Study of biochemical variability of potato cultivars by soluble protein, isoesterase, and isoperoxidase electrophoretic patterns

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ABSTRACT

Biochemical variability between thirteen European and five Czech potato (*Solanum tuberosum* L.) cultivars grown in the Czech Republic was studied by soluble protein, isoesterase, and isoperoxidase electrophoretic patterns. It was confirmed that cultivar differences in protein polymorphism can be revealed by applied electrophoretic patterns. It was shown that the different character of protein and isozyme profiles required different approaches to their evaluation. For complex patterns such as electrophoretic soluble protein spectra, it is more convenient to use the evaluation of their absorbance profiles and for simpler profiles of isozymes the evaluation based on the presence or absence of a band in a definite position (simple matching) should be used. In spite of the complexity of tetraploid disposition of analysed cultivars, the results suggested higher similarity of profiles between relative cultivars and they also indicated the existence of higher similarity between cultivars from the same breeding firm.

Keywords: potatoes; cultivars; electrophoresis; proteins; isoesterases; isoperoxidases

Electrophoretic patterns of soluble proteins and isozymes have been used as a powerful tool for the study of genetic variability of *Solanum* species since the sixties of the last century. The profiles of tuber soluble proteins, esterases, and peroxidases were applied in the studies of relationship and plant genetics of cultivated and wild potato species and their hybrids (Desborough and Peloquin 1965, 1966, 1968, Simon and Peloquin 1980, Giovannini et al. 1993, Kormuťák et al. 1999).

After successful invocation of protein systems in the study of genetic variability at an inter-species level, they were applied to studies of cultivar differences. The protein and isozyme electrophoretic patterns give important results for the cultivar characterisation and for the purposes of identification and verification of cultivar authenticity in potato trading. In Europe, collections of European potato cultivars were discriminated by their protein and esterase patterns (Stegemann and Loeschke 1976, Cook 1995). Collection of some Finnish cultivars was characterised by esterase isozyme patterns (Sontag et al. 1985), collection of Spanish cultivars by peroxidase patterns (Nieto et al. 1990), etc. Electrophoretic profiles of tuber proteins and isozymes of esterases (EST), peroxidase (PER), phosphoglucoisomerase (PGI), glutamic-oxalacetic transaminase (GOT), alcohol dehydrogenase (ADH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGDH) were used for discrimination of North-American (Oliver and Martínez-Zapater 1985, Douches and Ludlam 1991) and South-American (Contreras and Mansilla 1989, Macias et al. 1989) potato cultivars. The profiles of tuber soluble proteins and isozymes have been recommended for the identification and certification of potato cultivars, cultivar verification and seed purity control for a long time (Oliver and Martínez-Zapater 1985, Burton 1989) above all for their simple performance (Cook 1999). On the other hand, there are some methodological problems. Above all, protein patterns have some disadvantages: difficult interpretation of complex profiles, effects of gene dosage – manifested as differences in band intensities (tetraploid origin of the majority of cultivars), variation in protein profiles caused by differences in maturity, growing technology and storage conditions (Cook 1999).

In 2002, 108 potato cultivars were registered in the Czech Republic, out of which over 70% are foreign cultivars. The aim of this study was to compare genetic variability between Czech and European potato cultivars in a model set of tuber protein, esterase and peroxidase profiles.

MATERIAL AND METHODS

The list of cultivars used in this study as well as their origin (country, breeder and parental combination) are given in Table 1. Plant material for analyses (cultivar guaranteed tubers in grade of propagation C1) was obtained from the Central Institute for Supervising and Testing in Agriculture in Brno. Tubers were mature and healthy, stored for four months at +4°C. A tuber sap from the whole longitudinal tuber profile was used for analyses. Five tubers (as five replications) were analysed in each potato cultivar for evaluation of non-genetic variability. Run sample was composed of 20 µl of centrifuged

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Table 1. The list of cultivars subjected to PAGE of soluble proteins, isoesterases and isoperoxidases

Country of origin	Cultivar	Numerical code	Breeder	Parental combination
A	Krystala	1	a	KEq 11/41 × Anosta
A	Karin	2	a	Rita × Hera
A	Krasa	3	a	KEq 66/7 × Anosta
A	Kordoba	4	a	KE 79/155 × LP 53
A	Amylex	5	b	Zvíkov × 126/11-67
B	Rosara	6	c	Secura × ESH 2605/77
B	Cinja	7	d	Berolina × Omega
B	Filea	8	d	Cinja × Stamm 77/330
B	Rosella	9	e	Agria × 1071/79/2793 L
B	Marabel	10	e	Nema × M 75-364
C	Asterix	11	f	Cardinal × Svp. VE 70-9
C	Monalisa	12	g	Bierma A 128 × Colmo
C	Saturna	13	h	Marita × (Record × 1673-1)
C	Santé	14	i	SVP Y 66-13-636 × SVP AM 66-42
C	Impala	15	j	52/72/2206 T × Biranco
B	Agria	16	e	Quarta × Semlo
D	Folva	17	k	Miranda × Maris Piper
E	Lenka	18	l	Bintje × Quarta

A – Czech Republic, B – Germany, C – Netherlands, D – Denmark, E – Austria
a – Sativa Keřkov, a.s.; b – Selekta Pacov, a.s.; c – SAKA-RAGIZ Pflanzenzucht GbR, Hamburk;
d – Nordkartoffel-Zuchtgeselshcaft mbH, Lüneburg; e – Kartoffelzucht Böhm KG, Lüneburg; f – De Z.P.C. BA, Leeuwarden;
g – F.G. v.d. Zee en Zonen, Z.P.C; h – E. en J. Scholten; i – J. Vegter; j – Agrico Research B.V., Emmerloord;
k – Landbrugets Kartoffelfond, Vandel; l – N.Ö. Saatbaugenossenschaft GmbH, Windigsteig (Anonym 1994, Med 2001)

tuber sap (10 000 g, 3 minutes) + 5 µl loading buffer (40% v/v glycerol, 0.01% w/v Bromophenol blue in distilled water). PAGE of proteins and isozymes was performed by standard cooled dual vertical slab units SE 600 (Hoefer Scientific Instruments, San Francisco, USA) under conditions of 0.025M Tris, 0.192M glycine (pH 8.3) buffer system. The discontinuous gel system was used – 4% stacking gel (0.125M Tris-HCl, pH 6.8) and 7.5% separating gel (0.375M Tris-HCl, pH 8.8). The ratio of acrylamide to bisacrylamide was 30:0.8.

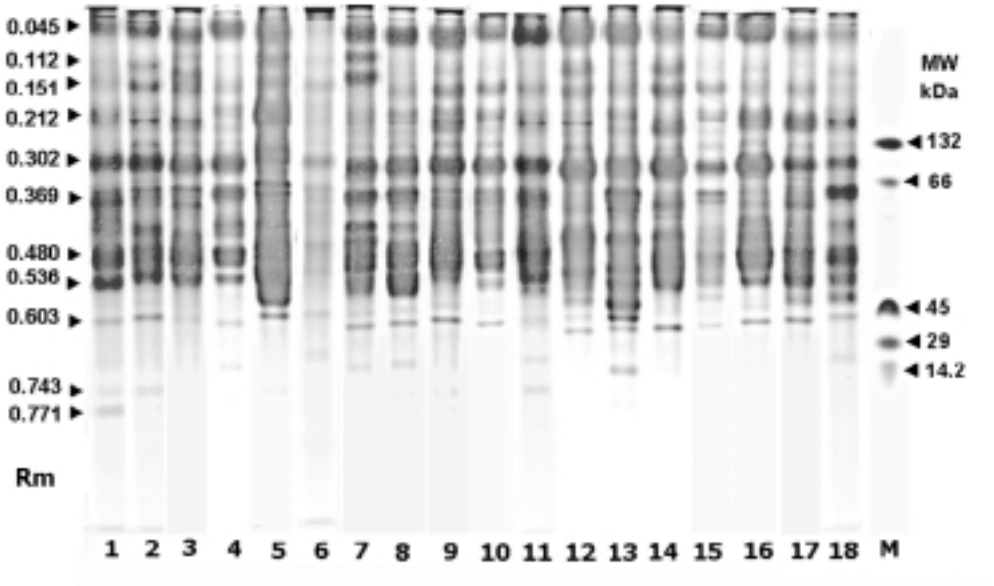

Figure 1. PAGE profiles of soluble tuber proteins of *S. tuberosum* cultivars (cultivar names are given in Table 1)
MW – molecular weight marker, Rm – relative mobility

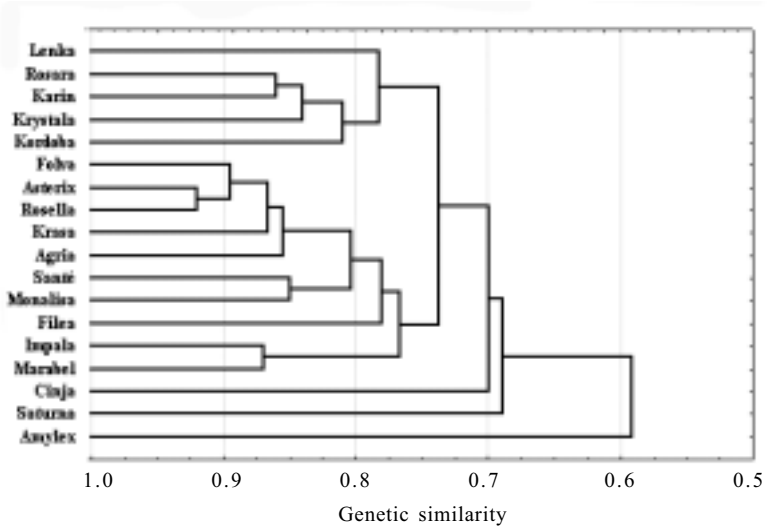

Figure 2a. Dendrogram based on PAGE profiles of soluble tuber proteins of *S. tuberosum* cultivars – evaluation A

Proteins were detected by staining the gels overnight in staining solution (25 ml solution I + 55 g trichloroacetic acid + 180 ml methanol, distilled water added to 1000 ml; solution I includes 250 mg Coomassie Brilliant Blue G and 750 mg Coomassie Brilliant Blue R-250 in 100 ml distilled water). Isoesterases were visualised by gel incubation (in dark, at a temperature 37°C) in 100 ml 0.1M Tris-HCl buffer, pH 7.2 containing 40 mg a-naphthyl acetate (dissolved in 5 ml acetone) and 100 mg Fast Blue RR Salt (Schenck and Wolf 1986). Peroxidases were detected by incubation of gels (in dark, at a temperature 30°C) in the solution of 50 ml 1M Na-acetate buffer (pH 4.7), 50 mg 3,3',5,5'-tetramethylbenzidine (TMBZ) dissolved in 50 ml methanol and 2 ml H_2O_2 (Vallejos 1983).

Electrophoretic data were processed by computerised image analysis (scanned gel record was used as input information for special software GELMANAGER FOR WINDOWS and BIOPROFIL 1D). Two ways of evaluation of genetic distances between the obtained profiles were used: A) correlation coefficient determination (Pearson's coefficient, Jackman 1994) and B) similarity coefficient determination (Nei and Li coefficient; Vilber Lourmat 1999). Similarity coefficients were processed by cluster analysis (UPGMA method, StatSoft, Inc. 2001).

RESULTS AND DISCUSSION

Each cultivar profile of protein or enzymatic pattern (Figures 1, 3 and 5) represents five replications from five analysed potato tubers. In all three analysed systems, non-genetic variability was not found between replicated profiles. Protein and enzymatic patterns of all tested cultivars were stable within the framework of replications.

In the range of analysed cultivars, 12–20 clearly distinguishable bands of soluble protein profiles were observed (Figure 1). On the contrary, Kormuťák et al. (1999) found 10–14 bands in soluble protein profiles of eight Slovakian potato cultivars analysed under similar conditions. The observed bands cannot be evaluated as equivalent because they have different thickness and staining intensities. It is difficult to evaluate soluble protein pro-

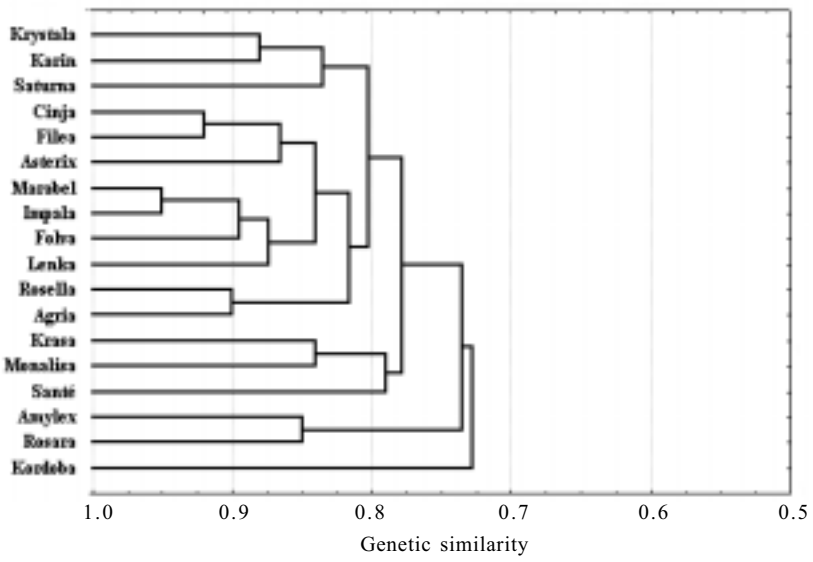

Figure 2b. Dendrogram based on PAGE profiles of soluble tuber proteins of *S. tuberosum* cultivars – evaluation B

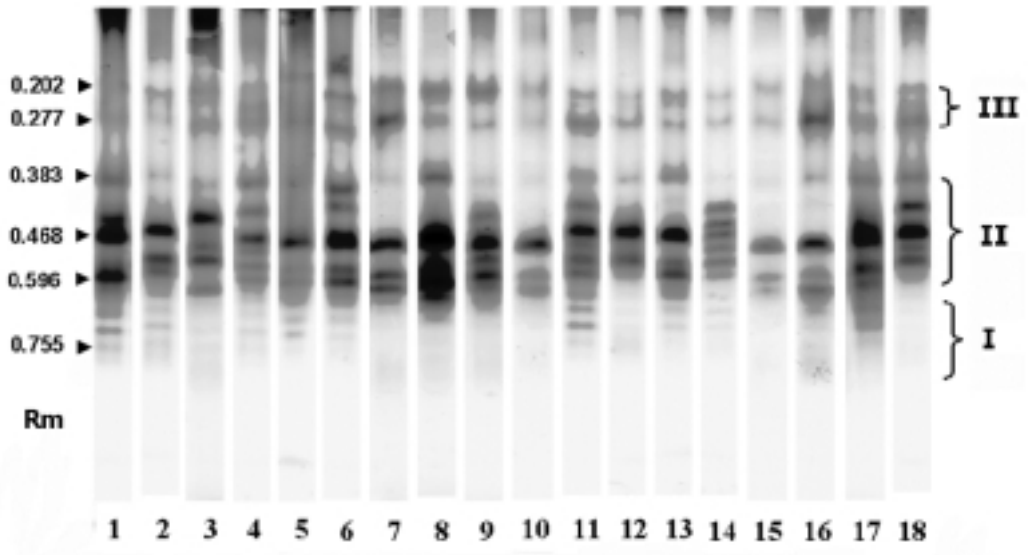

Figure 3. Isoperoxidase patterns of *S. tuberosum* cultivars (cultivar names are given in Table 1)

files due to their complex character. This observation confirms the results reported by Cook (1999). For this reason, a comparison of absorbance profiles by special software using Pearson's coefficient was chosen as a main evaluation (evaluation A). An evaluation based on the presence or absence of a band in a definite position was applied as alternative evaluation (evaluation B).

Two marked bands, invariable in the whole range of analysed cultivars (relative mobility [Rm] 0.045 and 0.302, respectively), were found in protein profiles of all tested cultivars. According to them, it was possible to divide the protein banding pattern into three regions. The first region included proteins bounded by the mentioned bands (high molecular weight proteins). The second region contained bands in a range of Rm 0.340–0.603. The third region was composed of weakly stained bands having the value of Rm higher than 0.603. This group of bands was not used for the evaluation of protein absorbance profiles. As shown in Figure 2a, cluster analysis revealed similarity in the range of 0.590–0.920. Two main clusters of cultivars were shown on a similarity level 0.750. The first cluster contained five cultivars (Lenka, Rosara, Karin, Krystala and Kordoba) and the second cluster included ten cultivars (Folva, Asterix, Rosella, Krasa, Agria, Santé, Monalisa, Filea, Impala and Marabel). The remaining three cultivars, especially Czech cultivar Amylex, had more different protein patterns. From the aspect of the country of origin and breeder, three Czech cultivars from the same breeder are in the first cluster, which could suggest some similarity in utilisation of genetic sources in potato breeding. It is interesting that the alternative evaluation of protein patterns (evaluation B, Figure 2b) showed high similarity between the profiles of more relative cultivars – Cinja and Filea (0.920). This result was not found in evaluation A. It could be explained by the fact that the greatest difference in the

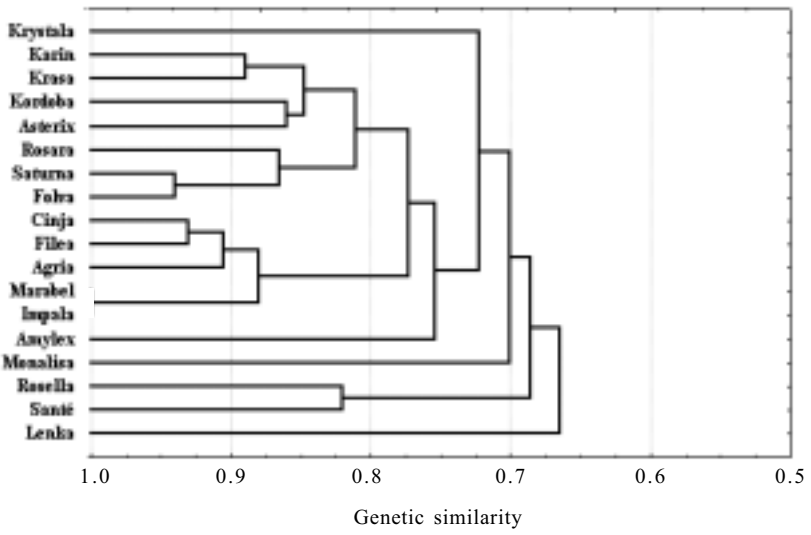

Figure 4. Dendrogram based on PAGE profiles of isoperoxidases of *S. tuberosum* cultivars – evaluation B

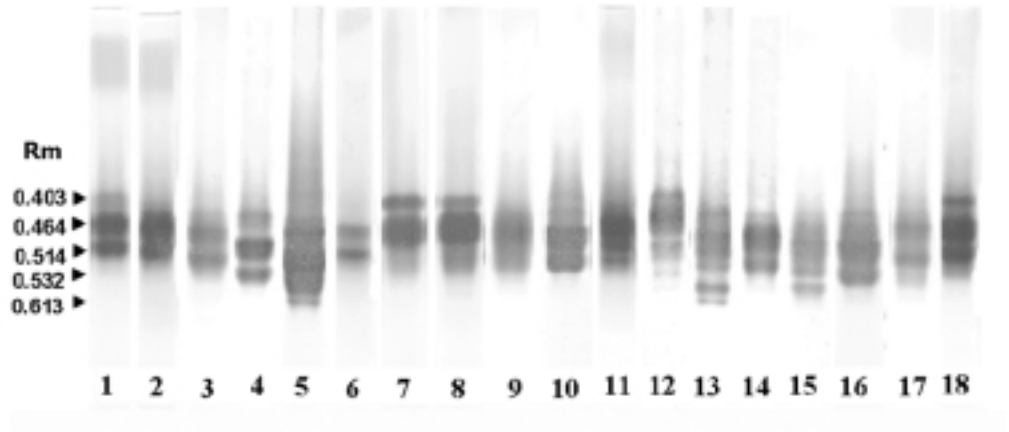

Figure 5. Isoesterase patterns of *S. tuberosum* cultivars (cultivar names are given in Table 1)

profiles of both cultivars is in the presence (Cinja) or absence (Filea) of two clear bands with Rm 0.112 and 0.151, respectively. The manifestation of the relationship between cultivars Rosella and Agria was similar (similarity 0.900). Very clear similarity was also observed between Dutch cultivar Impala and German cultivar Marabel; both evaluations showed high similarity (0.875 for A and 0.950 for B, respectively).

Although tuber peroxidases (considered to be a monomer enzyme, Oliver and Martínez-Zapater 1985) are an isozyme system, their patterns appear more complicated with complex character. According to Giovannini et al. (1993), the peroxidase pattern on polyacrylamide gel can be divided into three regions. Our peroxidase profiles had similar designs (Figure 3) even though we used gels with smaller pores (relative mobilities of bands of the mentioned regions were lower) and different hydrogenous donors for detection. However, Klisurska and Dencheva (1980) reported that hydrogenous donors TMBZ and O-dianisidin afford similar results. Region I included weakly staining bands with Rm in the range 0.600–0.760. It can be argued whether to use this region for pattern evaluation because the results could be invalidated under non-sensitive conditions of detection. However, we used this region for evaluation. Region II includes bands with Rm in the range 0.383–0.596. The band with Rm 0.383 is present in profiles of all tested cultivars. A banding region with decisive variability lies below this band. Region III is formed by two invariable bands with Rm 0.202 and 0.277, respectively. Other bands can be found toward the start but they are more difficult to detect with lower reliability. On the contrary, Giovannini et al. (1993) found a high level of polymorphism in region III in analysed wild *Solanum* species and their progenies.

Figure 4 shows relations between cultivars on a level of similarity in the range 0.660–1.000. Similarity is obviously higher between relative cultivars Cinja and Filea again, but this assumption was not confirmed in the relative pair Agria and Rosella. Peroxidase patterns of cultivars Marabel and Impala were evaluated as coincident.

Opinions of the profile character of esterase isozymes are different. Whereas Kormuťák et al. (1999) observed a relatively low number of isoesterases in a set of eight Slovakian potato cultivars, Douches and Lundlam (1991) evaluated the banding pattern created by EST-C locus in a set of 116 North American cultivars as very complicat-

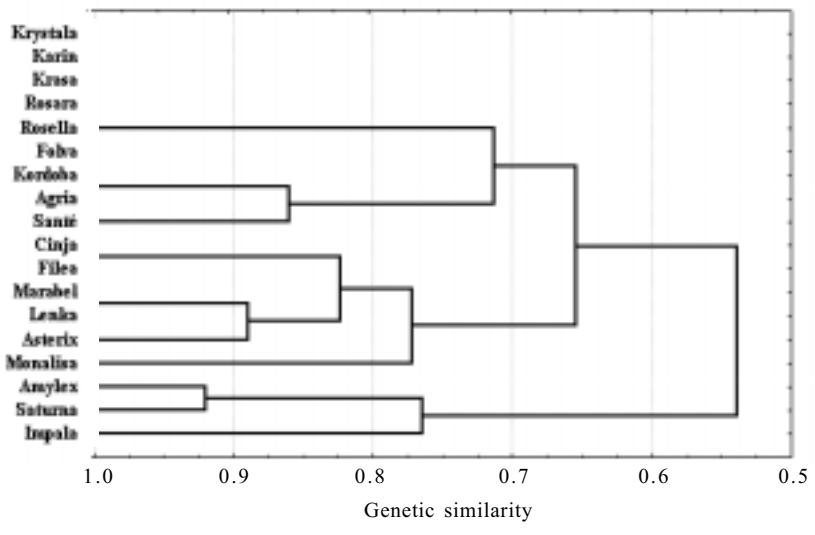

Figure 6. Dendrogram based on PAGE profiles of isoesterases of *S. tuberosum* cultivars – evaluation B

ed and difficult to interpret. In agreement with this opinion, Burton (1989) reported that esterases separated in polyacrylamide gel (pH 8.9) had a richer pattern. The bands of esterase activity are in a region with Rm from 0.403 up to 0.613 (Figure 5). Giovannini et al. (1993) and Kormuťák et al. (1999) reported the region occurrence for isoesterases on polyacrylamide gel in the range of Rm 0.600–0.900, however these authors used only 6% gel. Coefficients of similarity between the analysed cultivars were observed in the range from 0.540 up to 1.000 (Figure 6). Esterase patterns of cultivars Cinja and Filea were coincident as well as the patterns of cultivar pairs Kordoba, Agria, Marabel and Lenka, respectively. The relationship between Cinja and Filea (Cinja is the parent of Filea) can explain the high similarity of their electrophoretic profiles in used systems. The most abundant cluster including six cultivars (Krystala, Karin, Krasa, Rosara, Rosella and Folva) showed the appurtenance to the same electrophoretic phenotype, which is formed by two strong bands with Rm 0.464 and 0514, respectively. Three of these cultivars Krystala, Karin and Krasa are derived from the same breeder and cultivars Krystala and Krasa have the same parent (cultivar Anosta). On the contrary, the profiles of cultivars Amylex, Saturna and Impala differ from all cultivars and they can be evaluated as clearly dissimilar (level of similarity is only 0.540).

CONCLUSION

Based on the results, we can confirm that cultivar differences in protein polymorphism can be revealed by applied electrophoretic patterns. It is evident that the different character of protein and isozyme profiles requires different approaches to their evaluation. For complex patterns such as soluble protein patterns it is more convenient to use the complex evaluation of their absorbance profile (Jackman 1994) and for simpler profiles of isozymes the evaluation based on the presence or absence of a band in a definite position – simple matching (Oliver and Martínez-Zapater 1985) should be used. In spite of the complexity of tetraploid disposition of analysed cultivars, the results suggested higher similarity of profiles between relative cultivars and they also indicated the existence of higher similarity between cultivars from the same breeding firm.

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ABSTRAKT

Studium biochemické variability odrůd brambor pomocí elektroforetických spekter rozpustných bílkovin, izoesteráz a izoperoxidáz

Biochemická variabilita mezi třinácti evropskými a pěti českými odrůdami brambor (*Solanum tuberosum* L.) pěstovanými v ČR byla studována prostřednictvím elektroforetických spekter rozpustných bílkovin, izoesteráz a izoperoxidáz. Bylo potvrzeno, že rozdíly bílkovinného polymorfismu na odrůdové úrovni mohou být odhaleny elektroforetickými technikami. Ukázalo se, že odlišný charakter bílkovinných a izoenzymových profilů vyžaduje rozdílný přístup k jejich hodnocení. Pro komplexní spektra, jako je spektrum rozpustných bílkovin, je vhodnější hodnocení jejich absorbančních profilů a pro jednodušší profily izoenzymů hodnocení založené na přítomnosti nebo absenci pruhu v definované pozici. I přes složitost tetraploidního založení analyzovaných odrůd brambor naznačují výsledky vyšší podobnost profilů mezi příbuznými odrůdami. Rovněž byla nalezena vyšší podobnost mezi odrůdami, které pocházejí ze stejné šlechtitelské firmy.

Klíčová slova: brambory; odrůdy; elektroforéza; bílkoviny; izoesterázy; izoperoxidázy

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