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Elucidation of the Evolution and Taxonomy of Cultivated Potatoes with Electrophoresis

I. Groups Tuberosum, Andigena, Phureja and Stenotomum*

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Summary. A recently developed polyacrylamide gel electrophoresis technique for tuber proteins is used to help elucidate the evolution and taxonomy of some cultivated potatoes. The results substantiate the theory that Group Tuberosum evolved from Group Andigena, that Group Andigena evolved from a cultivated diploid x wild diploid hybrid, and that Group Phureja evolved from Group Stenotomum. Furthermore, the results suggest these groups are closely enough related to merit classification within a single species.

Key words: Page – Proteins – Cultivated potatoes – Evolution – Taxonomy

Introduction

The evolution and taxonomy of the cultivated potatoes is a subject of some controversy. Many (Dodds 1962; Glendinning 1975a; Hawkes 1972; Ugent 1970) agree that *Solanum tuberosum* L. Group Tuberosum Dodds evolved by natural and artificial selection from *S. tuberosum* Group Andigena, although Grun (1970b) does not believe this was necessarily the case. The general consensus now is that tetraploid *S. tuberosum* is not a true autopolyploid, but rather an allopolyploid resulting from *S. tuberosum* Group Stenotomum x wild diploid species (Grun 1970b; Hawkes 1972). The identity of this wild diploid remains unresolved although Hawkes (1972) favors *S. sparsipilum*. Mutation and artificial selection in Group Stenotomum for absence of tuber dormancy could have resulted in *S. tuberosum* Group Phureja (Hawkes 1972).

These relationships suggested have been based on morphology (Hawkes 1972; Simmonds 1964), on cytoplasm differences (Grun 1970a, 1970b), and on the creation of Neo-Tuberosum (Glendinning 1975b; Simmonds 1966, 1968). The problems of evolution and systematics have also been addressed biochemically through the use of immunological methods (Grigor'eva and Bukasov 1973). Polyacrylamide gel electrophoresis (PAGE) of tuber proteins has been suggested as a tool for use in taxonomic studies by Desborough and Peloquin who described species specific protein banding patterns in *Solanum* (1966, 1969a). A previous study using acid PAGE showed that certain protein bands occurred in different frequencies among haploids, selfs, and cultivars of Group Tuberosum (Desborough and Peloquin 1969b). We, therefore, decided to use an improved PAGE technique to examine the taxonomic relationship of some of the cultivated potatoes from a biochemical standpoint. Conceivably, the ancestral Group(s) might contain a greater variety of proteins (Hawkes and Lester 1966). Furthermore, closely related Groups would be expected to have more of the same proteins than those distantly related. If qualitative differences were few or non-existant, quantitative differences, or differences in the frequencies of proteins, might provide a means for delineating relationships.

Materials and Methods

Mature tubers were collected from 110 Andigena clones, supplied by Dr. Plaisted, Cornell University, and from 148 Phureja and 54 Stenotomum clones from Dr. Haynes, North Carolina University. Mature tubers from 66 Tuberosum cultivars were provided by Dr. Wildung, University of Minnesota.

Several methods of extracting tuber proteins were employed. Fresh tubers that had been in cold storage (5-8° C) for up to five months were chopped and macerated in 30% or 100% (v/v) DMSO. Alternatively, tubers were lyophilized and then powdered and passed through a 177 μ mesh sieve; proteins were extracted with 1 ml sodium hydrosulfite solution (7 g/l) per 100 mg tuber powder. In each case, following centrifugation of the protein extract at 16,000 x g for 30 minutes, the supernatant was either used immediately or frozen.

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7½% polyacrylamide slab gels, 0.75 mm thick, with a pH of 4.3 were formed in a Hoefer Scientific Instruments apparatus according to the formulations in Table 1 (after Peloquin et al. 1975) Twenty-five µl of extract was applied to each sample slot. Proteins were separated after 1½ hours of 60 mAmps current per gel slab. The protein bands were stained with 0.5% (w/v) Aniline Blue-Black in 7½% (v/v) acetic acid.

Results and Discussion

A total of twenty-eight bands were delineated in the gels with twelve to twenty bands appearing in any one clone (Fig. 1). Banding patterns were not altered by method of extraction nor by freezing the extract. No band was unique to a single Group (Table 2). Moreover, eleven bands (those numbered 3, 4, 5, 6, 7, 10, 11, 14, 14A, 14B and 15) appeared with approximately equal frequency in all four Groups as determined with a chi-square test using a significance level of P = 0.10 (Table 3). This similarity among Groups supports their classification as a single species. Some bands (15A, 15B and 16), however, were found in the tetraploid Groups, Andigena and Tuberosum, and scarcely, if at all, in the diploid Groups, Phureja and Stenotomum (Table 2). From this, one would expect that a wild diploid ancestor of the tetraploids must contain the genes for these proteins.

The Groups sharing a greater number of bands of equal frequency presumably are more closely related than those Groups which share a smaller number. Thus, from Table 4, Groups Phureja and Stenotomum are the most closely related pair. This may be due to the recent evolution of Phureja from Stenotomum and/or the continued introgression of each group into the other where cultivation overlaps. Also from Table 4 it can be seen that Andigena may as easily have Phureja ancestry as Stenotomum ancestry. Moreover, Tuberosum appears to have evolved from Andigena rather than directly from a cultivated diploid x wild diploid species hybrid.

At this point it would be well to consider some complicating factors. Ugent (1970) notes that the diploid Groups

Table 1. Formulations for slab PAGE at pH 4.3 as suggested by Peloquin et al. 1975

Stock solutions
A: 30.0 gm Acrylamide, 0.8 gm bis-acrylamide, H_2O to 100 ml
B: 48 ml 1 N KOH, 17.2 ml glacial acetic acid, H_2O to 200 ml
C: 48 ml 1 N KOH, 2.87 ml glacial acetic acid, H_2O to 200 ml

	Running gel	Stacking gel
A:	10.0 ml	1.5 ml
B:	10.0 ml	–
C:	–	2.5 ml
H_2O:	19.6 ml	5.9 ml
10% Ammonium persulfate:	0.2 ml	0.1 ml
TEMED:	0.2 ml	5.0 µl

Reservoir buffer: 31.2 gm β-alanine, 8.0 ml glacial acetic acid, H_2O to 10 liters.

Table 2. Frequencies of twenty-eight acid protein bands in Groups Andigena (And), Tuberosum (Tub), Phureja (Phu) and Stenotomum (Ste)

Group	Band number 3	3A	4	4A	4B	4C	5	5A	6	7
And	.98	.71	.75	.35	.86	.55	.76	.61	.93	.08
Tub	.98	.24	.91	.67	.77	.29	.98	.15	.92	.08
Phu	.91	.55	.66	.23	.58	.51	.88	.16	1.00	.07
Ste	.91	.59	.57	.26	.48	.37	.81	.07	1.00	.11

Group	Band number 8	9	10	10A	10B	11	11A	11B	12
And	.35	.22	.95	.93	.02	.65	.58	.01	.71
Tub	.52	.08	.98	.73	.20	.88	.32	.02	.97
Phu	.82	.50	.99	.22	.59	.74	.16	.34	.57
Ste	.63	.57	.96	.06	.59	.57	.09	.37	.48

Group	Band number 12A	13	14	14A	14B	15	15A	15B	16
And	.53	.58	.93	.02	.13	.67	.28	.19	.36
Tub	.12	.48	.77	.03	.21	.86	.14	.18	.15
Phu	.43	.93	.76	.07	.19	.69	.01	.01	.00
Ste	.22	.85	.78	.06	.30	.48	.00	.00	.00

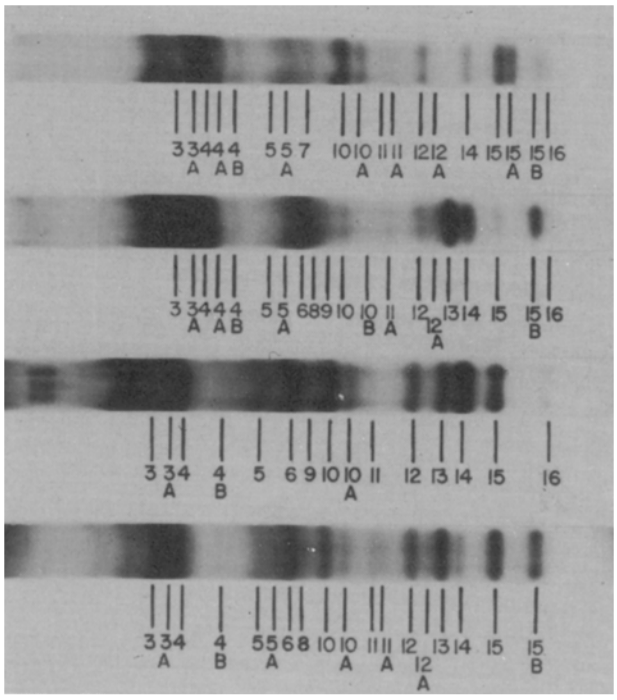

Fig. 1. Acid slab gels showing the numbering system employed. Bands occuring above band 3 were not analyzed due to extremely poor resolution in numerous gels

Table 3. Comparison of band frequencies in Groups Andigena (And), Tuberosum (Tub), Phureja (Phu) and Stenotomum (Ste)

Converging lines: bands appear with equal frequency in those groups
Straight line: bands appear with a unique frequency in that group
Differences significant at the 10% level as determined by a chi-square test

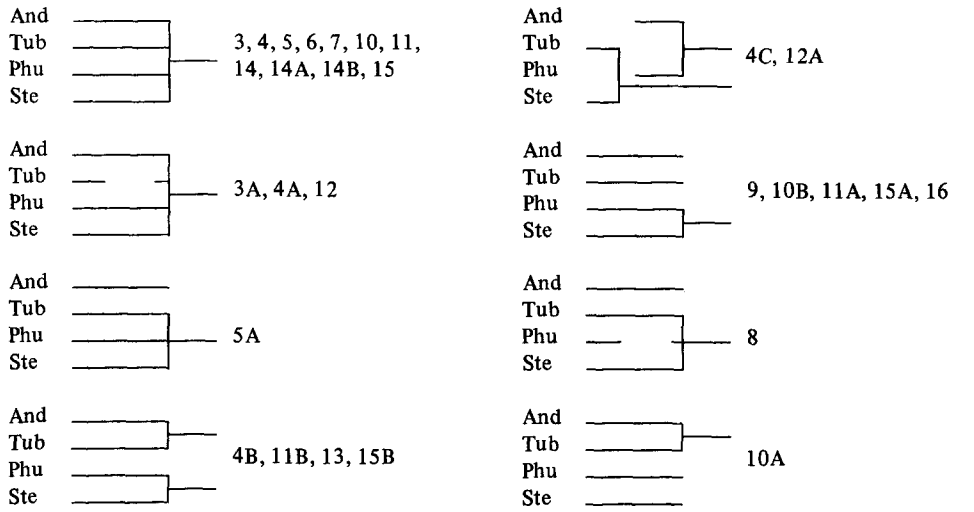

Table 4. A summary of the number of band frequencies shared by each pair of groups. Total number of bands is twenty-eight

Group pair	Number of bands appearing with equal frequency
Andigena – Tuberosum	16
Andigena – Phureja	16
Andigena – Stenotomum	14
Tuberosum – Phureja	12
Tuberosum – Stenotomum	15
Phureja – Stenotomum	24

Phureja and Stenotomum cross readily with many wild diploid *Solanum* species of section Tuberarium. Moreover, in the light of the many recorded instances of unreduced gametes in various *Solanum* species (Nijs and Peloquin 1977), it is quite possible that in South America there is continued introgression of the weedy wild species and diploid cultivated species into the cultivated tetraploids, further confusing the picture.

Further studies are planned which will include more Phureja, Stenotomum, and Andigena clones collected throuthout their ranges, as well as clones of some of the putative diploid ancestors of cultivated potatoes. The postulated occurrence of the three bands in a progenitor species of Groups Tuberosum and Andigena will be tested. The species *S. sparsipilum* is the one of choice to be examined in an attempt to verify Hawkes' theory of the origin of cultivated potatoes. The use of a basic gel system in addition to our acid system should provide further insights.

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