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Metabolism of excised embryos of *Lupinus luteus* L.

VII. An electrophoretic analysis of esterases and peroxidases in cultured embryos and normal seedling axes

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Abstract

A rich and active spectrum of esterases (*E*) exists already in the organs of a dry seed. The isolation of axes from the cotyledons exerts a considerable influence on the pattern of *E*. In the axial organs of a seedling the spectra and activity become impoverished starting from the 3rd day of culture in the dark. On the other hand in the axial organs isolated from the cotyledons from that moment onwards the spectra become richer and more active, particularily in the root. It appears that the enhancement of spectra in the detached axes is not dependent on the elimination with the cotyledons of a source of inhibitors, since in the cotyledons, until the end of the culture period (12 days), a rich pattern of *E* is maintained. Peroxidase (*P*) appears starting from the third day giving impoverished spectra, in general development similar to *E*.

The removal of the seed coat from dry seeds exerts during the first two days of imbibition a strong influence on the spectra of axial and cotyledonary enzymes.

This report is an extension of the previous paper (Czosnowski 1974) and concerns the dynamics of the occurrence and activity of esterase and peroxidase isozymes in the early developmental phases in decotyledonized embryo axes and in seedling organs in situ, of yellow lupine cultured in the dark for 12 days.

MATERIALS AND METHODS

Plant material. The origin of the seed material as well as the methods of culturing have been given in the previous publication (Czosnowski 1974), with the exception that the embryo axes have been isolated after 8 hours of imbibition under sterile conditions.

Main series I. For the enzymatic analyses samples have been collected from the following materials: embryo axes and cotyledons from dry seeds (on the graphs: 0^d) and after 1, 2, 3, 7 and 12 days of cultivation of the isolated embryo axes on the medium, or from normal seedlings grown on water (in the graphs Fig. 1. resp.: 1^d, 2^d, 3^d, 7^d and 12^d). The isolated embryos, starting from the first day of cultivation have been divided into root and shoot and the seedlings into a root, stem with developing apical bud and the cotyledons, except for the material 1^d in which the embryo axis was separated as a whole from the cotyledons. In this way 26 experimental elements were obtained, which were subjected to enzymatic analysis and designated on the diagram in Fig. 1 by numbers from *1* to *26*.

As a supplement to the main series a short series was added (series II), consisting of seedlings from seeds deprived of the seed coats at time 0^d, and germinated for 1 and 2 days. As a result 5 experimental variants were additionally obtained and designated in the diagram by numbers from *27* to *31*.

Preparation of extracts and electrophoresis have been detailed earlier (Czosnowski 1974). This time however smaller initial quantities of the material were obtained: 300 mg of axial elements (root, shoot) and cotyledons, and 8 young embryo axes regardless of the weight, which for technical reasons have not been split into a root and shoot portion. For the extraction of material each time 2 ml of the buffer solution sufficed.

Enzymatic assays

E Esterase (E. C. 3. 1. 1. 2)

P Peroxidase (E. C. 1. 11. 1. 7)

The letters *E* and *P* have been used to designate the individual enzymes on the diagrams in Fig. 1 and in the text below. Thus, for example, *"E-12"* indicates an esterase in a root isolated from an embryo in the 3rd day of cultivation.

The method followed for esterase assays was essentially that of Mitra et al. (1970). Gels were incubated in a solution of 50 ml 0,1 M phosphate buffer pH 6,0 containing 1 ml of 1% naphthylacetate in 60% acetone and 25 mg Fast Blue RR.

Peroxidase gels were prerinsed in a 0.2 M acetate buffer at pH 5.0 (5 min.) and then incubated for 30 min. in a substrate solution containing: 95 ml 0,2 M acetate buffer pH 5,0, 20 mg benzidine and 5 ml EDTA 5%. After rinsing in distilled water the gels were placed in 0,03% H_2O_2 in the same acetate buffer.

All enzymatic reactions were conducted at room temperature (20—22°C) for 30 minutes. The solutions without substrates served as controls. The graphic presentation of the results is the same as in previous communication (Czosnowski 1974).

RESULTS AND DISCUSSION

Series I

The normal, dry seeds (0^d)

Individual elements of the dry seed contain several active isoesterases. In the embryo axes and in the cotyledons the spectra are almost identical. There is no *P*.

Embryo 1^d, 2^d, 3^d, 7^d, 12^d

a) Shoot *E:* After 1 day of culture the spectrum is richer than in the dry seed (all 7 of the main *E*), later the spectrum becomes impoverished, but is generally similar; one can observe certain shifts in the activity of individual bands.
P: On the third day 1 band and from 7^d two bands of weak activity appear.

b) Root *E:* After 1 day of culture the spectrum is as in the shoot (somewhat lower activity), later it distinctly becomes impoverished, while from 3^d it strongly develops (*7, 12, 17, 22*).
P: Appears on 3^d, and later an increase in activity is maintained.

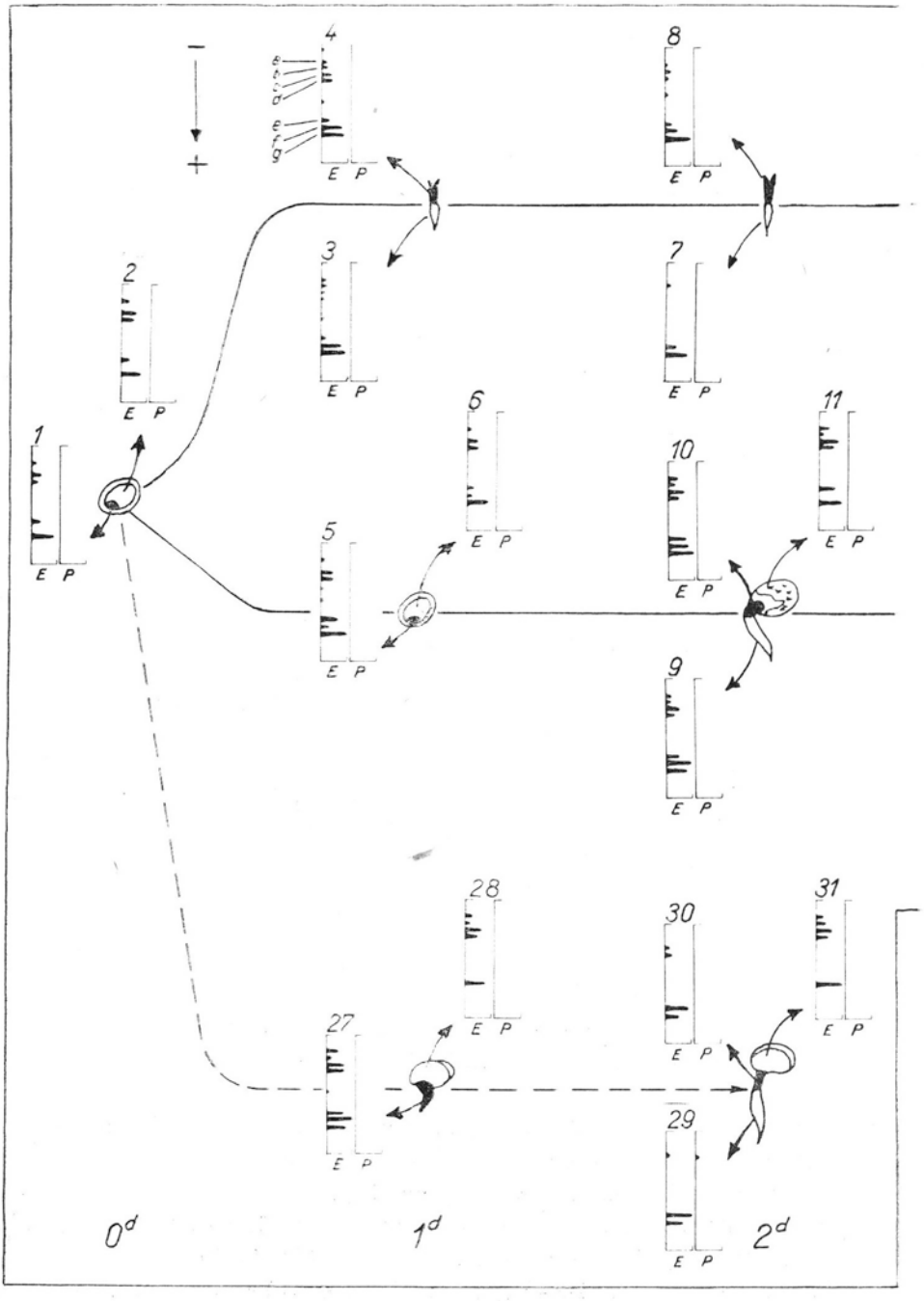
Seedling 1^d, 2^d, 3^d, 7^d, 12^d

After one day of culturing the axes were not split into shoot and root, but they were analysed as a whole. Comparing *5* with *1* an enrichment of the *E* spectrum is observable.
No *P*.

a) Shoot *E*: After 2^d the spectrum is full (7 bands) gradually becoming impoverished till 12^d (*10, 15, 20, 25*).
P: From the 7th day it shows up weakly.

b) Root *E:* After 2^d (*9*) the spectrum is similar to the shoot (*10*). From 3^d it rapidly becomes impoverished (*14, 19, 24*).
P: Appears in 3^d, showing a maximal development at 7^d — two strong bands (*19*). In 12^d there is one band of medium activity.

c) Cotyledons *E*: Throughout the culture period the spectrum is rich, while from 7^d it is full (7 bands) and very active.
P: A weak manifestation from the 7th day.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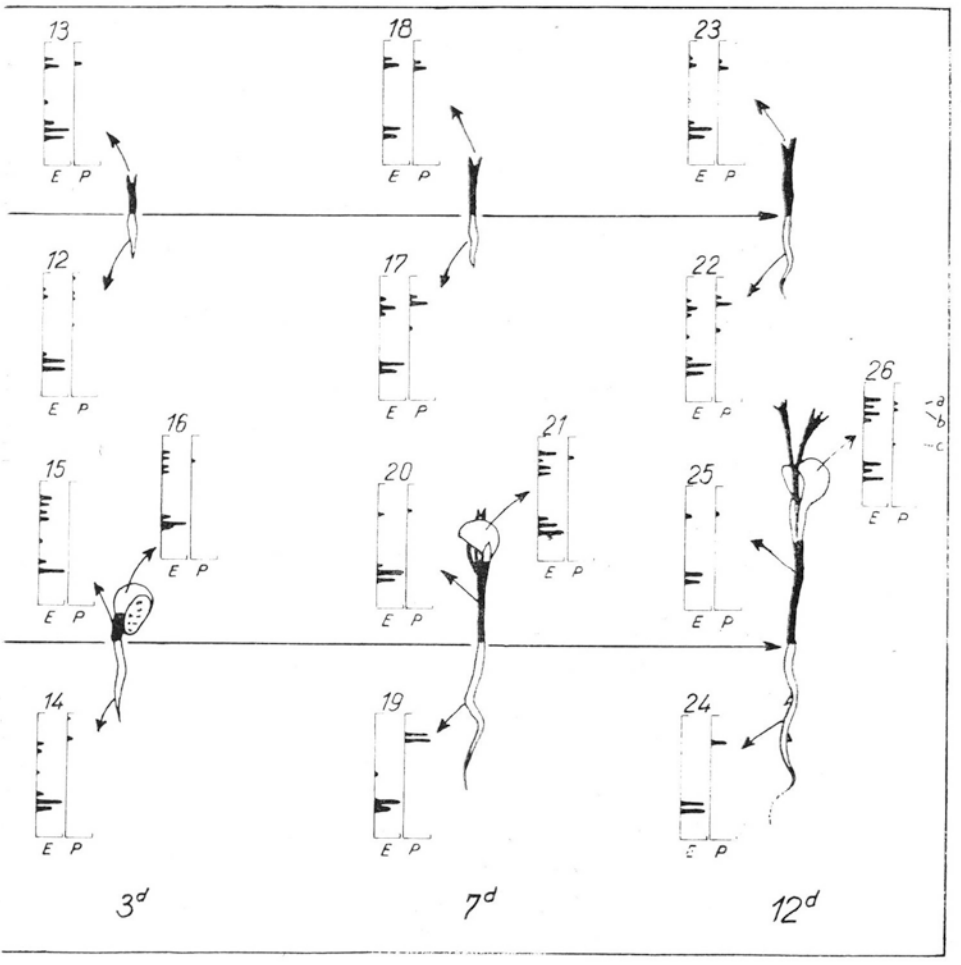

Fig. 1. Isozyme patterns of eserases (*E*) and peroxidases (*P*) in dry seeds (0^d), in isolated cultured embryo axes (upper row) and in normal seedlings (middle row) on the 1st, 2nd, 3rd, 7th and 12th day. The lower row (broken line) represents the isozyme patterns in the seedlings from decoated dry seeds on the first and second day of germination.

Series II

Seedlings 1^d and 2^d originating from decoated seeds at 0^d

a) Seedling axes *E*. 1^d (shoot and root together): full spectrum (*27*). 2^d in the shoot and in the root a strong impoverishment of the *E* spectrum, similar to that observed in normal seedlings after 7^d and 12^d (compare *29* and *30* with *19*, *20* and *24*, *25*).
P: Appeared as one weak band in the second day.

b) Cotyledons. After 1^d and 2^d the spectra are identical (*28, 31*), differing from corresponding material in series I (*6* and *11*) primarily in the reduction of the rapidly migrating isoesterase fraction to only one band.

The results of series II indicate, that the removal of the seed coats from the dry seeds has a very strong effect during the first two days of imbibition on the enzymatic spectra of axial organs and cotyledons.

A closer analysis of the results obtained in series I provides further data on the dynamics of enzymatic spectra in the early developmental phases of the studied material.

Esterase. The isoesterase spectra over 12 days of culturing of isolated embryos and seedlings are principally manifesting themselves on the basis of a constant "potential" formula for *E.* This consists of a group of 4 bands at low Rf values and a group of 3 bands at higher Rf values (respectively *a, b, c, d* and *e, f, g* in diagram *4*). During culturing the isolated embryo is characterized by the following properties: after 1^d there is great similarity of spectra for the shoot and root; after 2^d, 3^d differences between these two organs begin to appear in favour of the shoot; after 3^d the spectrum of the root becomes richer and more active than in the shoot. In the seedling from 2^d to 12^d one can observe a gradual impoverishment of the spectra in the axial organs, and their strong development in the cotyledons during the second half of the culture period (*21, 26*). A similar impoverishment of the isoesterase spectra during early ontogenetic phases has been observed by Macko et al. (1967) in wheat and by Mäkinen (1968) in the roots of onion.

The effect of the isolation of the embryo axes from the cotyledons is very distinct. After 1^d of culturing, in the axis of the seedling (*5*) and in the organs of the excised embryo (*3, 4*) the spectra are similar. After 2^d in the shoot, and primarily in the root of the seedling the spectrum is richer (*9, 10*) and more active than in the organs of the isolated embryo (*7, 8*). After 3^d the differences between the elements of the seedling axis (*14, 15*) and the elements of the isolated embryo (*12, 13*) tend to disappear. During further development, (after 3^d) the general pattern of spectra and of their activity is richer in the axial organs of the isolated embryos (after 12^d: *24, 25* and *22, 23*).

In the seedling the impoverishment of the spectra in the shoot and in the root is during the development similar, while in the isolated embryos the changes of spectra in the root are much more strongly accentuated than in the shoot.

Peroxidase. Peroxidase activity has been demonstrated by the methods employed for the first time in material collected on the third day of culture, namely in the shoot and root of isolated embryos and in the roots of seedlings.

In the organs of an isolated embryo the spectrum is richer and more active than in the seedling axes, where 7[d] root is an exception in that in one case it had 2 strong *P* bands. In all 3 bands were found (*P*) *a*, *b*, *c* — see diagram *26*.

Several authors have been finding active isozymes P in the resting seeds (eg. Mitra et al. 1970, Anstine et al. 1970). Siegel and Galston (1967) have been observing the onset of the appearance of P in pea seeds starting after 24 hours of imbibition. In our material this time is delayed considerably.

Gaspar et al. (1973) has shown (using among other methods strach gel electrophoresis) that in lentil embryo axes separated from the cotyledons there occurs during the first 72 hours of development an increase in total activity of peroxidase relative to normal axes from seedlings. This is caused by the enhancement of a certain group of isoperoxidases and by the synthesis de novo of some additional isozymes. On the other hand some isozymes reduce their activity in the isolated axes. The results of our studies show certain similarities in the development of the isoperoxidase system in a somewhat later phase of the development of lupine (from the third day onwards), though the use of a somewhat different electrophoretic technique does not permit a direct comparison of the data. The pattern of isoperoxidase dynamics described by Gaspar et al. (l.c.). has a much more close similarity to the pattern of changes within the isoesterase system in our material.

The results presented indicate that in the case of esterases and peroxidases the isolation of yellow lupine embryo axes from the cotyledons has a strong influence on the isozyme dynamics. This effect was not observable in the case of dehydrogenases (Czosnowski 1974) where shifts in the spectral patterns and fluctuations in the activity have shown similar patterns in the detached and attached organs. The purpose of further studies would be to explain, whether the dynamics, particularily of the esterases, in the axial organs is affected, totally or individually, by the inhibitive system originating from the cotyledons, or by the processes arising from the wounding effect during preparation of the material. Assuming that in the axes there operate inhibitors formed in the cotyledons, one would have to assume that they operate differently in the axes than in the cotyledons, since in the latter a rich and active spectrum of isoesterases is maintained with minor fluctuations throughout the duration of the experiment.

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Metabolizm izolowanych zarodków Lupinus luteus L. VII

Streszczenie

Bogate i aktywne spektrum esteraz (*E*) występuje już w organach suchego nasienia. Odizolowanie osi od liścieni wywiera duży wpływ na obraz *E*. W organach osiowych siewki spektra oraz aktywność ubożeją począwszy od trzeciego dnia hodowli w ciemności, natomiast w organach osiowych izolowanych od liścieni począwszy od tego momentu spektra są bogatsze i aktywniejsze, szczególnie w korzeniu. Wydaje się, że wzmożenie spektrów w izolowanych osiach nie polega na usunięciu (wraz z liścieniami) źródła inhibitorów, ponieważ w liścieniach do końca hodowli (12 dni) utrzymuje się bogaty obraz *E*. Peroksydaza (*P*) pojawia się począwszy od trzeciego dnia, dając spektra ubogie i w ogólnym charakterze rozwojowym podobne do *E*.

Zdjęcie okrywy nasiennej z suchych nasion wywiera w ciągu pierwszych dwóch dni imbibicji silny wpływ na spektra enzymów organów osiowych i liścieni.