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Characterisation of Potato (*Solanum tuberosum* L.) Varieties by Electrophoresis of Tuber Proteins

Světlana SÝKOROVÁ and Eva MATĚJOVÁ

Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

Abstract: The modified PAGE method (TRIS – Glycine buffer pH 8.9) was used for the characterisation of selected 25 registered potato varieties. This method enabled to identify each variety from the examined variety set. The calculation of identity indexes (proportion of common bands) helped to evaluate the similarity of varieties from these aspects. The examination of electrophoretic profiles of soluble tuber proteins, which are highly polymorphic and stable, can be considered as valuable for variety characterisation and identification.

Keywords: potato; variety; characterisation; tuber proteins; electrophoresis

It is generally known that the methods of potato variety identification based on morphological traits of plants and tubers are not precise because they are highly influenced by environmental conditions. For these purposes it is therefore reasonable to search for stable traits of tubers: among them soluble proteins, enzymes or their combinations were successfully used already in the sixties as genetic markers (Loeschcke & Stegemann 1966; Desborough & Peloquin 1968). Polyacrylamide or starch gel electrophoresis in alkaline or acid medium is a suitable and efficient method for the separation of these proteins. It is advantageous that the electrophoretic pattern of proteins does not change in tubers stored for several months in cold conditions and that they are consistent in potatoes from different localities and years (Desborough 1983). Šašek and Zelenka (1973) and Šašek (1974) described many Czechoslovak and foreign potato varieties on the basis of protein polymorphism after starch gel electrophoresis using the method published by Paulik (1957). Stegemann and Loeschcke (1977) worked out a catalogue of European potato varieties based on the variability of electrophoretic patterns of proteins, esterases, and peroxidases. Their methodical approach was also recommended by UPOV (International Union for the Protection of New Varieties of Plants) as the Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability and Checking Identity of Potato. The tubers should be mature, preferably harvested after senescence of foliage. Tubers stored at 4–10°C can be used regardless of the season as long as there is no or only light sprouting (UPOV 2002).

The objective of the paper was to demonstrate on a model set of selected registered potato varieties (most frequently grown in the CR) the possibility of the use of electrophoretic patterns of tuber proteins as a genetic criterion for the identification and characterisation of a variety.

MATERIAL AND METHODS

A set of 25 registered potato varieties was selected (Table 1). According to data of the Czech Central Institute for Supervising and Testing in Agriculture (CISTA) they took up at least 1% of the

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multiplication area in 2005. The included varieties represented all groups of earliness (7× very early, 6× early; 7× medium-early; 5× medium-late to late) and various usage (cooking) types (Catalogue of Czech Potato Varieties 2004). Ten varieties originated from Germany, nine from the Netherlands, five from the Czech Republic and one from Austria. Cultivar guaranteed tubers in the grade of propagation C1 were obtained from the Central Variety Testing Station of CISTA in Lípa near Havlíčkův Brod. Tubers were mature and healthy.

Modified polyacrylamide gel electrophoresis (PAGE) according to UPOV (2002) and Sýkorová *et al.* (2005) was used to obtain electrophoretic patterns of soluble tuber proteins (patatins). The modification of the above-mentioned methodology consisted in using the stock Tris-glycine tank buffer (0.039 mol/l of TRIS, and 0.047 mol/l of glycine; pH 8.9) directly for a run without any recommended twenty-fold dilution. OWL vertical dual slab gel system, power supply Desaga PS 600 and Desaga Frigostat were used for electrophoresis. Each gel well was filled with 7 µl of the sample, temperature during electrophoresis was kept at 5°C, and voltage was max. 300 V; migration distance was 6 cm. After run, staining and destaining the gels were dried between two layers of cellophane soaked in a 2% glycerol solution. The values of relative electrophoretic mobility (REM) were calculated for the particular bands, and electrophoretic patterns of the particular varieties were represented graphically. With the used methodology the intensity of band staining was not taken into account. To evaluate similarity of the patterns, the matrix of identity indexes (ii) was constructed for all pairs of varieties. Identity index (in %) for each variety pair was calculated as the proportion of common bands number to all bands number multiplied by 100. The greater the ii means, the greater the similarity of two varieties. The dendrogram of dissimilarity was created using the Statistica software.

RESULTS AND DISCUSSION

Electrophoretic patterns of examined varieties are shown in Figure 1. In total, 69 different protein bands were identified. The average number of protein bands detected per variety was 13. Only 8 protein bands were found in the pattern of Ramos while 18 in Velox variety (Figure 1). Relatively high polymorphism of tuber proteins indicated a possibility of their use for variety characterisation. Bárta *et al.* (2003) identified 12–20 clearly distinguishable bands of soluble protein profiles per variety in 18 registered potato varieties and Kormuťák *et al.* (1999) 10–14 bands per variety in 8 Slovakian potato cultivars.

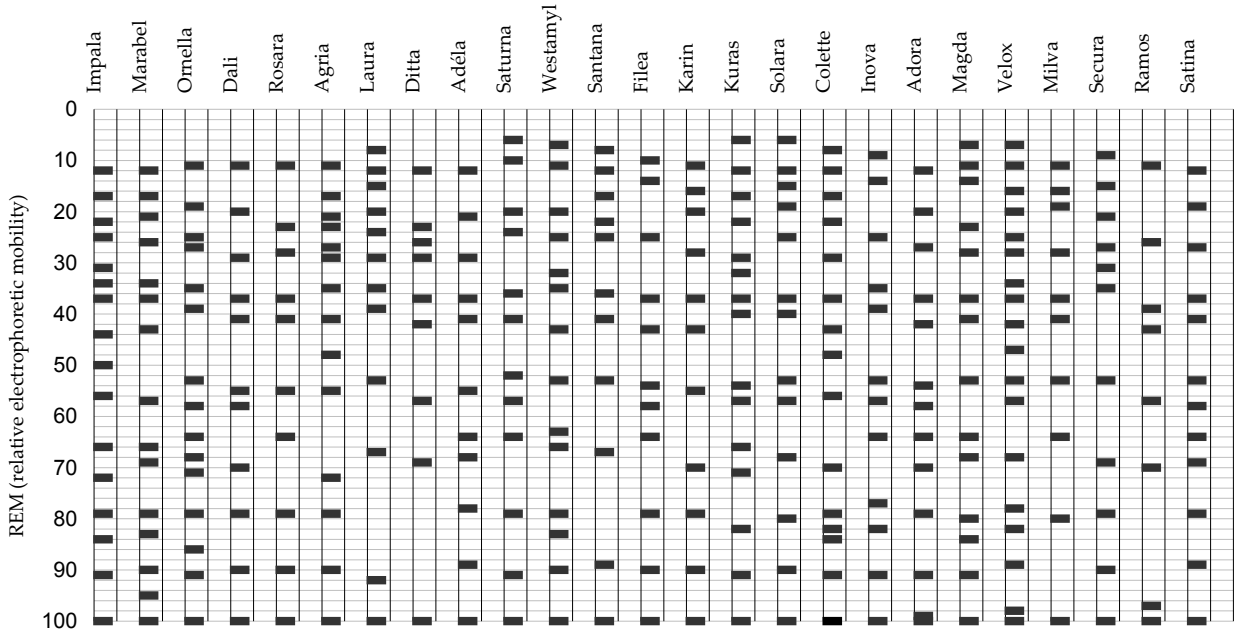

Figure 1. Electrophoretic patterns of tuber proteins (25 potato varieties)

Table 1. List of the examined potato varieties

Variety	Earliness*	Country of origin**	Cooking type, usage***
Impala	VE	NL	B
Marabel	E	DE	BA - B
Ornella	ML	CZ	mashed products, starch
Dali	E	NL	BA
Rosara	VE	DE	BA
Agria	ME	NL	B, French fries
Laura	ME	DE	B,BC, French fries
Ditta	ME	AU	AB
Adéla	E	CZ	B
Saturna	ML	NL	chips, starch
Westamyl	ML	CZ	starch
Santana	E	NL	C, French fries
Filea	ME	DE	BA
Karin	E	CZ	BA
Kuras	ML	NL	starch, unsuitable for consumption
Solara	ME	DE	B
Colette	VE	DE	BA
Inova	VE	NL	B
Adora	VE	NL	B,BC
Magda	VE	CZ	B, French fries
Velox	VE	DE	B, French fries
Milva	ME	DE	AB
Secura	ML	DE	B
Ramos	E	NL	BC, French fries
Satina	ME	DE	CB

*Earliness: VE – very early, E – early, ME – medium-early, ML – medium-late to late

**Country of origin: AU – Austria, CZ – Czech Republic, DE – Germany, NL – Netherlands; the varieties are listed in descending order according to the multiplication area in the Czech Republic in 2005

***Cooking type, usage according to CISTA: A – for salads, as a vegetable; B – for the preparation of dishes of all kinds, as a vegetable; C – mainly for the preparation of dough and mashed potatoes

The values of identity indexes (ii) were arranged into four groups 0–25%; 26–50%; 51–75%; 76–100% and the number of variety pairs belonging to each group was represented graphically (Figure 2). 300 values of ii ranged between 7 and 73%. The lowest ii value was found out in the pair Impala and Ramos and the highest ii value (that means the highest similarity) in the pair Karin and Dali. Most variety pairs (286) had ii lower than 50%, but no pair had ii higher than 75%, which implies a possibility of differentiating between varieties by this method.

The distinction of individual varieties based on protein electrophoretic profiles can be seen in a dendrogram of dissimilarity (Figure 3). However, we were not able to ascribe the similarity of elec-

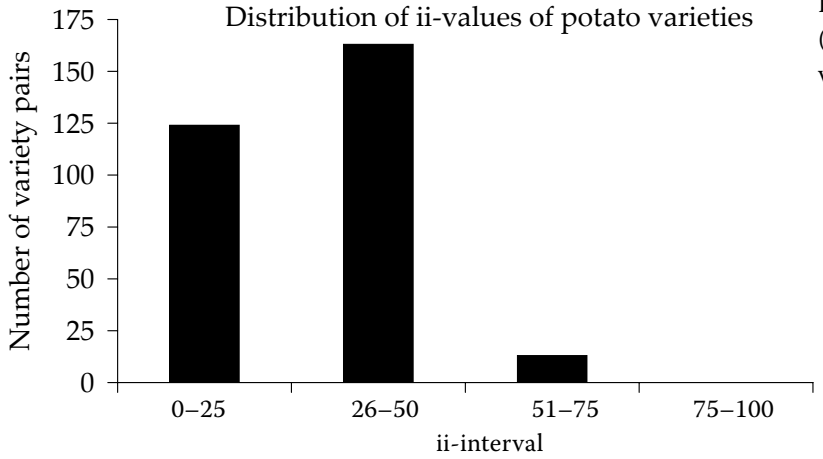

Figure 2. Distribution of identity indexes (ii) of tuber protein patterns of 25 potato varieties

trophoretic profiles e.g. to common country of origin, certain cooking type or earliness group.

Bárta *et al.* (2003) studied the biochemical variability between thirteen European and five Czech potato (*Solanum tuberosum* L.) cultivars grown in the Czech Republic and confirmed that cultivar differences in protein polymorphism could be revealed by applied electrophoretic patterns. Our results also document the suitability of PAGE method recommended by UPOV (2002) for the identification of potato varieties. In this method we have only modified the tank buffer using a stock solution without any dilution, which was necessary for achieving required conditions for an electrophoretic run (voltage, time of run). An advantage of the used method is the preparation of frozen extracts of tuber sap immediately after harvest and their gradual analyses. (The extracts

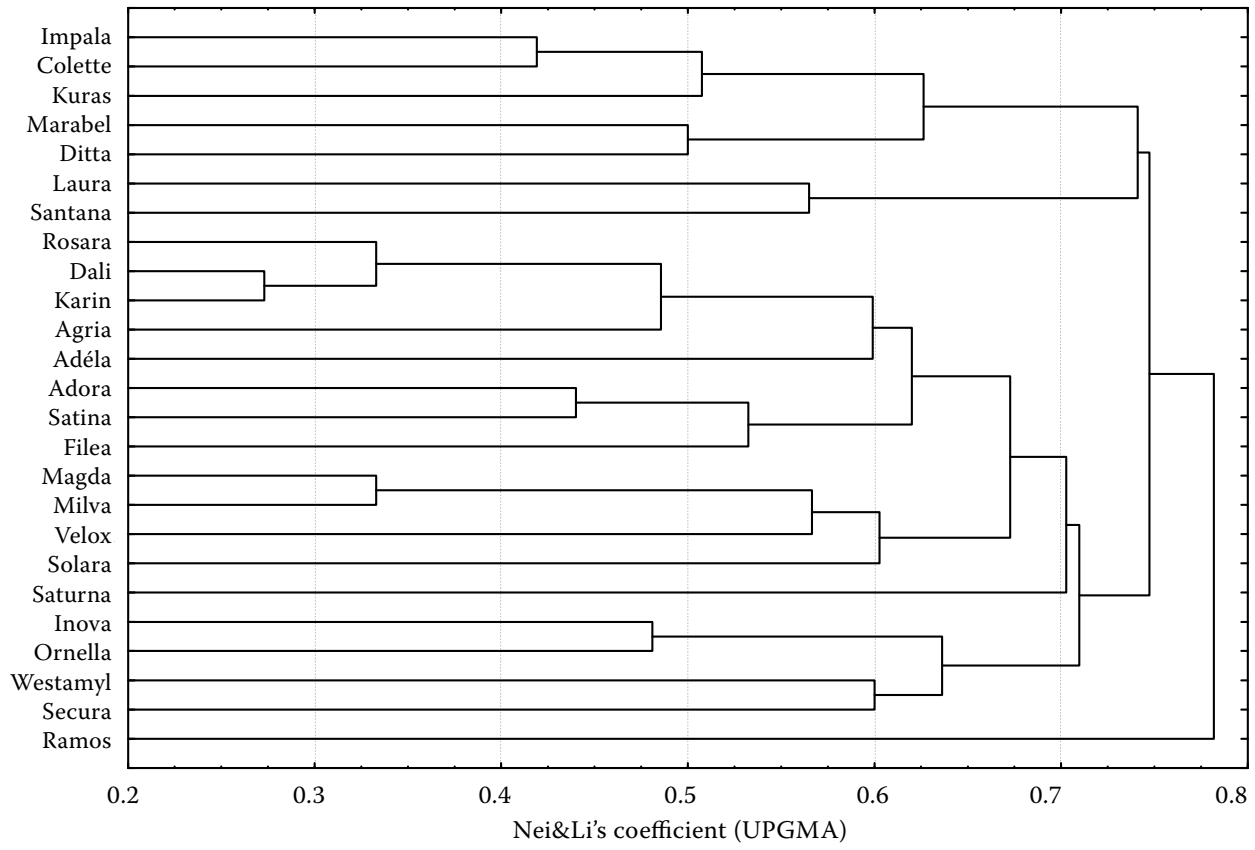

Figure 3. Dendrogram of dissimilarity of potato varieties; x-axis represents a measure of variety distinctness

could be stored in frozen condition for several months without any changes).

The methods of characterisation and verification of potato varieties by means of electrophoresis of tuber proteins can be appropriately applied both in breeding and in the control of variety trueness, e.g. in the marketing of ware potatoes.

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Corresponding author:

Mgr. Světlana Sýkorová, CSc., Výzkumný ústav rostlinné výroby, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 355; fax: + 420 233 310 636; e-mail: sykorova@vurv.cz