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Histochemical Demonstration of Non-specific Esterase in Wheat Root

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Abstract. Azo-coupling methods were used for demonstrating non-specific esterase in the wheat root in all parts of a transverse section, usually with the exception of the woody parts of the vascular bundle. The central cylinder gave a more intense reaction than the primary cortex and the rhizoderm. The reaction was not inhibited by dodecyl sulphate. A weakening of the reaction intensity was observed after application of $AgNO_3$. The Tween method did not yield reliable results.

The effect of humus substances on plant metabolism was investigated by a number of authors, e.g. ŠMÍDOVÁ (1960) who studied the effect of humate on the oxidase systems of the wheat root. The aim of the present work was to proceed from her results and pay greater attention to possible influences of humus substances on the hydrolytic enzymes of lipid metabolism. For studying this problem we have selected histochemical methods and thus the examination was extended to include carboxyl esterases in general.

Material and Methods

Seeds od winter wheat Pyšelka (*Triticum vulgare* VILL.) were germinated for 3 days on moist filter paper in Petri dishes at 25° C, the seedlings were then cultivated in aqueous cultures in the following solutions: (1) distilled water, (2) solution of the potassium salt of humic acid (Humussäure, Riedel-de Haen A. G.) at a concentration of 100 mg/litre, (3) Knop's nutrient solution diluted 1 : 3 with distilled water. Parts of roots about 7—11 mm from the root tip were fixed for 14 h in Baker's calcium formol at 3° C, washed for 2 days in Holt's sirup at the same temperature and saturated with glycerin-gelatin for 30 min at 37° C (BENEŠ 1962b). The material after such treatment was used for making thin transverse sections (20 μ), using a freeze-microtome.

A simultaneous azo-coupling method and the Tween method were used for demonstrating non-specific esterase.

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1. Simultaneous azo-coupling method. The substrates used were α-naphthylacetate, naphthol-AS-acetate and naphthol-AS-D-acetate; the diazonium salt Diazoechtblau BB (Rohner). The sections were incubated at 37° C either in 40 ml of 0·01M phosphate buffer at pH 6·4 with an addition of 1 ml of 1 % solution of substrate (α-naphthylacetate was dissolved in ethanol, the other substrates in dimethylformamide) and of 50 mg diazonium salt (BENEŠ 1962b), or else in 40 ml 0·05M veronal buffer of pH 7·4; concentrations of substrate and of the diazonium salt were identical. The solutions were filterd before incubations. Duration of incubation was 30 min. when using α-naphthylacetate and 60 or 120 min. when using the other esters. Control material was incubated in the corresponding buffer after adding the diazonium salt and the solvent. A positive reaction was revealed by formation of an azo-dye in the place of enzyme activity: it was blue-black for α-naphthylacetate, light-blue for naphtol-AS--acetate and light-purple for naphtol-AS-D-acetate.

2. The Tween method. The substrates used were Tween 40 and 60 (Lachema), Tween 20 and Span 80 (Light and Co.). We followed the procedure of GOMORI (1952) with the difference that the substrate was dissolved in a veronal buffer of pH 7·4 and the duration of incubation was 24 h. After incubation the sections were submerged in a 1 : 9 diluted commercial solution of yellow ammonium sulphide. The control material was incubated in a solution without substrate.

The following inhibition tests were carried out in both methods for demonstrating the nature of the enzyme involved: (1) Incubation in 10^{-2}M dodecyl sulphate (LOJDA 1958) in a veronal buffer of pH 7·4. After 60 min the other components of the incubation medium were added to the solution (either α-naphthylacetate and Diazoechtblau BB or Tween 20 or 40) and incubation continued for another 60 min with α-naphthylacetate or for 24h after adding Tween. (2) Incubation in $2 \cdot 10^{-2}$M $AgNO_3$ in water for 60 min and after adding α-naphthylacetate and diazonium salt for another 60 min. It was impossible to use a buffer on account of a precipitate of Ag^+ with Cl^- or PO_4^{3-} being formed.

After terminating the incubation the sections were washed and mounted in glycerin—gelatin.

Results

Azo-coupling methods revealed the presence of non-specific esterase in the protoplasts of almost all cells of a transverse section through the wheat root, usually with the exception of the woody elements of the vascular bundle. The positive reaction after incubation with α-napthylacetate appeared more intense in the central cylinder than in the primary cortex and in the rhizoderm. The difference was very pronounced after reaction with naphtol-AS-acetate. Under greater magnification the granular character of the precipitate was well--apparent. The reaction was very intense on using α-naphthylacetate, it was weaker with naphthol-AS-acetate and very weak with naphtol-AS-D-acetate. The course of the enzyme reaction did not depend on the buffer used. In the presence of a diazonium salt the cell membranes were stained yellow. In preparations with remnants of protoplasm inside the vessels a positive reaction appeared in these cells, too. The presence of dodecyl sulphate did not affect the course of the reaction. After incubation with $AgNO_3$ the reaction intensity was weakened.

The Tween method made it possible in some cases to demonstrate differences from the control but the results were mostly unreliable.

The enzyme localization was identical in all wheat roots cultivated under different nutrient conditions.

Discussion

Several substrates were used for demonstrating the non-specific esterase by an azo-coupling method. α-naphthylacetate and naphtol-AS-acetate were found to be suitable for histochemical demonstration of the enzyme in wheat roots. In all cases the central cylinder gave a higher response than the primary cortex and the rhizoderm; this difference was best apparent with naphtol-AS-acetate. The evaluation of the reaction intensity was complicated by the heavy vacuolization of the protoplasts of the cortex cells. The yellow stain of cell membranes after incubation with diazoechtblau BB was readily distinguished from a positive reaction. The differences in the reaction intensity in different parts of the section when using naphtol-AS-acetate were generally in agreement with the findings of BENEŠ (1962a, b) who used *Vicia faba* roots.

The substrates used here can be hydrolyzed by a number of enzymes (see e.g. BENEŠ 1962b). The nature of the enzyme involved could not be determined by applying various inhibitory agents. It may be concluded from the results with dodecyl sulphate that we were dealing here with a non-specific esterase rather than with a lipase. The enzyme examined could also have been a peptidase, as shown by the partial inhibition with silver nitrate.

Histochemical methods have been used only rarely for demonstrating non-specific esterase in plant materials. The particle-bound enzyme was described by BENEŠ and co-workers (1961). WAŁEK—CZERNECKA (1963) demonstrated histochemically the presence of non-specific esterase in the spherosomes of epidermal cells of Allium cepa. OLSZEWSKA and co-workers (1965) distinguished histochemically between several non-specific esterases in the spherosomes of cells of the epiderm and the root meristem of *Allium cepa* and in the epiderm of *Haemanthus lantharinae* and *Ornithogallum umbellatum*.

Special attention was not devoted in this article to the localization of the non-specific esterase on a subcellular level.

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Azokopulačními metodami byla nespecifická esteráza prokázána v kořeni pšenice ve všech částech příčného řezu, obvykle s výjimkou dřevních částí cévního svazku. Střední válec jevil intensivnější reakci než primární kůra a rhizodermis. Reakce nebyla inhibována dodecylculfátem. K částečnému zeslabení intenzity reakce došlo po aplikaci $AgNO_3$. Tweenovou metodou nebyly získány spolehlivé výsledky.

Я. ГАВРАНКОВА, Кафедра физиологии растений и биологии почвы факультета естествознания Карлова университета, Прага: **Открытие неспецифической эстеразы в корне пшеницы гистохимическим методом.** — Biol. Plant. 8 : 452—455, 1962.

Азокопуляционными методами доказана неспецифическая эстераза в корне пшеницы во всех частях поперечного среза, обычно с исключением ксилемной части сосудисто-волокнистого пучка. В осевом цилиндре реакция была более интенсивной чем в первичной коре и ризодерме. Реакция не ингибировалась додецилсульфатом. К частичному ослаблению интенсивности реакции привело применение $AgNO_3$. Твиновым методом не были получены надежные результаты.