Organ-specificity of esterase isoenzymes in *Brassica* seedlings

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Abstract. Esterase isoenzymes in roots, epicotyls and leaves of 5-, 6- and 7-week-old *Brassica* seedlings were studied. No variation in isoe sterase phenotypes was found between individuals within the examined cultivar. Differences in the number and intensity of isoe sterase bands between the investigated organs were observed. Distinctive isoe sterase patterns were evaluated for each organ. Some esterase isoenzymes are proposed as markers of particular organs: EST 1/1 and EST 1/2 for leaves, whereas EST 2/2 and EST 2/3 for roots. Further studies will be aimed at using different organo-specific isoe sterase forms as markers of early stages of in vitro organogenesis in *Brassica* callus tissue culture.

Key words: *Brassica oleracea*, electrophoresis, esterase, tissue-specific isoenzymes.

Different isoenzymes, mainly peroxidases, have been recommended as sensitive physiological and biochemical markers of embryogenesis and organogenesis in plant tissue cultures (THORPE, GASPAR 1978, RAWAL, MEHTA 1982, EVERETT et al. 1985). Esterase isozymes (E.C.3.1.1.1.), that act upon α- and β-naphthyl acetate can be readily assayed in plant extracts and have long been successfully used as genetical markers in studies of polymorphism within plant species (KIDAMBI et al. 1990). Moreover, isoe sterase profiles have been recently used in studying differentiation in plant tissue cultures (PRASANNA et al. 1983, EVERETT et al. 1985, CHAWLA 1989, 1991). EVERETT et al. (1985) suggest, that esterase isozymes can be useful markers of embryogenesis in tissue culture of maize.

In this study, an attempt to identify organ and tissue specific isoenzymes of esterase in *Brassica* seedlings was made. It is assumed that particular tissue specific isoenzymes may be used as early markers for differentiation of callus tissue, obtained from *Brassica* explants under in vitro conditions. Detection of these tissue specific
isoforms can help to control the process of callus tissue differentiation, before any morphological symptoms of regeneration are visible.

Materials and methods

Plant material

Seeds of *Brassica oleracea* L. var. *capitata* f. *alba* cv. Ditmarska Najwcześniejsza were germinated under non sterile conditions, on filter paper soaked in distilled water, at 23°C in continuous darkness. One-week-old seedlings were then cultured on liquid medium containing only 1/8-strength of Murashige Skoog inorganic nutrients (MURASHIGE, SKOOG 1962). The hydroponic cultures were maintained in a growth room, in continuous light (intensity 3000 Lux), at a temperature of 24°C. Samples of root, epicotyl and leaves were taken from single seedlings separately, after 5, 6 and 7 weeks of growth. Thirty individuals in each developmental stadium were investigated.

Polyacrylamide gel electrophoresis

Samples of plant material were homogenized at 0°C in 0.1 M Tris-HCL buffer at pH 7.5. The tissue fresh weight to extraction buffer volume ratio was 1:2 (w/v). The tissue homogenates were then centrifuged at 10 000 g for 10 minutes at 4°C. The crude supernatant was used for the assay of esterase isoenzymes.

Isoenzyme patterns were determined using the method of vertical 12.5% polyacrylamide gel electrophoresis, with Tris-glycine buffer (pH 8.3-8.5), according to LAEMMLI (1970). The volumes of 50 µl of the crude extracts were loaded, in non-denaturing conditions (without sodium dodecyl sulfate), in each lane. The electrophoresis was carried out for 4-5 hours with 30 mA/gel.

Staining of gels

Esterase isoenzymes were localized by staining the gels with the solution containing 10 ml of 0,1M phosphate buffer at pH 6.0, 0.2 ml of 1% α-naphtyl acetate in 60% acetone and 5.0 mg Fast Blue RR. The gels were incubated for 30-40 minutes at 230°C in darkness and fixed in 7% acetic acid.

Results

Extracts from roots, epicotyls and leaves of 5-, 6- and 7-week-old seedlings of *Brassica* were analysed. Typical 5-week-old seedlings of *Brassica oleracea* are presented in Fig. 1. In general, no variation in electrophoretic phenotypes of the
Esterase isoenzymes in *Brassica*

esterase system was observed between 30 separately investigated individuals in the same developmental stage. Moreover, no significant qualitative differences in isoenzyme patterns were noticed between 5-, 6- and 7-week-old seedlings were noticed (data not shown). Patterns of esterase isoenzymes in roots, epicotyls and leaves of four single 5-week-old seedlings are shown in Fig. 2. Total, schematic isoesterase patterns typical of root, epicotyl and leaves of *Brassica* seedlings tested in the present study, are presented in Fig. 3.

In all, a maximum number of 11 isoforms was noted. They were distributed in five regions of banding on the gels, marked EST 1, EST 2, EST 3, EST 4 and EST 5, with 0 to 3 bands in each region. The number of isoenzyme bands in different organs ranged from 5 (in epicotyl) to 10 (in leaves). Isoenzymes 1 and 2 in EST 2, isoenzyme 2 in EST 4 and slightly visible zone EST 5 with one isoenzyme band were present in the extracts of the all examined organs. In other cases, differences in the number and stain intensity of bands especially in EST 1 and EST 2 regions were observed between the investigated organs.

**Esterase pattern in leaves**

The leaf extracts contained 10 isoforms. The EST 1 region was the most active zone, with two bands of a high stain intensity. The EST 2 zone was represented by two bands: isoenzyme 1 with a high stain intensity and isoenzyme 2 with a low stain intensity. Three isoenzyme bands were detected in EST 3, the first band seeming to be relatively more intensive than the others. Two isoenzyme bands with a very low stain intensity were found in the EST 4 region. One slightly visible band represents the EST 5 region.

**Esterase pattern in root**

A total number of 8 isoenzymes was observed in the root extracts. The absence of any isoenzyme bands in the EST 1 region was noticed for all investigated seedlings.
In the EST 2 zone three isoenzyme bands were found: isoenzyme 1 and 2 with a high stain intensity and isoenzyme 3 with a low stain intensity. Isoenzyme 3 was present only in the extracts from roots. Three bands of a low stain intensity were noticed in the EST 3 region and only one band in EST 4 and EST 5 regions.

Esterase pattern in epicotyl

Only 5 isoenzymes, with a relatively low stain intensity were recorded in epicotyl extracts. In EST 1 only isoenzyme 2 was visible, whereas in EST 2, just as in leaf pattern, isoenzymes 1 and 2 were present. No isoenzyme band was found in the EST 3 region. Only one slightly visible isoenzyme band was observed in both EST 4 and EST 5 regions.

Discussion

Very little information is currently available on the tissue and organ specific expression of different isoenzymes during development and differentiation of higher plants. According to SCANDALIOS (1974) "distinct isozymes may be present in different tissues of a given organism; some isozymes may be present in a tissue at a given developmental stage but absent at another; genetically identical isozymes may be present in different tissues but in varying quantities". Such enzyme fluctuations seem to be the consequence of differential gene action during plant development.

Some common isoenzyme systems, like catalase or starch branching enzyme, show distinct tissue and organ specificity (SCANDALIOS 1974, YAMANOUCHI, NAKAMURA 1992, OTA et al. 1992). Although esterase isoforms have long been used only in studies of plant populations polymorphism, there are some evidences that they can also be used as biochemical markers of differentiation in plant tissue cultures (PRASANNA et al. 1983, EVERETT et al. 1985, CHAWLA 1989, 1991).

In our research, the number of esterase isoforms and the intensity of some of the bands differed from organ to organ. For every organ a specific isoesterase pattern was observed. Figure 3 summarizes differences in the isoesterase patterns of three various Brassica organs. The isoforms EST 1/1 and EST 1/2 were apparently specific of leaves, whereas in the case of other organs, bands with the same mobility as in the above isoforms were absent. Although the bands of EST 2/1 and EST 2/2 were detected in the extracts of all tested organs, their relatively high and equal stain intensities were marked only for the root. Moreover, it is noticeable that isoenzyme EST 2/3 can be found only in the root tissue. Therefore, we conclude that some esterase isoforms in Brassica seedlings can be evaluated as organ specific at particular developmental stages.
Fig. 1. *Brassica oleracea* seedlings after 5 weeks of cultivation on liquid medium containing 1/8-strength of Murashige and Skoog inorganic nutrients
Fig. 2. Comparison of the esterase patterns in roots (R), epicotyls (E) and leaves (L) of four separately investigated 5-week-old Brassica seedlings (marked with letters A, B, C, D, respectively)
Isoenzymes are distributed on the gel in five regions indicated as EST 1, EST 2, EST 3, EST 4 and EST 5
We suggest, that organ and tissue specific isoenzymes may be used as useful biochemical markers of early stages of organ differentiation in organogenetic Brassica callus tissue, cultured in vitro on regenerating media. Electrophoretical and immunological methods (using antibodies raised in rabbit against specific isoenzymes) would provide basis for early detection of tissue specific gene expression during plant cell differentiation. Such information can be very useful in monitoring and optimization of effective plant regeneration under in vitro culture conditions.

REFERENCES


Specyfika organowa isoenzymów esterazy w siewkach Brassica

Streszczenie

Analizowano izoenzymy esterazy w korzeniach, epikotylach i liściach 5-, 6- i 7-tygodniowych siewek Brassica oleracea. W badanej próbie 30 osobników w tym samym stadium rozwojowym nie
stwierdzono zmienności osobniczej izoenzymów esterazy. Zaoberwowano znaczne różnice w ilości i intensywności wybarwienia prążków izoesteraz pomiędzy badanymi organami. Wykazano, iż esteraza wykazuje specyfikę organową i na podstawie otrzymanych wyników ustalono wzory izoenzymów esterazy charakterystyczne dla korzenia, epikotyla oraz liści. Niektóre izoenzymy proponuje się jako formy markerowe dla poszczególnych organów: EST 1/1 oraz EST 1/2 dla liści, natomiast EST 2/2 i EST 2/3 dla korzeni. Dalsze badania będą zmierzały w kierunku zastosowania poszczególnych organo-specyficznych form izoesteraz jako markerów wczesnych stadiów orga- genezy w tkance kalusowej Brassica hodowanej in vitro.