STUDIES ON THE NITROGEN METABOLISM OF RICE (RÉSUMÉ OF THESIS FOR CAND. SC. BIOL.)

by

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A study of the mineral nutrition of higher plants is a problem of paramount importance both from theoretical and practical point of view since it has a bearing on all the major phases of plant physiology (photosynthesis, water regime, growth and development, differentiation of tissues etc.) and thereby greatly determines the yield. Studies on the mineral nutrition of rice plants are warranted, among others, by the wide occurrence and sometimes, however, little attention was devoted to a deeper understanding of the rice fields during the last years. According to the prevailing opinion the development of brusone is also affected by the mineral nutrition and especially by the nitrogen metabolism of the rice plant.

The problems of irrigation, particularly those connected with the determination of optimal water supply, are waiting for further elucidation. Observations made by practical rice growers on the effect of the thickness of the water layer, on that of the atmospheric humidity, soil structure, soil composition and soil cultivation (11, 12, 13) might and must be explained by studies on the mineral nutrition. The need for physiological studies is further accentuated by recent trends in the elaboration of a logical system of soil fertilization.

The irrigation procedures used nowadays are mostly empirical in nature and underwent little changes during the last decades (3). The »modern« rice growing is based essentially on the experience of ancient rice growers. The practice of rice cultivation has been improved in many respects, mostly as far as the use of machines and the whole set up of irrigation is concerned, however, little attention was devoted to a deeper understanding of the rice plant.

A number of important problems concerning the mineral nutrition of rice remained obscure, since in most experiments the attention was focused exclusively on the yield and a study of the reaction of the rice plant to various treatments was not undertaken. It is impossible to set up a reasonable system of soil fertilization without a deeper knowledge of the nutritional requirements of the rice necessitate a detailed investigation of a number of physiological

and biochemical processes. It is necessary to investigate the trend and direction of various metabolic processes at different developmental stages and to relate these processes with the basic problems of irrigation and soil fertilization.

In the present study the uptake of nitrogen and phosphorus by rice plants was investigated in experiments of short duration (4—12 hrs). Further studies were undertaken on the transformation of inorganic N into organic form, on transamination problems and on the translocation of the transamination products from the roots. This led to an investigation of various aspects of metabolism in the root system of rice seedlings such us the problem of primary amino acid synthesis and the comparison of some basic metabolic processes of rice with those occurring in other cultivated plants. We also studied the nature of organic N compounds transported from the root system to the shoot. Furthermore the effect of the level of irrigation water on the uptake and mobilization of substances was investigated.

Materials und Methods

The experimental plants were grown under semi-steril conditions for 14 days in washed sand. Before sowing the seeds were swollen for 12 hrs at room temperature and pregerminated on filter paper in Petri dishes until the seed coat was burst. Till the emergence of the seedlings (germination) the water content of soil was kept on 75—80% of the total water holding capacity. After the emergence the water content of soil was modified according to the requirements of the experiment in question and the water evaporated was supplied every day. The plants cultivated in this way exhibited well developed root and shoot system. Barley seedlings were grown as control plants under identical conditions without irrigation. Except the plants were subject to experimental starvation, the inorganic nutrients were mixed, according to MITSCHERLICH, with the sand before sowing. The amount of nutrient salt varied according to the requirements of the experiments.

The root system of plants was washed with streaming water in order to remove the sand particles. In the studies on ion uptake the seedlings were grown in a modified HOAGLAND solution for various periods according to the aim of the experiments. As a N-source only NH_4-N was supplied to the culture solution. The cultures were carefully checked for eventual bacterial contamination. The concentration of the nutrient solution and the length of the experiments was chosen to make the measurement of the amount of various elements also by the end of the experiments possible. Phosphorus was determined according to FISKE and SUBBAROW. NH_4-H was assayed by photometric nesslerization (7). Aerobic and anaerobic conditions were created by bubbling air or nitrogen through the nutrient solutions.

The extraction of amino acids was carried out by precipitating the proteins with 80% alcohol from a homogenate followed by centrifugation. Extracts corresponding to 20 cg fresh weight were chromatographed on Sch. & Sch. paper (No. 2043 b) dividing the ring chromatograms into 4 sectors. As a developing solvent a mixture of phenol: water: butanol: acetic acid (16 : 16 : 128 : 40) was chosen. The chromatograms were sprayed with 0,2% ninhydrin dissolved in acetone. Identification of the ninhydrin positive spots took place by cochromatography of standard amino acids. A photometric method developed by FISCHER was used for quantitative assays (5).

The plant material used for the various experiments consisted of 300 rice seedlings of average size. The average deviation from the mean in the various experiments did not exceed $\pm 2{,}5\%$.

Results und discussion

As may be seen from *Fig. 1.* the anaerobic environment of rice roots does not inhibit the nitrogen uptake of rice seedlings, whereas the absence of oxygen greatly inhibits the N-uptake by barley roots. This situation is profoundly changed when the shoot of rice seedlings is removed or submerged. In these cases aeration promotes the N-uptake of the rice plant as

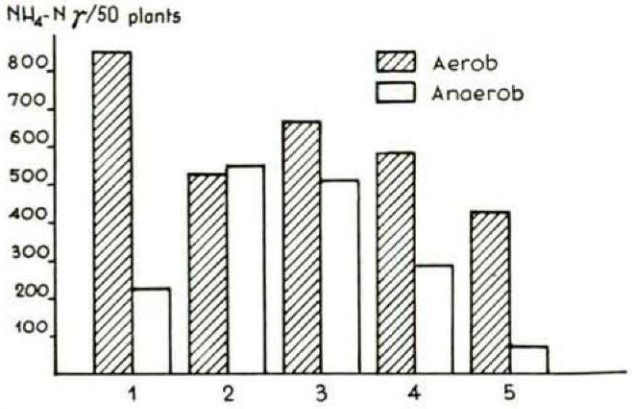

Fig. 1. The effect of aerobic and anaerobic conditions on the ion uptake of rice
1 = barley (control plant)
2 = rice root in nutrient solution
3 = rice root + shoot in $^2/_3$ nutrient solution
4 = whole plant in nutrient solution, submerged
5 = decapitated root in nutrient solution

well. Therefore the processes responsible for the uptake of nutrients are apparently promoted by the oxidative metabolism both in shoot and root, however the O_2 requirement of roots is fulfilled and mediated somehow by the leaves.

The same seems to be true for the uptake of phosphorus. The oxygen needed for P-uptake is supplied by the shoot. This process is extremely important in so far as after inundation the conditions in the soil are rapidly shifted towards anaerobiosis (9). In case of a sufficient O_2-translocation from shoot to root the ion uptake by the root system does not depend on the O_2 content of the surrounding medium. However, if the O_2-transport is for some reason inhibited (i. e. high water level) the synthetic processes of root tissues will decline with a concomitant increase in hydrolytic processes. Therefore, a too high water level is undoubtedly disadvantageous for the development of rice and inhibits the uptake of nutrients. The inhibitory effect of high water level can not be explained entirely by the absence of O_2. There are many indications to show that the water, even if enriched with oxygen, is not an optimal environment for the development of rice.

Studies on the uptake of nutrients indicated that the young rice seedlings absorb high quantities of NH_4-nitrogen which is then rapidly converted into organic compounds (14, 15). As shown by paper chromatographic studies an

unknown ninhydrin positive spot appeared around the 90th minute of the experiments (Rf 0,19). The unknown N-acceptor yielded the following amino acids upon hydrolysis with 6 n HCl: cystein, histidine, aspartic acid, glutamic acid, alanine, tyrosine, valine and leucine (Fig. 2). The conclusion was, therefore, reached that the N-acceptor is a peptide.

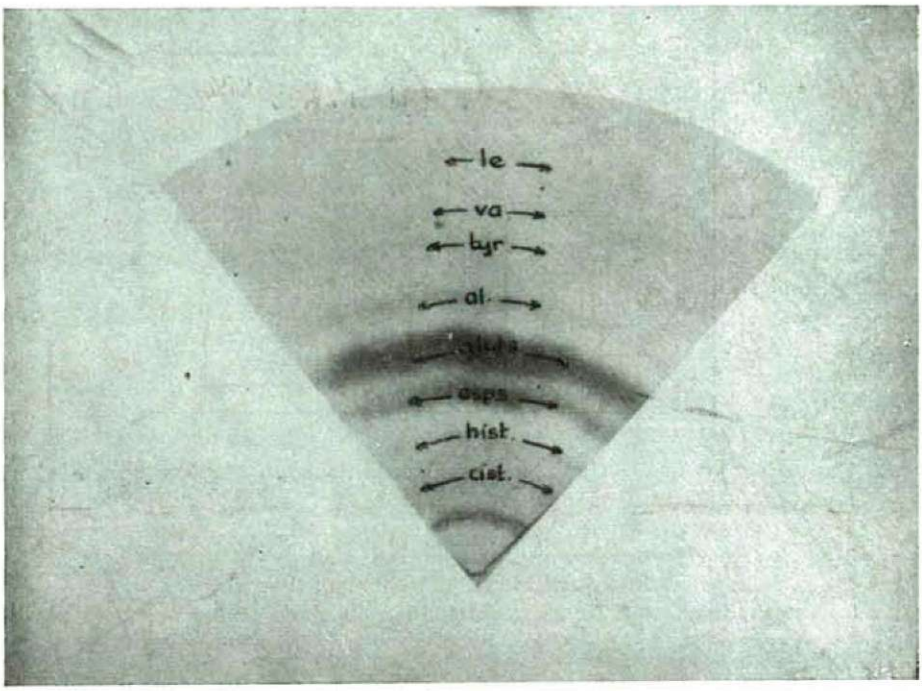

Fig. 2. Products of peptide hydrolysis

cist. = cysteine
hiszt. = histidine
aszp. = aspartic acid
gluts. = glutamic acid
al. = alanine
tyr. = tyrosine

Various explanations may account for the mechanism of N-accumulation in the peptide. A keto-peptide might be involved wich incorporates inorganic nitrogen as a direct NH_4-acceptor. On the other hand, the peptide might be a simple protein percursor synthesized via normal transamination processes. As to the first alternative a hypothesis put forward by LINDERSTROM—LANG (8) should be born in mind. According to the ideas of the Scandinavian worker the preexistence of all component amino acids is not a prerequisite of peptide synthesis. The synthesis of ketopeptides must also be taken into account supposing thereby a secondary incorporation of amino groups as a final step of peptide formation. This suggestion is, of course, highly hypo-

thetical and the use of N^{15} is indispensable for the decision of the problem. Also the detection of enzymes would be necessary which are capable of catalyzing amination or transamination with peptides. My own studies have shown transamination unequivocally only between glutamic acid and alanine.

The incorporation of inorganic nitrogen into organic compounds follows two main routes in higher plants:

1. α-ketoglutaric acid $\overset{+NH_3}{\underset{-NH_3}{\rightleftharpoons}}$ glutamic acid $\overset{+NH}{\underset{-NH_3}{\rightleftharpoons}}$ glutamine

2. oxaloacetic acid $\overset{+NH_3}{\underset{-NH_3}{\rightleftharpoons}}$ aspartic acid $\overset{+NH_3}{\underset{-NH_3}{\rightleftharpoons}}$ asparagine.

There is still a disagreement whether alanine is synthesized only by transamination or also by direct amination. Transamination seems to be more likely according to our paper chromatographic studies.

The relatively high amount of peptides found in the roots and leaves of rice seems to be of a high importance. Unfortunately little is know about the role of peptides in nitrogen metabolism especially as far as higher plants are concerned. A few peptides have been described, however, their biochemical properties and physiological role are very poorly understood (2, 4, 10).

Data obtained from the analysis of the solution secreted by guttation show that peptides might also play an important role in the translocation of N-compounds.

In order to gain some information as to the utilization of various nitrogen compounds the uptake and incorporation of NO_3 was studied. It may be seen from Table 1. that rice seedlings are able to the reduction and in-

Table 1. The utilization of various nitrogen compounds by rice seedlings

	Nitrogen compound	Total-N mg/50 seedlings				Mg N uptake compared to starving plants
		root	shoot	endosperm	whole seedling	
1	NH_4—N	2,0	7,7	7,4	17.1	5,7
2	NO_3—N	2,0	7,5	6,9	16,4	5,0
3	—	0,5	3,5	7,4	11,4	—

corporation of the consumed NO_3, an observation which is in contradiction to BONNER's ideas about the question (1). The reduced intermediate is mainly converted into organic form via glutamic and aspartic acid. The conversion of NO_3 is, however, much slower than that of NH_4.

Studies of the effect of water level on nutrient uptake and mobilization of substances in the endosperm indicate that an inundation as high as 10—15 cm, right after the emergence of the seedlings, in addition to root growth, also inhibits the accumulation of organic substances because of the distur-

bances in nitrogen metabolism clearly revealed by the accumulation of free amino acids (Table 2). In addition, the mobilization of substances and their translocation from the endosperm is also inhibited. These results are in accord with the findings of ERÜGIN and GOSH (3, 6). It is indicated that the carbohydrate metabolism is disturbed and in addition the translocation of N-compounds from the endosperm is inhibited. A detailed description of these results will appear later.

These observations outlined above also have a bearing on the practice of rice growing. The rice seedlings are flooded with water just at the beginning of their germination which may be regarded as the most sensitive

Table 2. Changes in the level of free amino acids in roots of rice seedlings as influenced by various water levels

	Extent of inundation	Alanine	Aspartic acid	Complex spot	γ-amino-butyric acid	Glutamic acid	Histidine	Leucine	Tyrosine	Valine
1	No inundation	++	++	++	+++	++	+	(+)	+	(+)
2	Shallow inundation	++	++	+++	++	++	(+)	++	+	+
3	High inundation	++++	+++	++++	+++	+++	—	+	—	++

\+ = demonstrated (in various amounts)
(+) = only in traces
— = absent
1) = complex spot containing asparagine, glutamine, and glycine

stage of plant development. This might exert a deleterious effect on the first phase of seedling growth but in addition it might affect the whole vegetation period of the rice plant.

An analysis of the data peresented in this paper suggests that an adequate equilibrium between the synthetic and hydrolytic processes resulting in a normal development of the rice seedlings, i. e. the preponderance of a synthetic trend in metabolism, is reached by a low water level (0,5—1 cm). Higher water level (15 cm), even if enriched somehow with O_2, does not favour the development of rice. Therefore any agrotechnical procedure, which stressing some other points (for example weed eradication) neglects the optimal conditions necessary for the undisturbed development of rice must be regarded as harmful.

Summary

Since the inundation of rice fields creates anaerobic conditions in the soil and thereby a special situation as far as uptake and transformation of nutrients by the rice plant are concerned the present study was undertaken to elucidate the effect of anaerobic conditions on the uptake and incorporation of nitrogen by rice seedlings. The studies were mainly concentrated on the uptake of nitrogen and its entry into organic matter. In this connec-

tion a special attention was paid to the investigation of transamination. The effect of aerobic and anaerobic conditions upon the uptake of inorganic nutrients was also studied. The influence of various water levels upon some biochemical processes of the rice plants and their connection was investigated. It has been observed that the anaerobic conditions in the soil do not affect the nitrogen and phosphorous uptake of intact rice plants. The removal of the shoot or its submersion, however, results in an inhibition which can be removed by aeration. Therefore, the biochemical processes of the rice root mediating the uptake of inorganic compounds depend on O_2. The O_2 is supplied by the shoot. If the translocation of O_2 is for some reason inhibited (too high water level) the synthetic processes of the roots slow down and the hydrolytic processes gain in importance. Therefore, a high inundation, especially at an early stage of rice development, affects infavourably the growth processes. Rice seedlings are able to convert both inorganic nitrogen sources (NH_4, NO_3) into organic form. Especially NH_4 appears rapidly in organic compounds. The question as to the physiological role of the peptide contained by rice roots is still waiting for further elucidation. The entry of inorganic nitrogen into organic compounds is mediated by glutamic acid in rice seedlings. The synthesis of alanine takes place, in our opinion, via transamination.

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