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Genetic bases of isozyme variation for alkaline phosphatase and glucosephosphate isomerase in *Solanum* *

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Summary. Genetic bases of isozyme phenotypes of alkaline phosphatase (AKP) and glucosephosphate isomerase (GpI) from tuber extracts of potato species of the genus *Solanum* were investigated by starch gel electrophoresis. Data were obtained from reciprocal F_1 matings of *S. tuberosum* × ssp. *andigena* (Juz. & Buk.) Hawkes and ssp. *tuberosum* × (*S. phureja* × *S. chacoense*) and BC_1 matings where ssp. *tuberosum* was the recurrent parent. AKP and GPI are dimeric enzymes and the variation observed for each was found to be coded by single tetrasomic loci (Akp and Gpi) with three (A, A′, A″) and five (G, G′, G″, G‴, G⁗) alleles, respectively. Although the G‴ and G⁗ encoded homodimers have similar electrophoretic mobilities, the specific enzymatic activity of the G⁗ encoded homodimer is approximately 25% that of the G‴. The predictable genetic bases for these two enzymatic polymorphisms make them suitable for use as genetic markers in the potato. Chromosome mapping of the loci which encode these enzymes is now possible.

Key words: Electrophoresis – Potato – Isozymes – Genetic markers – Inheritance

Introduction

Various biochemical procedures for clonal identification and taxonomic analysis of a number of tuber bearing species of *Solanum* have been reported (Gell et al. 1956; Hawkes and Lester 1966, 1968; Mecklenburg 1966). Electrophoretic examination of tuber proteins has proven useful not only in the discrimination between species and their interspecific hybrids (Desborough and Peloquin 1966, 1971; Siepmann and Stegemann 1967), but also between horticultural groups (Desborough and Peloquin 1968, 1971; Rickemann and Desborough 1978). Both esterase and peroxidase enzyme systems have been used in cultivar characterization (Desborough and Peloquin 1968).

It has been proposed that isozymes (Scandalios 1969; Shaw 1965) and total protein banding patterns (Desborough and Peloquin 1968; Stegemann and Loeschke 1977) be used as genetic markers. To be used as a genetic marker an enzyme system must have a predictable genetic basis. Genetic hypotheses have been formulated to explain the inheritance of banding patterns produced for esterase variation (Desborough and Peloquin 1967) and total protein staining (Rickemann and Desborough 1978).

In this paper we present inheritance data to explain the genetic bases of phenotypic variation for two specific enzymes, glucosephosphate isomerase (GPI) and alkaline phosphatase (AKP), and to investigate their potential for use as genetic markers.

Materials and methods

Solanum tuberosum L. ssp. *tuberosum* clones were obtained from Drs. Raymon Webb and Richard Cole, while a northern adapted ssp. *andigena* (Juz. & Buk.) was supplied by Dr. Robert Plaisted (Table 1). An induced tetraploid of an interspecific hybrid of *S. phureja* × *S. chacoense* was drawn from the potato genetics project at The Pennsylvania State University. For clarity of duscussion these subspecies have been assigned the capital letters T, A, and PC for ssp. *tuberosum*, ssp. *andigena* and the interspecific hybrid (*S. phureja* × *S. chacoense*), respectively. Subscript numerals indicate clones or individual accessions.

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Table 1. Identification and source of *Solanum tuberosum* parental material used in the production of reciprocal F_1 and BC_1 populations

Clone identification	Clonal[a] source	Taxonomic classification	Experimental designation
Norchip	A	ssp. *tuberosum*	T_1
	C		T_1
Red Pontiac	A	ssp. *tuberosum*	T_2
	C		T_2
Superior	A	ssp. *tuberosum*	T_3
	C		T_3
Kennebec	A	ssp. *tuberosum*	T_4
	C		T_4
4NJV2	D	Induced tetraploid of interspecific hybrid *S. phureja* × *S. chacoense*	PC
R85-4	B	ssp. *andigena*	A_1

[a] *A* = Dr. Raymon Webb, Beltsville, MD; *B* = Dr. Robert Plaisted, Ithaca, NY; *C* = Dr. Richard Cole, University Park, PA; *D* = Potato genetics project, The Pennsylvania State University

Reciprocal crosses of T×A and T×PC were made to produce four sets of F_1 populations (8 families). Individuals from the two T sources were used interchangeably in F_1 matings. From these F_1 reciprocals, plants containing either A or P (*S. phureja*) cytoplasm were selected for controlled pollinations to a recurrent T parent producing 7 sets of reciprocal BC_1 populations (14 families).

Extracts were collected from tubers from each F_1 and BC_1 family using the technique of Desborough and Peloquin (1966). For each extract 20 grams of freshly peeled tuber tissue were soaked for 0.5–4 h in 0.7% sodium hydrosulfite, rinsed in distilled water and blotted dry. The samples were ground with a mortar and pestle in 2.5–3.0 ml of 30% dimethyl sulfoxide and centrifuged for 30 min at 16,000 g.

Extracts were stored at −60 °C until they were examined by horizontal starch gel electrophoresis (May et al. 1979; May 1980). Gels consisting of 14% Electrostarch (Electrostarch Co., Madison, Wisconsin) were prepared in gel buffers described by Ridgway et al. (1970) and Clayton and Tretiak (1972) consisting of 0.04 M citric acid adjusted to pH 6.1 with N-[3-aminoprophy]morpholoine for tray buffer, diluted 1 : 10 for gel buffer, for separation of GPI and AKP isozymes, respectively. Filter paper wicks (3×8 mm, Schleicker and Schuell No. 470) were dipped in thawed tuber extracts, inserted in gels and an electric potential of 200 V (maximum of 75 mA) was applied for 4–6 h. Electrophoresis was stopped when the dye marker (red food coloring) applied with the samples had migrated to within 1 cm of the end of the gel. The gel was then sectioned into four horizontal slabs by use of a monofilament thread. Staining solutions of Shaw and Prasad (1970), Brewer (1970) and Allendorf et al. (1977) were modified and used to produce banding patterns of the isozyme. The staining mixture for GPI consisted of 10 ml of gel buffer, 60 μM G6PDH, 10 mg NADP, 10 mg tetrazolium bromide (MTT), 50 mg fructose-6-phosphate and 3 mg phenazine methosulphate (PMS) and 10 ml of 2% agar in Ridgway gel buffer (Ridgway et al. 1970) at 60 °C. The stain for AKP consisted of 100 mg β naphthyl acid phosphate and 50 mg fast garnet GBC salt in 100 ml of Ridgway gel buffer.

Six A, seven T, and 101 F_1 hybrid clones were surveyed to determine the variation present for GPI and AKP. Variation in mobility, relative intensity and number of bands was used to identify variant types. Each phenotype was assigned a number and only those bands which developed at the same time during staining were used. Photographs of gels were taken with a 35 mm single lens reflex camera utilizing Kodak Ektachrome (E-6) 160 tungstem film. A genotypic basis was assigned to each isozyme phenotype. The frequency of each genotype resulting from the crossing was tabulated and the degree of fit to chromosome and chromatid assortment was tested by contingency chi-square tests (Steel and Torrie 1960).

The allelic nomenclature follows the form described by Gottlieb (1977) and Richmond (1972) with additional details and modifications made to allow for the genetic nature of a tetraploid organism. The loci coding for GPI and AKP were designated as Gpi and Akp. Alleles were given the arbitrary designation of the upper case letters G and A for Gpi and Akp, respectively. Lower case letters g and a indicate subunit composition of the isozymes producing electrophoretic phenotypes. These letters represent the basic subunit and allele designations. Each additional allele is designated by a superscript prime(s) (′). Numerical subscripts are used to indicate the number of subunits or allelic copies.

Results

Genetic variation

Two facts were considered in the interpretation of banding patterns and construction of genetic models for AKP and GPI. The *Solanum* species and hybrids in this study are tetraploid and therefore have four gene copies at each locus, and it has been demonstrated that AKP and GPI are dimeric enzymes (Darnall and Klotz 1972).

Electrophoresis of potato tuber extracts revealed one anodal zone of AKP activity producing nine phenotypic variants. Some of these variants are presented in Fig. 1 along with the positions of the homodimeric isozymes noted. All variants are consistent with a hypothesized model involving a dimeric enzyme encoded by one tetrasomic locus with three alleles. The relative banding intensities of 15 possible phenotypes are given in Fig. 2 which represent the theoretical isozyme ratios expected from the random association of subunits for a dimeric enzyme in a tetraploid. Of the three homodimeric isozymes possible, only a''_2 (type 1; Fig. 2) and a'_2 (type 5) were observed in homozygous plants. The position of the a_2 homodimer was deduced from the isozyme patterns of the heterozygous plants (Types 2, 3, 4, 7, 10, 11, 12).

Staining for GPI produced several anodal zones of activity, each presumably coded by at least one additional locus. In one zone, the reactivity of the enzyme to the stain was uniform and the banding patterns were suitable for genetic study. The banding patterns pro-

duced in this zone suggest (Fig. 3) that GPI has a dimeric subunit structure and further that the allozymes in this zone are encoded by a single tetrasomic locus with five alleles. Only the g_2' and g_2'' homodimers were observed in plants homozygous for the genes that code for these isozymes (G_4' and G_4'', respectively). However, positions of the g_2, g_2''' and g'''' homodimers could be observed in the patterns produced by heterozygous plants which contained copies of these alleles. Typical examples of the g_2 homodimers appear in types 16–18, g_2''' appears in types 6, 7, 10, 11, 13, 14 and 15 and g_2'''' in types 29, 30, 31, 32.

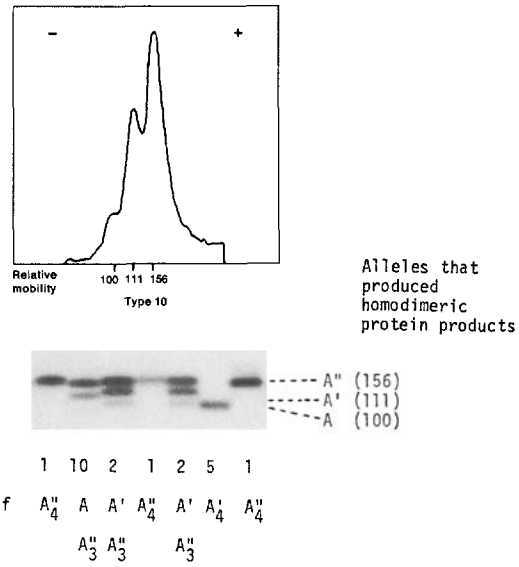


Fig. 1. AKP phenotypes observed in *Solanum* with the positions of the homodimeric protein product specified by each allele indicated to the right. Genotype and type number of individual phenotypes are listed below the gel pattern. A densitometer tracing of type 10 (A_1A_3'') is also provided. Although the cathodal – most band of the type 10 individual is not distinct, it was observed

Some of the variants of GPI which were observed are presented in Fig. 4 along with the position of the homodimeric protein products. The banding patterns observed agreed with the banding patterns predicted by a model with random subunit combinations. For example, the proposed genotype composition of type 20 (Figs. 3 and 4) is G, G_2', G''' in which subunits combine at random to produce a set of isozymes with a theoretical 1:4:2:4:4:1 banding intensity. The observed band intensities od the type 20 individuals shown in Fig. 4 are visually close to the predicted ratio. Also shown in Fig. 4 is the homodimeric product of the G' allele (type 1) and types 6 and 16 (9:6:1 banding ratio) resulting from the subunit associations in the heterozygotes $G_3'G_1''$ and G_1G_3', respectively. Among the variants observed though not shown in Fig. 4 was the tetra-allelic individual GG'G''G''' (type 3; Fig. 3) which produced a zymogram with a 1:2:3:4:3:2:1 banding intensity. An artifact band which occasionally appears above the position of the g_2''' homodimer is the result of storage degradation of GPI (Staub et al. 1982).

Studies in a number of different organisms have revealed the presence of isozymes which have reduced specific activity (Carter et al. 1972; Detter et al. 1968; Schwartz 1966). The banding patterns of types 29, 30, 31 and 32 can be explained by the presence of a fifth allele (G'''') having a homodimer with the same electrophoretic mobility as the G''', but is less active. If the homodimer of the G'''' allele were hypothesized to be 25% as active as the G''' homodimer, then a $G_3'G_1''''$ individual's (type 29; Fig. 3) protein subunits would recombine to produce a 3-banded isozyme pattern with a theoretical banding intensity ratio of 1:12:36. The anodal most band which migrates to the position of the G'''' homodimer would be too faint to be usable and the gene combination would be recorded as having two bands in an apparent 1:3 ratio. Likewise, a $G_1G_2'G''''$ individual (type 30; Fig. 3) would produce a banding pattern in a theoretical ratio of 1:10:5:16:

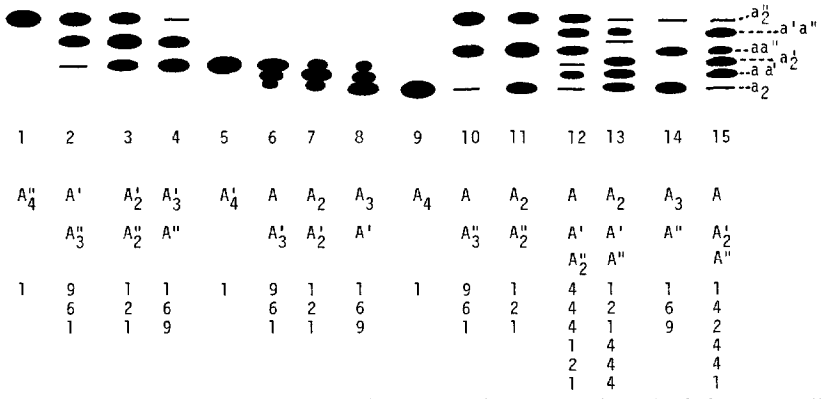
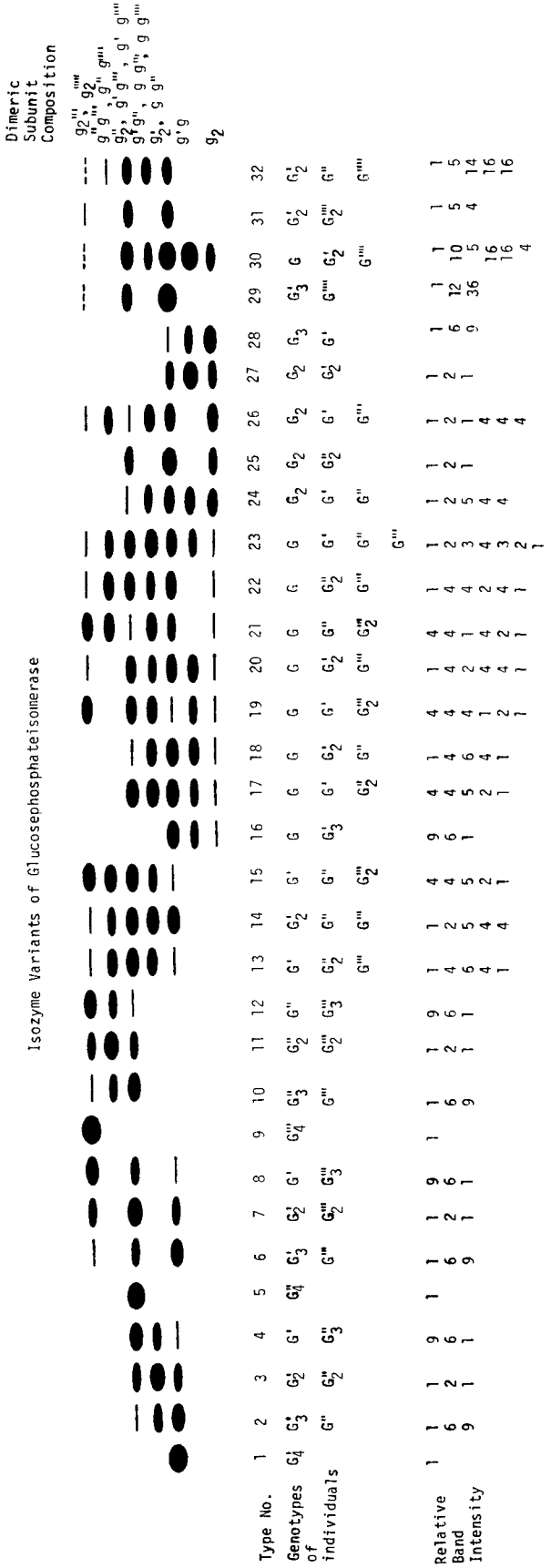


Fig. 2. Diagrammatic representation of alkaline phosphatase isozyme variants in *Solanum* predicted by a 1 locus – 3 allele model. Types 6, 8, 9, 13, 14 and 15 were not observed but are predicted by the model



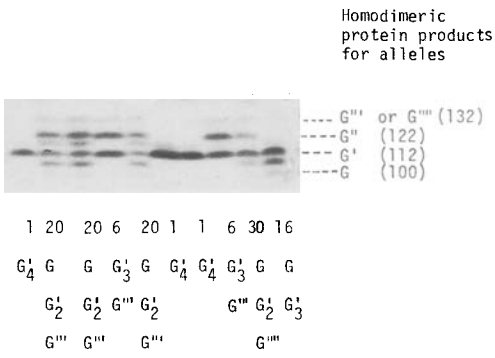


Fig. 4. GPI phenotypes observed in *Solanum* with positions of the homodimeric protein product specified by each allele indicated to the right. Subunits and type number of individual phenotypes are listed below the gel pattern. The bands appearing anodal to the homodimeric products of the G'' ' or G'' '' alleles are artifactual (see text)

16 : 4. The anodal most band again would be so faint that this genotype would be scored as having only five bands.

Readings with a scanning densitometer (Clifford Densicomp, Model 445, Clifford Instruments) at 505 nN of types 30 and 31 indicate that their band ratios are 1 : 11 : 7 : 13 : 13 : 7 and 1 : 2 : 2.7, respectively. These data support the hypothesis of a g'''' homodimer with reduced specific activity (approximately 25%). The appearance of G'_3G'''' individual (type 29) in 9 of 24 ssp. *andigena* clones obtained from Dr. Plaisted shows that this allele often occurs within ssp. *andigena* from this source. None of the 12 parental ssp. *tuberosum* clones that we observed during this study (Staub et al., unpublished) contained the G'''' allele.

Inheritance

Segregation ratios expected in F_1 and BC_1 populations produced by heterozygous tetraploid parents depend upon the proximity of the isozyme alleles to the kinetochore. If these alleles are close to the kinetochore, chromosome type segregations would be anticipated. If they are not close to the kinetochore chromatid type segregation would be expected. Progeny segregations were compared to these expected ratios and chi squares of goodness of fit were determined. Only the fits to chromatid and chromosome assortment are presented because combinations of chromosome and chromatid assortment did not significantly improve the chi square values.

Fig. 3. Diagrammatic representation of glucosephosphate isomerase isozyme variants in *Solanum* observed or predicted by a 1 locus – 5 allele model. Types 8, 9, 12, 22, 26, 27 and 28 were not observed in this study. Other variants not observed are possible with this model

Table 2. Segregation of isozymes of alkaline phosphatase in F_1 progenies from reciprocal crosses of *S. tuberosum* × spp. *andigena* and ssp. *tuberosum* × *S. phureja* × *S. chacoense*

Lot	Cross ♀ ♂	Parental isozyme genotypes		Genotypes of isozymes in F_1 individuals				Chi square analysis of type of assortment	
		T	A or PC	Type 1 *n*	A_4''	3 $A_2'A_2''$	12 $AA'A_2''$	Chromatid ($P \geqq$)	Chromosome ($P \geqq$)
EMI	$T_1 \times A_1''$	A_4''	A_4''	26	26	0	0	1	1
MF	$A_1 \times T_1$			57	57	0	0	1	1
Σ				83	83	0	0	1	1
EMX	$T_1 \times PC$	A_4''	A_4''	47	47	0	0	1	1
EMN	$PC \times T_1$			50	50	0	0	1	1
Σ				97	97	0	0	1	1
EQJ	$T_3 \times PC$	A_1A_3'	A_4''	68	0	32	36	0.10	0.5
ENE	$PC \times T_3$			79	0	36	43	0.25	0.5
Σ				147	0	68	79	0.005	0.5
EMZ	$T_2 \times PC$	A_4''	A_4''	43	43	0		1	1
ENH	$PC \times T_2$			68	68	0		1	1
Σ				111	111	0		1	1

Table 3. Segregation of isozymes of alkaline phosphatase in reciprocal backcross progenies from crosses of (*S. tuberosum* ssp. *andigena* × ssp. *tuberosum*²) and [(*S. phureja* × *S. chacoense*) × ssp. *tuberosum*²]

BC_1 Lot	♀ ♂	Parental isozyme		Genotypes in BC_1 individuals					Chi Square analysis of type of assortment	
		T	F_1 hybrid	Type *n*	1 A_4''	2 $A_1'A_3''$	3 $A_2'A_2''$	4 $A_3'A_1''$	Chromatid ($P \geqq$)	Chromosome ($P \geqq$)
EUI	$T_1 \times$ EMF 22 – 12	A_4''	A_4''	62	62	0	0	0	1	1
EVT	EMF 22 – 12 × T_1			69	69	0	0	0	1	1
Σ				131	131	0	0	0	1	1
EUJ	$T_1 \times$ EMF 22 – 13	A_4''	A_4''	59	59	0	0	0	1	1
EVY	EMF 22 – 13 × T_1			20	20	0	0	0	1	1
Σ				79	79	0	0	0	1	1
EUH	$T_1 \times$ EMF 23–31	A_4''	A_4''	31	31	0	0	0	1	1
EWF	EMF 23 – 31 × T_1			49	49	0	0	0	1	1
Σ				80	80	0	0	0	1	1
EUO	$T_1 \times$ EMN 92 – 52	A_4''	A_4''	16	16	0	0	0	1	1
EZJ	EMN 92 – 52 × T_1			16	16	0	0	0	1	1
Σ				32	32	0	0	0	1	1
EUN	$T_1 \times$ EMN 92 – 50	A_4''	A_4''	50	50	0	0	0	1	1
EZI	EMN 92 – 50 × T_1			53	53	0	0	0	1	1
Σ				103	103	0	0	0	1	1
ETJ	$T_4 \times$ EMV 96 – 37	$A_1'A_3''$	$A_2'A_2''$	37	11	13	3	10	0.001	0.001
FBB	EMV 96 – 37 × T_4			47	18	20	1	8	0.001	0.001
Σ				84	29	33	4	18	0.001	0.001
ETK	$T_4 \times$ EMV 96 – 41	$A_1'A_3''$	A_4'	42	27	15	0	0	0.30	0.05
FBE	EMV 96 – 41 × T_4			7	4	3	0	0	0.70	0.70
Σ				49	31	18	0	0	0.30	0.05

In Table 2 data from segregations of Akp alleles in reciprocal F_1 populations are presented. Parental and progeny isozyme genotypes are indicated along with parental genotypes listed. Each tetraploid parent contains four gene doses at a single structural locus. At Akp, three structural alleles A, A′, A″ have been identified which produce homodimeric protein products with relative mobilities of 100, 111, and 156, respectively (Fig. 1).

In reciprocal lots EMI, EMF, EMX, EMN, EMZ and ENH ssp. *tuberosum* (T_1 and T_2), ssp. *andigena* (A_1) and the *S. phureja* × *S. chacoense* (PC) parents

Table 4. Segregation of isozymes of glucosephosphate isomerase in F_1 progenies from reciprocal crosses of *s. tuberosum* ssp. *tuberosum* × spp. *andigena* and spp. *tuberosum* × (*S. phureja* × *S. chacoense*)

F_1 Lot	Cross ♀ ♂	Parental isozyme genotypes T	A or PC	Type *n*	Genotypes of isozymes in F_1 individuals 1 G'_4	2 $G'_3G''_1$	3 $G'_2G''_2$	6 $G'_3G'''_1$	7 $G'_2G'''_2$	11 $G''_2G'''_2$	13 $G'_1G''_2G'''_1$	14 $G'_2G''_1G'''_1$	15 $G'_1G''_1G'''_2$	16 $G_1G'_3$	18 $G_1G'_2G''_1$	19 $G_1G'_1G''_2$	20 $G_1G'_2G'''_1$	21 $G_1G''_1G'''_2$	23 $G_1G'_1G''_1G'''_1$	29 $G'_3G'''_1$	31 $G'_2G'''_2$	Chi square analysis of type of assortment: Chromatid ($P \geqq$)	Chromosome ($P \geqq$)
EMI	$T_1 \times A_1$	G'_4	$G'_3G'''_1$	26	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	2	0.10	–[a]
EMF	$A_1 \times T_1$			56	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	0	0.25	0.10
Σ				82	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.25	–
EMX	$T_1 \times PC$	G'_4	$G'_2G''_2$	50	5	0	0	38	7	0	0	0	0	0	0	0	0	0	0	0	0	0.03	0.25
EMN	$PC \times T_1$			50	10	0	0	38	2	0	0	0	0	0	0	0	0	0	0	0	0	0.005	0.06
Σ				100	15	0	0	76	9	0	0	0	0	0	0	0	0	0	0	0	0	0.005	0.09
EQJ	$T_3 \times PC$	$G_1G'_1G''_2$	$G'_2G''_2$	54	0	4	2	0	0	0	12	8	3	4	1	1	3	5	11	0	0	0.70	0.01
ENE	$PC \times T_3$			70	0	2	1	0	0	2	12	16	1	1	4	2	11	3	15	0	0	0.50	0.05
Σ				124	0	6	3	0	0	2	24	24	4	5	5	3	14	8	26	0	0	0.30	0.001
EMZ	$T_2 \times PC$	$G'_2G''_2$	$G'_2G''_2$	47	0	4	5	2	0	1	4	24	7	0	0	0	0	0	0	0	0	0.10	0.10
EMH	$PC \times T_2$			60	3	3	2	5	2	3	4	29	9	0	0	0	0	0	0	0	0	0.30	0.69
Σ				107	3	7	7	7	2	4	8	53	16	0	0	0	0	0	0	0	0	0.01	0.20

[a] *P* value not given since double reduction products were observed

Table 5. Segregation of isozymes of glucosephosphate isomerase in reciprocal backcross progenies (*S. tuberosum* ssp. *andigena* × ssp. *tuberosum*²) and [(*S. phureja* × *S. chacoense*) × ssp. *tuberosum*²]

BC_1 lot	Cross ♀ ♂	Parental isozyme genotypes T	Parental isozyme genotypes F_1 hybrid	Type *n*	Genotypes of isozymes in BC_1 individuals 1 G'_4	2 $G'_3G''_1$	3 $G'_4G''_4$	4 $G'_1G''_3$	6 $G'_3G''_1$	7 $G'_4G''_4$	10 $G'_3G''_1$	13 $G'_1G'_2G''_1$	14 $G'_2G'_1G''_1$	15 $G'_1G''_1G''_2$	16 $G_1G'_3$	17 $G_1G'_2G''_1$	Chi square analysis of type of assortment Chromatid ($P \cong$)	Chromosome ($P \cong$)
EUI	T_1 × EMF 22 – 12	$G_1G'_3$	G'_4	72	35	0	0	0	0	0	0	0	0	0	37	0	0.20	0.90
EVI	EMF 22 – 12 × T_1			77	50	0	0	0	0	0	0	0	0	0	27	0	0.10	0.01
Σ				149	85	0	0	0	0	0	0	0	0	0	64	0	0.10	0.10
EUK	T_1 × EMF 22 – 13	$G_1G'_3$	G'_4	59	31	0	0	0	0	0	0	0	0	0	28	0	0.30	0.70
EVY	EMF 22 – 13 T_1			19	12	0	0	0	0	0	0	0	0	0	7	0	0.70	0.30
Σ				78	43	0	0	0	0	0	0	0	0	0	35	0	0.30	0.30
EUL	T_1 × EMF 22 – 31	$G_1G'_3$	G'_4	31	18	0	0	0	0	0	0	0	0	0	13	0	0.70	0.30
EWF	EMF 22 – 31 × T_1			50	28	0	0	0	0	0	0	0	0	0	22	0	0.50	0.20
Σ				81	46	0	0	0	0	0	0	0	0	0	35	0	0.50	0.20
EUO	T_1 × EMN 92 – 52	$G_1G'_3$	$G'_3G''_1$	17	10	0	0	0	0	0	0	0	0	0	5	2	0.30	0.50
EZJ	EMN 92 – 52 × T_1			16	6	0	0	0	4	0	0	0	0	0	3	3	0.95	0.20
Σ				33	16	0	0	0	4	0	0	0	0	0	8	5	0.30	0.30
EUN	T_1 × EMN 92 – 52	$G_1G'_3$	$G'_3G''_1$	50	17	0	0	0	11	1	0	0	0	0	6	15	0.50	0.001
EZI	EMN 92 – 50 × T_1			47	7	0	0	0	8	0	0	0	0	0	16	16	0.10	0.001
Σ				97	24	0	0	0	19	1	0	0	0	0	22	31	0.10	0.001
ETJ	T_4 × EMV 96 – 37	$G'_2G''_2$	$G'_2G''_1G''_1$	49	1	4	10	5	1	0	0	13	15	0	0	0	0.90	0.50
FBB	EMV 96 – 37 × T_4			43	1	4	8	4	2	0	1	19	4	0	0	0	0.10	0.50
Σ				92	2	8	18	9	3	0	1	32	19	0	0	0	0.30	0.20
ETK	T_4 × EMV 96 – 41	$G'_2G''_2$	$G'_2G''_1G''_1$	40	2	7	6	3	2	0	1	12	7	0	0	0	0.90	0.50
FBE	EMV 96 – 41 × T_4			5	1	1	1	0	0	0	0	1	1	0	0	0	0.90	0.70
Σ				45	3	8	7	3	2	0	1	13	8	0	0	0	0.90	0.30

were characterized as type 1 (A'_4) and therefore were predicted to produce only type 1 progeny. In all cases progeny expectations were met. T_3 ($A_1 A'_3$ type 7; Figs. 1 and 2) and PC are parents in reciprocal lots EQJ and ENE. Progeny segregations (types 1 and 2) adequately fit chromosome assortment.

In the BC_1 segregations (Table 3), most progeny, as expected from parental genotypes, did not segregate. In the two lots that did segregate one (ETK and FBE) fit chromatid type of assortment. The other (lots ETJ and FBB) fit neither type of assortment. In these cases no unexpected variants were observed, though the genotypes A''_4 (type 1) and $A'_3 A''_1$ (type 4) occurred in high frequencies causing a lack of fit for chi square ($P \leqq 0.001$) (see "Discussion").

A hypothesized gene at one locus exists in the five structural allele form, G, G′, G″, G‴ and G‴′ for Gpi. These produce homodimeric protein products with relative mobilities of 100, 112, 122, 132, and 132, respectively (Fig. 4). All reciprocal F_1 and BC_1 progeny segregations adequately fit either one or the other type of assortment (Tables 4 and 5).

Discussion

Data presented here support the hypothesis that AKP and GPI are each coded by a single locus; Akp with three alleles (A, A′, A″) and Gpi with five (G, G′, G″, G‴, G‴′). The position of the G‴′ homodimer was determined in individuals which were heterozygous for combinations of the G, G′ and G‴′ alleles. From these banding patterns it was deduced that the G‴ and G‴′ alleles produced protein products with similar electrophoretic mobilities. The G‴′ allele apparently produces subunits which combine to produce an active enzyme which has approximately 25% the specific activity of the protein product of the other four alleles. Individuals with a GPI genotype $G'_2 G'_1{}'''$ (type 29), $G_1 G'_2 G'_1{}'''$ (type 30). and $G'_2 G'_1{}' G'_1{}'''$ (type 32) consequently produce isozyme banding patterns in which an extremely faint band appears at the G‴′ homodimer position.

Although unexpected AKP variants were not observed in BC_1 progenies of lots ETJ and FBB, low frequencies of $A'_1 A'_3$ (type 2) and $A'_2 A''_2$ (type 3) led to lack of fit (Table 3). In this case, the number of individuals examined (84) may have been too small to provide an adequate sampling of the population or there may have been some seedling selection which decreased the frequencies of types 2 and 3. Another possible explanation for this lack of fit lies in the fact that ssp. *tuberosum* parental clones from the two sources were used interchangeably during matings. In a survey of prominent potato varieties grown nationwide in the USA, May et al. (1982) found that intraclonal variation exists for AKP and GPI. If parental clones were genotypically different for AKP then progeny segregations resulting from crosses with these individuals may not fit either assortment type. Unfortunately, recharacterization of the parents used in ETJ and FBB was not possible since they were never propagated and were lost in storage.

Some reciprocal lots in which segregations fit either assortment type when taken individually, did not fit if the frequencies were combined. A possible reason for this disparity lies within the mechanics of the chi square test. In crosses where there are large numbers of classes with small expected frequencies, the chi square value could become inflated or distorted (Bryant 1966).

The relative frequency of chromatid vs. chromosome assortment can be influenced by the frequency of quadrivalents vs. bivalents and by variations in crossover frequency, both of which may be subject to environmental influences.

The segregation of Gpi and Akp in F_1 progeny showed no tendencies towards either assortment type. BC_1 segregations for Gpi more consistently fit chromosome assortment while Akp segregation showed no great tendencies towards either assortment type. More information on the segregation of these loci is needed to characterize their position in relation to the kinetochore.

To be used as a genetic marker an enzyme system must have a predictable basis and data must be reproducible. In this study isozymes of AKP and GPI have been found to form banding patterns which are predicted for dimeric enzymes encoded by a single tetrasomic locus. Other work has demonstrated the stability and reproducibility of these enzymes (Staub et al. 1982). Therefore, these enzymes could serve as genetic markers and chromosome mapping of these enzymes in potato is possible.

References

Allendorf FW, Mitchell N, Ryman N, Stahl G (1977) Isozyme loci in brown trout (*Salmo trutta* L.): Detection and interpretation from population data. Heretitas 86:179–190

Brewer GW (1970) An introduction to isozyme techniques. Academic Press, London New York

Bryant EC (1966) Statistical analysis. McGraw-Hill, New York

Carter ND, Hill MR, Weir BJ (1972) Genetic variation of PGI in some hystricomorph rodents. Biochem Genet 6:147–156

Clayton JW, Tretiak DN (1972) Amine-citrate buffers for pH control in starch gel electrophoresis. J Fish Res Board Can 29:1169–1172

Darnall DW, Klotz IM (1972) Protein subunits: a table (revised edition). Arch Biochem Biophys 149:1–14

Desborough SL, Peloquin SJ (1966) Disc electrophoresis of tuber proteins from *Solanum* species in interspecific hybrids. Phytochemistry 5:727–733

Desborough SL, Peloquin SJ (1967) Esterase isozymes from tubers. Phytochemistry 6:989–994

Desborough SL, Peloquin SJ (1968) Potato variety identification by use of electrophoretic patterns of tuber proteins and enzymes. Am Potato J 45:220–229

Desborough SL, Peloquin SJ (1969) Tuber proteins from haploids, self and cultivars of Group Tuberosum separated by acid gel electrophoresis. Theor Appl Genet 39:43–47

Desborough SL, Peloquin SJ (1971) Acid phosphatase isozymes in haploids and selfs of Group Tuberosum potato cultivars. Phytochemistry 10:571–572

Detter JC, Ways PO, Giblett ER, Baughan MA, Hopkinson DA, Povey S, Harris H (1968) Inherited variations in human phosphohexose isomerase. Ann Hum Genet 31: 329–338

Gell PGH, Wright STC, Hawkes JG (1956) Immunological methods in plant taxonomy. Nature (London) 177:573

Gottlieb TD (1977) Evidence for duplication and divergence of the structural gene for phosphoglucoseisomerase in diploid species of *Clarkia*. Genetics 86:289–307

Hawkes JG, Lester RN (1966) Immunological studies on the tuber-bearing *Solanums*. 2. Relationships of the North American species. Ann Bot (London) 30:269–290

Hawkes JG, Lester RN (1968) Immunological studies on the tuber-bearing *Solanums*. 3. Variability within *S. bulbocastanum* and its hybrids with species in Series *Pinnatisecta*. Ann Bot (London) 32:165–187

May BJ, Wright JE, Stoneking M (1979) Joint segregation of biochemical loci in *Salmonidae:* results from experiments with *Salvelinus* and review of the literature on the other species. J Fish Res Board Can 36:1114–1128

May B (1980) The Salmonid genome: evolutionary restructuring following a tetraploid event. PhD Dissertation, The Pennsylvania State University

May B, Staub JE, Kuhns LJ (1982) Potato cultivars: genetic variation within putative clones. Am Potato J 59:179–187

Mecklenburg HC (1966) Inflorescence hydrocarbons of some species of *Solanum* L. and their possible taxonomic significance. Phytochemistry 5:1201–1210

Ridgway GJ, Sherburne SW, Lewis RD (1970) Polymorphism in the esterases of Atlantic herring. Trans Am Fish Soc 99:147–151

Richmond RC (1972) Enzyme variability in the *Drosophilia williston* group. 3. Amounts of variability in the superspecies *D. paulistorum*. Genetics 70:87–112

Rickeman VS, Desborough SL (1978) Elucidation of evolution and taxonomy of cultivated potatoes with electrophoresis. 1. Groups Tuberosum, Andigena, Phureja, and Stenotomum. Theor Appl Genet 52:217–220

Rickeman VS, Desborough SL (1978) Inheritance of three electrophoretically determined protein bands in potato (*Solanum tuberosum* L.). Theor Appl Genet 52:187–190

Scandalios JG (1969) Genetic control of multiple forms of enzymes in plants: a review. Biochem Genet 3:37–79

Shaw CR (1965) Electrophoretic variations in enzymes. Science 149:939–942

Shaw CR, Prasad R (1970) Starch gel electrophoresis of enzymes – a compilation of recipes. Biochem Genet 4:297–320

Siepmann R, Stegemann H (1967) Enzym-Electrophorese in Binschulb-Polymerisaten des Acrylamids. A. Amylasen Phosphorylasen. Z Naturforsch 22:949–955

Staub JE, Kuhns LJ, Grun P, May B (1982) Stability of potato tuber isozymes under different storage regimes. J Am Soc Hortic Sci 107:405–408

Steel RGD, Torree JH (1960) Principles and procedures of statistics. McGraw-Hill, New York

Stegemann H, Loeschke V (1977) Das Europäische Kartoffelsortiment und seine Indexierung. Potato Res 20:101–110