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PAG electrophoregrams of six Finnish potato cultivars

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Abstract. The polyacrylamide gel electrophoretic (PAGE) patterns of soluble proteins and esterases of six Finnish potato cultivars (Jaakko, Pito, Hankkijan Timo, Hankkijan Tuomas, Hankkijan Tanu and Puikula) were determined. All cultivars are commonly grown in Finland. The PAGE procedure used yielded highly reproducible protein separation and good resolution. Samples studied had specific soluble protein and esterase PAGE patterns, indicating that electrophoregrams can be used for identifying Finnish potato cultivars. Only two cultivars, Hankkijan Tanu and Hankkijan Tuomas, which are close relatives, possessed very similar PAGE patterns. The electrophoretic pattern of Puikula was very similar to that of the Swedish cultivar Mandel when compared with the reference presented in the literature. Therefore a hypothesis is presented suggesting that these two local cultivars would be representatives of the same cultivar.

Introduction

In recent years electrophoresis has proved to be a useful chemotaxonomic tool in the classification of cultivars and breeding material of various organisms. By means of electrophoresis a potato variety can be identified in a couple of days and needs only a few milliliters of sap drained from tuber tissue. However, the identification requires that there are relevant reference electrophoregrams available.

Index tables of electrophoregrams of potato cultivars have been published to help in research work and control of cultivars. One of the first of these collections was the »Index of European Potato Varieties» by STEGEMANN and LOESCHCKE (1976). Their extensive work included electrophoregrams of 530 European potato varieties from almost all European countries. Later many workers have produced additional electrophoretic data on specific potato cultivars (e.g., MAIER and WAGNER 1981). However, no data on electrophoregrams of Finnish potato cultivars have been available so far.

It was the aim of the present study to produce reference data on electrophoregrams of the most common Finnish potato cultivars to be used in potato research work and varietal classification.

Index words: Potato, polyacrylamide gel electrophoresis, cultivar identification, protein, esterase

Table 1. Data of Finnish potato cultivars examined by polyacrylamide gel electrophoresis.

Potato	Breeder	Origin	Released on the market
JAAKKO	Jo[1]	Eigenheimer × Goldwährung	1951
PITO	»	Golden Wonder × Ella	1964
HANKKIJAN TIMO	Hja[2]	Fruhnudel × Katahdin	1975
HANKKIJAN TUOMAS	»	Amyla × Horsa	1975
HANKKIJAN TANU	»	Lori × Horsa	1981
PUIKULA		Finnish local cultivar	

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Materials and methods

Reagents

Acrylamide (for electrophoresis, 2 × crystallized) and Serva blau R-250 (min. 86 % purified) were obtained from Serva Feinbiochemica; N,N'-methylenebisacrylamide from BDH Chemicals Ltd; TRIS (hydroxymethyl)aminomethane 7—9 and α-naphthyl acetate from SIGMA Chemical Company; amido black 10 B, ammonium peroxodisulfate, boric acid, bromophenol blue, 3-(dimethylamino)-propionitril, sodium dihydrogen phosphate 1-hydrate, disodium hydrogen phosphate 12-hydrate, sodium disulfite dry, sodium sulfite anhydrous and trichloroacetic acid were obtained from E.Merck; fast blue B salt was obtained from Georg T. Gurr Ltd. All the reagents were analytical grade if not otherwise stated.

Sample preparation

Potato tubers were obtained from the cultivar collection maintained by the Finnish State Seed Testing Station. Tubers were harvested in September 1984, and stored at 10°C until analyzed in October. Breeder, origin and the year the cultivar was released on the market is shown in Table 1.

For sample preparation four tubers of each potato cultivar were washed, dried and frozen overnight. After thawing for 2 hours at room temperature the tubers were peeled and a 1 cm^3 piece was cut from each tuber. The juice, 1 ml, was pressed with a garlic squeezer and 20 μl of sulphite solution (1.0 g Na_2SO_3 + 0.75 g $Na_2S_2O_5$ to 20 ml H_2O) was added. The resultant slurry was centrifuged at 3000 rpm for 10 min at room temperature and the supernatant was separated. Supernatants (0.3 ml) were diluted with 0.3 ml of buffer solution (30 g saccharose + 10 mg amido black B 10 per 100 ml electrode buffer, 1:5 dilution). Sample solutions were stored frozen in sealed vials until use.

Preparation of gels

The gels for protein and esterase separation were prepared by the method of STEGEMAN and LOESCHCKE (1976) as applied by MAIER and WAGNER (1981). Details of the recipes used are given in Table 2.

Table 2. Recipes of gels and buffers used in potato protein and esterase PAGE (MAIER and WAGNER 1981).

Reagent		Amount required
Gel, ml		100
Acrylamide, g		5.76
N,N'-Methylenebisacrylamide, g		0.24
Buffer, ml		to 100
Catalyst		
3-(Dimethylamino)-propionitrile, ml		0.50
Sodium sulfite, 2 % solution, ml		0.25
Ammonium peroxodisulfate, 2 % solution, ml		1.60
Buffer, ml	for proteins pH 7.9 1000	for esterases pH 8.9 1000
Tris, g	3.79	15.13
Boric acid, g	4.56	1.15

The gels were polymerized in a tray constructed of acrylic plastic according to the measurements shown in Fig. 1. Up to 8 gels (140 mm × 180 mm × 1.5 mm) could be prepared simultaneously. Immediately after the catalyst solutions were added to the gel solution the solution was poured quickly into the gel tray through the inlet tubing, avoiding entry of air bubbles. After an hour the gels were removed from the tray, wrapped in household polyethylene film and stored at 4°C until used within a week.

Electrophoresis

The electrophoretic separation was carried out in a Pharmacia Gel Electrophoresis Apparatus GE-2/4 LS using a LKB 2103 Power Supply. For each run two gels of 140 mm × 180 mm × 1.5 mm were applied. Each gel accomodated 14 samples.

The sample solutions (20 μl) were placed in the gel slots with a microliter syringe. Duplicate electrophoresis runs were performed of each sample. Voltage was maintained at 400 V until the marker dye migrated to the bottom of the gel. Each run took 2—3 hours. Buffer temperature was maintained at 13—16°C by tapwater circulation.

Staining

Proteins were stained by immersing the gel overnight in 200 ml of 12 % TCA + 5 ml of Serva blau R 250 (1 % ethanol solution). Destaining found to be unnecessary. Esterases were stained as described by STEGEMANN and LOESCHCKE (1976).

Photography

The gel was placed on a glass plate on a light box and illuminated from below and photographed with Agfaorto 25 film.

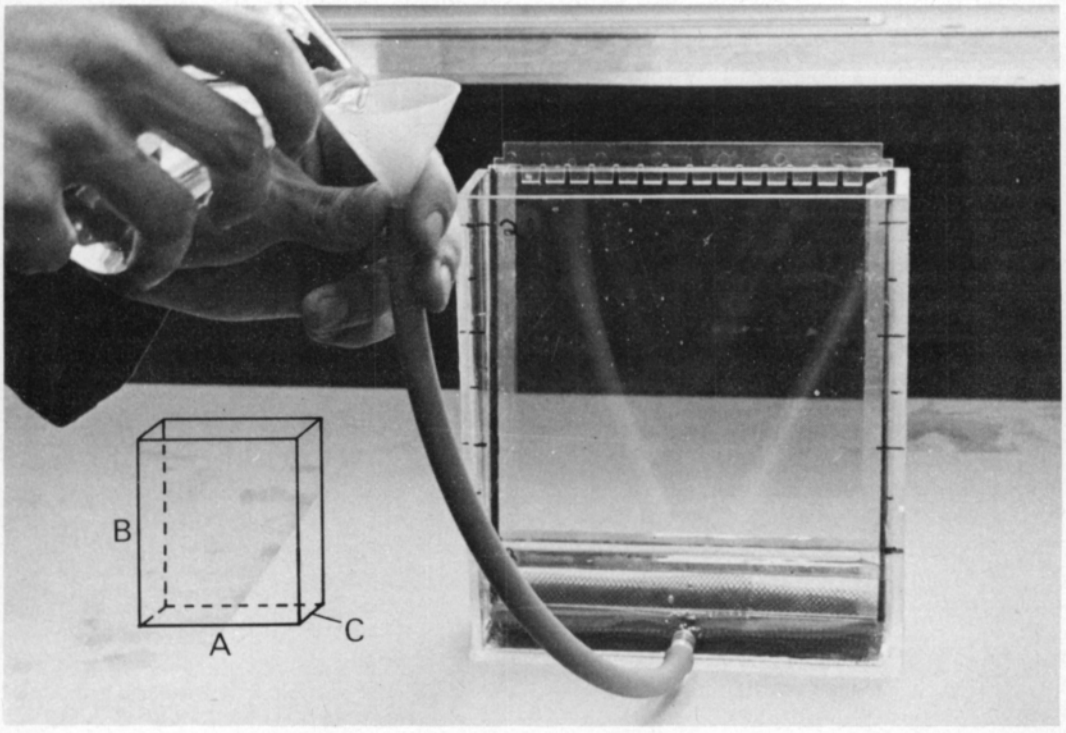

Fig. 1. Tray made of acryclic plastic (5 mm thick) for the preparation of gels. Tray dimensions a = 190 mm, b = 210 mm and c = 65 mm.

Identification

STEGEMANN and LOESCHCKE (1976) designated the four main protein bands in the cathodic part of the potato protein electrophoregram as A-, B-, C- and D-bands. By these 4 main bands it is possible to identify 9 different groups of potato cultivars (STEGEMANN and LOESCHCKE 1976). The final identification of a potato cultivar is based on its anodic protein band pattern and its esterase band pattern. As suggested and applied by MAIER and WAGNER (1981) we used the cultivar Bintje as an internal standard on each gel plate.

Determination of relative mobilities of protein bands

The mobilities and band positions of cathodic protein bands were determined from the photographic enlargement by measuring the distance from the origin (inner edge of sample slot) to the center of the band. The migration distance of a band divided by the migration distance of the reference band F (Fig. 2) equals the relative mobility of the band.

Reproducibility

Duplicate electrophoretic runs of the four replicate samples were performed to ensure visual similarity of the electrophoresis formula of the replicates. Reproducibility of the electrophoretic procedure used in this study was determined by the measuring relative mobilities of four main cathodic bands in the electrophoregram of a standard cultivar Bintje on 14 different gels. These data indicate that the relative band mobilities were highly reproducible from one gel to another, i.e., gel polymerization and electrophoresis are reproducible (Table 3).

Results and discussion

The electrophoretic patterns of the soluble proteins and esterases of the six Finnish potato cultivars studied are shown in Fig. 2 and 3. Based on a visual inspection of electrophoregrams the six cultivars could be divided in to three groups according to their four main cathodic bands of soluble proteins (Table 4). The cultivars Puikula and Pito were clearly differentiated from the other four cultivars by their main cathodic protein bands. In the nine-group systematics suggested by STEGEMANN and LOESCHCKE (1976) Puikula would go into group 5 and Pito into group 2, whereas the four other cultivars go into group 8.

In the anodic part of the gel of these four cultivars the position and number of protein bands differ from one another and therefore serve as characteristic fingerprints for their precise identification (Fig. 2). Two of these four potato cultivars, Hankkijan Tanu and Hankkijan Tuomas, have a common parent (Horsa) in their pedigrees (Table 1) which explains the similarity of their protein electrophoregrams. Although these close relatives have much of the same band pattern there are two specific anodic bands that can be used to

Table 3. An example of reproducibility of the electrophoregrams produced in the present study, expressed as relative mobilities (RM) of cathodic protein bands of the Bintje electrophoregram on 14 PA gels.

Cathodic band	A	B	C	D
n	14	14	14	14
RM	0.172	0.274	0.368	0.503
S	0.0037	0.0043	0.0048	0.0021
P = 0.95	$0.169 < \mu < 0.174$	$0.271 < \mu < 0.276$	$0.365 < \mu < 0.371$	$0.502 < \mu < 0.504$

S = Standard deviation
μ = Confidence interval

distinguish them from each other (Fig. 2).

Each cultivar possessed also a cultivar specific esterase electrophoregram (Fig. 3). The genetically closely related cultivars, Hankkijan Tanu and Hankkijan Tuomas, showed only a minute difference in their esterase patterns. Hankkijan Tuomas had an intense band in its esterase pattern (probably a double band) whereas Hankkijan Tanu had only a single band at the same position.

It was of special interest to compare the electrophoretic pattern of the cultivar Puikula with the pattern of the cultivar Mandel presented in the Index of European Potato Varieties by STEGEMANN and LOESCHCKE (1976). Mandel is a local cultivar which is grown in the Nordic countries. In Finland the names Puikula and Manteli (Mandel in Swedish) are used as synonyms of the same cultivar. In the comparison the cultivar Puikula was found to have much of the same protein and esterase patterns as Mandel. Figure 4 shows the protein and esterase electrophoregrams of these cultivars. Based on this data it would be necessary to make electrophoresis runs of Puikula and Mandel side by side on the same gel in oder to strengthen the hypothesis that these two local cultivars are actually representatives of one and the same cultivar.

The PAGE procedure used in the present study yielded reproducible protein separation and good resolution of protein bands for identifying purposes of Finnish potato cultivars. The six Finnish potato cultivars (Jaakko, Pito, Hankkijan Timo, Hankkijan Tuomas, Hankkijan Tanu and Puikula) could be

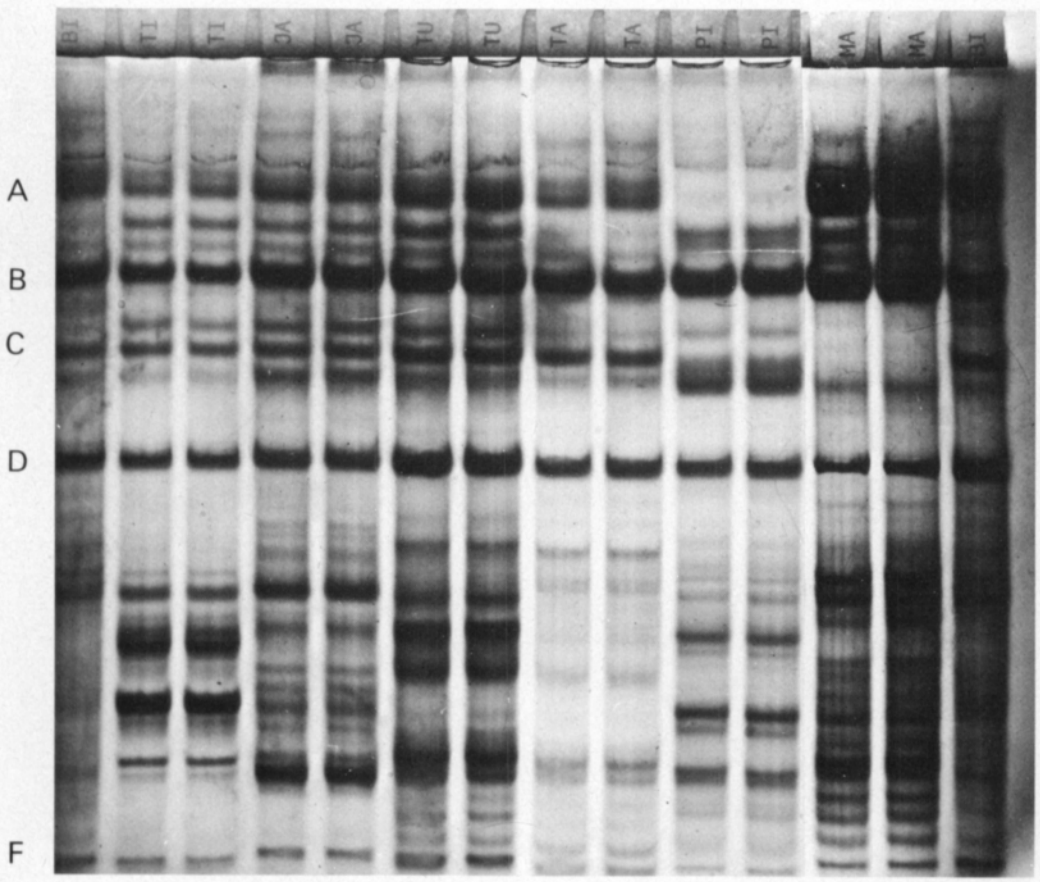

Fig. 2. Electrophoregrams of soluble proteins from six Finnish potato cultivars and cultivar Bintje. From left to right: Bintje, Hankkijan Timo, Hankkijan Timo, Jaakko, Jaakko, Hankkijan Tuomas, Hankkijan Tuomas, Hankkijan Tanu, Hankkijan Tanu, Pito, Pito, Puikula, Puikula and Bintje.

readily distinguished by their soluble protein and esterase PAGE patterns. However, two cultivars, Hankkijan Tanu and Hankkijan Tuomas, which are close relatives, possessed very similar soluble protein and esterase electrophoregrams. Special attention should be paid to the anodic bands of their soluble protein electrophoregrams when identifying either of these two cultivars.

The electrophoretic patterns of potato proteins and esterases are inherited independently and are stable for more than half a year when the tubers are stored at 4—10°C after harvest (STEGEMANN and SCHNICK 1982). The multiple forms of esterases change even less than the proteins during sprouting (STEGEMANN and SCHNICK 1982). Esterase electrophoresis is also a less time-consuming procedure than protein electrophoresis. For these reasons a determination from the esterase electrophoregrams would be useful prior to soluble protein electrophoresis.

Table 4. Grouping of six Finnish potato cultivars by applying the classification system suggested by STEGEMANN and LOESCHCKE (1976).

Cultivar	Cathodic band formula				Group
JAAKKO	A	B	C	D	8
PITO		B		D	2
PUIKULA	A	B			5
HANKKIJAN TANU	A	B	C	D	8
HANKKIJAN TIMO	A	B	C	D	8
HANKKIJAN TUOMAS	A	B	C	D	8

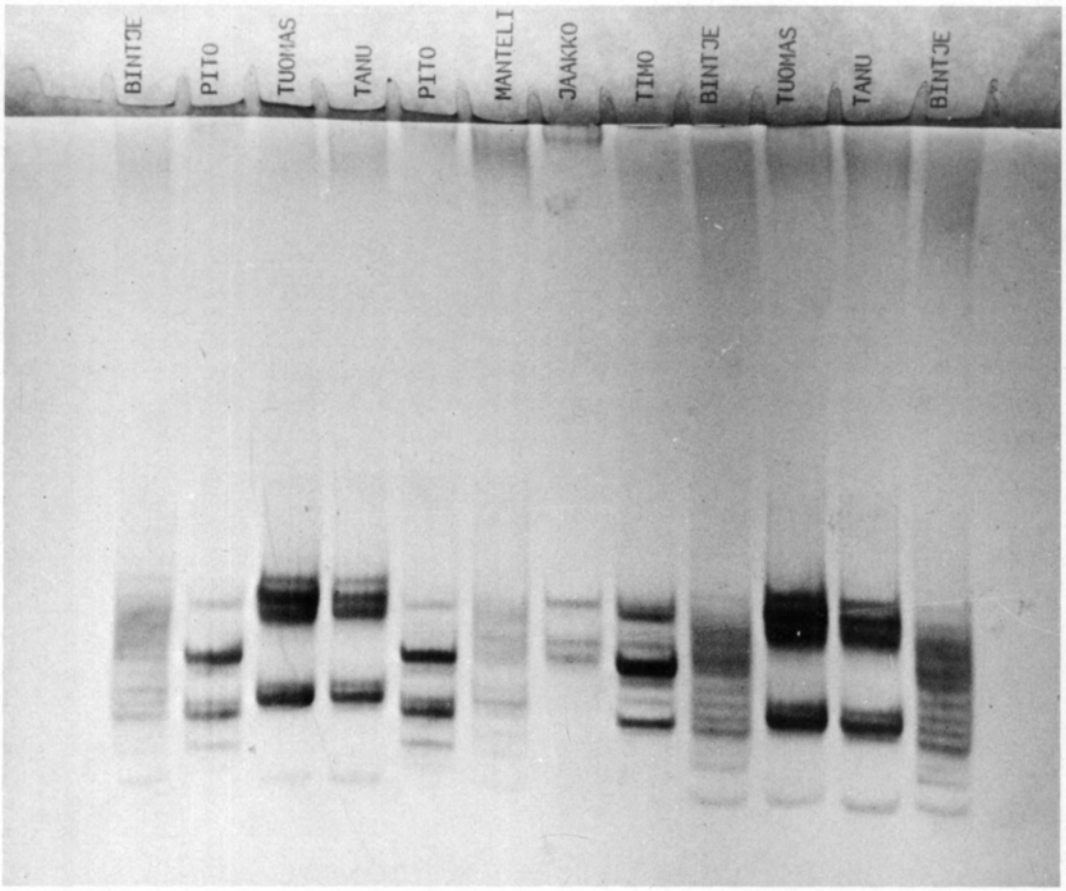

Fig. 3. Electrophoregrams of esterases from six Finnish potato cultivars and cultivar Bintje. From left to right: Bintje, Pito, Hankkijan Tuomas, Hankkijan Tanu, Pito, Puikula, Jaakko, Hankkijan Timo, Bintje, Hankkijan Tuomas, Hankkijan Tanu and Bintje.

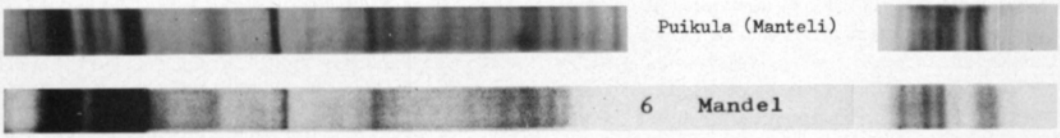

Fig. 4. Soluble protein and esterase electrophoregrams of Mandel (from STEGEMANN and LOESCSHKE 1976) and Puikula.

Conclusions

The procedure applied in this study can be used as a tool in the identification of questionable potato samples. The electrophoregrams given should be able to serve as reference in such work. However, one should bear in mind that in order to obtain more accurate proof of the identity of an unknown potato sample an electrophoretic analysis should also be performed with the unknown sample and an authentic reference sample side by side on a gel rather than only using the photographic presentation as references.

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SELOSTUS

Kuuden suomalaisen perunalajikkeen PAG-elektroforegrammit

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Perunalajikkeiden elektoforegrammeja käytetään hyväksi perunan tutkimus- ja tarkastustoiminnassa. Suomalaisista perunalajikkeista ei elektroforegrammiaineistoa kuitenkaan ole ollut käytettävissä.

Työn tarkoituksena oli määrittää yleisimpien suomalaisten perunalajikkeiden elektroforeettiset mallit eli elektroforegrammit.

Kuuden suomalaisen perunalajikkeen (Jaakko, Pito,

Hankkijan Timo, Hankkijan Tuomas, Hankkijan Tanu ja Puikula) liukoisten proteiinien ja esteraasien elektroforegrammit määritettiin polyakryyliamidigeelielektroforeesilla (PAGE).

Käytetyllä PAGE-menetelmällä proteiinien erotusmallien toistettavuus oli hyvä. Tutkituilla näytteillä oli spesifiset liukoisten proteiinien ja esteraasien PAGE-mallit, joita voidaan käyttää näiden lajikkeiden identifioinnissa. Kahden perimmältään lähekkäisen lajikkeen, Hankkijan Tanun ja Hankkijan Tuomaksen, elektroforegrammit olivat hyvin samantyyppiset. Näiden lajikkeiden elektroforegrammeista löytyi kuitenkin tietyt vyöhykkeet, joiden perusteella mallit voidaan erottaa toisistaan. Puikulan elektroforegrammi muistutti ruotsalaisen Mandel-lajikkeen kirjallisuudessa esitettyä elektroforegrammia. Tähän perustuen esitetään olettamus, että Puikula ja Mandel ovat saman lajikkeen edustajia.