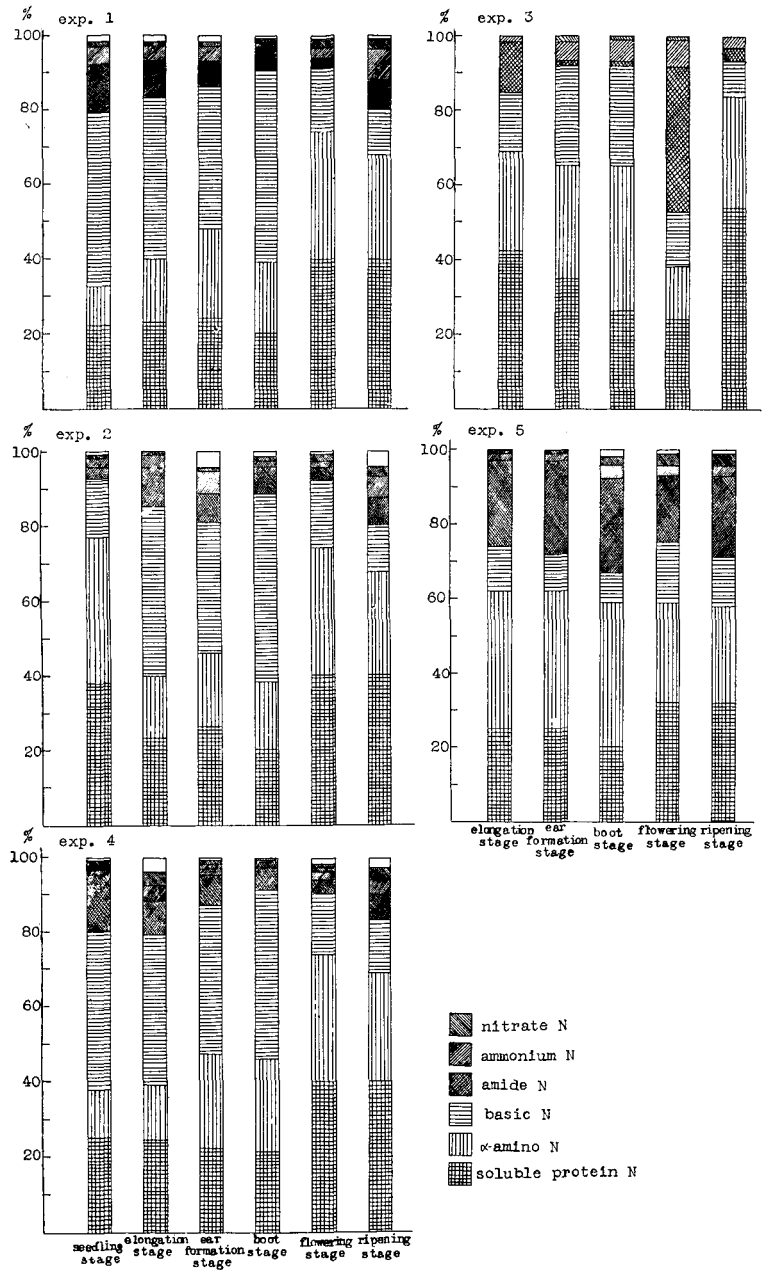


Fig. 4. Percentage of each fraction of soluble nitrogen in the rice plants of the various growing stages.

fractions, the soluble protein nitrogen, α -amino nitrogen and basic nitrogen, which supply the greater part of the total soluble nitrogen. In experiment 2 the contents of soluble protein nitrogen and α -amino nitrogen are pretty high in the seedling stage; this is because the seedlings raised in the hot bed nursery were employed as materials in that experiment. However, their contents become lower at the next elongation stage approaching to the values in the other experiments. In experiment 5, in which excess amount of nitrogen was supplied in the solution, the contents of every kind of soluble nitrogen are naturally higher than those in the other experiments; the increases of their content from elongation stage to the ear formation stage are not so remarkable. The percentage of each soluble nitrogen in the total soluble nitrogen are shown in figure 4. As will be seen in the figure, the most part of the soluble nitrogen is generally occupied by the three fractions: soluble protein, α -amino and basic nitrogen. After the flowering stage the percentage of soluble protein nitrogen and α -amino nitrogen become comparatively higher while the percentage of basic nitrogen becomes comparatively lower. In experiment 5, in which excess amount of nitrogen was applied, the percentage of amide is rather higher while the percentage of basic nitrogen is rather lower. Generally speaking the percentage of the basic nitrogen fluctuates inversely proportional to the percentage of the soluble protein nitrogen and α -amino nitrogen.

Then the experimental results in this section may be summarized as follows: total amount of nitrogen increases with the progress of the growing stages till the ear formation stage or the boot stage and after then the amount decreases gradually. Dividing the total nitrogen into two sorts, protein nitrogen and total soluble nitrogen, the content of the former decreases with the progress of the growing stages till the ear formation stage or boot stage and after then it begins to increase; on the contrary the content of the latter increases remarkably till the ear formation stage or the boot stage and after then it begins to decrease. Thus a rather high peak is seen in the fluctuation curve of the soluble nitrogen at the ear formation stage or the boot stage while a depression is seen in the curve of the protein nitrogen at the same growing stage. Moreover, the remarkable increase of the soluble nitrogen on that growing stage of ear formation is caused by the remarkable increase of the three fractions, soluble protein, α -amino and basic nitrogen, which supply the greater part of the soluble nitrogen.

It should be noticed here that if the high level nitrogen were supplied, the content of those soluble nitrogens becomes so high at the elongation stage as almost to equal those contents of ear formation stage.

On the other hand, naturally, the cause of the fluctuation of the content of each kind of nitrogen must be considered from various view points, but one of the reasons at least may be sought in the translocation of the nitrogen in the rice plant. In order to examine this point, the contents of total nitrogen in the following three parts, the leaf, the stem and the ear, were determined at each growing stage and the total nitrogen content in whole plant and in each part of a rice plant was calculated. The results are given in table 25.

TABLE 25. Increase or decrease of total nitrogen in the various parts of the rice plants between each growing stage.

growing stages	items	whole plant	leaf	stem	ear
seedling stage	nitrogen content	5.56 mg	5.56 mg	—	—
	difference from the preceding growing stage	—	—	—	—
elongation stage	nitrogen content	38.40 "	12.80 "	25.60 mg	—
	difference from the preceding growing stage	+32.84 "	+ 7.24 "	—	—
ear formation stage	nitrogen content	137.80 "	48.52 "	79.06 "	10.22 mg
	difference from the preceding growing stage	+99.40 "	+35.72 "	+53.64 "	+10.22 "
boot stage	nitrogen content	272.00 "	69.90 "	120.90 "	81.20 "
	difference from the preceding growing stage	+134.20 "	+21.38 "	+41.84 "	+70.98 "
flowering stage	nitrogen content	301.30 "	59.60 "	118.50 "	123.20 "
	difference from the preceding growing stage	+29.30 "	-10.30 "	- 2.40 "	+42.00 "
ripening stage	nitrogen content	320.60 "	46.10 "	118.50 "	156.00 "
	difference from the preceding growing stage	+19.30 "	-13.50 "	0.00 "	+32.80 "

As will be seen in the table, the increase of the nitrogen content in the whole plant is remarkable from the seedling stage to the boot stage while the rate of increase becomes comparatively smaller after the flowering stage. This may mean the greater absorption of nitrogen by the rice plant at the early growing stages and the less absorption after the flowering stage, the same as in the case of wheat which ISHIZUKA⁽⁴⁰⁾ has reported. Moreover, it is known that the nitrogen content in the leaf and the stem falls with the progress of growth after the boot stage, while the nitrogen content in the newly formed ear increases rather greatly in spite of the decrease of the absorption of nitrogen by the plant, as a whole. This may suggest the translocation of the nitrogen from the stem or the leaf to the newly formed ear, that may occur after the boot stage. Considering from such view point, the remarkable increase of the soluble nitrogen and the decrease of the protein at the ear formation stage as shown above may be explained as the preparation for the translocation of the nitrogen which will take place in the following stages; furthermore it may be attributed to the depression of the protein synthesis from the absorbed nitrogen by the plant and moreover to the decomposition of some protein at the ear formation stage.

5. Sugar content in the rice plants of each growing stage.

In the following an examination of the sugar content in the rice plants of each growing stage is described. Material used for the determination were the same as those used in the previous sections. And the method of analysis and the classification of the sugar were the same as those described in the previous chapter. The total sugar content is recorded in table 26.

TABLE 26. Total sugar content in the rice plants of the various growing stages.

growing stages	exp. 1	exp. 2	exp. 3	exp. 4	exp. 5
seedling stage	5.6 mg	2.8 mg	—	4.8 mg	—
elongation stage	2.3 "	0.9 "	5.8 mg	2.6 "	3.2 mg
ear formation stage	7.3 "	8.7 "	7.6 "	8.2 "	7.3 "
boot stage	4.7 "	9.7 "	7.8 "	5.1 "	6.9 "
flowering stage	10.6 "	4.6 "	5.2 "	9.8 "	6.0 "
ripening stage	18.5 "	17.2 "	13.7 "	16.6 "	11.5 "

It is true that there are some differences in the sugar content as shown by the different experiments, but the fluctuation of the values with the progress of the growing stages seems to be almost the same. Although the sugar content decreases from the seedling stage to the elongation stage, it increases remarkably from the elongation stage to ear formation stage. After that stage the increased sugar content in the leaves decreases once a little and then begins to increase again, showing high sugar content in the ripening stage. The sugars found in the rice plant may be the remainder subtracting the whole amount of sugar which is used as respiration material or constitutional material of the rice plant from the whole amount of sugar synthesized by photosynthesis. Although there are no data concerning the amount of sugar used as respiration material of the rice plant, the fluctuation above mentioned in the sugar content may be explained as follow by taking into consideration the experimental results on the fluctuation in the contents of the various kinds of nitrogen. In the elongation stage, when the transplanted seedlings are beginning active growth, the synthesis of constitutional substances is quite active consuming a great amount of the sugars, and this results in the lower content of sugar in the plant leaves. In the ear formation stage, when the rice plants are preparing for the translocation of some nitrogen from leaves to newly formed ears, the amount of sugar used as constitutional material becomes comparatively less, because the protein synthesis becomes weaker and even protein decomposition occurs. This is thought to be an explanation of the less sugar content in the rice leaves of that stage. Then translocation of some soluble nitrogen from leaves to the ear that takes place in accordance with the ear development comes to activate again the protein synthesis in the leaves using the greater amount of sugar as material. Therefore the sugar contents in the leaves decrease once at the boot stage. After the flowering stage the amount of the sugars which is used as constitutional material become less because of the decreased absorption of the nitrogen by the plant and at the same time the sugar amount used as the respiration material becomes probably less because the leaves had completed their growth at that time and thus the sugar content in the leaves comes to be comparatively higher. Then the sugars are divided into the reducing sugar and the non-reducing sugar. The content of the two kinds of sugars in each growing stage are given in table 27, and the percentages of each sugar in the total sugar are shown in figure 5.

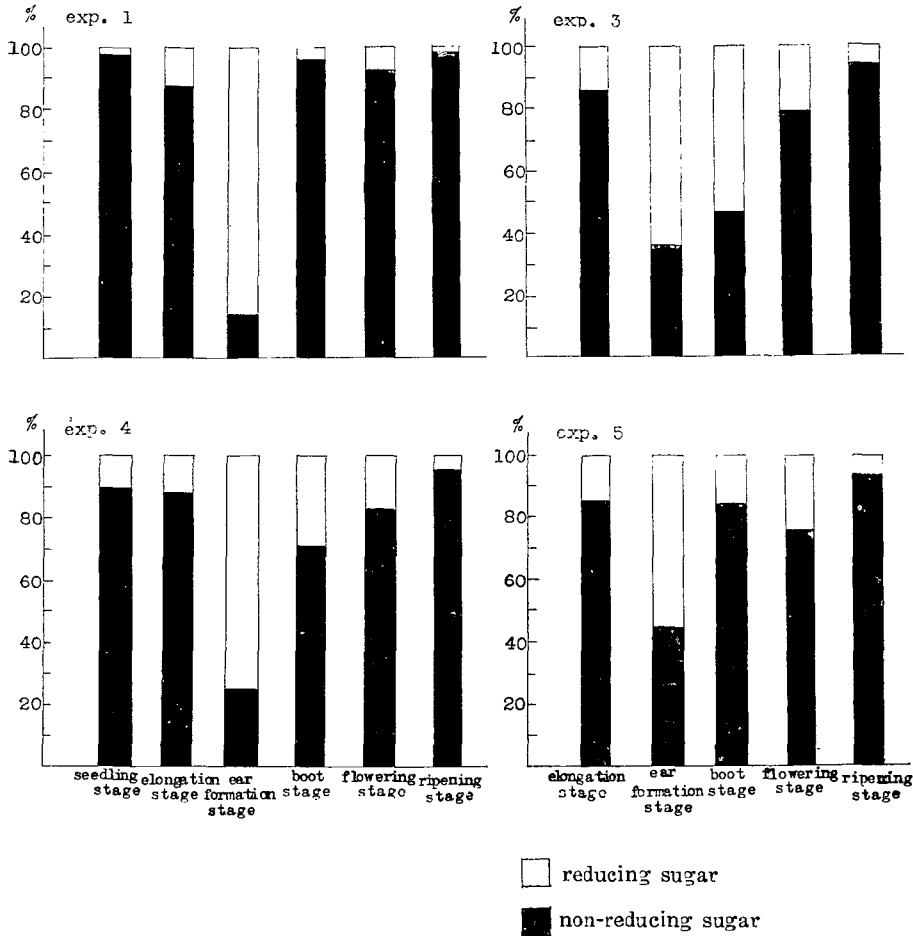


Fig. 5. Percentage of reducing and non-reducing sugar in the total sugar of the rice plant at the various growing stages.

On examining the table and the figure it comes to one's notice that the amount of reducing sugar is far beyond the amount of non-reducing sugar in the ear formation stage, while the relation is reversed in all the other growing stages. And it seems to be that such remarkable increase in the reducing sugar content in the ear formation stage is concerned with the changes which are taking place in the leaves as the preparation for the translocation of various substances to the newly formed ears.

TABLE 27. Contents of reducing and non-reducing sugar in the rice plants of the various growing stages.

growing stages	kinds of sugar	exp. 1	exp. 3	exp. 4	exp. 5
seedling stage	reducing sugar	0.7 mg	—	0.5 mg	—
	non-reducing sugar	4.9 "	—	4.3 "	—
elongation stage	reducing sugar	0.3 "	0.9 mg	0.3 "	0.5 mg
	non-reducing sugar	2.0 "	4.9 "	2.3 "	2.7 "
ear formation stage	reducing sugar	6.3 "	4.9 "	6.2 "	4.1 "
	non-reducing sugar	1.0 "	2.7 "	2.0 "	3.2 "
boot stage	reducing sugar	0.2 "	4.2 "	1.5 "	1.1 "
	non-reducing sugar	4.5 "	3.6 "	3.6 "	5.8 "
flowering stage	reducing sugar	0.9 "	1.1 "	1.7 "	1.5 "
	non-reducing sugar	9.7 "	4.1 "	8.1 "	4.5 "
ripening stage	reducing sugar	0.5 "	0.9 "	0.9 "	0.8 "
	non-reducing sugar	18.0 "	12.8 "	15.7 "	10.7 "

6. Conclusion.

In this chapter a description is given of how the rice plants were inoculated with the blast fungus at various growing stages and at the same time their morphological characters such as the thickness of the outer wall of the epidermal cells and also their contents of chemical components were examined. Although the experiments were made on those rice plants which were grown on the soil in the pot or in the water culture solutions or in the paddy field, such differences of the cultivation do not result in any important effects on the characters examined here. Also in these experiments two kinds of seedlings were used, the one those which were raised in the hot bed nursery and the other those seedlings raised in the ordinary nursery. And as shown already the former seedlings are rather susceptible against blast on comparison with the latter. But it is shown in these experiments that such characters of the high susceptibility in the former seedlings never remain long after their transplantation. In these experiments six growing stages were distinguished: seedling stage, elongation stage, ear formation stage, boot stage, flowering stage and ripening stage.

The moisture content in the rice leaves is greatest in the seedling stage and decreases with the progress of the growing stages. The content of organic matters in the rice leaves increases rather remarkably with the progress of the growing stages till ear formation stage or the boot stage but then such matters begin to decrease with the farther progress of the growing stages; the content of inorganic matter in the rice leaves decreases with the progress of the growing stages till the ear formation stage or the boot stage and thereafter begins to increase gradually. Such fluctuations are most remarkable in the content of SiO_2 , P_2O_5 and K_2O among the inorganic matters. According to the inoculation experiments rice plants are comparatively susceptible to the blast fungus in the seedling stage but when the seedlings were transplanted in the fields and they begin to grow vigorously, increasing their height and green color, their resistance against the attack of the fungus is enhanced greatly. Thus in the elongation stage the rice plants are most resistant against the blast disease amongst all the growing stages. However when the rice plants grow up to the ear formation stage or the boot stage, the rice leaves become very susceptible to the blast fungus, producing rather numerous diseased lesions in inoculation experiments. The presence of the acute type lesions comes to one's attention and also their size is generally greater than others. Thus it is certain that the rice leaves are most susceptible to the blast disease in the ear formation stage and the boot stage. The rice plants ordinarily reach the ear formation stage at the beginning of July in Hokkaido; it is clear that the weather in that season is most favourable for conidia formation by blast fungus, which overwintered in the straw. It must be noticed that the rice plants reach the growing stage of the highest susceptibility to the blast disease just when the weather is most agreeable for the formation and spread of the conidia of the blast fungus. But when the rice plants grow up to the flowering stage and to the ripening stage, they become again to be resistant against the blast invasion so far as the leaves are concerned. On the other hand it is known from the experiments that the outer wall of the epidermal cells of the rice leaves increase their thickness as well as the degree of their silification with the progress of the growing stages. Therefore the higher resistance of the rice leaves against the blast disease after the flowering stage may be explained by the difficulty the blast fungus experiences in penetrating through the thicker highly silicated walls. But such consideration

cannot explain the highest resistance to blast in the elongation stage when the thickness of the outer walls of the rice leaves and also their silification are not so great. However, it must be added here that the silica contents in the rice leaves of each growing stage always go parallel with the fluctuation of the blast susceptibility of these rice leaves, and some physiological functions of the silica, which may be concerned with the blast susceptibility, needs to be examined in future. It is quite impossible to lay all the responsibility of the susceptibility or resistance of the rice plant to blast disease on these morphological characters of the rice plants. Then the content of the various kinds of nitrogen and also the sugar content were determined on the rice plants of each growing stage. The total content of nitrogen increases with the progress of the growing stages till the ear formation stage and then decreases gradually. Classifying the total nitrogen into the protein nitrogen and the soluble nitrogen, a remarkably higher content of the soluble nitrogen and a rather less content of the protein nitrogen are seen in the ear formation stage or in the boot stage on comparison with the other growing stages. Such changes in the nitrogen which are seen in the ear formation stage may mean the preparation for nitrogen translocation, which takes place with the farther progress of the growing stages. By examination of the various fractions of the soluble nitrogen, it is known that the increase from the preceding stage at the ear formation stage is particularly remarkable in the following three, soluble protein nitrogen, α -amino nitrogen and basic nitrogen which supply in general the most part of the soluble nitrogen. It must be noticed here that the rice plants are most susceptible to the blast infection in the ear formation stage. On the other hand a rather higher content of the protein nitrogen and a rather lower content of soluble nitrogen are seen on the elongation stage. This is a good contrast to the amounts seen in the ear formation stage. Also the rice plants are least susceptible to blast infection in the elongation stage. Thus through all the growing stages, it is certain that the higher blast susceptibility goes parallel with the higher content of such soluble nitrogen as soluble protein nitrogen, α -amino nitrogen and the basic nitrogen. In the case of the high level nitrogen supply, the elongation stage of the rice plant loses its characteristic as the highest resistant stage against the blast disease and at the same time the nitrogen composition comes near to that of the ear formation stage, in which the rice plants are most susceptible against the blast. It is

worth notice that such parallelism between the high blast susceptibility of the rice plant and the accumulation of soluble nitrogen are seen likewise in the experiments of the previous chapter concerning the seedlings raised on the hot bed nursery and on the ordinary nursery. It must be added here that it is quite difficult to find out through all the experiments any direct correlation between the blast susceptibility and the sugar content.

Chapter III. Relations between the amount of nitrogen applied to the rice plant and their susceptibility to the blast disease.

It has long been well known that applications of high level nitrogen intensify the blast disease, being called popularly "Koe-imochoi" (high level manure blast). A great many researches concerning this problem have been reported, but there seems to be no definite opinion about the reason why the application of high level nitrogen intensifies the disease. In the previous chapter the present writer showed the fact that the rice plants supplied with three times as much nitrogen as the standard remain susceptible to the blast disease even in the elongation stage, in which the rice plants are ordinarily most resistant to the blast disease through all the growing stages. According to the above reported experiments also the nitrogen composition in the elongation stage becomes near to that in the ear formation stage as a result of the application of the high level nitrogen. In order to ascertain that point in detail, experiments were planned with experimental sections prepared with various nitrogen levels. The effect of the additional supply of nitrogen at the ear formation stage was also examined in the experiments described in this chapter. All through the experiments rice plants in the latter part of the boot stage were employed. The standard solution of water culture and the basal amount of nitrogen applied to the soil in the pot are the same as described already in the previous chapter.

1. Inoculation experiments.

Exp. 1: Seedlings were raised on quartz sand and were transplanted when their 3rd leaves were developing. After ten days they were divided into two sections, the one was allowed to continue to grow in the standard culture solution and the other was transferred

to the culture solution in which two times as much nitrogen as standard was added. Examination of their growth at the ear formation stage showed that the rice plants in the latter section were greater in height and in number of tillers than those in the former section. When they were grown to the latter phase of the boot stage, the plants were inoculated with the blast fungus. The diseased lesions were examined the two weeks after the inoculation. The results are given in table 28.

TABLE 28. Inoculations of the blast fungus on the rice plants of the different level nitrogen supply (1).

	standard amount of nitrogen	double amount of nitrogen
height	58 cm	61 cm
number of tillers	9.0	13.0
number of leaves examined	116	135
total length of leaves examined	2342 cm	2698 cm
total number of lesions	52	71
average number of lesions per leaf	0.45	0.53
average number of lesions per 100 cm length of leaves	2.2	2.7
length of lesions	max.	1.2 cm
	min.	0.2 "
	aver.	0.55 "
type of lesions	chronic, acute	acute

As will be seen in the table the number of lesions is a little greater in the rice plant applied with two times as much nitrogen than in the rice plant supplied with basal amount. Moreover all the lesions developed on the former are acute type, while on the latter both the acute type and the chronic type are seen. Likewise the size of the lesions is greater on the former than on the latter. Thus it is certain that the application of double amount of nitrogen favored the susceptibility to the blast disease.

Exp. 2: After the seedlings were grown in the standard culture solution for two weeks, they were divided into five sections in which the nitrogen level was different from the basal amount to five times as much as the basal. At the ear formation stage the height of the plants seems to be a little greater in proportion to the nitrogen level, while there were not so great differences in the number of tillers.

The inoculation with the blast fungus at the latter phase of the boot stage gave the results shown in table 29.

TABLE 29. Inoculations of the blast fungus on rice plants of different level nitrogen supply (2).

	standard amount of nitrogen	double amount of nitrogen	triple amount of nitrogen	quadruple amount of nitrogen	quintuple amount of nitrogen	
height	58 cm	58 cm	59 cm	59 cm	60 cm	
number of tillers	10.2	10.8	10.8	10.7	10.8	
number of leaves examined	126	131	126	127	131	
total length of leaves examined	2770 cm	2790 cm	2866 cm	2992 cm	3046 cm	
total number of lesions	48	62	68	66	73	
average number of lesions per leaf	0.38	0.47	0.54	0.52	0.56	
average number of lesions per 100 cm length of leaves	1.37	2.22	2.37	2.21	2.40	
length of lesions	max.	1.2 cm	1.3 cm	1.5 cm	2.1 cm	4.3 cm
	min.	0.2 "	0.2 "	0.5 "	1.0 "	1.0 "
	aver.	0.61 "	0.77 "	0.92 "	1.5 "	2.3 "
type of lesions	chronic, acute	chronic, acute	chronic, acute	acute	acute	

The number of the lesions was clearly greater in the section of the double amount nitrogen on comparison with those in the basal amount. But the differences were not so remarkable from the double amount section to the quintuple amount section. Examining the type of the lesions, all of them were the acute type without any traces of chronic type in the two sections of quadruple and quintuple amount nitrogen, while the chronic type was mixed with the acute type from the basal amount section to the three times amount section. Their sizes were larger in proportion to the nitrogen level and particularly greater size of the lesions in the sections of the quadruple and quintuple attracts one's attention. Thus it is safely said that the blast susceptibility of the rice plant was increased in accordance with the nitrogen level and it became most conspicuous in the nitrogen level beyond four times the basal. It must be noticed here that the results of the present experiment are a little different from the results in experiment 1, when the double amount nitrogen already favoured the susceptibility quite remarkably.

Exp. 3: In this experiment the rice plants were grown on soil

in the pot, and three sections which were different in nitrogen level from standard amount to three times amount were prepared. In the boot stage the height of the rice plants was greater in the sections of high level nitrogen on comparison with the basal section. The results of inoculation are shown in table 30. As will be seen in the table, both the numbers and the sizes of the lesions became greater with the increase of the nitrogen level. Moreover all the lesions developed in the sections of the high level nitrogen were acute type without any trace of chronic type, while in the standard amount section the chronic type lesions were mixed with the acute type lesions.

TABLE 30. Inoculations of the blast fungus on the rice plants of different level nitrogen supply (3).

	standard amount of nitrogen	double amount of nitrogen	triple amount of nitrogen
height	54 cm	60 cm	58 cm
number of tillers	10.5	11.0	11.3
number of leaves examined	118	120	126
total length of leaves examined	2610 cm	2715 cm	2743 cm
total number of lesions	32	53	56
average number of lesions per leaf	0.27	0.44	0.44
average number of lesions per 100 cm length of leaves	1.23	1.95	2.04
length of lesions	max.	1.2 cm	1.5 cm
	min.	0.2 "	0.2 "
	aver.	0.55 "	0.72 "
type of lesions	chronic, acute	acute	acute

Thus in this experiment the application of double as much nitrogen clearly favoured the blast susceptibility.

Exp. 4: The rice plants were grown on soil in WAGNER pots in which 0.8g ammonium sulfate, 2.8g superphosphate of lime and 0.7g potassium sulfate were applied as the basal manure. When those rice plants were grown up near to the ear formation stage, they were divided into two groups. In the one group 2g of ammonium sulfate was applied additionally in each pot and in other section they remained without change. The rice plants which received the additional supply of nitrogen seemed to become a little greater in height on comparison with the control plants already in two days after the additional supply,

and the differences became more remarkable at eight days after the treatment. The effects of the additional supply on the number of tillers were not certain. Then the rice plants in each section were divided again into two sections; the one was inoculated with blast fungus at two days after the treatment and the other was inoculated at eight days after the treatment. In each case the diseased lesions were examined at ten days after the inoculation. The results are given in table 31.

TABLE 31. Inoculations of the blast fungus on the rice plants which received additional supply of the nitrogen (1).

experimental section days after the treatment items	control		additional supply of nitrogen		
	2 days	8 days	2 days	8 days	
height	57.1 cm	58.0 cm	57.8 cm	62.4 cm	
number of tillers	8	9	8	9	
number of leaves examined	72	83	93	110	
total length of leaves examined	1044 cm	1250 cm	1448 cm	1870 cm	
total number of lesions	18	16	45	60	
average number of lesions per leaf	0.25	0.19	0.48	0.54	
average number of lesions per 100 cm length of leaves	1.7	1.3	3.1	3.7	
length of lesions	max.	1.2 cm	1.2 cm	1.2 cm	1.5 cm
	min.	0.2 "	0.2 "	0.2 "	0.2 "
	aver.	0.63 "	0.60 "	0.78 "	0.81 "
type of lesions	chronic, acute	chronic, acute	chronic, acute	acute	

As will be seen in the table, a far more lesions are developed on the rice plant which got the additional supply of nitrogen on comparison with the control plants, even when they were inoculated on the second day after the additional supply. Examining the types of the lesions, one sees that there are not so great differences between the control plant and those rice plants which were inoculated two days after the additional application of nitrogen, but the acute type lesion seems to be a little dominant in the latter. It must be noticed, however, that the developed lesions are all acute type when the rice plants were inoculated at 8th day after the additional supply of nitrogen. The size of the lesions was clearly larger even on those rice plants

other were inoculated two days after and the remainder were inoculated eight days after the treatment. In all the cases the diseased lesions were examined ten days after the inoculation. The results are summarized in table 32.

As will be seen in the table, it is certain that in the case of the untreated plants the differences of the inoculation date in this experiment do not result in any important effects on the number and the size of the lesions. But in the case of the plants treated with additional nitrogen the inoculation on the second day after the treatment resulted in larger and more lesions in comparison with the case of the control; such results were more remarkable in the case of the inoculation on 8th day after the treatment, while they were not so remarkable in the case of inoculation on the same day as the treatment. Examining the types of lesions, one notes that the acute type lesions are generally dominant through all the experiments of the additional supply section, while in the control both of the chronic type and the acute type are mixed uniformly. Particularly on the rice plants which were inoculated eight days after the treatment acute type lesions only are developed without any trace of the chronic type. Thus it is certain that the additional supply of nitrogen intensifies the blast susceptibility of those rice plants and the effects become more remarkable with passage of the days after the treatment.

Summarizing the experiments described in this section, it is evident that the apply of high level nitrogen favours the blast susceptibility of those rice plants, but it is rather difficult to find out the critical amount of the nitrogen by which the effect on the susceptibility becomes clear, because in some experiments the application of double quantity of nitrogen favoured the blast susceptibility quite remarkably while in other experiments the effect became barely remarkable on the supply of four times as much nitrogen as standard. On the other hand the application of the excess amount nitrogen at about the ear formation stage favoured the blast susceptibility of those rice plants already on the second day after application and the effect became more remarkable with the passage of the days after treatment.

2. Silification of the epidermal cells.

In this section the effect of high level nitrogen application on the number of silicated epidermal cells is examined. Materials were the

same as in the inoculation experiments, but the measurements on the rice plants of experiments 4 and 5 in the previous section were omitted because the effect on the silification of the epidermal cell seems to be uncertain at a few days after the additional apply of nitrogen. The procedures for the measurement were same as described in the previous chapter. Measurements were carried out on the following three kinds of epidermal cells: the motor cell, the short cell and the long cell. The percentages of silicated cells were calculated on each of these epidermal cells. The results are summarized in table 33.

TABLE 33. Percentage of silicated epidermal cells in the rice plants of different level nitrogen supply.

exp.	amount of nitrogen supplied	motor cell	long cell	short cell
1	standard amount of nitrogen	3.52 %	1.03 %	0.88 %
	double amount of nitrogen	3.22 "	1.10 "	0.82 "
2	standard amount of nitrogen	3.58 "	1.20 "	0.96 "
	double amount of nitrogen	3.20 "	1.28 "	0.86 "
	triple amount of nitrogen	3.46 "	1.05 "	1.10 "
	quadruple amount of nitrogen	3.18 "	1.23 "	0.52 "
	quintuple amount of nitrogen	3.04 "	1.19 "	0.69 "
3	standard amount of nitrogen	4.62 "	1.80 "	1.21 "
	double amount of nitrogen	3.31 "	1.24 "	0.98 "
	triple amount of nitrogen	3.04 "	1.39 "	1.18 "

Examining the table, one's attention is first directed to the fact the percentage of the silicated epidermal cells of the rice plant supplied with standard amount of nitrogen is higher in experiment 3, in which the rice plant were grown on the soil in the pot, than in the other experiments, in which the plants were grown in the water culture solutions. This suggests that the water culture of the rice plants is apt to be somewhat unfavorable for the silification of their epidermal cells. But it is clearly shown through the three experiments that the application of the greater amount of nitrogen decreases the percentage of silicated motor cells, while that is not always certain on the other kinds of epidermal cells. Further, it may be noticed again that the blast fungus penetrates the epidermal cells most frequently through the motor cells.

3. Content of various kinds of nitrogen in the rice plants supplied with different amounts of nitrogen.

The effects of the application of a high level of nitrogen on the content of various kinds of nitrogen in the rice plant are reported in this section. The materials were the same as those which were used in the previous experiments. The fractions of the examined nitrogen and the method of their analysis were all the same as described in the previous chapter. In all experiments the content of the nitrogen in the whole leaves of one rice plant was calculated besides the content of the nitrogen in the leaves of fresh weight 1 g. Table 34 summarizes the measurement of the total nitrogen content from experiment 1 to experiment 3. As will be shown in the table the total nitrogen content in the whole leaves of one rice plant increases with the amount of nitrogen applied to the rice plants. Likewise such content in the leaves of fresh weight 1 g increases in accordance with the nitrogen level in experiments 1 and 3. But only in experiment 2 the increase of nitrogen content accompanied by the high level nitrogen supply was not so clear.

TABLE 34. Total nitrogen in the rice plants given various level nitrogen supplies.

nitrogen level supplied	items	exp. 1	exp. 2	exp. 3
standard amount of nitrogen	in all leaves of one rice plant	21.79 mg	31.19 mg	26.40 mg
	in 1 g weight of fresh leaves	13.35 "	14.30 "	11.80 "
double amount of nitrogen	in all leaves of one rice plant	37.92 "	37.33 "	35.50 "
	in 1 g weight of fresh leaves	17.66 "	13.74 "	13.88 "
triple amount of nitrogen	in all leaves of one rice plant	—	50.65 "	43.10 "
	in 1 g weight of fresh leaves	—	14.39 "	14.10 "
quadruple amount of nitrogen	in all leaves of fresh leaves	—	45.52 "	—
	in 1 g weight of fresh leaves	—	13.01 "	—
quintuple amount of nitrogen	in all leaves of one rice plant	—	67.88 "	—
	in 1 g weight of fresh leaves	—	14.60 "	—

The results on those rice plants, which were furnished additional amount of nitrogen at the ear formation stage, (experiments 4 and 5)

are shown in table 35.

TABLE 35. Total nitrogen in the rice plants which received the additional nitrogen supply.

experimental section days after the additional supply experiments	control		treatment	
	after 5 days	after 13 days	after 5 days	after 13 days
exp. 4	11.01 mg	11.86 mg	13.45 mg	15.72 mg
exp. 5	12.15 "	12.41 "	13.24 "	14.37 "

As will be seen in the table, the increase of the content was certain already five days after the treatment and it became more remarkable thirteen days after the treatment.

Then the total nitrogen was divided into two parts, the protein nitrogen and the soluble nitrogen. The content of each kind of nitrogen from exp. 1 to exp. 3 is given in table 36. On inspection of the table, one sees that evidently the content of both kinds of nitrogen in all the leaves of one rice plant becomes higher when more nitrogen is supplied to the rice plant, although there is a little discrepancy in the value of the quadruple amount nitrogen section in experiment 2. The same fact holds in the content of the leaves of 1 g fresh weight. But examining the increasing rate of each nitrogen, one sees that it is rather greater in the content of soluble nitrogen than of the protein nitrogen. The percentage of each kind of nitrogen in the total nitrogen quantity is shown in figure 6. As will be seen clearly in the figure, the percentage of protein nitrogen decreases while the percentage of soluble nitrogen increases in accordance with the quantity of nitrogen applied. But in experiment 2 such tendency is seen barely in the section of quintuple amount of nitrogen. The higher percentage of soluble nitrogen which is accompanied naturally by a lower percentage of protein nitrogen is thought to be an index of the state of nitrogen excess in the rice plant. Further, in experiment 2, it is supposed that the rice plants are not really in the state of nitrogen excess for some uncertain reason till they were given more than four times as much nitrogen as the basal amount. It must be noticed here again that in experiment 2 the blast susceptibility was not so remarkably intensified till the quintuple amount nitrogen as the basal was applied.

TABLE 36. Contents of protein and soluble nitrogen in the rice plants of the different level nitrogen supply.

exp.	nitrogen level	items	proten-N	total soluble-N
1	standard amount of nitrogen	in all leaves of one rice plant	7.46 mg	4.33 mg
		in 1 g weight of fresh leaves	10.70 "	2.65 "
	double amount of nitrogen	in all leaves of one rice plant	27.27 "	10.65 "
		in 1 g weight of fresh leaves	12.70 "	4.96 "
2	standard amount of nitrogen	in all leaves of one rice plant	24.26 "	6.93 "
		in 1 g weight of fresh leaves	11.12 "	3.18 "
	double amount of nitrogen	in all leaves of one rice plant	30.29 "	7.04 "
		in 1 g weight of fresh leaves	11.15 "	2.59 "
	triple amount of nitrogen	in all leaves of one rice plant	40.55 "	10.10 "
in 1 g weight of fresh leaves	11.52 "	2.87 "		
3	quadruple amount of nitrogen	in all leaves of one rice plant	38.74 "	6.78 "
		in 1 g weight of fresh leaves	11.07 "	1.94 "
	quintuple amount of nitrogen	in all leaves of one rice plant	51.60 "	16.28 "
3	standard amount of nitrogen	in all leaves of one rice plant	21.85 "	4.55 "
		in 1 g weight of fresh leaves	9.77 "	2.03 "
	double amount of nitrogen	in all leaves of one rice plant	28.19 "	7.31 "
		in 1 g weight of fresh leaves	11.00 "	2.88 "
triple amount of nitrogen	in all leaves of one rice plant	33.30 "	9.80 "	
	in 1 g weight of fresh leaves	10.89 "	3.21 "	

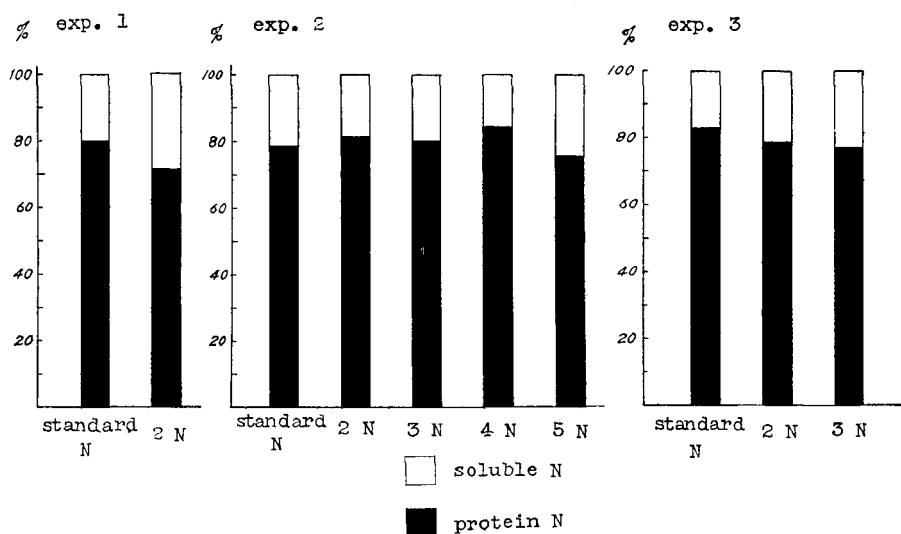


Fig. 6. Percentage of protein nitrogen and soluble nitrogen in the total nitrogen of the rice plant of the different level nitrogen supply.

Table 37 gives the results in experiments 4 and 5, in which the excess nitrogen was applied additionally at the ear formation stage. As will be seen in the table the increase in the content of each kind of nitrogen is evident already five days after the giving of the additional supply of the nitrogen; that increase becomes more remarkable at thirteen days after the treatment. Figure 7 shows the percentage of the two classes of nitrogen in the total. As shown in the figure, an increase of percentage of the soluble nitrogen accompanied with a

TABLE 37. Content of protein nitrogen and soluble nitrogen in the rice plants which received the additional supply of nitrogen.

exp.	fraction of nitrogen	control		additional supply of nitrogen	
		5 days	13 days	5 days	13 days
4	protein-N	9.24 mg	9.64 mg	11.40 mg	12.03 mg
	soluble-N	1.77 "	2.22 "	2.05 "	3.69 "
5	protein-N	10.30 "	10.28 "	10.43 "	11.24 "
	soluble-N	1.85 "	2.13 "	2.81 "	3.13 "

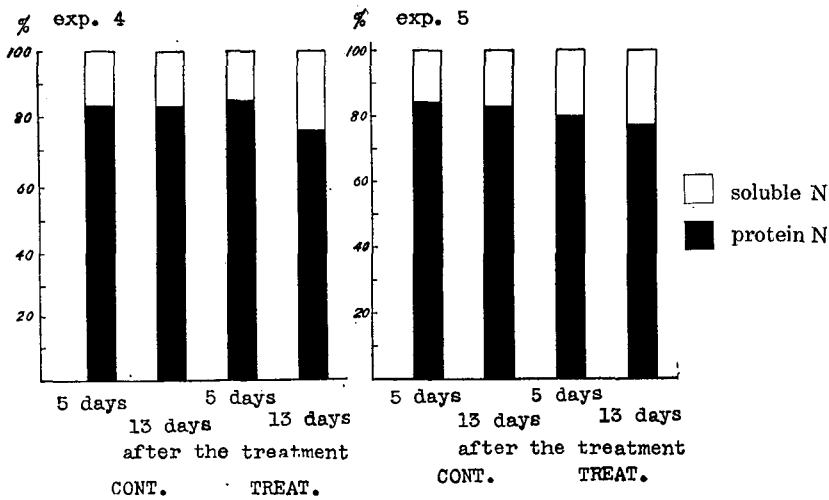


Fig. 7. Percentage of protein nitrogen and soluble nitrogen in the total nitrogen of the rice plants which received the additional supply of nitrogen.

decrease of percentage of the protein nitrogen is seen already five days after the additional application and that increase and decrease becomes quite remarkable on the thirteen days after the treatment.

Thus it is certain that the application of a large quantity of nitrogen causes remarkable increase of the soluble nitrogen in the rice plant. Then the fractions of the soluble nitrogen were examined, and the results in experiment 1-3 are shown in table 38. As will be seen in the table, the content of the α -amino nitrogen increases quite remarkably in accordance with the supply of the larger amount of nitrogen, while the content of some other kinds of soluble nitrogen shows even decreasing.

Table 39 gives also the results of experiments 4 and 5, in which the excess nitrogen were supplied additionally at ear formation stage. From the table, it is evident that the increase in the content of some kinds of nitrogen as α -amino nitrogen appears already on the 5th day after the additional supply of nitrogen, and it becomes even greater on the 13th day. Fig. 8 and fig. 9 give the percentage of each kind of soluble nitrogen in the total soluble nitrogen. As will be seen in the figure, the most part of soluble nitrogen in the rice plant supplied with excess nitrogen is occupied by α -amino nitrogen and the percentage of some other kinds of soluble nitrogen such as soluble protein

TABLE 38. Fractions of the soluble nitrogen in the rice plants of the different nitrogen levels.

exp.	nitrogen level	items	soluble protein N	α -amino N	basic N	amide N	ammonium N	nitrate N
1	standard amount of nitrogen	in all leaves of one rice plant	1.37	0.77	1.60 "	0.44 "	0.11 "	0.13 "
		in 1 g weight of fresh leaves	0.84 "	0.47 "	0.98 "	0.27 "	0.07 "	0.08 "
	double amount of nitrogen	in all leaves of one rice plant	1.08 "	7.05 "	0.39 "	0.45 "	0.18 "	0.24 "
		in 1 g weight of fresh leaves	0.50 "	3.28 "	0.18 "	0.21 "	0.08 "	0.11 "
2	standard amount of nitrogen	in all leaves of one rice plant	2.73 "	1.28 "	0.68 "	2.08 "	0.15 "	0.11 "
		in 1 g weight of fresh leaves	1.25 "	0.59 "	0.31 "	0.95 "	0.07 "	0.05 "
	double amount of nitrogen	in all leaves of one rice plant	2.32 "	0.86 "	2.74 "	0.93 "	0.17 "	0.22 "
		in 1 g weight of fresh leaves	0.86 "	0.32 "	1.01 "	0.34 "	0.06 "	0.08 "
	triple amount of nitrogen	in all leaves of one rice plant	1.44 "	1.90 "	5.67 "	0.46 "	0.28 "	0.21 "
		in 1 g weight of fresh leaves	0.41 "	0.54 "	1.61 "	0.13 "	0.08 "	0.06 "
	quadruple amount of nitrogen	in all leaves of one rice plant	1.53 "	2.94 "	1.57 "	0.50 "	0.25 "	0.31 "
		in 1 g weight of fresh leaves	0.44 "	0.84 "	0.45 "	0.14 "	0.07 "	0.09 "
quintuple amount of nitrogen	in all leaves of one rice plant	3.06 "	7.67 "	4.23 "	0.51 "	0.61 "	0.65 "	
	in 1 g weight of fresh leaves	0.66 "	1.65 "	0.91 "	0.11 "	0.13 "	0.14 "	
3	standard amount of nitrogen	in all leaves of one rice plant	1.95 "	0.78 "	0.45 "	1.14 "	0.13 "	0.09 "
		in 1 g weight of fresh leaves	0.87 "	0.35 "	0.20 "	0.51 "	0.06 "	0.04 "
	double amount of nitrogen	in all leaves of one rice plant	2.36 "	2.08 "	0.59 "	2.08 "	0.15 "	0.20 "
		in 1 g weight of fresh leaves	0.92 "	0.81 "	0.23 "	0.81 "	0.06 "	0.08 "

exp.	nitrogen level	items	soluble protein N	α-amino N	basic N	amide N	ammonium N	nitrate N
			mg	mg	mg	mg	mg	mg
	triple amount of nitrogen	in all leaves of one rice plant	1.96	3.92 "	0.98 "	2.44 "	0.40 "	0.34
		in 1 g weight of fresh leaves	0.64 "	1.28 "	0.32 "	0.80 "	0.13 "	0.11 "

TABLE 39. Fractions of the soluble nitrogen in the rice plants which received the additional supply of nitrogen.

exp.	fraction of nitrogen	control		additional supply of nitrogen	
		5 days*	13 days*	5 days*	13 days*
4	soluble protein-N	0.85 mg	0.94 mg	0.56 mg	0.52 mg
	α-amino-N	0.22 "	0.36 "	0.53 "	1.55 "
	basic-N	0.41 "	0.56 "	0.27 "	0.30 "
	amide-N	0.16 "	0.22 "	0.20 "	0.37 "
	ammonium-N	0.10 "	0.10 "	0.10 "	0.11 "
	nitrate-N	0.10 "	0.11 "	0.13 "	0.20 "
5	soluble protein-N	0.50 "	0.51 "	0.82 "	0.66 "
	α-amino-N	0.56 "	0.76 "	0.98 "	1.24 "
	basic-N	0.46 "	0.48 "	0.71 "	0.62 "
	amide-N	0.21 "	0.28 "	0.24 "	0.43 "
	ammonium-N	0.08 "	0.10 "	0.15 "	0.15 "
	nitrate-N	0.02 "	0.01 "	0.01 "	0.01 "

* days after the giving of the additional supply of nitrogen.

nitrogen or basic nitrogen are rather lower in the rice plants given excess nitrogen than in the control rice plant.

Summarizing the results described in this section, the effects of excess nitrogen supply on the content of various kinds of nitrogen in the rice plant are clearly seen in the latter phase of the boot stage*. When excess nitrogen is supplied, the total nitrogen content in the rice plant increases fairly, and the increasing rate of soluble nitrogen

* As was mentioned in the previous chapter, the effects of excess nitrogen supply on the content of nitrogen are not so clear in the ear formation stage.

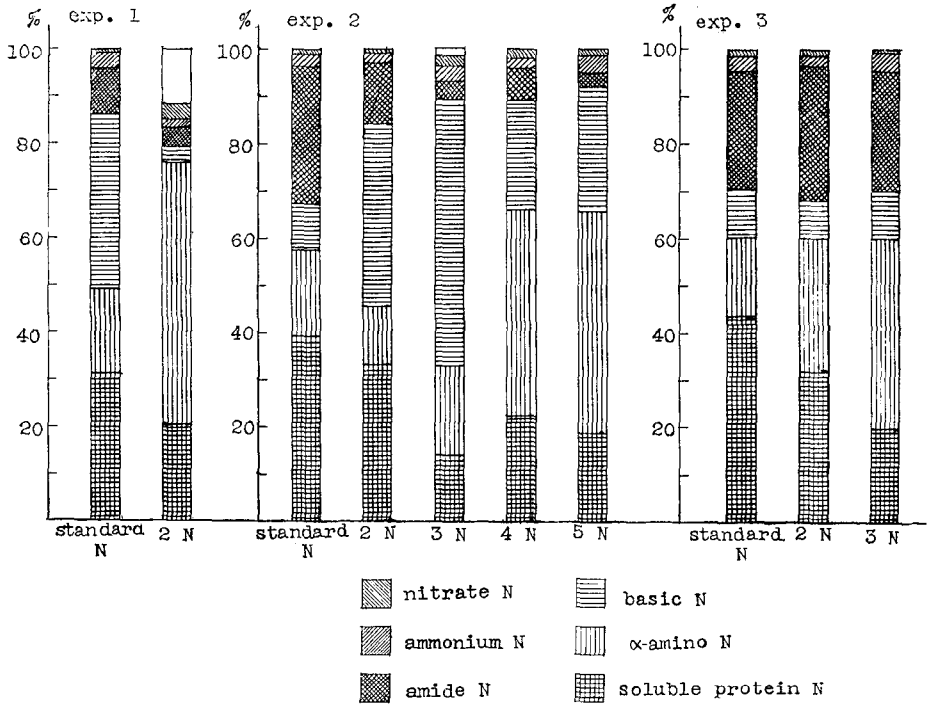


Fig. 8. Percentage of each fraction of the soluble nitrogen in the rice plants of different nitrogen levels.

is rather higher on comparison with that of protein nitrogen. In the percentage composition of total nitrogen, therefore, the percentage of protein nitrogen is rather lower while the percentage of soluble nitrogen is higher in the rice plant supplied with excess nitrogen on comparison with the percentages in the standard rice plant. Moreover the increase of α -amino nitrogen among all the kinds of soluble nitrogen is most remarkable. Such composition of the nitrogen in the rice plant seems to be an index of the excess accumulation of nitrogen in the rice plant. However, it is rather difficult to determine the critical amounts of nitrogen supply for the appearance of excess accumulation of nitrogen in the rice plant, as shown in the experiments.

When the excess nitrogen is supplied additionally during the course of plant growth, such excess accumulation of nitrogen in the rice plant as described above become evident already on the 5th day after the additional supply, becoming more eminent on the 13th day after the additional supply.

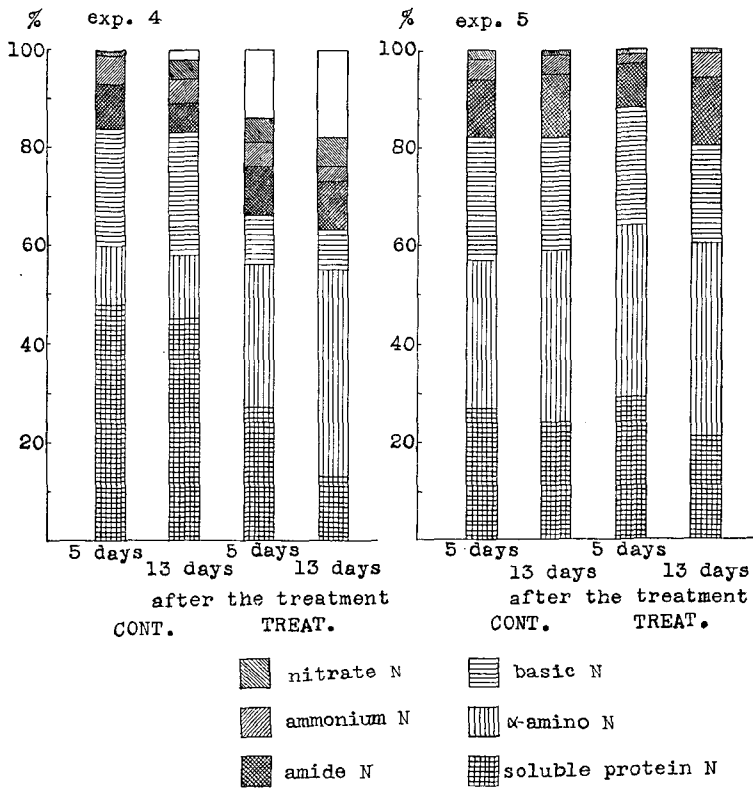


Fig. 9. Percentage of each fraction of the soluble nitrogen in the rice plants which received the additional supply of nitrogen.

It is worthy of notice, taking the results of inoculation experiment into consideration, that fluctuation of the blast disease susceptibility resultant from excess nitrogen supply is closely related with the appearance of such excess accumulation of nitrogen in the rice plant described above.

4. The kind of α -amino acids in the rice plant.

In the previous section it was shown that excess supply of nitrogen to the rice plant leads to the increase in quantity of α -amino acid in the plant. In this section an investigation on the effect of excess nitrogen supply on the quality of α -amino acid in the rice plant is described. The same rice plants as used in the previous experiments

TABLE 40. Kinds of amino acids found in the rice plants of the different nitrogen levels.

exp.	nitrogen levels	glu- tamic acid	aspar- tic acid	glu- tamine	aspara- gine	gly- cine	ala- nine	leu- cine	argi- nine	histi- dine
1	standard amount of nitrogen	+	-	+	+	+	+	+	+	+
	double amount of nitrogen	+	+	+	+	+	+	+	+	+
2	standard amount of nitrogen	+	+	+	+	+	-	-	+	+
	double amount of nitrogen	+	+	+	+	+	+	+	+	+
	triple amount of nitrogen	+	+	+	+	-	+	-	+	-
	quadruple amount of nitrogen	+	+	+	+	+	+	+	+	+
	quintuple amount of nitrogen	+	+	+	+	-	+	+	+	+
3	standard amount of nitrogen	+	+	+	+	+	-	-	+	+
	double amount of nitrogen	+	+	+	+	+	+	-	+	+
	triple amount of nitrogen	+	+	+	+	+	+	+	+	+

TABLE 41. Kinds of amino acids found in the rice plants which received the additional supply of nitrogen.

exp.	experi- mental section	days after the addi- tional supply	glu- tamic acid	aspar- tic acid	glu- tamine	aspara- gine	gly- cine	ala- nine	leu- cine	argi- nine	histi- dine
4	control	5 days	+	-	+	-	+	-	-	+	-
		13 days	+	-	+	-	+	+	-	+	-
	additional supply of nitrogen	5 days	+	+	+	+	+	+	-	+	-
		13 days	+	+	+	+	-	+	-	+	-
5	control	5 days	+	+	+	+	+	-	-	+	-
		13 days	+	+	+	+	-	-	-	+	-
	additional supply of nitrogen	5 days	+	+	+	+	-	+	-	+	-
		13 days	+	+	+	+	-	+	-	+	-

were used for the following experiments. Water extractions of the rice plant leaves were deproteinized and after the removal of ammonia by distillation in vacuum they were concentrated to a small quantity. The kinds of amino acids in the concentrated sap were determined by paper partition chromatography. Any one of phenol, butanol and lutidine were employed as the solvents of one direction development. All the amino acids which were detectable all through the experiments were the nine: glutamic acid, aspartic acid, glutamine, asparagine, glycine, alanine, leucine, arginine and histidine. In addition to them there were often found some undeterminable spots on the testing paper. Table 40 shows the presence of the amino acids in every section of experiments 1, 2 and 3. As will be seen in the table, the kinds of amino acids detectable are not always the same even in the control sections of each experiment. But it may safely be said that the number of kinds of amino acid detectable is apt to increase in company with the increasing of the amount of nitrogen supplied, though there are some discrepancies in experiment 2. Table 41 shows the results of experiments 4 and 5 when excess nitrogen was supplied additionally in the course of the rice plant growth. In these experiments the kinds of amino acid detectable were generally fewer in comparison with the results in the previous experiments. But the inclination for the number of amino acids to increase with the additional supply of nitrogen may be recognizable.

In conclusion it seems to be certain that the excess nitrogen supply exerts some effects on the amino acid composition in the rice plant, but they are rather indistinct in comparison with the effects on the quantity of amino acids in the rice plant.

5. Sugar content.

The effects of the excess nitrogen supply on the sugar content in the rice plant were investigated as described in this section. Use was made of the same rice plants as used in the previous experiments 1, 2 and 3. The sugar contents were determined by BERTRAND-method on the expressed sap of the leaves which had been previously deproteinized. The total sugar content is shown in table 42.

It will be seen from the table that the sugar content not only in the whole amount of leaves of one plant but also in 1g fresh weight of leaves increases fairly with the increase of nitrogen supply. On the other hand it is shown in the previous section that the increasing

TABLE 42. Total content of sugar in the rice plants of the different nitrogen levels.

nitrogen levels	items	exp. 1	exp. 2	exp. 3
standard amount of nitrogen	in all leaves of one rice plant	32.00 mg	28.41 mg	43.99 mg
	in 1 g weight of fresh leaves	19.61 "	13.03 "	18.66 "
double amount of nitrogen	in all leaves of one rice plant	44.68 "	76.02 "	64.31 "
	in 1 g weight of fresh leaves	20.80 "	27.95 "	25.12 "
triple amount of nitrogen	in all leaves of one rice plant	—	105.21 "	87.85 "
	in 1 g weight of fresh leaves	—	29.89 "	28.71 "
quadruple amount of nitrogen	in all leaves of one rice plant	—	119.70 "	—
	in 1 g weight of fresh leaves	—	34.20 "	—
quintuple amount of nitrogen	in all leaves of one rice plant	—	135.03 "	—
	in 1 g weight of fresh leaves	—	29.04 "	—

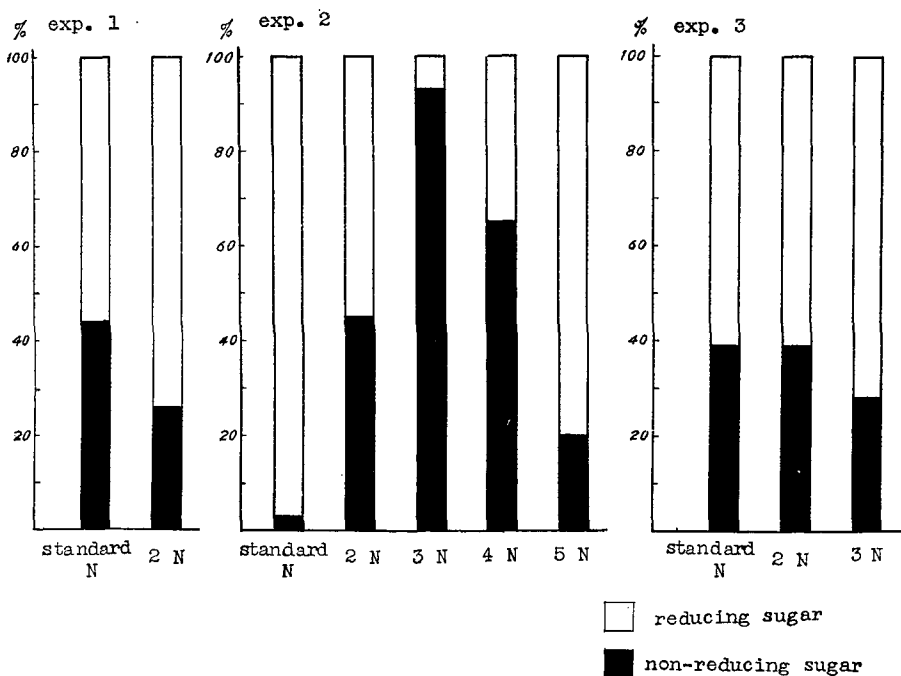


Fig. 10. Percentage of reducing and non-reducing sugar in the total sugar of the rice plants of the different nitrogen levels.

of the nitrogen supply to the plant results in the increasing of the content of amino acid in the rice plant. And the formation of the more amino acid has to be accompanied by the more consumption of sugar. Therefore the increase of sugar content shown in the table seems to mean the higher activity of photosynthesis in the rice plants supplied with excess nitrogen. The total sugar was divided into reducing sugar and non-reducing sugar. The content of each kind of sugar is given in table 43. Generally speaking it will be clearly seen from the table that the content of reducing sugar in the rice plant

TABLE 43. Content of reducing and non-reducing sugar in the rice plants of the different nitrogen levels.

Exp.	nitrogen levels	items	red. sugar	non-red. sugar
1	standard amount of nitrogen	in all leaves of one rice plant	17.92 mg	14.08 mg
		in 1 g weight of fresh leaves	10.98 "	8.63 "
	double amount of nitrogen	in all leaves of one rice plant	33.20 "	11.48 "
		in 1 g weight of fresh leaves	15.46 "	5.34 "
2	standard amount of nitrogen	in all leaves of one rice plant	22.94 "	5.47 "
		in 1 g weight of fresh leaves	12.61 "	0.42 "
	double amount of nitrogen	in all leaves of one rice plant	41.81 "	34.21 "
		in 1 g weight of fresh leaves	15.37 "	12.58 "
	triple amount of nitrogen	in all leaves of one rice plant	6.86 "	98.35 "
		in 1 g weight of fresh leaves	1.95 "	27.94 "
quadruple amount of nitrogen	in all leaves of one rice plant	41.37 "	78.33 "	
	in 1 g weight of fresh leaves	11.82 "	22.38 "	
quintuple amount of nitrogen	in all leaves of one rice plant	108.39 "	26.64 "	
	in 1 g weight of fresh leaves	23.31 "	5.73 "	
3	standard amount of nitrogen	in all leaves of one rice plant	25.83 "	18.16 "
		in 1 g weight of fresh leaves	11.53 "	7.13 "
	double amount of nitrogen	in all leaves of one rice plant	39.37 "	24.94 "
		in 1 g weight of fresh leaves	15.38 "	9.74 "
triple amount of nitrogen	in all leaves of one rice plant	62.97 "	24.88 "	
	in 1 g weight of fresh leaves	20.58 "	8.13 "	

increases with the increasing amount of nitrogen supply, though there are some discrepancies in experiment 2. The content of non-reducing sugar never increases in the same proportion and on the contrary there are sometimes found some decreases in its content. Therefore, taking the percentage composition of each kind of sugar into consideration (see figure 10), there are clearly shown the increase of reducing sugar percentage and the decrease of non-reducing sugar percentage accompanied by the increase of nitrogen supply, though there are some exceptions in experiment 2. This fact may be explained as follows. In the rice plant supplied with excess nitrogen the most part of the sugar is converted into the form of reducing sugar with which the organic nitrogenous substances such as amino acid are conveniently synthesized.

6. Conclusion

In this chapter the experiments have been described which were planned to learn the critical point of nitrogen supply at which the blast susceptibility of the rice plant comes to be enhanced, and also to learn the critical amount of nitrogen supplied by which the accumulation of soluble nitrogen in the rice plant begins to appear. The effects of the additional supply of excess nitrogen at about the ear formation stage upon the blast susceptibility of the rice plant as well as upon the chemical components were also examined. In addition the attention was paid on effect of supply of high level nitrogen on the silification of the epidermal cells of the rice plant.

As shown by the experiments, however, it is quite difficult to find out the critical point of the nitrogen supply just at which the blast susceptibility of the rice plant comes to be enhanced because in some instances the blast susceptibility of the rice plant is enhanced by the supply of double the amount of nitrogen as the standard while in other instance such effects do not appear so remarkably till the application of five times as much nitrogen as the standard. On the other hand the additional supply of excess nitrogen at about the ear formation stage begins to enhance the blast susceptibility of the plants on the second day after the additional supply and it becomes striking with the lapse of time till 8th day after the additional supply. On examining the degree of silification of the epidermal cell of those rice plants, one finds clearly, especially on the motor cell, that its silification becomes the less with the supply of the larger amount of nitrogen. It

is clearly known by observation of the number of diseased lesions developed by the inoculation experiment that their number is greater on the rice plant applied with the larger amount of nitrogen. Therefore, taking into consideration the fact that the blast fungus enters the rice plant mostly through the motor cells, it is certain that a part of the reason for the higher susceptibility of the rice plant supplied with the excess amount of nitrogen lies in the lower silification of the epidermal cells which is caused by the application of the excess amount nitrogen.

As for the nitrogen content in the leaves of these rice plants, the applications of high level nitrogen results in the increase of total nitrogen in their leaves. Among the parts of the total nitrogen, however, in that case the soluble nitrogen increase at a far greater rate in comparison with the protein nitrogen. Therefore, on observation of the percentage composition of both sorts of nitrogen in the total nitrogen, it is seen that protein nitrogen percentage decreases while the soluble nitrogen percentage increases in the rice plants supplied with high level nitrogen. It must be noted that, on such occasion, the increase of α -amino acid among the soluble nitrogens is most remarkable. Such a state of nitrogen composition in the rice plant which is caused by the application of the high level nitrogen appears already in the case of two times as much nitrogen apply as standard in some experiments while in other experiments it does not appear till five times as much nitrogen is supplied as standard. Thus it is rather difficult to find out the critical amount of the nitrogen level for producing the symptoms of excess nitrogen supply. It is quite the same as in the difficulties of finding the critical amount of nitrogen for the enhancement of the blast susceptibility. However it must be noticed that in these experiments the degree of blast susceptibility always goes parallel with the increase of such soluble nitrogen as α -amino acids.

Such parallelisms are also seen in the case of the rice plants which were given the additional supply of excess amount of nitrogen in the course of the plant growth. On the other hand it is ascertained that the increase in the amount of amino acid in the rice leaves which is caused by the supply of the excess amount nitrogen does not always mean the increase of the kinds of amino acids detectable in the rice leaves. The kinds of amino acids which are ordinarily found in the leaves, are following nine : glutamic acid, aspartic acid, glycine, alanine, leucine, arginine and histidine.

The effects of the excess amount nitrogen application on the sugar content in those rice leaves are quite complicated and it is rather difficult generally to find out any direct relations between the blast susceptibility and the sugar contents in these experiments. However there are some instances in which the high sugar content induced by the excess amount nitrogen application seems to be concerned with the induced high blast susceptibility.

Chapter IV

The kind of nitrogen supplied to the rice plants and their blast susceptibility.

It seems to be interesting to examine if the supply of the different kind of nitrogen to the rice plant gives some effects on their rice blast susceptibility and their nitrogen component. In experiments described in this chapter the rice plants were cultivated in water culture solutions of which nitrogen source were either of ammonium sulfate, ammonium nitrate, sodium nitrate or urea respectively, and they were inoculated with the rice blast fungus and at the same time their nitrogen contents were examined. It must be noted that the rice plant does not grow well in a culture solution of rather high pH value, and the pH value in the culture solution of which the nitrogen source is sodium nitrate is apt to rise on account of the unequivalent absorption of ions by the plant. Therefore in the culture solution of sodium nitrate nitrogen source the pH values were carefully adjusted to pH 5.0-5.4 by occasional addition of proper quantity of dilute hydrogen chloride. As for the quantity of the nitrogen supplied, two experimental sections were prepared with each nitrogen source, standard amount section and quintuple amount section. When the rice plants were grown up to the latter phase of the boot stage, they were employed for inoculation and for the chemical determinations.

1. Inoculation experiments.

Speaking first of the rice plants of standard nitrogen level, ammonium nitrate most favourably influenced the height of the rice plant and followed with a little differences by urea and sodium nitrate in order while the height was rather lower in the case of the ammonium sulfate. As for the number of tillers, the order was a little different

as follow: ammonium nitrate, urea, ammonium sulfate and natrium nitrate. The supply of high level nitrogen as great as quintuple amount seemed to be unfavorable to the growth of rice plants which showed lower height and smaller number of tillers in all cases of every nitrogen sources excepting urea.

TABLE 44. Inoculations of the blast fungus on the rice plants which were supplied with the different kinds of the nitrogen source.

nitrogen levels nitrogen sources items	standard amount of nitrogen				quintuple amount of nitrogen				
	NH ₄ NO ₃	NaNO ₃	(NH ₄) ₂ SO ₄	urea	NH ₄ NO ₃	NaNO ₃	(NH ₄) ₂ SO ₄	urea	
height	67 cm	65 cm	61 cm	66 cm	65 cm	64 cm	57 cm	67 cm	
number of tillers	11.5	8.3	9.3	10.0	10.0	7.3	10.0	11.0	
number of leaves examined	120	108	132	120	130	119	130	126	
total number of lesions	50	46	53	61	62	63	68	73	
average number of lesions per leaf	0.42	0.43	0.40	0.51	0.48	0.53	0.52	0.58	
average number of lesions per 100 cm length of leaves	1.8	2.0	1.9	2.2	1.9	2.2	2.5	2.3	
length of lesions	max.	1.2 cm	1.1 cm	1.4 cm	1.5 cm	1.3 cm	1.2 cm	1.5 cm	1.5 cm
	min.	0.2 "	0.2 "	0.2 "	0.2 "	0.2 "	0.2 "	0.3 "	0.4 "
	aver.	0.49 "	0.47 "	0.51 "	0.52 "	0.52 "	0.50 "	0.63 "	0.86 "
type of lesions	chronic, acute	chronic, acute	chronic, acute	chronic, acute	acute	acute	acute	acute	

Urea is the only nitrogen source of which a high level favors the plant growth.

The results of the blast fungus inoculation on those rice plants are given in table 44. In the case of the standard nitrogen level, there are not seen so great differences in the number of lesions developed between the sections of each nitrogen source, though the number is a little greater in the urea section than in the other sections of nitrogen sources.

The size of the lesions is somewhat larger in both the sections of urea and ammonium sulfate than in the other sections, while their types are chronic and acute through all the experimental sections. On the other hand the lesions which develop on the rice plant of high

level nitrogen are all the acute type irrespective of the kind of nitrogen source; also their number and size are greater than those which develop on the rice plant of standard nitrogen level. Such relations are specially prominent in the cases of both urea and ammonium sulfate nitrogen source. In conclusion it is certain that the difference of the nitrogen sources supplied to the rice plant does not exert any great effects on their blast susceptibility, though the employment of urea or ammonium sulfate as nitrogen source a little enhances their blast susceptibility. On the other hand the supply of excess amount of nitrogen always favors their susceptibility irrespective of the kind of nitrogen source.

2. Contents of the various kinds of nitrogen in the rice plants.

The contents of the various kinds of nitrogen in the previously employed rice plants are determined. The plants were collected two days after the inoculation and were immediately used for the determination. The results are summarized in table 45, the figures in the table showing mg of each kind of nitrogen in 1 g fresh weight of rice plant.

In the rice plants of standard nitrogen level, the total nitrogen content was greatest in the urea section followed by ammonium nitrate section, ammonium sulfate section and sodium nitrate section in order.

TABLE 45. Contents of each fraction of the nitrogen in the rice plants which were supplied with the different kinds of nitrogen sources.

nitrogen levels nitrogen sources items	standard amount of nitrogen				quintuple amount of nitrogen			
	NH ₄ NO ₃	NaNO ₃	(NH ₄) ₂ SO ₄	urea	NH ₄ NO ₃	NaNO ₃	(NH ₄) ₂ SO ₄	urea
	mg	mg	mg	mg	mg	mg	mg	mg
total-N	12.23	11.85	11.99	12.51	14.82	13.52	14.26	14.93
protein-N	10.42 "	9.68 "	9.51 "	9.96 "	11.23 "	10.15 "	9.89 "	10.25
soluble-N	1.81 "	2.17 "	2.48 "	2.55 "	3.59 "	3.37 "	4.37 "	4.68 "
soluble protein-N	0.63 "	0.54 "	0.64 "	0.73 "	1.08 "	1.02 "	1.38 "	1.35 "
α-amino-N	0.64 "	0.65 "	0.72 "	0.87 "	1.22 "	1.05 "	1.36 "	1.40 "
basic-N	0.36 "	0.48 "	0.50 "	0.51 "	0.72 "	0.66 "	0.77 "	0.84 "
amide-N	0.14 "	0.43 "	0.48 "	0.38 "	0.54 "	0.58 "	0.74 "	0.98 "
ammonium-N	0.03 "	0.04 "	0.10 "	0.04 "	0.03 "	0.04 "	0.10 "	0.08 "
nitrate-N	0.02 "	0.03 "	0.04 "	0.02 "	0.01 "	0.03 "	0.02 "	0.02 "

However, the differences between them are rather small. Dividing this nitrogen into the parts of protein and soluble nitrogen, the content of protein nitrogen was highest in the ammonium nitrate section followed by the sections of urea, sodium nitrate and ammonium sulfate in order respectively. The content of soluble nitrogen was highest in the experimental section of urea followed by the ammonium sulfate, sodium nitrate and ammonium nitrate in order. But the differences among the experimental sections were not very great.

The percentage composition of each nitrogen was calculated and the results are shown in figure 11. As will be seen in the figure, about 85% of total nitrogen is protein nitrogen while the remainder 15% is soluble nitrogen in the ammonium nitrate section; such distribution of each kind of nitrogen is thought to be normal. The similar distribution of each kind of nitrogen is shown in the sodium nitrate section. On the other hand in the urea section and also in the ammonium sulfate section there seems to be some tendency toward soluble nitrogen accumulation though not so clear, showing 80% of protein nitrogen and 20% of soluble nitrogen. Examining the amount of the each kind of nitrogen in the total soluble nitrogen, one sees that there are not so great differences in each nitrogen content through all experimental sections. However it must be noticed that the content of α -amino nitrogen and soluble protein nitrogen are comparatively greater in the

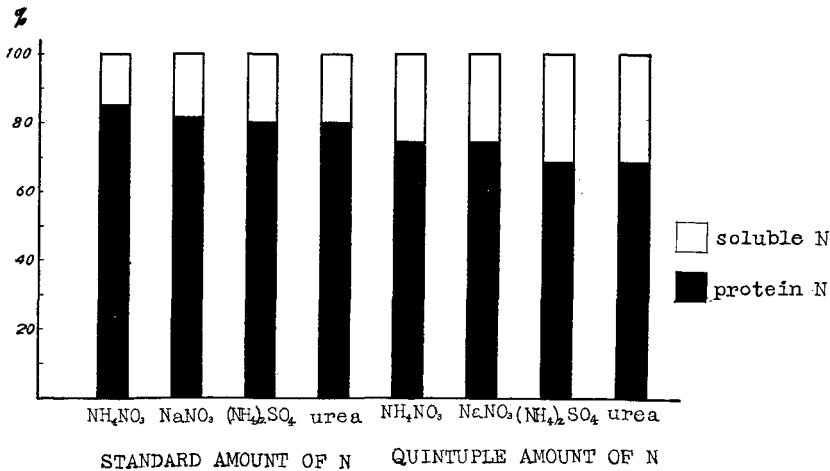


Fig. 11. Percentage of protein and soluble nitrogen in the total nitrogen of the rice plants which were supplied with different nitrogen sources.

urea section in which greater amount of total soluble nitrogen is also seen, and that the content of ammonium nitrogen is comparatively greater, while the content of amide is comparatively smaller in the ammonium nitrate section.

When the high level nitrogen is supplied, the content of the total nitrogen always becomes greater through every experimental sections than in the case of standard nitrogen level. Dividing this nitrogen into protein and soluble nitrogen, the increasing of the soluble nitrogen is prominent in every experimental section in comparison with the increase of protein nitrogen.

As will be seen in figure 11, the percentage of protein nitrogen constituent of the total nitrogen is smaller while the percentage of soluble nitrogen is greater in comparison with the case of standard nitrogen level. In short the symptoms of soluble nitrogen accumulation are clearly shown through all sections of high level nitrogen supply. Among the soluble nitrogen the content of such kinds of nitrogen as soluble protein, α -amino, basic and amide nitrogen are greater through all sections in comparison with the case of standard nitrogen supply. Especially in the urea section remarkably greater content of α -amino and soluble protein nitrogen attracts one's attention. At the same time, the contents of ammonium or nitrate nitrogen are not so greatly different from those in the plants of standard nitrogen level.

In conclusion, the difference of nitrogen source supplied to the rice plant does not result in remarkable effects on the content of any kind of nitrogen in the plants, with the one exception of the case of the urea in which some tendency toward soluble nitrogen accumulation is seen. The supply of a high level of nitrogen always leads to higher contents of soluble nitrogens in the rice plant such as soluble protein, α -amino, basic and amide nitrogen irrespective of the kind of nitrogen supplied to the rice plant as nitrogen source. It must be added here that such tendency is most remarkable in the case of the urea nitrogen source.

3. The kind of amino acid found in the rice plants supplied with different kinds nitrogen source.

In order to know whether the kind of nitrogen supplied to the rice plant exerts any effects on the kinds of amino acid found in the rice plant, the kinds of amino acid were examined by paper partition chromatography on those rice plants used in the previous sections.

The procedures of the measurement are quite the same as those described in the previous chapter.

The results are given in table 46.

TABLE 46. Kinds of amino acids which are found in the rice plants supplied with different nitrogen sources.

nitrogen levels nitrogen sources items	standard amount of nitrogen				quintuple amount of nitrogen			
	NH ₄ NO ₃	NaNO ₃	(NH ₄) ₂ SO ₄	urea	NH ₄ NO ₃	NaNO ₃	(NH ₄) ₂ SO ₄	urea
glutamic acid	+	+	+	+	+	+	+	+
aspartic acid	+	-	+	+	+	+	+	+
glutamine	+	+	+	+	+	+	+	+
asparagine	+	-	+	+	+	+	+	+
glycine	-	+	-	+	-	+	-	+
alanine	+	+	+	+	+	+	+	+
leucine	+	+	+	+	+	+	+	+
arginine	+	+	+	+	+	+	+	+
histidine	-	-	-	+	-	-	-	+

The full list of amino acids found in the rice plants through every experimental section is the following nine: glutamic acid, aspartic acid, glutamine, asparagine, glycine, alanine, leucine, arginine and histidine. Among them the five amino acids, glutamic acid, glutamine, alanine, leucine and arginine are always found in every rice plant no matter what the nitrogen source. Aspartic acid and asparagine are also recognizable all through the experimental sections with one exception of the case of standard amount supply of sodium nitrate. Glycine is not found in the rice plants supplied with ammonium nitrate or ammonium sulfate as nitrogen source irrespective of the amount of supply; histidine is found only in the rice plants supplied with urea as nitrogen source. As described in the previous section the content of amino acid is apt to become greater when urea is supplied as nitrogen source and it is shown in this experiment that the number of kinds of amino acid found in those rice plants also becomes greater.

However, it may attract one's attention that the larger amount of nitrogen supplied does not always result in the greater number of the amino acids found in the plants.

4. Conclusion.

The rice plant can utilize each kind of nitrogen: sodium nitrate, ammonium nitrate, ammonium sulfate and urea, as the nitrogen source for their growth as long as the pH values of the culture solutions are adjusted continuously to 5.0-5.4 with careful attention especially in the case of sodium nitrate. It is rather difficult to speak as to the superiority of some nitrogen source beyond the others for the rice plant growth, although the ammonium nitrate and the urea may be a little superior to ammonium sulfate and the sodium nitrate may be a little inferior to the ammonium sulfate as the best nitrogen source. Irrespective of the kinds of nitrogen sources applied, with one exception in the case of urea, the growth of the rice plants supplied with five times as much nitrogen is a little inferior to the growth of those given standard amount of nitrogen.

It is known from the experiments that the difference of the nitrogen source in the culture solution used does not result in any differences in the blast susceptibility of the rice plant, likewise it does not result in any important differences in their nitrogenous components with one exception in the case of the urea nitrogen source. The rice plants supplied with urea as their nitrogen source become a little more susceptible to the attack of the blast fungus, and at the same time there is seen some tendency of accumulation of the soluble nitrogen in those plants, on comparison with those supplied with other nitrogen sources.

Irrespective of the kind of nitrogen source, the application of the high level nitrogen as much as five times the standard amount makes the rice plant more susceptible to the blast disease while also the remarkable accumulation of the soluble nitrogen such as α -amino nitrogen accompanied by the increase of the total nitrogen, are seen in those rice plants. Among all the sources tested, such effects of the excess nitrogen supply are most remarkable in the case of the urea nitrogen source. It is also to be added here that the increase in the total amount of amino acids in the rice plant is not always accompanied by the increase in number of the kinds of amino acids detectable in the rice plants.

Chapter V

General conclusion.

In the previous chapters those instances, in which the blast susceptibility of the same rice variety "Eiko" fluctuates with the environmental conditions or with the differences of their growing stages, are ascertained. At the same time the morphological characters of the epidermal cells that may be concerned with the infection of the fungus and their principal chemical components are examined with the final purpose of finding out why the blast susceptibility in the same rice variety fluctuates with the environmental conditions.

All the experimental results described in the foregoing chapters are summarized in this chapter in an attempt to reach some general conclusions. The rice variety "Eiko" which was employed all through the experiments is originally medium in its susceptibility or resistance to the blast disease. The diseased lesions which develop usually on the rice plants cultivated under ordinary conditions are mostly of the chronic type. However if the procedure through which the diseased lesion develops is observed in detail, it is found that the lesion appears first as a very small brown spot. In a while an area which appears somewhat transparent develops around the brown spot, taking on the whole the appearance of the acute type lesion. Ordinarily they do not long retain such appearance but are soon converted into the typical chronic type lesion, disintegrated zone and necrotic zone coming clearly into appearance. In short, the development of the diseased lesion on the rice variety "Eiko" follows the course: brown spot→acute type→chronic type. If the susceptibility were enhanced under some conditions, the diseased lesions often continue to enlarge remaining in the acute form for a long. If the rice plant becomes more resistant under certain other conditions most of the lesions remain in the brown spot type for a long. According to the inoculation experiment, the rice seedlings grow up more susceptible to blast disease in the hot bed nursery on comparison with those which are raised in the ordinary nursery. The lesions developed on the former seedlings are either of chronic type or of acute type and never of brown spot type, while the lesions developed on the latter seedlings are either of chronic type or of brown spot type. The developed lesions are more numerous and their size is larger in general on the former seedlings than those on

the latter ordinary nursery seedlings. But it is clear that such characters in the seedling stage never remain long after the plants are transplanted to field from the nursery bed. The susceptibility of rice plants to the blast disease fluctuates rather greatly with their growing stages. In the elongation stage, irrespective of the kind of nursery in which they are raised, the rice plants are most resistant against the infection of the blast disease when they begin to grow vigorously after transplantation. The lesions developed in the course of inoculation experiment are comparatively few, and their sizes are generally smaller, while their type are mostly of the brown spot accompanied with some of the chronic type. On the contrary the next "ear formation stage" is the most favourable stage for the infection of the blast fungus. The number of lesions developed by the inoculation at that stage are quite numerous and their size is remarkably larger while their type is mostly acute accompanied by some of the chronic type. The rice plants are rather susceptible to the infection also at the next "boot stage". But it is evident that the leaves at the "flowering stage" become rather resistant against the infection of the blast disease, in view of the results of inoculation experiments. The leaves of the rice plant in the ripening stage are also clearly resistant against the infection. This is the general story of the fluctuation of the blast susceptibility during the development and growth of the rice plant, which is cultivated under ordinary conditions. On the other hand, when the rice plants are cultivated under the condition of excess nitrogen supply, the elongation stage loses its characteristic of the stage most resistant against blast disease, since the plants are infected as favourably as in the following "ear formation stage" or in the "boot stage". Both at the "ear formation stage" and at the "boot stage" the enhancement of the blast susceptibility by high level nitrogen supply is not so great as has been supposed, because plants in both stages are basically rather highly blast susceptible even when standard level of nitrogen is supplied. On the other hand, at the "flowering stage" and the "ripening stage" the tendency for the leaves of the rice plants to become rather resistant to the blast fungus remains, even when high level nitrogen is supplied. But it must be noticed that the high level nitrogen supply increases the length of the growing stages having the higher blast susceptibility as well as enhancing the blast susceptibility of each growing stage. It seems, however, to be quite difficult to know the critical amount of the nitrogen upon the supply of which the rice sus-

ceptibility to blast disease is clearly effected, because it is more or less concerned with other environmental conditions besides the nitrogen level. If the excess amount of nitrogen is applied a little before the ear formation stage, an eight day's interval is sufficient for the clear appearance of effects respecting the blast susceptibility of the rice plant. The furnishing of the nitrogen source as $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , NaNO_3 , urea respectively in the water culture of the rice plant does not result in any great effect on the blast susceptibility of the rice plant. However, the high level nitrogen supply always favours the blast susceptibility irrespective of the kind of nitrogen source; the effects are especially remarkable in the case of urea nitrogen source.

In order to ascertain whether the differences in blast susceptibility may be attributable to the differences in morphological characters of the rice plant epidermis, such characters were examined as the thickness of the outer layer of epidermal cell and the silification that may be concerned with blast fungus infection. According to these experiments, the outer wall of epidermal cells is generally thinner in the hot bed nursery seedlings which have been proven to be more susceptible than those of the ordinary nursery seedlings. The silification of the epidermal cells in the former seedlings is inferior to that in the latter seedlings. Moreover it is worthy of notice that such differences are most striking in the motor cells, through which the blast fungus has been proven to penetrate most frequently into the rice plant. Similar examples are seen in the case of excess amount nitrogen supply. The silification of the motor cells in the rice plants of which the susceptibility has been enhanced by the excess amount nitrogen supply is clearly higher than in the control rice plant. But on the contrary, the thickness and the silification of the outer layer of epidermal cells generally increase with the progress of the growing stages and therefore those characters do not always go parallel with the fluctuation of the susceptibility. Thus it is beyond question that the weakness of some morphological characters of rice epidermal cells favours the infection of the blast fungus but it is naturally impossible to attribute to them all the responsibility for the blast susceptibility.

Therefore the relations between the blast susceptibility of rice plant and the principal chemical components were examined on the rice plants described above. According to the experiments, the amounts of inorganic substances are comparatively lower and the moisture content is higher in the seedlings raised in the hot bed nursery than

in the ordinary nursery seedlings while there is not so great difference in the amounts of organic substances. Regarding the fluctuation of such contents with the growing stage, the moisture content in the rice plant decreases while the amount of organic substances increases generally with the progress of the growing stage. The amount of inorganic substances in the rice plant is least in both "the ear formation stage" and "the boot stage" when the rice plants are most susceptible to the blast disease. Thus it will be seen that the amount of inorganic substances is lower in the rice plants which are the rather blast susceptible. On examining the content of the inorganic substances, one finds the amount of the almost every kind of inorganic element such as P, K, Ca, etc. to be comparatively lower in the more susceptible rice plant while such a condition is most conspicuous in the amount of the silica. The relation between the susceptibility and the silica content of rice plants has been discussed since many years ago, and it has been thought that the silica acts as a preventive against the penetration of the causal fungus through the cuticle. But according to the present experiments, the correlation between blast resistance and the amount of silica in the leaves is always quite significant even when there exists a rather reverse relation between the silification of the epidermal cell and the blast resistance. This may suggest the existence of other functions of silica which may act against the infection of the blast fungus. In fact W. ENGEL (1953) suggested some physiological function of the silica from the fact that the most of that element in the rice plant is connected with galactose. This is a problem which needs to be examined fully in the future.

Estimations of the amount of sugar in the rice plants above mentioned, show that it is lower in the hot bed nursery seedling than in the ordinary seedling because of the presence of the frame cover in the former. But on the other hand the fluctuations of sugar content in rice plants with the progress of their growing stage are pretty intricate. It is rather difficult to find out any relationship between the blast susceptibility and the amount of sugar, while the fluctuation of the sugar content may be partly explained as dependent upon the nitrogen metabolism of the rice plant.

The amounts of the various kinds of nitrogen in the rice plants described above are reviewed in the following. First, on comparison between the hot bed nursery seedlings and the ordinary seedlings, the total amount of the nitrogen is higher in the former than in the

latter. When the total is divided between the protein nitrogen and the soluble nitrogen, the protein nitrogen percentage is found to be lower while the soluble nitrogen percentage is higher in the former seedling than in the latter seedling. Therefore the amount of soluble nitrogen in 1g fresh weight of the former seedlings is far beyond that of the latter seedling while the differences in the protein nitrogen amount are not so great. On inspecting the composition of the soluble nitrogen, one sees that the amount of every kind of such substance as soluble protein or α -amino acid and so on is always higher in the hot bed nursery seedlings than in the ordinary seedlings because the total amount of soluble nitrogen is great in the former, but the difference in the amount of soluble protein nitrogen is more striking.

Regarding the fluctuation of the nitrogen amount in the rice leaves with the progress of their growth, the total amount of nitrogen increases gradually with the progress of their growth till the ear formation stage and then decreases a little. On dividing this total nitrogen into its two compositions, protein nitrogen and soluble nitrogen, one notices that both the growing stages of "the ear formation stage" and "the boot stage" are quite different from the other growing stages in the percentage composition of the nitrogen. The percentage of soluble nitrogen in the total nitrogen is remarkably higher at both the stages just mentioned on comparison with any other growing stages. Further, the amount of soluble nitrogen in 1g fresh weight of rice leaves is greatest at "the ear formation stage" or at "the boot stage", while the amount of protein nitrogen is least at those two stages. Examining the details of the soluble nitrogen one sees certainly that the amounts of every kind of soluble nitrogen are always higher in the rice leaves of the two, ear formation and boot stages than in the rice leaves of the other growing stages, and among them difference in the amount of three nitrogen fractions, that is α -amino, soluble protein and basic nitrogen, is quite prominent. When the high level nitrogen is supplied to the rice plants, the amount of nitrogen found in the leaves of that rice plants become higher through every growing stage. Moreover it takes one's attention that the effects of the excess amount nitrogen supply are most noticeable at "the elongation stage". In that growing stage the percentage of soluble nitrogen in the total nitrogen, becomes higher and the percentage of protein nitrogen becomes lower as a result of the supply of the high level nitrogen, thus the conditions become nearer to those in "the ear formation stage" or "the boot stage".

It must be noted that the amount of α -amino nitrogen and soluble protein nitrogen among the fractions of the soluble nitrogen are caused to become remarkably greater by the supply of high level nitrogen during "the elongation stage", resembling the stage in the ear formation stage or the boot stage. When the excess amount of nitrogen is supplied just before "the ear formation stage", the effects on the nitrogen composition of the rice plant, as shown by the remarkable increase of the amount of soluble nitrogen such as α -amino acid, begin to appear on 5th day after the supply, becoming more evident on the 13th day after the supply. However it is quite difficult to find out the critical amount of nitrogen, of which supply just gives the typical effect of high level nitrogen supply on the nitrogen component in the rice plant.

The variation of the nitrogen source to the rice plant among any one of NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, NaNO_3 and urea does not result in any great effects on the nitrogen amount nor on its composition, excepting the case of urea in which the total amount of nitrogen is a little higher than when other nitrogen sources are given. It is same in each nitrogen source that the high level nitrogen supply gives always those effects as already mentioned on the nitrogen amount and on the nitrogen composition in the rice plant, while the effects seem to be more evident in the case of the urea nitrogen source.

On putting together the above summarized results of inoculation experiments and of the nitrogen analysis in the rice plants it is clearly evident that those rice plants of which nitrogen composition is comparatively lower in the percentage of protein nitrogen while comparatively higher in the percentage of soluble nitrogen are always more susceptible to the infection of the blast disease as far as the present experiments are concerned. Also as the amounts of total nitrogen in the rather blast susceptible rice plants are generally higher, the amount of soluble nitrogen in the susceptible plants becomes remarkably greater. Thus the parallelism between the greater amount of soluble nitrogen in the rice plant and their greater blast susceptibility draws one's attention. When the total amount of soluble nitrogen is comparatively greater in the rice plant, every fraction of the soluble nitrogen also seems generally to be greater. But it is important as well as interesting to find out what kinds of fractions of the soluble nitrogen in the rice plant are most closely correlated with the blast susceptibility of the plant. Therefore the correlation coefficient be-

tween the amount of every fraction of the nitrogen in the rice plant and the size of the lesions developed in the inoculation experiment were calculated on the 59 examples of the above described experiments.

As will be seen in table 47, there is found some positive correlation between the amount of total nitrogen and the size of the lesions, though not very distinct.

TABLE 47. Correlation coefficient between each fraction of the nitrogen in the rice plants and the sizes of the lesions which were developed by the inoculation experiments.

	correlation coefficient	probable error
total nitrogen	+ 0.26	± 0.08
protein nitrogen	- 0.02	± 0.08
total soluble nitrogen	+ 0.37	± 0.07
soluble protein-N	+ 0.15	± 0.09
α -amino-N	+ 0.46	± 0.06
basic-N	+ 0.32	± 0.07
amide-N	+ 0.02	± 0.09
ammonium-N	+ 0.35	± 0.08
nitrate-N	+ 0.29	± 0.08

On dividing the total nitrogen into protein nitrogen and soluble nitrogen, one finds with certainty a positive correlation between the soluble nitrogen amount and the lesion size, while there seems to be no correlation between the protein nitrogen amount and the lesion size. Among the every fraction of the soluble nitrogen, correlation coefficients are comparatively greater in the three fractions, that is α -amino nitrogen, basic nitrogen and ammonium nitrogen. Especially the correlation with the α -amino nitrogen is the strongest. Smaller values in the correlation coefficients of amide nitrogen and soluble protein nitrogen are rather contrary to the expectation. However it is important to pay attention to the fact that the soluble protein and amide are easily converted to amino acid, which has clear correlation with the susceptibility. It is also known from the experiments that the following kinds of amino acids or amides, viz., glutamic acid, aspartic acid, glutamine, asparagine, glycine, alanine, leucine, arginine and histidine are ordinarily found in the rice plant. And even though the total amount of these amino acids or amides varies, the numbers

of their kinds to be detected in the rice plant do not ordinarily vary so greatly. From the experimental results described above the following conclusions may be drawn with safety. The rice variety "Eiko", which is originally medium in its blast susceptibility, changes its blast susceptibility in accordance with the progress of the growing stages and with the differences in conditions of cultivation. It is certain that one part of the direct cause responsible for such fluctuations of blast susceptibility lies in such morphological characters as the thickness and the silification of the outer wall of the rice epidermal cells, but they are concerned chiefly with the penetration of the blast fungus through epidermis and have no connections with the proliferation of the fungus in the rice plant. The fungus proliferation in the rice plant is concerned rather with some chemical components of the plant. It is certain through all the experiments that the blast susceptibility of the rice plant fluctuates parallel with the increase or decrease of the soluble nitrogen amount contained in the plant. Among the fractions of the soluble nitrogen, amino acids or those fractions which are easily converted into amino acid are most closely correlated with the susceptibility.

Such correlations between the blast susceptibility and the soluble nitrogen amount may be explained from two different standpoints. The one is the standpoint which attaches much importance to the defensive reaction of the rice plant against the blast infection. On thinking from this standpoint, the accumulation of a large quantity of soluble nitrogenous substances such as amino acids may be supposed to weaken or make slower the development of such defensive reaction against the infection. From the other standpoint much importance is attached to the strength of the aggressiveness of the parasitic fungus in the rice plant. On thinking from this standpoint, the accumulation of much soluble nitrogenous substances may increase the strength of the aggressiveness of the causal fungus in the rice plant. Thus the problem needs to be investigated from the both standpoints. It is, however, worthy of notice that, as will be known from the developing process of the diseased lesion on the rice plant, the defence reaction against the blast infection is not originally very strong nor very quick in the rice variety "Eiko" which is used in these experiments. Therefore in the following part of the report on the present investigation, physiological studies on the blast fungus are carried on with the final purpose to follow the problem from the latter of the standpoints mentioned above.

PART B

Physiological studies on the rice blast fungus.

It is shown in the previous part that the accumulation of soluble nitrogen such as soluble protein, α -amino, basic and amide nitrogen in the rice plant favours their susceptibility to the blast disease. Truly there is need to examine from the various points of view why such accumulation of soluble nitrogen promotes the susceptibility. However the present writer believes that the first step to solve this problem lies in knowing what effects such accumulation does exert on the physiological characters of blast fungus, as the disease development is concerned with the struggle between the host plant and the parasite. The experiments which were planned from such view point are described in the following.

Chapter I

Growth factor of the blast fungus.

For the physiological studies of rice blast fungus it is quite necessary to culture the fungus on a synthesized culture medium. But the blast fungus does not grow well on the pure synthesized culture medium without the addition of such natural substances as rice straw or potato decoction and so on. The intentions of TOCHINAI and NAKANO (1940) to culture the fungus on complete synthesized media resulted in failure, while the very good growth of the fungus obtained on the media added with the peptone of which chemical component was rather ambiguous. Recently F. W. LEAVER, and his co-workers⁽⁵⁴⁾, TANAKA and KATSUKI,^(112,113) and also the present writer⁽⁷⁴⁾ have come to know that the lack of some growth factor results in the failure of the good growth of the fungus and have discovered that the addition of biotin and vitamin B₁ on the synthesized media improves remarkably the growth of the fungus.

Exp. 1: First, biotin was extracted from beef liver as its methylester according to GYÖRGY. After having been saponized some quantity of it was added to the standard culture media. The concentration of the extracted growth factor was determined by bioassay using "FLEISCHMAN'S *Saccharomyces cerevisiae*" as test organism according to SNEEL, and the concentration on which the growth of yeast in 12 hours became maximum was determined as two units. TOCHINAI and NAKANO'S

culture solution was employed as standard; its composition was as follows: KNO_3 2g, KH_2PO_4 1g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1g, sucrose 3.0g, trace of FeCl_3 and water added up to 1000cc. Each chemical used was biotin-free; if it was shown by the bioassay that the chemicals contained trace of biotin, it was removed by treatment with charcoal.

The fungus was of same strain as used in the inoculation experiments of previous part. Hydrogen ion concentration of the culture solution was pH 5.4 initially. After incubation of 20 days in the 28°C thermostat, the fungus was filtered out and pH of the filtrate was determined. The filtrated fungus was fully washed with hot water and its dry weight was determined.

The results are shown in table 48. As will be seen in the table, the fungus does not grow at all in the biotin-free culture solution, while in the culture solution containing one unit biotin and vitamin B_1 , the fungus growth reaches to 8.0mg in the dry weight. The addition of more biotin till 50 units improves the more the fungus growth, showing the growth of 37.6mg weight in 50 units biotin supply.

TABLE 48. Addition of biotin and vitamin B_1 and growth of the blast fungus.
(crude biotin)

experimental section	concentration of biotin added	addition of vitamin B_1	fungus growth	spore formation	final pH of medium
1	50 units	+	37.6 mg	±	7.4
2	30 "	+	37.9 "	±	7.4
3	10 "	+	34.5 "	±	7.6
4	5 "	+	13.0 "	—	7.6
5	1 "	+	8.0 "	—	5.8
6	1 "	—	3.8 "	—	5.8
7	0 "	+	0 "	—	5.4
8	0 "	—	0 "	—	5.4

However in the culture solution containing one unit biotin and no vitamin B_1 , the growth of the fungus remained 3.8mg dry weight. The addition of vitamin B_1 to the biotin-free culture solution was invalid. In the cultures in which the biotin of ten units and more were added, the formation of a few spores was observed. It is also shown that the pH value of the culture solution increases with the growth of the fungus because of the unequal absorption of ions. Thus it is clear

that the biotin is indispensable for the growth of the fungus and vitamin B₁ is a supplemental factor.

Exp. 2: In order to know the optimum concentration of biotin for the fungus growth and also to check the previous results the next experiments were planned using synthesized biotin.

Culture solution containing 0.5% of vitamin B₁ and 0, 1, 5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110 m γ of biotin respectively in each flask were prepared and the fungus was inoculated. The dry weight of the fungus, formation of the spore and final pH after 20 day's incubation are given in table 49. As will be seen in the table there is no growth in the biotin-free culture solution. The fungus growth is improved with the addition of biotin till 90 m γ , while the addition of too much biotin as 110 m γ seems to be unfavourable for the fungus growth.

TABLE 49. Addition of biotin and vitamin B₁ and the blast fungus growth.
(purified biotin)

biotin added	fungus dry weight	spore formation	final pH of medium
0 m γ	0 mg	—	5.4
1 "	46.0 "	—	6.0
5 "	55.4 "	±	6.2
10 "	64.8 "	±	6.2
15 "	76.3 "	±	6.2
20 "	84.0 "	±	6.4
25 "	95.5 "	±	6.4
30 "	107.6 "	±	6.6
40 "	142.3 "	±	6.4
50 "	197.4 "	±	6.8
70 "	205.5 "	±	7.2
90 "	202.0 "	±	7.2
110 "	183.4 "	±	7.2

Some spores, though quite few, are found in the culture solution containing biotin of 5 m γ and more. pH value of the culture solution on which the fungus growth was fair always increases, because of the unequivalent absorption of the ions. On calculating the optimum concentration of biotin from the results, one finds it to be in the 2.3–3.0 m γ /ml. In order to certify again the necessity of vitamin B₁ as

a factor supplementary to biotin, some cultures as shown in table 50 were prepared.

TABLE 50. Indispensability of biotin and vitamin B₁ for the blast fungus growth.

culture No.	addition of biotin	addition of vitamin B ₁	fungus dry weight	final pH of medium
1	+ *	+ **	203.5 mg	7.2
2	+	—	78.8 "	6.0
3	—	+	0 "	5.4
4	—	—	0 "	5.4

* showing the addition of 80 m γ biotin in 30 cc culture solution.

** showing the addition of 50 m γ vitamin B₁ in 300 cc culture solution.

It is clearly shown that the addition of vitamin B₁ to the biotin-containing culture solution greatly improves the fungus growth while its addition to the biotin-free culture solution is invalid.

To summarize the results, it is known that the presence of biotin is indispensable for the blast fungus growth and the optimum concentration lies in 2.30–3.00 m γ /ml. Moreover the vitamin B₁ acts as supplementary growth factor with biotin for the fungus growth.

Chapter II

Nitrogen sources of the blast fungus.

As shown in the previous chapter, the addition of biotin and vitamin B₁ to the pure synthesized culture solution made it possible to culture the blast fungus on the solution. So the experiments were planned first to learn the nitrogen source of this fungus in detail. The relations between the kinds of nitrogen sources and the fungus growth, the aspect of nitrogen absorption by the fungus and the relations between the level of nitrogen supplied and the fungus growth are described in the following. Through all the experiments 80 m γ biotin and 0.5 γ vitamin B₁ were added in each flask of 30 cc culture solution.

1. The kind of nitrogen source and fungus growth.

The value as nitrogen source for the fungus growth was compared on various kinds of nitrogen by replacing the nitrogen of standard

solution by each one respectively of the following nitrogenous compounds; KNO_3 , $\text{Ca}(\text{NO}_3)_2$, NaNO_3 as nitrate nitrogen; $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, ammonium oxalate as ammonium nitrogen; NH_4NO_3 as ammonium nitrate nitrogen; KNO_2 , NaNO_2 as nitrite nitrogen; glycocoll, l-alanine, dl- α -amino-butyric acid, dl-valine, l-leucine, dl-norleucine, l-cystine, dl-methionine, tyrosine, arginine-hydrochloride, dl-ornithine hydrochloride, aspartic acid, d-glutamic acid, creatine, taurine, urea, asparagine as amino-acid and amide nitrogen. All these chemicals were certified not to contain any trace of biotin by bioassay using *Saccharomyces cerevisiae* and if any trace of it were found, the traces were removed by charcoal absorption before use. In all cases same amount of nitrogen as in the standard solution was supplied.

As the addition of some kinds of nitrogenous compounds acidifies the solution, adequate quantities of 0.5-0.2N NaOH were added to adjust their pH at 5.4. The fungus growth (in dry weight), spore formation and the pH value after 20 day's incubation were examined. The results are given in table 51. From the table it is known that the supply of nitrate nitrogens always favours the fungus growth irrespective of their kind of anion and that the pH of the culture solution increases with the fungus growth. The fungus grows fairly in case of the supply of ammonium nitrogen or ammonium nitrate as nitrogen source, but the growth is clearly inferior to that in the nitrate nitrogen culture. In this case, however, the pH value in the solution decreases with the fungus growth. Therefore the decrease of pH value in the culture solution which probably is due to the unequivalent absorption of ions hinder the further growth of the fungus. It is evident from the table that any nitrite nitrogen is inadequate as nitrogen source for the fungus growth. The value of amino acids and amides as nitrogen source is different respectively. The supply of glycocoll, l-alanine, aspartic acid, dl-glutamic acid, asparagine in each case greatly favour the growth; each of urea, dl-valine, l-leucine, l-cystine, dl-norleucine, dl-methionine, tyrosine, arginine hydrochloride, dl-ornithine hydrochloride is inferior to the above noted amino acids or amides in value as nitrogen source, though rather fair growths are observed. Each taurine, creatine and dl- α -amino-butyric acid is rather an inadequate nitrogen source for the fungus growth. According to the daily observation on the fungus growth (see table 52) the growth rate seems to be greater in cultures of l-alanine, asparagine, aspartic acid, dl-glutamic acid respectively than in nitrate nitrogen cultures.

TABLE 51. Various nitrogen sources and the blast fungus growth.

nitrogen sources	dry weight of the fungus	the formation of spores	final pH of medium
Ca (NO ₃) ₂	181.7 mg	±	6.0
KNO ₃	208.7 "	±	8.0
NaNO ₃	215.4 "	±	7.6
NH ₄ NO ₃	64.2 "	—	4.4
(NH ₄) ₂ SO ₄	38.5 "	—	4.1
(NH ₄) ₂ HPO ₄	55.6 "	—	3.4
Ammonium oxalate	60.2 "	—	4.8
KNO ₂	0 "	—	5.6
NaNO ₂	0 "	—	6.0
glycocoll	249.5 "	±	5.4
l-alanine	230.4 "	±	5.4
dl- α -amino-butyric acid	0.6 "	—	5.4
dl-valine	64.2 "	—	5.2
l-leucine	90.6 "	—	5.4
dl-norleucine	153.5 "	±	5.4
l-cystine	45.5 "	—	5.0
dl-methionine	70.5 "	—	5.4
tyrosine	152.3 "	±	5.4
arginine hydrochloride	115.8 "	—	5.4
dl-ornithine hydrochloride	81.5 "	—	4.6
aspartic acid	218.7 "	±	7.6
dl-glutamic acid	216.2 "	±	7.4
creatine	7.7 "	—	5.4
taurine	19.9 "	—	5.4
urea	95.4 "	—	5.4
asparagine	269.6 "	±	5.4

With respect to the change of pH value in amino acid or amide nitrogen cultures, it increases up to 7.4 in aspartic acid or dl-glutamic acid culture while in dl-ornithine hydrochloride culture it decreases to 4.6. In the other cultures pH does not vary so greatly.

The formation of a few conidia is to be seen in each culture of Ca(NO₃)₂, KNO₃, NaNO₃, glycocoll, l-alanine, dl-norleucine, tyrosine, aspartic acid, dl-glutamic acid and asparagine.

TABLE 52. Speed of the blast fungus growth on the culture solutions of various nitrogen sources.

nitrogen sources	culture age (days)						
	1	2	3	4	5	6	7
KNO ₃	—	±	±	+	+	++	++
NaNO ₃	—	±	±	+	+	++	++
Ca (NO ₃) ₂	—	±	±	+	+	++	++
glycocoll	±	+	++	++	+++	+++	+++
alanine	±	+	++	++	+++	+++	+++
spartic acid	±	+	++	++	+++	+++	+++
glutamic acid	±	+	++	++	+++	+++	+++
asparagine	±	+	++	++	+++	+++	+++

2. Rate of growth of fungus on some culture solutions and the absorption of nitrogen by the fungus.

In the previous section it is described that the fungus seems to grow faster in some amino acid culture solutions than in the nitrate nitrogen culture solutions. In order to make certain of this point and to know the aspect of nitrogen absorption by fungus, the following experiments were planned on cultures of KNO₃, NaNO₃, glycocoll and asparagine respectively. All the handling for preparing culture solutions and inoculating the fungus were the same as described in the previous section; 5–15 cultures in accordance with the fungus growth were taken out to determine the dry weight of fungus at every two days from the 2nd till 20th or 26th day of culture. And at the same time the nitrogen amount left in each culture solution was determined by KJELDAHL method. From these results increased amount of fungus dry weight and the amount of nitrogen absorbed for every two day period were calculated. In all experiments the results per one flask are shown. The nitrogen amount supplied per one flask was 8.28 mg in all experiments.

i) KNO₃ culture: The results on the KNO₃ culture are shown in table 53 and figure 12.

A little fungus growth is seen already at 2 days after the beginning of the culture and the growth rate increases remarkably from 6th day till 16th day of culture. Then the growth becomes slow and reaches the maximum at 22nd or 24th day of culture. During the

TABLE 53. Increase of blast fungus dry weight measured at 2-day intervals and nitrogen absorption by the fungus.

(KNO₃ culture)

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24	26
fungus dry weight (mg)	1.8	7.9	34.1	68.3	90.9	130.2	164.1	180.0	189.1	201.6	203.0	207.9	206.5
increased weight of the fungus (mg)		6.1	26.2	34.2	22.6	39.3	33.9	15.9	9.1	12.5	1.4	4.9	-1.4
amount of nitrogen left in the culture solution (mg)	8.20	7.61	6.68	6.00	4.56	3.92	2.88	2.72	2.28	2.12	2.12	2.08	2.00
amount of nitrogen absorbed (mg)		0.59	0.93	0.68	1.44	0.64	1.04	0.16	0.44	0.16	0	0.04	0.08

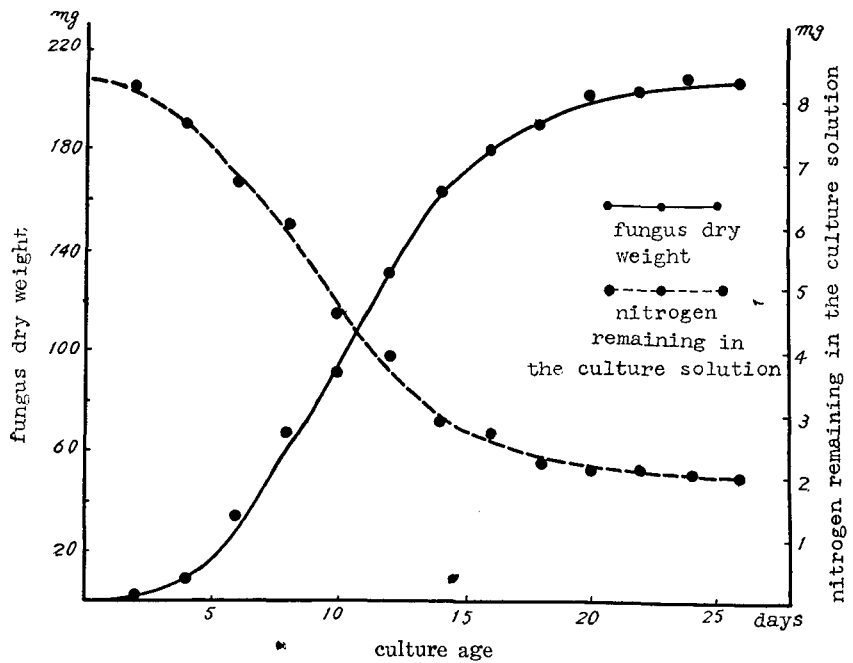


Fig. 12. Increase of blast fungus dry weight and the decrease of nitrogen in the culture solution at 2-day intervals.

(KNO₃ culture)

period of vigorous fungus growth, that is from 6th till 16th day of culture, most remarkable increase of the fungus dry weight is seen on the 10th-12th days of culture when an increase as great as 39.3 mg is recorded for this two days. In view of the absorption of the nitrogen by the fungus, it is evident that the fungus absorbs the nitrogen vigorously during the same period when vigorous growth is seen. But it is to be noticed that the maximum absorption of nitrogen as great as 1.44 mg occurs on 8th-10th days of culture which period precedes the days of the most vigorous growth, viz., the 10th-12th days. The absorption rate of the nitrogen drops once on 10-12 days of culture but vigorous absorption recovers again soon after. But after the 15th day of culture the nitrogen absorption decreases in accordance with the decrease of growth rate of the fungus. About 2.0 mg nitrogen remains in the culture solution when the fungus growth reaches the maximum on 22nd day of culture. From these results it will be said that the activity of the fungus is greatest at the rather earlier stage of fungus growth and the greater amounts of nitrogen are also absorbed at the earlier stage. Then immediately after the greatest amount of nitrogen has been absorbed, the most vigorous growth of the fungus occurs as a result of utilization of that nitrogen.

ii) NaNO_3 culture: The experimental results on NaNO_3 culture are shown in table 54 and figure 13. They are almost the same as in the KNO_3 culture. The rather vigorous growth begins on 6th day of culture and it continues till the 16th day.

TABLE 54. Increase of the blast fungus dry weight measured at 2-day intervals and nitrogen absorption by the fungus. (NaNO_3 culture)

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24
fungus dry weight (mg)	2.0	7.2	26.1	53.5	82.9	120.8	144.3	174.1	193.0	206.1	205.9	211.9
increased weight of the fungus (mg)	5.2	18.9	27.4	29.4	37.9	23.5	29.8	18.9	13.1	-0.2	6.0	
amount of nitrogen left in the culture solution (mg)	8.24	7.64	7.20	6.01	4.60	3.84	2.96	2.44	2.00	2.16	1.92	1.88
amount of nitrogen absorbed (mg)	0.60	0.44	1.19	1.41	0.76	0.88	0.52	0.44	-0.16	0.24	0.04	

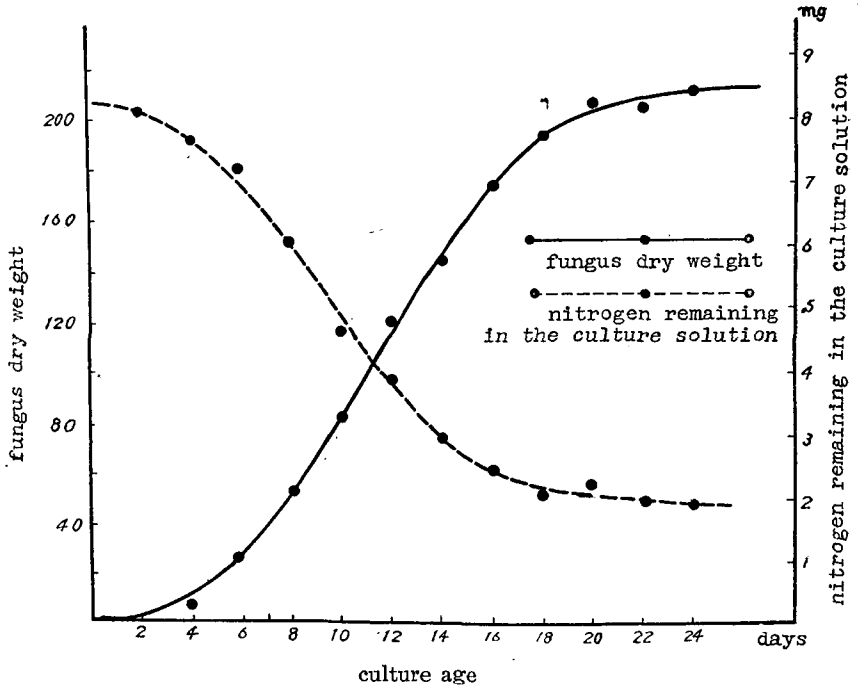


Fig. 13. Increase of the blast fungus dry weight and the decrease of nitrogen in the culture solution at 2-day intervals. (NaNO_3 culture)

The greatest increase of fungus dry weight, as great as 37.9 mg, is observed on the 10th–12th days of culture while the greatest absorption of nitrogen occurs on 8th–10th days which precede the time of the greatest increase of the fungus. The nitrogen absorption drops once on 10th–12th days of culture but recovers soon after. After the 16th day the fungus activity declines gradually accompanying the decrease of nitrogen absorption. The fungus dry weight finally on 24th day of culture is 211.9 mg.

iii) Asparagine culture: The experimental results on the asparagine culture are shown in table 55 and figure 14.

The aspects of fungus growth and the nitrogen absorption are almost same as in the case of nitrate nitrogen culture but the speed of fungus growth is clearly faster. The fungus shows a growth of 7.0 mg already on 2nd day of culture and remarkable increases in fungus weight are observed from 4th day till 12th day of culture. The greatest increase of the fungus weight among the two day periods

TABLE 55. Increase of the blast fungus dry weight measured at 2-day intervals and the nitrogen absorption.
(asparagine culture)

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24	26
fungus dry weight (mg)	7.0	11.6	34.8	114.1	153.7	186.6	209.0	213.4	220.1	222.3	224.5	222.0	222.6
increased weight of the fungus (mg)	4.6	23.2	79.3	39.6	32.9	22.4	4.4	6.7	2.2	2.2	-2.5	0.6	
amount of nitrogen left in the culture solution (mg)	8.24	7.52	6.48	5.68	4.42	3.07	2.64	2.12	1.64	1.44	1.04	1.04	0.96
amount of nitrogen absorbed (mg)	0.72	1.04	0.80	1.26	1.35	0.43	0.52	0.48	0.20	0.40	0	0.08	

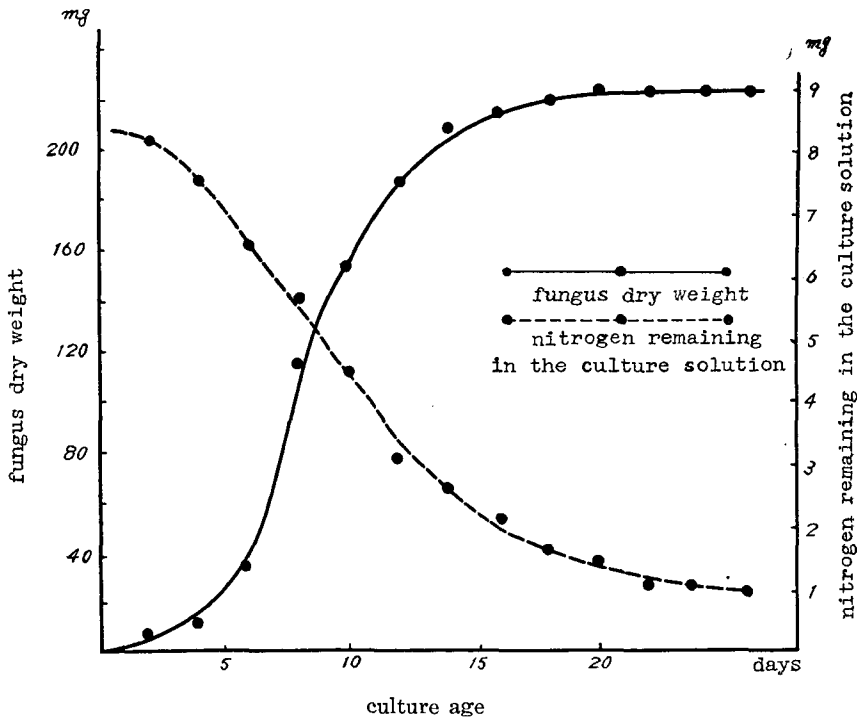


Fig. 14. Increase of the blast fungus dry weight and the decrease of the nitrogen in the culture solution at 2-day intervals.
(asparagine culture)

occurs on 6th-8th days of culture, that is about 4 days earlier than in the nitrate nitrogen culture. Moreover that greatest increase is as great as 79.3 mg which is far beyond the amount in the nitrate nitrogen cultures. After the 14th day of culture the increase of fungus weight becomes slow and it reaches its maximum of 222 mg in dry weight at the 20th day of culture. Viewing the aspect of nitrogen absorption by the fungus, one sees that much nitrogen is absorbed on 4th-6th days of the culture when the amount reaches to 1.04 mg. But the total amount of nitrogen absorbed before the greatest fungus growth, appearing on the 6th-8th days, is 1.76 mg which is far less in comparison with the 3.6 mg nitrogen absorbed in the case of nitrate nitrogen cultures. This may show the higher value of this amino acids as nitrogen source for this fungus on comparison with nitrate nitrogen. The nitrogen absorption drops once on the 6th-8th days of the culture when the greatest increase of the fungus weight occurs; but it recovers soon showing greater absorption of nitrogen, as great as 1.26 mg on 8th-10th days and 1.35 mg on 10th-12th days of the culture. These periods of greater nitrogen absorptions may be due to the remarkable increase of fungus mycelium volume. After the 12th day of the culture the nitrogen absorption declines accompanied by the decline of fungus activity.

iv) Glycocoll culture: The experimental results on the glycocoll culture are shown in table 56 and figure 15. In this case the speed of the fungus growth is also clearly faster than that in the nitrate nitrogen culture, though it does not attain to that in case of the asparagine culture. The greatest increase of fungus weight, as great as 51.5 mg for two days, appears on the 8th-10th days of the culture while greatest nitrogen absorption, as great as 1.60 mg for two days, occurs on 6th-8th days. The total nitrogen amount absorbed before the greatest fungus growth, which appears on 8th-10th days of the culture, is 2.52 mg that is less than the absorption in the case of the nitrate culture, revealing the higher value of this amino acid as nitrogen source for this fungus growth. Other aspects of the fungus growth and the nitrogen absorption are almost the same as in the asparagine culture.

On summarizing the results above set forth, the period of the fungus growth may be divided into three stages. The first is the "early stage" during which the increase of fungus weight is not so great, the second is the stage to be called "middle stage" during which the fungus growth is most vigorous and the last is the stage to be

TABLE 56. Increase of the blast fungus dry weight measured at 2-day intervals and nitrogen absorption by the fungus.
(glycocoll culture)

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24
fungus dry weight (mg)	2.8	18.0	50.1	99.8	150.9	183.5	209.9	227.5	233.3	234.0	242.1	240.9
increased weight of the fungus (mg)		15.2	32.1	49.7	51.1	32.6	26.4	17.6	5.8	0.7	0.8	-1.2
amount of nitrogen left in the culture solution (mg)	8.12	7.60	7.20	5.60	4.11	3.36	2.44	1.72	1.64	1.33	1.24	1.12
amount of nitrogen absorbed (mg)		0.52	0.40	1.60	1.49	0.75	0.92	0.72	0.08	0.31	0.09	0.12

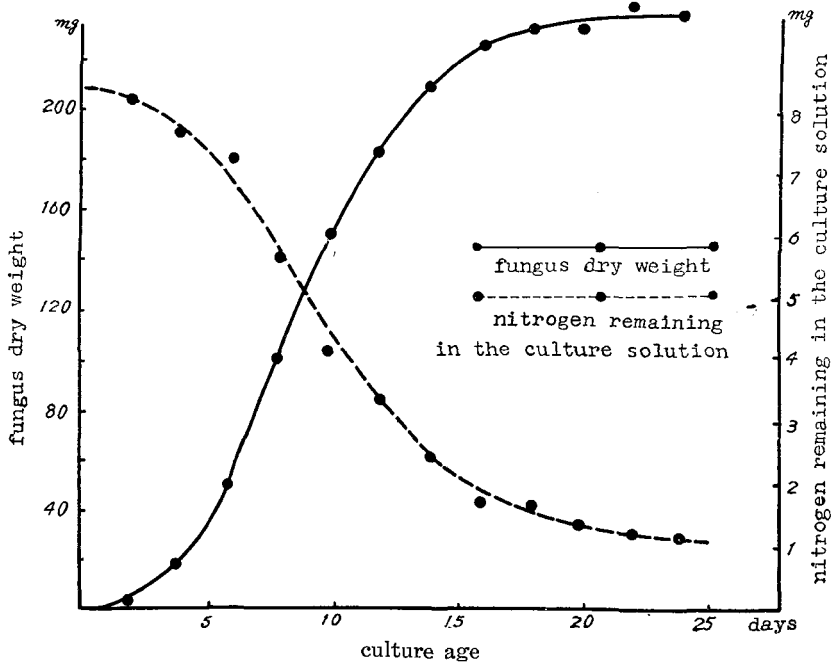


Fig. 15. Increase of the blast fungus dry weight and the decrease of the nitrogen in the culture solution at 2-day intervals.
(glycocoll culture)

called "latter stage" in which the activity of the fungus declines gradually. It is till the middle stage that the vigorous nitrogen absorption occurs and it is in the earlier part of middle stage that the greatest increase of fungus weight appears, the greatest amount of nitrogen having been absorbed just before. This earlier part of middle stage when the greatest increase of fungus weight appears may be called the "greatest activity period" of the culture. In table 57 the "greatest activity period" of the four cultures and the amount of increase in fungus weight during that period are given.

TABLE 57. Period of greatest activity in the culture of blast fungus on four kinds of nitrogen source and the increase of the fungus dry weight within that period.

nitrogen sources of the culture	greatest activity period	fungus weight increased during the greatest activity period
KNO ₃	10th-12th days	39.3 mg
NaNO ₃	10th-12th days	37.9 "
asparagine	6th- 8th days	79.3 "
glycocoll	8th-10th days	51.1 "

It is certain that the "early stage" of the fungus growth is shortened in the culture in which such better nitrogen sources as asparagine or glycocoll are supplied and consequently, the "greatest activity period" appears earlier. Also in those cultures fungus growth in the middle stage, especially in the greatest activity period is far more great. Thus it is known that the differences of fungus growth in cultures different in their nitrogen source are determined during the middle stage of growth.

The utilization ratios of various nitrogens for fungus growth, which are calculated by dividing the fungus weight grown by the nitrogen amount absorbed, are shown in table 58.

On comparing the ratios which are calculated at the end of the culture, one sees that they are a little greater in the nitrate nitrogen culture than in the amino acid nitrogen culture. But this is because the greater amount of nitrogen absorbed by the greater volume of fungus at the latter stage of the fungus growth comes into the calculation. The ratios which are calculated at the greatest activity period are clearly greater in the amino acid nitrogen cultures than in the

TABLE 58. Utilization ratio of four kinds of nitrogen source on the blast fungus culture.

nitrogen sources of the culture	at the end of the culture	at the greatest activity period
KNO ₃	33.5	2.98
NaNO ₃	33.1	27.2
asparagine	31.2	43.9
glycocoll	34.6	36.1

nitrate nitrogen cultures, showing the high utilization of such amino acids as asparagine and glycocoll by the blast fungus.

3. Transformations of the nitrogen supplied in the culture solution.

The nitrogenous compounds supplied in the culture solutions are generally transformed by the fungus into ammonium nitrogen before they are absorbed. In order to examine this point on blast fungus, the filtrates of the cultures were chemically analyzed on each culture of KNO₃, NH₄NO₃, aspartic acid and glycocoll.

i) KNO₃ culture: When nitrate nitrogen is supplied in the culture solution, it is usually presumed that the nitrate nitrogen is reduced to ammonium nitrogen by the fungus before it comes to be assimilated. In order to make certain of this point on the nitrate nitrogen culture, five cultures were taken out at 2-day intervals and the amounts of nitrate and ammonium nitrogen in the filtrate were examined. Nitrate nitrogen was determined colorimetrically by phenol-disulfonic acid method and ammonium nitrogen was determined colorimetrically by NESSLER's reagent. Table 59 and figure 16 give the experimental results. The figures in the table show the mg of nitrogen per one flask filtrate.

As will be seen in the table and the figure, ammonium nitrogen is not detected till 16th day of the culture. On the 18th day, when the fungus growth and the nitrogen absorption become smaller, the ammonium nitrogen becomes detectable, and after then the amount increases gradually, reaching to the amount of 0.48 mg on the 24th day of the culture. This may suggest that all the reduced nitrogen is immediately absorbed by the fungus while its growth is vigorous, and that the ammonium nitrogen becomes detectable when the nitrogen

TABLE 59. Decrease of the nitrate nitrogen and the appearance of ammonium nitrogen in the process of blast fungus growth.

(KNO₃ culture)

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24
nitrate-nitrogen	mg. 8.08	mg. 7.52	mg. 6.72	mg. 5.76	mg. 4.68	mg. 3.72	mg. 3.04	mg. 2.64	mg. 2.12	mg. 1.80	mg. 1.61	mg. 1.44
ammonium-nitrogen	0 "	0 "	0 "	0 "	0 "	0 "	0 "	0 "	0.24"	0.32"	0.48"	0.48"
total	8.08"	7.52"	6.72"	5.76"	4.68"	3.72"	3.04"	2.64"	2.36"	2.12"	2.09"	1.92"

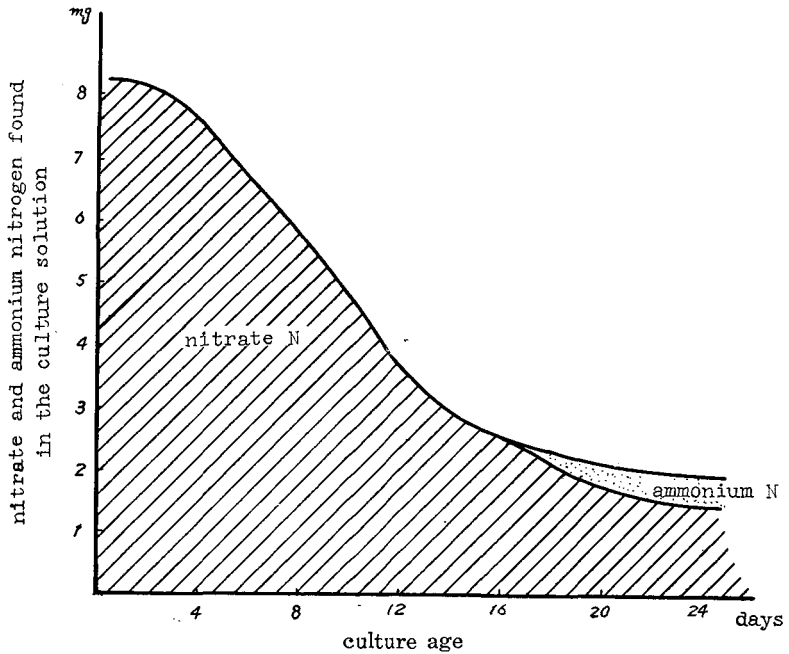


Fig. 16. Decrease of the nitrate nitrogen and the appearance of ammonium nitrogen in the process of blast fungus growth. (KNO₃ culture)

absorption comes to be rather smaller in the latter stage of growth. YOSHII (1936), TANAKA and KATSUKI (1951) reported that no trace of nitrite or ammonium nitrogen is found in the filtrate of nitrate nitrogen culture. This is perhaps because their experiments were carried on by using the filtrates of rather younger cultures.

ii) NH_4NO_3 culture: Though the fungus growth is not so good in the ammonium nitrate culture solution, it seems of interest to ascertain which kind of nitrogen, ammonium or nitrate, is absorbed mostly by the fungus when ammonium nitrate is supplied in the culture as nitrogen source. Therefore culturing the blast fungus in ammonium nitrate culture solutions, the writer determined both the ammonium and nitrate nitrogen in the filtrate on every other day of the culture.

TABLE 60. Decrease of nitrate and ammonium nitrogen in the course of blast fungus growth on the ammonium nitrate culture solution.

culture age (days)	2	4	6	8	10	12	14	16	18	20	22
nitrate-nitrogen	mg 4.16	mg 4.12	mg 4.08	mg 4.08	mg 4.04	mg 4.01	mg 4.00	mg 3.96	mg 3.91	mg 3.92	mg 3.98
ammonium-nitrogen	4.08"	3.88"	3.48"	3.04"	2.80"	2.40"	2.48"	2.52"	2.58"	2.62"	2.58"
total	8.24"	8.00"	7.56"	7.12"	6.84"	6.41"	6.48"	6.48"	6.49"	6.54"	6.56"

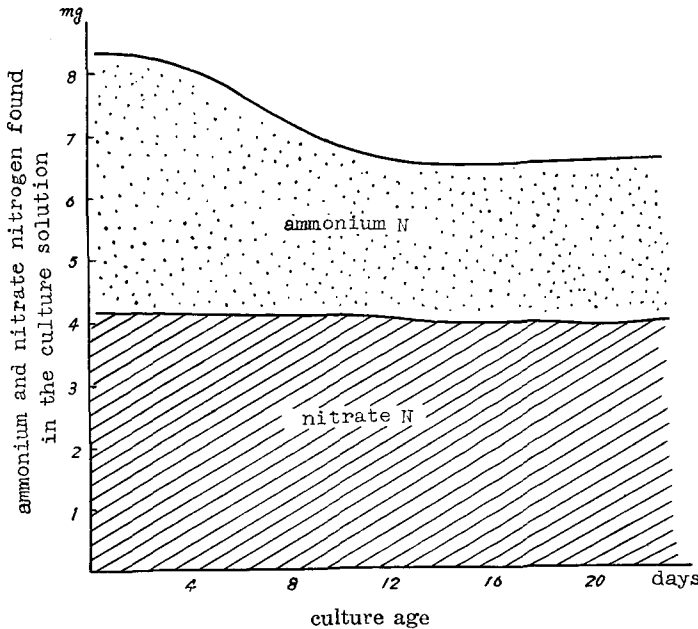


Fig. 17. Decrease of nitrate and ammonium nitrogen in the course of blast fungus growth (NH_4NO_3 culture)

The results are given in table 60 and figure 17. As will be seen in the table, ammonium nitrogen in the filtrate decreases clearly with the fungus growth while the decrease of nitrate nitrogen is rather uncertain. Thus it is clear that the fungus absorbs mostly the ammonium nitrogen when both kinds of nitrogen, ammonium and nitrate, are supplied. It is expected that the unequivalent absorption of the ammonium ion results in the depression of pH; the pH determination of the filtrate verifies this point (Table 61).

TABLE 61. Decrease of pH value in the culture solution of ammonium nitrate culture in the course of the blast fungus growth.

culture age (days)	2	4	6	8	10	12	14	16	18	20	22
pH	5.4	5.4	5.0	4.6	4.2	3.8	3.2	3.2	3.4	3.2	3.2

Therefore the reason, why the fungus growth is not so good in the ammonium nitrate nitrogen culture, lies in such depression of the pH value of the culture solution.

iii) Glycocoll culture: When amino acids are supplied in the culture solutions as nitrogen source, they may be decomposed by the fungus before the nitrogen is absorbed. Therefore the fluctuation of the amount of amino acid and ammonium in the filtrates of glycocoll culture were examined on every other day. The amino acid nitrogen was determined by VAN SLYKE'S method and the ammonium nitrogen colorimetrically by NESSLER'S reagent. Table 62 and figure 18 show the results. The figures in the table indicate the amount of nitrogen per one flask filtrate. While the amino nitrogen decreases with the days of culture, the ammonium nitrogen becomes detectable on the 6th day of the culture. The ammonium nitrogen in the filtrate reaches to the maximum 0.76 mg on the 10th-12th days of the culture and then decreases gradually. These facts may suggest the decomposition of the amino acid by oxidase which is produced by the fungus. As described in the following chapter, the blast fungus does produce the oxidase in fact and its activity is strong during 6th-14th days of the culture. As will be seen in the table, however, the ammonium nitrogen found in the filtrate is rather less even in the high activity period of the oxidase; this is because most of the ammonium produced

by the decomposition of amino acid is immediately absorbed by the fungus.

TABLE 62. Decrease of the amino nitrogen and the appearance of ammonium nitrogen in the glycocoll culture solution in the process of the blast fungus growth.

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24
amino-nitrogen	mg 8.32	mg 7.58	mg 6.40	mg 5.12	mg 3.98	mg 2.92	mg 2.01	mg 1.52	mg 1.20	mg 1.00	mg 0.88	mg 0.80
ammonium-nitrogen	0 "	0 "	0.40"	0.72"	0.72"	0.76"	0.64"	0.56"	0.44"	0.44"	0.44"	0.44"
total	8.32"	7.58"	6.80"	5.84"	4.70"	3.68"	2.65"	2.08"	1.64"	1.44"	1.32"	1.24"

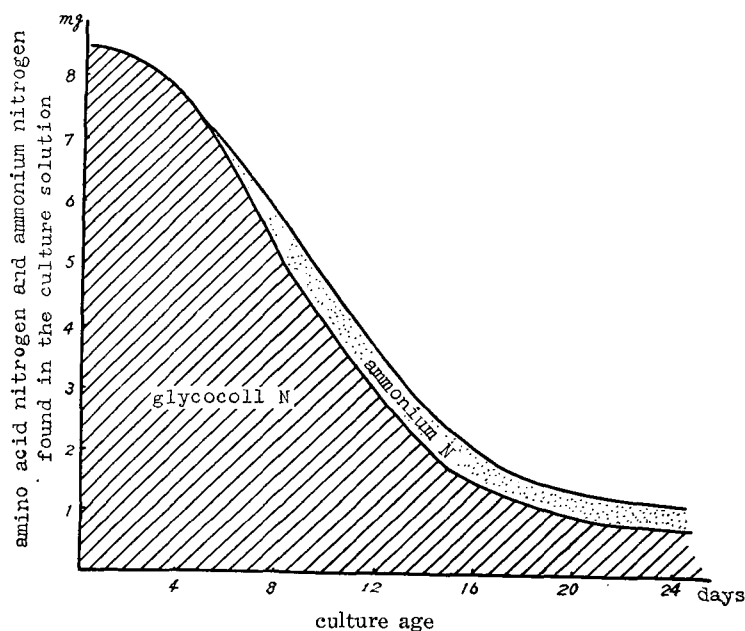


Fig. 18. Decrease of the amino nitrogen and the appearance of ammonium nitrogen in the glycocoll culture solution in the process of the blast fungus growth.

iv) Aspartic acid culture: Experiments were carried on the aspartic acid culture. The results are shown in table 63 and figure 19.

In this case the ammonium nitrogen becomes detectable on the 4th day of the culture and reaches maximum 0.64 mg on 8th day.

TABLE 63. Decrease of the amino nitrogen and the appearance of ammonium nitrogen in the aspartic acid culture solution in the process of the blast fungus growth.

culture age (days)	2	4	6	8	10	12	14	16	18	20	22	24
amino-nitrogen	7.96 mg	7.12 mg	5.89 mg	4.76 mg	3.68 mg	2.84 mg	2.08 mg	1.64 mg	1.36 mg	1.20 mg	0.98 mg	0.88 mg
ammonium-nitrogen	0	0.28	0.56	0.64	0.56	0.41	0.48	0.34	0.28	0.16	0.22	0.21
total	7.96	7.40	6.45	5.40	4.24	3.28	2.56	1.98	1.64	1.36	1.20	1.09

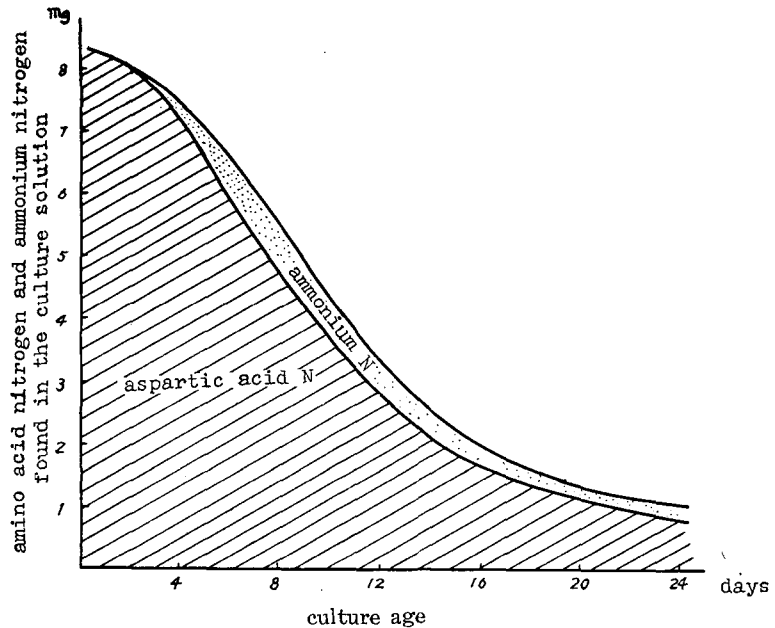


Fig. 19. Decrease of the amino nitrogen and the appearance of ammonium nitrogen in the aspartic acid culture solution in the process of the blast fungus growth.

It may be said that the amino acid is vigorously decomposed by the fungus producing ammonium in 6th-14th days of culture when the activity of the amino acid oxidase is most vigorous as described in the following chapter. But the amount of the ammonium nitrogen found in the culture solution during these period is rather small because the most of the ammonium produced by the fungus would be immediately absorbed by the fungus.

4. The nitrogen level and the fungus growth.

In all experiments above described the nitrogen given in each culture solution was always 8.28 mg per flask (30cc culture solution). In the followings the effects of the supply of greater amounts of nitrogen on the fungus growth are examined. In these experiments each KNO_3 , NaNO_3 , asparagine and glycocoll was employed as nitrogen source, the amount supplied being 8.28 mg, 16.56 mg, 33.12 mg, 49.68 mg, 66.24 mg, 82.80 mg and 99.36 mg respectively. After 20 days of culture in each culture solution, the fungus was filtrated out; the fungus dry weight and the amount of nitrogen absorbed were determined. The fungus dry weight per 1 mg nitrogen absorbed was calculated in each case. When the large quantity of nitrogen was supplied, it was found by other experiment undescribed to be often necessary to add sugar during the course of the culture; 3 cc of sugar solution, containing 6.0 g sugar in 100 cc, was added strilely on the 12th day of the culture.

i) KNO_3 culture: Experimental results on KNO_3 culture are given in table 64. Experimental sections 1, 2, 3, 4, 5, 6, 7, means the supply of nitrogen of 8.28 mg, 16.56 mg, 33.12 mg, 49.68mg, 66.24 mg, 82.80 mg and 99.36 mg per each flask (30cc culture solution) respectively (same in the following experiments).

TABLE 64. Blast fungus growth, nitrogen absorption, and the utilization ratio of the nitrogen in the culture solution of various nitrogen levels.

(KNO_3 culture)

experimental section	1	2	3	4	5	6	7
fungus dry weight	210 mg	227 mg	244 mg	256 mg	271 mg	281 mg	290 mg
nitrogen absorbed	6.40 "	13.16 "	26.24 "	40.25 "	53.87 "	67.22 "	79.48 "
utilization ratio	32.81 "	17.25 "	9.30 "	6.36 "	5.03 "	4.18 "	3.16 "

As shown in the table, the more the amount of nitrogen supplied in the culture solution, the greater the fungus growth becomes. On the other hand while the amount of nitrogen absorbed during 20 day's culture becomes greater with the greater amount of nitrogen supply, the ratio of nitrogen utilization is contrariwise reversely proportional

to the amount of nitrogen supply.

ii) NaNO_3 culture: Experimental results on the NaNO_3 culture are given in table 65.

TABLE 65. Blast fungus growth, nitrogen absorption and the utilization ratio of the nitrogen in the culture solution of various nitrogen levels.

(NaNO_3 culture)

experimental section	1	2	3	4	5	6	7
fungus dry weight	220 mg	229 mg	238 mg	247 mg	255 mg	261 mg	287 mg
nitrogen absorbed	6.50 "	13.57 "	26.61 "	39.24 "	52.19 "	65.27 "	77.46 "
utilization ratio	33.85 "	16.88 "	8.95 "	6.29 "	4.89 "	4.03 "	3.71 "

As in the case of KNO_3 culture, the greater amount of nitrogen supply favours larger fungus growth in company with the more nitrogen absorption. But the utilization ratio of nitrogen becomes less with the greater amount of nitrogen supply.

iii) Asparagine culture: Experimental results on asparagine culture are given in table 66.

TABLE 66. Blast fungus growth, nitrogen absorption and the utilization ratio of the nitrogen in the culture solution of various nitrogen levels.

(asparagine culture)

experimental section	1	2	3	4	5	6	7
fungus dry weight	235 mg	251 mg	264 mg	279 mg	290 mg	302 mg	327 mg
nitrogen absorbed	7.27 "	14.12 "	28.80 "	47.71 "	60.36 "	73.26 "	87.05 "
utilization ratio	32.32 "	17.78 "	9.17 "	6.24 "	4.80 "	4.12 "	3.76 "

As already described, asparagine is a nitrogen source more favorable for the fungus growth than nitrate nitrogen; the fungus growth reaches to 235 mg even in the solution with original amount (8.28 mg) of nitrogen supply. On the other hand the greater supply of the

asparagine favors more the plant growth, and supplying of 99.36 mg asparagine nitrogen results in a fungus growth as great as 327 mg. The amount of nitrogen absorbed by the fungus increases in proportion to the amount of nitrogen supply.

The ratio of nitrogen utilization is contrariwise reversely proportional to the amount of nitrogen supply, quite the same as in the case of the nitrate nitrogen cultures.

iv) Glycocoll culture: Experimental results on the glycocoll culture are recorded in table 67.

TABLE 67. Blast fungus growth, nitrogen absorption and the utilization ratio of nitrogen in the culture solutions of various nitrogen levels. (glycocoll culture)

experimental section	1	2	3	4	5	6	7
fungus dry weight	239 mg	259 mg	275 mg	289 mg	306 mg	310 mg	324 mg
nitrogen absorbed	7.06 "	14.18 "	29.23 "	45.15 "	60.69 "	74.67 "	89.15 "
utilization ratio	33.85 "	18.27 "	9.41 "	6.40 "	5.04 "	4.15 "	3.63 "

Glycocoll is one of the most favourable nitrogen sources for the fungus growth; the fungus growth is somewhat great even in the original amount of nitrogen supply as is also true in the case of the asparagine culture. The greater the amount of glycocoll nitrogen supplied, the more the fungus growth, accompanied a greater absorption of the nitrogen, quite the same as in the asparagine culture. The ratio of nitrogen utilization is reversely proportional with the greater amount of nitrogen supply.

Summarizing the results of the four experiments above described, it is evident that the nitrogen level in the standard culture solutions acts rather as a limiting factor for the fungus growth while the increase of the nitrogen level favors the fungus growth. So further experiments were planned to examine the relations between the growth rate of the fungus and the nitrogen level supplied. Asparagine cultures were employed. In these experiments three sections were prepared; the one was the standard nitrogen level (section I), the second was given half the amount of nitrogen of the standard (section II) and the third was given

eight times as much nitrogen as the standard (section III). Some of the cultures were filtrated each two days, and the fungus dry weight per one flask was determined. The results are given in table 68 and figure 20. The figures given between the adjacent culture days in the table show the increase of fungus dry weight during the two days.

Examining the fungus growth, one notes that the early stage continues till the 6th day of culture in the case of section I; in the case of the section III it is till the 4th day, while in the case of section II it is prolonged to 10th day of the culture. On the other hand "the greatest activity period" of the fungus growth, when the greatest growth for two days is seen, is found to be on the 6th-8th days period in the case of section I; in the case of section III it is distinguished

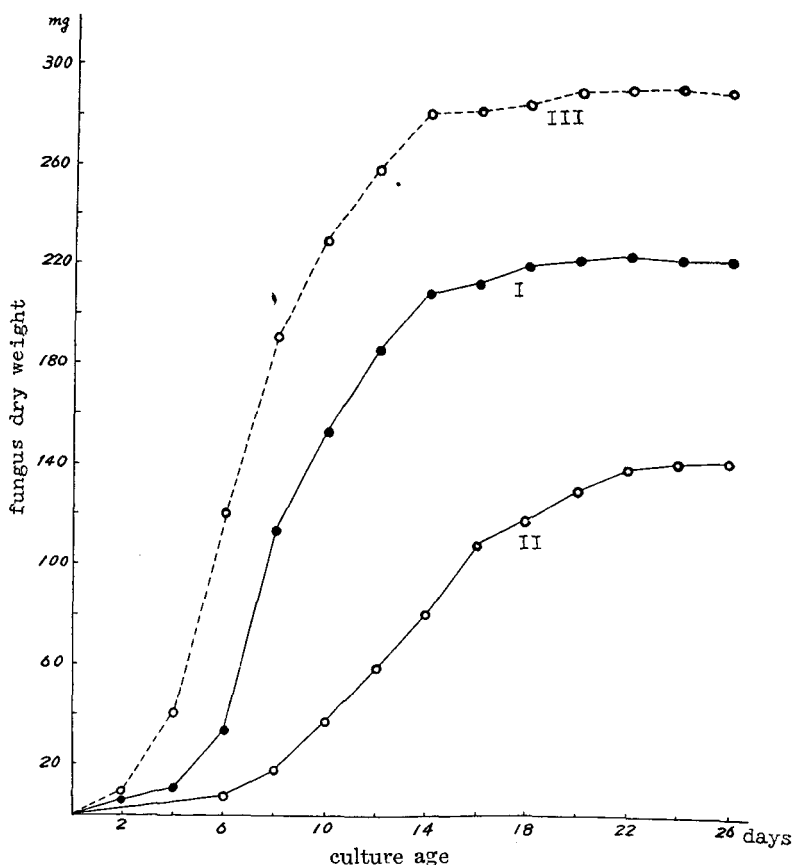


Fig. 20. Increase of the fungus dry weight by two-day periods in the culture solution of different nitrogen levels.

TABLE 68. Increase of the blast fungus dry weight by 2-day periods in the culture solutions of the different nitrogen levels. (mg)

culture age	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day	18th day	20th day	22nd day	24th day	26th day
I	7.0	11.6	34.8	114.1	153.7	186.6	209.0	213.4	220.1	222.3	224.5	222.0	222.6
	4.6	23.2	79.3	39.6	32.9	22.4	4.4	6.7	2.2	2.2	-2.5	0.6	
II			8.0	19.5	38.2	59.1	80.8	109.0	118.2	130.0	138.1	140.5	141.3
			11.5	18.7	39.6	21.7	28.2	9.2	11.8	8.1	2.4	0.8	
III	9.4	41.0	121.0	190.8	229.6	258.0	281.5	282.0	285.0	290.0	291.5	292.0	290.2
	32.0	80.0	69.8	38.8	28.4	23.5	0.5	3.0	5.0	1.5	0.5	1.8	

earlier on 4th-6th days of the culture; in the case of section II it is found later on 10th-12th days of culture. Thus it is evident that the duration of "the early stage" is shortened in the high level nitrogen section while it is prolonged in the low level nitrogen section; therefore the growth rate of the fungus becomes the greater in the solution having the greater amount of nitrogen supply. As will be seen, most of the fungus growth is completed in the middle stage which continues for about 10 days in every section. But the increase of the fungus dry weight during this stage is naturally greater in the case of the higher level nitrogen supply.

The amounts of nitrogen absorbed in each two-day period of the culture are shown in table 69.

TABLE 69. Amount of nitrogen absorbed in every other day in the culture solution of the different nitrogen level. (mg)

culture age	0-2nd day	2nd-4th day	4th-6th day	6th-8th day	8th-10th day	10th-12th day	12th-14th day	14th-16th day	16th-18th day	18th-20th day	20th-22nd day	22nd-24th day	24th-26th day
I	0.12	0.68	1.36	0.92	1.29	1.20	0.78	0.35	0.20	0.13	0.11	0.04	0.05
II	0.02	0.24	0.35	0.70	1.00	0.64	0.80	0.20	0.06	0.08	0.02	0	0.02
III	0.26	11.45	11.12	9.24	10.95	6.40	7.20	2.40	0.61	0.28	0.13	0.08	0.18

In all the experimental sections the greatest absorption of nitrogen occurs just before the appearance of the greatest fungus growth ("the greatest activity period") while the nitrogen absorption in the latter stage of the fungus growth is rather small. On the other hand the

amount of nitrogen absorbed by the fungus during the time from the early stage till the middle stage of growth is greater in the section of greater amount nitrogen supply while it is smaller in the section of lower level nitrogen supply.

The ratio of nitrogen utilization in each section, which was determined by dividing the fungus dry weight by the nitrogen amount absorbed, is recorded in table 70.

When the fungi completed their growth, the ratio is greater in the section of the lower level nitrogen supply. But on the contrary the ratio calculated at "the greatest activity period" of the fungus growth is greater in the first section (I) than in the second (II), while in the third (III), in which fungus was supplied with the greatest amount of nitrogen, the ratio is the least; this is because the amount of nitrogen absorbed is extremely greater in the third section (III). In other words the supply of a greater amount nitrogen does not always mean the more effective utilization of the absorbed nitrogen although the fungus growth is always the greater in the case of greater amount of nitrogen supply.

TABLE 70. Utilization ratios of nitrogen in the blast fungus cultures on the culture solution of different nitrogen levels.

	at the full growth of the fungus	at the greatest activity period
I	30.6	37.9
II	34.2	20.0
III	4.8	5.3

In conclusion of this section, it may be said that the nitrogen amount in the standard culture solution acts as a rather limiting factor for the fungus growth and therefore the increase of the nitrogen level supplied favors the fungus growth. And the greater amount nitrogen supply leads to the greater amount of nitrogen absorption at the early stage of the fungus growth, resulting in the faster growth of the fungus.

5. Conclusion.

There have been reported some studies on the nitrogen sources

of the blast fungus. Among them the studies by TOCHINAI and NAKANO (1940) using synthesized culture solutions go into detail. But at the time of those studies it was not yet known that biotin and vitamin B₁ are indispensable for the blast fungus growth and therefore the growth on the synthesized culture solutions was generally quite poor. As the fungus growth seems to have been hardly maintained by the trace of biotin contained in the supplied chemicals such as sucrose in those experiments, the nitrogen source of the blast fungus needs to be re-examined by means of the use of synthesized culture solutions which contain both biotin and vitamin B₁. According to the present studies, some amino acids or amides such as glycocoll, alanine, aspartic acid, glutamic acid and asparagine are quite excellent nitrogen sources for promotion of the fungus growth, while the fungus can synthesize the inorganic nitrogen also. When each one of the above mentioned amino acids or amides are supplied, the growth of the blast fungus is quite improved, the duration of the "lag phase" shortened and the increase of the fungus dry weight in "the middle stage" of the fungus growth becoming quite large. But the fungus growth is rather poor on the amino acids other than those mentioned above. This is because the action of the amino acid oxidase which is produced by the blast fungus is specific for those amino acids as described in the following chapter. Examining the value of the inorganic nitrogen as the nitrogen source of the blast fungus growth, one learns that nitrate nitrogen is superior to ammonium nitrogen. But it is evident that the depression of pH value of the culture solution by the unequivocal absorption of ions in the ammonium nitrogen culture is the direct cause of these difference in their value as the nitrogen source.

In view of the fact that the ammonium becomes detectable in the culture solution in which the amino acids are supplied as nitrogen source, it is certain that the amino acids are decomposed to ammonium by the blast fungus before the assimilation. Studies on the amino acid oxidase of the blast fungus are described in the following chapter.

On the other hand it is certain, in view of the fact that the ammonium becomes detectable in the culture solution in which the nitrate nitrogen is supplied as sole nitrogen source, that the nitrate nitrogen is reduced to ammonium nitrogen preceding the assimilation.

The nitrogen absorption by the blast fungus is generally vigorous at the rather earlier growing stage and the greatest nitrogen absorption by the fungus always occurs just before the greatest increase in fungus

dry weight appears.

On calculating the utilization ratio that means the increase of fungus dry weight by 1mg nitrogen absorption, one finds that the better nitrogen sources are not always higher in utilization ratio. But this is because the ratio was calculated at the end of the culture (at about 24 days culture). When the ratio is calculated at the earlier part of "the middle stage" of the fungus growth in which it is quite vigorous, it is certain that the better nitrogen sources are also higher in their ratio of utilization. No matter what kind of nitrogen is supplied as nitrogen source in the culture solution, nitrogen concentration, 0.275mg/ml, that is original in standard solution, is a rather minimum concentration for the fungus growth. A greater nitrogen supply clearly favours the more vigorous growth up to the concentration of 3.312mg/ml. When a greater amount of nitrogen is supplied, much of it is absorbed early and the duration of "the lag phase" is quite shortened; moreover the fungus growth in "the middle stage" becomes remarkably vigorous. But on the other hand, the utilization ratio of the nitrogen is rather lower in the case of greater nitrogen supply, though the fungus growth is remarkably greater.

Chapter III

Amino acids oxidase of rice blast fungus.

As described above, the ammonia becomes detectable in the culture solution in which only amino acid is supplied as nitrogen source. This naturally means the decomposition of amino acid by the blast fungus. The decomposition of amino acid by the micro-organisms is a catalysis by either oxidase, reductase or hydase; decomposition by oxidase is most common. The presence of amino acid oxidase in the blast fungus culture was first reported by the present writer (1954); NAKAMURA, SHIMOMURA and SUGIMOTO (1955) reported the production of l-amino acid oxidase by the fungus. In the following the experiments on that enzyme produced by the blast fungus are described. As every effort to extract the pure enzyme from the fungus has not come to success yet, the thoroughly washed fungus mat itself was used in the following experiments. The oxygen absorption by the fungus, which was cultured in the standard culture solution for ten days, was determined by WARBURG manometer. Each glutamic acid, aspartic acid or glycocoll

was employed as substrate for this determination; their pH values were adjusted to 7.0 with phosphate buffer and the temperature of the water bath was adjusted to 30°C. At the same time the oxygen consumption by natural respiration by the fungus was determined in distilled water. The value obtained by subtracting oxygen consumption by natural respiration was treated as the oxygen consumption by the oxidase of amino acid. On the other hand, the decrease of the amount of amino acid in the vessel of WARBURG manometer was determined by VAN SLYKE'S method, and the increase of the ammonia in the vessel was determined colorimetrically by NESSLER'S reagent. The results are shown in table 71. The figures in the table show the consumption of oxygen by the fungus of dry weight 100mg in one hour. As will be seen in the table, about 65mm³ oxygen are consumed by the oxidase of the blast fungus, while the estimated value of decreasing amount

TABLE 71. Amino acid oxidase in the blast fungus.

substrate	oxygen consumption by oxidase	decrease of amino acid		increase of ammonia	
		estimated	calculated *	estimated	calculated *
glutamic acid	63.3mm ³	0.082 mg	0.079 mg	0.098 mg	0.095 mg
aspartic acid	60.8 "	0.071 "	0.076 "	0.093 "	0.092 "
glycocoll	69.5 "	0.085 "	0.087 "	0.102 "	0.105 "

* calculated from the oxygen consumption.

of amino acid and also of increasing amount of ammonia are quite near to the value calculated from the oxygen consumption. Thus the production of amino acid oxidase by the blast fungus is quite certain, and the activity of this enzyme is rather weak on comparison with that of bacteria, while the value is rather strong on comparison with saprophytic fungus such as *penicillium*, *aspergillus*, *neurospora*. Some characters of amino acid oxidase caused by the blast fungus are determined in the following. In all the experiments the oxygen absorption was determined by WARBURG manometer under the conditions of 30°C and pH 7.0.

i) Specificity of the amino acid oxidase: The activities of the enzyme in relation to some kinds of amino acid were examined. In these experiments the fungus which was cultured on the standard culture solution for ten days was employed as the test organism.

The results are given in table 72. The enzyme does not act against each dl-methionine, dl-valine or arginine hydrochloride; these amino acids are valueless nitrogen sources for the fungus growth as described already. The activity against the dl-tyrosine is doubtful. The activity toward the each of glycocoll, l-aspartic acid, l-glutamic acid, d-glutamic acid, dl-glutamic acid, l-alanine, l-leucine, dl-leucine is certain; all these amino acids are good nitrogen sources for the fungus growth.

TABLE 72. Specificity of the amino acid oxidase of the blast fungus.

substrate	activity	substrate	activity
glycocoll	+	dl-tyrosine	±
l-aspartic acid	+	l-leucine	+
l-glutamic acid	+	arginine hydrochloride	—
d-glutamic acid	+	dl-leucine	+
dl-glutamic acid	+	dl-valine	—
l-alanine	+	dl-methionine	—

ii) Effect of KCN addition: Employing the fungus cultured in glutamic acid culture solution for ten days as the test organism, the effects of the addition of 1/1000M KCN on the enzyme action were examined. The results are shown in table 73 and figure 21.

TABLE 73. Effect of KCN addition on the amino acid oxidase of the blast fungus.

exp. section \ time	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
cont.	11.6 mm ³	28.5 mm ³	41.0 mm ³	59.9 mm ³	66.3 mm ³	82.5 mm ³
addition of KCN	5.8 "	12.0 "	18.0 "	22.3 "	29.4 "	35.8 "

As will be seen, the addition of M/100 KCN obstructs the action of the oxidase as much as about 57%. As the amino acid oxidase itself is originally indifferent to the addition of KCN, this result may suggest the intermediation of cytochrome system in the living cell of the fungus on the transfer of oxygen from outside to the oxidase as USAMI, KANEKO and SASAKI (1954) suggested on living cell of *proteus*.

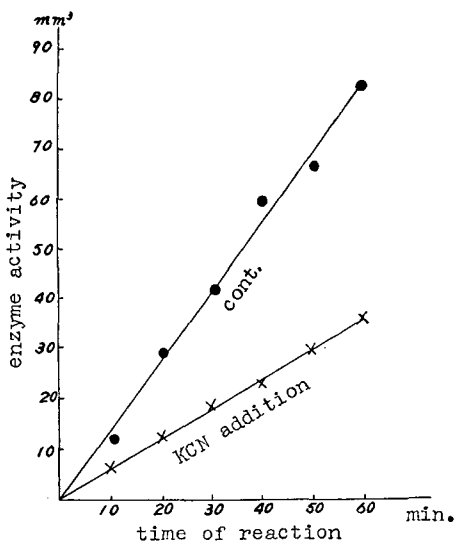


Fig. 21. Effect of KCN addition on the amino acid oxidase of the blast fungus.

iii) The age of the culture and the oxidase activity: Some of the cultures in the glutamic acid culture solution were filtered out on every other day from the 2nd till 26th day of the culture, and the oxidase activity against glutamic acid was examined by WARBURG manometer. The results are given in table 74 and figure 22 (curve I). The figures in the table show the amount of oxygen which was absorbed in one hour by the fungus of 100 mg dry weight. As noted

TABLE 74. Culture age of the blast fungus and the activity of the amino acid oxidase.
(standard culture)

age of culture	oxygen absorption	age of culture	oxygen absorption
2nd day	9.8mm ³	16th day	60.1mm ³
4th "	25.0 "	18th "	34.9 "
6th "	62.6 "	20th "	22.1 "
8th "	75.1 "	22nd "	14.2 "
10th "	82.7 "	24th "	9.2 "
12th "	82.8 "	26th "	6.3 "
14th "	77.0 "		

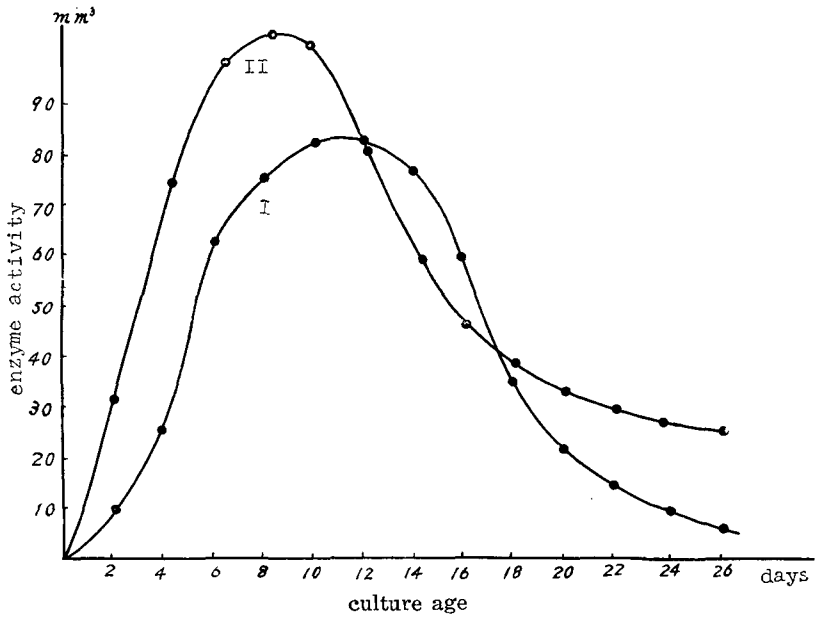


Fig. 22. Fluctuation of amino acid oxidase activity with advance of age of culture of the blast fungus.

already the fungus growth is most vigorous in the period of the 6th-10th days of the culture when glutamic acid is supplied as nitrogen source. On the other hand, as will be seen in the table and the figure, the activity of amino acid oxidase is also most vigorous on 6th-10th days and then its activity falls gradually. As mentioned in the previous chapter, the greatest amount of nitrogen is absorbed on 4th-6th days of the culture when glutamic acid is supplied as nitrogen source. And it must be noticed that the greatest increase of the oxidase activity also appears on the 4th-8th days of culture. The same kind of experiments were carried out on those fungi which were cultured in culture solution of double nitrogen level (16.56 mg per flask). The results are shown in table 75 and figure 22 (curve II). In this case the activity of the amino acid oxidase was rather vigorous already on the 2nd day of the culture, and the oxygen consumption reached to 103.5 mm^3 on the 8th day. This is in accordance with the faster and greater growth of the fungus in the case of the high level nitrogen supply. Thus it is known that the blast fungus growth is closely connected with the activity of the amino acid oxidase produced by this fungus.

TABLE 75. Culture age of the blast fungus and the activity of the amino acid oxidase.
(high level nitrogen culture)

age of culture	oxygen absorption	age of culture	oxygen absorption
2nd day	30.8 mm ³	16th day	45.0 mm ³
4th "	75.3 "	18th "	35.1 "
6th "	98.9 "	20th "	31.1 "
8th "	103.5 "	22nd "	28.4 "
10th "	101.1 "	24th "	26.3 "
12th "	80.0 "	26th "	24.1 "
14th "	89.8 "		

iv) Nitrogen source of the culture and the activity of amino acid oxidase: The differences of nitrogen source in the blast fungus culture may exert some influences on the activity of the amino acid oxidase. In order to examine this point, five kinds of cultures, in which each KNO₃, l-aspartic acid, l-glutamic acid, l-leucine or l-alanine were given as nitrogen source, were prepared; the activity of amino acid oxidase of each culture was examined employing l-aspartic acid, l-glutamic acid, l-leucine, l-alanine as substrate. The measurements were carried on with the fungus of 10th-14th day's culture, as the activity of the oxidase was high at that age of the culture. The results are given in table 76. As will be seen in the table, the activity of the amino acid oxidase is most vigorous on the same kind of amino acid as given as nitrogen source of the culture, and it is clear that the activity of

TABLE 76. Nitrogen sources of the blast fungus culture and the activity of amino acid oxidase produced by the fungus.

nitrogen source for the fungus	substrate for enzyme action			
	l-aspartic acid	l-glutamic acid	l-leucine	l-alanine
KNO ₃	60.8 mm ³	63.3 mm ³	62.9 mm ³	58.8 mm ³
l-aspartic acid	90.6 "	70.4 "	74.2 "	71.5 "
l-glutamic acid	72.3 "	82.5 "	69.5 "	67.4 "
l-leucine	70.2 "	75.5 "	80.8 "	72.1 "
l-alanine	69.3 "	63.8 "	70.2 "	80.1 "

the enzyme is rather adaptive to the same kind of amino acid given as nitrogen source of the culture. However the activity of the oxidase is clearly detectable even when only inorganic nitrogen is given as nitrogen source, although it is comparatively lower than that noted in the case of amino acid nitrogen source. When the amino acid is given as nitrogen source of the culture, the difference of the kind of the amino acid supplied does not make so great differences in respect to the production of the enzyme, as long as the amino acid provides adequate nitrogen source for the fungus growth.

Summarizing the experimental results in this section, the oxidase activity of the blast fungus is far beyond that of such saprophytic fungi as *penicillium*, *aspergillus* or *neurospora* while the activity is far inferior to that of bacteria. The enzyme is inactive for some kinds of amino acid such as dl-methionine, dl-vealine, arginine-hydrochloride which are also inadequate nitrogen sources for the fungus growth. But the activity is quite evident for such kind of amino acids as glycocoll, l-aspartic acid, l-glutamic acid, d-glutamic acid, dl-glutamic acid, l-alanine, l-leucine, dl-leucine, each of which is a good nitrogen source for fungus growth. The activity of the enzyme is detectable even when only inorganic nitrogen is given as nitrogen source, but its activity is comparatively lower than that in the fungus supplied with some amino acids nitrogen source. When the some kind of amino acid is given as nitrogen source the action of the enzyme is greatest against the same kind of amino acid as employed as nitrogen source, suggesting the adaptive production of the enzyme to the amino acid given as nitrogen source. Considering from the fact that the action of the oxidase is hindered by the addition of KCN, the cytochrome system may intermediate the transfer of oxygen from outside to the oxidase in the living fungus. On seeing the fluctuation of the enzyme activity with the age of the culture, one notes that the activity begins to increase immediately after the fungus growth begins and it is most vigorous at the earlier part of "middle stage" when the fungus growth is most vigorous, after which it begins to fall. If the fungus growth rate is favoured by the high level nitrogen supply, the peak of enzyme activity appears the earlier and its activity becomes remarkably high. It must be noticed here that the growing stage of the most vigorous enzyme activity is always in accordance exactly with the growing stage of the most vigorous nitrogen absorption.

Chapter IV

The proteolytic enzyme of the blast fungus.

It seems to be quite natural to suppose that the proteolytic enzyme produced by the phytopathogenic fungi may exert some important effects on the process of the disease development.

In this chapter, therefore, examination of the proteolytic enzyme produced by the blast fungus is reported. As every effort to extract the enzyme from the filtrate or the mycelium has not yet come to success, all the experiments were carried on by employing the filtrate or mycelium itself as the enzyme source. Five cultures were filtered out after some days of the culture and the filtrate was concentrated to the total volume 25 cc by vacuum distillation at temperature of 38°-40°C. Five cc portions of the material were used as the enzyme source in the following experiments. On the other hand the fungus mats remaining on the filter paper were thoroughly washed with distilled water and stood still at room temperature for some time in order to drain off the water. Some parts of them, supposed about 20 mg in dry weight, were also employed as enzyme source in the followings. In this case the accurate dry weight of the used fungus was determined at the end of each experiment and the activity on the fungus of dry weight 20 mg was calculated on the basis of the supposition that the enzyme activity is proportional to the amount of the fungus. Six percent gelatine or six percent casein were employed as substrates for the determination of the enzyme activity. Five cubic-centimetres of the substrate were added with 5 cc of phosphate buffer of each hydrogen ion concentration. The enzyme was allowed to act on that solution with a drop of toluol in the thermobath of 40°C, and at some interval the increase of amino nitrogen was determined on 2 cc of the solution by VAN SLYKE's method. Thus the enzyme activity was shown by the increase of the amount of amino acid in the substrate. All the vessels used for the experiments were sterilized previously and all attention was paid during the whole procedure to avoid contamination by bacteria.

1. The proteolytic enzyme detectable in the filtrate (exo-enzyme).

i) The time of reaction and the kind of substrate: The enzyme source was prepared from the filtrate of 15 day's cultures and gelatine

was employed as substrate, the pH being adjusted to 5.4. The increase in amount of amino acid in the solution was determined at 2 hours, 4 hours, 8 hours, 18 hours, 24 hours, 32 hours and 48 hours of the reaction respectively. Table 77 shows the results.

TABLE 77. Proteolytic exo-enzyme of the blast fungus.

time of reaction	amount of amino-N
2 hrs.	0.01 mg
4 "	0.04 "
8 "	0.08 "
18 "	0.14 "
24 "	0.22 "
32 "	0.31 "
48 "	0.40 "

As will be seen in the table, the increase of amino acid in the solution becomes detectable already at 2 hrs. of the reaction, and the amount continue to increase with time, reaching to 0.40 mg at 48 hrs.. On the basis of these observations, the activity of the proteolytic enzyme in the filtrate of blast fungus may be said to be not so great though its activity is evident. A part of the cause of rather lower activity in this experiment may lay in the rather inadequate pH of the solution as described below. The enzyme activity was next examined on substrates of each gelatine, casein, glycyl-glycine and chloracetyl-l-tyrosine. The pH value was adjusted to 7.0 in these experiments, and the increases of the amino acid in the solution were examined at 24 hrs. of the reaction. The results are given in table 78.

TABLE 78. Specificity of proteolytic enzyme of the blast fungus.

(exo-enzyme)

substrate	activity
glycyl-glycine	+
chloracetyl-l-tyrosine	-
casein	+
gelatine	+

The enzyme is active against each one of glycyl-glycine, casein, and gelatine, while it is inactive toward the chloracetyl-l-tyrosine.

ii) pH and the enzyme activity: In order to know the optimum pH for the enzyme activity, each prepared of pH 2.2, 2.6, 3.6, 4.6, 5.6, 6.6, 7.0, 8.6, and 9.6 were made. Gelatine was employed as substrate and the increasing amounts of amino acid were determined at 12, 24 and 48 hrs. of the reaction. All the results are shown as comparative values by treating the increased amount of amino acid at 48 hours of reaction in the section of pH 7.0 as 100. The results are given in figure 23. The enzyme activity is remarkably low in every sample below pH 5.6. When the pH was adjusted to 7.6, the enzyme activity was quite vigorous till 12 hrs. of the reaction but at the longer reaction times activity began to fall. The same kind of tendencies are also seen in both sections of pH 8.6 and 9.6; on the other hand, the increasing state of the amino acid in both sections of pH 7.0 and 6.6 seems to be normal and the amino acid found in the section of pH 7.0 at 48 hours of the reaction was greatest of all.

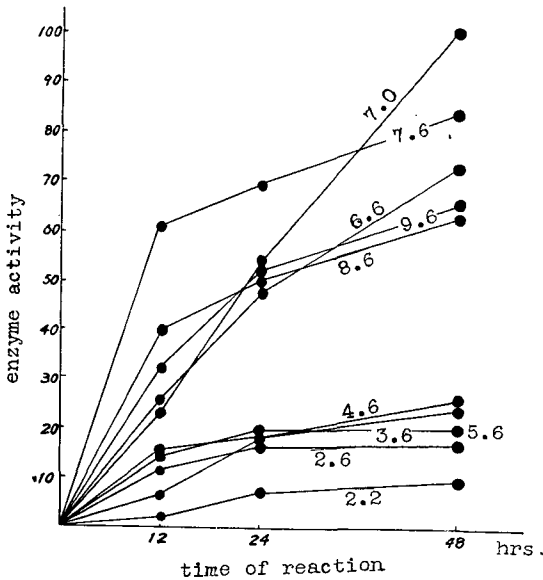


Fig. 23. Relations between pH value and the proteolytic enzyme activity. (exo-enzyme)

Therefore it is certain that the optimum pH for the enzyme action lies at the pH 7.0 and the activity of the enzyme is rather high on

the side of decreasing alkalinity.

iii) Temperature for enzyme activity: In order to know the optimum temperature for enzyme action, sections of each 20°C, 30°C, 40°C and 50°C were prepared. Casein was employed as substrate and the pH was adjusted to 7.0. The determination of the amino acids in the solution was carried on at 12, 24 and 48 hours of reaction. All the results are shown as comparative values by treating as 100 the amount of amino acid in the section 40°C at 48 hrs. of reaction. The results are given in figure 24.

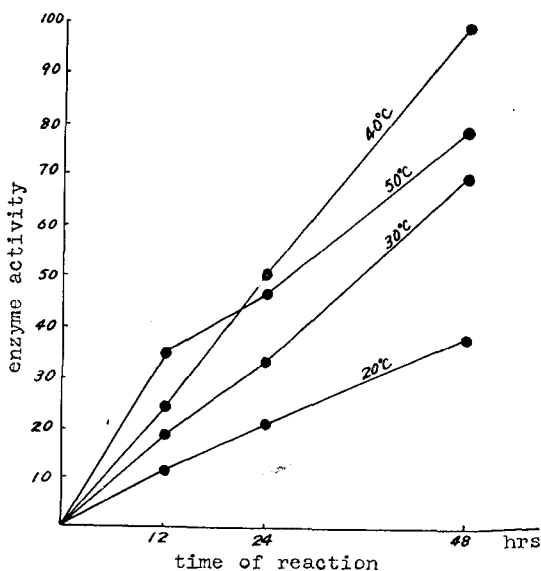


Fig. 24. Relations between the temperature and the proteolytic enzyme activity. (exo-enzyme)

As will be seen in the figure, the temperature of 20°C is too low for the enzyme action while the temperature of 50°C is rather too high. And it is clear that the temperature of 40°C is the optimum for the enzyme action.

iv) Addition of cysteine or KCN and enzyme activity: In some proteolytic enzymes as papain their action is activated by the addition of some SH-compounds or cyan-compounds. It is important to know if the activity of the proteolytic enzyme in question is influenced by the addition of such chemicals. Therefore the effects of the addition of cysteine or KCN on the enzyme activity were examined. The

concentrations of added chemicals were each 1/100, 1/200, 3/1000, 1/1000, 1/2000M, and casein was employed as the substrate. The pH and the temperature of the reaction were adjusted to 7.0 and 40°C respectively. The effects of the addition of cysteine are shown in table 79.

TABLE 79. Effect of cysteine addition on the proteolytic enzyme.
(exo-enzyme)

concentration of cysteine	duration of reaction		
	24 hrs.	48 hrs.	72 hrs.
cont.	70	95	100
1/2000 mol	68	90	97
1/1000 "	62	93	98
3/1000 "	57	91	105
1/ 200 "	41	105	172
1/ 100 "	43	127	191

The results are calculated by treating the amount of amino acid at 72 hrs. of reaction in control section as 100. The effect of the addition of cysteine is evident at the concentrations above 1/200 M, while there are no effect at the concentration below 3/1000M. When the cysteine is added at the concentration above 1/200M, the amount of amino acid in the cysteine added section is less than that in the control at 24 hrs. of reaction, but with the longer duration of reaction time the amino acid amount in the cysteine added section comes to get ahead of the amount in control. And it is clear that the activity of the enzyme in the blast fungus is encouraged by the addition of cysteine at concentration above 1/200 M. The reason why action of the enzyme is inhibited temporarily at 24 hrs. of the reaction by the addition of

TABLE 80. Effect of KCN addition on the proteolytic enzyme.
(exo-enzyme)

concentration of KCN	duration of reaction			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
0 mol	66	93	98	100
1/2000 "	59	86	90	96
1/1000 "	57	79	92	98
3/1000 "	46	83	94	105
1/ 200 "	43	80	101	104

cysteine is uncertain, but a part of the reason may lie in the employment of unpurified material as enzyme source instead of the purified one. The effect of the addition of KCN on the enzyme action is summarized in table 80. The results are calculated by treating the amount of amino acid at 96 hrs. of reaction in the control as 100.

There seems to be some tendency for the addition of KCN to inhibit the action of the enzyme at 24 hours of reaction and on the longer duration of reaction for the activity of the enzyme to be activated a little. But the effort to get the clearer evidence that the action of enzyme is promoted by the addition of KCN has not come to success, though the determination was continued to 96 hrs. of the reaction. The proteolytic enzyme that is activated by the addition of SH-compound is to be also activated by the addition of KCN. The reason why the enzyme is not activated by the addition of KCN in this experiment, while it is activated by the addition of cysteine, is uncertain. A part of the reason may lie in the employment of unpurified material as the enzyme source.

v) Age of the culture and the activity of the enzyme: The activity of the enzyme fluctuates with the age of the culture. In order to know the details, four cultures of KNO_3 nitrogen source were filtered out at each 4, 7, 9, 12, 14, 16, 21, 26 days of the culture and the filtrate were concentrated respectively to 50 cc by vacuum distillation. Employing 5 cc of this concentrated filtrate as the enzyme source, the enzyme activity was determined at each age of the culture. The substrate was gelatine and the pH was adjusted to 7.0. The enzyme activities were determined by the measurement of amino acid at 24 hours of the reaction. The results are shown in figure 25. The results were calculated by treating the activity of 9 day's old culture as 100. As will be seen in the figure, the enzyme activity increases gradually till 9th day of the culture and after then begins to fall suddenly. The activity in the 21 day's old culture is quite weak. It is noticeable that the enzyme activity reaches maximum on the 9th day of the culture when the most remarkable fungus growth appears.

vi) The kind of nitrogen source of the culture and the enzyme activity: It seems to be of interest to know if differences of nitrogen source of the culture exert any effect on the activity of the proteolytic enzyme produced by the fungus. In order to find out about such relations, the following described experiments were planned. The nitrogen sources employed in these experiments were asparagine,

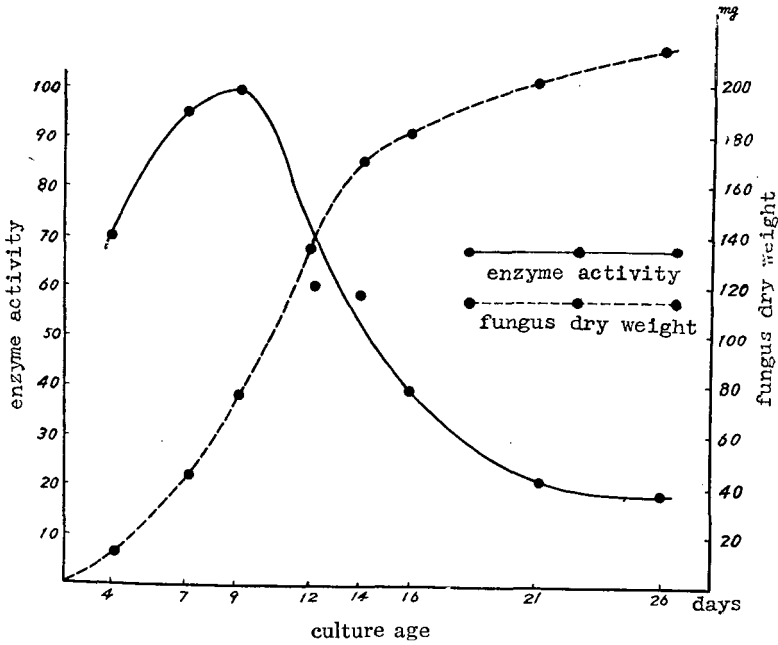


Fig. 25. Culture age and the proteolytic enzyme activity. (exo-enzyme)

glycine, l-alanine, aspartic acid, glutamic acid, urea and KNO_3 , which have all been proved to be good nitrogen sources for the fungus growth. Gelatine was used as substrate and the pH was adjusted to 7.0. The enzyme activity was determined by the increasing of amino acid in the substrate at 24 hours of the reaction. As shown in the previous section the enzyme activity fluctuates with the age of the culture in every experimental sections of each nitrogen source. Therefore the age of the culture when the peak of the enzyme activity appears, and the value of maximum enzyme activity are shown in table 81. The figures which represent the enzyme activity in the table were calculated by giving the amino acid in KNO_3 section a value of 10. The enzyme activity of the urea section was inferior to that of the KNO_3 section; at the same time the urea is a rather inferior nitrogen source to KNO_3 . The peak of the enzyme activity appears earlier in the KNO_3 section than in the urea section. All the other amino acids and amides, with the one exception of glycine, were excellent for the enzyme production and the enzyme activity was generally greater in such amino acid or

TABLE 81. Relations between the nitrogen source of the culture and the enzyme activity.

(exo-enzyme)

N-sources	activity of the enzyme	age of culture when the activity reached maximum	dry weight of mycelium when activity examined
KNO ₃	10	9th day	70.6 mg
asparagine	17.3	9th "	120.1 "
glycine	5.8	9th "	105.1 "
l-alanine	16.4	16th "	195.5 "
aspartic acid	19.1	12th "	163.2 "
glutamic acid	17.8	9th "	83.4 "
urea	7.9	16th "	75.1 "

amide cultures than in KNO₃ culture. But only in the glycine culture the enzyme activity was exceptionally weak, although glycine is an adequate nitrogen source for the fungus growth. The age of culture at which the enzyme activity reaches maximum seems not to have any correlation with the greatness of the enzyme activity or the growth rate of the fungus. This statement takes into consideration the facts that the peak of the enzyme activity appears on the 9th day of the culture both in the KNO₃ and asparagine sections while the difference in the value of the maximum activity of the enzyme is rather great between the two sections and also the facts that in the case of aspartic acid culture the peak of the enzyme activity appears on 12th day of culture which is 3 days later than in the other amino acid sections while the greatest value of the enzyme activity is seen in that culture.

2. The proteolytic enzyme detectable in the mycelium (endo-enzyme).

The endo proteolytic enzyme of the blast fungus was examined by employing the mycelium as the enzyme source.

i) The reaction time and the substrate: The fungus which was cultured for 15 days in standard culture solution was employed as the enzyme source. The substrate was gelatine and the pH was adjusted to 5.4. The results are shown in figure 26. The relation between the time and the proteolytic enzyme is almost the same as in the case of the exo-enzyme, but the activity is a little greater in the mycelium

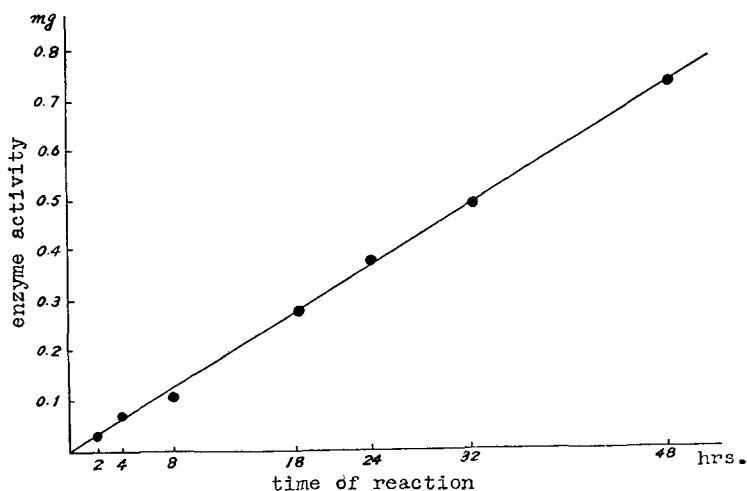


Fig. 26. Proteolytic endo-enzyme of the blast fungus.

of dry weight 20 mg than in the 5 cc of concentrated filtrate. The specificity of the enzyme was examined for each one: gelatine, casein glycyl-glycine and chloracetyl-l-tyrosine. The results are shown in table 82. Like as in the exo-enzyme, the endo-enzyme is active against each gelatine, casein and glycyl-glycine while it is inactive toward chloracetyl-l-tyrosine.

TABLE 82. Specificity of proteolytic enzyme of the blast fungus.
(endo-enzyme)

substrate	enzyme activity
glycyl-glycine	+
chloracetyl-l-tyrosine	-
casein	+
gelatine	+

ii) pH and the enzyme activity: Employing the mycelium as the enzyme source, the writer examined the relations between the pH of the solution and the enzyme activity in the same way as in the previous section. The results are calculated by treating the amino acid at 27 hrs. of reaction in the section of pH 7.0 as 100. Figure 27 shows the results.

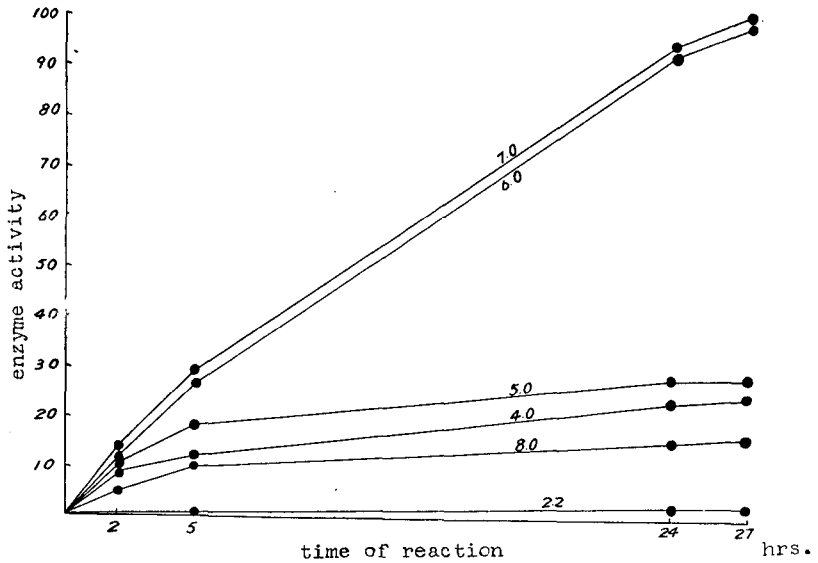


Fig. 27. Relations between pH value and the activity of the proteolytic enzyme.
(endo-enzyme)

It is quite same as in the previous section that the optimum pH for the enzyme action lies in 7.0. But the range within which the enzyme action pretty fair, is rather narrow. In the pH below 5.0 and above 8.0 the enzyme action becomes quite weak.

iii) Addition of KCN or cysteine and the enzyme activity: The effects of the addition of cysteine or KCN on the enzyme activity were examined by adding 1/100 N either of cysteine or of KCN.

The results, which were calculated by treating the amino acid at 29 hrs. of reaction in the control section as 100, are given in figure 28.

As will be seen in the figure, the enzyme action is clearly promoted by the addition of the cysteine, and there is not any such tendency as a temporary inhibition in the beginning of the addition as was seen in the previous experiments where the concentrated filtrate was employed as the enzyme source. Moreover the activation of the enzyme action was clearly shown also by the addition of KCN, while any efforts to prove such fact never came to success in the case of the exo-enzyme.

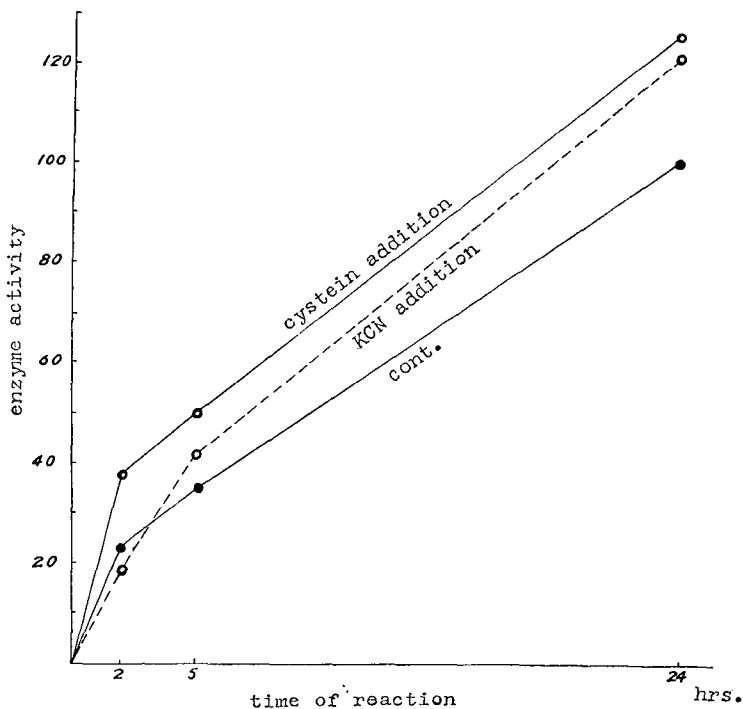


Fig. 28. Effects of cysteine or KCN addition on the proteolytic enzyme. (endo-enzyme)

iv) The age of the culture and the enzyme activity: The relations between the age of the culture and the enzyme activity are quite the same as in the experiments of the previous section. At first the enzyme activity increases gradually with the duration of the culture and it begins to fall rather suddenly immediately after the activity reaches its maximum. Figure 29 graphs the results in the culture of KNO_3 nitrogen source.

v) The nitrogen source of the culture and the enzyme activity: In order to know the relations between the nitrogen source of the culture and the enzyme activity, nine various cultures were prepared in which one each respectively KNO_3 , asparagine, glycine, l-alanine, aspartic acid, glutamic acid, urea, casein and gelatine was supplied as the nitrogen source. The experiments were carried on in the same way as in the previous section. The experimental results are summarized in table 83. The activity of the enzyme in the table was

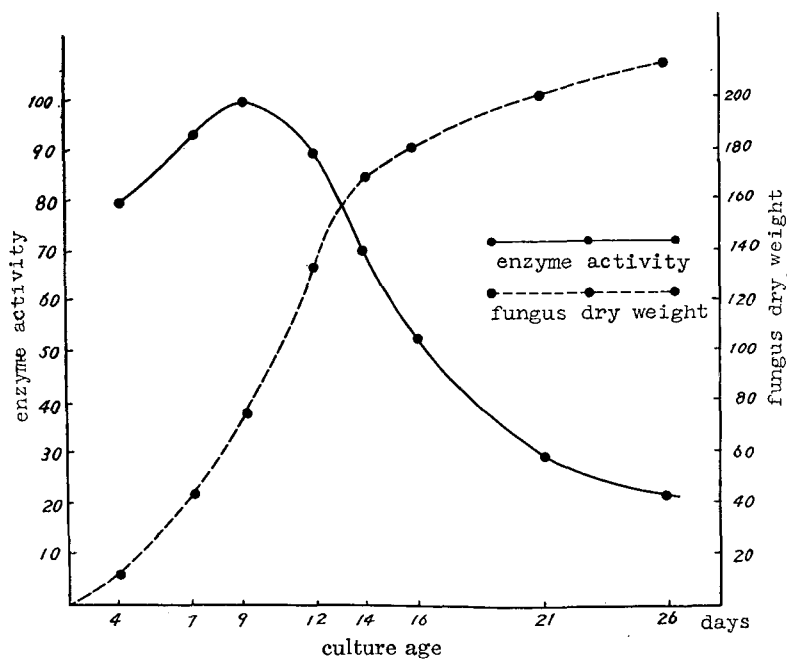


Fig. 29. Culture age and the proteolytic enzyme activity.
(endo-enzyme)

TABLE 83. Relations between the nitrogen source of the culture
and the proteolytic enzyme activity.
(endo-enzyme)

nitrogen sources	enzyme activity	days of culture when enzyme activity reaches to maximum	fungus dry weight when the activity was examined
KNO ₃	10	9 days	70.6 mg
asparagine	16.8	9 "	120.1 "
glycine	6.5	9 "	105.4 "
l-alanine	17.2	14 "	195.5 "
aspartic acid	18.4	14 "	163.2 "
glutamic acid	17.1	9 "	83.4 "
urea	5.8	16 "	75.1 "
casein	25.6	9 "	215.0 "
gelatine	26.1	9 "	221.0 "

calculated by treating the amino acid at 24 hrs. of the reaction in the KNO_3 section as 10. Like as in the case of the exo-enzyme, the enzyme activity is generally greater in the section in which the fungus growth is the better; in the urea nitrogen section in which the fungus growth is not so good the enzyme activity is also rather inferior to the other sections; the enzyme activity is exceptionally weak in the glycine section. In the both sections of casein and gelatine nitrogen source the enzyme activity is also remarkably high while the fungus growth is also excellent; this may suggest the adaptive production of the proteolytic enzyme caused by the presence of protein in the culture solution. As in the case of the exo-enzyme the culture age when enzyme activity reaches maximum seems not to have any correlation with the growth rate or the greatness of the enzyme activity.

Summarizing the above stated results on the proteolytic enzyme of the blast fungus, the enzyme activity is clear both in the culture filtrate (exo-enzyme) and in the mycelium (endo-enzyme) though each of them is not always very strong. There seem to be a few observable differences in the characters of the endo-enzyme and the exo-enzyme, but the responsibility for the observation of these differences seems to rest upon the employment of unpurified filtrate or mycelium itself as the enzyme source in these experiments. The characters of the proteolytic enzyme of the blast fungus may, in general, be summarized as follows: the enzyme is active against each casein, gelatine, and glycyl-glycine while it is inactive against the chloracetyl-l-tyrosine; the optimum pH for the reaction lies in 7.0; the optimum temperature is about 40°C and the action of this enzyme is promoted by the addition of either cysteine or KCN at the concentrations above 1/100 N. Thinking from this characters, it seems to the writer the enzyme in question here is similar to papain. The activity of the enzyme fluctuates with the age of the culture. It reaches generally to its maximum at the age when the growth rate of the fungus begins to be great and thereafter it decreases rather suddenly. It is evident that fungus supplied with a good nitrogen source as some amino acids produces the enzyme abundantly. Moreover the supply of the protein as casein or gelatine in the culture media favors adaptively the production of the proteolytic enzyme by the fungus.

Chapter V

Carbon sources of the blast fungus.

Since the indispensability of biotin and vitamin B₁ for the growth of the blast fungus has been proved, the studies on the carbon source of the blast fungus should be re-examined. This chapter consists of a description of experiments on the carbon source which were carried on by employing the culture solution containing biotin (80 m γ in 30 cc culture solution) and vitamin B₁ (0.5 γ in 30 cc culture solution). Carbon sources examined are the following—sugars: viz., glucose, fructose, galactose, sucrose, lactose, maltose; polysaccharides: viz., soluble starch, inulin; higher alcohols: viz., glycerine, mannit; and organic acids: viz., formic acid, acetic acid, oxalic acid, succinic acid, lactic acid, and citric acid. The amount of the carbon source supplied was always the same as the amount of carbon contained in the standard culture solution*. All the chemicals used in these experiments were tested

TABLE 84. Carbon sources and the blast fungus growth.

carbon sources	fungus dry weight	spore formation	final pH of medium
glucose	113.6 mg	±	6.6
fructose	57.0 "	±	5.8
galactose	17.7 "	—	5.6
sucrose	129.8 "	±	7.0
lactose	32.5 "	—	6.4
maltose	134.7 "	±	7.2
soluble starch	56.5 "	±	6.2
inulin	100.1 "	±	6.8
glycerine	1.8 "	—	5.4
mannit	96.4 "	±	6.8
formic acid	0 "	—	5.4
acetic acid	0 "	—	5.4
oxalic acid	1.3 "	—	5.6
succinic acid	57.5 "	—	6.2
lactic acid	1.0 "	—	5.4
citric acid	27.0 "	—	5.6

* It is the same as described in chapter I of this part.

previously for contamination of the growth factor by the bioassay of "*Saccharomyces cerevisiae*" and if even a trace of contamination of the growth factor in the chemicals were proven it was completely removed by absorption with charcoal before material was used. The pH of the culture solution was adjusted to 5.4 in original. The cultures were filtered after two week's culture and fungus dry weight, spore formation and the final pH were determined. The results are summarized in table 84. As will be seen in the table, maltose and sucrose were the most excellent carbon sources for the fungus growth while lactose was far inferior to them. When glucose was given as carbon source, the fungus growth was fairly good though a little inferior to that of sucrose. On fructose carbon source, the fungus growth remained at half of that in the case of glucose, while galactose, though one of the hexoses, was a rather inadequate carbon source for the fungus growth. Inulin among polysaccharides was a rather good carbon source while soluble starch was not so good. Mannit among higher alcohols was also a rather good carbon source but the fungus growth was very scanty on glycerine. As to the organic acids, the fungus growth of 57.5mg in dry weight, which corresponds to the growth on fructose or soluble starch, was to be seen on succinic acid carbon source; some growth could be seen also on citric acid, while no growth was seen on formic acid, acetic acid, oxalic acid and lactic acid. The pH value of the culture solution rose with the duration of the culture whenever the fungus growth was fairly good, because of the unequivalent absorption of ion. Slight spore formation was seen in each of the cultures of glucose, fructose sucrose, maltose, soluble starch, inulin and mannit.

To summarize the results, the carbon sources are enumerated in the order of their nutritiousness as follows: 1. maltose, 2. sucrose, 3. glucose, 4. inulin, 5. mannit, 6. succinic acid, 7. fructose, 8. soluble starch, 9. lactose, 10. citric acid, and 11. galactose. The excellency of maltose as the carbon source for the blast fungus was reported already by TOCHINAI and NAKANO (1940); glucose and mannit were also reported as good carbon sources by them. But the present writer can not agree with their conclusions that glucose is a carbon source superior to sucrose or that soluble starch and glycerine are fairly good carbon source. Their results might have been disturbed by the trace of biotin contaminated in the used chemicals. Last of all it is worthy of notice that the fungus growth is pretty good on such organic acids as succinic acid and citric acid which are included in T. C. A. cycle.

Chapter VI

Respiration of the blast fungus.

It is quite natural that the activity of the fungus growth is concerned with the intensity of the respiration of the fungus. In this chapter experiments on the respiration of the blast fungus are described. The fungus was cultured on the standard culture solution, which contained 80 μ biotin and 0.5 γ vitamin B₁ in 30 cc culture solution of each flask. Nitrogen source of the standard culture solution was sometimes replaced by either glycocoll, alanine, asparagine, creatine or taurine. The experiments were performed on the fungus of 10-20 day's culture. Previous to the experiments the mycelia to be used were transferred into sterilized distilled water and were left to stand still in the thermostat for 24 hours to allow for consumption of the respiration materials which might be stored in the mycelium. The oxygen absorption by the fungus of about dry weight 50 mg was determined by WARBURG manometer at each ten minute interval for one hour and Q_{0₂} was calculated.

i) Temperature and respiration intensity: In order to know the optimum temperature for respiration, the intensity of respiration was determined in each temperature of 10°, 15°, 25°, 30°, 35°, 40°, 50°C. The used fungus was that which had been cultured in glycocoll nitrogen culture solution for 15 days. One cubic centimetre of M/6 glucose was given as substrate in the WARBURG vessel. The pH of the substrate was adjusted to 6.0 with phosphate buffer. One half cubic-centimetre of 10% KOH was employed as the absorbent. The results are shown

TABLE 85. Temperature and the respiration intensity of the blast fungus.

	10°	15°	25°	30°	35°	40°	50°
10 min.	1.6 mm ³	15.3 mm ³	6.4 mm ³	19.3 mm ³	21.2 mm ³	27.5 mm ³	8.4 mm ³
20 "	3.4 "	24.9 "	22.0 "	38.2 "	41.5 "	56.4 "	19.0 "
30 "	4.2 "	39.8 "	33.1 "	56.6 "	66.2 "	76.0 "	28.1 "
40 "	5.7 "	53.2 "	47.1 "	73.3 "	91.0 "	89.1 "	39.0 "
50 "	7.0 "	63.5 "	69.8 "	90.0 "	113.9 "	108.9 "	45.2 "
60 "	8.3 "	73.0 "	95.0 "	109.0 "	136.8 "	129.0 "	57.1 "
Q _{0₂} "	0.2 "	2.0 "	2.6 "	3.0 "	3.7 "	3.5 "	1.6 "

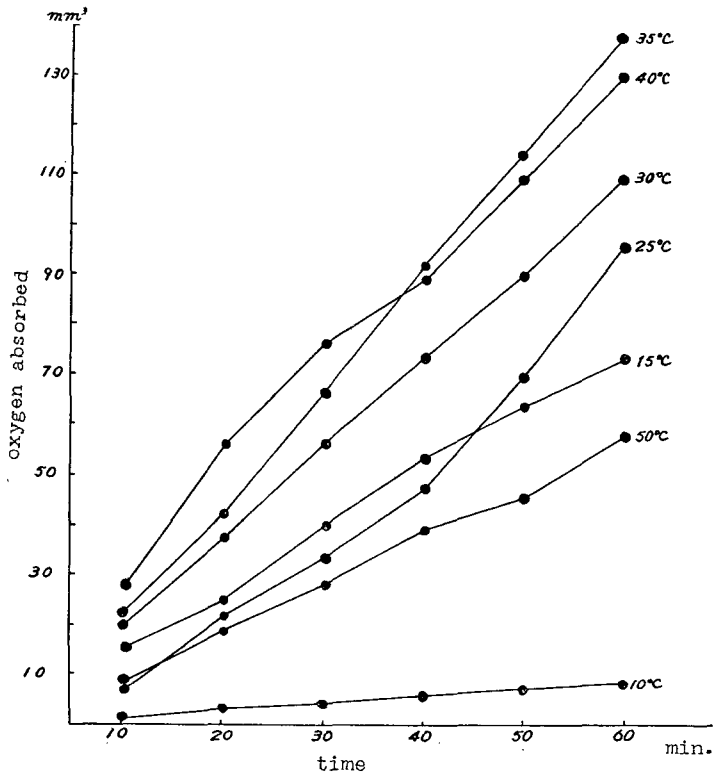


Fig. 30. Temperature and the respiration intensity of the blast fungus.

in table 85 and figure 30. In this table the oxygen absorption by the fungus of dry weight 36.6mg is shown. As will be seen in the table and figure, the optimum temperature for the respiration lies at 35°C and Q_{O_2} is 3.7 in that temperature. At 40°C the oxygen absorption is rather greater than at 35°C up until 30 minutes of the determination but it becomes smaller with passage of time, giving finally Q_{O_2} 3.5. This may suggest the effect of the high temperature on the respiration. For respiration 50°C is clearly too high, Q_{O_2} being only 1.6. The respiration is also fairly good in 30°C though the Q_{O_2} is a little smaller than at 35°C; it is pretty good even at 15°C while 10°C is too low. It is worthy of notice that the optimum temperature for respiration, 35°C, is higher than the optimum temperature for the fungus growth, 28°-30°C.

ii) pH and the intensity of respiration: In order to know the

optimum pH for the respiration, the experiments were performed using culture solution of each pH 2.6, 4.0, 5.0, 6.0, 7.0, 8.0, which were prepared by adding phosphate buffer solution. The used fungus was

TABLE 86. Relations between the pH value and the respiration intensity of the blast fungus.

time \ pH	2.6	4.0	5.0	6.0	7.0	8.0
10 min.	3.32 mm ³	15.0 mm ³	11.9 mm ³	26.2 mm ³	19.1 mm ³	2.4 mm ³
20 "	8.29 "	17.5 "	28.8 "	45.5 "	33.4 "	14.3 "
30 "	11.6 "	22.0 "	50.1 "	64.5 "	45.5 "	23.9 "
40 "	15.0 "	29.0 "	62.2 "	86.0 "	64.5 "	26.2 "
50 "	20.0 "	31.5 "	79.0 "	105.0 "	79.0 "	40.6 "
60 "	25.0 "	38.2 "	98.0 "	126.6 "	93.3 "	47.8 "
Q _{O₂} "	0.49 "	0.76 "	1.78 "	2.53 "	1.87 "	0.96 "

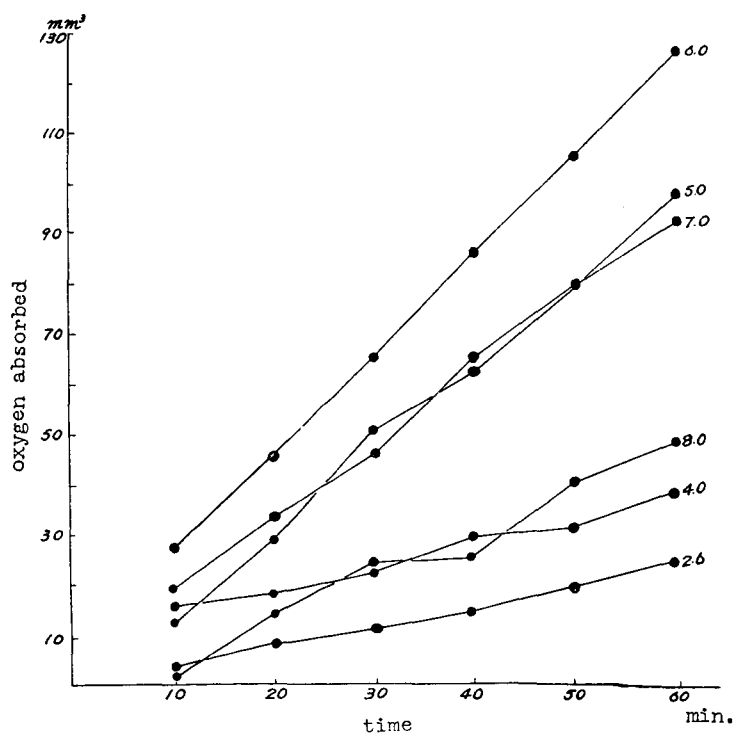


Fig. 31. Relations between pH value and the respiration intensity of the blast fungus.

that which was cultured in alanine culture solution for 20 days. Substrate was M/6 glucose and the temperature of experiment was 35°C. The results are given in table 86 and figure 31.

As will be seen, fairly good respiration is seen in the pH range of 5.0-7.0 with optimum 6.0, showing greatest Q_{O_2} 2.53. The optimum pH for the respiration, 6.0, is about the same with the optimum pH for the fungus growth.

iii) Respiration material: The suitabilities of various substrates for the respiration of the fungus were examined. The tested substrates are as follows: glucose, fructose, galactose, sucrose, maltose, inulin, mannit, succinic acid, citric acid, oxalic acid. The fungus samples employed for the experiment were those which were cultured in KNO_3 culture solution for ten days. Experimental temperature was 35°C and the pH was 6.0. The concentration of the substrates tested was always M/6. The results are given in table 87.

TABLE 87. Respiration intensity of the blast fungus on the various kinds of carbohydrates.

substrate	Q_{O_2}
glucose	4.52
fructose	0.33
galactose	0.28
sucrose	2.00
maltose	0.22
inulin	1.38
mannit	1.25
succinic acid	2.40
citric acid	1.82
oxalic acid	1.66

As will be seen in the table, glucose is the most excellent respiration substrate for the fungus, while fructose and galactose are rather bad. The respiration intensity is unexpectedly low on the substrate of sucrose and maltose, especially on the latter. Inulin and mannit are not very good respiration substrates. On the other hand, succinic acid, one of the organic acids, is a fairly good respiration substrate for the fungus; citric acid and oxalic acid are also pretty good substrates, while succinic acid and citric acid, are good carbon sources

for the fungus growth as described in the previous chapter. It is worthy of notice that the three organic acids above mentioned are those which are included in the T. C. A. cycle. As the comparatively lower values of Q_{O_2} on the substrates of sucrose and of maltose were so unexpected, the determination on the substrates of sucrose, maltose, inulin and mannit were continued for three hours and Q_{O_2} values were calculated at each one hour. The results are shown in table 88.

TABLE 88. Respiration intensity of the blast fungus on some kinds of disaccharide and of higher alcohol.

	Q_{O_2}		
	0-1 hr.	1-2 hrs.	2-3 hrs.
sucrose	2.00	3.07	4.32
maltose	0.22	0.96	2.11
inulin	1.38	1.50	1.80
mannit	1.25	1.20	1.18

As will be seen in the table, the Q_{O_2} becomes greater with the longer duration of time on each of the substrates of sucrose, maltose, and inulin, while on the substrate of mannit it becomes rather smaller. This may suggest that the sucrose, maltose and inulin are utilized after they were decomposed by saccharase, maltase, and inulinase respectively.

iv) Effect of KCN on the respiration: In order to learn whether the respiration is inhibited by the addition of KCN, the following experiments were planned. The used fungus was that which had been cultured in asparagine nitrogen culture solution for 14 days. The experiments were performed under the conditions of temperature 35°C , pH 6.0; glucose was employed as the substrate. The M/1000 KCN was added at 30 minutes after start of the determination. The results are shown in figure 32. As will be seen in the figure, the respiration is clearly inhibited by the addition of KCN, inhibition percentage being calculated as 59.04%. The higher concentration of KCN added makes the inhibition percentage the greater. A inhibition of 77.26% is seen as result from the addition of 5/1000 M KCN and a inhibition of 91.12% from addition of 1/100 M KCN. From these experiments it is evident that the respiration of the blast fungus is concerned with the cytochrome system.

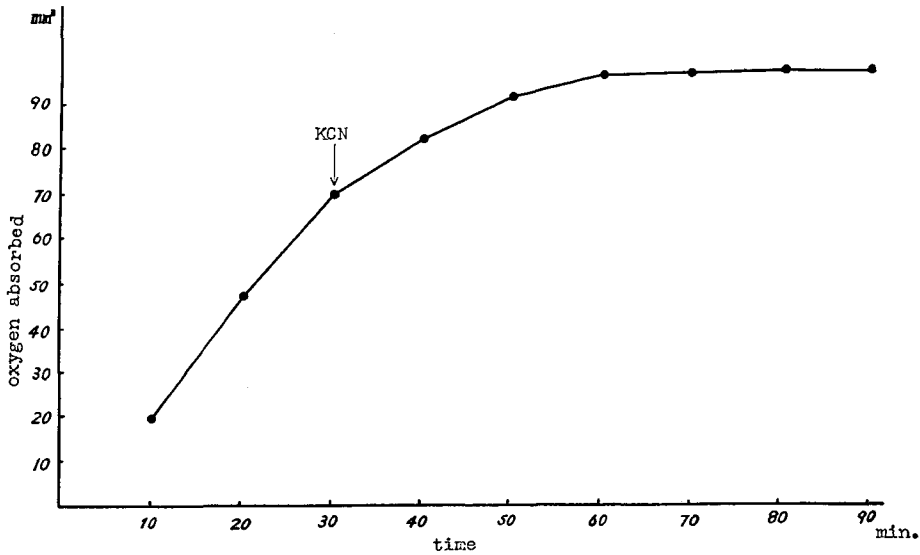


Fig. 32. Effect of KCN addition on the respiration of the blast fungus.

v) The fluctuation of the respiration intensity with the age of the culture: The fluctuation of the respiration intensity with age of the cultures was examined. The nitrogen sources of the cultures employed were each KNO_3 , asparagine and alanine. At each chosen culture age some cultures were filtrated and they were used for the determination of the respiration after one hour's starvation in distilled water as usual. Substrate was M/6 glucose, the temperature was 35°C and pH was 6.0. The results are shown in figure 33.

On the KNO_3 culture, Q_{O_2} increases gradually with the duration of the culture till 15th day and thereafter it falls gradually. Maximum Q_{O_2} on 15th day of culture is 7.70. On the asparagine culture, Q_{O_2} increases till 15th day of culture and then begins to fall. Maximum Q_{O_2} on 15th day is as great as 16.43. On the alanine culture, Q_{O_2} increases till 9th day and then begins to fall rather suddenly. The maximum Q_{O_2} on 9th day is 14.07. Through all the experiments the peak of the fluctuation appears in the rather latter phase of "the middle stage" of fungus growth.

vi) Nitrogen source and the respiration intensity: As shown in the previous section, there is a tendency for the maximum value of Q_{O_2} and the culture age when the maximum Q_{O_2} appears to be different

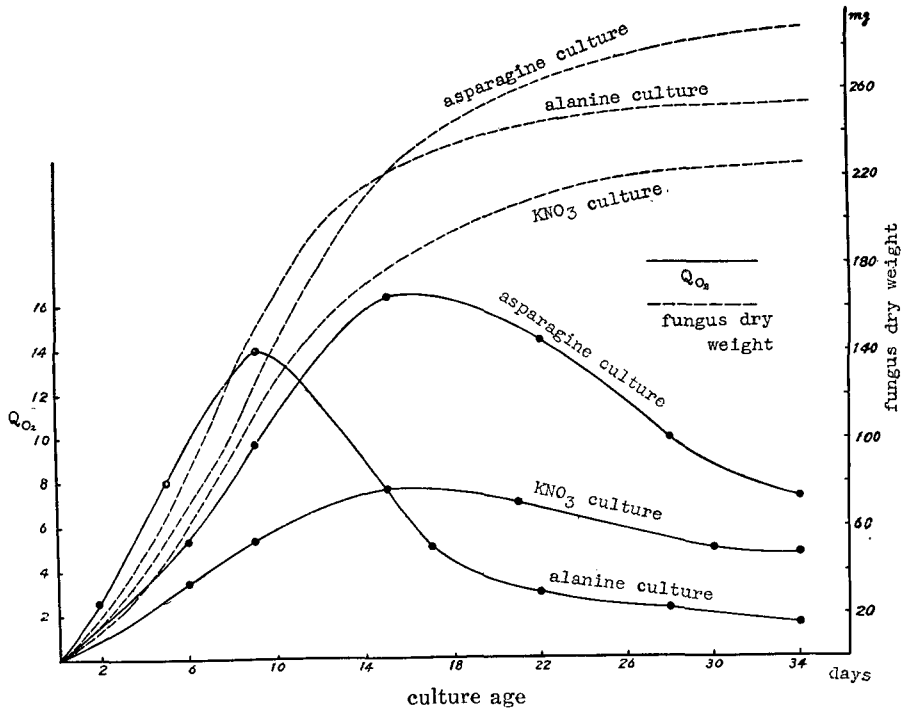


Fig. 33. Fluctuation of the respiration intensity with the progress of the culture age.

TABLE 89. Relation between the nitrogen sources of the culture and the respiration intensity.

nitrogen sources	Q_{O_2}	culture age when max. Q_{O_2} appears
asparagine	16.43	15th day
glycocoll	12.63	9th "
alanine	14.07	9th "
aspartic acid	16.91	9th "
glutamic acid	4.41	9th "
KNO_3	7.70	15th "
norleucine	6.85	13th "
arginine hydrochloride	4.64	26th "

in the cultures different in their nitrogen sources. In order to ascertain this point, experiments were planned employing cultures of 8 kinds of nitrogen source. In table 89 the maximum Q_{O_2} through the entire

duration of the culture and the culture age when the maximum Q_{O_2} values appear are shown. In the table the nitrogen sources are arranged in the order of superiority for the fungus growth from above downward. The comparatively lower value of Q_{O_2} in the glutamic acid culture is an unexpected result. But in general the respiration intensity is greater in the cultures of the excellent nitrogen sources such as asparagine, glycocoll, alanine and aspartic acid on comparison with the cultures of norleucine or arginine which are not so good nitrogen source for the fungus growth. In the case of the asparagine culture, the maximum value of Q_{O_2} appears on the 15th day of the culture which is six days later than the values in the cases of the other amino acid nitrogen sources. This is also one of the unexpected results. Although there are some discrepancies like these, it may be generally said that the greater respiration intensity is seen on the culture of the better nitrogen source and the maximum value of Q_{O_2} appears the earlier in culture age.

Summarizing the experimental results on the respiration of the blast fungus, the optimum temperature lies at 35°C which is a little higher than the optimum temperature for growth ($28^{\circ}\text{--}30^{\circ}\text{C}$), and the optimum pH lies at 6.0 which is as same as the optimum pH for the growth. Glucose is the most excellent substrate for the respiration while fructose and galactose among hexoses are not good substrates. Sucrose, maltose and inulin are utilized as substrates after the decomposition by saccharase, maltase and inulinase respectively, that may be produced by the fungus. Mannit, one of the higher alcohols, is a pretty good substrate for the respiration though a little inferior to the polysaccharides. Some organic acids, succinic acid, citric acid and oxalic acid which are all included in the T. C. A. cycle are also pretty good substrates for the respiration of the blast fungus while they are likewise pretty good carbon sources for the fungus growth. It is also evident that the respiration is inhibited by the addition of KCN suggesting the fact that the respiration is connected with the cytochrome system. The respiration intensity fluctuates with the culture age of the fungus. It increases with the duration of the culture and reaches to the maximum generally at the latter part of "the middle stage" of the fungus growth, which is a few days before the age when the fungus growth reaches to the maximum. Thereafter the respiration intensity begins to fall rather suddenly. The maximum Q_{O_2} and the culture age when the maximum appears are different according to differences in

the nitrogen source of the cultures. Generally speaking the greater respiration intensity in the earlier culture age is seen on the cultures of the better nitrogen sources.

Chapter VII

General conclusion.

While a great many investigations have been reported on the blast disease, the physiological studies on the blast disease fungus have been rather few. The reason seems to lie chiefly in the fact that the blast fungus has been difficult to culture on the pure synthesized media. Recently, however, it has come to be known by such investigators as F. W. LEAVER and the coworkers⁽⁵⁴⁾, TANAKA and KATSUKI^(112,113), and the present writer⁽⁷⁴⁾ that biotin and vitamin B₇ are indispensable for the blast fungus growth. And the fungus has come to be cultured fully on the pure synthesized culture solution containing the growth factors above mentioned. The blast fungus never develops on the complete lack of biotin, but only a trace of it improves the growth. Greater amounts of it favour the more the growth till the dose of 2.3-3.0m μ /ml; the excess biotin has no effect upon the fungus growth. The other growth factor vitamin B₇, on the other hand, is a complementary one to the biotin. As biotin and vitamin B₇ are almost always detectable in slight amounts in such organic compounds as sucrose even of the highest quality, the results of the experiment which were made without paying any attention to the growth factors might have been disturbed by the small amount of biotin which was contaminated in the used chemicals. Therefore the studies on the nitrogen source or the carbon source of the blast fungus need to be re-examined. The present studies were carried on by employing the culture solutions in which the adequate quantities of both growth factors were supplied. According to these studies some amino acids or amides as glycocoll, alanine, aspartic acid, glutamic acid, asparagine are the comparatively more excellent nitrogen sources for the fungus growth while the blast fungus can synthesize the inorganic nitrogen. On such nitrogen sources as the amino acids or amides named above, "the early stage" of the fungus growth is shortened and "the greatest activity period" of the fungus growth appears earlier. Moreover the fungus growth in "the middle stage" is remarkably great. In short, the fungus grows faster

and to a larger size on the nitrogen source of above-named amino acids or amides. Among the inorganic nitrogens, nitrate nitrogen appears to be superior to ammonium nitrogen as the nitrogen source of the blast fungus, but the responsibility lies in the falling of pH value in the ammonium nitrogen culture solution because of the unequivalent absorption of the ion. Whatever the nitrogen source may be, the nitrogen given in the culture solution is generally absorbed vigorously till "the middle stage" of the fungus growth. The absorption of the greatest amount of nitrogen in each two day period occurs in the early part of "the middle stage" just before the greatest increase of fungus dry weight appears. Fairly great absorption of the nitrogen is often seen also in the latter part of "the middle stage" especially on the cultures of rather excellent nitrogen sources. But such greater absorption is attributed to the greater amount of mycelium developed in the earlier part of "the middle stage". Thus it is evident that the absorption of nitrogen in the earlier growing stage is remarkably vigorous considering the comparatively smaller amount of the mycelium. On calculating the utilization ratios of the various nitrogen sources, by measurement of the increase of the fungus dry weight by 1 mg nitrogen absorption, one finds that the value is always higher on the better nitrogen sources, if it is calculated at the earlier part of "the middle stage". In order to compare the efficiency of the various nitrogen sources in furthering the fungus growth, it is quite reasonable to calculate the utilization ratios at the earlier part of "the middle stage" in which the fungus activity is most vigorous. Thus it is certain that the better nitrogen sources, such as above named amino acids or amides, are also utilized in higher efficiency by the fungus. When nitrate nitrogen is given in the culture solution of the blast fungus, it is first reduced to ammonium by the blast fungus as in the case of the other micro-organisms. This is shown by the fact that ammonium comes to be detectable in the culture solution in which the nitrate nitrogen is supplied as the only nitrogen source, but the detection of ammonia is often quite difficult in the culture of rather early growing stage. This is because the fungus is vigorously active at the earlier growing stage, and the resulted ammonia is absorbed immediately after the reduction. YOSHII (1936), TANAKA and KATSUKI (1951) reported that they could never find out any trace of nitrite or ammonia in the blast fungus culture solution in which nitrate nitrogen was supplied, but this discrepancy in the results may be attributed to the fact that

their experiments might have been made by employing rather young cultures. It was already stated that some kinds of amino acids or amides are good nitrogen sources for the blast fungus, and when such amino acids or amides are supplied as only nitrogen source in the culture solution of the blast fungus, ammonia comes to be found in that culture solution. This will suggest naturally the secretion of an amino acid oxidase by the fungus. And in fact its production by the fungus was ascertained in the present experiments. According to the experiments, the enzyme is active against the following amino acids; glycocoll, l-aspartic acid, l-glutamic acid, d-glutamic acid, dl-glutamic acid, l-alanine, l-leucine, dl-leucine, while it is inactive for the amino acids: arginine-hydrochloride, dl-valine, dl-methionine. The amino acids for which the oxidase is active are those which were proved to be good nitrogen sources, and it is certain that effectiveness of various amino acids as nitrogen source of this fungus is attributable to the fact that those amino acids are acted upon by the oxidase. The activity of the oxidase fluctuates with the age of the culture: generally it increases gradually with the growth of the fungus till "the middle stage" of the fungus growth and begins to decrease from the latter part of "the middle stage." As mentioned already, the greatest absorption of the nitrogen in each two days period occurs in the early part of "the middle stage", and the oxidase activity reaches also to the maximum at about the same age of the culture. Thus the greater decomposition of the amino acid at that stage stimulates the greater absorption of the nitrogen by the fungus, which encourages the greater increase of the fungus growth. While the activity of the amino acid oxidase is proved even in the culture solution in which only inorganic nitrogen is supplied as the nitrogen source, far greater activity is found in the culture solutions in which some kinds of amino acids as named above are given. It is certain that the activity is always greatest toward the same kind of amino acid which is given as the nitrogen source, suggesting their adaptive production to the kind of the given amino acid as the nitrogen source of the culture. In these experiments in which the living mycelium itself was employed as the enzyme source, the action of the amino acid oxidase was inhibited by the addition of KCN. This may suggest intermediation of the cytochrome system between the oxidase and the oxygen outside. Examining the relations between the amount of nitrogen given to the fungus and the fungus growth, the nitrogen concentration of 0.275 mg/ml in TOCHINAI-NAKANO'S

culture solution was never the optimum concentration for the fungus growth irrespectively of the kind of nitrogen given as source. It is clear that the fungus growth increases with the increase of the nitrogen amount given in the culture solution at least to the concentration of 3.312 mg/ml. On the other hand, the high level nitrogen supply stimulates a greater absorption of the nitrogen by the fungus especially at the earlier age of the culture, and shortens the duration of "the early stage" of the fungus growth. Thus the growth rate of the fungus is quickened and especially the growth in "the middle stage" becomes remarkable. It must be noticed here that those relations are more conspicuous on the better nitrogen sources of the fungus growth. On the other hand utilization ratio of the nitrogen is not always the greater in the case of greater nitrogen supply, because of the too much absorption of the nitrogen. The blast fungus has a proteolytic enzyme, which is active toward gelatine, casein and glycyglycine while inactive toward chloracetyl-L-tyrosine. Optimum temperature for the enzyme action lies at 40°C and the optimum pH at 7.0. The activity of the enzyme is promoted by the addition of cyan-compounds at a concentration above 1/200 M. Considering from these characters of the enzyme, one may safely conclude that the enzyme is one closely related to papain. The activity of the proteolytic enzyme fluctuates with the age of the culture; the peak appears generally in "the middle stage" of the fungus growth. It seems to be that the matter of fluctuation with the culture age is not affected greatly by the kind of nitrogen sources given in the culture solution nor by the amount of the nitrogen given. But the maximum value of the enzyme activity within the culture period is greatly affected by the kind of the nitrogen source. The employment of some amino acids or amides as nitrogen source makes the enzyme activity greater, while rather smaller activity results even in the case when inorganic nitrogen is given as only nitrogen source. Generally speaking greater activity of the proteolytic enzyme is seen in the cultures of the better nitrogen sources, although there are some exceptions as in the case of urea and glycine. Examining the carbon sources of the blast fungus, one finds that sucrose and maltose are the most excellent carbon sources for the blast fungus growth while lactose, among the disaccharides, is far inferior to them as carbon source. Glucose among hexoses is fairly good carbon source and fructose is also a pretty good one while galactose is inadequate. Inulin is a fairly good carbon source but soluble starch is not so good.

The fungus grows fairly with mannit as the carbon source while it does not grow with glycerine. Among organic acids, succinic acid is a pretty good carbon source and fungus growth as great as in the case of fructose carbon source is seen. Some fungus growth is seen also when citric acid is supplied as carbon source, but growth is not seen when such organic acids as acetic acid, oxalic acid and lactic acid are supplied. As well known, the carbon sources given in the culture are used as the substrate for respiration and also as the constitutional material of the mycelium. It is the excellent carbon sources which are used well both as the respiration substrate and as the constitutional material of the mycelium. There are no data to indicate the superiority of one carbon source over another as supplying material for the constitution of the mycelium. However on comparing the superiority of the carbohydrates as respiration materials, one finds glucose to be the most excellent one for the blast fungus while both fructose and galactose are rather inadequate substrates. Sucrose and maltose come to be utilized as the respiration substrate after they are decomposed by saccharase and maltase respectively. While maltose is the most excellent carbon source for the fungus growth as described above, its value as the respiration substrate is far inferior to glucose. Inulin and mannit are fairly good respiration substrates as well as good carbon sources for the fungus growth. As for the organic acids, succinic, citric and oxalic acids are proved all to be good substrates for encouraging the respiration of the blast fungus, suggesting that the T.C.A. cycle is included in the respiration mechanism of the blast fungus. Optimum temperature for the blast fungus respiration lies at 35°C which is a little higher than the optimum temperature for growth; optimum pH lies at 6.0 that is as same as the optimum pH for growth. Respiration is inhibited by the addition of cyan at concentrations above 1/1000 M and thus it is certain that the blast fungus respiration is concerned with the cytochrome system. The respiration intensity fluctuates with the age of the culture: it increases gradually with the duration of the culture till "the middle stage" and then begins to fall. It is certain that the respiration intensity becomes greatest at the culture age when the most active increase of the fungus dry weight per two-day period is occurring. The maximum value of respiration intensity during the culture and the culture age when that maximum intensity appears are affected by the nitrogen source given in the culture. Generally the better nitrogen sources such as some

amino acids or amides make the maximum value of respiration the greater and makes the culture age when it appears the earlier. In other words it is certain that greater growth is always accompanied by greater respiration.

DISCUSSION

As well known, blast susceptibility is not only different between the rice varieties, but also even in the same one variety it is caused to fluctuate by some differences of the environmental conditions under which the rice plants are grown. The present writer tried first to find some cases in which the rice variety "Eiko" varies in blast susceptibility. The rice variety "Eiko", used all through the present study, is originally medium in its susceptibility and develops ordinarily the chronic type lesions accompanied by a few of the acute type ones upon infection of the blast fungus. On observing the process of the lesion development one sees first appear a small brown spot, around which an area looking somewhat transparent or yellowish appears soon, to produce thus the acute type lesion. The most of the lesions do not remain ordinarily in such form for long, but soon the disintegrated zone and the necrotic zone come into appearance transforming the acute type lesion into the chronic type one. Such process of lesion development may suggest that the so-called "defence reaction" is neither very strong nor very quick in this rice variety. When the blast susceptibility of this variety is enhanced for some reason, not only do both the number and the size of the developed lesions become greater, but also the period of remaining in the acute type in the process of lesion development becomes longer, producing a greater number of acute type lesions. Such course is found in the case of examining the lesions at about 10 days after the inoculation. By examining the lesions developed by the inoculation experiments, the present writer ascertained the following five points: 1. when the rice seedlings are raised on the hot bed nursery, they become more susceptible to the blast disease in comparison with those which were raised on the ordinary nursery bed. 2. The rice plants are most resistant to the blast disease at "the elongation stage" and most susceptible to the blast disease at both the "ear formation stage" and the "boot stage" through all their growing stages. 3. The high level nitrogen supply lengthen the highly susceptible growing stage of the rice plant and the characteristic of

the "elongation stage" to be most resistant against the blast infection is completely lost. 4. Although it is quite difficult to find out the critical level of nitrogen at which the blast susceptibility just begins to rise, two times as much nitrogen amount as the standard often remarkably raises the blast susceptibility. 5. When the excessive nitrogen is supplied additionally just before the "ear formation stage", the blast susceptibility of the rice plant is enhanced clearly at 7 days after the additional supply. The reason why the same rice variety varies in respect to blast susceptibility as mentioned above is to be sought from different two sides. The one is research from the side of the host (rice plant) and the other is research from the side of the parasite (blast fungus). And finally it is desirable to discuss the problem by synthesizing the results of the researches from both sides. Researches from the side of the host plant (rice plant) may be also divided into two parts. The one is those studies on the rice plant characters which are concerned with the susceptibility in the process from the spore germination till the completion of the penetration of the fungus through the cuticle; the other part includes studies on the rice plant characters which are concerned with the proliferation of the fungus in the rice plant. Studies on the morphological characters of the rice epidermis, through which the blast fungus penetrates into the plant, are one of the most important items in the former classification. Studies concerning the chemical components of the rice plant, which may favor or inhibit the fungus growth in the plant, are important subjects in the latter. In line of the former studies H. SUZUKI (1933~1940) studied the relations between the blast susceptibility of the rice plant and degree of silification and also the thickness of the outer wall of those rice leaves. He emphasizes the parallelisms between the thinner outer wall of the epidermal cell or the inferior silification of the wall and the greater blast susceptibility. According to the present investigations the leaves of the hot bed nursery seedlings which were proved to be more blast susceptible are thinner in their epidermal cell walls and are inferior in the silification of epidermal wall, in comparison with those of ordinary nursery seedlings. Especially such relations are remarkable in the motor cells (bulliform cells) through which the penetration of fungus occurs most frequently. It is also known that the rice plants supplied with high level nitrogen are inferior in silification of at least the motor cells in their leaf epidermal cells while they become more susceptible to the blast disease by this supply of nitrogen. On the

other hand it is rather difficult to find out any such correlations between the fluctuation of the blast susceptibility with the advance of their growing stages and those morphological characters of the epidermal wall. Generally there are tendencies for the silification and the outer wall thickness of the epidermal cell to increase with the advance of growth. The susceptibility is smallest at the "elongation stage" and greatest at the next "ear formation stage" as mentioned already. At any rate it is certain that such morphological characters of the epidermal cell, as outer wall thickness and silification, are concerned with the blast susceptibility by determining the difficulties or facilities of epidermal penetration of the causal fungus, but it is impossible to attribute all the responsibility to such morphological characters. Thus the relations between the susceptibility and the amount of various chemical components in the rice plant come to be discussed. According to the present research, the contents of the inorganic substances are smaller in the hot bed nursery seedlings than in the ordinary nursery seedlings and also the leaves of the rice plant contain smaller amount of inorganic substance both at the "ear formation stage" and at the "boot stage" comparing with those at the other growing stages. Thus the higher blast susceptibility is concerned with the lower content of inorganic substances in the rice plant. On examining the composition of the inorganic substance, one finds that the content of every kind of inorganic element, such as P, K, Ca etc., becomes smaller on the more blast susceptible rice plant. As almost half of the total inorganic substance is supplied by silica, the differences in the content of silica are most clear between the rice plants different in their blast susceptibility. H. SUZUKI (1943) reported that the rice plants of high blast resistance contain the greater amount of potassium in their leaves. Also finding out the facts that the presence of potassium ion is unfavourable for the formation of appressoria of the blast fungus and that the potassium ion is apt to come out through epidermis when the potassium content in the rice leaves is comparatively greater, SUZUKI tried to explain the high blast resistance by the failure of appressoria formation which is due to the presence of potassium ion. The fact that rather numerous lesions are developed on the rice plant low in its potassium content, as shown in the present investigation, may be partly explained from such a view point. The relation between the silica content in the rice plant and the blast susceptibility has been discussed by many investigators and the silica has been thought to play an important role in

enabling the rice plant mechanically to resist the fungus penetration. As shown in the present investigation a lower silica content in the rice plant is always very closely correlated with greater blast susceptibility, even when no relations between the silification of the epidermal cells and the blast susceptibility can be seen from the morphological observations. This may suggest some physiological role of silica in addition to the mechanical role for preventing the blast fungus invasion. In fact W. ENGEL (1953) wrote recently that most silica contained in the plant exist in connection with galactose and is supposed to have some physiological function. At any rate this is a problem to be solved in future.

The relations between sugar content in the rice plant and blast susceptibility have been often discussed. According to the present studies, however, it is rather difficult to find out direct correlations between them. Even if there seems to be some correlation between them, the blast susceptibility will be explained somewhat rightly by the fluctuation of the nitrogen amount which is closely connected with the sugar content in the rice plant. The relations between blast susceptibility and nitrogen content in the rice plant have also been studied by many investigators (SAWADA 1936, '39; TAHARA 1937; SEMPLO 1950; KRISHNASWAMI 1952; TANAKA and KATSUKI, 1952). Among them TAHARA states that the blast susceptibility of the rice plant is favoured by such unbalance of nitrogen composition in the rice plant as the extraordinary increase in the amount of non-protein nitrogen, which is often accompanied by the remarkable increase of the total amount of nitrogen in the plant. TANAKA and KATSUKI notice the greater content of amino acid in those rice plants which are highly blast susceptible. According to the present studies, those rice seedlings which are raised with high blast susceptibility on the hot bed nursery contain the greater amount of total nitrogen comparing with those rice seedlings which are raised on the ordinary nursery. Dividing this total nitrogen into the protein nitrogen and the soluble nitrogen, the percentage of the latter in the total nitrogen is comparatively greater and the percentage of the former is comparatively lower in the hot bed nursery seedlings than in the ordinary nursery seedlings. Accordingly the hot bed nursery seedlings contain the greater amount of soluble nitrogen. It is also known that the content of soluble nitrogen in the leaves is comparatively greater while the content of protein nitrogen is comparatively lower both at the "ear formation stage" and at the "boot stage" comparing

with the contents respectively in the cases of the other growing stages. Moreover the rice plants of which blast susceptibility has been intensified by the high level nitrogen supply come always to contain much amount of soluble nitrogen in their leaves. Thus through all the present experiments it is clearly shown that the rice leaves become highly blast susceptible when the content of soluble nitrogen in leaves becomes greater accompanied by the comparatively smaller content of protein nitrogen. On examining the composition of the soluble nitrogen in the leaves, one learns that the amounts of every kind of the soluble nitrogen, such as soluble protein nitrogen, α -amino nitrogen, amide nitrogen and so on, become greater with increase of the amount of the total soluble nitrogen. However, calculating the correlation coefficient on 59 samples between the size of the lesions developed and the amount of the various kinds of soluble nitrogen, one finds that a positive correlation between the lesion sizes and amount of amino acids in the rice plant is most certain. Thus it will be concluded that the higher blast susceptibility of the rice plant is closely connected with the increase of the soluble nitrogen such as α -amino acid in the plant.

Now it is quite important as well as interesting to seek the reason why the accumulation of the soluble nitrogen such as amino acid favours the blast susceptibility of the rice plant. Recently the so-called "defence reaction" which appears in the cell of the host plant on the infection of the causal fungus has attracted attention, and the problem of the disease resistance of the plant is often discussed from the angle of this reaction. It is natural that the degree of the severity of invasion by the fungus is determined by the interaction between the aggressive force of the parasite and the defence reaction of the host plant. Therefore the present problem mentioned above may be discussed from the two standpoints. Considering from the standpoint which places much importance upon the defence reaction, the accumulation of the soluble nitrogen is supposed to weaken or to retard the development of the defence reaction of the rice plant. While from the other standpoint which puts much importance to the aggressive force of the blast fungus, the accumulation of the soluble nitrogen in the rice plant is supposed to activate the aggressive force of the causal fungus which has already penetrated the epidermis of the rice plant. As described already, however, the rice variety "Eiko" is originally medium in its blast susceptibility and it must be noticed that the lesions develop through first the form of the acute type into the

chronic type lesion even on those rice plants which are grown under the ordinary conditions. This means that the defence reaction of the rice variety "Eiko" is originally weak. In order to solve the present problem, therefore, investigations from the standpoint which puts importance on the aggressive force of the causal fungus seems to be rather important. Then for this purpose the physiological characters of the blast fungus must be examined in detail by cultural studies on this fungus. It will be noticed here that cultural study of the blast fungus has been made easier by the recent discovery of its growth factors. Examination of the growth of the blast fungus leads to the distinguishment generally of three growing stages: they are the "early stage" (lag phase) in which the increase of the fungus weight is rather small, the "middle stage" in which the fungus is most vigorous and increases in weight greatly, and the "latter stage" in which the fungus activity declines. In the earlier part of the middle stage the fungus activity becomes greatest and the largest increase of the fungus dry weight per two day periods is seen at that time. On the other hand, the most part of the supplied nitrogen is generally absorbed from the "early stage" to the "middle stage" of the fungus growth. The greatest absorption of nitrogen in each two-day period is seen just before the greatest increase of the fungus dry weight per two-day period appears in the earlier part of the "middle stage". According to the present study on the nitrogen sources of this fungus, the blast fungus can utilize inorganic nitrate or ammonium nitrogen as its nitrogen source; the nitrate nitrogen is reduced to ammonium nitrogen as usual by the fungus previous to utilization by synthesis. But, on the other hand, some amino acids, such as glycocoll, alanine, aspartic acid, glutamic acid, asparagine are certainly superior to those inorganic nitrogens as nitrogen source for this fungus. The amino acids applied to the blast fungus are first decomposed by the action of the amino acid oxidase, of which production is proved by the present study. Further, this enzyme is active only on such amino acids as glycocoll, alanine, aspartic acid, glutamic acid and asparagine. This is the reason why only these amino acids provide a good nitrogen source for the fungus growth. When one of those amino acids is supplied as nitrogen source, the duration of the "early stage" (lag phase) in the blast fungus growth is shortened. This is explained by the fact that the activity of the amino acid oxidase increases with the supply of those amino acids and the greater amounts of nitrogen come to be absorbed earlier.

Moreover the fungus activity is increased by the supply of those amino acids as clearly shown by the fact of the greater respiration intensity of those fungi, which are supplied with those amino acids as their nitrogen source. The greater activity is naturally accompanied by the greater increase of the fungus dry weight. Thus it is certain that upon the application of some kinds of amino acid as mentioned above, the fungus activity as well as the activity of the amino acid oxidase is enhanced earlier in the fungus growth and the greater amount of nitrogen comes to be absorbed in the earlier growing stage, resulting in the improvement of the rate as well as the amount of growth. It is known also that the nitrogen concentration of 0.275 mg/ml in the standard culture solution is a somewhat limiting factor for the fungus growth and the greater amount application of nitrogen up to the concentration of 3.312 mg/ml makes greater the growth rate and the total growth as long as the amount of carbon source is sufficient. This relation is most manifest in the case of amino acid nitrogen source; it must be added also that the greater amount application of amino acid makes greater the activity of the amino acid oxidase produced by the fungus in the earlier stage of the fungus growth. It will be noticed here that the blast fungus produces a proteinase which is closely related to papain. The activity of this enzyme also becomes greater when the fungus growth is improved by the supply of a better nitrogen source. According to the studies on the carbon source of the blast fungus, sucrose and maltose are most excellent carbon sources for the fungus growth while glucose, inulin and mannit are also utilized well by the fungus. Some growth of the fungus is seen also on the carbon source of such organic acids as succinic and citric acid, which are included in the T. C. A. cycle. It is shown in the experiments that these carbon sources can be used as substrates for the respiration of the fungus. On the other hand, NAKAMURA and SHIMOMURA (1953) reported that the blast fungus grows well on the sugar-free but amino-acid-containing culture solution, and suggested that the organic acids which are produced by the decomposition of some amino acids are also utilized as the respiration material and as the constituent material for the fungus growth. Thus the superiority of some amino acids for the growth of the blast fungus may be explained partly at least by those considerations.

In the following the results of the above mentioned culture studies will be discussed in connection with the problem of the blast suscepti-

bility of the rice plant. As mentioned above, sucrose and glucose are excellent carbon sources for the blast fungus growth. The content of those sugars in the rice plants fluctuated actually with the differences of the environmental conditions under which the plants grow. But it may be safely concluded that the sugar content in the rice plant is always sufficient for the vigorous growth of the invader as the sugars are being synthesized continuously in the day time by the carbon assimilation. Thus it is naturally difficult to find out any direct relations between the sugar content in the rice plant and the blast susceptibility. On the other hand state of affairs are different concerning the relations between the nitrogen content and the blast susceptibility. The nitrogen contents in the rice plants are caused to fluctuate rather largely by the differences of the environmental conditions. Classifying the nitrogen into the each kind of nitrogen, far more remarkable fluctuations are seen in the soluble nitrogens such as amino acid nitrogen. At the same time, as mentioned above, some amino acids as glycocoll, alanine, aspartic acid, glutamic acid, asparagine are especially excellent nitrogen sources for the fungus growth. Increased application of those amino acids intensifies the fungus activity, including the activity of the amino acid oxidase as well as the fungus respiration, and results in the faster and greater growth of the fungus. Such more vigorous growth of the fungus will naturally suggest the greater aggressiveness of the fungus against the rice plant, if the fungus has penetrated into the plant. This is supported also by the fact that the activity of the protease, that is certainly one of the most important factors of the aggressive force of the fungus, becomes more vigorous with the more vigorous growth of the fungus. It must be noticed here that those amino acids as cited above are ones which are found ordinarily in the rice plants. Therefore the accumulation of those amino acids in the rice plant will naturally make greater the growth of those blast fungus hyphae which come into the rice plant and give rise to the vigorous invasion of the plant by this fungus producing lesions of greater size.

It is true in the present discussion that no consideration is paid to the defence reactions which may occur in the cells penetrated by the blast fungus. But it must be noticed again that the rice variety "Eiko" which is used all through the present experiments, does not react originally very sensitively to the blast invasion as will be understood from the fact that the diseased lesion developed ordinarily through

the process of acute type into the chronic type. Therefore if the activity of the fungus which comes into the cell of the rice plant is vigorous and the growth in the plant cell is rapid, the defence reaction of the invaded cell would be unable to keep place with the fungus growth. This is supported by the fact that the diseased lesions continue to enlarge remaining in acute type for rather long, when the rice plants become highly blast susceptible resultant from the accumulation of such soluble nitrogen as amino acid. Thus it is reasonable enough to believe that the activity of the fungus, once it has penetrated into the rice plant, is influenced by the quantity of such chemical components as amino acid in the plant; likewise it is reasonable that the condition of the disease is determined by this activity of the fungus as long as the problems are concerned with those rice plants which do not react so sensitively or rapidly to the fungus invasion. It must be confessed here that the differences in blast susceptibility between the various rice varieties (varietal susceptibility) are not always explained by the differences of the quantity of soluble nitrogen such as amino acid in the rice plant according to the other experiments of the present writer. This is because the development of the disease is determined rather by the rapid defence reaction in the case of the higher blast resistant rice variety.

Recently TAKAHASHI (1956) compared under microscope the growth of the penetrated blast fungus, both in living cells and in cells killed by heating, by means of sheath inoculation experiments. He reported that he could clearly recognize the differences of the fungus growth in the living cells of the rice plants different in their susceptibility because of the cultural conditions. In the case of killed cells, however, TAKAHASHI found that the fungus growth was rather greater in every kind of rice plants and he could not find any differences in the fungus growth in the rice plants clearly different in their susceptibility to the blast fungus. From these observations TAKAHASHI came to the conclusion that the blast susceptibility of the rice plant is not so much due to the differences of the quantities of their chemical components as to the degree of the defence reaction of the rice plant. This consideration seems to be based on the opinion that there are always sufficient amounts of nourishing elements for the fungus growth in the cells of the rice plant. The present writer has tried to gather some data, employing those rice plants as material of which the blast susceptibility was varied by the supply of nitrogen

at different levels. In experiment 1 the rice plants were grown in water culture solutions: in experiment 2 they were grown on the soil in the pot. When the rice plant had grown up to the "boot stage", sheath of the second leaf from above on the chief stem was cut off and used for the inoculation. All the procedures followed TAKAHASHI, but the cells of the sheath were killed by freezing instead of heating. Those cells were proved previously to be killed by standing them in -25°C room for 5 hours. The degree of fungus growth in the sheath cells was determined at 24-30 hours after the inoculation according to TAKAHASHI'S method. The results are given in table 90. All the figures in the table show the average of the observations on 80-100 appressoria in each 5 sheaths.

TABLE 90. Comparison between the growth of the blast fungus mycelium in living cells and growth in killed cells of the rice plant.

	amount of nitrogen supplied to rice plants	living sheath	sheath killed by freezing
exp. 1	standard amount of nitrogen	2.5	3.1
	triple amount of nitrogen	3.4	3.9
	quintuple amount of nitrogen	4.2	5.7
exp. 2	standard amount of nitrogen	1.4	1.6
	double amount of nitrogen	2.6	2.9
	triple amount of nitrogen	3.1	3.8

As already shown, the rice plants supplied with high level nitrogen are the more susceptible to the blast disease and also, in the present experiments, the degree of fungus growth in the living cells of the rice sheath is always greater on the rice plant supplied with the more nitrogen. The relations are quite the same also in the killed cells and the degree of the fungus growth is the greater in the rice plants supplied with the more nitrogen, although the values of the measurements are a little higher in the killed cells on comparison with those in the living cells. These are somewhat different results from TAKAHASHI'S. According to him, in the case of the cells killed by heating, the germinating hyphae often grow long over the cells of the sheath

previous to the formation of the appressoria and moreover the germinating hyphae come into the cells frequently without formation of normal appressoria, or even if the penetrations were carried on normally the state of the growth of the fungus in the penetrated cells is quite often irregular. On the other hand, it is noticed often truly, that the germinating hyphae grow a little longer over the cells killed by freezing previous to the formation of the appressoria on comparison with the case of the infection on the living cells, but any other irregular growth of the germinating hyphae is not seen in the case of the freeze-killed cells. Although it is quite difficult to explain conclusively the reason why there appeared some differences between the present results and those of TAKAHASHI, it may be said safely at least that heating is not the most suitable way of killing in order to observe the state of the growth of the fungus in the killed cells. Moreover taking into consideration the fact that the contents of those soluble nitrogens in the rice plant such as amino acids, which are quite excellent nitrogen sources for fungus growth, are not always so great as to have no connection with the growth of the fungus penetrated into them, the present writer can not agree with TAKAHASHI's conclusion that the amount of some chemical components such as soluble nitrogen in the rice plant is quite seldom responsible if at all, for the determination of the rice susceptibility to blast infection.

Summarizing all the experiments and considerations above described, it is concluded safely as follows. So long as the problems are concerned with those rice varieties which are cultivated rather widely in the field but are originally not so highly blast resistant, the cause of the fluctuations in blast susceptibility on account of some environmental conditions is quite often attributable to the accumulation of such soluble nitrogen as amino acids, while some parts of the responsibility are certainly attributed to the variations in those morphological characters of their epidermal cells which are concerned with difficulties of fungus penetration. The accumulation of the soluble nitrogen as amino acids makes active the growth of the penetrated fungus and makes greater its aggressive force against the rice plant, strengthening its respiration and the activity of such enzymes as amino acid oxidase and protease. On the other hand the defence reaction of the invaded cells of a plant of an originally not so highly resistant variety, can hardly come up with such vigorous growth and the greater aggressive force of the penetrating fungus, so greater invasions by the fungus

result finally in such rice plants producing numerous diseased lesions of greater size and of the acute type.

SUMMARY

1. The fluctuations of blast susceptibility which are induced by differences of the cultural environment of the rice plant or by differences of the growing stages are determined by the inoculation experiments on the one rice variety "Eiko" which is rather widely cultivated in Hokkaido and is thought originally to be medium in its blast susceptibility. At the same time some morphological characters of the epidermal cells which are thought to be concerned with the difficulties of fungus penetration and also the quantities of some principal chemical components in those rice plants were determined, the final purpose being to inspect the cause of blast susceptibility or resistance of the rice plant.

2. The diseased lesions which develop on the leaves of rice variety "Eiko" grown up under normal environmental conditions are commonly chronic in type. In that rice variety, however, the chronic-type lesion develops always through the process of the brown spot and the acute type lesion. It is ordinarily for a quite short period that the lesion remains in the acute type, but in those cases when the rice plants become the more blast susceptible, the lesion continues to enlarge remaining in the acute type for rather long.

3. Through all the inoculation experiments, the degree of blast susceptibility or resistance of the rice plant is determined by examining the numbers of lesions developed, their sizes and their types at about 10 days after inoculation.

4. Seedlings raised on the hot bed nursery are more blast susceptible than those raised on the ordinary nursery bed. But this character of the former seedlings never remains long after transplantation to the field.

5. Through all the growing stages the rice plant is most resistant against attack by the blast fungus at the "elongation stage" and is most susceptible at the "ear formation stage" or the next following "boot stage". But in the case of the high level nitrogen supply the "elongation stage" loses its characteristic of high resistance against the blast fungus.

6. It is certainly beyond question that the high level nitrogen

supply enhances the susceptibility of the rice plant but it is rather difficult to find out the critical amount of nitrogen at which the blast susceptibility begins to be enhanced.

7. The additional supply of excess nitrogen at about the "ear formation stage" begins to exert effects on the susceptibility of the rice plant at two days after application; the effects become greater with the lapse of days till eight days after application.

8. The replacement of the nitrogen source applied to the rice plant whether natrium nitrate, ammonium nitrate, ammonium sulfate or urea does not result in very important effect upon the blast susceptibility of those rice plant with the one exception of urea. The rice plant grown on the nitrogen source of urea seems to be a little more susceptible to the blast disease on comparison with plants grown on the other nitrogen sources.

9. Examining the thickness of the epidermal cell walls and the number of silicated cells in the epidermis of the leaves on those rice plants which were proved by the above mentioned experiments to be different in their blast susceptibility, one learns as to the motor cells, at least in general, that the outer wall of the epidermal cell is thinner and the number of silicated cells are less on the more susceptible rice plants. It is certain, therefore, that those morphological characters of the epidermal cells are concerned to some extent with the defence against the blast fungus penetration through the epidermal cells.

10. Comparison of the amount of some principal chemical components in the leaves of those rice plants which were proven to be different in their blast susceptibility, proves that those plants of higher blast susceptibility are the higher in their moisture content and are the lower in the content of their inorganic substances but that there are not so important difference in their total content of organic substances. The contents of all kinds of inorganic elements such as phosphorus, potassium, calcium etc. are the less in the rice plant of higher blast susceptibility; the differences in the silica contents are specially striking. The contents of those inorganic elements may be concerned mostly with the blast susceptibility through their function of strengthening the tissues of the rice plant.

11. The sugar contents in the leaves of the rice plant is quite variable. It is rather difficult in the present experiments to find out any direct relations between the sugar content and blast susceptibility.

12. While there is a tendency for the total nitrogen content to

be a little higher in the rice plants of higher blast susceptibility, it is certain that in those rice plants the percentage of protein nitrogen in the total nitrogen is less and that of soluble nitrogen is higher on comparison with the cases of the rice plants of low blast susceptibility. It is quite remarkable in these experiments that the higher content of soluble nitrogen in the rice plant always goes parallel with the higher blast susceptibility, and also that the content of α -amino acid itself or the content of some organic soluble nitrogens, which are easily changeable to amino acid, is concerned most closely with the blast susceptibility.

13. Calculating the correlation coefficient between the amounts of various kinds of nitrogen in the rice plants and the sizes of the lesions developed by the inoculation experiments on the 59 examples above described, the correlation between the size of lesions and the amount of the soluble nitrogen is perfect showing the correlation coefficient of 0.37. Among the soluble nitrogens, the amount of the amino acids is most closely correlated with the size of the lesions showing a correlation coefficient of 0.46.

14. The kinds of amino acids which are found commonly in the rice plant are the following nine: glutamic acid, aspartic acid, glutamine, asparagine, glycine, alanine, leucine, arginine and histidine. Greater amount of the amino acids in the rice plant is not always accompanied by a greater number of kinds of amino acids.

15. By culturing the blast fungus on synthesized media some physiological characters of the fungus are examined.

16. For the growth of the blast fungus biotin is always indispensable and vitamin B₁ is also a supplementary factor for the biotin. The optimum concentration of biotin for the blast fungus growth lies in the range of 2.3 m γ -3.0 m γ /ml.

17. While the blast fungus can assimilate the inorganic nitrogen such as ammonium salt or nitrate salt, the application of either one of the following amino acids: glycocoll, alanine, aspartic acid, glutamic acid, or asparagine, as a nitrogen source improves the fungus growth greatly.

18. Regarding the process of fungus growth, the following three growing stages are distinguishable: the early stage (lag phase) in which the fungus growth rate is rather slow, the middle stage in which the fungus growth is most vigorous and the latter stage in which the growth is weakened. In the earlier part of the middle stage the fungus

activity becomes greatest; the greatest increase of the fungus dry weight in each two day period is seen at that time.

19. When the above mentioned amino acids are supplied respectively as the nitrogen source, the period of the lag phase is shortened and the period of the greatest activity comes to appear earlier; also the increase of fungus dry weight during the middle stage is quite remarkable.

20. On observing the state of nitrogen absorption by the fungus, one sees vigorous absorption from the earlier stage till the middle stage; the greatest absorption of nitrogen per two-day period appears just before the greatest increase per two-day period of the fungus dry weight occurs.

21. Calculating the ratio of the nitrogen utilization at the end of the culture on the various kinds of nitrogen source, one finds it rather difficult to find out differences in the ratio between the cultures of the different nitrogen sources. But when the ratios are calculated at the time when the fungus is in the greatest activity showing the greatest increase of its dry weight per two-day period, it is clearly known that the amino acids above named are utilized rather efficiently as the nitrogen sources on comparison with the other nitrogen sources.

22. The blast fungus has the ability to reduce nitrate nitrogen; the nitrate nitrogen supplied in the culture solution is reduced to ammonium nitrogen previous to its assimilation.

23. The blast fungus produces amino acid oxidase and the amino acids applied in the culture solution are decomposed by the action of this oxidase previous to assimilation by the fungus.

24. The amino acid oxidase produced by the blast fungus is effective on the following amino acids: glycocoll, l-alanine, l-leucine and dl-leucine. The specificity of the amino acid as the nitrogen source of the blast fungus may be explained by the specificity of the activity of the oxidase upon those amino acids.

25. In the living cells of the blast fungus, cytochrome system may mediate the transportation of the oxygen to the oxidase.

26. The activity of the amino acid oxidase is seen apparently even when inorganic nitrogen is given as only nitrogen source, but the activity becomes quite vigorous when some amino acids as mentioned above are supplied as nitrogen source. Moreover the activity of the oxidase is most vigorous toward the same kind of amino acids as supplied in the culture solution as nitrogen source of the fungus; it

is certain that the oxidase is produced adaptively to the amino acids given.

27. The oxidase activity increases remarkably at the time when the nitrogen absorption by the fungus is most active.

28. On observing the relation between the fungus growth and the amount of nitrogen given to the fungus, one finds that the nitrogen concentration of 0.275 mg/ml in the original culture solution is rather a limiting factor for the fungus growth and that it is improved by the greater amount of nitrogen supply up to the nitrogen concentration of 3.312 mg/ml at least.

29. The greater amount nitrogen supply allows the blast fungus to absorb the greater amount of nitrogen in the earlier stage. The duration of the early stage of the fungus growth is shortened and moreover the fungus growth in the middle stage becomes quite large.

30. On the application of the greater amount of nitrogen the activity of the amino acid oxidase increases greatly at the earlier part of the fungus growth.

31. On the other hand the utilization coefficient of the nitrogen becomes rather low as a result of the greater amount of nitrogen supply.

32. The blast fungus has a protease which may related to papain. It is secreted in the culture solution.

33. The activity of the protease fluctuates with the advances of the fungus growth. Its maximum appears ordinarily at about 10 days after the beginning of the culture.

34. It is certain that the activity of the protease is seen even when the inorganic nitrogen is given as the only nitrogen source, but the supply of the amino acids or amide as the nitrogen source greatly improves its activity.

35. For the growth of the blast fungus sucrose and maltose are the most excellent carbon sources. Besides them glucose, inulin and mannit are also fairly good carbon sources for the fungus growth. It is known that the blast fungus can utilize some organic acids such as succinic acid and citric acid as the carbon source of their growth.

36. The respiration of the blast fungus is rather vigorous on comparison with that of an ordinary saprophytic fungus such as *aspergillus* or *penicillium* while it is far behind that of bacteria.

37. The optimum temperature for the respiration of the blast fungus lies at 35°C and the optimum pH lies at 6.0. It is found that

the respiration is inhibited by the addition of KCN of the concentration beyond 1/1000 M.

38. Those carbohydrates which are proved to be excellent as the carbon source for the fungus growth are generally utilized favourably as the respiration material of the fungus.

39. The respiration intensity of the fungus fluctuates with the progress of the growing stages of the fungus; its maximum appears ordinarily in the early part of the middle stage of the fungus growth.

40. On comparing the fluctuation of respiration intensity of those fungus samples which are different in their growth and their growth rate because of the difference of the kind of nitrogen source applied, their respiration intensity shows increase earlier in the growing stage when the growth rate becomes the greater as a result of the supply of some superior nitrogen source.

41. The proliferation of the fungus in the host plant is determined by the correlative relations between the aggressive force of the fungus which has penetrated into the host plant and the defence reactions which appear in the host cells invaded by the fungus. It must be noted that the rice variety "Eiko" which has been employed through all the present experiments is not a rice variety which reacts sensitively to the fungus invasion and develops a defence reaction immediately after the penetration of the fungus. It is often supposed in "Eiko" rice variety that the defence reaction can hardly come up with the fungus growth in the host cells, considering from the fact that the acute type lesions continue to enlarge for rather long when the blast susceptibility of the rice plant is enhanced by some environmental conditions.

42. Summarizing the above described experimental results, it may be concluded that as long as the problems are concerned with those rice varieties which are originally medium in their blast susceptibility, such as variety "Eiko" employed through all the present experiments, the enhancement of their blast susceptibility caused by some environmental conditions or their growing stages is quite often concerned with the accumulation of the soluble nitrogen such as amino acids in the rice plant. That form of nitrogen favours the growth of the fungus in the plant as well as increases the activity of the enzymes produced by the fungus such as amino acid oxidase or protease and also promotes the respiration intensity of the fungus in the plant. Such high activities of the fungus in the rice plant which may mean the strong

aggressive force against the host plant go far beyond the defence reaction which may appear in the cells penetrated by the fungus and result at last in the even greater invasion by the blast fungus.

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