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Peroxidase Activity and Isoenzyme Patterns in Wheat during Ontogenesis

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Abstract. Protein content, total and specific peroxidase activity and isoperoxidase patterns were determined in crude protein preparations from individual parts of field-grown wheat (*Triticum aestivum* L., cv. Jubilar). Protein content in roots, leaves, and stalks increased at the beginning of ontogenesis and then decreased from 6th, 9th, and 10th development phase (according to Feekes), respectively. Steady increase of the protein content in the ears was observed.

Highest peroxidase activity was found in the roots; it diminished from the onset of ontogenesis till maturity of the plants. In the leaves and stalks a slight decrease of peroxidase activity till the 10th development phase and then an increase till maturity was found. The ears exhibited a gradual increase of peroxidase activity. The course of specific peroxidase activity was found to be very similar to that of total activity.

Isoperoxidase patterns did not change significantly. In the leaves, a decrease of activity of C_4 and C_5 isoperoxidases was recorded. In the stalks, C 1 isoenzyme emerged at the end of ontogenesis. A gradual increase of A_1 and A_5 isoperoxidase intensity took place both in the leaves and stalks.

Peroxidase is a widespread enzyme within plant tissues. Almost in all tissues studied a considerable number of peroxidase isoenzymes was detected (*e.g.* STAFFORD and BRAVINDER-BREE 1972, MARKLUND *et al.* 1974, HOYLE 1977). A number of studies have dealt with changes of peroxidase activity and isozymic patterns during some growth processes (WOLTER and GORDON 1975, LABERGE 1975, CHAPPET and DUBOUCHET 1972, GASPAR *et al.* 1974, GASPAR 1979) and with isolation and characterization of some isoperoxidases (PAUL and STIGBRAND 1970, MORITA *et al.* 1970, 1971, KIM *et al.* 1980). The subcellular localization of peroxidase has also been extensively studied (ALVARES and KING 1967, HALL and SEXTON 1972, OSBORNE *et al.* 1972, DARIMONT and BAXTER 1973, RAA 1973). In spite of these efforts it is still not clear which peroxidase activities found *in vitro* (*e.g.* oxidation of various phenolics, IAA, and NADH, H_2O_2 production) are true in *in vivo* functions. Also detailed knowledge of the *in vivo* functions of individual isoenzymes is still lacking.

Parallel studies of activities and properties of individual isoenzymes *in vitro* and of the changes in occurrence and activities of these isoenzymes

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during the plant ontogenesis might be helpful in completing our knowledge. Recently, we isolated and characterized isoperoxidase B 1 from wheat seedlings (ZMRHAL and MACHÁČKOVÁ 1978). Isolation and characterization of other isoenzymes is in progress. The present paper reports on the changes of the peroxidase activity and of its isoenzyme patterns in wheat plants during ontogenesis and discusses the possible dependence of growth processes on these changes.

MATERIAL AND METHODS

Winter wheat (*Triticum aestivum* L., cv. Jubilar) was grown in the field (in Praha-Ruzyně region). The field was fertilized in autumn with 120 kg N, 90 kg P, and 120 kg K per ha. Sampling was performed in the following phases of development (according to Feekes's scale): 2, 3, 4, 5, 6, 7, 8, 10, 11.1, 11.3, 11.5, 12, and 12.2. At the onset of ontogenesis plant samples were separated into leaves and roots. Starting from the 6th phase of development the stalks and from the 11th phase, the ears were analyzed separately.

Enzyme Isolation

Plant material was homogenized and at the same time cell sap was pressed out of homogenized material (with the exception of roots) on a pre-cooled stainless fruit press in the presence of 0.5 M potassium phosphate buffer pH 6.0 with 1% thiourea to inhibit polyphenoloxidase (MACHÁČKOVÁ *et al.* 1975). The roots were homogenized with sand using a mortar and pestle. Both methods of homogenization were previously shown to give equal yields of protein material. Homogenates were filtered through cheesecloth and centrifuged for 20 min at 20 000 *g*. Proteins of supernatant were precipitated with solid $(NH_4)_2SO_4$ to 90% saturation. The precipitate was dissolved in 1% thiourea solution and chromatographed on a Sephadex G-25 column. The elution was performed with redistilled water. Peroxidase activity of the eluted fractions was controlled by *o*-phenylenediamine-H_2O_2 reagent (1 : 1 mixture of saturated solution of *o*-phenylenediamine and 1% H_2O_2). All isolation procedures were conducted at 4 ± 1 °C. The protein fraction exhibiting peroxidase activity was freeze-dried and used for determination of protein content, peroxidase activity and electrophoretic isoperoxidase patterns.

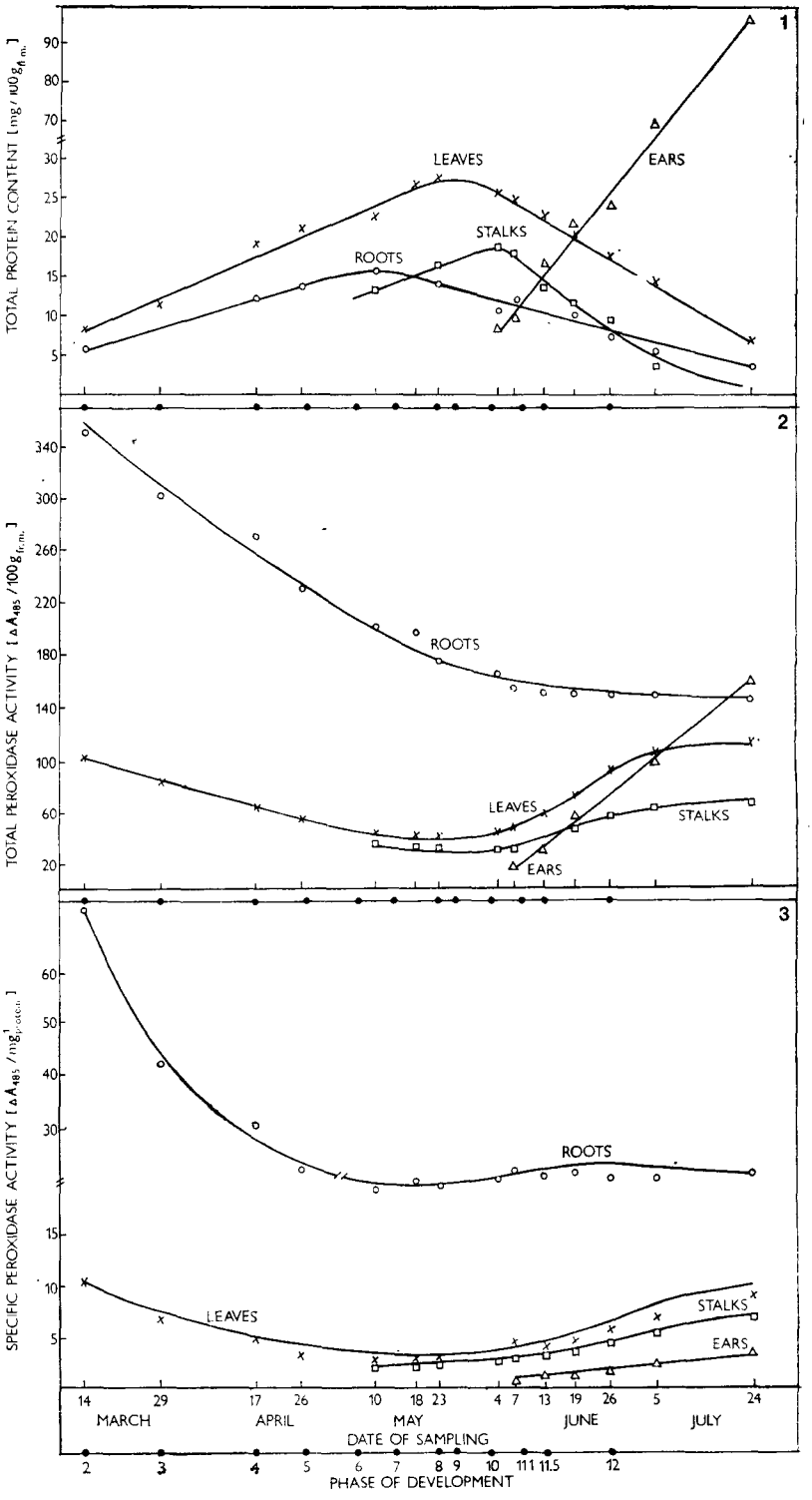
Electrophoresis

was run in 10% starch gel in 0.01 M borate buffer pH 9.0 at 15 V cm^{-1} for 3 h at 0 °C. Freeze-dried protein samples were stored at 4 °C and electrophoresis was run in large starch-gel blocks with all samples at once. Detection was accomplished using *o*-phenylenediamine reagent (see above). Peroxidases were detectable as yellow-brown zones, the intensity of which was estimated visually.

Peroxidase Activity

was determined spectrophotometrically at $\lambda = 485$ nm with 10^{-4} M *o*-phenylenediamine as substrate using 10^{-4} M H_2O_2 at 30 °C. Specific enzyme

Abbreviations used: IAA = indole-3-acetic acid; NADH = reduced nicotinamide adenine dinucleotide.


1
TOTAL PROTEIN CONTENT [mg/100 g fr.m.]
LEAVES
EARS
STALKS
ROOTS
2
TOTAL PEROXIDASE ACTIVITY [ΔA485/100 g fr.m.]
ROOTS
LEAVES
STALKS
EARS
3
SPECIFIC PEROXIDASE ACTIVITY [ΔA485/mg protein]
ROOTS
LEAVES
STALKS
EARS
14 29 17 26 10 18 23 4 7 13 19 26 5 24
MARCH APRIL MAY JUNE JULY
DATE OF SAMPLING
2 3 4 5 6 7 8 9 10 11 11.5 12
PHASE OF DEVELOPMENT

activity is expressed as the change of A_{485} caused by 1 mg enzyme preparation within 60 s. Total peroxidase activity is the activity (ΔA_{485}) of enzyme preparation gained from 100 g of fresh plant material.

Proteins

were assayed according to LOWRY *et al.* (1951) with crystalline serum albumin as a standard. The freeze-dried protein preparation was dissolved in 0.05 M phosphate buffer.

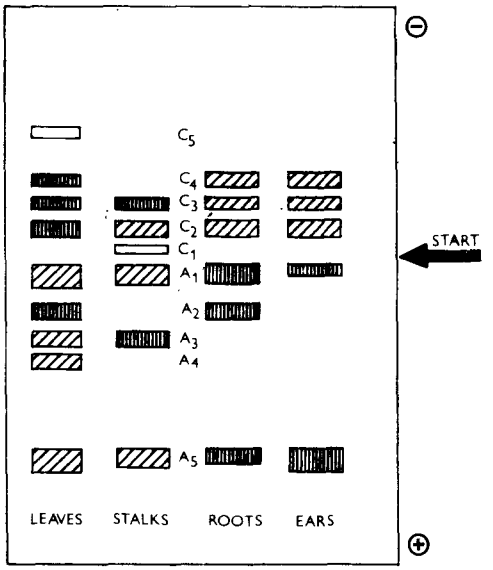


Fig. 4. Representative isoperoxidase patterns in various plant parts. — Order of increasing activities: blank, obliquely hatched, and vertically hatched areas.

RESULTS

At the onset of ontogenesis, protein content was higher in the leaves than in the roots (see Fig. 1.). In the roots, protein content increased to 6th development phase and then decreased till maturity of the plants. A similar pattern was found in the leaves and stalks, but the decrease started at 9th and 10th development phase, respectively. A steady increase of protein content was observed in the ears (see Fig. 1).

The pattern of total peroxidase activity in the course of ontogenesis in different plant parts may be seen in Fig. 2. The roots exhibited high peroxidase activity while the stalks and leaves a relatively low one. Peroxidase activity in the roots was very high at the onset of ontogenesis and then gradually decreased till maturity. A slight decrease of peroxidase activity till the 10th development phase and then an increase till maturity was

Figs. 1, 2, 3. The course of total protein content (Fig. 1), total peroxidase activity (Fig. 2) and specific peroxidase activity (Fig. 3) in individual parts of wheat plants during ontogenesis.

recorded in the leaves and stalks. The ears exhibited a gradual increase of peroxidase activity.

The course of specific peroxidase activity was found to be similar to that of total activity (see Fig. 3). The specific activity decrease in the roots is very rapid and there is almost no further change from 6th development phase. Changes of specific activity in the leaves, stalks, and ears are less pronounced than those of total activity.

Representative isoperoxidase patterns of individual plant parts are shown in Fig. 4. Isoperoxidase patterns did not change significantly during ontogenesis, only smaller, mostly quantitative differences were observed. A gradual decrease of intensity of C_4 and C_5 isoperoxidases took place in the leaves. These isoenzymes were no more detectable after the 10th phase of development. On the other hand, intensities of A_1, A_5, C_2, and C_3 bands increased. A new isoenzyme C_1 emerged at the end of ontogenesis in the stalks and also a slight increase of intensity of A_1 and A_5 bands was recorded. The intensity of C_2 and C_3 isoenzymes gradually increased and C_4 emerged in the last two samplings in the ears. At the same time, the intensity of anodic bands A_1 and A_5 diminished. A gradual decrease of intensity of all bands, especially of cathodic ones, was recorded in the roots.

DISCUSSION

In spite of some doubts about the reliability of gel electrophoresis for studies of isoperoxidase patterns (JENNINGS and STREET 1974), it is quite evident today that many isoperoxidases occur in plant tissues. Their different subcellular localization together with different substrate specifities point to differences of functions between various isoenzymes.

High peroxidase activity (especially of cathodic isoenzymes) in roots at the onset of ontogenesis might be explained as a protection against supra-optimal IAA level. It is well known that some isoperoxidases act in some cases as IAA oxidase (MACHÁČKOVÁ and ZMRHAL 1974). GASPAR and VERBEEK (1974) ascribed the role in auxin catabolism in lentil and barley to the most cathodic isoperoxidase. Moreover, it was found that rhizogenesis is accompanied by the appearance of specific isoperoxidases (GASPAR *et al.* 1974).

The occurrence of peroxidase activity in the ears was to be expected, as it is known that dry kernels contain peroxidase (KRUGER and LABERGE 1974).

Often a direct correlation between peroxidase activity and growth intensity was found (CHAPPET and DUBOUCHET 1975, WOLTER and GORDON 1975). On the other hand, GARDINER and CLELAND (1974) described that cessation of pea epicotyl elongation was accompanied by an increase in activity of some soluble and cell wall isoperoxidases. We observed similar phenomenon: cessation of growth of wheat plants was accompanied by increase of total peroxidase activity in the stalks and leaves. Moreover, the intensity of A_1 and A_5 isoperoxidases was found to increase in the stalks and leaves during ontogenesis. These two isoenzymes were recently studied in detail (ZMRHAL and MACHÁČKOVÁ 1978) — in that work they were designated B 1 and D 4, respectively. It was shown that D 4 (A_5) is an isolation artefact formed through B 1(A_1) decomposition. It was further proposed that cell wall-bound B 1(A_1) isoenzyme might be very active in lignin formation. This isoenzyme

was found to be concentrated in xylem sclerenchyma tissue either in the leaves or in the stalks (SEIDLOVÁ and ZMRHAL — in preparation). Thus, it is evident that cessation of growth in wheat plants is accompanied by an increase of total peroxidase activity (which might cause increased IAA oxidation) and by an increase of occurrence of an isoperoxidase active in lignin formation.

To better understand the role of cathodic and slow anodic isoperoxidases, further studies on these isoenzymes are needed.

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BOOK REVIEW

BOTHE, H., TREBST A. (ed.): BIOLOGY OF INORGANIC NITROGEN AND SULFUR. — Springer Verlag, Berlin—Heidelberg—New York, 1981. 384 pp., 144 figs. Cloth DM 89.—; approx. US $ 46.80.

The book contains all the contributions to the Conference held in May, 1981, in Bochum (Federal Republic of Germany), where all aspects of both the nitrogen and the sulfur cycles were discussed. In addition, important data were communicated by German scientists of the National Program on the Metabolism of Inorganic Nitrogen and Sulfur Compounds.

Both the nitrogen and the sulfur cycles are interconnected with the biological cycles of matter, with the production of biomass by plants and bacteria, as well as with the routes of mineralization.

In the majority of plants, the inorganic nitrogen requirement is met by uptake of ammonium and/or nitrate from the soil solutions. Besides these forms of nitrogen, certain plants, in symbiotic association with various microorganisms, are able to use atmospheric nitrogen to indirectly fulfill the need of nitrogen. In all these symbiotic associations nitrogen fixation is apparently mediated by the enzyme nitrogenase.

Nitrate taken up by plants must be reduced to ammonia before the nitrogen can be assimilated into organic form. Nitrate reduction to ammonium depends on the activities of enzymes. These topics have been discussed in the presentation of Losada *et al.* in this volume.

Ammonium taken up from the soil solutions or produced as a result of nitrate reduction or symbiotic nitrogen fixation is incorporated into organic form in plants. Formerly it was considered that this conversion occurs through the activities of the enzyme glutamic acid dehydrogenase which in the presence of α-ketoglutarate and reduced pyridine nucleotide and ammonium, catalyzes the synthesis of glutamate. It is now generally considered that under most physiological conditions the initial incorporation of ammonium is into glutamine through the activity of glutamine synthetase and a subsequent conversion of glutamine to glutamate. This problem is widely discussed by Beevers.

The genetics of dinitrogen fixation, some aspects of the physiology of dinitrogen fixation, its biochemistry, pathways and regulatory aspects of N_2 and NH_4^+ assimilation in N_2-fixing bacteria have been summarized in contributions of Brill, Neumann, Postgate *et al.*, Zumft, Kleiner and Both, in a special chapter.

The roles of sulfur and nitrogen in cell nutrition show many similarities. Both must be reduced by living systems to form proteins and other macromolecules enriched by macroelements required by plants and plant-like microorganisms. Nutritional interaction between the two elements has been described and both of them show a rather uniform distribution throughout the tissues and organs of higher plants and among cells of microorganisms. The present state of knowledge of the assimilatory sulfate reduction is presented in a special chapter which also contains data on enzymatic reactions which may connect sulfur and nitrogen metabolism. There are also included papers dealing with the role of thioredoxins in enzyme regulation in cyanobacteria, which are involved in assimilatory sulfate reduction in higher plants.

Aspects of S- and N-metabolism in tobacco cell suspension cultures have been described by Bergmann.

Each chapter is finished by the list of references.

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