CHANGES IN THE AMINO ACID AND PROTEIN METABOLISM OF RICE AS A RESULT OF DISEASES

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(Received December 1, 1966.)

Introduction

Hungary is the northest area in Europe where rice can be grown. There are years when, owing to the prolonged cool weather and the lack of sunshine in summer, the rice diseases are rather frequent. In such years even a 40—75 p. c. lowering of productivity was observed (e. g., in 1949, 1954. 1955). After 1950 the rice growing has taken more and more areas. For a long time there had prevailed a wrong view alleging that rice grows well also in our alkali soils. Our experimental results, obtained so far, added, however, a lot to make it clear that our Hungarian-bred rice sorts were not salt-resistant and were unsuitable for alkali soils (Pálfi 1965 b). At rice growing in alkali soils, in case of unfavourable summer weather, the occurrence of, and the damage caused by, diseases is much more expressed than among plants growing in other solis. Just the widespread appearance of rice-diseases makes possibile to study the damaging factors.

In leaves of diseased rice plants and of plants overfed with N, a compound of unknown composition was demonstrated by paper chromatography (PÁLFI 1964 a, 1965 a).

After the reaction with ninhydrine and fixation by copper salt, this substance gives a blue colour instead of the red characteristic for the amino acids. In further investigations it appeared that the size and intensity of the blue spot increased parallel to the extent of unilateral N-nutriton before sowing (PÁLFI 1964 b, c).

The appearance of the blue spot is connected with disturbances in the metabolism (probably in the amino acid and protein metabolism) since the spot could always be detected in diseased rice, namely in an amount proportional to the extent of disease (PÁLFI 1965 a). During studies of healthy rice grown in the fields throughout the former years, this spot has not been demonstrated in any case (PÁLFI 1963).

The present paper set itself the task of ascertaining whether or not the blue spot, named by us „blue substance" and designed by „β", can be demonstrated in other parts of the rice plant apart from the leaves. Our previous results had been obtained on a single rice sort, the Hungarian-bred Dunghan shali. In the course of the present experiments other Hungarian and Soviet rice sorts have been investigated as well. If the appearence of the blue spot is connected with similar con-

ditions in other rice sorts, as well, its demonstration, i. e., the „blue-test" has a general validity.

In the course of our previous experiments it had already been demonstrated that the amino acid (especially the glutamine and asparagine) content in the rice leaves may increase not only as a result of an abundant N-nutrition but this increase may appear also as a consequence of any influences disturbing the protein synthesis (PÁLFI 1965 b). Similar results have also been published by other authors (PLESKOV 1957, FARKAS 1963, BIDWELL et al. 196, FELDMANN and HANKS 1964, SEHGAL and BOONE 1964).

Material and methods

The experiments were carried out by the researchers of the State Institute for Qualification of Sorts and Agricultural Technology (Országos Mezőgazdasági Fajta- és Termeléstechnikai Minősítő Intézet; OMFTMI). Physiological experiments were performed in the State Plantation, Kopáncs. The purpose of experiments made by OMFTMI was to provoke rice diseases by addition enormous amount of nitrogen before sowing, by excluding the light artificially and through infections (VÁMOS 1959, ZSOLDOS 1962). This method is suitable to select brusone-resistant sorts (PODHRADSZKY 1961).

Dunghan shali was grown without fertilizer (control) and having 3 and 6 q ammonium sulphate per cadastrial acre, respectively (1 cadastrial acre = 0, 5755 ha). Moreover, samples were taken from the following sorts: *„Pannoryza 2"*, *„Bánát 725"*, *„ H6"*, *„H 9"*, *„Káka 203"*, *„Szarvas 63"* Hungarian-bred rices, and *„Cuban 3"*, *„Krasnoarmenskij 312"*, *„Dubovskij 129"*, *„Krasnodarskij 424"*, *„Uzoros 17"* Soviet sorts. Samples were taken six times: during shooting, blooming, embryogenesis and further three times during ripening. Samples were also taken in the previous year from Dunghan shali in the State Plantation, Pale.

As published by us (PÁLFI *1964 b*) and others as well. (MOTHES *1960*, YOSHIDA *1961*, COIC et al. 1963, BOODSON et al. 1964, LIZANDR and BROVCUNA 1964), the nutrient (NPK) content of leaves differs in shoot by leaf storeys. Therefore, the different leaf storeys were separatly analyzed, and the results obtained on the upper second leaves compared with. Most of the seedlings had only three living leaves.

In each case fresh leaves fixed and dried at 65 C° were analysed. The ascending paper-chromatographic analysis of the 50 p. c. ethanol extracts was carried out on Sch.-Sch. 2041 b and WHATMAN No 1. paper. Butanol-glacial acetic acid — water solvent has been applied in a ratio of 2 to 1 to 1. The running was slowered by cooling. (2 C°) and sometimes the running was repeated several times. In two-dimensional development phenol-water (4 to 1) served as a second solvent. The demonstration of the compounds was performed with ninhydrine, isatine, alioxan and folin reagents. In the identification of spots the method of the universal standard mixture, elaborated by us, had been applied (see Figs. 1 and 2;) (PÁLFI 1964 b, c and 1965 a). For the quantitative determination the spots were fixed by copper-salt, eluted and then subjected to photometry (SZALAI 1957). The extinction value of the eluted standard series yielded the calibration curve. Hydrolysis has been performed with 6 n hydrochloric acid for 24 hours at 105 C°, the samples being sealed in glass tubes.

Results

The Hungarian-bred Dunghan shali, as it is well known, is not resistant to diseases. The weather was unfavourable enough for the occurrence of different diseases. There were quite a number of rainy, cool days and the clear days were followed by a strong cooling at nights. Sunshine hardly intruded the plantstand, which was very dense owing to the great N-content. The flooding water was very cold (15—17 C°) even in the sunny hours at noon. Leaves of rice supplied with great amount of N before sowing have been longer and wider than those of the controll. Their colour was dark green, their tissue loose, fragile. During clustering

there appeared lodged, diseased spots in the rice fields. The leaves of such shoots, as in the former years (PÁLFI 1964 b, c), showed well the brownish-grey spots of brusone *(„Piriculariosis")*. Diseased shoots and smaller spots of diseased plants appeared as well in the rice fertilized with 3 q N per cad. acre.

In Fig. 1 three samples of Dunghan shali, supplied with different amounts of N are to be seen. The blue spot framed with broken line („β") on the chromatograms is situated between valine and γ-aminobutyric acid. As it can be seen, the blue spot appeared only in rice supplied with 3 and 6 q N per cad. acre ammonium sulphate but not in the control. In Fig. 1. the formerly described universal standard mixture (PÁLFI 1964 b, c, 1965 a) can be found in three concentrations and it is possible from them to make quantitative estimation of the investigated extracts.

Starting from identical amounts of the different parts of rice and using the same amount of extracts for chromatography, it was impossible to compare with one another the results due to the very different intensity of the spots. Therefore a simple amount of the leaf- and cluster-extracts, twofold amount of the stem-extracts and a fourfold amount of the root-extracts were carried on the paper. In Fig. 2 it can .very well be seen that the blue substance appears in a larger amount in the leaf and in the stem, less in the root and the least in the cluster. Furthermore it is striking to see in this cromatogram the large asparagine content of the cluster which may refer to a strong protein synthesis.

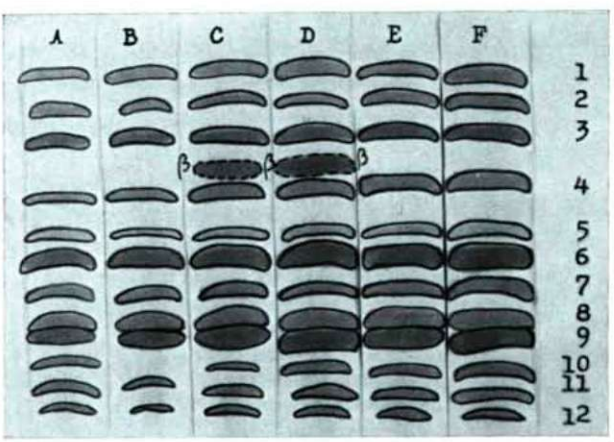

Fig.1. Chromatogram of leaf-extracts of Dunghan shali with different N-supply. Blooming. „A" : universal standard with 25 μg total amino acid content. „B" : rice without fertilizer. „C": 3 q ammonium sulphate per cad. acre. „D": 6 q ammonium sulphate fertilizer par cad. acre. „E" and „I": universal standard mixtures with 50 and 75 μg total amino acid content. „β": blue spot, the unknown substance.
Composition of the universal standard in the case of 50 μg total amino acid:

1. Leu	1,5 μg	7. Glu+Ser	6,0 μg
2. Phe	3,0 „	8. Gly+Glu—NH_2	3,5 „
3. Val	1,5 „	9. Asp	10,0 „
4. γ-Amb.	1,5 „	10. Asp—NH_2	8,0 „
5. Pro	5,0 „	11. Lys	1,0 „
6. Ala	5,0 „	12. Cys	4,0 „

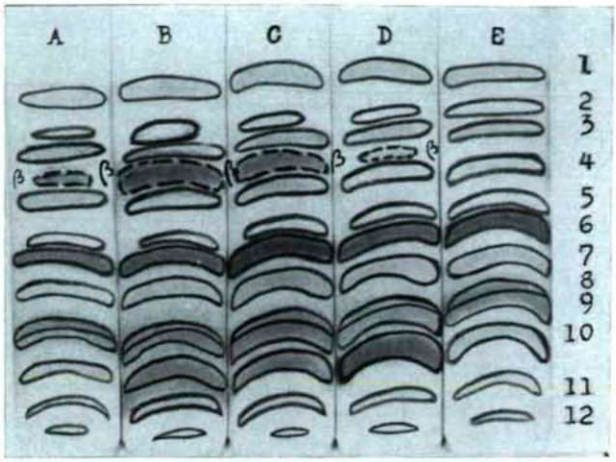

Fig. 2. Chromatogram of extracts of different parts of the rice plant in case of a great amount of N-supply. Dunghan shali in blooming. „A": root. „B": stem. „C": leaf. „D": cluster. „E": universal standard with 50 μg total amino acid content. „ß": blue spot. Numbers as in Fig. 1.,

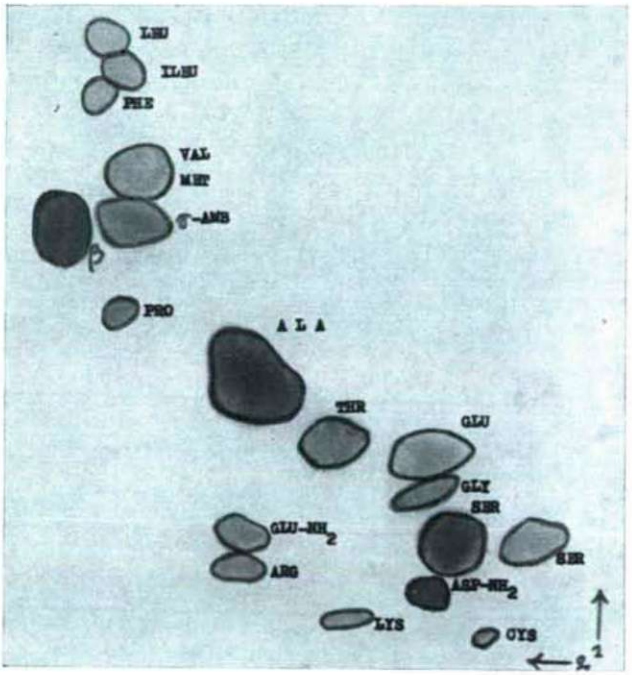

Fig. 3. Chromatogram of the leaf extract of rice with abundant N-supply. Dunghan shali, blooming. „ß": blue spot.

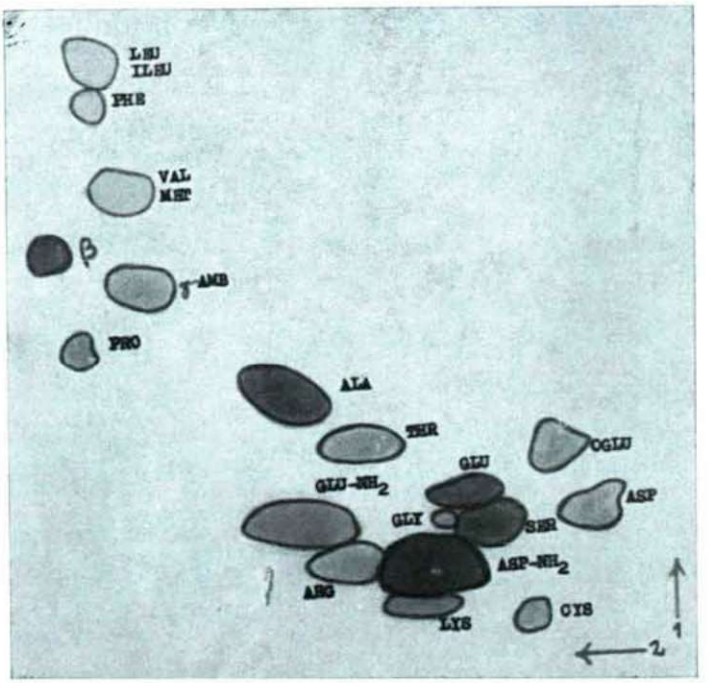

Fig. 4. Chromatogram of the cluster extract of rice with abundant N-supply. Dunghan shali, blooming. „ß": blue spot.

Two-dimensional chromatograms were also prepared from the different parts of rice and of these we show in Figs. 3 and 4 the chromatograms of the leaf and cluster extracts. It is true, that from two-dimensional chromatograms the quantitative deductions are less valid, however, it may be considered that there was a much smaller blue spot in case of the cluster extracts than in that of leaf-extracts. This fact seems to be supported by one-dimensional determinations of the eluted spots, too. Asparagine yields a strikingly large spot in the two-dimensional chromatogram of the cluster, and also the glutamine spot is considerable. In the chromatograms of the cluster there appeared, above aspartice acid, oxyglutamic acid, as well, the demonstration of which is rather difficult.

The one-dimensional chromatogram in Fig. 5 was made with hydrolysed samples. In the free amino acid extracts of rice supplied with large amount of N before sowing there is a large blue spot. Leaves purified from the free amino acids do not containt the blue substance in their hydrolysate. In the chromatogram of hydrolysed free amino acid extracts the blue spot appeared unchanged.

Fig. 6 shows that the blue spot refers to disturbances not only in the case of the Hungarian-bred Dunghan shali but with the Soviet sorts, as well. Provoked by heavy N-supply, the blue substance appeared in each of the Soviet sorts studied by us, however, in an amount smaller than in the Hungarian sorts.

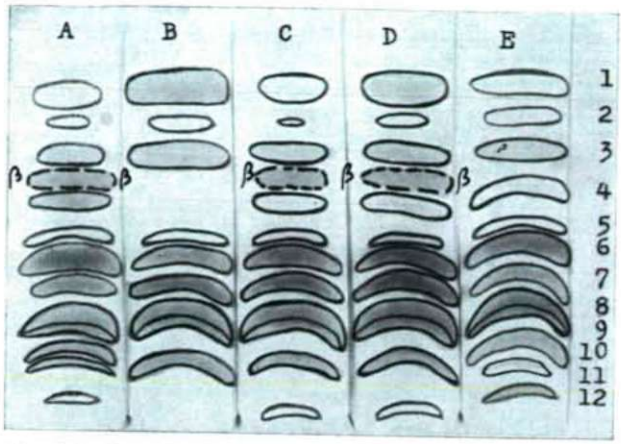

Fig. 5. Amino acids of leaf extracts and hydrolysed leaf proteins of rice (Dunghan shali) in blooming. Supplied with large amount of N. „A": free amino acids. „B" hydrolyzed leaf protein purified from free amino acids. „C" and „D": hydrolyzed free amino acid extract, simple and double amount. „E": universal standard with 50 μg total amino acid content. Number as in Fig. 1.

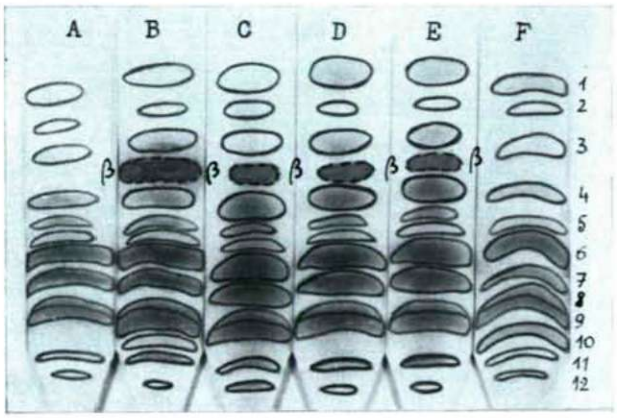

Fig. 6. Chromatograms of rice leaves, Hungarian and Soviet sorts. Blooming. „A" : Dunghan shali without fertilizer. „B"; Dunghan shali with abundant N-supply. „C": Dubovskiy 129 with large amount of N. „D": Krasnodarskiy 424 with large amount of N. „E": Krasnoarmenskiy 313 with large amount of N. „F": universal standard with 50 μg total amino acid. „ß": blue spot. Numbers as in Fig. 1.

At the same time no blue spot was demonstrated in the plants without a fertilizer.

In Fig. 7 the leaf-extracts of three Hungarian-bred and three Soviet-bred rices are compared. It can be seen that in the one-dimensional chromatogram developed in phenol-water solvent the blue spot framed with broken line showed the highest Rf value. From the chromatogram it appears that in the Hungarian

sorts supplied with great amount of N the blue substance is more abundant than in the Soviet-bred rice supplied with a similar fertilizer. Several hundred chromatograms were prepared and the result was always the same. In the control of the Hungarian Dunghan shali there was no blue substance or it appeared only in traces. Diseased samples, however, occurred in the control, as well, probably due to the unfavourable weather conditions.

Fig 7 shows that the blue spot appears starting both from the fresh substance and from the dry one. Thus the blue substance is not a decomposition product formed during drying.

In each of the three years of investigation the blue substance could be demonstrated in the lower leaf storeys of rice plants, but in an amount less than in the upper storeys.

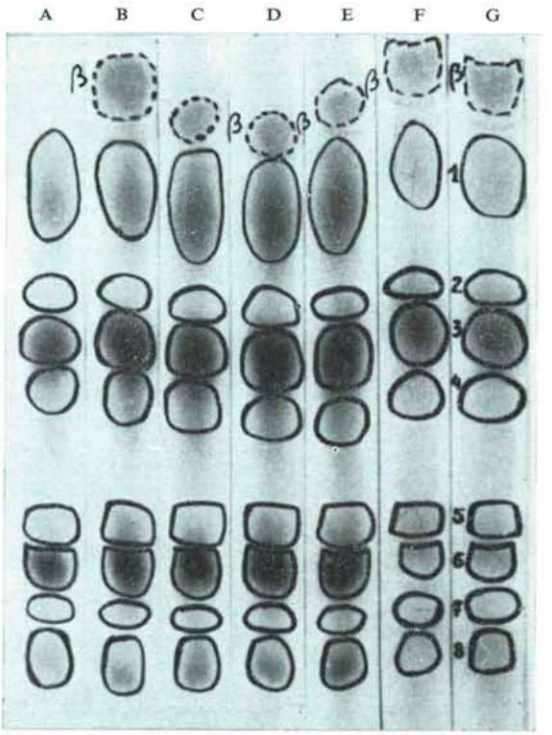

Fig. 7. Chromatograms of leaf extracts of Hungarian and Soviet rice sorts developed with phenol-water solvent. First strip on the left is the extract of unfertilized control, others of that supplied with large amounts of N. The left five strips are made from fresh substance, the two ones on the right from dry substance. „A": and „B": Dunghan shali. „C": Krasnoarmenskij 313. „D": Dubovskij 129. „E": Krasnodarskij 424. „F": Szarvasi 63. „G": Kákai 203.

1. Phe, Leu, Val
2. „-Amb.
3. Glu-NH_2, Ala, Arg
4. Thr, Lys
5. Asp-NH_2
6. Ser, Glu
7. Cys
8. Asp

„ß"-blue spot

In Fig. 8 one can see the clusters with barren flowers of rice attacked by brusone, partly or wholly whitened. The leaves of these plants are green but with brownish-grey spots. As to yield, we must note that in case of Dunghan shali, with abundant fertilizer before sowing, the decrease of yield ihad been of about 80 p. c. with other Hungarian sorts 30—70 p. c. and there was no decrease in the yield of the Soviet-bred rices.

The rice plants of the State Plantation, Pale were not unilaterally supplied with N before sowing, yet, several diseased spots appeared the previous year. The plants of the diseased spots contained a considerable amount of blue substance, but the healthy ones did not. Thus only plants changed by the unilateral N-supply give the blue spot but so does rice diseased in other ways, too.

Our experiments showed that, the unknown substances does not give the alloxane and foline reactions of the amino acids, it is not decomposed by L-amino acid oxydaze (information from G. FARKAS), therefore its amino acid character is doubtful.

Fig. 8. Barren clusters of diseased Dunghan shali supplied with a great amount of N. They are partly or wholly whitened.

Discussion

Dunghan shali with abundant supply of N before sowing has very loose tissues and is susceptible to diseases. Such an abnormal physiological state can equally be caused by prolonged cool weather, lack of sunshine, cold soil or flooding water or by lack of phosphorus. The blue spot, first demonstrated by us (PÁLFI 1964a) refers to such a weakened state and probably disturbances in the protein metabolism. The blue substance can be demonstrated in any part of such a rice plant, although in different amounts — even from apparently healthy plants. The upper leaves of rice, that are still green at the complete ripening, contain the blue substance only in traces, and in such leaves the protein synthesis is already rather weakened.

During the first two years only Dunghan shali was investigated. In the third year beside it six Hungarian and five Soviet rice sorts were included.

The results show that in the leaf extracts of plants abundantly supplied with N or of plants diseased in brusone the blue spot could be demonstrated in all of the twelve sorts. On the contrary, it cannot be demonstrated in the case of the healthy controls. The „blue substance" content of the Soviet sorts is much less than that of the Hungarian ones. The brusone susceptibility of the Soviet sorts is much less, as well. This is supported by last year's results, since being provoked, all of the Hungarian sorts became more or less contaminated by *Piricularia* in contrast to the Soviet sorts. It must be mentioned, however, that Dunghan shali, having nice long grains, is far the best of all the studied sorts as regards to nutritional value, physical and cuisine properties, as well.

The blue substance had been demonstrated from each leaf storey of the plants but to a less extent from the lower leaves. This is in agreement with our former statement (PÁLFI 1963, 1964 b, 1965 a) that free amino acids, as well, can be found in different concentrations in the different leaf storeys and that the free amino acid content of the leaves is the less in the lowest storey. The same had been established by FRENYÓ (1961) in respect to inorganic nutrients of rice. In the leaves of the diseased rice not only the blue substance appears in large amounts but free amino acids and amides, too.

In the experiments with wheat diseased with mycosis FARKAS (1963) and others with different plants (FELDERMANN and HANKS 1964, GRINEVA 1964, SEHGAL and BOONE 1964) obtained similar results.

Summary

1. During paper chromatographic analysis of amino acids of rice leaves growing in the field an unknown substance had been demonstrated. This substance („blue substance") after being treated with ninhydrine and a following copper-salt fixation gives a blue spot instead of a red one, characteristic of the amino acids.

2. Investigations of samples collected in three years showed that the appearence of the blue substance indicates an abnormal physiological state, probably a disturbance in the amino acid and protein metabolism.

3. The blue substance occurs not only in the leaves but in the root, stem and cluster of rice, as well. It was found in the greatest amount in the leaves and the stem, less in the roots and the least in the cluster.

4. The blue substance content of the single leaf-storeys of rice plants is different: there is less of it in the lower leaves than in the upper, younger ones. Thus the blue substance appears in a larger quantity where the protein metabolism is the most intensive. At total ripening of the grains, when the protein metabolism decreases even in the upper green leaves still alive, no blue substance could be demonstrated there.

5. The demonstration of the blue substance had been performed on plants grown under varied conditions: in cool weather, artificially performed lack of sunshine, *Piricularia* infection and addition of abundant N-fertilizers. Results obtained in case of six Hungarian and five Soviet sorts prove that the blue-test can be applied for the demonstration metabolic disturbances (probably disturbances of the protein metabolism) of rice.

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