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Tuber Proteins from Haploids, Selfs, and Cultivars of Group Tuberosum Separated by Acid Gel Disc Electrophoresis*

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Summary. Tuber extracts of 46 cultivars (American varieties), and 350 haploids ($2n = 24$) and selfs ($2n = 48$) from four parents ($2n = 48$), were analysed by acid gel disc electrophoresis. This system separated proteins into 12–14 bands. Twelve cultivars possessed unique patterns of bands, and the remaining cultivars could be placed in groups on the basis of 8 different banding patterns. Distinctions between varieties within groups was accomplished by either esterase or peroxidase isozyme patterns. The usefulness of basic gel proteins, esterase, and peroxidases for varietal identification is known; the acid gel protein patterns described provide a fourth system.

Proteins from haploids and selfs were examined for variation in frequency and presence of bands. Differences among bands of the 4 parents were minor. Most haploids and selfs possessed the same bands as their parents, but there were interesting exceptions. The frequency of certain bands was significantly higher in selfs than in haploids. The results fit what would be expected if the parent tetraploid is simplex for a dominant allele controlling the production of each protein. Other bands are more frequent in haploids than selfs and some bands are present in haploids and not in the parent. Suppressor genes in the tetraploids may account for these latter results.

I. Introduction

Solanum species have been distinguished by patterns of tuber proteins electrophoretically separated in an acid gel system (DESBOROUGH and PELOQUIN, 1968b). Intra-species comparisons of fourteen bands detected certain bands which occurred in different frequencies. The species-specific banding patterns provide a criterion for taxonomic differentiation. Genetic investigation can be directed at those bands segregating within a species.

Tuber proteins resolved in basic pH electrophoretic systems have been characterized for 59 Dutch (ZWARTZ, 1966), 21 German (LOESCHCKE and STEGEMANN, 1966) and 45 American varieties (DESBOROUGH and PELOQUIN, 1968a). Proteins identified as esterase isozymes analysed from *Solanum* species and interspecific hybrids did not appear related to taxonomic status or ploidy level (DESBOROUGH and PELOQUIN, 1967). However, haploids ($2n = 24$) and selfs ($2n = 48$) of Group Tuberosum cultivars ($2n = 48$) and haploid-species hybrids ($2n = 24$) yielded simpler esterase patterns for which a genetic hypothesis was formulated. When these same esterase isozymes along with peroxidase isozymes and soluble proteins are considered, identification of 45 American varieties was possible (DESBOROUGH and PELOQUIN, 1968a).

The acid gel system appears useful for both cultivar comparisons and genetic analyses with haploids and selfs. Results obtained with this system may supplement the protein and enzymes presently available for variety identification. More important, however, are the patterns for which genetic information may be obtained. The acid gel system resolves from twelve to fourteen protein bands, whereas the basic gel system resolves about twenty-five protein bands, thus it is easier to compare gels with the acid system. Group Tuberosum clones are well situated for genetic studies since haploids representing populations of gametes, and selfs representing populations of zygotes are available in large numbers.

II. Materials and Methods

Tubers used were from the variety collection or research material maintained at the University of Wisconsin Potato Research Farm, Rhinelander, Wisconsin. Tuber extracts were prepared from (1) forty-six American cultivated varieties ($2n = 48$) and, (2) 151 haploids ($2n = 24$), 199 selfs ($2n = 48$) obtained from four Group Tuberosum cultivars (Ag231, Chippewa, Katahdin and Merrimack). These extracts were the same ones used in previous studies (DESBOROUGH and PELOQUIN, 1967 and 1968a). Electrophoretic procedures were the same as reported in DESBOROUGH and PELOQUIN (1968b). Duplicate and sometimes triplicate gels of the same sample were usually compared for accuracy of band designation.

III. Results

Acid gel protein bands from forty-six American varieties were determined. Only twelve bands were considered for comparisons between varieties because bands 1 and 2 were not resolved in these gels. Bands 3 to 14 were numbered to correspond to *Solanum* species patterns (DESBOROUGH and PELOQUIN, 1968b). Representative acid gel patterns of six varieties are given in Figure 1. The patterns were unique for each of twelve varieties (Group 1, Table 1). The prefix "A" before each pattern is used to denote acid gel protein band; this designation parallels E for

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Table 1. *Nine Groups of acid gel patterns of forty-six varieties. Prefix A denotes acid gel protein band*

Group 1	
Anoka	A—5—89——12—14
Blanco	A345—89——121314
Boone	A–45—910—1213—
Delus	A—5—8910—12—14
La Salle	A—5—8910—1213—
Monona	A345—89——12—14
Navajo	A–45—89——12——
Platte	A———8910—121314
Pungo	A—5—89——121314
Red Warba	A–45—9——121314
Waseca	A–45–789101112——
White Rose	A345–78910—121314
Group 2	
Haig	
Plymouth	A–45—89——12—14
Group 3	
Beauty of Hebron	A–45—89——121314
Sheridan	
Group 4	
Rushmore	
Saco	A345—8910—12—14
Group 5	
Early Ohio	
Early Red	A–45—891011121314
Fundy	
Group 6	
Early Rose	
La Rouge	
Red LaSoda	A–45—89—111213—
Sequoia	
Group 7	
Arenac	
Russet Arenac	
La Chipper	
Reliance	A–45—8910—121314
Russet Rural	
Sebago	
Group 8	
Early Gem	
White Early Gem	
Golden Chipper	
Kennebec	A–45—8910111213—
Keswick	
Russet Burbank	
Viking	
Group 9	
Canus	
Green Mountain	
Huron	
Norgold Russet	A–45—8910—12—14
Ona	
Snowflake	
Superior	
Tawa	

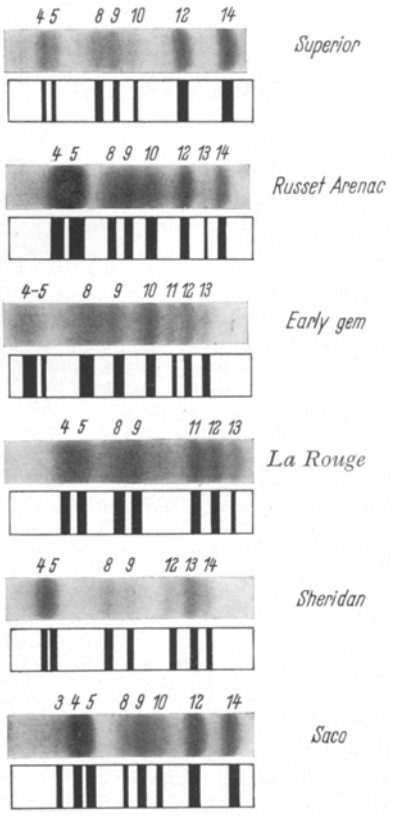

Fig. 1. Acid gels of tuber proteins from six varieties

Table 2. *Identification of Varieties by Acid gel pattern (A; and Esterase (E) or Peroxidase (P) isozyme pattern*

Group 2 A–45—89——12—14	
Haig	$E45^267^2$
Plymouth	$E–5^267$
Group 3 A–45—89——121314	
Beauty of Hebron	$E4^25$—
Sheridan	$E–5^26^27$
Group 4 A345—8910—12—14	
Rushmore	P67—10111213
Saco	P678910111213
Group 5 A–45—891011121314	
Early Ohio	$E45^267$
Early Red	$E4^25^3$—
Fundy	$E–5^267$
Group 6 A–45—89—111213—	
Early Rose	$E–5^36$–
La Rouge	$E4^25^26$–
Red La Soda	$E4^25^26$–
Sequoia	$E4^25^26^2$–
Group 7 A–45—8910—121314	
Arenac	P6–8910111213
Russet Arenac	P6—910111213
La Chipper	P678–10111213
Reliance	P678910111213
Russet Rural	P6789101112—
Sebago	P67–910111213
Group 8 A–45—8910111213—	
Early Gem	$E4^25$—
White Early Gem	$E4^25^26^27$
Golden Chipper	$E4^256$–
Kennebec	$E4^25^26$–
Keswick	$E–5^26$–
Russet Burbank	$E4^256$–
Viking	$E–5^36$–
Group 9 A–45—8910—12—14	
Canus	$E4^256$–
Green Mountain	$E4^25^267$
Huron	$E–5^3$—
Norgold Russet	$E4^25^2$—
Ona	$E–5^26$–
Snowflake	$E45^36$–
Superior	$E4^25^26$–
Tawa	$E–5^26$–

esterase isozyme and P for peroxidase isozyme bands used in DESBOROUGH and PELOQUIN (1968a). Anoka with pattern A5891214, Navajo with A459812 and Pungo with A589121314 are three examples with few bands; an example of a more complex pattern is White Rose with 10 bands. Three Groups, 2, 3 and 4, each contain a pair of varieties with the same pattern. The other five Groups with additional patterns contain from three to eight varieties. Nevertheless, each of these varieties can be uniquely classified after they have been grouped by their A-pattern from their esterase or peroxidase isozymes pattern (Table 2).

Fourteen proteins from Group Tuberosum haploids and self tuber extracts were resolved by the acid gel system. These protein bands were also numbered from one to fourteen and correspond to those described for *Solanum* species (DESBOROUGH and PELOQUIN, 1968b). Acid gel protein banding patterns were examined from 151 haploids ($2n = 24$) and 199 selfs ($2n = 48$) from tuber extracts of four cultivars. Twenty duplicate tubers of the four cultivars were also included to ascertain the reliability of band classification. Cultivar Ag231 was represented by 89 haploid and 126 self tubers. Chippewa by 29 haploid and 34 self tubers, Katahdin by 21 haploid and 28 self tubers, and Merrimack by 12 haploid

Table 3. *Frequency of Acid Gel Bands from parents, haploids and selfs of four Group Tuberosum cultivars*

	Total Samples	Acid Gel Protein Bands													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ag 231 parent	8	8	8	8	8	8	8		8	8	8	8	8	8	8
haploids	89	62	39	64	55	83	11	3?	86	81	70	38	83	4	75
selfs	126	62	95	106	114	124	11		118	115	113	71	117	26	95
Chippewa parent	7	7	7	7	7	7		7	7	7	7		7	7	7
haploids	29	26	24	25	12	27	8	21	26	26	22	8	25	15	25
selfs	34	23	30	27	29	30		29	31	31	31		31	18	31
Katahdin parent	3	3	3	3	3	3		3	3	3	3	3	3	3	3
haploids	21	9	14	17	17	17		17	17	17	16	9	16	11	15
selfs	28	18	26	16	27	28		26	27	27	26	20	28	19	25
Merrimack parent	2			2	2	2		2	2	2	2	2	2	2	2
haploids	12			4	11	12		10	12	12	10	4	10	6	10
selfs	11			4	6	6		7	7	7	7	2	8	2	7

Table 4. *Frequency (%) of Acid Gel Protein Bands in haploids and selfs from four Group Tuberosum cultivars*

	Total samples	Acid Gel Protein Bands													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ag 231 haploids	89	70	44	72	62	93	12		97	91	79	43	93	.04	84
selfs	126	49	75	84	91	98	9		94	91	90	56	93	20	76
Chippewa haploids	29	90	83	86	42	93	24	73	90	90	76	28	86	52	28
selfs	34	68	88	80	85	88		85	91	91	91		91	53	91
Katahdin haploids	21	43	67	81	81	81		81	81	76	43	43	76	52	72
selfs	28	78	93	57	97	100		93	97	97	93	72	100	68	89
Merrimack haploids	12			33	92	100		84	100	100	84	33	84	50	83
selfs	11			35	55	55		64	64	64	64	18	73	18	73

and 11 self tubers (Table 3). The protein patterns of three tuber extracts of Katahdin were found to be identical, gels contained all bands except band 6 (Fig. 2). Band identity was also determined for several tuber extracts of Ag231, Chippewa and Merrimack cultivars and found to be the same among tuber extracts of any one parent (Table 3).

The Katahdin haploid pattern shown at the top of Figure 2 illustrates that a haploid can have the same bands as its parent tetraploid; this gel was run for $1^1/_2$ hours compared to 2 hours for the other gels, therefore the bands are not as widely spaced. The bottom haploid pattern illustrates segregation for band 13 between Katahdin haploids. Four examples of Ag231 extracts demonstrate 1) the parent extract with all but band 7, 2) a haploid with fewer bands than its parent, 3) segregation between selfs for 4 bands (Fig. 3). Two acid gels of Chippewa demonstrate that a haploid can have more bands than a self from the same cultivar (Fig. 3).

A summary of band frequencies for all parents, haploids and selfs of the four cultivars is given in Table 3. Each of the parent cultivars differed only by the presence or absence of one, two or three bands: Ag231 lacks band 7, Chippewa lacks bands 6 and 11, Katahdin lacks band 6 and Merrimack lacks bands 1, 2 and 6. All of the other bands (3, 4, 5, 8, 9, 10, 12, 13 and 14) are common to the four cultivars. The identity of these bands was checked by artificila mixtures of pairs of extracts run in the same gel.

In general, bands that were present in haploids and selfs were the same ones observed in their tetraploid parent. Forty-one haploid vs. self comparisons of a specific band frequency can be made for Ag231, Chippewa and Katahdin. Of these, no differences were noted in thirteen comparisons among haploids and selfs. Twenty-two comparisons reveal a higher band frequency among selfs rather than haploids; however, there are five instances of band frequency higher in haploids than in selfs. Eight Chippewa haploids had band 6 or 11 and the parent or self tetraploids did not (Table 3). Band 1 in Ag231 and Chippewa haploids and band 3 in Katahdin haploids occurred in higher frequency than in corresponding selfs, but these bands did occur in the parent tetraploids. The presence of band 7 in three Ag231 haploids is questionable because the bands were faint. Merrimack haploids are exceptional in that ten of the eleven haploid vs. self comparisons reveal a higher band frequency than observed in selfs.

IV. Discussion

An earlier survey of *Solanum* species demonstrated that four to fourteen protein bands were separated with the acid gel system. There was extensive inter-

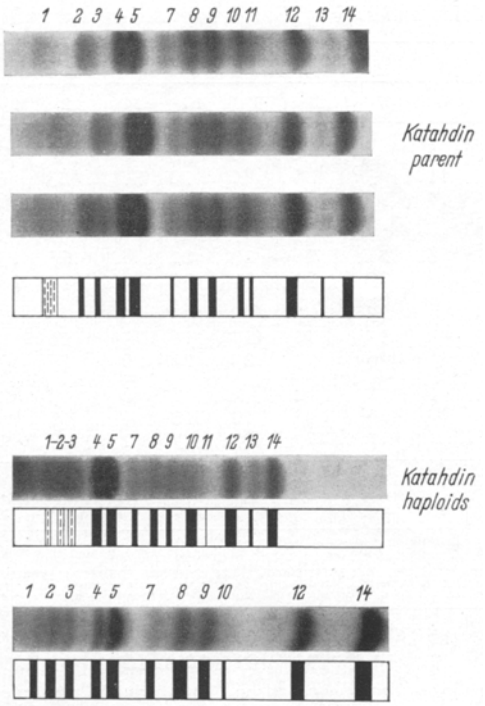

Fig. 2. Acid gels of tuber proteins from three tubers of Katahdin grown in different field areas, the protein patterns are the same. Acid gels of tuber proteins from two Katahdin haploids, bands 11 and 13 are lacking in lower gel

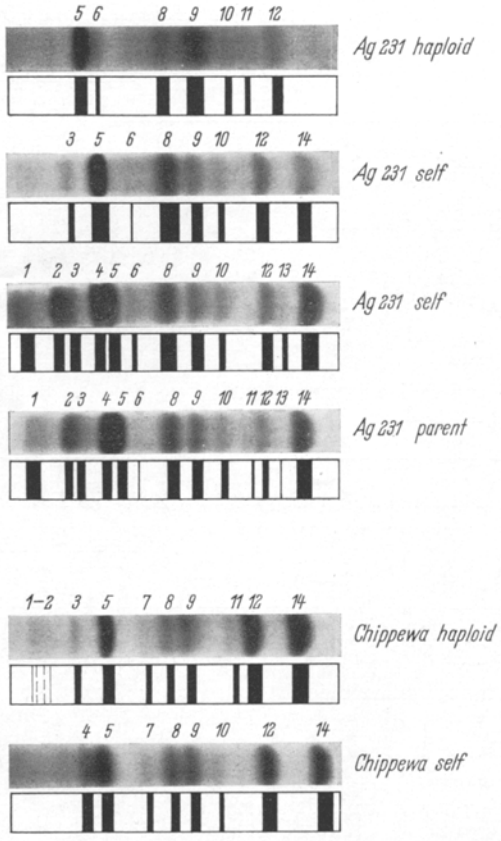

Fig. 3. Acid gels of proteins from a haploid, two selfs and parent tuber of Ag231, the haploid lacks bands 1, 2, 3, 4, 13 and 14, the selfs differ in bands 1, 2, 4 and 13. Lower two gels of a Chippewa haploid and self, the haploid has bands 1—2, 3 and 11 and the self does not

species as well as intra-species variability for occurrence of these bands. However, certain band patterns were unique to a species and could be considered species-specific. Those bands which did segregate within a species were of interest for further genetic studies. Group Tuberosum, well represented by varieties, haploids and selfs, provided unique materials for this type of study.

Distribution of twelve bands within forty-six American varieties was not as extreme as the distribution of bands among the species. There was, however, adequate variation to divide varieties into nine Groups; Group 1 consists of 12 varieties with unique patterns, Groups 2 to 4 each had two varieties with similar patterns, and Groups 5 to 9 had from 3 to 8 varieties with similar patterns (Table 1). The varieties in Groups 2 to 9 can be identified by a second system, either their esterase or peroxidase isozyme pattern (Table 2). The varieties in all Groups except 4 and 7 needed only their acid gel pattern and esterase isozyme pattern for a non-overlapping band combination. Varieties in Groups 4 and 7 could be distinguished by their unique A plus P pattern. It is of interest that Arenac and Russet Arenac or Early Gem and White Early Gem do have the same A patterns but differed in their isozyme patterns. These results confirm the usefulness of the acid gel protein bands for varietal identification, particularly since 12 varieties had unique patterns. Thus four systems: acid and basic gel proteins, esterase and peroxidase isozymes, permit confirmation of variety identification.

Tuber extracts of haploids and selfs from four Group Tuberosum cultivars analysed for their acid gel protein patterns provide information amenable to genetic analyses. Differences among fourteen bands of the parent cultivars were established to be very minor: band 7 was missing in Ag231, bands 6 and 11 in Chippewa, band 6 in Katahdin and bands 1, 2 and 6 in Merrimack. These differences were not nearly as great as inter-species differences.

Comparisons of haploids and selfs with their parent tetraploid were made to determine if bands occur in different frequencies within these "gamete" and "zygote" populations. Most bands were in common with the parent cultivar, but there are some interesting frequency comparisons among haploids and selfs. In eight Chippewa haploids band 6 or 11 was present while these were lacking in their parent and self tetraploids (Table 3). The frequencies of band 1 in Ag231 and Chippewa and band 3 in Katahdin haploids are greater than in their selfs (Table 4). Merrimack haploids are especially unusual in this

aspect with ten bands of higher frequency than its selfs (Table 4). One might speculate that suppressor genes in the tetraploid parent and selfs prevent production of these proteins; while suppressor genes have segregated and thus are not present in certain haploids. This is a strong possibility particularly for bands 6 and 11 observed in Chippewa haploids but which are absent in the parent or self tetraploids. An unlikely possibility is that recessive genes control the production of proteins.

It should be noted that aniline blue black does not detect all proteins, since more sensitive enzyme techniques resolve additional protein bands. More information is required about the protein bands discussed here, specifically if these bands are single proteins or enzymes. However, distinct protein band which vary in frequency among haploids compared to selfs can be selected for further study.

It is tempting to interpret any protein band which occurred in about 50 percent of the haploids and about 75 percent of the selfs as possibly being controlled by a single dominant allele that was simplex in the parent tetraploid. There are several examples of this that will be worthwhile to analyze closely. They are band 2 in Ag231, band 4 in Chippewa and bands 1, 11 and 13 in Katahdin (Table 4). Other bands occurring in 80 to 90 percent of the haploids and selfs of any one cultivar are less interesting genetically, but of importance in cultivar to cultivar and cultivar to species comparisons.

Ploidy level seemingly had no effect on the total band pattern, haploids could have the same bands as its parent tetraploid or they could have fewer bands (the only case of more bands is discussed above). Haploids could also have fewer, greater or the same bands as selfs from any one cultivar (Figure 3).

Zusammenfassung

Knollenextrakte von 46 Klonen amerikanischer Kartoffel-Sorten sowie von 350 Haploiden ($2n = 24$) und Selbstungen ($2n = 48$) von vier Eltern ($2n = 48$) wurden mit Hilfe von Disk-Gel-Elektrophorese in saurem Milieu analysiert. Dieses System trennte die Proteine in 12—14 Zonen auf. Zwölf Klone wiesen einheitliche Proteinmuster auf. Die übrigen Klone konnten auf Grund von acht verschiedenen Proteinmustern in Gruppen eingeteilt werden. Unterscheidungen zwischen Sorten innerhalb dieser Gruppen wurden entweder anhand von Esterase- oder von Peroxydase-Isozym-Mustern vorgenommen.

Die Brauchbarkeit von basischen Gel-Proteinen, Esterasen und Peroxydasen für die Kennzeichnung von Sorten ist bekannt; die beschriebenen Protein-Muster nach Gel-Elektrophorese im sauren Medium stellen ein neues, viertes System dar.

Proteine von Haploiden und Selbstungen wurden im Hinblick auf Variationen in Häufigkeit und Auftreten von Zonen untersucht. Die Unterschiede innerhalb der Protein-Muster der vier Eltern waren geringfügig. Die meisten Haploiden und Selbstungen wiesen die gleichen Zonen auf wie ihre Eltern; jedoch kamen interessante Ausnahmen vor. Gewisse Zonen traten beträchtlich häufiger in Selbstungen als in Haploiden auf.

Diese Ergebnisse sind zu erwarten unter der Annahme, daß der tetraploide Elter simplex für ein dominantes Allel ist, das die Synthese eines jeden Proteins kontrolliert.

Andere Zonen zeigten sich häufiger in Haploiden als in Selbstungen. Einige Zonen traten nur in Haploiden, nicht aber im Elter auf. Suppressor-Gene in den Tetraploiden könnten für diese Ergebnisse verantwortlich sein.

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