A NEW INDICATOR OF ABNORMAL AMINO ACID AND PROTEIN METABOLISM IN DISEASED PLANTS: RICE, POTATO, SOYBEAN, TOBACCO

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Introduction

The free amino acid content of plant organisms may be increased by means of a large N or P supply, or in consequence of certain diseases (BIDWELL et al., 1964; FARKAS and KIRÁLY, 1961). By paper chromatography we have looked for a physiological indication to demonstrate beyond any doubt if or not the increase of the free amino acid content is a consequence of some infection.

An unknown substance, lying at 0,64 Rf between the spot of γ-amino butyric acid and valine, was detected first in the chromatogram of rice (PÁLFI, 1964). Fixed with copper nitrate, this substance, in distinction to amino acids, gives a blue colour instead of a red one. Therefore, the unknown substance has been designated by us as „blue subtance" and its indication as the „blue test." In the case of rice, it appeared that under conditions promoting the occurence of diseases (PÁLFI, 1956) or even provoking them artificially (PODHRADSZKY, 1961) the more resistant foreign varieties contained less of the „blue substance" than the less resistant Hungarian types. Therefore, the test may be suggested as a physiological indication to select disease-resistant varieties.

By preparing an amino acid-free protein extract of diseased leaves and hydrolizing it with hydrochloric acid, the „blue substance" cannot be found among the amino acids produced. Thus it is not a component of protein. When the free amino acid extracts containig the „blue substance" are hydrolized, all the amides and peptides in them decompose but the „blue substance" remains. Thus it is neither an amide nor a peptide. Since the „blue substance" has been detected in wheat, as well (PÁLFI, 1965), our investigations were extended to other plants, too.

Materials and methods

Ascending one-dimensional papers (WHATMAN No 1) have been slowly developed by being cooled in a 2:1:1 solvent of butanol-acetic acid-water. In the second dimension a phenol-water mixture (4:1) has been applied. Final development has been secured by ninhydrine at 98 °C. The alcoholic extract of 1 g fresh substance or 200 mg substance dried at 65 °C served as starting material. Leaves of healthy and diseased plants were collected from the

same parcels during flowering. 12 Hungarian and foreign rice varieties were studied among others the *Hungarian Dunghan shali*. Potato was from the sort of *„Tompa rózsa"*; soy-bean *„Iregi korona"* and tobacco *„Szabolcsi"*. The diseases were: *„blast disease"* at rice *(Piricularia oryzae* CAV.), *„leaf roll virus"* (*Corium solani* HOLMES) at potato; *„soybean mosaic"* at soy-bean and *„Marmor tabaci var. vulgare Holmes"* at tobacco.

Results

Figure 1 shows that the „blue substance" is present besides rice in the leaf-extracts of diseased potato and tobacco, as well. At the same time, the extracts of healthy plants collected from the same parcels as the diseased ones did not contain the „blue substance". Figure 2 illustrates that the blue spot can be found in the chromatogram of the leaf extracts of virus-infected soy-bean, too. And the „blue substance" couldn't be found in the case of the healthy control, either. It can be seen that „blue sbustance" reaches the largest Rf value in the second dimension, i. e., in phenol-water solvent.

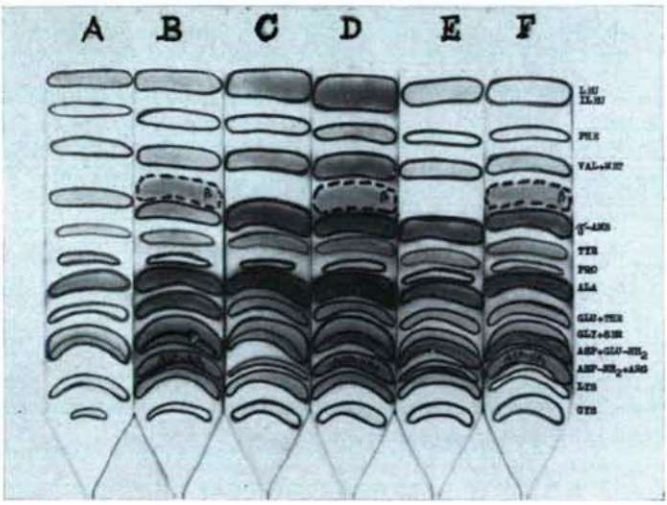

Fig. 1. Chromatogram of leaf extracts of healthy and diseased plants. A = healthy rice; B = Piricularia infected rice; C = healthy potato; D = virus infected potato; E = healthy tobacco; F = virus infected tobacco. β = blue spot, unknown.

Figure 3 shows the two-dimensional development of the leaf extracts of virus-infected tobacco — the „blue substance" appears here, too. In this figure as well as in the previous ones it is obvious that the „blue substance" appearing, also asparagine can be found in a considerable amount. Further it was experienced that i diseased plants where γ-amino butyric acid was present in a larger amount, more „blue subtance" could be detected.

It may be seen in figures 4 and 5 that the „blue substance" appears only in the thin layer chromatogram of the diseased potato.

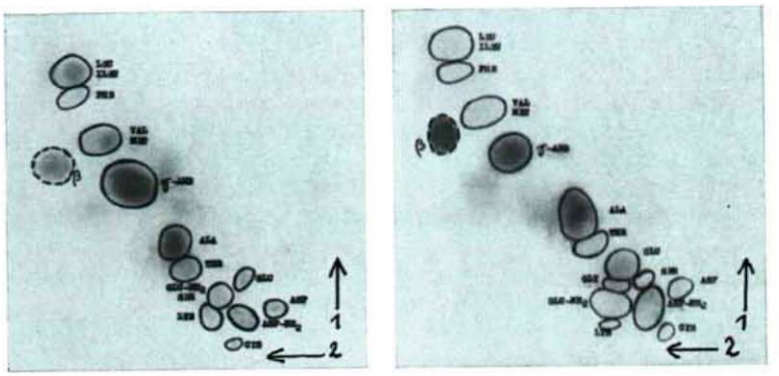

Fig. 2. Chromatogram of extract from virus-infected soybean leaf. β = the „blue spot".
Fig. 3. Chromatogram of extract from virus-infected tobacco leaf. β = the „blue spot".

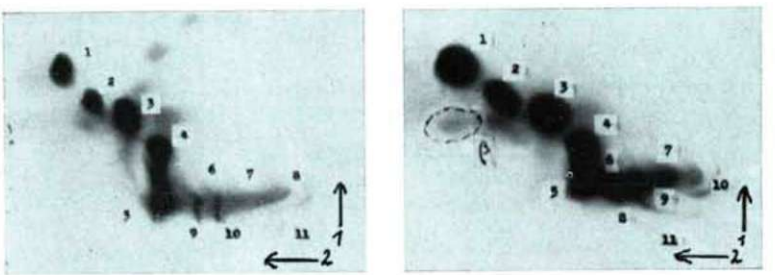

Fig. 4. Thin layer chromatogram of healthy potato. 1 = Leu + Ileu; 2= Val + Met; 3 = — Amb; 4 = Ala + Thr; 5 = Glu — NH_2 + Arg; 6 = Glu; 7 = Ser + Gly; 8 = Asp; 9 = Lys; 10 = Asp — NH_2; 11 = Cys.

Fig. 5. Thin layer chromatogram of diseased potato. 1—7. numbers as in Fig. 4; 8 = Lys; 9 = Asp. — NH_2; 10 = Asp; 11 = Cys; ß = blue spot.

From the leaves of rice containing „blue substance" a tapwater extract has also been prepared and inoculated with a pure culture. Bacterii of seven species, among them *Bac. subtilisATCC 6633* and *Escherichia coli 0 111* consumed the amino acids at 30 C° in seven days but the „blue substance" remained unchanged. It follows from this that this substance is no 1-amino acid and is not toxic.

It was detected that the largest amount of the „blue substance" could be found in the leaves of plants, the stem containing less and the roots and seed the least of it. In the lower leaf storeys of diseased plants there was less „blue substance" than in the younger upper leaves. Thus the „blue substance" appeared in a large amount where the protein metabolism was more intense. In the case of rice, during ripening of the corn when the protein synthesis was markedly decreassed in the still green but already old upper leaves, too, the „blue substance" could hardly be pointed out from the extracts. These data indicate that the appearance of the „blue substance" is connected with an increased protein metabolism.

The blue spot can be detected not only in plants mentioned in this paper but on the leaves of other diseased plants, as well. Thus recently it has been detected also in the chromatograms of extracts of virus-infected bean, sunflower and virus and *Phytophtora* infected *Solanum laciniatum* AIT. medicinal herb. These plants, however, have not been studied in detail.

Summary

In the course of the study of the free amino acid spectrum of diseased rice, soybean, potato and tobacco leaves an unknown compound has bean detected, the appearance of which is correlated with an abnormal physiological state of the plants, or with some disturbances in the amino acid — protein metabolism.

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