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CHANGES OF SEVERAL ENZYME ACTIVITIES OF CARBOHYDRATE CATABOLISM IN BEAN LEAF TISSUES INFECTED WITH *UROMYCES PHASEOLI TYPICA*

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Abstract

Primary leaves of bean plant (*Phaseolus vulgaris* L.) were infected with *Uromyces phaseoli typica* and activities of several enzymes of carbohydrate metabolism in extracts of "pustule tissues", "ring tissues", "interior tissues" and "healthy tissues" were followed from sixth to tenth day after inoculation. The activities of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase hexokinase, phosphoglucomutase and phosphoglucoisomerase, but not aldolase, were all much higher in the first three leaf tissues than in the healthy tissue.

Introduction

Infection of higher plant tissues by obligate parasites results in changes in glucose catabolism (Daly *et al.*, 1957; Daly *et al.*, 1962; Frič, 1964; Lunderstädt, 1964; Farkas *et al.*, 1964; Scott, 1965; Fuchs, 1961; Shaw, 1963). Histochemical studies of carbohydrate metabolsim of rust infected bean leaf tissues showed that the distributions of the enzyme activities in leaf tissues were not uniform (Tschen and Fuchs 1968; 1970). Distribution of enzymes of carbohydrate catabolism in bean leaf tissues infected with *Uromyces phaseoli typica* was studied by means of a leaf disk technique (Tschen, 1966) and reported here.

Materials and Methods

Growth conditions and infection of plants

Garden beans (*Phaseolus vulgaris* L.) of the susceptible "Favorit" variety were grown from seeds in a growth chamber equipped with incandescent lamp

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with 8 hours dark and 16 hours light periods, 6,000–8,000 lux at the level of top of the plants at 25±2°C. Ten-day-old plants were inoculated by spray on the leaves with uredospores of *Uromyecs phaseoli typica* (1 mg of spores and 2 mg talc in 20 ml water for infection of 30 plants). The inoculated plants were incubated in a moisture chamber at 21°C in the dark for 24 hours, then the shoot apexes of plants were decapitated and the plants grown in a growth chamber, Five days later the shoot apexes of the plants were removed again. The infection of the plants was evident under these conditions on the 3rd day after infection as light fleck on the leaves. On the 6th day the pustules were fully developed. On the 7th day fungus began to sporulate and the sporulation came to a climax on the 9th or 10th day. Infection density amounted to 6 pustules per centimeter square on the leaves.

Preparation of leaf disks

Different leaf disks were removed from healthy and infected primary leaves of beans with a 4 mm diameter corkborer at different stages after infection, namely:

1. Leaf disks from healthy leaves, 4 mm in diameter ("healthy tissues").
2. Leaf disks, 4 mm in diameter, interjacent between fungal pustules of infected leaves ("interior tissue").
3. A tissue ring from infected leaf, 4 mm in diameter, in the center of which the fungal pustule and the mycelial area were removed with a 1 mm diameter corkborer ("ring tissue"). Some "ring tissues" were stained with acetic acid-aniline blue (Romeis, 1968) and examined under microscope. On 13th day after infection, only about 3% of total mycelial mass in the leaf disks would be observed.
4. A leaf disk from infected leaf, 4 mm in diameter, in the center of which the fungal pustule and mycelia were contained ("pustule tissue").

The "ring tissue" and the peripheral leaf tissue of pustule in "pustule tissue" developed into the green island in the later stages of infection.

Preparation of crude extracts

Two hundred mg of leaf tissues (about 10 leaf disks) were ground in a mortar and pestle at 4°C with 2 ml of fresh grinding medium which consisted of 1 ml of 0.3 M KCl and 1 ml of 2×10^{-3} M EDTA in 0.5 M cysteine (adjusted to pH 7.5 with 3 N NaOH). The homogenate was subjected to centrifugation at 30,000×g for 20 minutes to removed particulate system. The supernant liquid was collected for protein and enzyme assays respectly.

Protein assay

Protein was precipitated by a concentration of 0.3 M trichloroacetic acid

in the sample and assayed by the methods of Lowry *et al.* (1951) with ctystalline bovine serum albumin as the standard.

Enzyme assay

The enzyme activities (O.D.×1,000/min/mg protein) were assayed by following the conversion of pyridine nucleotides in the reaction mixtures at a wave length of 340 nm by a spectrophotometer by the methods of Bergmeyer (1962) as modified by Lunderstädt (1964).

Chemicals

Glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), 6-phosphogluconate (6PG), fructose 1, 6-diphosphate (FDP), fructose 6-phosphate (F6P), adenosine triphosphate (ATP), reduced nicotinamide adenine dinucleotide (NADH), glucose 6-phosphate dihydrogenase (G6PDH) and glyceraldehyde 3-phosphate dehydrogenase/triose phosphate isomerase (G3PDH/TPI) nicotinamid adenine dinucleotide phosphate (NADP) were purchased from Boehringer & Soehne Co. Mannheim, Germany.

Reaction mixtures for enzyme assays

The following reaction mixtures (Tschen, 1966) were freshly prepared before use.

Glucose 6-phosphate dehydrogenase: 0.6 ml 0.05 M triethanolamine, 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.023 M G6P, 0.1 ml 0.013 M NADP, 1 ml plant extract.

6-phosphate dehydrogenase: 0.6 ml 0.05 M triethanolamine, 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.023 M 6PG, 0.1 ml 0.013 M NADP, 1 ml plant extract.

Hexokinase: 0.4 ml 0.05 M triethanolamine, 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.08 M NaF, 0.1 ml 0.1 M glucose, 0.1 M ATP, 0.1 ml 0.013 M NADP, 0.02 ml 0.1 mg/ml G6PDH, 1 ml plant extract.

Aldolase: 0.7 ml 0.05 M triethanolamine, 0.1 ml 0.1 M FDP, 0.1 ml 0.00425 M NADH, 0.02 ml 0.2 mg/ml G3PDH/TPI, 1 ml plant extract.

Phosphoglucoisomerase (PGI): 0.6 ml 0.05 M triethanolamine, 0.2 ml 0.1 M $MgCl_2$, 0.1 ml 0.1 M F6P, 0.1 ml 0.013 M NADP, 0.02 ml 0.1 mg/ml G6PDH, 1 ml plant extract.

Phosphoglucomutase (PGM): 0.6 ml 0.05 M triethanolamine, 0.2 ml 0.1 M $MgCl_2$, 0.2 ml 0.07 M G1P, 0.1 ml 0.013 M NADP, 0.02 ml 0.1 mg/ml G6PDH, 1 ml plant extract.

Results

From the beginning of development of pustules to the climax stages of fungal sporulation, the activities of enzymes of carbohydrate metabolism in

different leaf tissue disks were measured. The representative results of each enzyme system from two independent experimental series are shown in the figures (Fig. 1–6).

With the exception of aldolase, all investigated enzymes are enhanced from the beginning of pustule development to the climax stage of fungal sporulation, and the activities of enzyme are increased in proportion to the infection; the highest enzyme activities are in the "pustule tissues", the lowest activities in "healthy tissues". The activities of the "ring tissues" and of the "interior tissues" lie between these extremes; and the enzyme activities in the "interior tissues" are higher than that of "heathy tissues".

The activities of enzymes of hexose monophosphate shunt, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in "pustule tissues" are about 200% higher than in the "heathy tissues" (Fig. 4 and 5); the activities of glycolytic enzymes, phosphoglucoisomerase and aldolase, are only about 150–200% higher.

Activities of enzymes of the carbohydrate metabolism (except aldolase), all reached a maximum almost on 9th to 10th day after inoculation, and the maximal enzyme activities in the infected leaf tissues depended clearly on the climax of fungal sporulation, especially pronounced for the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

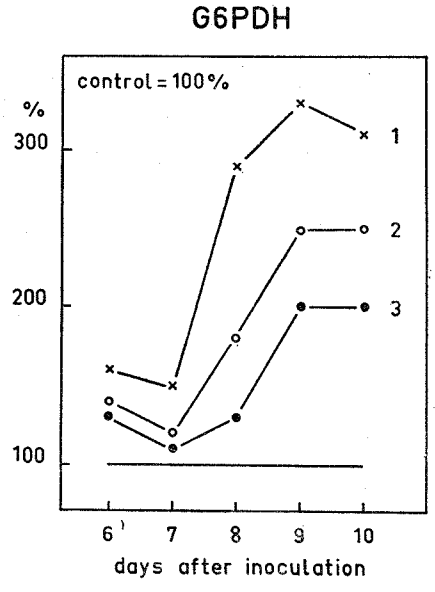

Fig. 1. Relative activities of glucose 6-phosphate dehydrogenase in different leaf tissues of bean after infection with *Uromyces phaseoli*. 1: Pustule tissue; 2: Ring tissue; 3: Interior tissue.

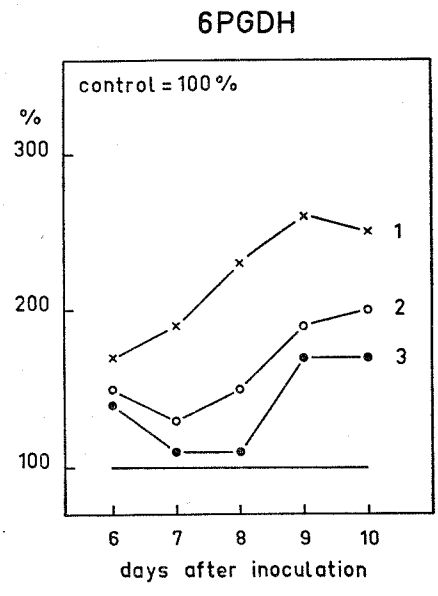

Fig. 2. Relative activities of 6-phosphogluconate dehydrogenase in different leaf tissues of bean after infection with *Uromyces phaseoli*. 1: Pustule tissue; 2: Ring tissue; 3: Interior tissue.

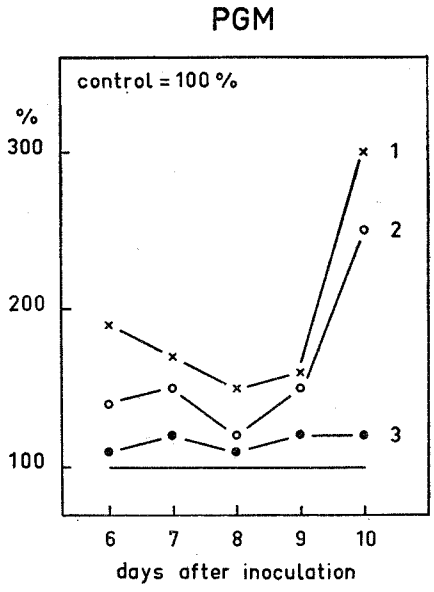

Fig. 3. Relative activities of phosphoglucomutase in different leaf tissues of bean after infection with *Uromyces phaseoli.* 1: Pustule tissue; 2: Ring tissue; 3: Interior tissue.

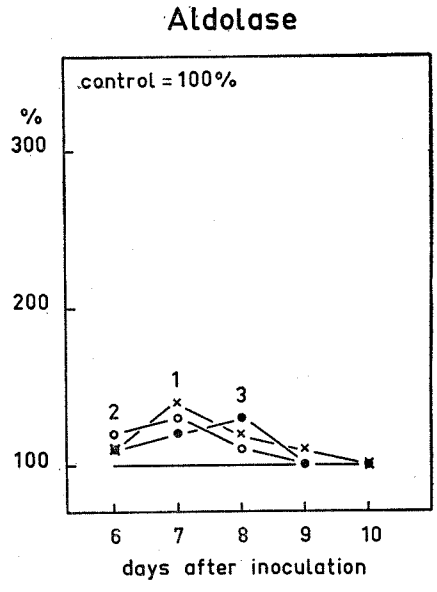

Fig. 4. Relative activities of aldolase in different leaf tissues of bean after infection with *Uromyces phaseoli.* 1: Pustule tissue; 2: Ring tissue; 3: Interior tissue.

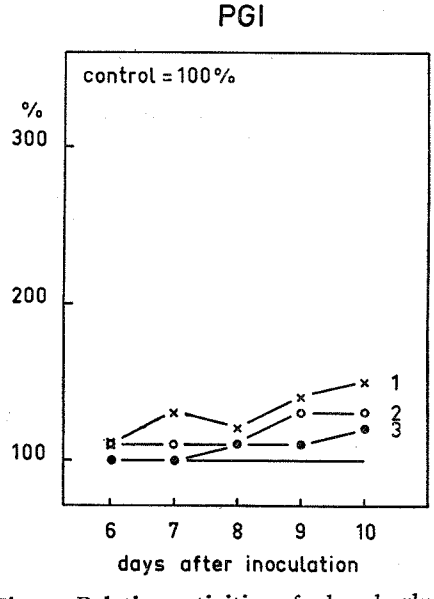

Fig. 5. Relative activities of phosphoglucoisomerase in different leaf tissues of bean after infection with *Uromyces phaseoli.* 1: Pustule tissue; 2: Ring tissue; 3: Interior tissue.

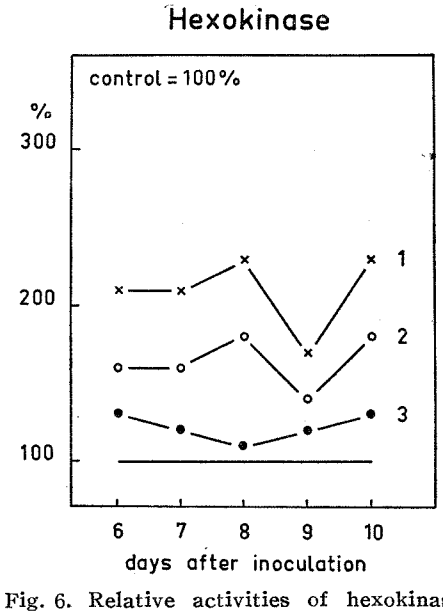

Fig. 6. Relative activities of hexokinase in different leaf tissues of bean after infection with *Uromyces phaseoli.* 1: Pustule tissue; 2: Ring it ssue; 3: Interior tissue.

Disscussion

Enhancement of the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase was found in cereal leaves after infection with rust (Kiraly and Farkas, 1962; Lunderstädt, 1964) or powdery mildew fungus (Scott and Smillie, 1962), while the high enzyme activities in "pustule tissues" are mostly due to the presence of pathogen, the higher enzyme activities in "interior tissues" or "ring tissues" should be explained by the response of these cells to pathogen. The exact nature of this influence of pathogen on plant cells is not yet known (Heitefuss, 1970).

Time course of enzyme activities in this study paralleled the respiration in rust infected bean leaf tissues prepared the same way (Tschen, 1966); the activities of phosphoglucomutase and hexokinase activities were highest at the time of respiration maximum.

Those results are in consistence with the assumption that intense enhancement of activities of phosphoglucomutase, hexokinase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in host tissues under the influence of obligate fungal pathogens results in an accelerative carbohydrate catabolism via hexose monophosphate shunt (Lunderstädt, 1964; Scott, 1965; Shaw and Samborski, 1957).

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銹菌誘導豆葉組織醣代謝酵素的變化

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四季豆（*Phaseolus vulgaris* L.）的初生葉在接種銹菌（*Uromyces phaseoli typica*）之後的第六天到第十天，每天取下各種盤形葉組織，分別分析一些醣類代謝酵素的活動性。

感染銹菌的豆葉組織，它的酵素活動性大體上隨著感染程度的增進而逐漸升高；升高的程度以 phosphoglucomutase, hexokinase, glucose 6-phosphate dehydrogenase 和 6-phosphogluconate dehydrogenase 比較顯着，phosphoglucoisomerase 和 aldolase 增加很少。各種葉組織當中以含有眞菌的「膿疱葉組織」的酵素活動性爲最高，其次是感染組織中眞菌已被除去的「環狀葉組織」再其次是膿疱間的「中間葉組織」，而以沒有感染的健康葉組織爲最低。