CHANGES OF FREE AMINO ACIDS IN RICE SEEDLINGS DUE TO THE EFFECT OF FACTORS RENDERING THEM SUSCEPTIBLE OF THE BROWNING DISEASE

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One of the major problems of rice cultivating countries is the browning disease (»bruzone«, »Aki-ochi«, »Hie-imochi«) periodically appearing. As for its appearance the general opinion so far was that it may be produced alone by the *Piricularia oryzae,* at any rate a considerable role is to be attributed to the factors rendering the plant susceptible of the browning disease, (abundant *N*, lack of radiation, gloomy weather, low temperature, increased H_2S in the soil). In recent years numerous papers have been published supporting this conception 3, 5, 6, 7). In such cases the fungal infection is likely to appear secondarily following a »physiological drought« of fairly high degree (3). To control and follow the disease changes of physiological nature — taking place in the rice plant due to the effect of unfavourable factors — must be learnt. Of the factors rendering the plant susceptible of the disease an account is given first of the low temperature, secondly of the change of the free amino acid due to the effect of H_2S.

Material and method

The experimental plants were cultivated in sand from 14 to 18 days. Seeds were pre-germinated in Petri-dishes. The humidity of the sand, preceding germination, was secured 80%, thereafter the young rice plants were »slightly« flooded. Flooding, when the experiments necessited, was made with the modified HOAGLAND-solution.

The low temperature treatment (10—12 C°) lasted for 24—36 hours. Here the pots were placed in water bath of the corresponding temperature. Temperature of the air ranged from 24° to 26 C°.

To examine the effect of H_2S the young plants were placed in HOAGLAND-solution and the H_2S was kept bubbling in the solution for 3 hours. In the plants thus treated, beside the change of the amino acid, the quantitative changes of *N* and *P*, occurring of the solution, has also been observed.

For the determination of the free amino acid *Schleicher-Schüll* 2043 B paper was used. In the first dimension butanol-acidum aceticum-water (2 : 1 : 1), in the second mixture of phenol-water (4 : 1) was employed. Spots of the amino acids were produced with 0,2% acetone ninhydrin.

Results and discussion

It is commonly known that the *N*-metabolism in unhealthy plants is unbalanced, consequently that ratio of *soluble N: protein N* is strongly shifted to the favour of the former (2). In the examinations — contrary to previous practice — the quantitative change of the free amino acids were primarily taken into consideration.

The changes due to the effect of the low temperature show the *N*-metabolism disturbances characteristic in general of the unhealthy plants. It is especially important to emphasize the abnormally strong accumulation of the basic amino acids playing a considerable role in the protein synthesis (aspartic acid, glutamic acid, respectively their amids). At the same time the quantity of some of the amino acids (alanine, γ-aminobutyric-acid, tyrosine etc.) hardly changed following a 24-hour cooling. *(Fig. 1—2).*

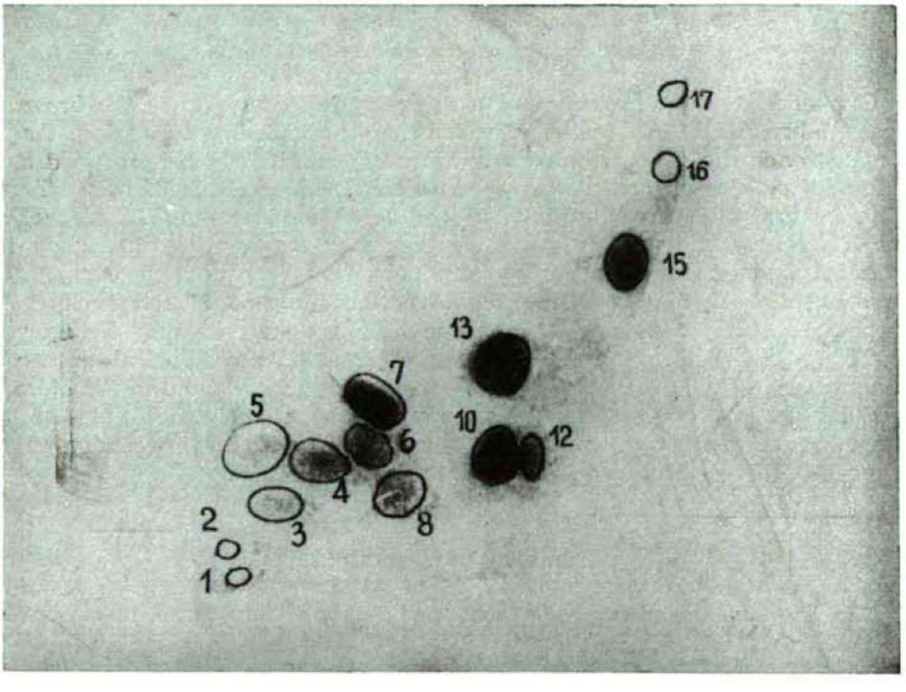

Fig. 1. Free amino acids in root tissues (at 23 C°)

Considering that a *N*-uptake — even in a small degree — could be demonstrated in our experimental circumstances, it seems that the protein synthesis is primarily inhibited by the low temperature while the conversion of inorganic *N* into amino acids is less inhibited. The accumulation of the free amino acid notable in the endospermium indicates that the transport too, is injuriously affected by the low temperature while the mobilization is not affected significantly *(Fig. 3.).*

The H_2S, however, had quite a different effect on the quantitative changes of the free amino acids of the rice plant kept bubbling through the solution for 3 hours. The free amino acid content of the plants thus treated are shown in *Fig. 4*. As no *N*-uptake could be demonstrated from the solu-

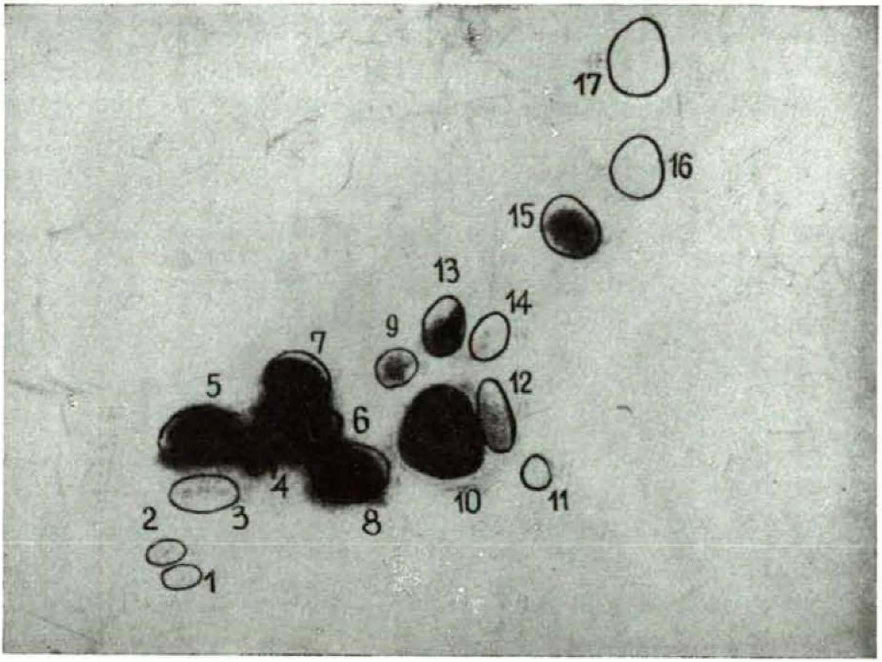

Fig. 2. Free amine acids in root tissues (at 11 C°)

1 = cysteine,	10 = glutamine,
2—3 = unidentified,	11 = histidine,
4 = serine,	12 = unidentified,
5 = aspartic acid	13 = alanine,
6 = glycine,	14 = tyrosine,
7 = glutamic acid	15 = γ-aminobutyric acid,
8 = asparagine,	16 = valine,
9 = threonine,	17 = leucine

tion treated with H_2S, the comparison of the strips of the *A*, *C* and *D*, *F* paperchromatogramms is reasonable. It can be stated that some of the amino acid spots on the *C* and *F* chromatogramm (aspartic acid, glutamine, glutamic acid) almost entirely disappear during the very short time of the experiment whereas the quantity of the alanin, γ-aminobutyric acid — though slightly — increases. It may be assumed that the quantity of the last two amino acids increased through transamination process, the amino groups being transferred, from glutamine and aspartic acid. Thus the transamination — under our experimental circumstances — has not been blocked by H_2S.

According to our observations both the low temperature and the presence of H_2S affect the water-uptake (Guttation ceased rapidly by low temperature respectively H_2S). This phenomenon draws our attention to the so-called »physiological drought« mentioned also in the literary data (4). The rice as compared to other plants — due to its anatomical structure — is much more sensitive to the water-loss. Minimal lack of water alone, i. e. a

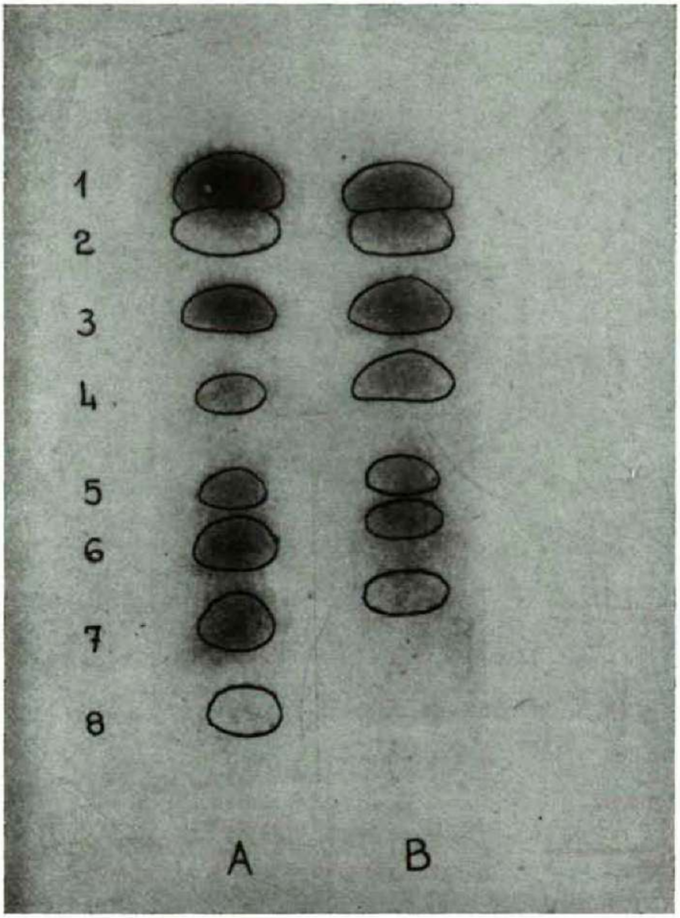

Fig. 3. Changes of free amino acids in the endosperm

A = at 11 C°, B = at 23 C°

1 = leucine,
2 = phenyl-alanine,
3 = valine,
4 = γ-aminobutyric acid
5 = alanine,
6 = glutamic acid,
7 = aspartic acid + glutamine,
8 = unidentified

continued disturbance in the water supply may produce irreversible changes. This condition is peculiarly dangerous in the field when a long-lasting cool period is followed suddenly by dry, warm weather. The water and soil grow warm very slowly — as it is known from WAGNER's investigations — resulting in significant disturbances in the water supply. It is evident that the »physiological drought« occurs sooner in plants of poor roots, overfed with *N*, and developing in anaerobic soil. For this reason, the formation of the brown disease, in our opinion, can be explained by the simultaneous appearance of several factors.

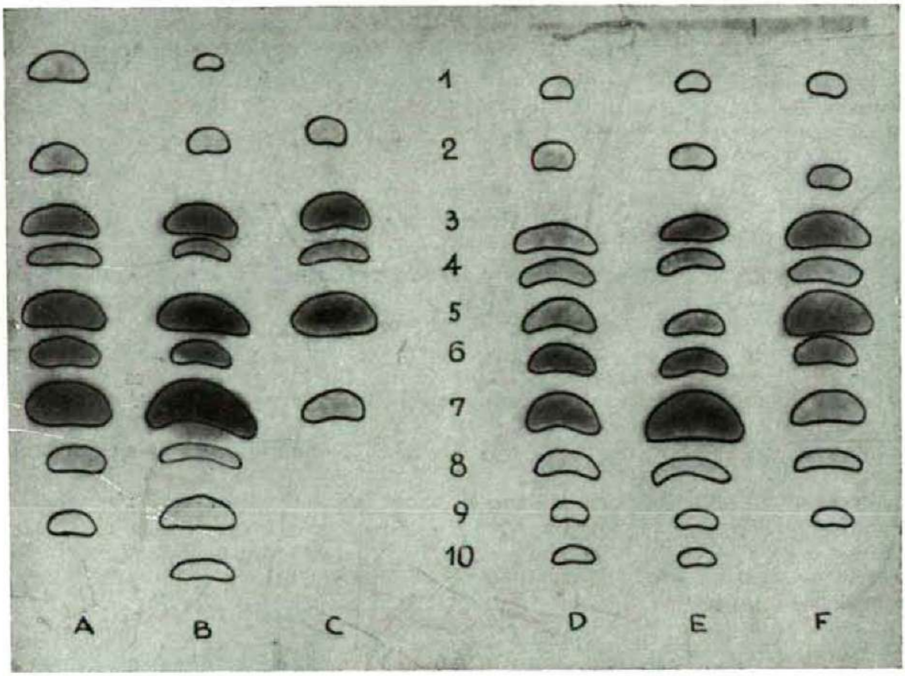

Fig. 4. The amino acids of root and leaf tissue.

ABC root
DEF shoot
AD control (start of experiment, before the transfer of seedlings to the nutrient solution)
BE Plants in nutrient solution aerated for 3 hours
CF plants in nutrient solution treated with H_2S for 3 hours

1 = leucine,
2 = valine,
3 = γ aminobutyric acid
4 = tyrosine,
5 = alanine,
6 = glutamic acid,
7 = aspartic acid + glutamine + + asparagine,
8 = histidine,
9 = glutathione,
10 = cysteine

Summary

The periodical appearance of the brown disease of the rice indicates that the weather is an important factor in the formation of this disease. According to our experimental results both the low temperature — especially

under unfavourable radiation conditions — and the H_2S produce considerable disturbance in *N*-metabolism. Moreover the factors, rendering the plant susceptible of the disease, may produce also disturbance in the water supply, primarily in plants of poor roots, with excess *N* developing in bad anaerobic soils. This phenomenon directs the attention to the important role played by the so-called »physiological drought«.

This condition may be assumed to play a decisive role in the production of a disease of such physiological origin.

On the basis of the above mentioned facts may be supposed that the species resistant to the browning disease — likely due to their *anatomical structure* — can better tolerate also the scarcity of water. These experimental results may present some basis for the breeders.

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