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ELECTROPHORETIC ENZYME STUDIES IN *SCILLA SIBIRICA* ANDR.

The finding that a number of enzymes may exist in more than one molecular form has led to an increased experimental prominence of such isoforms, particularly with respect to their genetic variation and tissue specificity. Up to the present time, several authors have pointed out the tissue and organ specificity of protein and enzyme complements in plants (Scandalios, 1964, 1969; Steward et al. 1965; Barber, Steward, 1968; Shaw, 1968; Jaaska, Jaaska, 1969; Guzmán et al. 1971; etc.). The present communication deals with the results of polyacrylamide gel electrophoretic studies of the isoform systems of acid phosphatase, esterase, leucine aminopeptidase, peroxidase, glucose-6-phosphate and 6-phosphogluconate dehydrogenases in the vegetative and floral organs of the squill, *Scilla sibirica* Andr., with regard to their organ specificity.

Materials and methods

The plant material used in this investigation was the squill *Scilla sibirica* Andr., which had grown in the open. The following organs of the squill — the bulb, root, leaf, stem, petal, anther, stigma-style and ovary — were analyzed at the stage of flowering.

A 500 mg sample of different organs was homogenized in a prechilled mortar with quartz sand and 2.0 ml of cold homogenization buffer, containing 0.05M tris-hydroxymethylaminomethane (Tris), 0.04M ascorbic acid, 0.001M EDTA—Na₂Mg and 0.005M cysteine. The resulting homogenates were centrifuged at 18,000 g during 30 minutes. About 50 mg Sephadex G-200 and 200 mg sucrose were added to the supernatants, and the extracts were stored frozen at -10 °C.

Enzyme extracts were subjected to vertical electrophoresis in polyacrylamide gel tubes. The gel for the anionic and cationic systems was composed as described by Jaaska and Jaaska (1969), and enzyme staining was carried out according to the procedure of Jaaska (1972).

Results

Acid phosphatases. Figure 1A represents diagrammatic interpretations of electrophoretic separation on the polyacrylamide gel of anodically moving acid phosphatases from different organs of *Scilla sibirica* Andr. All the enzymograms in the figure show an electrophoretically constant slow-migrating doublet with a different staining intensity (i. e. activity) and only a slightly faster-moving fraction. In addition to the above-mentioned fractions, some bands were also detected in different organs.

Two weak, scarcely distinguishable bands were observed in extracts of bulbs and roots near the origin of the enzymogram. A weak zone was observed near the middle of the gel both in the vegetative (leaves, stems) and the floral organs (anthers, petals). The anther tissues revealed an intensely stained band near the doublet and a weak fast-moving zone. Comparison of the enzymograms in Fig. 1A clearly demonstrates that the above-mentioned intensive and weak phosphatase isoforms were characteristic or specific of the anther tissue only, whereas they were absent from the enzymograms of the remaining organs. Some enzymatic activity remained at the site of sample application in the extracts of the petals and the anthers. At the same time, it should be noted that our electrophoretic acid phosphatase patterns were essentially similar in their main fractions, whereas phosphatase enzymograms of the various organs differed only in the diminutive bands.

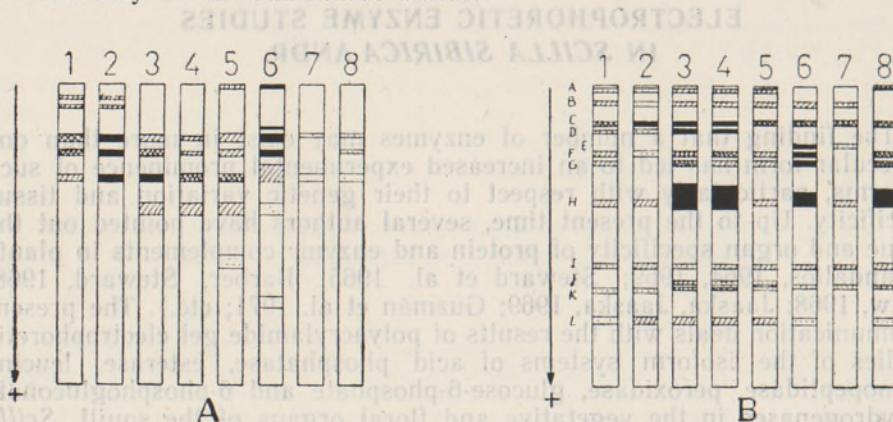


Fig. 1. Polyacrylamide gel electrophoretic patterns of acid phosphatase (A) and esterase (B). Enzymograms: 1 — bulb, 2 — root, 3 — leaf, 4 — stem, 5 — petal, 6 — anther, 7 — stigma-style, 8 — ovary.

Esterases (Fig. 1B) were represented in different organs of the squill by a rather complex series of fractions, and the number of bands varied from six to eleven, depending on the particular organ. The stigma-style tissue exhibited the lowest number of isoforms, and the stem tissue showed the highest number.

Although the isoform complex varied with different organs, it can be seen from the figure that the enzymograms of different organs were also similar. Five electrophoretically distinct esterase isoforms (Fig. 1B, bands C, F, H, I, K) proved to be common to all the investigated organs of the squill, with the exception of one band (F), which was absent from the stigma-style tissue. Comparison of the enzymograms presented in Fig. 1B shows that esterase isoform D, which is characteristic of the vegetative organs, is absent from the floral organs. Esterase isoform H near the middle of the gel shows considerable quantitative variation in the staining intensity in the organs studied: namely, the bulb, root, anther, and stigma-style had a similar distinct fraction with a weak staining intensity, whereas the leaf, stem, petal and ovary had a broad intense staining zone. Isoform E is characteristic only of the anther and stigma-style tissues, whereas this band is totally lacking in the enzymograms of the others. Thus, certain isoforms were common to all the organs studied, but showed differential activities, whereas others were specific to either one or two of the tissues.

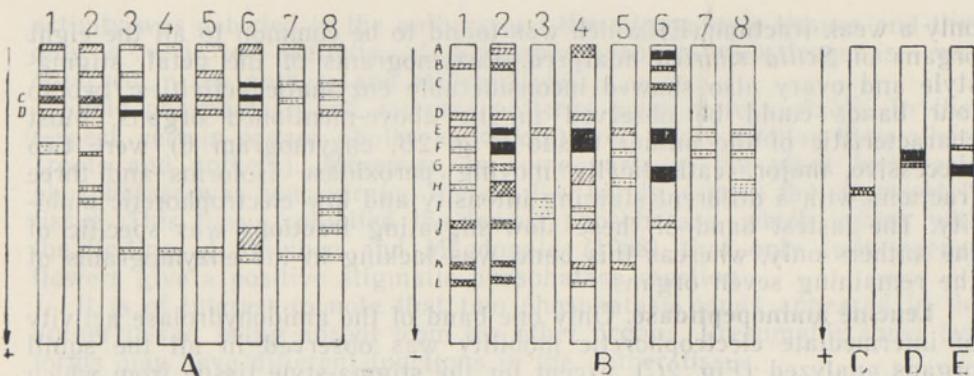


Fig. 2. Polyacrylamide gel electrophoretic patterns of anodical peroxidases (A), cathodical peroxidases (B), leucine aminopeptidase (C), glucose-6-phosphate dehydrogenase (D), and 6-phosphogluconate dehydrogenase (E). Designations see under Fig. 1.

Peroxidase enzymograms in the anionic electrophoretic system (Fig. 2A) showed 3 to 8 fractions, depending on the organ. Two fractions (C and D in Fig. 2A) found in the enzymograms proved to be common not only to all the vegetative organs studied, but also to the floral organs, except for the stigma-style tissue. The squill leaf, stem and petal had basically similar anodically moving peroxidase enzymograms, consisting of two above-mentioned fractions and a weak fast-moving band; the leaf and the petal also revealed a more slowly moving band identical in electrophoretic mobility. At the same time, several fractions found to be present in the root tissue were absent from the remaining tissues. In the enzymograms of roots, up to eight electrophoretically distinct fractions could be distinguished. Enzymograms of the anther, stigma-style and ovary revealed two slow-migrating anodical peroxidase fractions and a weak fast-moving one, which were identical in electrophoretic mobility, but differed in the relative staining intensity. In addition, the enzymogram of the ovary showed the presence of some electrophoretically distinct fractions migrating slightly faster from the middle of the gel, and the enzymogram of the anther tissue revealed the presence of an additional organ-specific zone of higher electrophoretic mobility, which was never seen in the enzymograms of any other analyzed organs of *Scilla sibirica*. All the enzymograms in the figure show a staining area near the origin at the site of sample application.

The cathodically moving peroxidase enzymograms (Fig. 2B) are more complex than those of anodically moving peroxidase, showing the presence of numerous isoform bands of a different staining intensity and electrophoretic mobility. In all, up to 12 electrophoretically distinct cathodically migrating peroxidase isoforms were present in the squill organs, but no single tissue possessed all the enzyme fractions. The bulb, root, and stem revealed essentially similar peroxidase patterns consisting of eight to ten electrophoretically distinct fractions, with the exception of one slow-migrating band which was lacking in the bulb tissue and band I, characteristic of the stem and leaf only, whereas it was clearly absent from the enzymograms of the other organs. The remaining seven cathodic peroxidase fractions were common to all the three organs compared, but differed in the relative staining intensity and in the zone broadness, which were characteristically different in each organ. At the same time the pattern of the leaf, in addition to zone I (Fig. 2B, enzymogram 3), revealed the presence of

only a weak fraction (E) which was found to be common to all the eight organs of *Scilla sibirica* analyzed. Enzymograms of the petal, stigma-style and ovary also showed inconsiderable enzymatic activities; two to four bands could be observed in the above-mentioned organs. Most characteristic of the anther tissue (Fig. 2B, enzymogram 6) were two successive, major, cathodically moving peroxidase isoforms and three fractions with a different staining intensity and low electrophoretic mobility. The fastest band of these slow-migrating fractions was specific of the anthers only, whereas this band was lacking in the enzymograms of the remaining seven organs.

Leucine aminopeptidase. Only one band of the amidohydrolase activity of intermediate electrophoretic mobility was observed in all the squill organs analyzed (Fig. 2C), except for the stigma-style tissue from which this fraction was totally absent or in which it was very faint.

Glucose-6-phosphate dehydrogenase appeared as two closely spaced bands (Fig. 2D) in the squill vegetative organs (bulb, root, leaf, stem) and also in the petal and in the ovary. Comparison of these doublet bands showed that the first fraction revealed a high enzymatic activity as evidenced by its intense staining, and the second band was distinct, electrophoretically variable, and essentially similar in activity, whereas the intensity of the major fraction varied in different organs. On the whole, the bulbs and ovaries showed the highest activity, the leaves and stems exhibited a lower, and the petals the lowest activity.

6-Phosphogluconate dehydrogenase enzymograms revealed two closely spaced fractions (Fig. 2E). The main band is faster-moving as well as electrophoretically invariable, and the slower one is light, with a small variability in its electrophoretic mobility. The intensity of these bands varied in different organs. The 6-phosphogluconate dehydrogenase activity was higher in the vegetative organs and lower in the floral ones. In the corolla of the squill, 6-phosphogluconate dehydrogenase activity was distributed in the following way: the ovary tissue extracts exhibited the highest activity, the anther showed a rather low activity, and the stigma-style revealed the lowest activity of all. Visual observations did not show any slower fraction in the enzymogram of the stigma-style.

Discussion

The data obtained in the course of the present investigation confirm the observations that several enzymes exist in multiple molecular forms in different organs of the squill and that the degree of heterogeneity varies considerably, depending on the particular kind of the enzyme or the tissue.

Leucine aminopeptidase (LAP) was the most conservative enzyme, showing no significant variation between the different organs, apart from the fact that no LAP activity was detected in stigma-style extracts. Neither could Mäkinen and Macdonald (1968) determine whether LAP isoforms exist in the stigma and the style of *Oenothera organensis*. LAP is widely distributed both in plants and animals. Although the exact function of LAP is not yet understood, it appears to be a hydrolytic enzyme that is probably of considerable importance in protein degradation (Scandalios, 1964; Mallery, 1971).

The two dehydrogenases studied here were also quite conservative enzymes. Both glucose-6-phosphate and 6-phosphogluconate dehydrogenases revealed two closely spaced fractions in different organs of the squill, with the only exception that no glucose-6-phosphate dehydrogenase

activity was detected in the anthers and the stigma-style tissues and that only a very low activity of 6-phosphogluconate dehydrogenase was observed in the anthers and stigma-styles.

Acid phosphatase and esterase patterns were more variable. In that respect, certain organs (bulbs and roots) were more similar than others (roots and anthers). Moreover, in some parts of the plant body acid phosphatase was concentrated in very few bands, e.g. in the stigma-style tissue. This tissue exhibited the lowest phosphatase, which agrees with the finding of Mäkinen and Macdonald (1968) that only fully opened flowers give a positive stigmatic phosphatase reaction.

It is of interest to note that two phosphatase bands appeared in the anther tissue, being absent from the other organs. Presumably these isoforms may have different functions in the anther tissue.

It is also significant to point out that the anther and stigma-style tissue had an esterase band which was absent from the others. Despite the existence of extensive animal and plant literature concerning the presence of esterases, the physiological function and the significance of these ubiquitous enzymes have remained almost totally unknown.

Both anodically and cathodically moving peroxidases varied in the organs from which they had been extracted. Tissue or organ specificity was most apparent in the cathodically moving peroxidases. In a given organ there were 2 to 10 isoforms of cathodic peroxidases, a total of 12 isoforms being detectable. Certain organs were more similar (e.g. bulb, root and stem) than others. There was a very distinct difference between the anther and the stigma-style; the anthers had up to six cathodic peroxidase forms, only two bands appeared in the stigma-style, and only one of these bands corresponded to a band of the anther.

It is interesting to note that the leaf and stem tissues showed one band which was characteristic only of these organs and was absent from the remaining ones. The anther tissue also revealed a slow-migrating peroxidase form which was totally lacking in the other organs.

Although the variability of isoforms, as shown by anodic peroxidases, was not so striking, nevertheless, the root, anther and ovary contained one or more isoforms which were characteristic or specific of this organ, only.

Our results also confirm the fact that some enzymes were rather conservative, while the isoform composition of other enzymes was more variable. Thus, some isoforms could be found in all or in several analyzed tissues, while others were restricted to one particular organ, only. The existence or the appearance of organ-specific isoforms may be interpreted as evidence suggesting a differential activity in various organs of separate genes controlling the biosynthesis of individual isoforms. The reason for the presence or absence of certain isoforms in some organs is not yet clear, but it may be explained by assuming activation or repression of the genes controlling their biosynthesis.

Summary

Polyacrylamide gel electrophoresis was applied to study the isoform composition of six different enzymes in various organs of the squill *Scilla sibirica* Andr.

No variation in different squill organs was observed in the leucine aminopeptidase pattern consisting of one band of invariable electrophoretic mobility, apart from the fact that no leucine aminopeptidase activity was detected in stigma-style extracts. Glucose-6-phosphate and 6-phospho-

gluconate dehydrogenases both revealed two closely spaced fractions in the organs analyzed, while no glucose-6-phosphate dehydrogenase activity was observed in the anther and stigma-style tissues.

Peroxidase, esterase and acid phosphatase were found to be electrophoretically multicomponent; certain isoforms were common to all tissues, while others were specific of either one or two organs, only.

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REFERENCES

- Barber J. T., Steward F. C., 1968. The protein of *Tulipa* and their relation to morphogenesis. *Developm. Biol.* **17** (3) : 326—349.
- Guzmán C. A., Ferri M. V., Trippi V. S., 1971. Isoperoxidases in organs of two species of the genus *Datura* (*Solanaceae*). *Phytochem.* **10** (10) : 2389—2391.
- Jaaska V., 1972. Electrophoretic enzyme studies in the genus *Secale* L. *Eesti NSV TA Toim. Biol.* **21** (1) : 61—70.
- Jaaska Vilve, Jaaska Vello, 1969. Heterogeneity and tissue specificity of some enzymes in kidney bean. *Eesti NSV TA Toim. Biol.* **18** (4) : 408—416.
- Mallery C. H., 1971. Protein metabolism of *Allium* radicle tips during germination. *Ibid.* **25** : 448—455.
- Mäkinen Y., Macdonald T., 1968. Isoenzyme polymorphism in flowering plants. II Pollen enzymes and isoenzymes. *Physiol. Plant.* **21** : 477—486.
- Scandalios J. G., 1964. Tissue specific isozyme variations in maize. *J. Heredity* **55** (6) : 281—285.
- Scandalios J. G., 1969. Genetic control of multiple molecular forms of enzymes in plants. *A Rev. Biochem. Gen.* **3** : 37—79.
- Shaw C. R., 1968. Electrophoretic variation in enzymes. *Science* **149** : 936—942.
- Steward F. C., Lyndon R. F., Barber J. T., 1965. Acrylamide gel electrophoresis of soluble plant proteins: a study on pea seedlings in relation to development. *Am. J. Bot.* **52** (2) : 155—164.

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SCILLA SIBIRICA ANDR. ENSÜÜMIDE ELEKTROFOREETILINE UURIMINE

Resümee

Polüakrüülamidigelektroforeesi abil uuriti hariliku siniliilia *Scilla sibirica* Andr. erinevate organite happele fosfataasi, esteraasi, leutsiini aminopeptidaasi, peroksüdaasi, glükuoso-6-fosfaadi ja 6-fosfoglükonaadi dehüdrogenaaside fraktsioonilist koostist. Mõleinad dehüdrogenaasid andsid eletroforeesil kaks fraktsiooni, mis teineteisest erinesid elektroforeetilise liikuvuse poolest, olid aga ühisest peaaegu kõigil uuritud organeil. Glükuoso-6-fosfaadi dehüdrogenaasi aktiivsust ei tähdeldatud tolmukapeade ja emakasuudmete-kaelte ekstraktides. Leutsiini aminopeptidaas andis elektroforeesil ainult ühe põhifraktsiooni, mis puudus emakasuudmete-kaelte ekstraktides.

Peroxüdaas, esteraas ja happele fosfataas jaotusid elektroforeesil paljudeks fraktsioonideks, millest mitmed olid ühised kõigile uuritud kudedede, teised omased vaid mõnele või isegi ainult ühele taimeorganile.

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15. XI 1973

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ЭЛЕКТРОФОРЕТИЧЕСКОЕ ИЗУЧЕНИЕ ФЕРМЕНТОВ В *SCILLA SIBIRICA* ANDR.*Резюме*

С помощью электрофореза в полиакриламидном геле исследовался фракционный состав кислой фосфатазы, эстеразы, лейцинаминопептидазы, пероксидазы, глюкозо-б-фосфат- и б-фосфоглюконатдегидрогеназ в отдельных органах пролески сибирской

Дегидрогеназы глюкозо-б-фосфата и б-фосфоглюконата встречались в виде двух фракций, которые отличались друг от друга электрофоретической подвижностью, но были общими почти для всех исследованных органов. Глюкозо-б-фосфат дегидрогеназной активности не наблюдали в экстрактах пыльников и рыльце-столбиков. Лейцинаминопептидазная активность выявлялась как одна дискретная фракция с постоянной электрофоретической подвижностью.

Пероксидаза, эстераза и кислая фосфатаза присутствуют в различных органах пролески сибирской в виде множественных молекулярных форм, из которых многие фракции были общими для всех исследованных тканей, а некоторые свойственны только определенным органам.

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