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NONSPECIFIC ESTERASE ISOZYME POLYMORPHISM IN NATURAL POPULATIONS OF *GOSSYPIUM THURBERI**

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Abstract—Examination of seed samples of *Gossypium thurberi* by means of acrylamide gel electrophoresis was undertaken to detect the presence of molecular polymorphism in nonspecific esterases. Although little morphological variation is observed within and between populations of this species, six different nonspecific seed esterase zymograms were demonstrated suggesting that high molecular polymorphism is present. Studies using urea indicated that the specific isozyme patterns did not result from secondary alterations in structures of polypeptide species during extraction and therefore are not *in vitro* artifacts.

INTRODUCTION

DIFFERENCES in genotype may be reflected by an alteration in chemical structure and behavior of a given enzyme. Therefore, the utilization of physico-chemical characteristics of enzymes for taxonomic studies is possible. In electrophoretic studies concerning the phenomena of isozyme formation by higher plants, Schwartz *et al.*,[1] Macho *et al.*,[2] Desborough and Peloquin,[3] Williamson *et al.*,[4] and Hall *et al.*,[5] published data showing the presence of multiple esterase forms. These latter forms varied between species, within strains of the same species, and in different parts of the same plant.

A recent electrophoretic analysis by Scogin[6] demonstrated the existence of a genetically based polymorphism with respect to isozymic patterns within natural populations of *Baptisia* (Leguminosae). Therefore, to predict a possible taxonomic or physiological relationship between species with regard to a given isozyme pattern, intraspecific variation in the latter sense should be evaluated.

The results described in this paper attempt to display the occurrence of isozymic polymorphism for a wild species of cotton, *Gossypium thurberi.*

RESULTS AND DISCUSSION

The isozyme polymorphism in the four natural populations of *G. thurberi* for nonspecific seed esterases is shown in Fig. 1. Six different zymograms (A–F) were observed for the samples of seeds analyzed.

The upper half of the gels (1–5 cm) show large diffused banding areas. Jooste and

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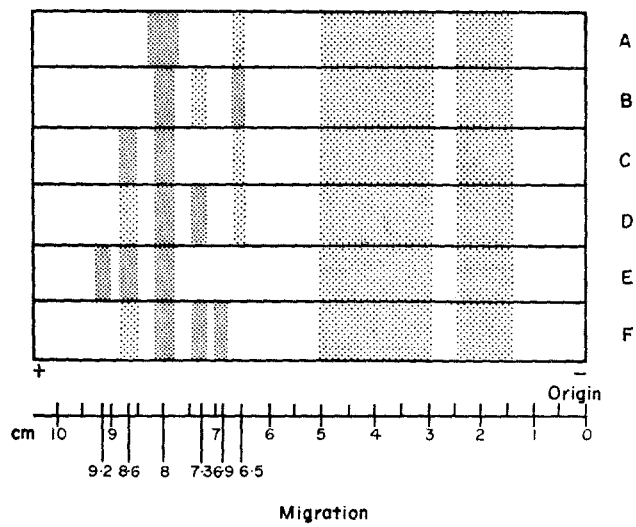

FIG. 1. DIAGRAM OF ACRYLAMIDE GEL ELECTROPHORETIC SEED NONSPECIFIC ESTERASE SPECTRA OF A COMPARISON BETWEEN DIFFERENT ZYMOGRAMS (A–F) FOUND WITHIN AND BETWEEN DIFFERENT NATURAL POPULATIONS OF *G. thurberi.*

Moreland,[7] examining seed ester hydrolases, attributed this upper region to enzymes that were not completely freed from particulate matter. Macho *et al.*,[2] investigating nonspecific proteins of seeds, also observed similar results. Analysis of this protein by column chromatography indicated it to be a nonspecific aggregate of proteins. These observations seem to coincide with those of Berthet and De Duve,[8] who have shown that many of the hydrolytic enzymes are contained within packet-like organelles. Consequently, even after extraction and purification, a portion of these enzymes could be adsorbed to small fragments of organelles. The complex fragments, although not large enough to be sedimented during the 4300 *g* centrifugation, could still effectively cause an apparent increase in mol. wt. This in turn could account for the diffuse area in the top half of our gels.

Of major interest in this study are the nonspecific esterases located in a relatively narrow region, 6·5–9·2 cm. Within this interval distinct variation (qualitative and quantitative) of bands occur. With the exception of a band at 8 cm, which is present in all cases, zymograms B, D, and F contain a band at 7·3 cm, and F has a band at 6·9 cm. In addition band 8·6 cm is located in zymograms C, D, E and F; and E contains a band at 9·2 cm. A band at 6·5 cm, present in zymograms A–D, completes the banding pattern of this variable region. A comparison of the relative frequencies of seed expressing a specific zymogram from each of the four populations analyzed is shown in Table 1. In general, zymograms A–D occur much more frequently in the natural populations than do zymograms E and F. These data suggest that isozymic variation may be expressed both within and between populations.

To account for the origin of these isozymic patterns, several distinct possibilities must be considered: (a) isozymic variants of a specific esterase gene in homozygous or hetero-

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TABLE 1. FREQUENCY OF OCCURRENCE OF NONSPECIFIC ESTERASE PATTERNS IN ZYMOGRAMS A–F OF THE NATURAL POPULATIONS OF *G. thurberi*

Location	Total seeds analyzed	Zymograms					
		A	B	C	D	E	F
Santa Catalina Mountains	78	0·718	0·000	0·179	0·090	0·026	0·000
Rincon Mountains	72	0·125	0·264	0·083	0·486	0·042	0·000
Santa Rita Mountains	72	0·805	0·083	0·000	0·111	0·000	0·000
Baboquivari Mountains	45	0·175	0·066	0·355	0·222	0·111	0·066
Totals	268	0·488	0·104	0·134	0·224	0·037	0·011

zygous combination; (b) a number of different and similar polypeptide subunits in different combinations and derived from closely related nonallelic genes; (c) enzymes completely unrelated and not derived from a common source; and (d) a selective combination involving all of these possibilities. As to which of these alternatives is most likely, it can be noted from the data that the 8 cm band is the only one present in all of the zymograms and is also present in greater amounts than any of the other nonspecific esterases. Thus, all of the latter may have arisen from this main esterase through mutation within a single gene (intragenic variation) or through duplication of a single gene, which then underwent mutation (intergenic variation). Hybridization studies of isogenic lines which produce each of these nonspecific esterase bands, however, are needed to test this hypothesis.

As suggested by Shaw,[9] specific isozyme patterns may result from secondary conformational alterations in the structure of polypeptide species and/or disaggregation of certain enzymes into active subunits. To test for such *in vitro* artifacts, experimental disruption and reaggregation of nonspecific proteins and esterases were undertaken using urea. The following experimental conditions were used: (a) extraction of seed in 5M urea Tris-glycine buffer followed by an electrophoretic assay for nonspecific proteins and esterases; and (b) dialysis overnight of extractions in 5M urea and no urea Tris-glycine buffers.

Extraction of seed in urea followed by analysis on urea-treated gels produced an absence of esterase activity for the normal staining period of 1 hr (Fig. 2). After 8–16 hr, however, diffuse areas of light staining appeared in regions 1–5 cm and 6·5–10 cm indicating some activity of the altered enzymes. Examination of the nonspecific protein stained gels showed that the urea-treated gel contained a number of new bands in region 4–10 cm.

The urea-treated samples dialyzed in Tris-glycine buffer and examined on no urea gels showed a small distinct band corresponding in mobility (8 cm) to that of the controls (Fig. 3). Also, a diffuse region (6·5–8 cm) of staining was located on the origin side (i.e. greater mol. wt. and/or exposure of more side groups with positive charges due to conformational changes or breakdown of enzymes) of this distinct band. The urea treated samples dialyzed in urea Tris-glycine buffer exhibited no enzyme activity during the normal staining period. Dialysis of the untreated control in Tris-glycine buffer did not affect the enzyme activity.

Schwartz[10] indicated that such inactivations occur either through an alteration of the active site or by an actual breakdown of the intact enzymes into smaller polypeptide sub-

[9] C. R. SHAW, *Intern. Rev. Cytol.* **25**, 297 (1969).
[10] D. SCHWARTZ, *Genetics* **49**, 373 (1964).

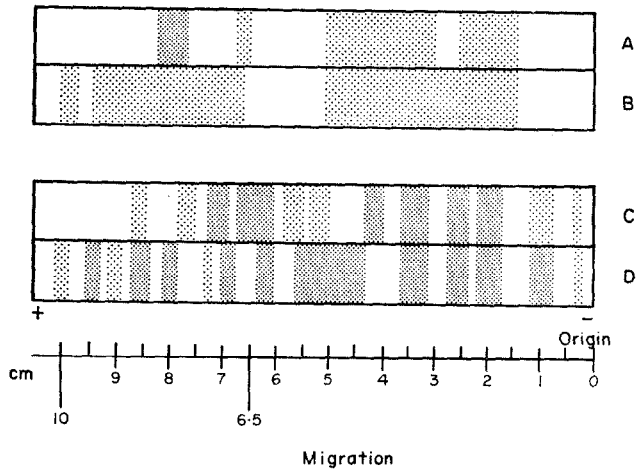

FIG. 2. UPPER SPECTRA COMPARISON. DIAGRAM OF ACRYLAMIDE GEL ELECTROPHORETIC SEED NONSPECIFIC ESTERASE SPECTRA.
A, no urea treatment; 1 hr nonspecific esterase staining. B, urea treatment of samples and gels; 8–16 hr nonspecific esterase staining.
LOWER SPECTRA COMPARISON. DIAGRAM OF ACRYLAMIDE GEL ELECTROPHORETIC SEED NONSPECIFIC PROTEIN SPECTRA STAINED WITH BUFFALO BLACK.
C, no urea treatment. D, urea treatment of samples and gels.

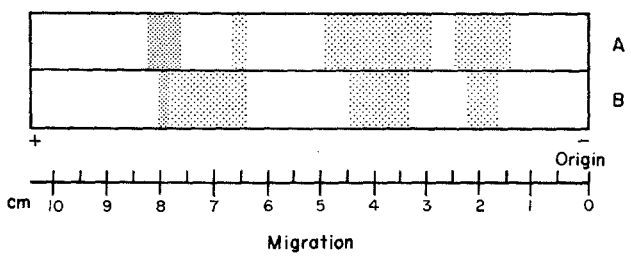

FIG. 3. DIAGRAM OF ACRYLAMIDE GEL ELECTROPHORETIC SEED NONSPECIFIC ESTERASE SPECTRA.
A, no urea treatment; 1 hr nonspecific esterase staining. B, urea treatment of samples only, followed by dialysis overnight in Tris-glycine buffer; 1 hr nonspecific esterase staining.

units. The results from the non-specific urea-treated protein gel indicated a breakdown of protein material into smaller subunits. The possibility of conformational changes exposing specific charged groups which in turn would alter protein mobility cannot be excluded. Removal of urea by dialysis allowed some of the enzyme to reaggregate and/or rectify any conformational changes to produce an active esterase band along with partially active forms (diffuse area). These data, using one type of denaturant, tend to indicate that any significant alteration of intact esterases during the process of extraction would be expected to greatly inactivate them. Therefore, during the enzyme assay discrete bands would not be expected.

Except for differences in leaf petal pigmentation and boll size, Bryan[11] has observed very little morphological variation within and between populations of *G. thurberi* throughout southern Arizona and Sonora, Mexico. The presence of six types of zymograms for

[11] W. E. BRYAN, personal communication (1969).

G. thurberi, occurring at varying frequencies within and between the four locations studied indicates that notable genetic variability does exist at the molecular level. Williamson *et al.*,[4] found nonspecific seed esterase variability within and between specific varieties of *Avena sativa*. A variety (Orbit), believed to be widely adapted, contained more variability than (Putnam 61), a narrowly adapted variety. They suggested that if isozyme variation were controlled genetically, then it may be related to adaptability of a variety. This observation may also be applied to *G. thurberi* on account of the observed variation in isozymic patterns occurring within and between the population areas studied. Further analysis of a larger number of samples from each given area is needed before any definite conclusions can be drawn with respect to the frequency data.

Our results with the nonspecific esterases suggest, as did those of Scogin,[6] that intraspecific variation should be evaluated before any interspecific analyses are undertaken. Work is presently under way to analyze the interspecific nonspecific seed esterase relationships between all available species within the genus *Gossypium*. This esterase analysis should give further support to the taxonomic relationships developed from examination of nonspecific seed proteins of species and allotetraploids in the genus *Gossypium*.[12]

EXPERIMENTAL

Collection. Seeds for analyses were collected from a large number of *G. thurberi* plants at four population sites distributed north, east, south, and west of Tucson, Arizona, U.S.A. These sites include: Santa Catalina Mountains, at Molina Basin (north); Rincon Mountains, at the Madrona Ranger Station area (east); Santa Rita Mountains, at Madera Canyon (south); and the Baboquivari Mountains, on the road to Kitt Peak (west). Hanson[13] has described the plant *G. thurberi* and its habitats and locations within Arizona.

Extraction and Separation. All procedures were carried out at 0–4°. One seed was ground for each extraction in 0·4 ml of Tris-glycine buffer (0·1 m; pH 8·3) with a mortar and pestle. Partial purification by centrifugation for 10 min at 4300 *g* in a Sorvall refrigerated centrifuge followed. A Canalco Model 1200 Bath electrophoretic apparatus and a Beckman Model RD-2 Duostat power source were used. The method of preparation of the gels and electrophoretic runs was similar to that of Steward *et al.*,[14] with the modifications of Cherry *et al.*[12]

Staining. Nonspecific esterases were stained using the technique of Johnson *et al.*,[15] except that the gels were stained for 1 hr at room temp. and fast blue RR was used as a stain. The gels were destained overnight in HeOH–HOAc–H_2O (50:10:50, v/v) followed by storage in 50% MeOH. The nonspecific protein staining procedure was that of Cherry *et al.*[12]

Urea treatment. 5M urea Tris-glycine buffer solution was used to extract the proteins.[10] The acrylamide gels were made as 5M urea solutions, when urea treated gels were used for this analysis (Fig. 2). Since the Santa Rita Mountains sample showed the least frequency of polymorphism (Table 1) seed from this sample were treated with urea for nonspecific protein and esterase analyses. The banding patterns for all the seeds were the same in their respective analyses (Figs. 2 and 3).

Acknowledgement—We thank Miss Vickie L. Day for her assistance in the electrophoretic analysis of the replicates of seed from the natural populations.

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