Electrophoretic Band Patterns of Phosphoglucoisomerase and Malate Dehydrogenase in the Prickly Pear Cactus *Opuntia cymochila* Engelm. and *O. phaeacantha* Engelm. (Cactaceae) from Southwestern Oklahoma

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Electrophoretic band patterns of phosphoglucoisomerase (PGI) and malate dehydrogenase (MDH) enzymes for populations of the prickly pear cactus, Opuntia cymochila **Engelm. and** O. phaeacantha **Engelm., in southwestern Oklahoma are discussed. Identical electrophoretic PGI band patterns were found in** O. cymochila **and** O. phaeacantha. **The pattern consisted of 10 bands and is explained by a three-gene model. Two or more gene loci were involved in the control of MDH. A difference was noted in the MDH band patterns for** Opuntia **species, suggesting that this enzyme might be taxonomically useful.**

INTRODUCTION

Morphological and genetic information concerning the *Opuntia* is limited, and the systematics of the genus is unclear. This is partly because cacti are not found in areas where extensive botanical research was done in the past (1,2), and because herbarium specimens are difficult to prepare. Most studies of *Opuntia* have been nomenclatural, morphological, or anatomical (2-5). Few studies have examined its genetics. Wallace and Fairbrothers (6) using isoelectric focusing studied genetic diversity in populations of *O. humifusa.* Pinkava and Parfitt (7) determined the chromosome number of 95 taxa of cacti and indicated that polyploidy was common in *Opuntia.* This study used starch gel electrophoresis to examine two enzymes, phosphoglucoisomerase (PGI) and malate dehydrogenase (MDH), for populations of *O. cymochila* and *O. phaeacantha* located in southwestern Oklahoma. This is the first examination of the band patterns of these enzymes from a large number of individuals from a single population of *O. cymochila.*

MATERIALS AND METHODS

A total of 382 pads of *O. cymochila* and 24 pads of *O. phaeacantha* were sampled between September, 1986, and February, 1987 from a field located approximately 8 km southwest of Hollis, Harmon Co., Oklahoma. The 1.6-km-square field was divided into 100 plots by lines running north-south and east-west at 0.16 km intervals. At each intersection of these lines, the nearest patch of *O. cymochila* southeast of the intersection was located, and four pads were collected as samples. Two patches contained only two pads, which were sampled. Twenty-four pads of *O. phaeacantha* were obtained from various places in the field for comparison purposes. A section of the stem epidermis measuring 10 mm × 30 mm × 3 mm was cut from each pad. The sample with 0.5 mL of distilled water was placed in a microcentrifuge tube in an ice bath and ground with a metal pestle. The homogenized sample was centrifuged at room temperature for 10 min at 8000 × g. A No. 3 Whatman filter paper wick (2 mm × 20 mm) was inserted into the sample and allowed to absorb fluid for 20 min. Starch gel electrophoresis was conducted utilizing standard procedures (8) with the following variations. Starch concentration was 12.8% (25.6 g of Sigma potato starch and 12.8 g of Electrostarch in 300 mL of gel buffer). The System I of Gottlieb's (9) buffer systems was used for characterizing the PGI band patterns. The gel was placed in an electric field at constant 35 mA at 4 °C for 6 hr during which time the tracking dye migrated approximately 9 cm. The buffer system of Warwick and Gottlieb (10) was used for the MDH analyses. A constant 7.5 W at 4 °C for 4 hr was used for electrophoresis of the

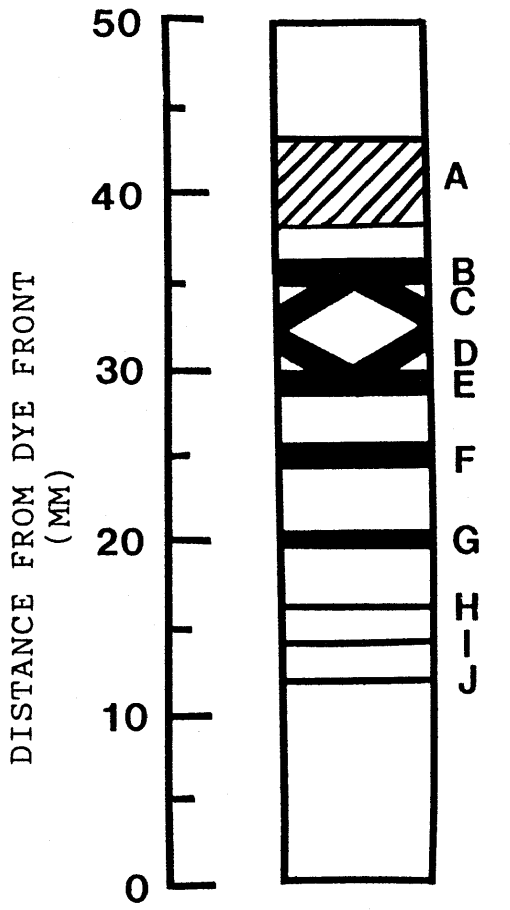

FIGURE 1. PGI enzyme band patterns for *O. cymochila* and *O. phaeacantha*. Thickness of the lines indicates the relative band width. The crosshatching of band A indicates it was diffuse. Curved lines for bands C and D indicate where the bands were difficult to distinguish from their neighboring band.

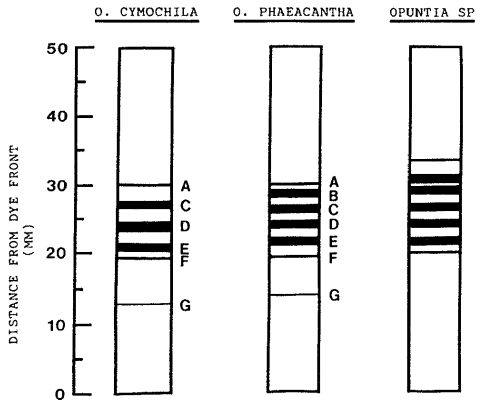

FIGURE 2. MDH enzyme band pattern for *O. cymochila*, *O. phaeacantha*, and another *Opuntia* sp. from northern Oklahoma. Thickness of the lines indicates relative band width.

MDH gel. MDH and PGI enzymes were stained using standard assays of Shaw and Prasad (11) with some modification: for PGI, 50 mL 0.1 M Tris-HCl (pH 8.0), 5 mg $NADP^+$, 40 mg $MgCl_2$, 0.5 mg PMS, 5 mg MTT, 45 units G6PD, and 80 mg fructose 6-phosphate; for MDH, 30 mL 0.2 M Tris-HCl, 75 mg l-malic acid, 5 mg NAD, 5 mg MTT, and 0.5 mg PMS. PGI and MDH were used in this study because the staining procedures produced relatively distinct bands in comparison to other enzymes examined.

RESULTS

The PGI bandpatterns of both *O. cymochila* and *O. phaeacantha* were identical and consisted of ten bands (Fig. 1) which were labeled A (fastest) through J (slowest). The fasted band (A) was diffuse but present on all gels. Bands B, C, D, and E always stained intensely. In some cases bands B and C were difficult to distinguish from each other, as were bands D and E. Bands F and G were distinct, while the slowest three bands H, I, and J were the most faintly staining. Differences in migration rates seemed to occur when pads were diseased or more dehydrated than normal. Seven diseased ones (apparently infected by a fungus) completely lacked enzyme activity.

The MDH electrophoretic band pattern (Fig. 2) consisted of a lightly staining band as the fastest (A), three sharply staining bands (C, D, and E) in *O. cymochila*, or four sharp bands (B, C, D, and E) for *O. phaeacantha*, and a lighter staining band (F) in both species. In 17% of the *O. cymochila* and 67% of the *O. phaeacantha* pads analyzed, another slower band (G) was noted. This band was faint and difficult to resolve on every gel. If recognition of the band was not conclusive, it was not recorded as being present. An *Opuntia* sp. (species not confirmed) sampled from 10 km SW Freedom, Woodward, Co. had five sharply staining bands and two faint bands (Fig. 2).

DISCUSSION

Phosphoglucoisomerase (PGI) is a dimeric molecule (12). In almost all verte-

brates it has been found to be controlled by a single gene locus (13). Plants usually have one isozyme (a polymer produced from monomeric units specified by one locus) in the cytoplasm and a different one in the plastids (14). The enzyme catalyzes the reversible isomerizations of glucose 6-phosphate and fructose 6-phosphate.

A three-gene model has been used to explain the multiple band patterns of PGI in *Clarkia* (Onagraceae) (15). It is briefly presented here for comparison to the model we propose for the PGI band pattern in *Opuntia.* In the genus *Clarkia,* more advanced species possess two cytosolic isozymes, in addition to the one found in the chloroplasts, and the second cytosolic gene has seemingly originated by gene duplication (15). In *Clarkia,* electrophoresis reveals band numbers for the PGI system ranging from four to ten. The fastest migrating band (PGI-1) lacks electrophoretic variability and is considered to be specified by a single monomorphic gene. For the other two genes (PGI-2 and PGI-3), the polypeptide subunits coded by the genes may associate to form isozymes which have mobilities intermediate to the PGI-2 and PGI-3 enzymes. For example, if the genes for PGI-2 and PGI-3 are homozygous, three electrophoretic bands result (1A1A, 1A2A, and 2A2A). If both genes are heterozygous (1A1B, 2A2B), ten bands result (1A1A, 1A1B, 1B1B, 1A2A, 1A2B, 1B2A, 1B2B, 2A2A, 2A2B, and 2B2B). In the case of *Clarkia,* the maximum number of bands was nine due to an overlapping of two bands. The heteromeric isozymes always migrated a distance exactly halfway between the two homodimers.

The PGI banding pattern (Fig. 1) observed in *O. cymochila* and *O. phaeacantha* can be explained by a three-gene model similar to that in *Clarkia*. The fastest PGI band, A, is probably produced by a single gene (PGI-1) and lacks electrophoretic variability. Bands B through J in *Opuntia* represent allele combinations from two additional genes (PGI-2, PGI-3). Bands B, D, H, and J are analogous to the homomeric isozymes (1A1A, 1B1B, 2A2A, and 2B2B). Bands C, E, F, G, and I are the heteromeric isozymes (1A1B, 1A2A, overlapping 1A2B-1B2A, 1B2B, and 2A2B). Table 1 provides a comparison with the three-gene *Clarkia* model using mean R_f values exactly midway between the R_f values for bands H and J, which correspond to the homomeric isozymes. The R_f values for the other bands corresponded rather closely, but not exactly, to those proposed by the *Clarkia* three-gene model. The differences are probably due in part to difficulty in clearly distinguishing and measuring bands B-C, and D-E. The R_f values for bands C and D were estimated as closely as possible to make the comparison to the *Clarkia* model.

If a three-gene model explains the PGI band pattern of *Opuntia,* why do these cacti populations lack variation in the PGI system and how is the heterozygote condition maintained? Seemingly if the cacti are reproducing sexually, typical Mendelian variation would be expected, resulting in band numbers ranging from four to 11. The absence of differences in the PGI band pattern suggests that these *Opuntia* populations may be reproducing vegetatively, as has been shown in populations of *O. fragilis* in southeastern Manitoba (16). No cactus seedlings have been noticed for *O. cymochila* or *O. phaeacantha* in the study area. If sexual reproduction does occur, it is unclear how the heterozygote condition is maintained in these cacti populations. Reciprocal translocation may be maintaining heterosis in a fashion similar to that occurring in *Oenothera lamarckiana* (17). An examination of the chromosomes may help to determine if reciprocal translocations are involved.

Malate dehydrogenase (MDH) is also a dimer (18). The number of loci involved in its control in plants is unclear (19). MDH exists in the mitochondria as a component of the Krebs cycle and also in the extramitochondrial portion of the cell, where it is involved in several metabolic pathways (18). Plant studies generally report three or four isozymes (14) that function in the mitochondria, peroxisomes, and cytoplasm. Mukerji and Ting (20) reported that MDH is present in the chloroplasts of cactus. In their study, anion exchange column chromatography was used to separate tissue samples into mitochondrial, chloroplast, and supernatant forms. Starch gel electrophoresis was then

TABLE 1. Comparison of observed and expected average R_f values for the three-gene PGI system of *Opuntia*.

Band	Observed R_f values	Enzyme subunits	Expected R_f values[a]	Difference
A	0.43			
B	0.36	2A2A		
C	0.34	2A2B	0.335	0.005
D	0.31	2B2B		
E	0.29	2A3A	0.260	0.030
F	0.25	2A3B, 2B3A	0.240,0.235	0.010,(0.015)
G	0.20	2B3B	0.215	0.015
H	0.16	3A3A		
I	0.14	3A3B	0.140	0.000
J	0.12	3B3B		

[a]Expected R_f values calculated based on homomeric values.

performed on the fractions. The study concluded that different MDH isozymes were involved in malate metabolism in respective plant cell compartments. Maize has been described (21) as having three unlinked mitochondrial gene loci which code for as many as 21 different isozymes. Leech and Ellis (22), Zelitch and Barber (23), and Mukerji and Ting (24) report that chloroplasts contain an MDH isozyme. Yamazaki and Tolbert (19) concluded that spinach chloroplasts do not contain any MDH activity and suggested that previous reports were probably due to contamination.

This confusion about MDH makes it difficult to determine how many loci are involved in MDH production in *O. cymochila* and *O. phaeacantha.* The slowest, lightly stained band, G, seemingly corresponds to bands which were attributed to glyoxisomal MDH bands (25). However, nonspecific alcohol dehydrogenase bands occasionally appeared on MDH gels during a study of maize (25). This could also be responsible for band G. Longo and Scandalios (25) also showed a distinct, three-banded pattern similar to that found *O. cymochila*. They attributed the slowest band to a mitochondrial enzyme and the fastest to a supernatant enzyme, with an unexplained middle band. In *O. phaeacantha,* the additional band could possibly have arisen through polyploidy, a phenomenon common in *Opuntia* (19). Polyploid plants often have increased numbers of isozymes (14).

Twenty-four pads of *O. phaeacantha* were analyzed and four distinctive MDH bands (B, C, D, and E of Fig. 2) were noted. The third species of *Opuntia* (species not confirmed) from northern Oklahoma had the same general MDH pattern, but five sharp bands (Fig. 2). The different MDH band patterns noted among these three species of *Opuntia* suggest that these distinct differences in the number of MDH bands may be taxonomically useful.

In summary, the three-gene model used to explain the PGI electrophoresis band pattern seems consistent with the data. However, more sophisticated genetic studies are needed to substantiate these preliminary conclusions. Additional studies are needed to determine the number of loci involved in MDH production.

ACKNOWLEDGMENTS

We thank Dr. Weniger for assistance in identifying the various species of cacti. In the field and laboratory we were assisted by Jack Herring, Monte Thies, and Jade Pung. The research was supported in part by Faculty Research Grants to William Caire and Jenna Hellack from Central State University.

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