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ESTERASE ISOZYMES FROM *SOLANUM* TUBERS*

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Abstract—The variation and distribution of esterase isozymes in tuber-bearing Solanums was determined by disc electrophoresis. Esterases obtained from tubers of wild species, interspecific hybrids, haploids ($2n=24$) and selfs ($2n=48$) of Tuberosum cultivars ($2n=48$) and haploid-species hybrids revealed a diversity of patterns among fifteen isozyme bands. Simple and complex patterns occur in species and interspecific hybrids; they do not appear related to taxonomic status or ploidy level. Within cultivated potatoes, Group Phureja and Group Tuberosum have simple patterns as compared to Group Stenotomum and Group Andigena. The haploids and selfs of Tuberosum specifically contain simple patterns amenable to biochemical and genetic analysis. A hypothesis based on the active enzyme being a tetramer composed of one, two or three types of monomers accounts for the results. It is assumed that three alleles E^a, E^b and E^c each control the production of a different monomer which combine to give fifteen esterase isozymes. More critical biochemical and genetic data are required to test these hypotheses.

INTRODUCTION

MANY enzyme systems have been investigated in higher plants by the use of electrophoretic techniques. Detailed biochemical and genetical studies of esterases from maize have been accomplished by Schwartz *et al.*[1] Some other plant enzymes having isozyme patterns that have been genetically analysed are leucine aminopeptidase,[2] catalase[3,4] and peroxidase[5]. Schwartz *et al.*[6] reported on the occurrence of multiple esterases in a variety of plant genera including *Solanum*, however, no extensive genetic or biochemical studies were undertaken. The plant tissue containing these enzymes has varied. One advantage of the *Solanum* material is that the tuber represents a relatively homogeneous storage tissue, whereas most plant tissue is more complex.

Soluble tuber proteins have been reported in *Solanum*.[7] The proteins were demonstrated by disc electrophoresis and the resulting patterns usually consisted of fewer than twenty proteins. Variation between species of both major and minor bands was most prominent in a group having the fastest migration rate. Furthermore, these proteins segregated in certain interspecific hybrids. These proteins are esterases active at pH 7·0 on the substrate alpha naphthyl acetate. This report is concerned with the identification and distribution of these esterases in cultivated potatoes and related wild species.

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RESULTS

Identification of the faster-migrating proteins was accomplished by running duplicate gels of the same extract and staining one gel for proteins and the other for esterases. Figure 1 illustrates sets of paired gels representing different soluble protein patterns and patterns of esterase isozyme bands. *Solanum brachistotrichum* contains four esterase isozyme bands, *S. cardiophyllum* subsp. *ehrenbergii* two, Group Phureja one and *S. pinnatisectum* two.

A survey of fifty-three species revealed that esterase isozyme patterns were very diverse ranging from simple patterns composed of as few as one band to more complex patterns with up to nine bands. Nearly one hundred different patterns were found among the species. In most species the pattern was not species-specific, however, similar patterns were observed in some species where the number of isozymes was five or less (Table 1). An example of this similarity is *S. pinnatisectum* (Figs. 1 and 2); these two different introductions contain the same esterase pattern. Other examples include *S. bulbocastum*, *S. canasense*, *S. demissum*, *S. fendleri*, *S. megistrobolum*, *S. sparsipulum*, and Group Phureja. More complex patterns were observed in *S. acaule*, *S. chacosense*, *S. multidissectum*, *S. raphanifolium* and Group Stenotomum. Complexity of patterns, in addition to the number of bands, consists of variation in band width (Fig. 2). The broad bands previously reported which were distinctive of many species were not found in *S. tuberosum*.

The interspecific hybrids also contain a large variety of esterase patterns. In general, bands were found in hybrids that were present in either parental species. However, no simple biochemical or genetical hypothesis can account for the complexity of patterns from the interspecific hybrids. Esterase patterns of four hybrids, *S. demissum* × Group Phureja, *S. verrucosum* × Group Stenotomum, *S. kurtzianum* × *S. multidissectum* and *S. kurtzianum* × *S. raphanifolium*, are given in Fig. 2.

Within the cultivated species *S. tuberosum*, Group Phureja and Group Tuberosum have simple esterase patterns consisting largely of bands 4, 5 and 6 and less frequently of bands 3 and 7. In contrast, Group Andigena and Group Stenotomum have more complex patterns (Table 1). One hundred and fifteen Phureja-haploid hybrids and their derivatives were available for esterase determination; these included F_1 (Phureja × haploid), $F_1 \times F_1$ and haploid × F_1 hybrids. Table 2 indicates the patterns of isozyme bands in the hybrids which range from a simple one-band type to five-band types. A total of nineteen different patterns was observed. The third group of gels in Fig. 2 represents four examples of these simple isozyme patterns.

There was no essential difference in esterase patterns of fifteen Phureja-haploid F_1 hybrids ($2n=24$) and their clonally doubled tetraploids ($2n=48$).

Haploids ($2n=24$) can be obtained with relative ease from tetraploid Group Tuberosum cultivars ($2n=48$).[8] In addition, the tetraploids can be selfed. These procedures can be used to obtain two populations which essentially represent the gametes and zygotes from a tetraploid cultivar. This unique situation was taken advantage of for the esterase study. Nine esterase isozyme patterns of one hundred and fourteen haploids ($2n=24$) and one hundred and sixty-five selfs ($2n=48$) from four cultivars are given in Table 2. Representative gels of haploids and selfs from the cultivars Katahdin and Merrimack are given in Fig. 3; in general, these patterns are the simplest observed thus far.

The occurrence of fewer isozyme bands in haploid extracts, in some only one, made haploids convenient to check classification of the bands. The usual technique involved gel to

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TABLE 1. ESTERASE IZOSYME FREQUENCY IN *Solanum* SPECIES AND CULTIVARS

Series	Species	Samples tested	Esterase band								
			1	2	3	4	5	6	7	8	9
Acaulia	*S. acaule*	9		6	6	7	8	8	6	1	2
Bulbocastana	*S. bulbocastum*	9			2		9		5		
Circaeifolia	*S. capsicibaccatum*	1					1	1	1	1	
Commersonia	*S. chacoense*	9	1	1	1	1	2	7	9	7	4
	S. tarijense	5			1	1	1	3	4	5	
Cuneoalata	*S. infundibuliforme*	1	1		1	1		1			
Demissa	*S. demissum*	12		5	5	5	9	11	12	6	1
	S. verrucosum	6		2	4	6	4	2			
Longipedicellata	*S. fendleri*	12			2	5	2	12	5		
	S. hjertingii	4	1	1	2	2	2	2	2	1	
	S. papita	2					2	2	1		
	S. polytrichon	10	2		4	3	8	9	7	5	
	S. stoloniferum	12		6		2	6	9	9	8	6
	S. × *vallis-mexici*	1						1	1	1	1
Megistacroloba	*S. boliviense*	3			1	3	3	2	2	3	2
	S. megistacrolobum	6		1	6	6	1				
	S. raphanifolium	5	4	3	5	4	5	4	3	1	3
	S. sanctae-rosae	2	1		2	1	2	2	1		
	S. toralapanum	1		1	1				1	1	1
Pinnatisecta	*S. brachistotrichum*	1		1	1	1	1	1	1		
	S. cardiophyllum	2	1	1			1	1	1	1	1
	subsp. *ehrenbergii*	3			2	1	1		2	2	1
	S. jamesii	11			2	7	5	11	6	1	
	S. michoacanum	2		2	1	1		1	1		
	S. pinnatisectum	6			1	4	5				
	S. × *sambucinum*	1						1	1	1	1
	S. stenophyllidium	1									1
Tuberosa	*S. alandiae*	1			1		1		1		1
	S. × *berthaultii*	2			2		2		2		2
	S. brevicaule	2		1	1	1	1	2	2	1	
	S. cajamarcense	1			1	1	1	1	1	1	1
	S. canasense	6			5	1	5	3	3		
	S. candolleanum	1				1	1	1	1		
	S. chiquidenum	1						1	1		
	S. coelestipetalum	1						1	1		
	S. gandarillasii	2					2	2	2		
	S. gourlayi	3	1	2	3	2	2	2	2		
	S. kurtzianum	4	1	2	4	4	3	3	2		
	S. lignicaule	1	1		1	1		1			
	S. marinasense	2				1	1	2	1		
	S. medians	2			1	1	2	2	1		
	S. microdontum	4	2	4	4	2	4	3	4		
	S. mochicense	1	1	1	1	1	1				
	S. multidissectum	3	1	1	3	2	2	3	2	3	1
	S. pampasense	1		1	1	1					
	S. spegazzinii	2	1				1	2	1	1	
	S. sparsipilum	5		4	3	4	5				
	S. sucrense	2			2		2	2	2		
	S. vernei	3			1	1		3	2	1	1
	S. tuberosum										
	Group Andigena	7			6	6	6	3	5	3	1
	Group Phureja	10			1	3	5	3			
	Group Stenotomum	10	5	10	5	7	9	6	9	1	2
	Group Tuberosum	26			2	26	26	26	7	1	

gel comparisons of the bands and subsequent numbering by their relative position. Artificial mixtures proved particularly useful to indicate whether isozyme bands were similar or different. Figure 4(a) illustrates four examples of this type of experiment.

TABLE 2. ESTERASE ISOZYME PATTERNS OF SELFS ($2n=48$) AND HAPLOIDS ($2n=24$) FROM GROUP TUBEROSUM CULTIVARS ($2n=48$), F_1 (PHUREJA-HAPLOID), $F_1 \times F_1$ AND HAPLOID $\times$ F_1 HYBRIDS

	Samples tested	Esterase isozyme patterns									
		4	5	45	456	56	4567	57	567	3456	457
Ag 231 selfs	41			2	9		19		1		2
Ag 231 haploids	29	2	1		8	2	2	1	5		
Chippewa selfs	44			2	34	5					
Chippewa haploids	33		7	6	11	5					
Katahdin selfs	40				32	1	1		1		
Katahdin in haploids	23	2		2	13	6					
Merrimack selfs	40			14	16	3	2				
Merrimack haploids	29	2		12	4	1	5				
F_1 (Phureja-haploid)*	97	1	2		33	11	4		16	1	
$F_1 \times F_1$†	4				6	1			1		
Haploid $\times$ F_1	14				2	2	3			1	

* In addition this group gave one sample with a 34 pattern; six with 345; one with 678; four with 3457; seven with 34567; one with 4568; and one with 45678.

† In addition one sample had a 46 pattern.

The esterases, following treatments with M NaCl for 12 hr plus freezing and thawing, gave variable results. Among the haploids no evidence of new bands was found after treatment (Fig. 4b). However, in certain hybrids new bands were found indicating that the method is not destroying the enzyme activity or sites of combination.[9] The lack of new bands in the haploids may indicate that monomer B is the predominant type in bands, 4, 5 and 6, thereby limiting new combinations.

It can be seen from Table 1 that there is no apparent simple or direct taxonomic relationship between esterase patterns and a series or species. For example, *S. tuberosum* Group Stenotomum resembles some unrelated species in degree of complexity. It is also clear that the isozyme patterns are not dependent on ploidy level (Fig. 3). However, within Group Tuberosum one finds the distribution of isozyme bands is limited to 3, 4, 5, 6 and 7. This is fortunate since these plants are available with known genetical background in larger number.

DISCUSSION

The esterase isozyme patterns allow two hypotheses to be formulated. The biochemical hypothesis is based on the active isozyme being a tetramer composed of one, two or three types of monomers. Other dimer, tetramer or octomer combinations do not result in an adequate number of bands. This model is similar to that proposed by Goldberg[10] for lactate dehydrogenase of trout. Thus, at least three monomer types are required to give the fifteen distinct bands observed (Fig. 5). This does not assume that narrow bands have equal frequency with broad bands or that they can be precisely classified. It appears that one unit difference in the relative mobility rate permits separation into the nine main bands. The

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[10] E. GOLDBERG, *Science* **151**, 1091 (1966).

double and triple bands may differ to a less degree, i.e. arising from a composition of tetramers with only two monomers different such as ACCC and BBCC, which both have a relative mobility of ten.

It may be assumed by a genetic hypothesis that an allele E^a controls the production of monomer A, E^b controls monomer B and E^c controls monomer C, where the relative mobility of A is one, of B is two and of C is three. The three alleles postulated may be at independent loci. That the isozyme patterns are genetically controlled is illustrated most directly by the presence of similar isozymes in diploid hybrids and their doubled tetraploid counterparts. Further evidence is that bands are not found in haploids that were not in their tetraploid

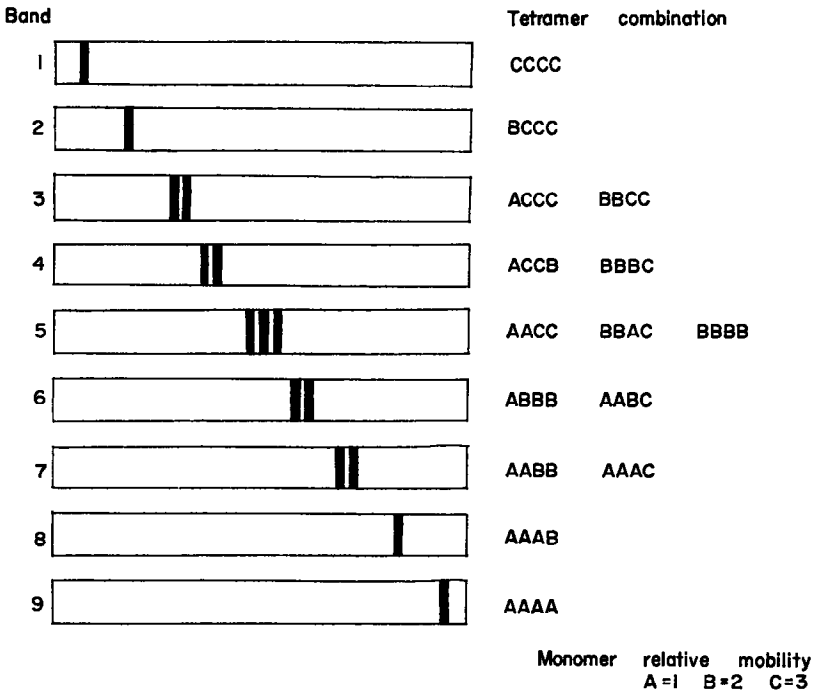

FIG. 5. HYPOTHETICAL TETRAMER COMBINATION(S) REPRESENTING FIFTEEN ESTERASE ISOZYMES.

parent, and bands found in hybrids are similar to those found in either parent. Figure 5 indicates the fifteen tetramers which result if A, B and C monomers can combine to yield an active isozyme. These assumptions explain the double bands observed for 3, 4, 6 and 7 and the triple band for 5. The classification of these bands awaits precise biochemical and genetical analyses.

MATERIALS AND METHODS

The tubers used were from the collection of the Potato Introduction Station, Sturgeon Bay, Wisconsin, or from our research material. Tubers from fifty-three *Solanum* species, thirty-two interspecific hybrids, one hundred and fourteen haploids ($2n=24$) of *S. tuberosum* Group Tuberosum ($2n=48$), one hundred and sixty-five selfs ($2n=48$) from four Group Tuberosum cultivars ($2n=48$), one hundred and fifteen hybrids ($2n=24$) between haploids ($2n=24$) and Group Phureja ($2n=24$) and fifteen vegetatively doubled clones ($2n=48$) of Group Phureja-haploid hybrids ($2n=24$) were used for esterase determination. Classification of wild species

is according to Hawkes[11] and of cultivated potatoes, Dodds.[12] Tubers were stored at 38° until protein extractions were made.

Preparation of protein extracts and disc electrophoretic conditions were the same as previously reported.[7] The method of esterase determination was adapted from Schwartz[13] except that disc electrophoresis with acrylamide gel was used instead of starch gel electrophoresis. Esterases were visible in gels after incubation for 5–20 min in phosphate buffer, pH 7·0, containing alpha naphthyl acetate and tetrazolized fast blue as a dye coupler. Stained bands were stable and suitable for comparison even after several weeks of storage in distilled water. Protein staining was the same as before with Aniline Blue Black.

Preliminary identification of the faster migrating proteins was accomplished by slicing gels longitudinally and staining one-half for proteins and the other half for esterases. The method of disc electrophoresis did permit direct comparisons between whole gels. The esterase stain revealed bands not detected by the protein stain; this has also been observed by Paul and Fottnell.[14] But in the majority of tuber extracts there was agreement between the two stains and an equal number of bands in this region were visible.

The isozyme bands were classified by their relative mobility rates into nine main types. In four types double bands and in one type a triple band were present; when these are included there are fifteen bands (Fig. 5). The nine main bands were numbered according to their relative mobility; for example, the slowest band was called number one and the fastest number nine.

When similarity of isozyme bands was suspected, two extracts from different tubers were mixed and run in the same gel. In this way identity or difference of isozymes was checked. Densitometer graphs were employed to confirm mobility rates of isozyme bands.

The number of subunits contained in the active izozyme has been determined in other enzyme systems.[9,15] The usual procedure consists of dissociation of the complex molecule by treatment with NaCl along with freezing and thawing. The tuber esterases were treated with M NaCl for 12 hr frozen and thawed. Zymograms obtained were compared with the original gel.

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