Peroxidase Zymograms at Constant and Gradient pH Electrophoresis as an Analytical Test in the Identification of Potato Varieties

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Many of the thousand potato varieties cultivated around the world show similar organoleptic features, raising commercial problems for variety identification. We propose the peroxidase zymogram of tuber sap, obtained after polyacrylamide electrophoresis at pH 7.9, as an identification test among the about 50 potato varieties marketed in Spain. In addition, the peroxidase pattern obtained after electrofocusing in a 3.5–9.5 pH gradient can be used to confirm any doubtful interpretation of the above constant pH zymograms. Sap samples were prepared by freezing and thawing of potato tubers, followed by a gentle squeeze by a hand press; after addition of a proper antioxidant, sap samples can be maintained frozen without apparent changes in the peroxidase isozymes. The peroxidase pattern is not associated with the geographical source of potato tubers, which suggests an independence from soil, climatic, or agronomical factors. The peroxidase zymogram does not depend of the size of the tubers, which must be healthy and free of sprouts.

In addition to wheat, rice, and corn, potato (*Solanum tuberosum* L.) is one of the four crop species grown worldwide. Its prevailing tendency to genetic polymorphism, as well as the easiness of hybridization with some other *Solanum* species, has given rise to an outstanding number of potato varieties. The germplasm bank in the International Potato Centre (Lima, Peru) keeps up to near of 10 000 different potato genetic lines. Such a varietal diversity often raises difficulties of commercial identification, especially when the specific organoleptic features of potato tubers are not clearly different or when they are masked because of climatic or nutritional factors.

However, tuber proteins can be potentially used as variety biochemical markers. Because of its technical simplicity and speed, and the possibility of simultaneous analysis of many samples, the electrophoresis pattern of cell proteins appears as a suitable technique for such a purpose, under either nondenaturating (Parnell, 1984; Singh et al., 1973; Stegemann, 1977) or denaturating (Cooke and Cliff, 1984; Stegemann et al., 1980) conditions. In addition, the electrophoretic patterns of some enzyme proteins have been also employed as variety markers. By use of starch or polyacrylamide gels as supporting media, some isozyme patterns have been used with more or less success in variety identification of different crop plants (Almgård and Clapham, 1975; Bassiri and Adams, 1978; Salinas et al., 1982; Singh et al., 1973). A review of the subject has been made by Cooke (1984).

Concerning potato, the electrophoretic pattern of some tuber sap enzymes has been earlier applied, in association with the protein spectrum, to the resolution of variety identification (Desborough and Peloquin, 1967, 1968; Stegemann and Loeschke, 1976). In this work we applied the electrophoretic pattern of peroxidase isozymes to the identification of more than 50 potato varieties, which have been marketed in Spain for many years.

MATERIALS AND METHODS

Materials. We have assayed the 55 potato varieties listed in Table I. These varieties are pure genetic lines provided by the Certified Seed Potato Producers indicated in the table. Tubers were selected at random, without any symptom of phytopathological attack. After the tubers were washed with tap water to remove the remaining sand, they were externally blotted dried and frozen at −20 °C for 24 h. Samples were then air thawed for a night, and after peeling, a set of 10–15-mm side cubes were cut from the internal portion of the tubers. About 2–3 mL of tuber sap were collected by pressing the cubes with a small Teflon hand-managed press. A 1% (v/v) of a 10% Na_2SO_3 and 7.5% $Na_2S_2O_5$ reductive solution was added to 1 mL of the squeezed sap, and the mixture was centrifuged at 15000*g* for 30 min. Sap proteins were determined according to the method of Potty (1969), which overcomes interferences due to the abundance of phenolics existing in this material.

Constant pH Electrophoresis. Electrophoresis runs were made on 11 × 24.5 cm plates of 1.5-mm thickness, which can fit up to 20 samples. Plates were made of 6% acrylamide and an acrylamide/bis(acrylamide) ratio of 19:1 in 0.03 M Tris–borate buffer, pH 7.9. A small crystal of about 10 μg of bromophenol blue was added to each sap sample as a tracking dye, and then 10-μL samples were layered on gel wells placed at 3 cm from the cathode. Electrophoresis was performed in a Multiphor II (LKB, Stockholm) horizontal tank, thermostated at 8–10 °C by a circulator cooling bath; the starting conditions were a constant current of 20 mA for 10 min, which was then changed to a 620-V constant voltage for an additional 2 h.

Electrofocusing. Isoelectric focusing developments were made on 11 × 24.5 cm PAGE plates (LKB) of 1-mm thickness and a 3.5–9.5 pH gradient, which can fit up to 24 samples. Plates were made of 5% acrylamide and a 97:3 acrylamide/bis(acrylamide) ratio, with 2.4% (p/v) ampholines. Fifteen-microliter samples were soaked up in small pieces (4 × 8 mm) of Whatman No. 1 paper and layered in the central position of each row. Anode and cathode solutions were 1 M H_3PO_4 and 1 M NaOH, respectively. Electrophoresis was also performed in a Multiphos II (LKB) tank thermostated at 8–10 °C, at 30-W constant power and an initial current of 50 mA; the electrofocusing time was 1.5 h. pH markers were trypsinogen (9.30), lectins (8.65, 8.45, and 8.15), myoglobin (7.35 and 6.85), carbonic anhydrase (6.55 and 5.85), β-lactoglobulin (5.20), soybean trypsin inhibitor (4.55), and amyloglucosidase (3.50).

Peroxidase and Protein Staining. For peroxidase staining plates were dipped in a fresh solution containing, in 100 mL, 0.5 g of benzidine dihydrochloride, 4.5 mL of glacial acetic acid, and 0.1 mL of 30% (p/v) hydrogen peroxide (Kuhns and Fretz, 1978a). The peroxidase isozymes were developed within 10 min as brilliant blue bands, which in 5–6 min slowly shift to less sensitive brown bands. After 10 min of staining, the plates were rinsed with water and then immediately color photographed.

A second set of plates were stained for proteins after a pre-

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Table I. Source and Tuber Sap Protein of the 55 Potato Varieties Analyzed in This Work

variety	source[a]	protein, mg/mL	variety	source[a]	protein, mg/mL
Alava	Vitoria	7.3	Kennebec	Vitoria	7.3
Alava	Palencia	4.9	Kennebec	Burgos	7.3
Allard	Burgos	14.4	Kennebec	Palencia	7.7
Alpha	Granada	7.3	King Edward	Palencia	6.3
Apollo	Granada	5.9	Kondor	Burgos	12.8
Ariane	Burgos	4.9	Lamia	Burgos	7.1
Arran Banner	Vitoria	3.7	Larga	Palencia	7.6
Arran Banner	Burgos	5.2	Lola	Burgos	8.9
Arran Banner	Palencia	6.3	Lora	Burgos	4.5
Baraka	Vitoria	5.2	Marfona	Burgos	6.8
Baraka	Burgos	5.1	Mariana	Burgos	9.9
Baraka	Palencia	6.7	Marijke	Vitoria	5.2
Baraka	Granada	5.7	Monalisa	Burgos	6.3
Belda	Burgos	7.1	Nicola	Granada	7.6
Belda	Vitoria	9.1	Notted Gum	Vitoria	3.9
Bintje	Navarra	9.6	Olinda	Burgos	5.3
Blanca	Burgos	5.2	Osirene	Burgos	4.9
Blanca	Vitoria	10.5	Ostara	Vitoria	7.5
Cardinal	Vitoria	5.2	Ostara	Burgos	6.5
Casparo	Burgos	8.0	Pumbila	Burgos	7.3
Chieftain	Burgos	4.9	Red Pontiac	Vitoria	6.1
Chieftain	Palencia	7.8	Red Pontiac	Burgos	3.9
Cima	Burgos	5.5	Red Pontiac	Palencia	7.1
Claustar	Vitoria	5.5	Red Pontiac	Granada	5.5
Claustar	Palencia	4.5	Rosalie	Vitoria	5.2
Concurrent	Burgos	8.7	Sahel	Palencia	7.3
Desirée	Vitoria	4.9	Semana	Burgos	6.3
Desirée	Burgos	4.5	Shepody	Palencia	12.7
Desirée	Palencia	5.1	Spunta	Vitoria	7.1
Desirée	Granada	4.9	Spunta	Burgos	5.1
Diamant	Vitoria	6.1	Spunta	Palencia	8.1
Draga	Burgos	4.1	Spunta	Granada	5.1
Edzina	Burgos	4.1	Tobique	Burgos	4.5
Edzina	Palencia	7.5	Turia	Vitoria	6.7
Eureka	Burgos	7.3	Turia	Burgos	5.7
Famosa	Burgos	7.6	Turia	Palencia	7.8
Faraona	Palencia	3.1	Vakon	Burgos	6.3
Frisia	Palencia	10.4	Vekaro	Burgos	6.9
Jaerla	Vitoria	4.7	Victor	Burgos	6.5
Jaerla	Burgos	3.5	Vulkano	Burgos	8.5
Jaerla	Palencia	6.7	Zubeldia	Vitoria	7.1
Jaerla	Granada	5.7			

[a] Vitoria: Agrupación de Productores de Patata de Siembra de Alava (AGRUPAL). Palencia: Cultivadores de Patata Valdivia SA (CULPAVAL, Aguilar de Campóo). Burgos: Cooperativa Santa Isabel (PROPASI). Navarra: Organización de la Patata en el Pirineo Occidental SA (OPPOSA, Noain). Granada: Cooperativa San Francisco (Huétor Tájar).

vious fixation step by dipping the plates for 45 min in an 11% trichloroacetic acid and 3.4% sulfosalycilic acid solution, followed by a 30-min washing with ethanol/acetic acid/water (25:8:67 v/v). Proteins were visualized by 10 min of staining in an 0.11% solution of Coomassie Brilliant Blue 250R in ethanol/acetic acid/water (25:8:67 v/v); the colored background was destained by successive washings with the above ethanol/acetic acid/water solvent. Plates were then dried at room temperature after the gel was covered with a cellophan sheet previously wet with an ethanol/acetic acid/glycerine/water (3:1:1:5 v/v) mixture.

RESULTS AND DISCUSSION

The application of electrophoretic techniques for varietal identification of potato tubers was engaged in the 1960s by Desborough and Peloquin (1966, 1967, 1968), who showed the feasibility of protein and esterase patterns for differentiation between *S. tuberosum* cultivars and hybrids with other related *Solanum* species. Other contributions to the subject were those of Zwartz (1966) and Zacharius et al. (1971), followed by the vast work of Stegemann and Loeschke (1976), who studied the electrophoretic patterns of protein and esterase isozymes of about 1000 European potato varieties.

Table I shows the sources of the 55 potato varieties that are analyzed in this work, as well as the protein content of tuber sap. We have found a broad range in sap proteins, even within the same variety, as a function of the geographic origin of the tuber, which in turn must depend on soil, climatic, or agronomical factors. A close reliance on such a factors in the protein content of cereal grain had earlier been found by many authors (Whitehouse, 1973). The peroxidase patterns of tuber sap after constant 7.9 pH electrophoresis are shown in Figure 1. The diagrams below the plates show the coding used for identification of each isozyme band. Besides some weakly marked bands, there are three outstanding peroxidase fractions (A–C), whose presence or absence, in addition to their relative intensity, constitutes the main reference point for the dichotomic key of Table II. Peroxidase is normally a very heterogeneous isozyme system, and because of this the peroxidase zymogram has been often used for varietal identification of some cereals and legumes (Almgård and Clapham, 1975, 1977; Bassiri and Adams, 1978; Salinas et al., 1982; Singh et al., 1973). However, in many cases the intervarietal differences in peroxidase zymograms are not relevant. Almgård and Clapham (1977) find the same isozyme patterns in 11 Swedish winter wheat and in some spring ones; similarly, 3 rose cultivars from among a set of 8 appear indistinguishable with this method (Kuhns and Fretz, 1978b). As is shown in Table II, the pairs Marfona–Vulkano and Apollo–Edzina showed the same banding patterns, with similar band intensities, and could not be distinguished by constant pH electrophoresis. Surprisingly, Eureka, Lamia, and Mariana varieties did not show any peroxidase band.

As was stated in an earlier work (López Gorgé et al., 1982), the horizontal technique has the advantage over the vertical one used by Stegemann and Loeschke (1976) of showing the cathode migrating proteins, some of them outstanding from an identification point of view as the peroxidase fractions C and L. The electrophoretic step for each variety is well reproduced (Figure 2), whereas significant differences were not observed in the peroxidase patterns in relation to the geographic source. The density band of a determined fraction can change from one sample to another, but its relative intensity with regard to the other fractions remains constant; this was earlier demonstrated on the protein electrophoregrams of common bean (Adriaanse et al., 1969) and potato (López Gorgé et al., 1982) cultivars. Moreover, some authors have shown the independence of the protein pattern in relation to climatic and fertilization factors (Adriaanse et al., 1969; Wrigley and Sheppherd, 1973). However, viral infections have been decribed as a disturbing factor concerning protein pattern in potato tubers (Loeschke and Stegemann, 1966a), and accordingly samples must be free of any pathological attack. Figure 2 shows the overlap of the main peroxidase isozymes A–C with the protein bands earlier named C, D, and 2, respectively (López Gorgé et al., 1982; Stegemann and Loeschke, 1976). The peroxidase bands E–J coincide with the "d" tail of the protein electrophoretic pattern, which did not show any relevance in the protein-supported identification keys (López Gorgé et al., 1982; Stegemann and Loeschke, 1976).

The higher resolution of electrofocusing in comparison with that at constant pH electrophoresis, prompted many authors to apply this technique to varietal identification. The isoelectric focusing of grain proteins (Cooke and Cliff, 1984; Wrigley and Sheppherd, 1973), as well as the electrofocusing zymogram of esterases and acid phosphatases (Almgård and Clapham, 1977; Stegemann, 1977) has been

Table II. Dichotomic Key for Varietal Identification of Potato Tubers through the Peroxidase Zymogram Obtained at Constant pH 7.9 Polyacrylamide Gel Electrophoresis

bands A + B +	
band D+	Jaerla
band D–	
band C the strongest	
band C slightly > band A ~ B	
band E > band A ~ B	Shepody
band E < band A ~ B	
bands EG the strongest in the set E–J	Vekaro
band F the strongest in the set E–J	Notted Gum
band C much more > band A ~ B ~ E	
prominent EF bands	
bands EF > bands AB	Alava
bands AF > bands EB	Rosalie
bands EB > bands AF	Cima
normal EF bands	
band E > bands AB	Vakon
band E < bands AB	Kennebec
band C much more > band A ~ B, small E band	
band L+	Frisia
band L–	Draga
band B the strongest	
sharp C band, undefined bands E–I	Pumbila
sharp C band, bands E–I	Concurrent
undefined C band, sharp bands E–I	Desirée
band A the strongest	
sharp bands E–J	
strong EF bands, band E slightly < band F	Turia
normal EF bands, band E < band F	Spunta
undefined bands E–J	
bands A much more > band B	
undefined C band	Claustar
sharp C band	Tobique
band A slightly > band B	
band L+	Lola
band L–	Larga
band F the strongest	Lora
band A ~ B the strongest	
band C–	Victor
band C+	
homogeneous bands E–J	
sharp bands E–J	Blanca
sharp bands E–J, tail between bands AB and bands E–J	Red Pontiac
bands EG slightly > band F, bands HI–	Alpha
bands EG slightly > band F, preband E+	Nicola
bands EF slightly > band G, band I+	Zubeldia
bands GH slightly > bands EF slightly > bands IJ	Arran Banner
bands EF much more > bands G–J	
bands EF in 3–4 subfractions	Cardinal
sharp EF bands	Baraka
band A ~ C the strongest	Kondor
band A ~ B ~ C the strongest	
sharp and very strong EF bands	Ostara
sharp EFI bands	Chieftain
undefined EFI bands	Ariane
bands E–J–	Osirene
band A ~ B ~ C ~ E the strongest	
band L+	King Edward
band L–	Marfona, Vulkano
band A ~ B ~ C ~ preband A the strongest	Monalisa
bands A + B –	
band C–	Olinda
band C +	
band A > band C	
tail between band A and bands E–J	Semana
bands E–J–	Apollo, Edzina
band A ~ C, band E+, bands F–I–	Faraona
band A < band C	
band C slightly > band A, band L+	Sahel
band C much more > band A, band L–	Bintje
bands A – B +	
band B ~ C	Allard
band B > band C	
bands E–I–	Casparo
band E+, bands F–I–	Famosa
bands E–I +	
band K+	Marijke
band K –	
sharp B band, bands EI > bands F–H	Belda
undefined B band, undefined E band > bands F–I	Diamant
all bands –	Eureka, Lamia, Mariana

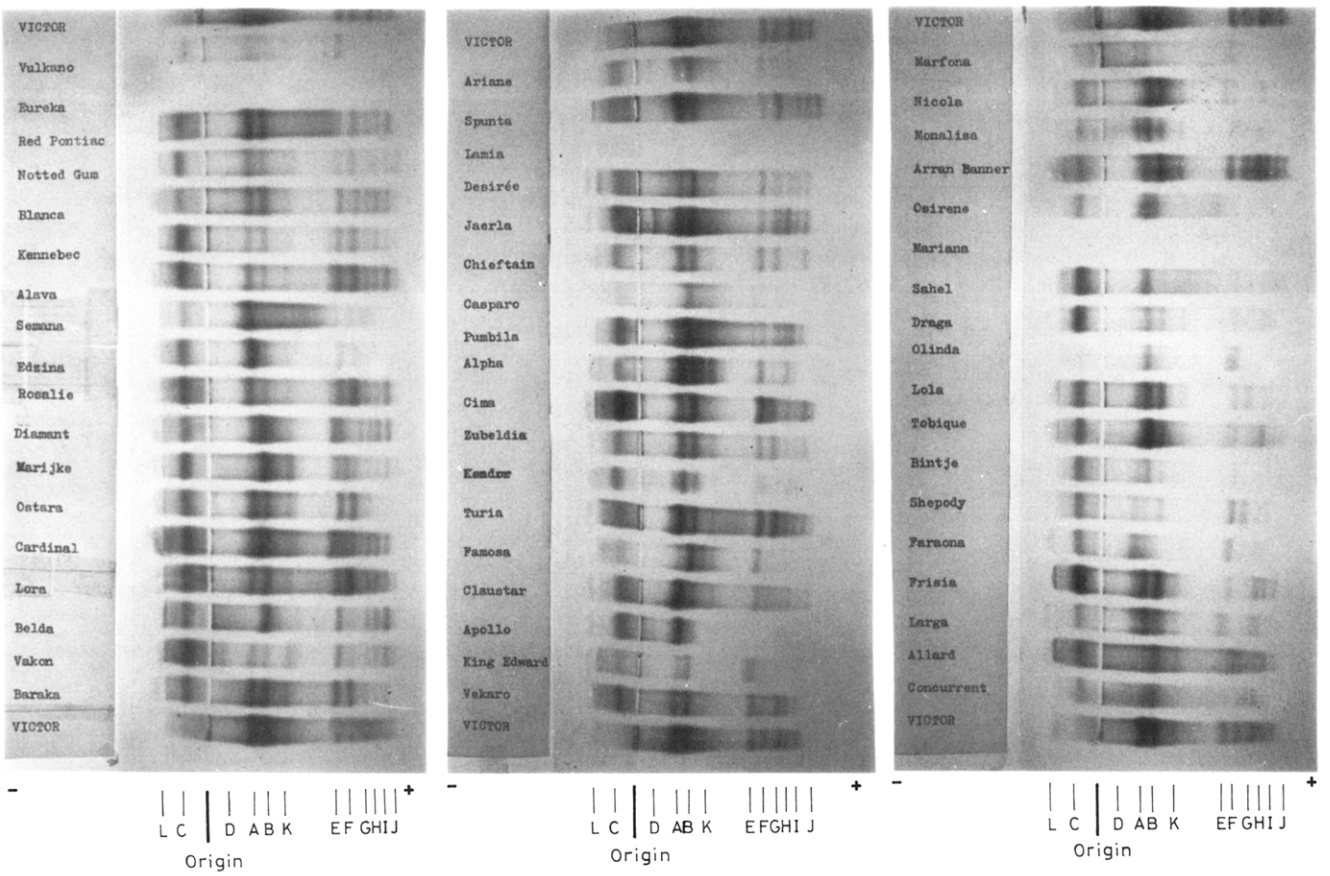

Figure 1. Peroxidase zymograms at constant pH (7.9) electrophoresis of tuber sap obtained from 55 potato varieties. Diagrams below the zymograms show the names of the different peroxidase isozymes.

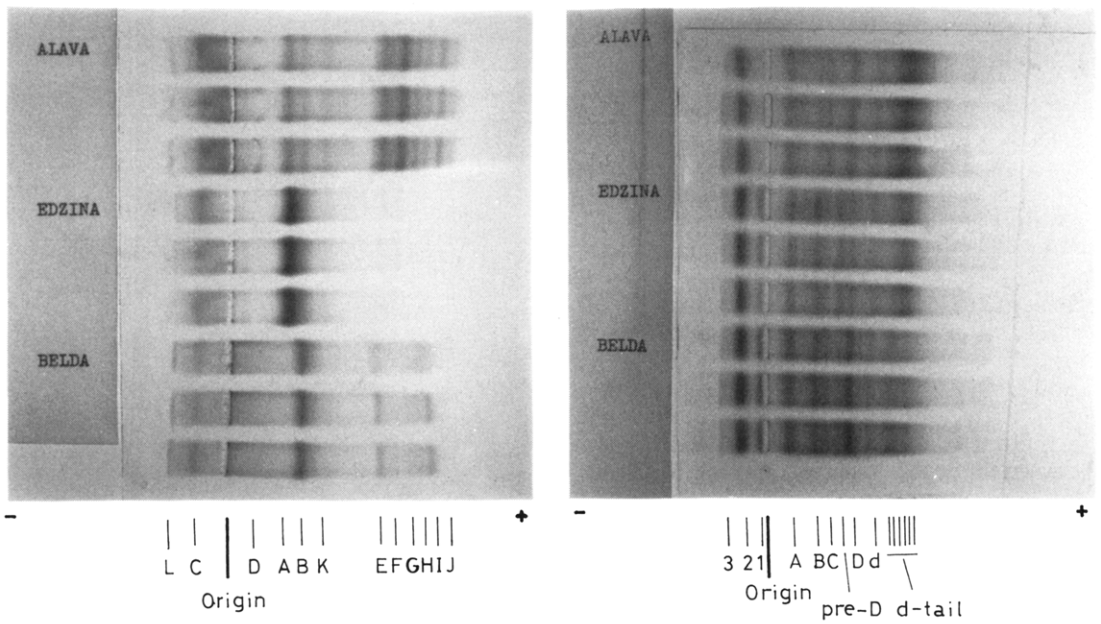

Figure 2. Peroxidase zymograms (left) and protein staining (right) after constant pH (7.9) electrophoresis of tuber sap obtained from the potato varieties Alava, Edzina, and Belda. Each sample was run in triplicate. Diagram below the peroxidase zymograms shows the names of the peroxidase isozymes, and that below the protein staining indicates the protein fraction mentioned in earlier works (López Gorgé et al., 1982; Stegemann and Loeschke, 1976).

used in the identification of varieties and hybrids of some crop species. The peroxidase patterns of potato sap after electrofocusing at 3.5–9.5 pH gradient are shown in Figure 3. Up to nearly 20 peroxidase fractions can be detected in some varieties, a very similar number to the 20 isozyme bands found in leaf extracts of *Festuca arundinacea* cultivars (Hicks et al., 1982). Different sets of these bands are characteristics of some potato varieties, but the strong heterogeneity of peroxidase zymograms makes difficult the setting up of any type of dichotomic key. Hicks et al. (1982) did not find significant differences in the peroxidase patterns of cultivars and wild forms of tall fescue (*F. arundinacea*) when leaf extracts were electrofocused at 3.5–9.5 pH gradient. A limited variability of peroxidase isoforms has also been observed in tobacco tissue cultivars (Rucker and Radola, 1971).

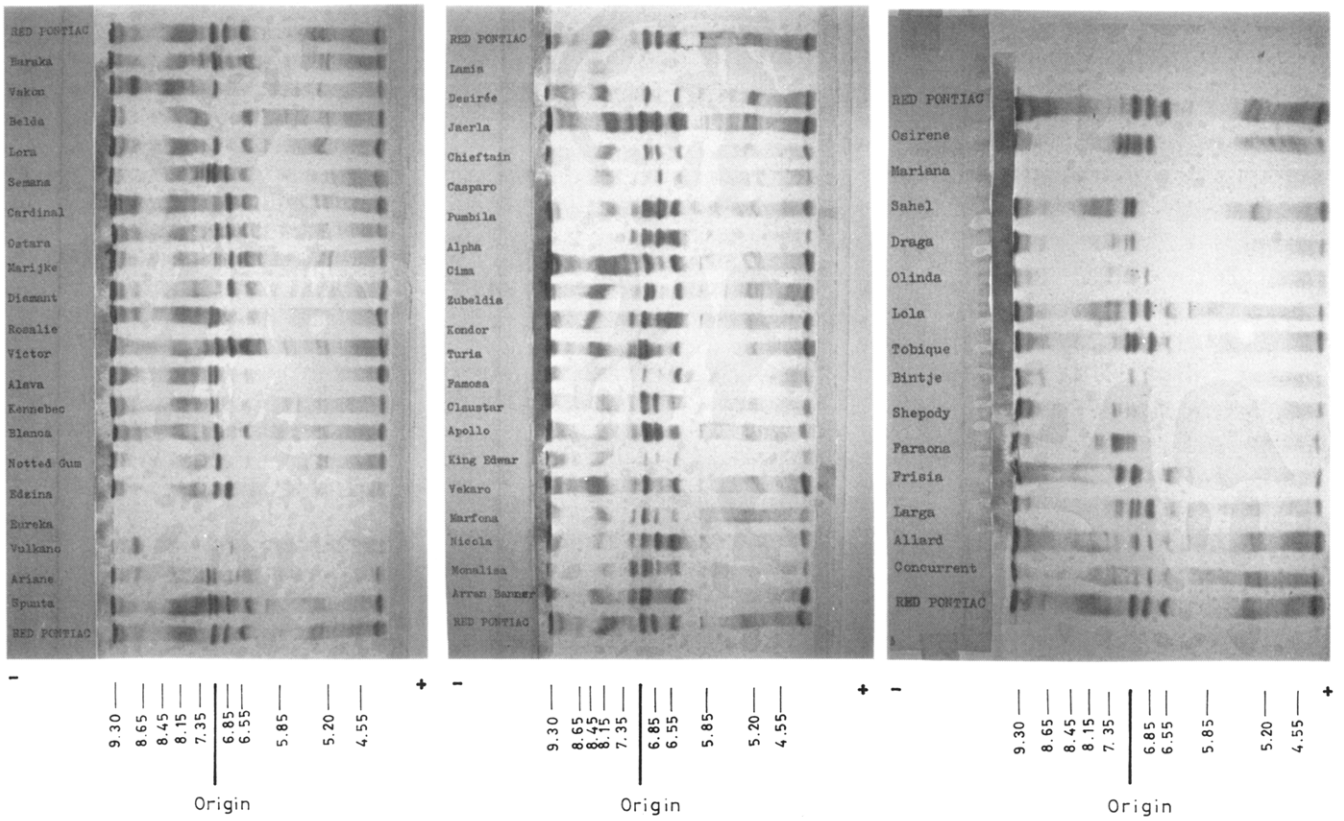

Figure 3. Peroxidase zymograms at gradient pH (3.5–9.5) electrophoresis of tuber sap obtained from 55 potato varieties. Diagrams below the zymograms show the position of the isoelectric point markers indicated under Material and Methods.

On the contrary, the peroxidase pattern obtained after isoelectric focusing of potato sap shows a great variability, which makes this technique a good complement to the constant pH peroxidase zymogram. The fingerprinting of peroxidase isozymes after pH 3.5–9.5 electrofocusing shows a characteristic pattern for some potato varieties; the pairs Apollo–Edzina and Marfona–Vulkano, earlier mentioned as indistinguishable at constant pH, show clear different peroxidase patterns at gradient pH 3.5–9.5 electrophoresis. Even though the peroxidase isozymes appear throughout the pH wide range 3.5–9.5, the most characteristic fractions are focused in the pH 6.0–7.5 zone (Figure 3). Hicks et al. (1982) found in leaf extracts of tall fescue that the basic p*I* peroxidase bands are the most interesting for cultivar differentiation.

As it occurred at constant pH electrophoresis, the isoelectric focusing pattern of peroxidase isozymes is well reproduced (Figure 4), and there are no significant differences between the patterns of geographically different samples of any variety. Because of the high content of phenolics, sap samples must be protected against protein oxidation, which can modify the stability and electrophoretic behavior of proteins, as well as the activity of peroxidase fractions (Loeschke and Stegemann, 1966b). The use of the reductant mixture Na_2SO_3–$Na_2S_2O_5$ stops the oxidative browning of sap without any additional effect on the peroxidase pattern, both at constant and gradient pH electrophoresis. Potato peroxidases are indeed very resistant to chemical treatments, even to SDS attack (Stegemann et al., 1973). Finally, sap samples can be maintained frozen at −15 °C for 2 months without any difference in the peroxidase zymograms, just as also occurs with the protein patterns (López Gorgé et al., 1982; Ruiz Nieto et al., 1989).

In conclusion, the peroxidase zymogram obtained by polyacrylamide electrophoresis, both at constant pH and under a pH gradient, is now used for the first time as unique evidence in the identification of a set of 55 potato varieties marketed in Spain.

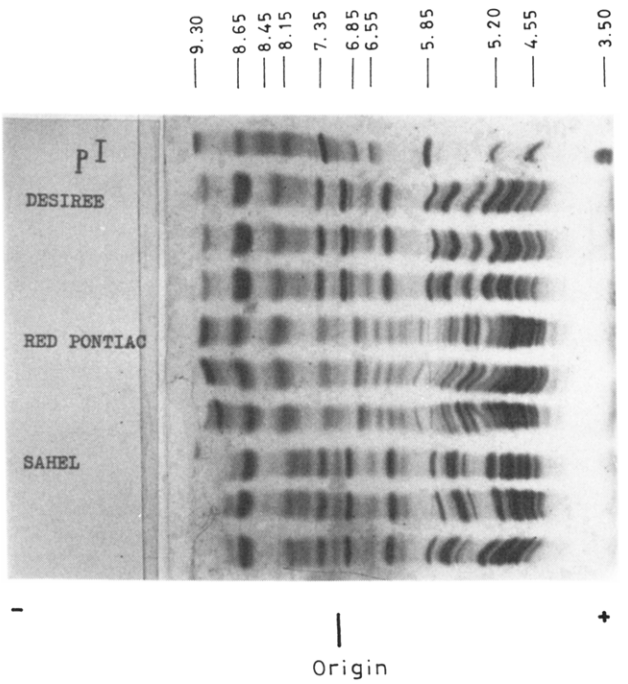

Figure 4. Protein staining after gradient pH (3.5–9.5) electrophoresis of tuber sap obtained from the potato varieties Desirée, Red Pontiac, and Sahel. Each sample was run in triplicate. The top row shows the position of the isoelectric point markers listed under Materials and Methods.

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LITERATURE CITED

Adriaanse, A.; Klop, W.; Robbers, J. E. Characterization of *Phaseolus vulgaris* cultivars by their electrophoretic patterns. *J. Sci. Food Agric.* **1969**, *20*, 647.

Almgård, G.; Clapham, D. Isozyme variation distinguishing 18 *Avena* cultivars grown in Sweden. *Swed. J. Agric. Res.* **1975**, *5*, 61.

Almgård, G.; Clapham, D. Swedish wheat cultivars distinguished by content of glyadins and isozymes. *Swed. J. Agric. Res.* **1977**, *7*, 137.

Bassiri, A.; Adams, M. W. Evaluation of common bean cultivar relationships by means of isozyme electrophoretic patterns. *Euphytica* **1978**, *27*, 707.

Cooke, R. J. The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* **1984**, *5*, 59.

Cooke, R. F.; Cliff, E. M. The characterization of oat cultivars by electrophoresis. *J. Natl. Inst. Agric. Bot.* **1984**, *16*, 415.

Desborough, S.; Peloquin, S. J. Disc electrophoresis of tuber proteins from *Solanum* species and interspecific hybrids. *Phytochemistry* **1966**, *5*, 727.

Desborough, S.; Peloquin, S. J. Esterase isozymes from *Solanum tuberosum*. *Phytochemistry* **1967**, *6*, 989.

Desborough, S.; Peloquin, S. J. Potato variety identification by use of electrophoretic patterns of tuber proteins and enzymes. *Am. Potato J.* **1968**, *45*, 220.

Hicks, P. H.; Sleper, D. A.; Randall, D. D.; Crane, C. F. Peroxidase isozyme differences in tall fescue cultivars and allopolyploid accessions. *Euphytica* **1982**, *31*, 175.

Kuhns, L. J.; Fretz, T. A. Distinguishing rose cultivars by polyacrylamide gel electrophoresis. I. Extraction and storage of protein and active enzymes from rose leaves. *J. Am. Soc. Hort. Sci.* **1978a**, *103*, 503.

Kuhns, L. J.; Fretz, T. A. Distinguishing rose cultivars by polyacrylamide gel electrophoresis. II. Isozyme variation among cultivars. *J. Am. Soc. Hort. Sci.* **1978b**, *103*, 509.

Loeschke, V.; Stegemann, H. Protein in potato tubers in relation to variety and virus infection (polyacrylamide electrophoresis). *Phytochemistry* **1966a**, *5*, 985.

Loeschke, V.; Stegemann, H. Separation of potato tuber proteins by polyacrylamide electrophoresis. *Z. Naturforsch.* **1966b**, *21B*, 879.

López Gorgé, J.; Chueca, A.; Vilchez, M. *An. Edafol. Agrobiol.* **1982**, *41*, 1491.

Parnell, A. A short note describing the electrophoregrams of new wheat varieties. *J. Natl. Inst. Agric. Bot.* **1984**, *16*, 431.

Potty, V. H. Determination of proteins in the presence of phenols and pectins. *Anal. Biochem.* **1969**, *29*, 535.

Rucker, W.; Radola, B. J. Isoelectric patterns of peroxidase isoenzymes from tobacco tissue cultures. *Planta* **1971**, *99*, 192.

Ruiz Nieto, A.; Vilchez, M.; Chueca, A.; López Gorgé, J. *Rev. Agroquim. Tecnol. Aliment.* **1989**, *29*, 340.

Salinas, J.; Pérez de la Vega, M.; Benito, C. Identification of hexaploid wheat cultivars based on isozyme patterns. *J. Sci. Food Agric.* **1982**, *33*, 221.

Singh, R. S.; Jain, S. K.; Qualset, C. O. Protein electrophoresis as an aid to oat variety identification. *Euphytica* **1973**, *22*, 98.

Stegemann, H. *Z. Acker- Pflanzenbau* **1977**, *144*, 157.

Stegemann, H.; Loeschke, V. *Index of European Potato Varieties. Identification by Electrophoretic Spectra*; Arno Borynda GmbH: Berlin, 1976.

Stegemann, H.; Francksen, H.; Macko, V. Potato proteins: Genetic and physiological changes, evaluated by one- and two-dimensional PAA-gel-techniques. *Z. Naturforsch.* **1973**, *28C*, 722.

Stegemann, H.; El-Tabey Shehata, A.; Hamza, M. Broad bean proteins (*Vicia faba* L.). Electrophoretic studies on seeds of some German and Egyptian cultivars. *Z. Acker- Pflanzenbau* **1980**, *149*, 447.

Whitehouse, R. N. H. The potential of cereal grain crops for protein production. In *The Biological Efficiency of Protein Production*; Jones, J. G. W., Ed.; Cambridge University Press: Cambridge, U.K., 1973; p 83.

Wrigley, C.; Sheppherd, K. W. Electrofocusing of grain proteins from wheat genotypes. *Ann. N.Y. Acad. Sci.* **1973**, *209*, 154.

Zacharius, R. N.; Krulic, S.; Porter, W. L. Constancy of the protein electrophoretic pattern of a potato tuber variety. *Am. Potato J.* **1971**, *48*, 57.

Zwart, J. A. Potato varieties and their protein electropherogram characteristics. *Eur. Potato J.* **1966**, *9*, 111.

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