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TAXONOMIC DISTRIBUTION OF ISOENZYMES OF DEHYDROQUINATE HYDROLYASE IN THE ANGIOSPERMS

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Key Word Index—Angiosperms; monocotyledons; isoenzymes; shikimate pathway; 5-dehydroquinate hydrolyase; regulatory patterns; molecular evolution.

Abstract—The distribution of a dehydroquinate hydrolase (E.C. 4.2.1.10) isoenzyme activated by shikimic acid was studied in angiosperms through a simple diagnostic test connected with the regulatory properties of the enzyme. This form was present only in monocotyledons and essentially in Juncaceae, Gramineae and Cyperaceae. The use of regulatory patterns as taxonomic and phylogenetic indicators and the evolution of enzymatic potentialities in plants are discussed.

INTRODUCTION

The taxonomic value of regulatory patterns has been demonstrated in micro-organisms by comparative studies on the metabolism of aromatic compounds [1, 2], amino acids of the aspartate family [3] and also on the regulation of bacterial citrate synthase [4]. Specific regulatory mechanisms are stable characters shared by the members of large taxa for which a common evolutionary origin can be postulated.

In plants, different types of chemical or biochemical information including variability of proteins and enzymes [5–7] have been currently used for taxonomic purposes. However few examples are related to the taxonomic value or the evolutionary significance of metabolic control systems.

Aspartate kinase activity has been examined in some plant seedlings [8, 9] and there seems to be a great variety of regulatory patterns, but the results so far are of little taxonomic significance [10]. Again, after a survey of different angiosperms, Davies *et al.* [11] conclude that the presence or absence of allosteric properties associated with malic enzyme cannot usefully be applied to problems of taxonomy.

Recently for the first time in higher plants we have identified [12] two dehydroquinate hydrolyases (DHQ-ases) (the third enzyme of the shikimate pathway). One isoenzyme is associated with the shikimate: $NADP^+$ oxidoreductase (SH.ORase) (E.C. 1.1.1.25) in a complex which is at least bifunctional and which seems widely distributed in plants [13]. The other isoenzyme is a free form specifically activated by shikimic acid. This last property has been studied in detail in corn [14] but the present investigation was undertaken to determine the distribution of this regulatory system in angiosperms and the possible taxonomic interest thereof.

RESULTS

First we attempted to separate the two isoenzymes of DHQase using DEAE-cellulose chromatography and to check the regulatory properties of the free form. This allowed us to identify plants with and without the isoenzymes as shown on the figure 1. However, such a tedious procedure is not convenient for a systematic survey. An alternative method based on polyacrylamide

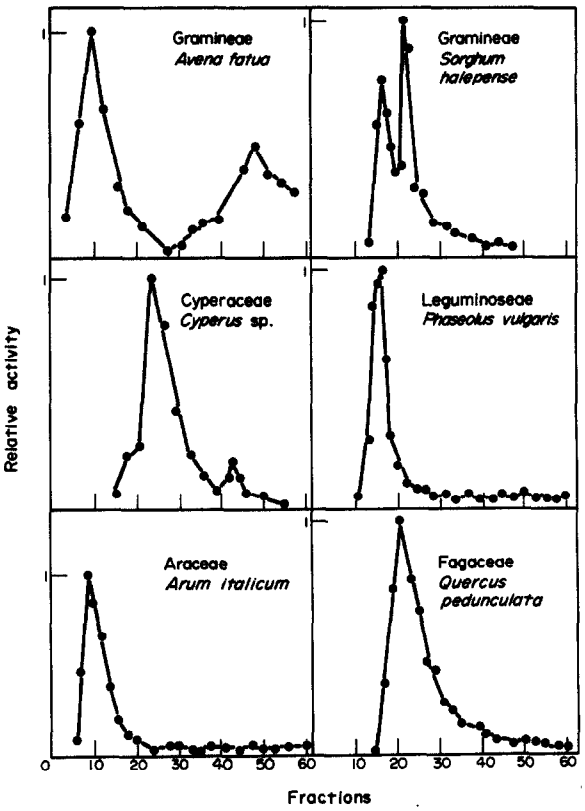

Fig. 1. Elution patterns of DHQase activity from different Angiosperms on DEAE cellulose column.

gel electrophoresis was also not possible since we have not succeeded in developing a specific staining reaction for the enzymes. Nevertheless, if the specific activation of DHQase 2 is clearly demonstrated on the isolated free form, this property is also detectable in a crude extract (before or after Sephadex G 25) containing both isoenzymes. Accordingly, we have carefully checked that a stimulatory effect of shikimic acid on the DHQase activity of the crude extract is only obtained in the cases where two forms are separable after DEAE-cellulose (Table 1).

A strict correlation enabled us to develop a rapid diagnostic test (see Experimental) as proof of the existence of two isoenzymes of DHQase, one of them being activated by shikimic acid. Using this procedure, we surveyed 65 species of 52 dicotyledonous families and 57 species of 19 monocotyledonous families. The data (tables 2 and 3) divide the plants into three groups: all dicotyledons and most of the monocotyledons tested are insensitive to shikimic acid action; in monocotyledons a positive response is obtained for all species examined in the Juncaceae, Gramineae and Cyperaceae; in two other monocot. families, Liliaceae and Iridaceae, only some species possess the two isoenzymes.

Table 1. Presence of multiple forms of DHQase and correlative activation of the activity by shikimic acid in crude extracts

Families and species	Forms characterized after chromatography on DEAE cellulose	Stimulation of DHQase activity from crude extract in presence of S.A. (in per cent of activity without modifier)
Monocotyledons		
Gramineae		
Avena fatua L.	2	64
Arundo donax L.	2	100
Panicum maximum Jacq.	2	120
Sorgum halepense (L.) Brot.	2	150
Triticum vulgare Vill.	2	170
Zea mays L.	2	90
Cyperaceae		
Cyperus sp.	2	90
Araceae		
Arum italicum Mill.	1	0
Dicotyledons		
Fagaceae		
Quercus pedunculata Ehrh.	1	0
Leguminosae		
Cercis siliquastrum L.	1	0
Phaseolus vulgaris L.	1	0
Solanaceae		
Solanum lycopersicum L.	1	0

Table 2. Dicotyledons studied showing no activation by shikimic acid

Families	Species
Juglandaceae	*Juglans regia* L.
Salicaceae	*Salix* sp.
Fagaceae	*Alnus glutinosa* (L.) Gaertn.
	Quercus pedunculata Ehrh.
Urticaceae	*Parieteria officinalis* L.
Polygonaceae	*Fagopyrum esculentum* Moench.
Caryophyllaceae	*Saponaria officinalis* L.
	Dianthus caryophyllus L.
Chenopodiaceae	*Spinacia olearacea* L.
Cactaceae	*Opuntia vulgaris* Miller
Magnoliaceae	*Magnolia soulangeana*
Lauraceae	*Persea americana*
Ranunculaceae	*Helleborus niger* L.
	Clematis viticella L.
Nympheaceae	*Nuphar luteum* Sm.
Berberidaceae	*Mahonia aquifolia* (Pursh) Nutt.
Cruciferae	*Alyssum saxatile* L.
	Cheiranthus cheiri L.
Theaceae	*Camellia japonica* L.
Guttiferae	*Hypericum perforatum* L.
Hammamelidaceae	*Liquidambar styraciflua*
Crassulaceae	*Sedum* sp.
Saxifragaceae	*Hydrangea hortensia* Sieb.
Rosaceae	*Potentilla* sp.
	Pyracantha coccinea Roemer
Leguminosae	*Wisteria sinensis* (Sims) DC.
	Phaseolus vulgaris L.
	Pisum sativum L.
	Vicia faba L.
Geraniaceae	*Pelargonium zonale* L.
Euphorbiaceae	*Ricinus communis* L.
Rutaceae	*Ruta graveolens* L.
Hippocastanaceae	*Aesculus hippocastanum* (Tourn.) L.
Celastraceae	*Evonymus vulgaris* Miller
Vitaceae	*Vitis vinifera* L.
Malvaceae	*Althea* sp.
Violaceae	*Viola* sp.
Passifloraceae	*Passiflora* sp.
Tamaricaceae	*Tamarix* sp.
Cucurbitaceae	*Cucumis melo* L.
Punicaceae	*Punica granatum* L.
Araliaceae	*Hedera helix* L.
Ericaceae	*Rhododendron* sp.
Primulaceae	*Primula auricula* L.
Ebenaceae	*Diospyros kaki* L.f.
Oleaceae	*Forsythia* sp.
Apocynaceae	*Nerium oleander* L.
	Vinca major L.
Asclepiadaceae	*Vincetoxicum officinale* Moench.
Rubiaceae	*Gallium cruciata* L.
Polemoniaceae	*Phlox paniculata* L.
Boraginaceae	*Echium vulgare* L.
	Pulmonaria affinis Jord.
Verbenaceae	*Lippia canescens* Humb. Bonp. et K.
Labiatae	*Salvia officinalis* L.
	Mentha rotundifolia L.
Solanaceae	*Solanum lycopersicum* L.
	Petunia hybrida
Buddleaceae	*Buddlea variabilis* Hemsley
Scrophulariaceae	*Antirrhinum majus* L.
Bignoniaceae	*Campsis radicans* (L.) Seemen
Plantaginaceae	*Plantago lanceolata* L.
Valerianaceae	*Centranthus ruber* (L.) DC.
Compositae	*Achillea millefolium* L.
	Cichorium endivia L.

Table 3. Effect of shikimic acid on the activity of DHQase in various species of Monocotyledons

Families	Species	Activation in presence of S.A.
Alismataceae	*Sagittaria sagittifolia* L.	0
Butomaceae	*Butomus umbellatus* L.	0
Liliaceae	*Asparagus officinalis* L.	+
	Convallaria maialis L.	0
	Polygonatum odoratum (Mill.) P.F.	0
	Ruscus aculeatus L.	0
	Asphodelus aestivus Brotero	+
	Hemerocallis sp.	+
	Hyacinthus orientalis L.	0
	Scilla liliohyacinthus L.	0
	Allium schoenoprasum L.	0
	Lilium sp.	0
	Colchicum autumnale L.	0
Agavaceae	*Yucca filamentosa* L.	0
	Agave americana L.	0
Amaryllidaceae	*Amaryllis* sp.	0
	Narcissus sp.	0
Dioscoreaceae	*Tamus communis* L.	0
Iridaceae	*Crocus versicolor* Ker G.	0
	Iris germanica L.	+
	Iris pseudacorus L.	0
	Iris pumila L.	+
	Iris xiphium (L.) Ehrh.	0
Juncaceae	*Juncus glaucus* Ehrh.	+
	Juncus obtusiflorus Ehrh.	+
Bromeliaceae	*Ananas sativus* Schult.	0
Commelinaceae	*Tradescantia zebrina* Loud.	0
Gramineae	*Avena fatua* L.	+
	Arundo donax L.	+
	Panicum maximum Jacq.	+
	Sorgum halepense (L.) Brot.	+
	Triticum vulgare Vill.	+
	Zea mays L.	+
	Lolium italicum A. Br.	+
	Dactylis glomerata L.	+
	Festuca arundinacea Schreb.	+
	Arrhenaterum elatius (L.) Mert. et K.	+
	Chloris gayana Kunth	+
	Poa annua L.	+
	Ammophila arenaria (L.) Link.	+
	Phyllostachys mitis Riviére	+
	Arundo phragmites L.	+
	Spartina maritima (Curt.) Fernald.	+
	Gynerium argenteum Nees	+
Palmae	*Phoenix* sp.	0
	Kentia sp.	0
Araceae	*Arum italicum* Mill.	0
Lemnaceae	*Lemna minor* L.	0
Typhaceae	*Typha latifolia* L.	0
Cyperaceae	*Carex pendula* Huds.	+
	Schoenus nigriscans L.	+
	Cyperus sp.	+
	Cyperus papyrus	+
Musaceae	*Musa* sp.	0
Cannaceae	*Canna edulis*	0
Orchidaceae	*Anacamptis pyramidalis* (L.) Rich.	0
	Platanthera bifolia (L.) Rich.	0

DISCUSSION

Although our methodology is not perfect, the homogeneity of the results obtained suggests that this approach to taxonomy is a valid one. Three phylogenetically closely linked families Juncaceae, Gramineae, Cyperaceae have in common a free DHQase isoenzyme activated by shikimic acid. Moreover, the results obtained from some species of Liliaceae and Iridaceae indicate a relation with these first three families. These data emphasize the relationships between the distribution of biochemical markers and the present phylogenetic schemes constructed by using mainly morphological characters. It is likely that following this broad survey, a more precise examination of species appertaining to the Liliaceae or the Iridaceae or related families could be helpful in determining the taxonomic position of organisms the affinities of which are uncertain or disputed.

Moreover the results described here give a good example of correlation between phylogeny and the evolution of biochemical organization. Previous data in animals [6] and plants [15] have shown that new enzymatic potentialities and new control mechanisms are found in the more advanced orders. These modifications (wider specificity, side reactions, continuance of biosynthetic chains, new isoenzymes) would arise from divergent evolution of duplicated genes [16, 6] and seem to give the mutant an advantage over the parent strain. The presence of two isoenzymes of DHQase, one of them activated by shikimic acid, is mainly found in advanced orders of monocotyledons and might represent a favourable solution for the plant. However the possible physiological role of this particular isoenzyme still remains to be elucidated [14].

EXPERIMENTAL

Plant material. Wild and cultivated plants were collected in the countryside around Toulouse and identified taxonomically. Some species were provided by the Botanical garden of the University; others were grown under controlled conditions. Enzymatic studies usually concerned the chlorophyllous organs and were in all cases performed on fresh material.

Chemicals. Dehydroquinic acid was prepared by chemical synthesis [17]. Shikimic acid puriss was obtained from FLUKA.

Enzymatic techniques. General techniques for extraction, purification and estimation of enzyme activity have already been published [13] but a routine procedure was designed especially to survey plants for the presence of the regulatory system. 5 g of plant material were ground in a chilled mortar with a phosphate buffer (pH: 7.5, 0.1 M) 0.2% mercaptoethanol, 20% glycerol, containing 2 mM EDTA. The homogenate was squeezed through 2 layers of muslin and centrifuged at 100000 *g* for 30 min. The supernatant was used as a source of enzyme or passed through a Sephadex G 25 column equilibrated with a phosphate buffer (pH 7.5, 0.1 M).

DHQase activity was determined on crude and (or) desalted extracts with and without shikimic acid in the medium. The modifier (final concentration: 5.10^{-4} M) was directly added to the reaction mixture without previous incubation.

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