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ISOENZYMES OF *DATURA METELOIDES* (SOLANACEAE). DEVELOPMENTAL PATTERNS AND TISSUE SPECIFICITIES

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INTRODUCTION

There exists at present no consensus as to what biological parameters qualify a taxon to be designated weedy or even as to a definition of the term "weed" (King, 1966). By most of the generally used criteria for weediness the species of *Datura* occurring in the southwestern United States qualify as weeds. To test Baker's (1972) hypothesis of a genetic basis for weediness (viz., sufficient genetic flexibility to allow preadaptation to a variety of habitats) I attempted to determine the genetic variation among individuals of *Datura meteloides* A. DC. It is desirable to determine if the extent of an organism's genetic variation influences its ability to successfully invade the variety of habitats in which weeds typically are found. This report presents results of studies preliminary to extensive investigation of populational isoenzyme variation.

MATERIALS AND METHODS

Fresh plant material was collected from the field or on the grounds of the Rancho Santa Ana Botanic Garden or was grown from seed in a controlled-environment chamber. Appropriate fresh tissue was diced with a razor blade and homogenized in a small volume of 20% (w/v) sucrose solution using a Ten-Broek tissue grinder. The homogenate was centrifuged 15 min at $10,000 \times g$ and the clear supernatant was subjected to electrophoresis in 7½% acrylamide gels at room temperature. Except where otherwise noted, gels were stained using incubation mixtures tabulated by Shaw and Prasad (1970).

RESULTS AND DISCUSSION

ISOENZYME PROFILE

The isoenzyme band patterns for five enzyme systems from mature leaf tissue of *Datura meteloides* are illustrated in Fig. 1. The following enzymes could not be histochemically detected following electrophoresis: acid and alkaline phosphatases, leucine aminopeptidase, alcohol and alpha-glycero-

Fig. 1. *Datura meteloides*. Isoenzyme band patterns for five enzyme systems from mature leaf tissue: esterase (EST, E.C. 3.1.1.2); glutamate dehydrogenase (GDH, E.C. 1.4.1.2); lactate dehydrogenase (LDH, E.C. 1.1.1.27); glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.10); and peroxidase (PER, E.C. 1.11.1.7).

phosphate dehydrogenases, and isocitrate lyase (Reeves and Volk, 1972). Malate dehydrogenase was detectable only as a single, very wide band (R_p 0.43–0.54) and consequently is not useful as a genetic marker.

Only one enzyme, glutamate-oxaloacetate transaminase, exhibited a polymorphic banding pattern among individuals sampled (see Fig. 1). One polymorphic locus out of five (20%) constitutes much less genetic variation than has been observed in similar studies of animals and other higher plants in which 26–55% of loci are found to be polymorphic (e.g., Ayala et al., 1972; Levin et al., 1972). Further study now under way of numerous natural populations may reveal additional polymorphs within these enzyme systems, but these results argue provisionally against the theory that large genetic variability allows weediness in *Datura*.

DEVELOPMENTAL PATTERNS

Different isoenzyme patterns have been observed in single tissues at various developmental stages in wheat (Bhatia and Nilson, 1969), maize (Hamill and Brewbaker, 1969) and *Allium* (Mallery, 1972). These changes in isoenzyme pattern are presumed to reflect fundamental processes of differentiation (Scandalios, 1974). Only one (peroxidase) of the five enzyme systems examined in *Datura meteloides* varied with plant age over a range of maturation from 10-da-old seedlings grown in the laboratory to mature plants collected in the field. It is concluded that these enzymes (except peroxidase) exhibit no developmental dependence in *Datura*.

The dependence of peroxidase isoenzyme pattern upon ontogenetic stage was studied in 10 species of *Datura* by Conklin and Smith (1971). *Datura*

AMYLASE

PER

Fig. 2. *Datura meteloides*. Tissue-specific isoenzyme patterns. a. Amylase band pattern from seeds. b. Isoperoxidase band patterns from leaves, stems, and roots of 1-mo-old seedlings.

meteloides was reported by these workers to be among the least variable in isoperoxidase pattern, varying only in relative band intensity in leaves of various ages (except for the most apical stem leaf which had fewer bands). This observation was borne out in the present study in that the basic isoperoxidase pattern does not change qualitatively in nonapical leaves from age 3 wk to maturity.

TISSUE SPECIFICITIES

Tissue specificities with respect to isoenzyme band patterns have been reported in barley (Upadhyya and Yee, 1968), onion (Makinin, 1968), maize (Efron, 1970) and rice (Pai, Endo and Oka, 1973). Six isoenzyme systems (EST; GOT; GDH; LDH; PEROX and Amylase, E.C. 3.2.1.1) were analysed from the following tissues: seed (endosperm plus embryo), root, stem, and leaf. In only two enzyme systems, peroxidase and amylase, were tissue specificities noted. Amylase is tissue specific in that it is detectable only in seed (see Fig. 2a).

Tissue-specific isoperoxidase patterns of root, stem, and leaf extracts from 1-mo-old seedlings are shown in Fig. 2b. Specificities between leaf and stem tissue consist only of relative staining intensity in one band (R_p 0.59), but are immediately recognizable and reproducible. Root-tissue homogenates exhibit an intense, additional band at R_p 0.40 which is entirely absent or very weak in extracts of other tissues. Root extracts have been observed

to contain additional isoperoxidase bands (relative to leaf tissue extracts) in *D. ferox* L. and *D. innoxia* Mill. (Guzman, Ferri and Trippi, 1971).

NATURE OF THE *DATURA* PEROXIDASE ENZYME SYSTEM

True peroxidase enzymes require as substrates for their catalytic action a hydrogen donor (an organic substrate) and hydrogen peroxide as an oxygen donor. The enzymes classified as peroxidases in *Datura* spp. yield colored bands in acrylamide gels with a variety of traditional peroxidase stains (e.g., benzidine plus hydrogen peroxide). In the present work identical isoperoxidase banding patterns were observed when a variety of hydrogen donors (benzidine, guaiacol, catechol, and quercetin) were used. An identical isoenzyme banding pattern is also obtained when the staining mixture is that for an oxidase system requiring only dissolved molecular oxygen as an oxygen source. A pattern identical to that for isoperoxidases was obtained with 0.01 M dihydroxyphenylalanine (a tyrosinase assay) and with 0.1% syringaldehyde (Harken and Obst, 1973; a laccase assay). These results suggest that members of this ill-defined system of oxidative enzymes in *Datura* are better classified as oxidases (E.C. 1.10.3.1 or E.C. 1.10.3.2) rather than peroxidases because of the lack of obligate dependence on H_2O_2 as an oxygen donor. This set of oxidative enzymes has very broad substrate specificities and serves a general oxidative function.

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