THE EFFECT OF H_2S ON THE IAA CONTENT OF THE RICE PLANT AND ON THE DEVELOPMENT OF ITS ADVENTITIOUS ROOTS

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H_2S formed in heavy, acidic degraded soils may damage the roots of the rice plant and impairs its metabolism. The hydrogen sulphide may, on the one hand, cause total destruction of roots, while its presence in smaller quantities inhibits the uptake of water, oxygen and nutrients, especially of potassium and phosphorus through blocking the enzymes containing heavy metals (BABA et al., 1952; VÁMOS, 1954; 1959; MORI, 1955; OKAJIMA, TAKAGI, 1955; YAMADA, OTA, 1958; PONNAMPERUMA, 1955; ZSOLDOS, 1959; MITSUI et al, 1962). The damaging effect of hydrogen sulphide is promoted by the soil and climatic conditions. First among the promoting soil conditions is the prevailing nitrogen abundance of the soil which causes the roots to spread horizontally under the surface of the soil (VÁMOS, 1959). It is just in this surface layer that the redox processes take place which cause damage to the roots and the whole plant. Among the climatic factors the scarcity of sunshine (VÁMOS, 1959) and cloudy, cool weather must be mentioned in the first place. In the case of scarcity of sunshine the synthetic processes slow down, the inner oxidizing power of the root is reduced, the growth of new roots becomes slow or stops altogether. Sudden cooling promotes quick release of H_2S from the mud, black with ferrous sulphide (VÁMOS, 1964). This is so because with the cooling the oxygen content of the flood-water increases, the redox-level sinks and the ferrous sulphide which is still in the anaerobic layer of the mud becomes oxidized and transformed into sulphuric acid. The sulphuric acid releases hydrogen sulphide from its environment which may reduce the sulphuric acid and then yellow colloidal sulphur precipitation which spreads like a veil, may be observed and SO_2 forms also. The SO_2 reduces the permeability of the cell wall and so not only the H_2S but also the electrically charged SH-ions can get through the cell wall unhindered, and damages the roots (VÁMOS, 1964). The destruction of roots is followed by tissue browning (MORI, 1955) and the development of adventitious roots, while on the leaves *Piricularia oryzae* and *Helminthosporium oryzae* (BABA et al., 1952) or possibly other fungi may appear thereby making the disease picture complete.

In the course of further investigation of the pathological processes we have made researches in order to elucidate the connection between H_2S effect, root destruction, node browning, development of adventitious roots and the possible IAA content of the rice plant (Fig. 1).

Fig. 1. Adventitious roots of rice plants suffer from root rot.

Materials and Methods

For our investigations we used a kind of Dunghan Shali susceptible to the browning disease but of excellent quality. Hydrogen sulphide was demonstrated from the plant tissues with the help of a solution of 1% of para-amino-dimethyl-anilin, in 5% hydrochloric acid, and 1% of ferrous chloride (TAKAGI and OKAJIMA, 1956). To demonstrate IAA, 20 g of fresh leaves mash was used in each test from which we made a 10 ml alcoholic extract. The extract was evaporated and transferred on to Schleicher-Schüll 2043 b paper and run in a mixture of isopropanole, ammonia and water at the ratio of 10 : 1 : 1. Development was made with EHRLICH's reagent.

Results

Examination of the IAA content of plants with damaged and undamaged roots gave the following results.

IAA could not be demonstrated with the above method in plants with undamaged roots living in favourable conditions. To find out whether the damage caused by H_2S had an influence on the development of adventitious roots or on the IAA content of the plant, the following investigations were carried out. Rice seedlings grown in sand culture were gently washed out and a quantity necessary for one test was placed for 3 days in tap-water containing H_2S of 1–2–4–5 and 10 ppm concentration, at pH–7. At this value 63,6% of the sulphide as H_2S and 36,3% as HS^- is present (RUBENTSCHIK, 1947). The solution was changed for a fresh one twice a day. The plants were worked up on the fourth day and in the case of the treatment at 4 and 5 ppm concentrations 5 μg of IAA was found in the leaves of the plants. When concentrations of 1, 2 and 10 ppm were used, a minimal IAA content and in the control plants no IAA content could be found

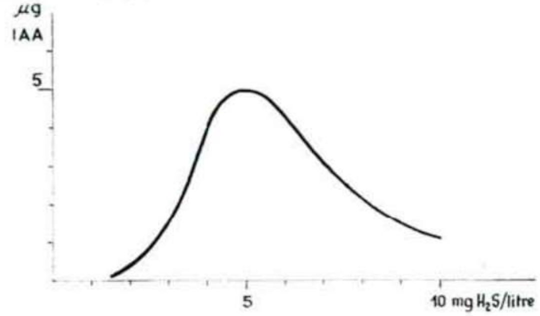

Fig. 2. IAA content of leaves of rice plant affected by H_2S. Solution changes twice daily.

with this method (Fig. 2). The test was repeated using fresh 1, 2, 3, 4, 5 and 10 H_2S ppm solutions every eight hours. Using this method, on the fourth day at a concentration of 3 ppm we got the above result, that is a content of 5 μg IAA from 20 g of fresh matter extract (Fig. 3). From these experiments, which we

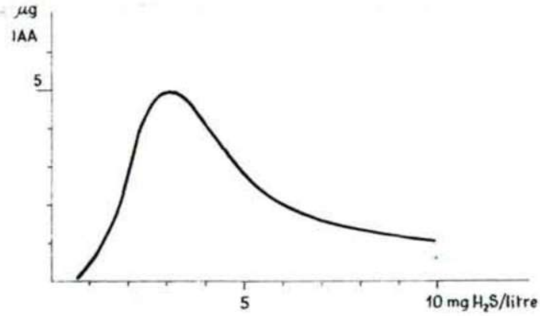

Fig. 3. IAA content of leaves of rice plant affected by H_2S. Solution changes thrice daily.

repeated several times with nearly identical results, we could conclude that H_2S plays a part in the formation of IAA. We made the same sort of tests also with plants in the field, but the presence of IAA could be demonstrated from the nodes and leaves of these plants only at the time of root rot. In the interest of the success of the experiment we promoted the destruction of roots artificially and therefore we treated the soil known as favourable for the development of the disease with 0,4, 0,6 and 1,0 tons/ha ammonium sulphate tamped it down and used surface sowing. The weather conditions also contributed to the success of the experiment and the expected root rot set in accordingly on Aug. 1, 1963. From 20 g collected during this time we could demonstate 10 μ g of IAA while from the control plants we could demonstate none. At this time adventitious roots began to develop vigorously not only from the submerged nodes but also from those above the water surface. The adventitious roots were negatively geotropic and the leaves showed sporadic contamination with *Piricularia oryzae.* From the luxuriantly developed plants those treated with 1,0 ton toppled down, while those treated with 0,4 ton and the control plants did not. The plants that toppled down, came into contact again with the soil by means of their adventitious roots and so could take up the necessary nutritive elements, the heavy metals, e. g., *Fe, Cu, Zn* and *Mn* which insure enzymatic activity, as the water contains none of these elements (VÁMOS, 1957). After the normalization of metabolism of these plants demonstration of IAA failed. The leaves developed showing no fungous contaminations. These plants yielded corn in contrast to the upright-standing diseased plants which showed the symptoms of the browning disease, violet-red drying of leaves and browning of nodes.

Presence of IAA and increased development of adventitious roots and contamination with *Piricularia oryzae* Cav. was found in the case of the plants shaded with white sheet. After shading was removed, the leaves developed, in abundant sunshine showed no IAA content and the fungous contamination did not spread on to the new leaves.

In order to demonstrate the connection between negative geotropy and the effect of hydrogen sulphide we carried out laboratory experiments. For this purpose we placed the rice plants which were cut off below the basal part of the stem into a measuring cylinder of 1 litre capacity at 5 ppm H_2S concentration. The roots which developed from the browned nodes showed negative geotropy. Consequently the cause of negative geotropy may be a chemotropy which again may be connected with the effect of H_2S.

GORDON and PALEG (1961) have demonstrated that IAA auxin may be formed from tryptophan without enzymes when quinones are present:

$$\text{phenol} \xrightarrow[\text{phenolase}]{O_2} \text{quinone}$$

By histological examinations we ascertained that the quinones cause browning in the nodes and the roots when the poliphenoloxidase containing copper is blocked by hydrogen sulphide (VÁMOS, 1959). This is so because the reduction of the coloured poliphenols then, does not take place, and the tissue browning characteristic of the browning disease occurs where the presence of hydrogen sulphide can be demonstated. Thus, in the presence of quincnes, as proved by the in vitro experiments of GORDON and PALEG, the formation of IAA may take place also without enzymes.

$$\text{quinone} + \text{triptophan} \rightarrow \text{indole pyruvic acid} \rightarrow \text{IAA}$$

The H_2S which is formed in the soil and gets into the tissues of the plant promotes the transformation of tryptophan into IAA by inactivating the enzymes containing heavy metals (catalase, poliphenoloxidase, peroxidase) or by inhibiting the IAA oxidase and the oxidizing system, it hinders the decomposition of IAA. Instead of impeded or checked synthetic processes the hydrolytic processes come into prominence, the free amino acids (ZSOLDOS, 1959) and the reducing sugars accumulate in the leaves and this circumstance promotes the appearance of *Piricularia oryzae*. When the rice plant luxuriantly grown in nitrogen abundance toppled down by means of its adventitious roots it got into contact with the soil. The uppermost stem section grew again vertically, and the metabolism became normal because the plant received from the soil all the necessarry elements that so far had been needed HILLMAN and GALSTON (1956) attribute important role to the manganese the absence of which inhibits, whereas its abundance increase the decomposition of IAA. Rice soil in general possesses abundant available manganese. In the plants shaded with a white sheet the oxidizing capacity was lowered owing to the lack of light energy and this resulted in intensified root rot thereafter the formation of IAA started in the leaves of the plants.

Summary

H_2S which is formed in the flooded paddy soils causes characteristic tissue browning at the basal part of the stem and in the nodes. This phenomenon is due to the fact that the H_2S hinders the reduction of the coloured polyphenol, by blocking the copper ions of polyphenol oxidase and so the quinones accumulate. In the presence of quinones the transformation of tryptophan into IAA takes place also without enzymes. Since the rice plant cannot take up certain indispensable ions from the soil in consequence of the destruction of its roots and the water does not contain them, the activation of the IAA oxidase which decomposes the IAA does not take place. So the IAA accumulates and adventitious roots develop from the nodes, often even from the nodes just below the panicle, that is, even from nodes remote from the water surface. The development of the adventitious roots is, presumably owing to the influence of H_2S, negatively geotropic.

In our field experiments we could demonstrate IAA from the leaves of luxuriantly developed rice plants damaged with root rot. After the damping of the plants when the adventitious roots came into contact with the soil, where from the necessary nutrients, e. g. manganese can be taken up, no IAA could be demonstrated.

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