Phytochemistry, 1969, Vol. 8, pp. 425 to 429. Pergamon Press. Printed in England

ACID GEL DISC ELECTROPHORESIS OF TUBER PROTEINS FROM *SOLANUM* SPECIES*

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(*Received* 20 *July* 1968, *in revised form* 30 *August* 1968)

Abstract—Twenty-two *Solanum* species were compared for possible species-related tuber protein patterns with the use of an acid gel system. From four to fourteen protein bands were present and both inter-species and intra-species band variations were apparent; several bands were present in most species while other bands were more frequent within certain species. Specific banding patterns for sixteen South American and Mexican species were determined. However, no correlation between band patterns and geographic distribution of the species was noted. Preliminary results with the acid gel system are in agreement with the earlier finding, using basic gels, of species-specific tuber protein patterns.

INTRODUCTION

THE VALUE of electrophoretic techniques in taxonomic and genetic studies of plants has been established.[1-6] Many protein and enzyme systems used in such studies from a variety of tissues, mainly storage tissues, in contrast to green tissues, have been successfully employed for reflecting genetic differences. Johnson[4] was one of the first to use an acid gel system for higher plant studies. He characterized twelve wheat species by seed protein patterns and was able to attribute certain protein bands to specific genomes.

Specific patterns of soluble tuber proteins from twenty-six *Solanum* species have been reported.[7] An expanded study confirmed the results and extended the number of species-specific patterns to fifty-six.[8] These electrophoretic patterns consist of up to twenty-five or thirty proteins which migrate at pH 8·3 in acrylamide gels. Disc electrophoresis also permits proteins to be separated at pH 4·3.

The purpose of this study is to evaluate acid gel patterns of soluble tuber proteins from Mexican and South American *Solanum* species. Proteins resolved in this acid system could provide information, in addition to data obtained in the basic gel system, helpful in attacking taxonomic problems of the tuber-bearing Solanums.

RESULTS

Preliminary electrophoretic separations to determine optimal running time and to screen for possible species variation in banding patterns were done for several samples. Pairs of

* Paper No. 1231 from the Laboratory of Genetics, University of Wisconsin. Supported in part by a grant from the Rockefeller Foundation.

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gels from two introductions of each of six species were examined. Segregation for a single slow- or fast-migrating band was observed in *Solanum acaule*, *S. canasense*, *S. demissum* and *S. kurtzianum* gel pairs; these gels were run for about 2 hr. A single band difference was noted between Group Stenotomum gels, but no band differences were observed in *S. chacoense* gels (these gels were run 1 hr). A standard running time of 2 hr was adapted and usually an

TABLE 1. CHROMOSOME NUMBER, SERIES AND DISTRIBUTION OF TUBER-BEARING *Solanum* SPECIES SAMPLES

Solanum species	Chromosome number, $2n=$	Series	Distribution
S. bulbocastanum	24	Bulbocastana	C. and S. Mexico
S. cardiophyllum subsp. *ehrenbergii*	24	Pinnatisecta	C., N.W. and W. Mexico
S. jamesii	24	Pinnatisecta	N.W. Mexico, S.W. United States
S. pinnatisectum	24	Pinnatisecta	C. Mexico
S. chacoense	24	Commersoniana	S. Bolivia, Argentina, Paraguay, Uruguay and S. Brazil
S. tarijense	24	Commersoniana	Bolivia and N.W. Argentina
S. acaule	48	Acaulia	Peru, Bolivia, N.W. Argentina
S. demissum	72	Demissa	Mexico
S. verrucosum	24	Demissa	Mexico
S. fendleri	48	Longipedicellata	N.W. Mexico, S.W. United States
S. papita	48	Longipedicellata	N.W. Mexico
S. stoloniferum	48	Longipedicellata	C. Mexico
S. polyadenium	24	Polyadenia	C. Mexico
S. megistacrolobum	24	Megistacroloba	S. Peru, N.W. Argentina
S. raphanifolium	24	Megistacroloba	S. Peru
S. tuberosum		Tuberosa	
Group Andigena	48		Venezuela, Colombia, Equador, Peru, Bolivia, N.W. Argentina—few Guatemala and Mexico
Group Phureja	24	Tuberosa	Venezuela, Colombia, Ecuador, Peru and N. Bolivia
Group Stenotomum	24	Tuberosa	S. Peru and C. Bolivia
S. canasense	24	Tuberosa	S. Peru
S. lignicaule	24	Tuberosa	S. Peru
S. marinasense	24	Tuberosa	S. Peru
S. multidissectum	24	Tuberosa	S. and C. Peru
S. sparsipilum	24	Tuberosa	C. Peru and C. Bolivia
S. vernei	24	Tuberosa	N.W. Argentina

extract was examined in three separate electrophoretic runs before final band classification was assigned.

Electrophoretic analyses of proteins revealed relatively simple patterns composed of up to fourteen bands in samples representing twenty-two species from nine Mexican and South American series (Table 1). Protein samples representing any single species did not separate into identical patterns; however, in many species certain bands were found to occur in all samples (Table 2). Introductions of *S. stoloniferum* possessed the most variation in band

frequency, but all samples had bands 8, 9, 10 and 12. An example of a species in which different introductions had many bands in common is *S. polyadenium* with bands 2, 4, 5, 7, 8, 9, 10 and 12 present in all samples. The bands which do segregate within any one species may be those of interest in genetic studies and will be discussed later.

In general, the degree of variability between species from series Tuberosa or species from other series was about the same. Protein band frequencies of species from series Tuberosa are given in Table 2; all fourteen bands also occur within this series. *S. marinasense* and *S.*

TABLE 2. ACID-GEL PROTEIN BAND FREQUENCY FROM SPECIES OF EIGHT SERIES AND SERIES TUBEROSA

Solanum species	Total samples	Acid-gel band													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Series other than Tuberosa															
S. bulbocastanum	10			6	10	5	7	10	9	10	9	5			
S. cardiophyllum subsp. *ehrenbergii*	10			7	7	7	2	4	10	10	10	6	8	3	4
S. jamesii	11			6	11	8	11	11	11	11	6	3	3		
S. pinnatisectum	6			1	6		6	6	6		1	5	6	6	
S. chacoense	6		3		6	3	2		5	6	5	5	6		6
S. tarijense	3		1	3	3	3		2	3		2	1	2		3
S. acaule	11			7	10	9	5	10	9	10	11	8			
S. demissum	11			11	11		11	11	6	11	9				
S. verrucosum	5			2	5		5	5	5	5	5				
S. fendleri	9				1	1		3	9	9		2	9		5
S. papita	2			2	2	2	1	2	2	2	1	1	2		2
S. stoloniferum	10	2	7	1	5	9	2	9	10	10	10	5	10	4	8
S. polyadenium	10	5	10	3	10	10	4	10	10	10	10	6	10		
S. megistacrolobum	7				7	7		7	7	5	4				
S. raphanifolium	3		2		3		3		3	2		2	3	3	
Series Tuberosa															
S. tuberosa															
Group Andigena	6				6	5		4	5	6	6	6			
Group Phureja	8			7	4	8	2	2	8	7			8	3	
Group Stenotomum	9		1	3	6	9	8	5	5	8	8	6			
S. canasense	7		3	3	7	4	4	5	7	7	4				
S. lignicaule	2						2	1	1	1		2	2	2	
S. marinasense	1				1	1	1		1						
S. multidissectum	3				3	2	3	2	2	3	1		3	1	
S. sparsipilum	5	1	2	5	1		1	5	4		5				
S. vernei	4				2	4			4	4	4	1	4	4	4

lignicaule appear to have very distinctive patterns. If is of interest that Group Phureja contained bands 12 and 13 and not 10 or 11; thus it differs from Groups Andigena and Stenotomum. The number of samples is too small to adequately verify these species patterns; however, preliminary comparisons indicate there is band variation between the species.

To illustrate the types of banding patterns in six species, one sample from each is shown in Fig. 1. Several bands occur in some selections of all species, whereas other bands may be present or absent within a species. Particularly striking is the presence of bands, 11, 12, 13 or 14 in some and not in other species (Table 2). Attempts were made to correlate geographic distribution with protein banding patterns, but no apparent correlations were found. For

example, when the fastest group of bands (12–14) were considered; Argentine species had bands 12 and 14, but several Peruvian species had bands 12 and 13, while Mexican species were about equally divided for the presence and absence of bands 12, 13 or 14.

Artificial mixtures of extracts from two species were run in the same gel to check band classification. Thirty mixtures were examined from various species. *S. canasense* with only five bands were chosen to check band identity with *S. acaule*, *S. bulbocastanum* and subsp. *ehrenbergii*. *S. stoloniferum*, with nine bands, was compared to a second clone of *S. bulbocastanum*. No additional bands were observed in the mixtures that had not been seen in the single extracts. Band classification thus appears to be valid for interspecies comparisons.

DISCUSSION

Data presented about protein banding patterns separated in an acid gel system (pH 4·3) for twenty-two *Solanum* species (Table 1) indicates this is a useful system to supplement more conventional taxonomic approaches. Separation of tuber proteins by this method produces fewer bands, four to fourteen, compared with the basic gel system (pH 8·3). In all probability these fourteen bands are among the 25–30 bands observed in basic gels. The acid gel excludes many proteins and thus allows fewer to migrate, resulting in simpler patterns. Other advantages of the acid gel systems are that it requires about one-sixth the concentration of tuber extract that basic gels do and bands are very distinct and evenly spaced, permitting easier gel to gel comparisons.

Sixteen species represented by five or more samples were selected for calculation of sample band frequency (Table 2). In two species only one band was always present, *S. acaule* (band 10) and Group Stenotomum (band 5); *S. bulbocastanum* had two bands in all introductions (bands 4 and 7) and the remaining species had from three to eight bands in all samples. Each of these sixteen species had a unique pattern when bands occurring in 100 per cent of their samples are considered (see below). However, bands which occur in 80 to 99 per cent of samples from a given species may contribute to the species pattern and should also be considered.

Solanum species	100% Bands	80–99% Bands
S. bulbocastanum	47	810
S. cardiophyllum subsp. *ehrenbergii*	8910	12
S. jamesii	4678	—
S. pinnatisectum	46781213	11
S. chacoense	491214	81011
S. acaule	10	45789
S. demissum	4679	10
S. verrucosum	46789	—
S. megistacrolobum	4578	—
S. fendleri	8912	—
S. stoloniferum	891012	5714
S. polyadenium	2457891012	—
S. tuberosum		
Group Andigena	491011	58
Group Phureja	5812	39
Group Stenotomum	5	6910
S. canasense	489	—
S. sparsipilum	3710	8

Bands that occur in all samples of one species strongly suggest species-specific patterns similar to those found with the basic gel system. Species-specific patterns are even apparent when bands which occur to 80–99 per cent are combined with those which occur in 100 per cent of all samples of one species. It is recognized that many additional introductions of species may be required to substantiate these species-specific patterns.

There are two main categories of bands found in this survey of *Solanum* species. The first category of bands are those that are clearly present or absent in a species; these bands may be of value for taxonomic studies. For example, protein bands in known or suspected species hybrids may be correlated with bands present in parent species. A second category of bands are common to many species but occur in different frequencies within species. These bands are of interest for genetic studies. *S. tuberosum* materials, especially selfs and haploids of Group Tuberosum cultivars, and hybrids between haploids and Group Phureja, are being extensively analysed for possible band differences for which the genetic control may be investigated.[9]

MATERIALS AND METHODS

The tubers used were from the collection of the Interregional Potato Introduction Station, Sturgeon Bay, Wisconsin. Tubers from 145 introductions of twenty-two species were used. The number of available clones from each species varied from two to eleven. The *Solanum* species, their chromosome number, series and geographic distribution are given in Table 1. Plant Introduction numbers of selected species clones are given in Desborough and Peloquin,[7] except for *S. cardiophyllum* subsp. *ehrenbergii*, *S. lignicaule*, *S. marinasense*, *S. tarijense* and *S. papita* which are given in Desborough.[8] Taxonomic classification of species was according to Hawkes.[10] Group Tuberosum clones are not included because a separate study of these is under way.

Protein extracts were the same ones prepared for the previous studies. The protein concentration of most extracts was more than adequate and only 0·01–0·02 ml of extract per gel was required.

Acrylamide gel was prepared for a 7·5 per cent separating gel, spacer gel, and sample gel to stack the proteins at pH 5·0 and run at pH 4·3 according to directions of Williams and Reisfeld.[11] The gel solutions A and B contain KOH and glacial acetic acid and the electrode buffer contains beta alanine. Each gel received 5 mA for about 2 hr at 5°. A standard refrigerator was modified in order to run seventy-two gels simultaneously at this temperature. Gels were stained with 1% aniline blue black and destained with 7% acetic acid. They could be stored in acid solution or photographed.

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