CHARACTERISATION OF SELECTED DIPLOID GENETIC RESOURCES OF GENUS *SOLANUM* INTENDED FOR SOMATIC HYBRIDIZATION WITH POTATO DIHAPLOIDS

CHARAKTERIZACE VYBRANÝCH DIPLOIDNÍCH GENETICKÝCH ZDROJŮ RODU *SOLANUM* URČENÝCH PRO SOMATICKOU HYBRIDIZACI S DIHAPLOIDY BRAMBORU

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136 diploid genotypes of genus *Solanum* and diploid potato were selected and characterised as the potential resources of resistance genes against potato late blight. For a consideration of infectious tests by inoculation of leaf discs and *in vitro* plants, 15 highly resistant genotypes of wild species were selected; 6 of which showed no symptoms. In contrast, all 11 tested diploids of *Solanum tuberosum* were susceptible. Finally, 20 clones (10 clones of *Solanum bulbocastanum*, 1 clone of *Solanum verrucosum* 9 clones of diploid *Solanum tuberosum*) were selected and characterised in detail which were useful for somatic hybridisation by electrofusion. The protoplast yield ranged between 1×10^5 and 3.5×10^6 per ml from on average 325 mg of plant leaf material and it was variable within single repetitions of one genotype too. The positive correlation between yield and vitality of isolated protoplasts was detected, r = 0.476 (mesoscale dependence). The regeneration from cultivated protoplasts to the complete plant has been taken approximately in range 170 and 200 days. Whole 136 genotypes were then analysed by RAPD for the purpose of the differentiation of individual species. The series of OPN and OPG decamers were used from them were selected primers OPG 8, OPN 3, 5, 8, 11, 15 and 18. The statistical results evaluation of RAPD analysis was realised by UPGMA analysis by software Quantity One (Bio-Rad, USA). This analysis showed, that 9 genotypes primarily classified as *Solanum pinnatisectum*, showed totally different RAPD bands spectrums compared with reference clone *Solanum pinnatisectum* PI275235. According to the morphological comparison of plants in the greenhouse these 9 genotypes were identified as *Solanum polyadenium*. These results indicate the detailed characterisation requirement of European collections of genetic resources of genus *Solanum* introduced from gene banks in the USA.

Key words: potato, genetic resources, resistance against *Phytophthora infestans*, infectious tests, somatic hybridisation, RAPD.

Potato late blight is economically the most important and destroying potato disease. Bradshaw and Mackay (1994) describe genetic resources resistant to *Phytophthora infestans*. There were many of potato resistance major-genes to *Phytophthora infestans* described. To

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this group belong for example *S. berthaultii* – *Rpi-ber* gene (Rauscher et al. 2006), *S. bulbocastanum* – *Rpi-blb1* (Van der Vossen et al. 2003), *Rpi-blb2* (Van der Vossen et al. 2005), *Rpi-blb3* (Park et al. 2005a) and *Rpi-abpt* genes (Park et al. 2005b), *S. demissum* – *R1* – *R11* genes (Bradshaw and Mackay 1994), *S. phureja* – *Rpi-phu1* gene (Śliwka et al. 2006), *S. pinnatisectum* – *Rpi1* gene (Kuhl et al. 2001), *S. mochiquense* – *Rpi-moc1* gene (Smilde et al. 2005), *S. circaeifolium*, *S. microdontum*, *S. polyadenium*, *S. stoloniferum*, *S. tariense*, *S. tuberosum* subsp. *andigena*, *S. vernei* and *S. verrucosum*.

Prezygotic and postzygotic barriers of inter-specific hybrids production often embarrass or prevent resistance genes utilisation to the potato gene pool (Orczyk et al. 2003). It is possible to overcome these problems by means of somatic hybridisation (Helgelson 1992). Presently, there is a tendency to clone and transfer these resistance genes to the potato cultivars. However, this process is complicated in light of EU legislation and it is financially and technologically intensive. Most of the other important plant traits used in breeding are polygenic and presently isolated and characterised sequences for GMO development are not available. In contrast, protoplast fusion provides transfer of these polygene traits (Orczyk et al. 2003).

There are many options how to describe variability among genetic resources of genus *Solanum* by means of molecular methods of DNA polymorphisms detection. RAPD method (Random Amplified Polymorphic DNA) promises to be a more cost-effective diagnostic technology for plant breeding (Szczerbakowa et al. 2005). The use of short arbitrary primers for random amplification in studies of DNA polymorphism was first reported by Williams et al. (1990). The molecular characterisation of inter-specific and intra-specific somatic hybrids of potato by RAPD markers has already been reported (Szczerbakowa et al. 2005).

The objective of this study was to test the suitability of selected diploid genetic resources of genus *Solanum* to somatic hybridisation for the purpose of resistance increasing of potential somatic hybrids to *Phytophthora infestans*. The second objective was the description of DNA variability among genetic resources of genus *Solanum* and diploid potato by means of RAPD analysis for the purpose of potential somatic hybrids detection.

MATERIAL AND METHODS

Plant material

A total of 136 genetic resources of genus *Solanum* and diploid potato were obtained from the gene bank at the Potato Research Institute in Havlíčkův Brod Ltd. Selected genetic resources of genus *Solanum* are characterised as potential resources of potato resistance genes against potato late blight. They are diploid (2n = 24) and so optionally useful to the somatic hybridisation with diploid potato providing the creation of tetraploid source breeding material resistant to *Phytophthora infestans*.

This collection obtained 94 genotypes of *S. bulbocastanum* Dun. (PI243345, PI243512, PI275188, PI275192) from which 79 genotypes (PI243510) were declared as potential donors of resistance gene *Rpi-blb1* against *Phytophthora infestans*. Then 12 genotypes were evaluated; *S. berthaultii* Hawkes. (PI265857, PI265858, PI310925, PI265858, PI498109), singly genotype of *S. pinnatisectum* Bitt. (PI275235) and *S. microdontum* Bitt. (PI458356), 9 genotypes of *S. polyadenium* Greenm. (PI310963, PI320342), 3 genotypes of *S. vernei* Firbas and Ross. (BGRC 015451, BGRC 024732, 07S0300234), 4 genotypes of *S. verrucosum* Schlechtd. (PI161173) and 11 diploid genotypes *S. tuberosum* L. (DH 165, 185, 315, 318, 322, 323, 324, 329, 387, 388, 447).

Plants were cultivated *in vitro* in cultivation box SANYO on agar medium according to Murashige and Skoog (1962) on a photoperiod of 16 hours of light at a temperature of 25°C and 8 hours in darkness at a temperature of 18°C. Part of the collection was transferred to the greenhouse with the goal to obtain leaf discs and for morphological evaluation.

Phenotypical evaluation of resistance to Phytophthora infestans

Two types of infectious tests were used – inoculation of leaf discs in Petri dishes and plants *in vitro* (Fig. 1a, b). In the case of inoculation of leaf discs, four leaves in size approximately 30 × 15 mm were used. Three leaves were inoculated on the bottom side of the

leaves by two 25µl drops of sporangia suspension containing 20 000 sporangia per ml of *Phytophthora infestans* strain with complex virulence to *S. demissum* R genes (*R*1, *R*2, *R*3, *R*4, *R*6, *R*7, *R*8, *R*9, *R*10, *R*11). The *Phytophthora infestans* strain originated from Valečov, Czech Republic. The fourth leaf was used as a negative control. Leaves were turned after 24 hours. After the following two days, the manifestation of resistance or susceptibility in phenotype of plants was evaluated. The methods of inoculation of the whole *in vitro* plants consisted in the application of inoculum by spraying according to Horáčková et al. (2008). The *in vitro* plants were evaluated on the fourth day too. Genotypes were evaluated as resistant (0–25% injury of leaf discs area), medium resistant (25.1–50%) and susceptible (50.1–100% or mycelium sporulation).

Usability of selected genotypes for production of somatic hybrids

Selected resistant genotypes of *Solanum* species and diploid potatoes were evaluated to estimate the usability for production of somatic hybrids by electro fusion (Fig. 1c). Protoplasts were isolated by the modified method according to Cheng and Saunders (1995) and Carlberg et al. (1987). Yield and viability of protoplasts were determined in Bürker cell. 10 ml of 0.25% methylene blue was given, dissolved in 0.47 M mannitol solution to the 0.5 ml of protoplast suspension in mannitol solution in the same molarity. Regression and correlation relations of viability in dependence on yield of protoplasts were evaluated by Statistica CZ 2007.

Furthermore, regeneration protoplast cultures ability was evaluated. The cultivation proceeded on the media according to Cheng and Saunders (1995). The ability and rate of cell wall regeneration, mitosis and calluses generation as far as regeneration to the whole plant were observed.

DNA analysis

DNA was isolated by means of the DNeasy Plant Mini Kit (Qiagen, GER). 12.5 ml of PCR reaction mixture contained 1x reaction buffer, $MgCl_2$ in 2.5 mM concentration, dNTP in 0.3 mM concentration, 15 ng of primer, 0.5 unit of *Taq* polymerase (Fermentas, Lithuania) and 10 ng of DNA. The time and temperature profile of the reaction was as follows: 180 s at 94°C for the first denaturation followed by 40 PCR cycles (20 s at 94°C for denaturation, 45 s at 36.5°C for primer annealing, 105 s at 72°C for extension) and 360 s at 72°C for final extension. There were tested two sets of decameric primers, OPG and OPN. RAPD polymorphisms were then evaluated by means of decamers OPG 8, OPN 3, 5, 8, 11, 15 and 18.

The separation of amplification products proceeded on the 1.5% horizontal agarose gel visualised by ethidiumbromide in concentration 0.5 mg per ml of electrophoretic buffer. Electrophoreograms were by GelDoc™XR (Bio-Rad, USA) documented. Statistical assessment of similarity among RAPD profiles was realised by bulk analysis UPGMA (unweighted pair group method with arithmetic mean) by computer programme Quantity One (Bio-Rad, USA).

RESULTS AND DISCUSSION

Phenotypical evaluation of resistance to Phytophthora infestans

The evaluation of the collection of 136 genotypes showed a high variability in the reaction to the presence of *Phytophthora infestans*.

100% susceptibility was detected and evaluated by means of the leaf discs inoculation method in the collection of all 11 diploid genotypes of *S. tuberosum*. A high susceptibility showed also in all genotypes of *S. polyadenium*

Table 1

Statistical evaluation of infectious tests of 66 genotypes *Solanum bulbocastanum*

	High and moderate resistance	Susceptible genotype
Inoculation of leaf discs	49	17
Inoculation of *in vitro* plants	53	13
Correlation coefficient	R = 0,5465	
Association intensity	Q = 0,8432	

(99.5% injury of leaf discs area) and *S. vernei* 068 (57%). Medium resistant was for example *S. microdontum* (27%). Resistant were *S. verrucosum* (10%) and most of *S. berthaultii* clones. *S. berthaultii* 260 showed no symptoms of disease.

Results of evaluation in the collection of 66 clones of *S. bulbocastanum* (PI243510) showed a very high variability in the range of necroses of leave discs or plants *in vitro*. Nevertheless, none of the tested leaf discs facilitated development of *P. infestans* and its sporulation. In our opinion the leaf injuries were in many cases caused by intensive hypersensitive reaction of plant tissues. Four of clones, SB PIS 17, 23, 59 and 70, didn't show any symptoms of disease. In our mind it was a very good signal of potential immunity or optimal gene background of the *Rpi-blb1* gene. But we anticipated well usable donors of the gene in higher numbers in this collection. Now, the injury of leaf discs area in cause of hypersensitive reaction ranged between 2% and 96%. It is possible to claim that all of the clones are resistant, but the majority of them is not usable for the utilisation in potato breeding, or somatic hybridisation, because a large range of necroses rapidly reduces photosynthetic activity and subsequently decreasing of plant yield efficiency. According to these results, we used in the next experiments only *S. bulbocastanum* clones that had showed injury of leaf area to the limit of 25%.

We statistically compared the results of both methods used (method of leaf discs and *in vitro* inoculation) by means of non-parametric regression. The results of the evaluation are presented in Table 1. The results show, that methods are partially comparable, but in 11% of cases was estimated the "fatal error", when resistant genotype was sorted by the second method to group of susceptible plants and conversely. The factors causing the error can have its nature in actual physiological status of tested plants in very different environments, because results of single methods were very well reproducible.

Usability of selected genotypes for production of somatic hybrids

15 resistant genetic resources and 11 diploid potato presented in Table 2 that are potentially useful material for production of somatic hybrids were selected from 136 genotypes of described

Table 2

Genotypes selected for somatic hybridisation by means of infectious tests

Genotype	Injury of leaf discs [%]	Resistance level (leaf discs)	Resistance level (*in vitro* plants)	Correspondence	Genotype	Injury of leaf discs [%]	Resistance level (leaf discs)
SB PIS 17[a]	0.00	R	R	YES	S. BER 260	0.00	R
SB PIS 23	0.00	R	R	YES	S. VERU 299[a]	9.00	R
SB PIS 40[a]	2.20	R	R	YES	DH 165[a]	100.00	S
SB PIS 41[a]	0.00	R	M	NO	DH 185[a]	100.00	S
SB PIS 47[a]	19.00	R	R	YES	DH 315[a]	100.00	S
SB PIS 59	0.00	R	M	NO	DH 318[a]	100.00	S
SB PIS 60[a]	8.33	R	R	YES	DH 322[a]	100.00	S
SB PIS 61[a]	47.00	M	M	YES	DH 323	100.00	S
SB PIS 66[a]	13.33	R	R	YES	DH 324[a]	100.00	S
SB PIS 70[a]	0.00	R	R	YES	DH 329[a]	100.00	S
SB PIS 71[a]	23.00	R	R	YES	DH 387[a]	100.00	S
SB PIS 73[a]	6.67	R	R	YES	DH 388[a]	100.00	S
SB PL 14	0.00	R	no value	no value	DH 447	100.00	S

SB PIS / PL – *S. bulbocastanum*, S. BER – *S. berthaultii*, S. VERU – *S. verrucosum*, DH – diploids of *S. tuberosum*, R – resistant, M – medium resistant, S – susceptible

[a] successful cultivation of protoplasts

collection. From approximately 325 mg of plant leaf material were isolated protoplasts. The protoplasts yield ranged between 1 × 10^5 and 3.5 × 10^6 per ml from on the average in cause

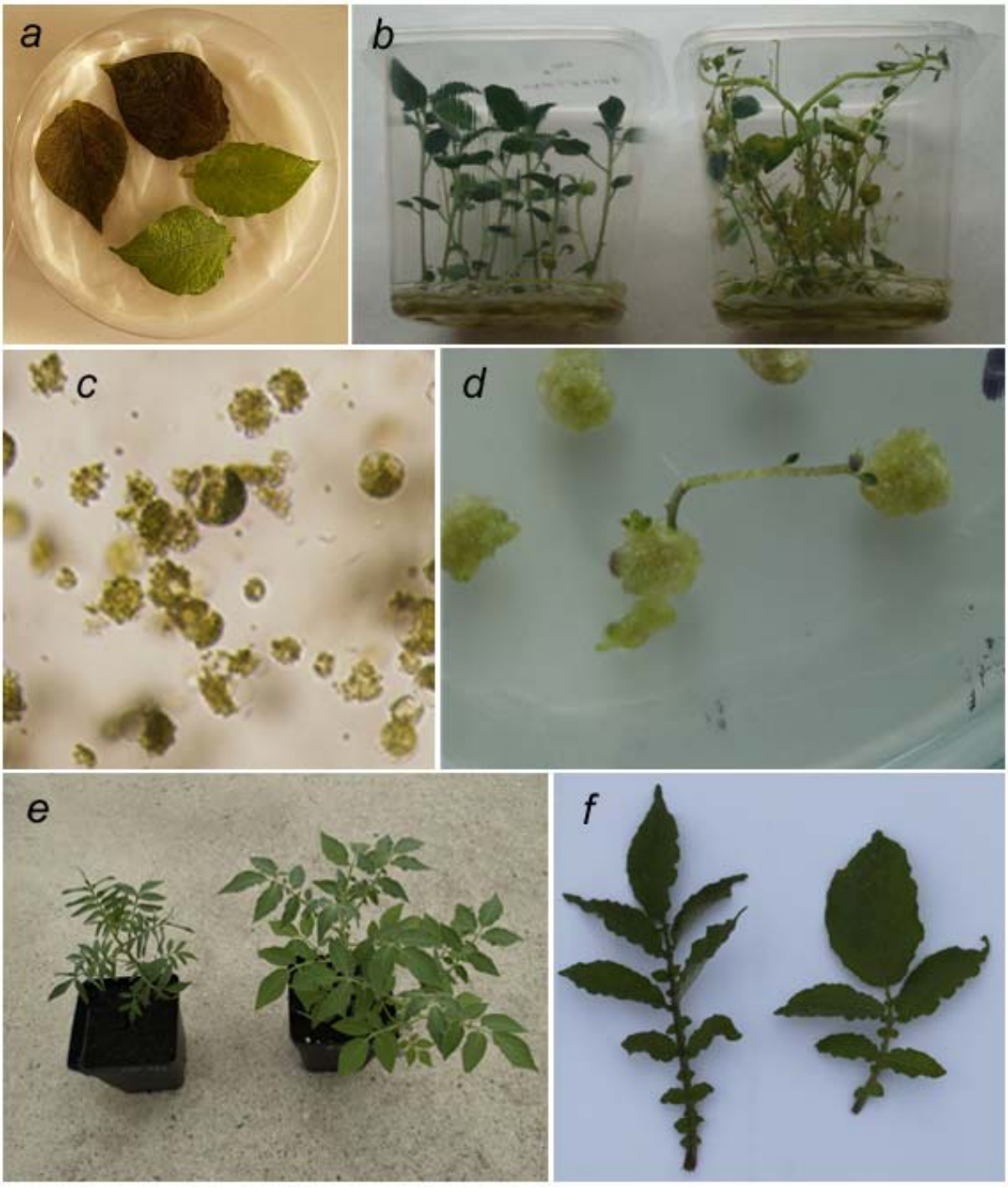

Fig. 1. a – Leaf discs damaged by *Phytophthora infestans*; b – *in vitro* plants damaged by *Phytophthora infestans*; c – fused protoplast; d – callus regeneration; e – comparison of *S. pinnatisectum* PI275235 (left) and *S. polyadenium* PI310963 (right); f – comparison of *S. vernei* BGRC 015451 (left) and 07S0300234 (right) leaves morphology

of variable amount of plant material in single cases of isolation. These results coincide with the results of Greplová and Polzerová (2007). The protoplasts yields were not usually well reproducible in the frame of one genotype. It was discovered that the positive correlation (r = 0.476) in dependence of vitality on the yield of isolated protoplasts. The value of correlation coefficient indicates well that the viability is not only dependent on the quantity of protoplasts in the sample, but also processes of protoplasts extraction from plant tissues or actual physiological status of plants influence its.

The cell wall was usually regenerated during 2–3 days. The cells went through first mitosis approximately to 8–12 days from the beginning of cultivation as well as in experiments by Fish et al. (1988), while Mattheij and Puite (1992) allege the start of first mitosis after 3 days of cultivation. Differences in time length can be probably caused by differences in used genotypes or cultivation conditions. Approximately after 60 to 90 days of cultivation calluses reached the size of 2 mm in diameter. On the first of these calluses regeneration of shoots started after 150 days (Fig. 1d). According to Cooper - Bland et al. (1996) the regeneration from isolated protoplast to the whole plant takes 4 to 5 month (150 days). In our case the time in reaching of the first regen-

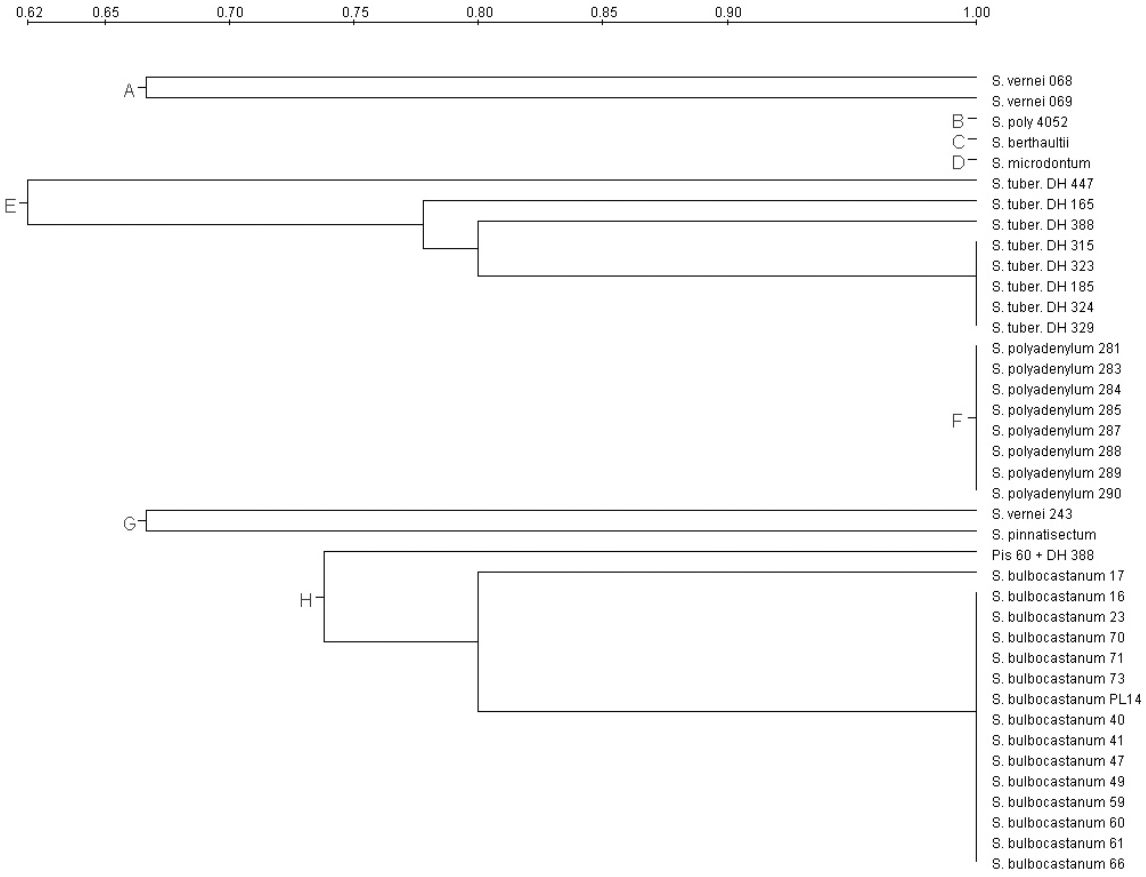

Fig. 2. Result of UPGMA analysis for primer OPN 11
PIS 60 + DH 388 – hypothetic somatic hybrid originated from mixture of individual isolated DNA of potential parents

erating plant was 2 to 3 month longer (170–200 days). Our results concur with the results by Greplová and Polzerová (2007).

DNA analysis

On the basis of DNA analysis by means of RAPD primer OPN 11 several species of genus *Solanum* were reliably differentiated. Therefore, this primer is showed as acceptable for the differentiation of potential inter-specific somatic hybrids. Results were statistically compiled to the cladogram form (Fig. 2). Other primers used for purpose of detection of interspecific variability were decamers OPG 8, OPN 3, 5, 8, 15 and 18.

These analyses resulted, that 9 genotypes primarily classified as *S. pinnatisectum*, showed totally different RAPD bands spectrums compared with reference clone *S. pinnatisectum* PI275235. According to the morphological comparison of plants in greenhouse (Fig. 1e) and their confrontation with literature (Correll 1962) these 9 genotypes were identified as *S. polyadenium*. These RAPD analyses resulted also differences between *S. vernei* 07S0300234 and second two genotypes BGRC 015451 and BGRC 024732. Comparison of their leaves is shown in Figure 1f. The error rate in the identification of genotypes species classification is not rare. Similarly, for example, Dinu et al. (2005) showed *S. bulbocastanum* with identification code (accession number) belonging to *S. polyadenium*. These results indicate the requirement of a detailed characterisation of European collections of genetic resources of genus *Solanum*, which were introduced from gene banks in the USA.

CONCLUSION

Applied methods were showed as acceptable for the selection of plant material intended for potato somatic hybridisation with the view of resistance increasing to *Phytophthora infestans*. These methods are applicable generally in genus *Solanum*.

Six genotypes (*S. berthaultii* 260 (PI265858) and *S. bulbocastanum* 17, 23, 59, 70 and PL 14) showed no symptoms of disease, whereas all diploid potatoes showed 100% susceptibility during the phenotypical evaluation of resistance to *Phytophthora infestans*.

The other 63 clones of *S. bulbocastanum* with declared presence of resistance gene *Rpi-blb1* corresponded to disease attack by the highly variable hypersensitive reaction.

Results of infectious tests could be especially tasked with incidence of cultivation conditions.

Finally 20 clones of genus *Solanum* were selected that were suitable for the developing of somatic hybrids highly resistant to *Phytophthora infestans*.

RAPD method is acceptable for the preliminary detection of potato somatic hybrids in the case of described conditions meeting.

RAPD analyses led to the discovering of some mistakes in gene bank collection. Consequently 9 genotypes primary classified as *S. pinnatisectum* (PI275235) were reclassified as *S. polyadenium*.

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REFERENCES

BRADSHAW, J.E. – MACKAY, G.R. (1994): Potato genetics. Cambridge: CAB International, 1994, 552 p.

CARLBERG, I. – KARLSSON, S. – ERIKSSON, T. (1987): Improved culture techniques for potato protoplasts. In: Bajaj, Y.P.S.: Biotechnology in Agriculture and Forestry 3, Potato. Berlin Heidelberg: Springer-Verlag, 1987, pp. 187–194.

COOPER-BLAND, S. – DE MAINE, M.J. – STEWART, H.E. – FLEMING, M.L.M.H. – PHILLIPS, M.S. – KUMAR, A. (1996): Intraspecific somatic and sexual hybridisation between dihaploid lines of *Solanum tuberosum* L.: evaluation of morphological traits and resistance to late blight *Phytophthora infestans* (Mont.) de Bary in the foliage. In: Euphytica, vol. *90*, 1996, N. 2, pp. 209–216.

CORRELL, D.S. (1962): The potato and its wild relatives. Texas: Texas research foundation Renner, 1962, p. 606.

DINU, I.I. – HAYES, R.J. – KYNAST, R.G. – PHILLIPS, R.L. – THILL, C.A. (2005): Novel inter-series hybrids in *Solanum*, section Petota. In: Theor. Appl. Genet., vol. *110*, 2005, N. 3, pp. 403–415.

FISH, N. – KARP, A. – JONES, M.G.K. (1988): Production of somatic hybrids by electrofusion in *Solanum*. In: Theor. Appl. Genet., vol. *76*, 1988, N. 2, pp. 260–266.

GREPLOVÁ, M. – POLZEROVÁ, H. (2007): Shortening of time of the plant regeneration from protoplast-derived calli in genus *Solanum*. In: Scientific studies – 15, Havlíčkův Brod: Potato research institute, 2007, pp. 61–69.

HELGELSON, J.P. (1992): New genes for disease resistances through somatic hybridization. In: Eur. J. Plant Pathol., vol. *98*, 1992, N. 2, pp. 223–229.

HORÁČKOVÁ, V. – DOMKÁŘOVÁ, J. – KREUZ, L. (2008): Metodika biologického testu *in vitro* pro účely selekce náchylných materiálů vůči plísni bramboru (*Phytophthora infestans* Mont de Bary) (Methodology of *in vitro* biology test for purpose of selection of susceptible materials against potato late blight (*Phytophthora infestans* Mont de Bary). In: Metodické postupy využitelné ve šlechtění (Methodically procedures available in breeding). Havlíčkův Brod: Potato research institute, 2008, pp. 27–30.

CHENG, J. – SAUNDERS, J.A. (1995): Protoplast electrofusion and regeneration in potato. In: Methods in Molecular Biology: Plant Cell Electroporation and Electrofusion Protocols, vol. *55*, 1995, N. 1, pp. 181–188.

KUHL, J.C. – HANNEMAN JR., R.E. – HAVEY, M.J. (2001): Characterization and mapping of *Rpi1*, a late-blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. In: Mol. Genet. Genomics, vol. *265*, 2001, N. 6, pp. 977–985.

MATTHEIJ, W.M. – PUITE, K.J. (1992): Tetraploid potato hybrids through protoplast fusions and analysis of their performance in the field. In: Theor. Appl. Genet., vol. *83*, 1992, N. 6–7, pp. 807–812.

MURASHIGE, T. – SKOOG, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. In: Physiol. Plant., vol. *15*, 1962, N. 3, pp. 473–497.

ORCZYK, W. – PRZETAKIEWICZ, J. – NADOLSKA-ORCZYK, A. (2003): Somatic hybrids of *Solanum tuberosum* – application to genetics and breeding. In: Plant Cell. Tissue Organ Cult., vol. *74*, 2003, N. 1, pp. 1–13.

PARK, T.H. – GROSS, J. – SIKKEMA A. – VLEESHOUWERS, V.G.A.A. – MUSKENS, M. – ALLEFS, S. – JACOBSEN, E. – VISSER, R.G.F. – VAN DER VOSSEN, E.A.G.B (2005a): The late blight resistance locus *Rpi-blb3* from *Solanum bulbocastanum* belongs to a major late blight *R* gene cluster on chromosome 4 of potato. In: Mol. Plant Microbe Interact., vol *18*, 2005a, N. 7, pp. 722–729.

PARK, T.H. – VLEESHOUWERS, V.G.A.A. – HUTTEN, R.C.B. – VAN ECK, H.J. – VAN DER VOSSEN, E.A.G. – JACOBSEN, E. – VISSER, R.G.F. (2005b): High resolution mapping and analysis of the resistance locus *Rpi-abpt* against *Phytophthora infestans* in potato. In: Mol. Breed., vol. *16*, 2005b, N. 1, pp. 33–43.

RAUSCHER, G.M. – SMART, D.C. – SIMKO, I. – BONIERBALE, M. – MAYTON, H. – GREENLAND, A. – FRY, W.E. (2006): Characterisation and mapping of *Rpi-ber*, a novel potato late blight resistance gene from *Solanum berthaultii*. In: Theor. Appl. Genet., vol. *112*, 2006, N. 4, pp. 674–687.

ŚLIWKA, J. – JAKUCZUN, H. – LEBECKA, R. – MARCZEWSKI, W. – GEBHARDT, C. – ZIMNOCH-GUZOWSKA, E. (2006): The novel, major locus *Rpi-phu1* for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. In: Theor. Appl. Genet., vol. *113*, 2006, N. 4, pp. 685–695.

SMILDE, W. D. – BRIGNETI, G. – JAGGER, L. – PERKINS, S. – JONES, J. D. G. (2005): *Solanum mochiquense* chromosome IX carries a novel late blight resistance gene *Rpi-moc1*. In: Theor. Appl. Genet., vol. *110*, 2005, N. 2, pp. 252–258.

SZCZERBAKOWA, A. – BOLTOWICZ, D. – LEBECKA, R. – RADOMSKI, P. – WIELGAT, B. (2005): Characteristics of the interspecific somatic hybrids *Solanum pinnatisectum* (+) *S. tuberosum* H-8105. In: Acta Physiol. Plant., vol. *27*, 2005, No. 3A, pp. 265–273.

VAN DER VOSSEN, E. A. G. – GROSS, J. – SIKKEMA, A. – MUSKENS, A. – WOUTERS, D. – WOLTERS, P. – PEREIRA, A. – ALLEFS, S. (2005): The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. In: Plant J., vol. *44*, 2005, pp. 208–222.

VAN DER VOSSEN, E.A.G. – SIKKEMA, A. – HEKKERT, B. – GROSS, J. – STEVENS, P. – MUSKENS, M. – WOUTERS, D. – PEREIRA, A. – STIEKEMA, W. – ALLEFS, S. (2003): An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. In: Plant J., vol. *36*, 2003, N. 6, pp. 867–882.

WILLIAMS, J.G.K. – KUBELIK, A.R. – LIVAK, K.J. – RAFALSKI, J.A. – TINGEY, S.V. (1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. In: Nucleic Acids Res., vol. *18*, 1990, N. 22, pp. 6531–6535.

SOUHRN

Bylo hodnoceno 136 diploidních genotypů rodu *Solanum*, jež jsou charakterizovány jako potenciální zdroje genů rezistence k plísni bramboru a diploidů bramboru. Na základě infekčních testů inokulací listových terčíků a celých *in vitro* rostlin bylo vybráno 15 odolných genotypů planých druhů z nichž 6 vykazovalo bezpříznakovost, naopak všech 11 diploidů *S. tuberosum* bylo náchylných. Nakonec bylo vybráno a podrobněji charakterizováno celkem 20 klonů (10 genotypů *S. bulbocastanum*, 1 genotyp *S. verrucosum* and 9 genotypů dihaploidů *Solanum tuberosum*) vhodných pro somatickou hybridizaci elektrickým polem. U těchto genotypů byla hodnocena výtěžnost a vitalita vyizolovaných protoplastů. Výtěžnost kolísala v rozmezí 1×10^5 a $3{,}5 \times 10^6$ protoplastů . ml^{-1}

získaných průměrně z 325 mg listů rostlin a byla variabilní i v rámci jednoho genotypu. Dále byla zjištěna pozitivní korelace mezi výtěžností a vitalitou získaných protoplastů. Jednalo se o lineární regresi, kde korelační koeficient r = 0,476 (středně silná závislost). Všech 136 genotypů bylo dále analyzováno metodou RAPD s cílem odlišit jednotlivé druhy. Byly použity sady dekamerů OPG a OPN, ze kterých byly vybrány primery OPG 8, OPN 3, 5, 8, 11, 15 a 18. Statistické vyhodnocení výsledků RAPD analýzy bylo provedeno shlukovou analýzou a zjištěno, že 9 genotypů, uváděných jako *S. pinnatisectum*, vykazovalo zcela odlišné elektroforetické profily RAPD markerů, ve srovnání s referenčním klonem *S. pinnatisectum* PI275235. Morfologickým srovnáním rostlin ve skleníku bylo zjištěno, že jde o 9 genotypů *S. polyadenium*. Výsledky naznačují potřebu detailní charakterizace evropských sbírek genetických zdrojů rodu *Solanum*, které byly introdukovány z genových bank v USA.

Klíčová slova: brambor, genetické zdroje, rezistence proti *Phytophthora infestans*, infekční testy, somatická hybridizace, RAPD.