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ISOENZYME POLYMORPHISM IN FLOWERING PLANTS—VII.

ISOENZYME VARIATIONS IN TISSUES OF BARLEY SEEDLING*

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Abstract—The isoenzyme characteristics of several enzymes in barley (*Hordeum vulgare*) var. Blanco Mariout, in relation to tissue differences at an early stage of plant development were studied. Extracts of root, coleoptile, and the first leaf of 8-day-old seedlings were analyzed by the use of starch and polyacrylamide horizontal gel electrophoresis techniques. The isoenzyme patterns of the following enzymes were studied: peroxidases, catalases, esterases, acid and alkaline phosphatases, leucine aminopeptidases, amylases, and glutamic-oxal-acetic transaminases. The data regarding the similarities and differences in the isoenzyme composition of the enzymes in the three tissues are presented.

INTRODUCTION

EXTENSIVE studies have been made of the multiple forms and differential electrophoretic mobilities of enzyme components during growth and development of various animal tissues,[1] but limited information is available concerning plant tissues. Electrophoretic studies of enzyme components would give information concerning the changes in the form of the enzymes concerned with the variations in the morphology and physiology during plant development.

The isoenzymic variation as well as changes in the soluble proteins, both immediately after seed germination and during seedling growth and development, have been studied in wheat.[2–4] However, information on similar changes in barley is rather limited. The purpose of this paper is to report the electrophoretic patterns of some of the enzymes present in different tissues during the early development of barley (*Hordeum vulgare*) var. Blanco Mariout.

RESULTS

Isoenzymes of certain enzyme systems were studied to investigate differences between root, coleoptile and first leaf tissue extracts of the 8-day-old seedlings of barley.

Esterases (Est.)

The characterization of esterase isoenzymes was made using two different substrates, α-naphthyl and β-naphthyl acetate. In all, eighteen bands were observed on the anode side of the gel using α-naphthyl acetate (Fig. 1, Table 1). The first leaf tissue showed the highest

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level of esterase activity and had fourteen bands; isoenzymes 6a, 9a, 15a, and 18a being absent. The root also had fourteen esterase isoenzymes; bands 2a, 13a, 14a, and 15a being absent in this case. The coleoptile showed sixteen isoenzymes; bands 13a and 18a being

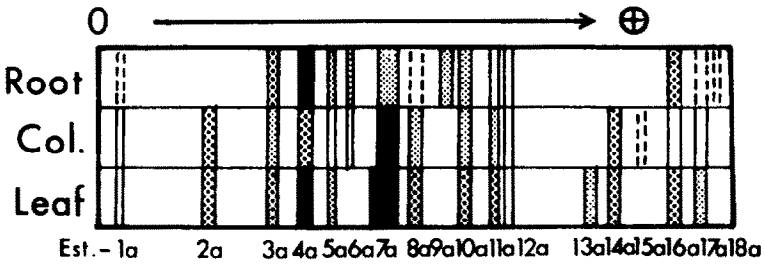

FIG. 1. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF ESTERASES.

Key to the shading in Figs. 1 to 7: (Band intensities classified as 1 to 5, 5 for intense staining, as in Table 1)

Traces of activity, band intensity 1.

Band intensity 2.

Band intensity 3.

Band intensity 4.

Band intensity 5.

TABLE 1. NUMBER AND INTENSITIES OF NONSPECIFIC ESTERASE ISOENZYME IN BARLEY TISSUES UTILIZING ALPHA- OR BETA-NAPHTHYL ACETATES AS SUBSTRATES

		Isoenzymes*																
Substrate	Tissue	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12a	13a	14a	15a	16a	17a	18a
Alpha-naphthyl acetate	Root	1	—	4	5	3	3	3	1	3	3	2	2	—	—	—	4	1	1
	Coleoptile	2	4	3	4	2	2	5	3	—	3	3	2	—	4	1	2	2	—
	First leaf	2	4	4	5	3	—	5	4	—	4	4	2	3	4	—	4	3	—
Beta-naphthyl acetate	Root	—	—	3	5	3	—	2	1	1	3	2	—	—	—	—	4	—	—
	Coleoptile	1	—	2	2	2	—	4	1	1	2	1	—	—	2	—	—	—	—
	First leaf	2	—	4	5	3	—	5	3	2	3	2	—	3	4	—	4	—	—

* Isoenzyme bands were classified by different intensities of staining: — no reaction to 5 for intense staining.

missing. Certain isoenzymes were common to all three tissues, but showed differential activities; others were specific to either one or two of the tissues. Thus, isoenzymes Est-9a and 18a were found only in the root, Est-15a was specific to the coleoptile, and Est-13a was found to be present only in the leaf. Est-2a and 14a were common to both coleoptile and leaf but absent in the root; Est-6a was common to both root and coleoptile but absent in the leaf.

When β-naphthyl acetate was used as the substrate, some difference was noted in the number of bands and their intensities (Table 1). In the root extract, isoenzymes Est-1a and 18a did not show any activity, and Est-15a and 16a showed no activity in coleoptile extract. Isoenzymes Est-2a, 6a, 12a, and 17a did not show any activity in any of the three tissue extracts. These observations clearly indicate that the isoenzymes Est-2a, 6a, 12a, 15a, 17a, and 18a were only active with α-naphthyl acetate as a substrate. There are a number of reports on the study of multiple isoenzymes of esterases in plants which indicate variation in the isoenzymes of esterases among different parts of the same species and among different species in a genus and among different genera.[5–8]

Alkaline Phosphatases (Alph.)

No alkaline phosphatase activity was detected in any of the three tissues studied.

Acid Phosphatases (Adph.)

Acid phosphatase isoenzymes in the three extracts are shown diagrammatically in Fig. 2. All the isoenzymes showing acid phosphatase activity were found to move towards the anode.

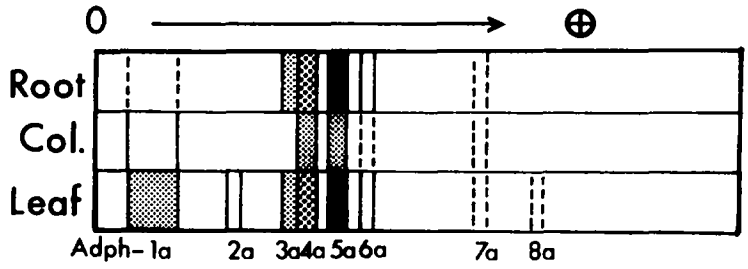

FIG. 2. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF ACID-PHOSPHATASES.

In all, there were eight acid phosphatase isoenzymes in the three tissues, all of which were found in the leaf. Isoenzymes Adph-2a and 8a were absent in roots, and Adph-2a, 3a, and 8a were absent in the coleoptile. These differences in the number of acid phosphatase isoenzymes in the three tissues are related to the minor bands only; the major bands Adph-4a and 5a were present in all three tissues.

Leucine-Aminopeptidases (LAP)

The three LAP isoenzymes detected were also found to move toward the anode (Fig. 3). In the root and coleoptile tissues isoenzyme LAP-3a was absent, whereas in the first leaf all three isoenzymes were found. The activity of isoenzyme LAP-1a was strongest in the root and leaf tissue. In general, the coleoptile had the lowest activity of the leucine-aminopeptidase isoenzymes.

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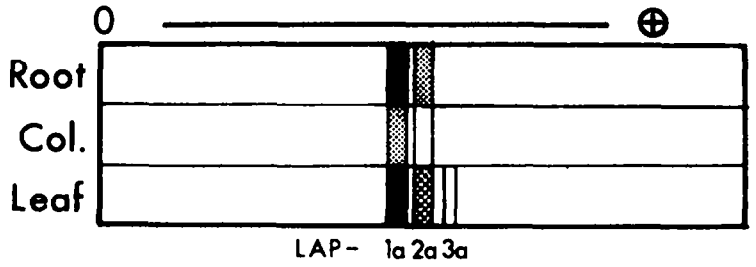

FIG. 3. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF LEUCINE AMINOPEPTIDASES.

Peroxidases (Px)

The isoenzyme pattern of the peroxidases stained with *o*-dianisidine as the hydrogen donor is shown in Fig. 4. The peroxidase isoenzymes were detected on both anode and cathode sides of the gel. On the anode side, ten isoenzymes were present in the three tissues, whereas on the cathode side there were seven. In root, Px-1a, 2a, 9a, 10a and Px-2c was absent. The coleoptile contained all but one of the isoenzymes, and the first leaf extracts all but five. The isoenzymes Px-6a, 7a, and 8a were found to be specific to the coleoptile; Px-3a, 4a, and 5a were common to both first leaf and the coleoptile; the coleoptile had Px-1c in common with the root and Px-2c in common with the first leaf; and isoenzyme Px-5c was found to be specific to the root tissue. In general, the coleoptile showed highest peroxidase activity and the first leaf tissue the lowest of the three tissues.

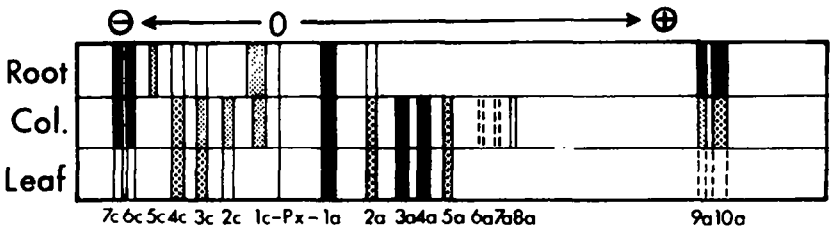

FIG. 4. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF PEROXIDASES, DONOR: O-DIANISIDINE.

Catalases (Cat.)

The catalases were resolved on the starch gels, and are shown diagrammatically in Fig. 5. Although the isoenzyme bands did not show gross differences among the three tissues, the gels did give evidence regarding the differential activity of catalases. The first leaf tissue extracts exhibited the strongest catalase activity, the coleoptile had rather low activity, and the root the lowest activity.

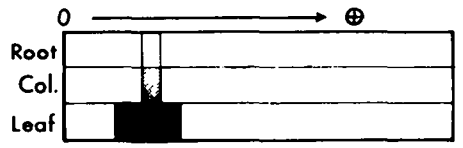

FIG. 5. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF CATALASES.

Amylases (Amy)

The diagrammatic pattern of the amylase isoenzymes is shown in Fig. 6. The amylase isoenzymes were found to move towards the anode. Seven isoenzymes were detected in the tissues and the root and the coleoptile had all seven and similar levels of activity. The leaf tissue, however, showed lower amylase activity, and Amy-2a was missing. Two of the seven bands, Amy-1a and 2a, differed distinctly from the rest of the bands in giving a reddish tinge with the detecting reagent and suggest that they might be β-amylases.

Frydenberg and Nielsen[9] studied amylase isoenzymes in germinating barley seeds of ninety-eight varieties. The amylase isoenzymes were found by them to move towards the cathode in agar gel (pH 7·3) although the amylases are expected to be anodic, considering their isoelectric points (5·70–5·75), which are lower than the buffer pH of the agar gels. The cathodic movement was explained by them to be due to the excessive endosmotic flow characteristic of agar gel electrophoresis[10] and apparently this flow proceeds faster towards the cathode than the amylases move anodally in relation to the buffer. They were able to detect nine bands showing amylase activity, seven of which could be classified either as α- or β-amylases. The two fastest-moving bands were characterized as β-amylase isoenzymes and the next five as α-amylases.

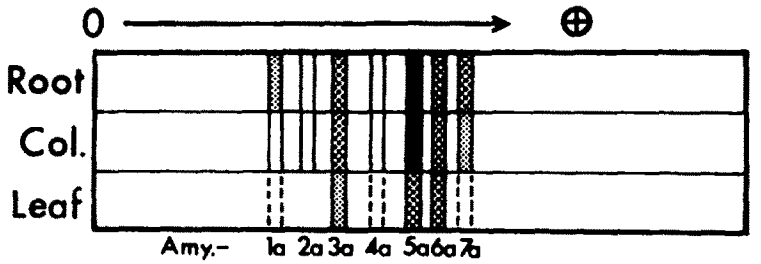

FIG. 6. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF AMYLASES.

The present observations on polyacrylamide gel (pH 8·3) showed the amylases to move towards the anode. Of the seven amylase isoenzymes recorded during the present studies, the slower two showed β-amylase characteristics, while the remaining five had α-amylase activity. These observations seem to be in agreement with those of Frydenberg and Nielsen.[9] However, the two additional unidentified bands observed by Frydenberg and Nielsen[9] were not detected during the present studies. It could be possible that these isoenzymes are specific to the germinating seeds, and are missing in the seedling tissues.

Glutamic-Oxalacetic Transaminases (GOT)

Six GOT isoenzymes were detected in the extracts of the three tissues of barley seedlings (Fig. 7). The leaf extracts had the highest activity of the isoenzymes, while the extracts from the root and the coleoptile had lower activities. However, the glutamic-oxalacetic transaminases showed a uniform distribution maintaining the same type and number of isoenzymes among the three tissues. It is interesting to note that Leonard and Burris[11] had found higher percentage of GOT transamination activity in the leaf extracts as compared to the root extracts of 2-week-old barley seedlings.

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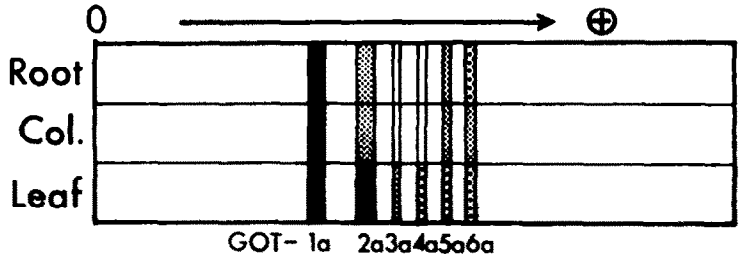

FIG. 7. ZYMOGRAM SHOWING THE ELECTROPHORETIC PATTERNS OF GLUTAMIC-OXALACETIC TRANSAMINASES IN ROOT, COLEOPTILE AND FIRST LEAF OF 8-DAY-OLD SEEDLINGS OF BARLEY (*Hordeum vulgare*) VAR. BLANCO MARIOUT.

It has been postulated that GOT is of considerable significance due to its role in glutamate oxidation.[12] Later studies of Smith and Williams[13] have also indicated that in plants the transaminases may play an indirect role in protein synthesis by their action on the interconversion of amino and keto acids.

DISCUSSION

The electrophoretic characterization of isoenzyme variation has been applied in genetics, morphogenesis, physiology, biochemistry and pathology in both plants and animals.[1,5–8,14–16] These studies illustrate that although all the cells in different tissues of a plant carry the same genetic information, the manifestation of the phenotype as expressed in the synthesis of enzyme proteins is specific and well-regulated for each stage of development.

The differential activation of genes in developing cells is an essential aspect of cell differentiation.[17] Although hormones have been suggested to play a role in the depression of the genes,[18] the mechanisms by which the genes are differentially activated are still not known and thus offer an interesting area of research.

The isoenzymic differences between root, coleoptile and first leaf of 8-day-old seedlings presented in this report further confirm the observations that different tissues have their own specific isoenzymic pattern. These results also show that certain isoenzymes in each of the enzyme systems are present in all the tissues, probably at all the stages of development; whereas certain others appear or disappear in different tissues during differentiation and growth. The study of the biochemical characteristics of such isoenzymes could lead to an understanding of the mechanisms which lead to the turning off and turning on of the synthesis of these isoenzymes by the controlling gene or genes, depending upon the needs of the different tissues during different phases of growth and development.

MATERIALS AND METHODS

Dry seeds of barley (*Hordeum vulgare*), var. Blanco Mariout, were germinated on wet filter paper in Petri dishes at 20° for 48 hr and then grown under continuous illumination in

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a control environment. The seedlings were harvested after 8 days. The leaf, the coleoptile and the roots were separated from each of the seedlings and similar tissue bulked together from all the seedlings. The tissue samples were either used fresh for extraction or were frozen at $-10°$ for later use.

Extraction was done by grinding in a mortar, 1 g of the tissue with 100 mg of acid-washed sand, and 0·1 ml of a freshly prepared mixture of two parts of 12·5 per cent glucose in 0·02 M Trizma (tris(hydroxmethyl)aminomethane-HCl) base solution (adjusted to pH 7·5 with HCl) and one part of an aqueous solution of 0·8 per cent NaCl + 0·02 per cent $NaNO_3$. The extracts were drawn into suitable size wicks cut from Beckman filter paper wicks (No. 319329) and applied directly to the macerated tissue.

The general horizontal gel electrophoretic procedures of Brewbaker *et al.*[19] were followed for isoenzyme separation and staining in starch and polyacrylamide gels. The procedure of Decker and Rau[20] was employed to stain for the glutamic-oxalacetic transaminases. The enzymes LAP, catalases and GOT were resolved and stained on starch gel and the rest on the polyacrylamide gel.

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