BIOLOGIA PLANTARUM 37 (3): 375-379, 1995

Tissue-specific expression of esterase isoenzymes in *Linum usitatissimum* L.

S.I. YURENKOVA, L.V. KHOTYLEVA and Y.V. TSEBRIKOV

Institute of Genetics and Cytology, Academy of Sciences of Belarus, F. Skorina str. 27, 220072, Minsk, Belarus

Abstract

Esterase isoenzyme spectra of different organs of seedlings and field-grown plants of fiber flax (*Linum usitatissimum* L., cv. Belinka) were studied by electrophoresis in polyacrylamide gel for estimating ontogenetic variability of gene expression. Formation of individual isoesterases depended on the type of tissue and the stage of its development. Isoesterases characteristic of exclusively one or some tissues of the same developmental stage were revealed simultaneously with basic esterase isoforms active in all analysed parts of seeds, seedlings and field-grown plants. The revealed changes of esterase isoenzyme spectrum during germination show tissue and time specificity of the endogenous regulation of genes controlling their formation.

Keywords: electrophoresis, isoesterases, ontogeny, polymorphism.

Introduction

A great number of investigations have been devoted to polymorphism of esterase. The research carried out by Schwartz *et al.* (1965) has shown the presence of multiple allelism of this enzyme in maize. Chromosome localization of genes controlling esterase isoenzymes was determined in wheat (Jaaska 1980, Petchey *et al.* 1990) and barley (Kahler and Allard 1983). The results of Schmidt-Stohn and Wehling (1983) and Jaaska (1983) enabled to use esterase spectra as genetic markers in research of population variability of a given crop. Only some papers have concerned isoenzyme analysis. Investigations of electrophoretic separation of esterase in flax have shown that there are differences only in electrophoretic mobility of fractions between genotrophs (Fieldes and Tyson 1973).

Due to the degree of multiplicity of esterases and diversity of their physiological functions in the cell, they are a convenient model for studying changes in their isoenzyme patterns during plant ontogeny. The object of our investigations was to

Received 20 September 1994, *accepted* 8 February 1995.

Abbreviations: Tris - tris(hydroxymethyl) aminomethane; EDTA - ethylene diamine tetraacetic acid

Acknowledgements: This work was supported by the Fundamental Investigation Foundation of the Academy of Belarus.

study esterases by electrophoresis in cotyledons and various other organs of seedlings and field-grown plants of fiber flax for estimating ontogenetic variability of expression of genes controlling this enzyme.

Materials and methods

Plant material of our investigations was fiber flax (*Linum usitatissimum* L.) cv. Belinka (Netherlands), seeds of which were kindly given to us by collaborators of the Pellagras Research Institute for Flax.

Tissue extraction for enzyme determination: Seeds and organs of seedlings of different age and field-grown plants were used to prepare crude extracts for analysis. Dry seeds, cotyledons and embryos of 1-d-old seedlings were homogenized in pre-chilled mortar with pistil using ice-cold 50 mM Tris-glycine buffer, pH 8.3, containing 5 mM EDTA and 10 mM 2-mercaptoethanol and left for 24 h at 4 °C for extraction (50 mg per 0.1 cm^3). Extracts of cotyledons, hypocotyls and roots of 4-d-old etiolated and 6 to 7-d-old-green seedlings were prepared by homogenizing in buffer (50 mg per 0.5 cm^3), those of leaves, stems and roots of field-grown plants (bud- and flowering stages) in ratio 1 g per 2 cm^3.

Gel electrophoresis and enzyme detection: Polyacrylamide gel electrophoresis was performed as described by Davis (1964) at 4 °C using 7.5 % gel and 10 mM Tris-glycine (pH 8.3) as electrode buffer in vertical *PV-15* apparatus (*Biotekh*, Minsk, Belarus). Enzyme samples (0.04 cm^3) mixed with concentrated saccharose solution were layered on the top of gel. Bromophenol blue was used as marker dye. Electrophoretic run was performed using a current of 40 mA per plate 12 × 10 cm for 2 h. The esterase isozymes (hydrolase of carboxylic acid ethers, EC 3.1.1.1) were visualized by diazo-method according to Yeh and O'Malley (1980).

Results and discussion

After separation in Davis's system esterase spectrum of fiber flax includes up to 16 bands greatly differing in staining rate (Fig. 1).

Enzymograms of the fast anodic isoesterases are a series of bands of enzymatic activity. However, all fast enzyme isoforms are absent on enzymograms of dormant seed cotyledons. Loci determining them are probably completely inactivated in dormant tissues. In tissues of cotyledons and embryos of germinating seeds already one and two isoesterases, respectively, are observed, what suggests that germination may be accompanied by formation of a number of new esterases in this zone. In cotyledons and hypocotyls of etiolated 4-d-old seedlings the same enzyme isoforms which are typical also for embryos of germination seeds (Rf 0.46 and 0.84) were detected. These two isoesterases do not show divergence and variability in electrophoretic mobility in any organs of either dark- and light-grown seedlings or field-grown flax plants of the developmental stages studied. However, relative

activity of the isoform with Rf 0.84 increases during seedling development, and the activity of the fraction with Rf 0.46 does not change. Two additional components, besides the above mentioned isoforms, emerge in rootlets. As they are absent in other organs, this can indicate their organ specificity and particular time regulation of expression of genes coding for them. One more isoesterase emerges in organs of green seedlings in comparison with etiolated ones, *i.e.* their spectrum consists already of three isoforms. However, electrophoretic mobility and relative activity of isoesterases are different depending on the tissue type. Thus, the fraction with Rf 0.5 which is also found in spectra of leaves, stem and roots of field-grown plants at the flowering stage, was revealed in cotyledon leaves. There is no such isoform in hypocotyls and rootlets and the component with Rf 0.75, which is not found any more on electrophoretograms, emerges.

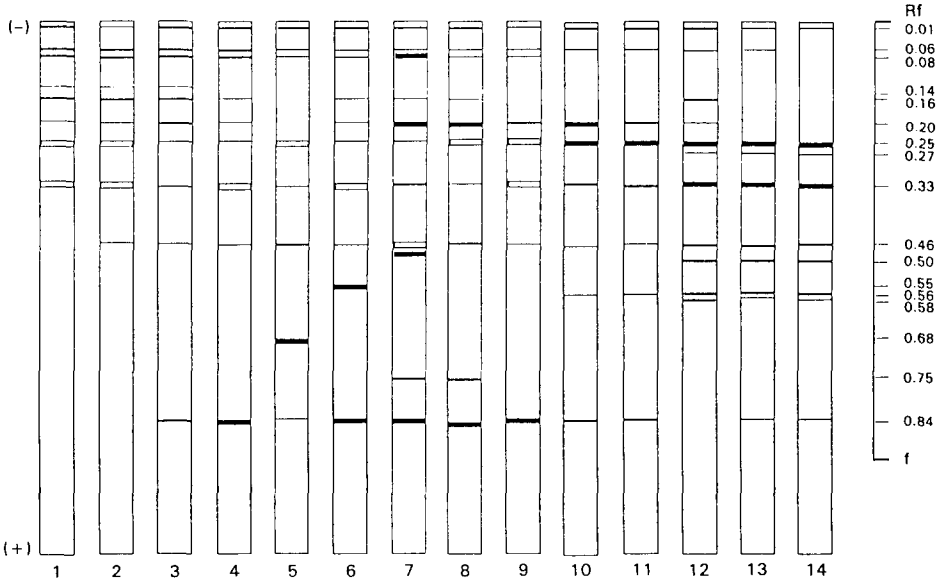

Fig. 1. Enzymograms of esterases in polyacrylamide gel (7.5%) from *Linum usitatissimum* cv. Belinka. *1, 2* - cotyledons of dormant and germinating (24 h) seeds, *3* - embryo of germinating seeds, *4, 5, 6* - cotyledon leaves, hypocotyls and roots of etiolated 4-d-old seedlings, *7, 8, 9* - cotyledons leaves, hypocotyls and roots of 6 to 7-d-old green seedlings, *10, 11* - leaves and stems of field-grown plants at the bud stage, *12, 13, 14* - leaves, stems and roots of field-grown plants at the flowering stage.

The next part of spectrum with medium mobility contains 2 - 5 relatively active isoesterases. A special feature of this region is occurrence of the components with Rf 0.25 and 0.33 in enzymograms of all tested tissues of different age. However there were differences in the rate of manifestation of these esterases - relative activity of isoenzymes increases during germination, the most intensive fractions being detected in organs of field-grown plants. The data show that at early developmental stages there is already a set of basic esterases which is subjected mainly to quantitative

shifts in the relative activity during further plant growth. On the contrary, less mobile isoforms of this region (Rf 0.14, 0.16 and 0.20) are visible in the spectra of organs of dormant and germinating seeds. In tissues of etiolated seedlings they represent minor components or are absent. One of them with Rf 0.20 is active at bud stage and in organs of field-grown plants. On spectra of plant tissues at flowering stage these isoesterases are not revealed, as a rule.

Cathodic isoesterases having the lowest electrophoretic mobility, are represented by 1 - 3 fractions. Special features of this part of the spectrum are the presence of the component with Rf 0.01, enzymatic activity of which is the same in enzymograms of all analyzed tissues, as well as the change of manifestation of other two fractions depending on the plant age. These two isoesterases have a considerable activity only at early stages of germination, they are revealed as minor components in green seedlings and only one low-activity fraction with Rf 0.06 is detected in organs of field-grown plants. This is indicative of a high activity of alleles determining the given isoesterases in tissues of young seedlings and suppression of realization of their genetic information in field-grown plants.

The comparison of the above-mentioned data with the results obtained by us earlier (Yurenkova *et al.* 1992) has shown that esterase spectra of cotyledons of 6 to 7-d-old seedlings and those of green leaves of 14-d-old flax plants differ greatly particularly in isoenzyme group with medium electrophoretic mobility in the range of Rf values from 0.14 to 0.43. In the latter, six additional isoesterases from this Rf range, some of which are detected also in organs of field-grown plants, are revealed. Such quantitative changes in the structure of electrophoretically revealed esterase components in green leaves of 14-d-old seedlings can be accounted for by the assumption that the genes encoding different isoesterases are expressed at different time owing to which isoenzyme spectra change depending on the stage of development.

It follows from the data obtained that transition of seeds from the dormant stage to germination is accompanied by fast and essential rearrangement of the enzyme apparatus. Already within 24 h of seed germination new esterases are formed in seedling tissues and reduction of activity and even disappearance of some isoenzymes typical for dormant seeds are observed. Formation of new enzyme isoforms during germination can be caused either by their biosynthesis *de novo*, or by transformation of active or inactive precursors into new molecular forms peculiar to seedling tissues.

References

Davis, B.J.: Disc electrophoresis. II. Method and application to human serum protein. - Ann. New York Acad. Sci. **121**: 402-427, 1964.

Fieldes, M.A., Tyson, H.: Activity and relative mobility of peroxidase and esterase isozymes of flax (*Linum usitatissimum*) genotrophs. I. Developing main stems. - Can. J. Genet. Cytol. **15**: 731-744, 1973.

Jaaska, V.E.: Electrophoretic survey of seedling esterases in wheats in relation to their phylogeny. - Theor. appl. Genet. **56**: 273-284, 1980.

Jaaska, V.E.: *Secale* and *Triticale*. - In: Isozymes in Plant Genetics and Breeding. Part 2. Pp. 79-101. Elsevier, Amsterdam 1983.

Kahler, A.L., Allard, R.W.: Worldwide pattern of genetic variation among four esterase loci in barley (*Hordeum vulgare* L.). - Theor. appl. Genet. **64**: 109-115, 1983.

Petchey, E.M., Koebner, R.M.D., Gale, M.D.: Genetic characterization of a further homeoallelic series of grains esterase loci, Est-6, in wheat. - Theor. appl. Genet. **79**: 294-296, 1990.

Schmidt-Stohn, G., Wehling, P.: Genetic control of esterase isoenzymes in rye (*Secale cereale* L.). - Theor. appl. Genet. **64**: 109-115, 1983.

Schwartz, D., Fuchsman, L., McGrath, K.N.: Allelic isozymes of the pH 7.5 esterase in maize. - Genetics **52**: 1265-1268, 1965.

Yeh, F.C.E., O'Malley, D.: Enzyme variations in natural populations of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. I. Genetic variation patterns in coastal population. - Silvae Genet. **29**: 83-92, 1980.

Yurenkova, S.I., Khotyleva, L.V., Zhuchenko, A.A. : [Comparative study on isoenzyme spectra of fiber flax cultivars.] - Dokl. Akad. Nauk Belarusi **36**: 473-475, 1992. [In Russ.]