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Changes of Esterase Electrophoretic Patterns during the Ontogenetic Stages of Diploid Native Greeke Species of the Genus Aegilops

By

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With 5 figures

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Abstract

Esterases of five native Greek varieties of the genus *Aegilops* were studied at their early ontogenetic germination stages, by the use of horizontal starch electrophoresis. We noticed a quantitative and qualitative increase in the number of bands after a lapse of 3—4 days. Besides we observed a high degree of intraspecies similarity among the electrophoretic patterns in contrast to the relative interspecies deviations. The most kindred varieties from the morphological and cytological point of view show approximately the same enzyme activity during their initial development stages. The experimental data thus obtained, enable us to use them for collecting information regarding taxonomic studies on molecular level.

Zusammenfassung

Mittels der Stärkegel-Horizontalelektrophorese werden die Esterasen von fünf in Griechenland beheimateter Varietäten von *Aegilops* während der frühen Entwicklungsstadien der Keimlinge untersucht. Es konnte nach Ablauf von 3—4 Tagen eine Zunahme der Banden sowohl hinsichtlich Zahl wie Intensität festgestellt werden. Weiters besteht eine große Ähnlichkeit der Elektrophoresemuster innerhalb der einzelnen Spezies im Gegensatz zu den interspezifischen Unterschieden. Auf Grund morphologischer wie cytologischer Merkmale eng verwandte Varietäten stimmen während der ersten Entwicklungsstadien auch hinsichtlich ihrer Enzymaktivitäten überein. Derartige Daten vermögen auf molekularer Ebene Informationen für taxonomische Studien zu liefern. (übers. vom Editor)

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Introduction

The differentiation and development of multicellular organisms during ontogeny is generally considered nowadays to be due to the coordinated activity of particular enzymes at each developmental stage. The result of this activity is the production of special enzymes and proteins, that are characteristic of the cells of the different tissues (BONNER & VARNER 1966). The tissues of a plant differ in the nature and time of formation of their enzymes (SCHWARTZ 1962, FIRENZUOLI, VANNI & BACCARI 1968, KESWANI & UPADHYA 1969). During the germination of seeds, the breakdown of the reserve material begins as a result of enzyme activity. The production and activity of these enzymes are regulated by their respective genes. The activity of the said enzymes manifests itself in stages during the course of ontogenetic evolution (SUSSMAN 1964, BHATIA & NILSON 1969) in such a manner, that concentration of new enzyme molecular forms and disappearance of old ones take place. The varied activity of the enzymes thus revealed, may be either qualitative or quantitative or even a combination of both (BHATIA & NILSON 1969).

Numerous investigators have recently been engaged in solving and establishing various genetic problems of the genus *Aegilops* by means of enzyme analysis. The genus *Aegilops* is mainly endemic to the mediterranean countries. It is particularly interesting, because it has played a considerable part in the phylogenetic evolution of the genus *Triticum* (RILEY, UNRAU & CHAPMAN 1958, RILEY & CHAPMAN 1960).

The evidence produced by JOHNSON (1967) using electrophoretic methods confirmed KIHARA's view (1954), that *Ae. cylindrica* resulted from the chromosome duplication of the product of cross-fertilization between *Ae. caudata* and *Ae. squarrosa*. In order to ascertain the interspecific phylogenetic relationships of the *Triticinae*, BHATIA (1968) combined electrophoretic, cytological and morphological data. NAKAI (1972) using a polyacrylamide disc isoelectrofocusing technique, established the genome correspondence between *Ae. triuncialis* (C^uC^uCC), *Ae. caudata* (CC) and *Ae. umbellulata* (C^uC^u) respectively. Using combined cytological and enzyme studies a degree of interspecific and intraspecific phylogenetic relationship among the native Greek representatives of the genus *Aegilops* was found (KARATAGLIS 1973).

Studies of differential gene action or gene expression during development are basic to the understanding of the developmental processes. A good approach to the problem is to study the ontogeny of enzymes characterestic of a particular system since this provides a sensitive index of the basic changes occuring during development and differentiation.

The purpose of this investigation is to study the electrophoretic patterns of esterases and their change during early ontogenetic germinating stages in some native Greek species of the genus *Aegilops*.

Materials and Methods

Five native Greek varieties of the genus *Aegilops* (*Ae. caudata* var. *typica*, *Ae. caudata* var. *polyathera*, *Ae. comosa* ssp. *eucomosa* var. *thessalica*, *Ae. comosa* ssp. *heldreichii* var. *biaristata* and *Ae. comosa* ssp. *heldreichii* var. *subventricosa*) were studied at their initial ontogenetic stages.

Seeds of all these varieties were allowed to germinate in Petri dishes at $24 \pm 1°$ C in the dark. Seedlings were harvested after 2, 4, 6, 10, 14 days and were homogenized with distilled water in the ratio of 2 : 1 w/v. The extracts were subsequently electrophorized or were frozen at $-15°$ C for later use. The extracts to be electrophorised were placed on Whatman 3MM filter paper strips (dimensions 7×6 mm) according to the technique used by BECKMAN and JOHNSON (1964). Horizontal starch gel electrophoresis was carried out as employed by SMITHIES (1955) and modified by KARATAGLIS (1973). During electrophoresis the potential difference was kept constant at 240 V, whereas the current intensity fluctuated between 60 and 90 mA. Electrophoresis was interrupted when the front had migrated a distance of 8—8.5 cm from the origin. During the whole process of electrophoresis the temperature of the gel was kept constant at 7—8° C by means of circulating cold water. The gel bearing the segregated enzymes was stained at room temperature for about ½—1 hour in a solution having the following composition: 40 ml of distilled water, 50 ml 0.2 M NaH_2PO_4 (PH = 4.6), 10 ml 0.2 M Na_2HPO_4 (PH = 8.8), 2 ml 1% a-naphthyl-acetate in an aqueous solution of 50% acetone (v/v), and 20 mg of dye Fast Red TR salt (BREWBAKER *et al.* 1968).

Areas of gel where esterase activity was observed were stained brown red.

Results

After electrophoresis of shoot extracts of the above mentioned species, bands were observed which can be classified in four main areas: a) areas of slow electrophoretic mobility, b) of medium, c) of fast, and d) of very fast mobility. Among them area (b) showed the greatest number of bands and variations of intensity. The bands of areas (a) and (d) were extremely faint and hardly discernible.

All the enzymes showing esterase activity were found to move towards the anode.

The total number of bands found in the investigated plants, is shown in Table 1.

A. *caudata* group

Ae. caudata var. *typica* — *Ae. caudata* var. *polyathera*.

The anodal bands showed a significant change in their number and intensity during germination period.

Bands I and II showed the same intensity and mobility in the first two ontogenetic stages in both *Ae. caudata* varieties and finally disappeared at the third stage. The same also happened with bands X and XI, however in the variety *polyathera* band X remained at the 6 day-old stage and then disappeared (Fig. 1, 2).

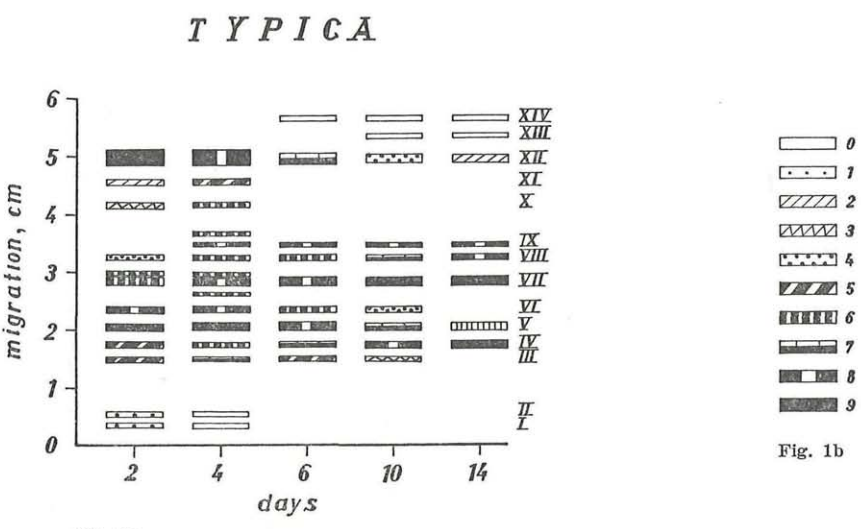

Fig. 1a

Fig. 1b

Fig. 1a. Schematic representation of zymograms showing the electrophoretic patterns of esterase in *Ae. caudata* var. *typica*.
Fig. 1. (on the right hand). Key to shading, in order of increasing intensity.

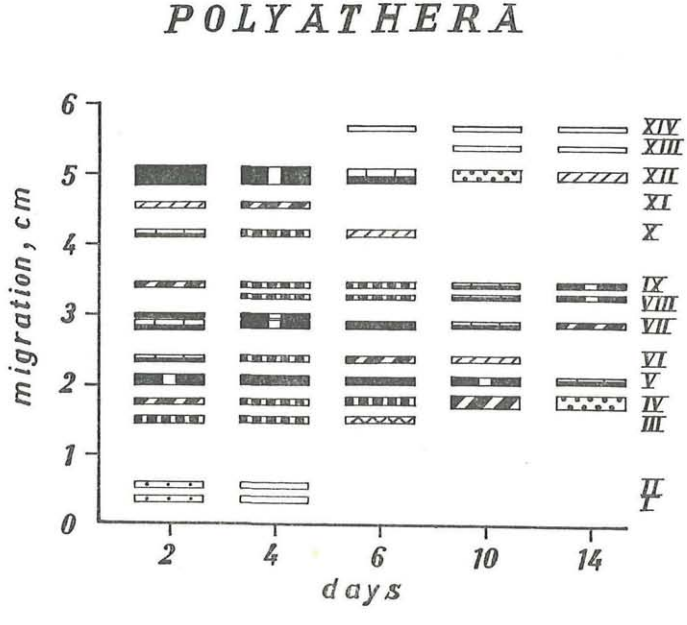

Fig. 2. Schematic representation of zymograms showing the electrophoretic patterns of esterases in *Ae. caudata* var. *polyathera*. (Symbols see Fig. 1b).

In both varieties the very fast electrophoretic mobility of bands XIV and XIII appeared after 6 and 10 days of germination respectively. Band IX in *typica* appeared after 4 days, whereas the corresponding band of *polyathera* occured in the first stage. The exact opposit was found to be the case for band VIII. Bands III and VI, appeared at the first stage and then disappeared at the 14 days stage.

The rest of the bands remained from the first until the last stage with a variation in intensity.

B. *comosa* group

1. ssp. *heldreichii* a) var. *biaristata*
   b) var. *subventricosa*

The esterase activity of the seedlings increased progressively during the first four days of growth and showed the highest number of bands in the 4 day stage in both varieties of *heldreichii*.

Table 1

Total number of bands

Species	Days				
	2	4	6	10	14
Ae. caudata var. *typica*	12	15	9	10	8
Ae. caudata var. *polyathera*	12	13	10	9	8
Ae. comosa ssp. *heldreichii* var. *biaristata*	10	13	10	10	11
Ae. comosa ssp. *heldreichii* var. *subventricosa*	10	12	8	8	8
Ae. comosa ssp. *eucomosa* var. *thessalica*	10	12	11	11	11

In 2 day old plants, bands I and II showed a very low intensity and mobility, hardly became visible at the next stage and untimately disappeared (Fig. 3, 4).

In 4 and 6 day old plants the bands with very fast electrophoretic mobility and very low intensity appeared first in *biaristata* and *subventricosa* respectively. In var. *biaristata* band IX first appeared after 4 day with a very low intensity and remained unchanged during the next stages.

2. ssp. *eu-comosa* a) var. *thessalica*

In 4 day-old plants the bands of slow mobility, I and II, have olready disappeared, while the appearence of IV, VI and XIII was visible for the first time. From all the bands only XIII showed a very faint intensity and remained unchanged during the next stages (Fig. 5).

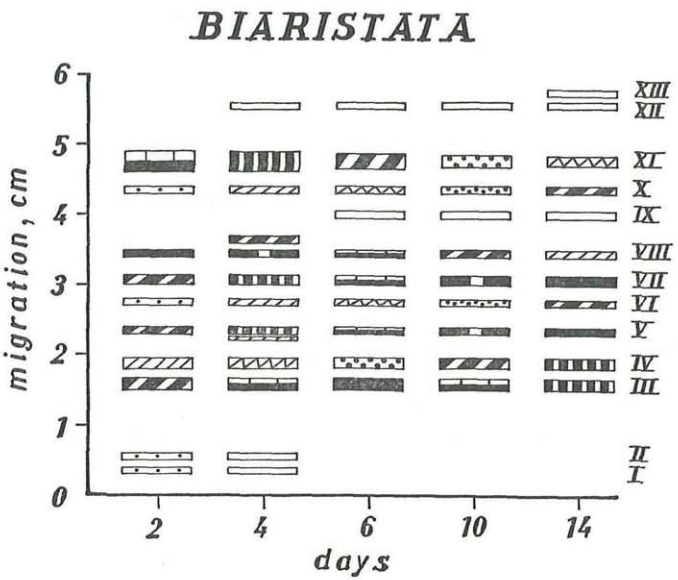

Fig. 3. Schematic representation of zymograms showing the electrophoretic patterns of esterases in *Ae. comosa* ssp. *heldreichii* var. *biatistata*. (Symbols see fig. 1b).

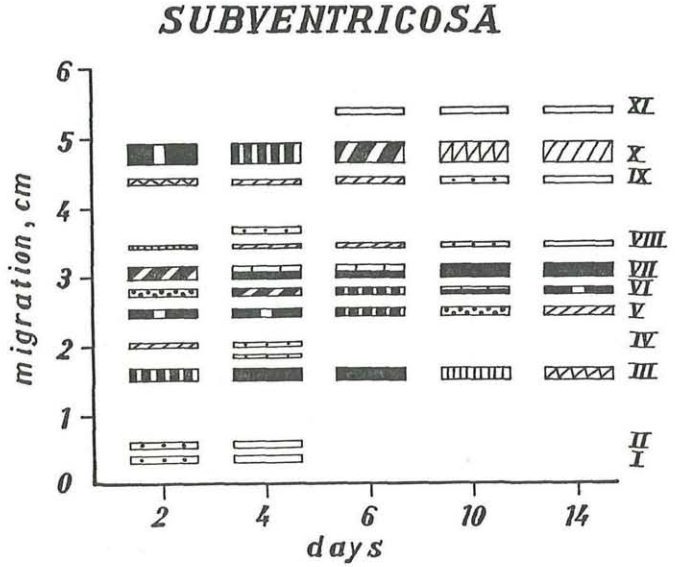

Fig. 4. Schematic representation of zymograms showing the electrophoretic patterns of esterases in *Ae. comosa* ssp. *heldreichii* var. *subventricosa* (Symbols see fig. 1b).

Discussion

The vital functions of seeds in general are in a latent state, although they are provided with the necessary nutrients for the growth and development of the plant during its early stages. During the germination of seeds, the breakdown of reserve materials and their transport from the endosperm to the embryo, occurs by means of enzyme activity. These specific enzymes

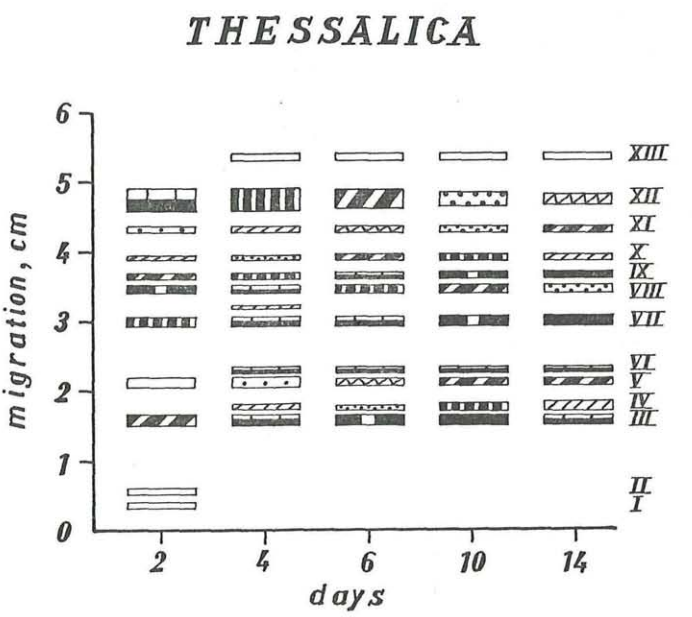

Fig. 5. Schematic representation of zymograms showing the electrophoretic patterns of esterases in *Ae. comosa* ssp. *eu-comosa* var. *thessalica* (Symbols see fig. 1b).

are either activated or synthesized de novo at the appropriate ontogenetics stage within the seeds (Bonner & Varner 1966, Keswani & Upadhya 1969).

During the germination of seeds and, simultaneous with the growth and development of the seedling, morphological changes are observed which are correlated with parallel changes in enzyme activity. In the varieties studies the number and intensity of esterase bands show a considerable increase during the 48—96 hour period of germination. This involves an increased activity of esterases during the said vegetative period, i. e. a more intensive simultaneous gene activity. It is thus concluded, that there is a close relationship and interdependence between the development and synthesis of enzymic forms, as was further demonstrated by Priesley & Fowden (1965), Mäkinen (1968), Keswani & Upadhya (1969), Efron &

SCHWARTZ (1968). This increase in the number of enzymes is due probably to the further breakdown of the reserve materials and to the synthesis of new ones for the growing and developing seedling (BONNER & VARNER 1966).

The presence or absence of a band and its intensity on the gel is a reflection of the quantity of the active enzyme present in the extract sampled. Possible regulatory control of tissue specific patterns have been considered by SHANNON (1968) and EFRON & SCHWARTZ (1968). So it is possible to say that the differentiation of enzyme patterns undoubtedly underlies ontogenetic changes and differences of tissues; hence, the enzyme complement of a tissue may be indicative of its stage of development and physiological function.

Intra- and inter-specific relationships:

During the course of ontogeny it was further observed that the materials showing the closest affinity, from the phenotypic and chromosomal point of view (KARATAGLIS 1974), appear with approximately the same enzyme activity during the first stages. This constitutes a criterion of the degree of the intra- and inter-specific relationship. This phylogenetic relationship can be calculated by means of a method applied by SMITH *et al.* (1970), CONKLIN & SMITH (1971), and KARATAGLIS & TSEKOS (1974) in the genera *Nicotiana*, *Datura* and *Aegilops* respectively. The equation for the hypergeometric series which they used is:

$$P_{(i)} = \frac{\binom{K_2}{i}\binom{n-K_2}{K_1-i}}{\binom{n}{K_1}}$$

Where $P_{(i)}$ is the probability of obtaning i common bands under random matching; i is the number of common bands; K_1 is the number of bands in one species; K_2 is the number of bands in the other species; n is the total number of bands possible.

Table 2

Probability of random band matching intra- and interspecies of *Ae. caudata* and *Ae. comosa*

varieties	Ty	P	S	B	Th
typica (Ty)	0	0.002	0.992	0.926	0.934
polyathera (P)		0	0.974	0.860	0.970
subventricosa (S)			0	0.010	0.407
biaristata (B)				0	0.263
thessalica (Th)					0

Using this method it is possible to illustrate the existing intra- and interspecific relationships among 4 day-old plants, and these are shown in table 2. By means of this table we can draw the general conclusion that the variation within a species is much less than between species.

A more detailed zymogram study of all the investigated varieties of the genus *Aegilops*, has led us to the conclusion, that certain molecular forms of esterase are present in all the germination stages observed. The variations of intensity and width of certain bands during the various germination stages, are due to differences in quantity and activity of the enzyme which is present in the extracted samples. Furthermore, it was observed that during the disappearance of the bands of low electrophoretic mobility, bands of very fast mobility appeared. The respective genes controlling the bands which show very fast electrophoretic mobility act, most probably, in my opinion, as inhibitors of the respective genes controlling in the bands of slow electrophoretic mobility (compare fig. 1, 2, 3, 4, 5).

These quantitative and qualitative changes in the zymograms, probably illustrate the activation or inactivation of those genes which determine the formation and development of the plant form (Danielli 1963, Steward, Lyndon & Barber 1965, Scandalios 1965, Gupta & Stebbins 1969).

References

Beckman L. & Johnson F. M. 1964. Esterase variations in *Drosophila melanogaster*. Hereditas 51: 212—220.

Bhatia C. R. 1968. Electrophoresis of analogous enzymes in *Triticinae*. Proc. 3rd Int. Wheat Genet. Symp. Canberra, 111—115.

— & Nilson J. P. 1969. Isoenzyme changes accompanying germination of wheat seeds. Biochem. Genetics 3: 207—214.

Bonner J. & Varner J. E. 1966. Plant Biochemistry. New York and London.

Brewbaker J. L., Upadhya M. D., Mäkinen Y. & MacDonald T. 1968. Isoenzyme polymorphism in flowering plants. III. Gel electrophoretic methods and applications. Physiol. Plant. 21: 930—940.

Conklin M. E. & Smith H. H. 1971. Peroxidase isozymes: A measure of molecular variation in ten herbaceous species of *Datura*. Amer. J. Bot. 58: 688—696.

Danielli J. F. 1963. Cytochemistry. New York.

Firenzuoli A. M., Vanni P. & Baccari V. 1968. Changes in enzyme levels during germination of seeds of *Triticum durum*. Plant Physiol. 43: 260—264.

Efron Y. & Schwartz D. 1968. In vivo inactivation of maize alcohol dehydrogenase by a twofactor system. Proc. N. A. S.

Gupta V. & Stebbins G. L. 1969. Peroxidase activity in hooded and awned barley at successive stages of development. Biochem. Genet. 3. 15—24.

Johnson B. L. 1967. Confirmation of the genome donors of *Aegilops cylindrica*. Nature 216: 859—862.

KARATAGLIS S. 1973. Comparative research upon the karyotype and electrophoretic enzymes of esterases from some Greek native species of the genus *Aegilops*. Ph. D. Thesis, University of Thessaloniki.

— & TSEKOS I. 1975. Electrophoretic variation in esterases of native Greek *Aegilops* species (*Ae. ovata, Ae. biuncialis* and *Ae. triuncialis*). Protoplasma (in press).

KESWANI C. L. & UPADHYA M. D. 1969. Isoenzyme changes during seed germination of saguaro cactus (*Carnegiea gigantea*). Physiol. Plant. 22: 386—391.

KIHARA H. 1954. Considerations on the evolution and distribution of *Aegilops* species based on the analyser-method. Cytologia, 19: 336—357.

MÄKINEN Y. 1968. Isoenzyme polymorphism in flowering plants. VI. Variation of isoenzyme patterns in onion seedlings. Physiol. Plant. 21: 858—865.

NAKAI Y. 1972. Isoenzymes of *Aegilops triuncialis* and its parental species. Wheat Inf. Service 33—34: 15—19.

PRIESLEY H. J. & FOWDEN L. 1965. Acid phosphatase and isocitritase production during seed germination. Phytochem. 4: 169—176.

RILEY R. & CHAPMAN V. 1960. The D genome of hexaploid wheat. Wheat Inf. Service II, 18.

RILEY R., UNRAU J. & CHAPMAN V. 1958. Evidence of the origin of the B genome of wheat. J. Hered. 49: 91.

SCANDALIOS J. G. 1965. Leucine aminopeptidase in maize development. J. Hered. 56: 177—180.

SHANNON L. M. 1968. Plant isoenzymes. Ann. Rev. Plant Physiol. 19, 187—210.

SCHWARTZ D. 1962. Genetic studies on mutal enzymes in maize. III. Control of gene action in the synthesis of pH 7,5 esterases. Genetics 47, 1609.

SMITH H. H., HAMILL D. E., WEAVER E. A. & THOMPSON K. H. 1970. Multiple molecular forms of peroxidases and esterases among *Nicotiana* species and amphiploids. J. Hered. 61: 203—212.

SMITHIES O. 1955. Zone electrophoresis in starch gels: Group variation in the serum proteins of normal human adults. Biochem. J 61: 629—641.

STEWARD F. C., LYNDON R. F. & BARBER J. T. 1965. Acrylamide gel electrophoresis of soluble plant proteins: A study on pea seedling in relation to development. Amer. J. Bot. 52: 155—164.

SUSSMAN M. 1964. Growth and Development. 2nd ed. New Jersey.