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Esterase Isozyme Patterns of Some Tropical and Subtropical Herbaceous Legumes[1]

K. H. Chow[2] and L. V. Crowder[3]

Herbaceous legumes play an important role in soil conservation and animal feeding in the tropics and subtropics. There are about 500 species in the genus *Desmodium* (Younge, Plucknett, and Rotar 1964), mostly distributed in the tropics and subtropics. A few of them, such as *D. canum*, *D. intortum*, and *D. uncinatum*, are good pasture plants (Bryan 1969; Younge, Plucknett, and Rotar 1964); others, such as *D. heterophyllum*, *D. ovalifolium*, and *D. triflorum*, are good soil cover crops (Whyte, Nilsson, and Trumble 1953). Breeding and hybridization among some *Desmodium* species have been carried out in Australia (Hutton and Gray 1967, McWhirter 1969), Hawaii (Rotar and Chow 1971), and other parts of the world (Compere 1961, Chow and Crowder 1972, 1973).

Isozymes, the different molecular forms of a protein having the same enzymatic specificity (Markert and Møller 1959), are separable by zone electrophoresis. The combination of zone electrophoresis and different enzyme staining methods has facilitated the possibility of studying different isozymes in living organisms. Polymorphic esterase variations have been found in a number of plants and animals and are potentially useful in genetic, breeding, taxonomic, and evolutionary research (Beckman and Johnson 1964, Bingham and Yeh 1971, Cherry and Katterman 1971, Chow and Crowder 1973, Fawcett and Patterson 1970, Frankel and Garber 1965, Nakai 1970, Wu and Li 1970). Recently, Chow and Crowder (1974), using isozyme patterns (esterase and peroxidase), identified a natural hybrid between two *Desmodium* species (*D. intortum* and *D. sandwicense*).

There is little information on isozyme patterns of tropical and subtropical herbaceous legumes. This paper reports the esterase isozyme patterns of some commonly found herbaceous legumes in the tropics and subtropics, including a range of *Desmodium* species. This study also examines, through the differences and similarities in esterase isozyme patterns, the relationships among the *Desmodium* species studied and should facilitate future breeding and hybridization work in this genus. Electrophoretic patterns of leaves and seed from one plant are compared to show that different organs of one plant may have different isozyme systems.

Materials and Methods

Esterase isozyme patterns were determined for 30 species in 12 genera. Plants were grown in a glasshouse at Cornell University, Ithaca, New York, during the winter and spring of 1971.

Fresh harvested leaves, the fourth from the growing tip, or swelled seeds were ground and squeezed in a mortar and pestle to obtain a suspension, which was then centrifuged at 10,000 r.p.m. for 15 to 20 minutes. The supernatant thus obtained was decanted into the small wells of a sample pickup tray. The isozymes were separated on electrophoresis strips, with the serum protein electrophoresis system designed by Photovolt Corporation (model 542) being used. The electrophoresis strips were made of cellulose acetate with a layer of starch on one surface. They were immersed for 5 minutes in a buffer solution containing 20 ml Ashton A: 2.6 g $LiOH \cdot H_2O$, 45.8 g boric acid, 3.8 liters H_2O; and 180 ml Ashton B: 6.08 g citric acid, 23.56 g Trizma (*tris*[hydroxymethyl]aminomethane) base, 3.8 liters H_2O (formulations obtained from

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[2] University of Singapore, Department of Botany, Singapore 10, Republic of Singapore.

[3] Cornell University, Department of Plant Breeding and Biometry, 252 Emerson Hall, Ithaca, New York 14853.

Dr. G. C. Ashton, University of Hawaii, personal communication). After being removed and blotted, the strips were transferred to a holding chamber, and a charge of 110 volts was applied for 1 minute. A 5 μl sample of the supernatant was then micropipetted onto the negative edge of the strip, and 110 volts were applied for 50 minutes.

For staining, the strips were placed in a solution containing 2.8 g NaH_2PO_4, 1.1 g Na_2HPO_4, 200 mg Fast Blue RR (Sigma Chemical Co., St. Louis, Missouri), 3 ml of solution containing 1-percent γ-naphthyl acetate and 99-percent ethyl alcohol by weight, and 200 ml H_2O. After 20 to 30 minutes at 37° C, they were immersed for 10 min in a wash solution composed of 10 parts methyl alcohol, 10 parts H_2O, 2 parts acetic acid, and 1 part ethyl alcohol, all by volume.

Electrophoresis was performed twice for each plant. Six plants each of *Desmodium intortum* 'Greenleaf', *D. intortum* 'Medellin', and *D. uncinatum* 'Silverleaf' and two plants each of all other species were examined. Patterns were identified by number, migration rate, and density of the bands, with the Densicord being used to facilitate quantitative analysis.

RESULTS AND DISCUSSION

The esterase molecules migrated only toward the positive pole, and the brown-stained bands were well separated. The bands remained on the strips for several days when stored in the wash solution. The number of esterase bands varied from two to four. Zymograms of the esterase isozyme patterns are shown in Figures 1–3. The width of the bands in the figures corresponds to the density or intensity of the sites of enzymatic activity, which was measured quantitatively by the Densicord. The short line at the left marks the origin; the light line at the right marks the front zone of the migrating materials. These two sites do not represent isozyme bands.

No two genera had identical patterns although several had common bands (Figure 1). This was particularly noticeable for the frontal region, which might have been modified with a different voltage and time. Within a genus, similar patterns were observed for *Centrosema*

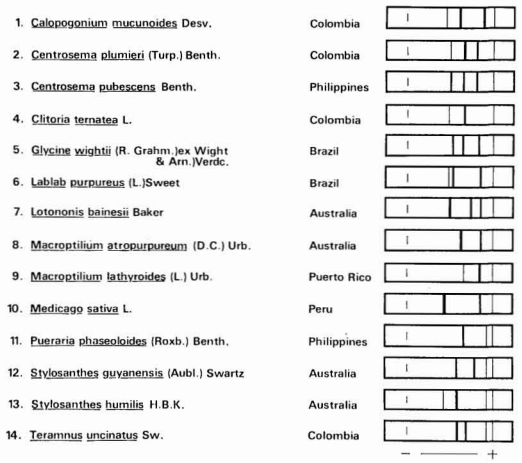

FIGURE 1. Esterase isozyme patterns of tropical legume species. Note that several genera have common bands; *Centrosema* and *Macroptilium* have similar bands; *Stylosanthes* spp. have different bands.

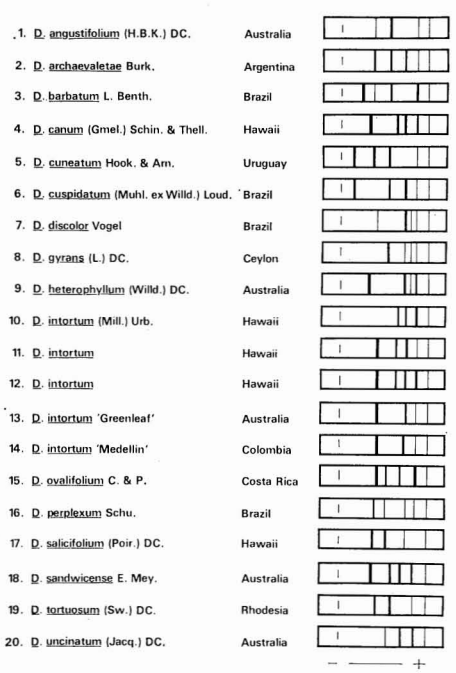

FIGURE 2. Esterase bands of *Desmodium* species. Common bands are found for all species but note variation among *D. intortum* entries.

pubescens and *C. plumieri*, as well as for *Macroptilium atropurpureum* and *M. lathyroides*. Considerable differences in esterase patterns were noted, however, among the *Desmodium* species (Figure

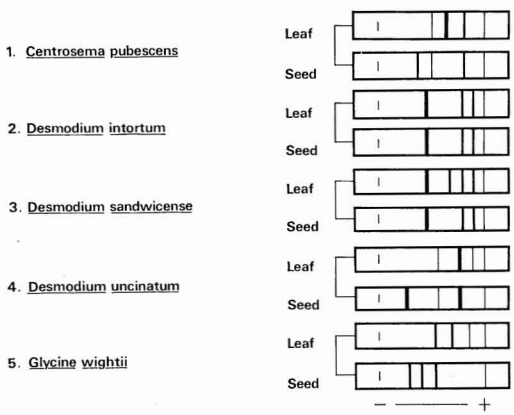

FIGURE 3. Esterase patterns of seed and leaf extracts of five tropical legumes. Note the similar banding of *Desmodium*, common bands of other species, but divergence of *Glycine*.

2). A wider range of banding patterns occurred within the *Desmodium* genus than within the other genera examined.

Ten of the 16 *Desmodium* species had four bands and the others had three bands (Figure 2). Selections of *D. intortum* had both three and four bands, which may indicate introgression effects, chance mutation, misclassification, or geographical isolation and natural ecotypic selection. Morphologically, the *Desmodium intortum* selections differed considerably with respect to growth habit, stem color, and the presence or absence of silver leaf markings and of brown leaf-spots (Imrie 1973, Rotar and Chow 1971). Variation in electrophoretic patterns have been shown among species of *Brassica* and *Raphanus* (Nakai 1970), *Hordeum* (Mitra, Jagannath, and Bhatia 1970), and *Nicotiana* (Hart and Bhatia 1967). Isozyme variation within one species has also been reported among *Glycine max* selections (Hymowitz 1973) and varieties (Larsen 1967) and among varieties of *Medicago sativa* (Bingham and Yeh 1971), *Pisum sativa* (Frankel and Garber 1965), and *Solanum tuberosum* (Desborough and Peloquin 1968).

In the present study, introductions of *D. canum* from Peru and Argentina showed almost identical electrophoretic patterns as that from Hawaii, the latter being illustrated in Figure 2. The three *D. canum* introductions probably were of the same origin. Two different ecotypes of *D. sandwicense* from Australia and Hawaii showed very similar esterase patterns. There is a close affinity between them. Two *D. uncinatum* selections from Australia and Hawaii had almost the same pattern. It was later learned that the Hawaiian selection of the species had been introduced from Australia under the name of silverleaf desmodium.

Each isozyme of a given *Desmodium* species—except for a single band each of *D. barbatum* and of *D. ovalifolium*—occurred in one or more other species. This banding similarity would be expected among species within a genus. Such relationships were also found to exist within various genera of the Leguminosae by Verdcourt (1970), who used electrophoretic patterns to modify taxonomic classifications within this family. In the present study, three of the *D. intortum* isozymes occupied the same site on the strips as those of *D. sandwicense*. The affinity of these two species has been shown by the ease of hybridization under controlled and natural conditions (Hutton and Gray 1967; McWhirter 1969; Rotar and Chow 1971; Chow and Crowder 1973, 1974). Hybrids of both with *D. uncinatum*, all having two common bands, have been reported (Hutton and Gray 1967, Rotar and Chow 1971, Chow and Crowder 1973) as well as have hybrids between *D. canum* and *D. uncinatum*, also having two common bands (Chow and Crowder 1972).

With each of the three *Desmodium* selections —*D. intortum* 'Greenleaf', *D. intortum* 'Medellin', and *D. uncinatum* 'Silverleaf'—plants showed almost identical esterase patterns with only a slight difference appearing in the density of some bands. The uniformity of isozyme patterns of different plants from one selection indicates a high degree of self-pollination within each selection and suggests that a strong selection pressure has occurred for a given biotype under natural conditions.

Because of availability, ease of storage, and commercial importance of seed, seed extracts frequently have been used to determine isozyme patterns. The esterase patterns of leaves and seeds from one plant are shown side by side in Figure 3. Among the five species studied, only *Desmodium intortum* had almost identical patterns for the leaves and seeds from the same plant. The other four species had different esterase

patterns for the leaves and seeds from the same plant, although common bands were observed. The results indicate that more than one group of esterase isozymes is present in different organs of one plant. The same phenomenon has also been observed by Hall (1970) in *Phaseolus vulgaris* and by Wu and Li (1970) in *Oryza sativa*. Developmental variation in isozyme patterns has been reported by Macko, Honold, and Stahmann (1967) in *Triticum vulgare* during germination and early growth period and by Mills and Crowden (1968) in *Pisum sativa*.

SUMMARY AND CONCLUSIONS

Esterase isozyme patterns in extracts from leaves and seeds of 30 different tropical and subtropical herbaceous legumes were studied by use of a serum protein electrophoresis system. From two to four bands with different mobilities and densities were detected in the starch layer that was placed on cellulose acetate strips. Differences and similarities in band patterns were found both within and between genera. A wide variation in esterase isozyme patterns occurred among *Desmodium* species. Within *Desmodium intortum*, different selections had different esterase isozyme patterns that were consonant with their morphological variation. Among the different ecotypes within each of *Desmodium canum*, *D. sandwicense*, and *D. uncinatum*, esterase patterns were almost identical or very similar. This indicates that the ecotypes within each of the species were of the same origin or of a close affinity.

Within each of the three selections of *Desmodium intortum* and *D. uncinatum*, plants showed almost identical esterase patterns, which indicates a high degree of self-pollination for these two species. The electrophoretic patterns of leaves and seeds from one plant were different, but common bands occurred. This indicates that more than one group of esterase isozymes is present in different plant organs in a single plant.

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