PALACKÝ UNIVERSITY OLOMOUC
FACULTY OF SCIENCE
DEPARTMENT OF BOTANY

Marie Greplová

ISOLATION, CULTIVATION AND FUSION OF PROTOPLASTS OF *SOLANUM* GENERA

PH.D. THESIS
Ph.D. Program of Biology – Botany

Supervisor: RNDr. Božena Navrátilová, Ph.D.

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> If we observe life processes in great detail, we will destroy them.
>
> Niels Bohr

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Marie Greplová

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Abstract:

Wild *Solanum* species are valuable because of resistances to a range of diseases, pests and certain abiotic stresses. However, they are not readily crossable with tetraploid *S. tuberosum*. For transfer their desired features, somatic hybridization is used as a method for bypassing sexual incompatibility in *Solanum* genus. This method uses protoplasts that behave as gametes under special conditions due to characteristics of the plasma membrane. In this thesis, the conditions for protoplast isolation, fusion and cultivation were amended. The organogenesis on calli was initiated and forwarded via concentration and ratio of plant growth regulators. The technique of protoplast fusion was used for the creation of intraspecific somatic hybrids on model materials *S. tuberosum* cv. Bintje and *S. tuberosum* dihaploid (dh) 243. The same condition was successfully applied to interspecific protoplast fusion with wild species *S. bulbocastanum* and *S. pinnatisectum*. As a result, there were achieved somatic hybrids *S. bulbocastanum* 8003 + *S. tuberosum* dh 243, *S. bulbocastanum* PI243345 + *S. tuberosum* cv. Korneta, *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje, *S. pinnatisectum* 8166 + *S. tuberosum* dh 299, *S. pinnatisectum* PI320342 + *S. tuberosum* cv. Keřkovské rohlíčky. Conditions for asymmetric somatic hybridization were tested and established. Using optimal parameters, asymmetric somatic hybrids *S. verrucosum* PI161173 + *S. tuberosum* cv. Karin were obtained. All hybrid plants were identified by means of flow-cytometry and random amplification of polymorphic DNA (RAPD). The hybrids *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje were also confirmed using simple-sequence repeat (SSR) analysis, chloroplast DNA and mitochondrial DNA analysis and their morphology *in vitro* was characterized according to leaf morphology and plant growth vigour. Nine of these hybrids were found to be highly resistant to *Phytophthora infestans* based on *in vitro* test. Acclimatization of somatic hybrids was successfully accomplished as well. The hybrids *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje and *S. bulbocastanum* 8003 + *S. tuberosum* dh 243 were evaluated in field conditions with respect to agronomic traits. The hybrids *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje flowered and offered good yield and quality of tubers. Their resistance to *P. infestans* was moderately increased. The somatic hybrids *S. pinnatisectum* 8166 + *S. tuberosum* dh 299, *S. pinnatisectum* PI320342 + *S. tuberosum* cv. Keřkovské rohlíčky and asymmetric somatic hybrid *S. verrucosum* PI161117 + *S. tuberosum* cv. Karin were evaluated in term of resistance to *P. infestans* in a laboratory test. Three interspecific somatic hybrids showed high level of resistance in comparison with control *S. tuberosum* cv. Asterix. Somatic hybridization through protoplast fusion proved itself to be an auspicious method for creating new breeding materials.

Keywords: potato, protoplast, somatic hybrid, *Solanum tuberosum*, *S. bulbocastanum*, *S. pinnatisectum*, *S. verrucosum*

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CONTENTS

1. INTRODUCTION

The potato, *Solanum* L. section Petota (Dumortier), is a group of species possessing extensive biological diversity and wide geographical distribution in the Americas. Wild *Solanum* species are found in various environments, isolated in nature by geographical and ecological barriers. These species represent euploid series with ploidy ranging from diploid ($2n = 2x = 24$) to hexaploid ($2n = 6x = 72$) level (Masuelli et al. 2009, Camadro et al. 2004, Jansky 2006). The cultivated potato, *S. tuberosum* L. ($2n = 4x = 48$) is a basic vegetable crop throughout the world. To achieve optimal quality and yield, this crop-plant however requires a large number of inputs owing to a complex of different economically important diseases. Many wild *Solanum* species is employed in breeding for durable resistance but barriers exist for sexual hybridization not only in area isolation. Some species share the same habitat and do not readily cross (Camadro et al. 2004). In sexual hybridization, success can be predicted from the endosperm balance number (EBN). Differences in EBN result in failure of crossing between the cultivated potato and most wild species (Jansky 2006). Crossing barriers can be overcome inter alia by means of biotechnological procedures such as somatic hybridization through protoplast fusion or ploidy manipulations (Shi et al. 2006, Jansky 2006). Knowledge of these techniques makes access possible to a plentiful gene pool although recalcitrant genotypes are common and this limits the success of protoplast fusions and somatic hybrid regeneration (Chen et al. 2008, Jansky 2006, Davey et al. 2005).

Somatic hybridization is a strategy for the symmetric or asymmetric combination of the genomes of two parents and includes both nuclear and semiautonomous organelle genomes. Somatic hybridization has proved to be a valuable tool in the production of breeding materials with disease and insect resistance (Thieme et al. 2008, Chen et al. 2008). The characteristic features of both parents are combined in each somatic hybrid and there can be induced the synthesis of the new metabolites as has been shown through analysis of glycoalkaloids by Savarese et al. (2009). The gene pool of the potato can be also extended through this method.

However, symmetric somatic hybrids are burdened with a plethora of undesirable traits from wild parent. This problem can be resolved using asymmetric somatic hybridization. Asymmetric protoplast fusion enables new combinations of nuclear and cytoplasmic elements. This method can produce cybrids with cytoplasm of wild species and nucleus of cultivated one (Liu et al. 2005). This offers the potential for potato improvement via cytoplasm transfer inclusion. Apart from asymmetric somatic hybridization, microprotoplast fusion is the other method for producing interspecific hybrid plants with only one or a few alien chromosomes. This method overcomes large-scale gene transfer. Antimicrotubular compounds, such as amiprophos-methyl, oryzalin, colchicine, propyzamide and other, are used to produce the microprotoplasts (Ramulu et al. 1991, Yemets and Blume 2008, Saito and Nakano 2002, Saito and Nakano 2001, Binsfeld et al. 2000).

The thesis “Isolation, cultivation and fusion of protoplasts of *Solanum* genera” were carried out within a doctoral study programme Biology, study field Botany in the Botany Department of the Science Faculty of Palacký University in Olomouc. This research was an integral part of a research programme to create new breeding materials through biotechnological techniques (somatic hybridization and/or polyploidization *in vitro*) of research projects OC 843.20, OC 843.90, QF4108, QF4133 and MSM 6010980701 conducted at the Potato Research Institute Havlíčkův Brod, Ltd. The main aim of the research, recapitulated in this Ph.D. thesis, was to establish a method for somatic hybridization of *Solanum* genera and create new breeding materials using wild species which are not crossable with cultivated potato and carrying interesting quality especially the resistance to *Phytophthora infestans*, the most serious pathogen of the cultivated potato. The diploid wild species with EBN 1 i.e. *Solanum bulbocastanum* and *Solanum pinnatisectum* and diploid wild species with EBN 2 *Solanum verrucosum* were used.

The results of this Ph.D. thesis are summarised in 7 chapters. They include a review and 10 scientific papers. The eighth chapter is a brief extension of the results. It includes one published paper which offers a view on relevant themes – *in vitro* polyploidization.

The first part shortly covers the history of somatic hybridization and summarises knowledge of the results achieved in the field of protoplast fusions of *Solanum* genera especially in the last five years.

The second part describes the culture conditions for donor plants, isolation of protoplasts using enzymatic solutions, culture media and culture conditions for protoplasts, cells, calli and plant regenerations, parameters of the electric field for fusion, electrofusion of model materials of *S. tuberosum* (dihaploid 243 and cv. Bintje) with confirmation of regenerant hybridity and verification of different dose and ratio growth regulators for earlier organogenesis on protoplast originated calli.

The third part of the thesis describes creation of somatic hybrids *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje and *S. bulbocastanum* 8003 + dihaploid *S. tuberosum* 243. The efficiency of flow-cytometry and RAPD analysis is compared. The cultivation of somatic hybrids in greenhouse points out to preservation of the morphological characteristics under both *in vitro* and *in vivo* conditions.

Introduction to the cybridization of *Solanum* genera, i.e. conditions for organelle inactivation using iodoacetamide and iodoacetic acid and conditions for nuclei inactivation using UV irradiation, is the subject of the fourth chapter. A creation of new materials– asymmetric somatic hybrids *S. verrucosum* PI 161173 + *S. tuberosum* cv. Karin is described.

Verification hybridity using SSR analyses and characterisation of morphology *in vitro* of somatic hybrids *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje, the variability with regard to both ploidy level and cpDNA and mtDNA composition are concluded in the fifth subheading. The morphology of somatic hybrids *S. bulbocastanum* PI 243345 + *S. tuberosum* cv. Korneta is also described. This combination demonstrates high yield of somatic hybrids but without future prospect.

The sixth subheading focuses on the acclimatization of somatic hybrids. It also acquaints with the results of cultivating somatic hybrids in the field which is a necessary part of their characterization. This is related to two somatic hybrids *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje and six somatic hybrids *S. bulbocastanum* 8003 + dihaploid *S. tuberosum* 243.

A reliable and readily available test is necessary for testing the resistance transfer from chosen wild species into somatic hybrids. The seventh part describes the development of a laboratory test on Petri dish and shows the first results for the resistance testing of the breeding materials.

Somatic hybridization is performed together with polyploidization *in vitro* as another method for the creation of breeding materials using the chosen wild *Solanum* species. For this reason the eighth chapter serves as documentation for this part of the author‘s work over the most recent years.

This research led to creation of original somatic hybrids that could serve as new breeding materials and confirmed the somatic hybridization as method suitable for incorporation features from wild species into the cultivated potato.

References

Binsfeld PC, Wingender R, Schnabl H (2000) Characterization and molecular analysis of transgenic plants obtained by microprotoplast fusion in sunflower. Theor Appl Genet 101(8):1250-1258

Camadro EL, Carputo D, Peloquin SJ (2004) Substitutes for genome differentiation in tuber-bearing *Solanum*: interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and endosperm. Theor Appl Genet 109(7):1369-1376

Chen Q, Li HY, Shi YZ, Beasley D, Bizimungu B, Goettel MS (2008) Development of an effective protoplast fusion system for production of new potatoes with disease and insect resistance using Mexican wild potato species as gene pools. Can J Plant Sci 88(4):611-619

Davey MR, Anthony P, Power JB, Lowe KC (2005) Plant protoplasts: status and biotechnological perspectives. Biotechnol Adv 23(2):131-171

Jansky S (2006) Overcoming hybridization barriers in potato. Plant Breeding 125(1):1-12

Liu J, Xu X, Deng X (2005) Intergeneric somatic hybridization and its application to crop genetic improvement. Plant Cell Tiss Org (2005) 82:19–44

Liu JH, Xu XY, Deng XX (2005) Intergeneric somatic hybridization and its application to crop genetic improvement. Plant Cell Tiss Org 82(1):19-44

Masuelli RW, Camadro EL, Erazzu LE, Bedogni MC, Marfil CF (2009) Homoploid hybridization in the origin and evolution of wild diploid potato species. Plant Syst Evol 277(3-4):143-151

Ramulu KS, Verhoeven HA, Dijkhuis P (1991) Mitotic blocking, micronucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. Protoplasma 160:65-71

Saito F, Nakano M (2001) Partial synchronization of cell division and micronucleation in suspension-cultured cells of *Hemerocallis hybrida*: The effects of hyroxyurea and various spindle toxins. Breeding Sci 51(4):285-291

Saito H, Nakano M (2002) Preparation of microprotoplasts for partial genome transfer via microprotoplast fusion in Liliaceous ornamental plants. JARQ-Jpn Agr Res Q 36(3):129-135

Savarese S, Andolfi A, Cimmino A, Carputo D, Frusciante L, Evidente A (2009) Glycoalkaloids as Biomarkers for Recognition of Cultivated, Wild, and Somatic Hybrids of Potato. Chem Biodivers 6(4):437-446

Shi YZ, Chen Q, Li HY, Beasley D, Lynch DR (2006) Somatic hybridization between *Solanum tuberosum* and *S. cardiophylium*. Can J Plant Sci 86(2):539-545

Thieme R, Rakosy-Tican E, Gavrilenko T, Antonova O, Schubert J, Nachtigall M, Heimbach U, Thieme T (2008) Novel somatic hybrids (*Solanum tuberosum* L. plus *Solanum tarnii*) and their fertile BC1 progenies express extreme resistance to potato virus Y and late blight. Theor Appl Genet 116(5):691-700

Yemets AI, Blume YB (2008) Antimitotic drugs for microprotoplast-mediated chromosome transfer in plant genomics, cell engineering and breeding. Plant cytoskeleton: a key tool for agro-biotechnology. In: Nato Science for Peace and Security Series C – Environmental Security (eds Blume YB, Baird WV, Yemets AI, Breviario D), pp 419-434

2. AIMS OF THE PH.D. THESIS

Acquire knowledge and carry out experiments in somatic hybridization. Obtain somatic hybrids of *Solanum* genera, i.e. breeding material.

There are following areas of research more specifically:

Search information on somatic hybridization of *Solanum* genera.

Study the conditions for protoplast isolation, cultivation and regeneration; study of the conditions for fusions by electric field; confirm the ability of the designed conditions.

Create somatic hybrids with the purpose of transferring important traits. Verify suitable methods for identification of somatic hybrids.

Study conditions for asymmetric somatic hybridization.

Characterize somatic hybrids under *in vitro* conditions.

Evaluate somatic hybrids in the field.

Evaluate the resistance of parental genotypes and somatic hybrids to *Phytophthora infestans*.

3. RESULTS

3.1 Review of somatic hybridization of *Solanum* genera

3.2 Isolation, cultivation and fusion of protoplasts

3.3 Creation of breeding materials through protoplast fusion of *S. tuberosum* and wild species *S. bulbocastanum* and *S. pinnatisectum*

3.4 Creation of breeding materials through asymmetric protoplast fusion of *S. tuberosum* and *S. verrucosum*

3.5 Evaluation of somatic hybrids in *in vitro* conditions

3.6 Acclimatization and evaluation of somatic hybrids in *in vivo* conditions

3.7 Characterization of breeding materials in the light of resistance to *Phytophthora infestans*

3.8 Creation of breeding materials through simultaneously solved biotechnological approach – *in vitro* polyploidization

3.1 Review of the somatic hybridization of *Solanum* genera

Introduction

The first protoplast isolation was achieved by Klecker in 1892 from the leaves of *Stratioles aloides* by mechanical release of protoplasts into hypertonic solution. More systematic study of protoplasts began in the second half of the 20th century (Davey et al. 2005). A comprehensive review of somatic hybridization of the potato was published by Orczyk et al. in 2003 and methods for overcoming hybridization barriers in this species were described in considerable details by Jansky (2006). This review followed the recent status of somatic hybridisation in the potato and related problems.

Gene resources for potato improvement

A wide variety of pathogens affect potato production, with an estimated 22 % annual yield reduction world wide (Aversano et al. 2007). As a result, wild *Solanum* species are now being used for their potential pest-disease and abiotic stress resistance.

For somatic hybridization, the following EBN1 species were tested: *S. brevidens* (Dong et al. 2005, Vaananen et al. 2005), *S. bulbocastanum* (Boltowitz et al. 2005, Brown et al. 2006, Lovene et al. 2007, Yermishin et al. 2006, Yermishin et al. 2008, Greplová et al. 2008, Savarese et al. 2009), *S. cardiophyllum* (Shi et al. 2006, Chen et al. 2008), *S. etuberosum* (Gillen and Novy 2007), *S. pinnatisectum* (Chen et al. 2008, Greplová et al. 2008) and *S. tarnii* (Thieme et al. 2008). Some diploid species with EBN2 were also employed in somatic hybridization: *S. chacoense* (Chen et al. 2008, Kovacs et al. 2003), *S. stenotomum* (Fock et al. 2007), *S. spegazzinii* and *S. multiinterruptum* (Rasmussen et al. 2000) and *S. vernei* (Trabelsi et al. 2005).

Wild species were studied for their disease resistance with particular attention to the fungal disease, late blight caused by *Phytophthora infestans*. This was tested in *S. bulbocastanum*, *S. cardiophyllum*, *S. commersonii*, *S. pinnatisectum*, *S. trifidum*, *S. berthaultii*, *S. megistacrolobum*, *S. microdontum*, *S. verrucosum* *S. fendleri*, S. *stoloniferum*, *S. guerreroense* and *S. polyadenium* (Chen et al. 2003, Zlesak and Thill 2004, Colton et al. 2006, Aversano et al. 2007) and in *S. nigrum*, *S. villosum* and *S. scabrum* (Lebecka 2008). Resistance to *Alternaria solani* (the causal agent of early blight) was examined for *S. bulbocastanum* and *S. commersonii* (Jansky et al. 2008). Aversano et al. (2007) investigated resistance to the fungal pathogen, *Fusarium solani* in *S. bulbocastanum* and *S. cardiophyllum*. Resistance to the bacterium *Ralstonia solanacearum* in *S. commersonii* was explored by Siri et al. (2009). Chen et al. (2003) and Aversano et al. (2007) tested *S. pinnatisectum*, *S. bulbocastanum* and

S. cardiophyllum for resistance to the bacterium *Erwinia carotovora*. Pest resistance was investigated to: *Meloidogyne chitwoodi* in *S. bulbocastanum, S. fendleri* and *S. ougasii* (Brown et al. 2004, Brown et al. 2006); *Globodera* in *S. bulbocastanum* and *S. cardiophyllum* (Aversano et al. 2007) and to *Leptinotarsa decemlineata* in *S. pinnatisectum*, *S. cardiophyllum* and *S. chakoense* (Chen et al. 2003, Chen et al. 2008, Jansky et al. 2009).

Resistance to abiotic stress is relevant as well. Carputo et al. (2000) and Bamberg et al. (2005) considered transfer of tolerance to frost from *S. commersoni*. Bradeen and Mollov (2008) studied resistance to some herbicides in *S. pinnatisectum*. In addition, less common species were subjected to somatic hybridization with *S. tuberosum*: *S. cardiophylium* (Shi et al. 2006), *S. villosum* (Tarwacka et al. 2009) or *S. rickii* (Matveeva et al. 2008).

Protoplast isolation

Isolation of *Solanum* protoplasts and regeneration of plants became successful in the nineteen seventies (Shepard and Totten 1977, Binding et al. 1978). A system of regeneration from protoplasts to plants was subsequently developed. Protoplast regeneration ability was later proven to be genotype dependent (Orczyk et al. 2003), a fact which necessitated optimization of protoplast isolation and cultivation especially in wild *Solanum* species. More recently, Chen et al. (2008) developed a systematic method for protoplast isolation and regeneration for several Mexican diploid wild potato species followed by successful protoplast fusion. In general, new approaches were tested in order to get high efficiency protoplast cultures using supplements such as haemoglobin, spermidin (Rakosy-Tican et al. 2007), or physical stimulation especially electrostimulation as described by Davey et al. (2005) for protoplasts of different plant species.

Protoplast fusion

Solanum somatic hybrids were successfully achieved in the nineteen eighties (Binding et al. 1982, Barsby et al. 1984, Gressel et al. 1984, Austin et al. 1985, Gleddie et al. 1986, Puite et al. 1986). Somatic hybridization became popular owing to the generation of novel germplasm. This germplasm arises as combination of nuclear and cytoplasm genes (Davey et al. 2005, Liu et al. 2005).

The fusion of protoplasts is commonly achieved by chemical or physical means.

The former is mediated by polyethylene glycol (PEG) and the latter using electric fields – alternating current to formation of protoplast chains and direct current pulls for fusion of protoplasts. Both methods were used to date but electrofusion seems to be preferred as the following citations suggest: Johnson et al. (2001) realized chemical and electrofusion at potato monoploids. Szczerbakowa et al. (2005), Trabelsi et al. (2005) and Przetakiewicz et al. (2007) worked with PEG to mediate fusion. In contrast, Kovacs et al. (2003), Nouri-Ellouz et al. (2006), Shi et al. (2006), Borgato et al. (2007), Lightbourn and Veilleux (2007), Lightbourn et al. (2007), Thieme et al. (2008) and Greplová et al. (2008) availed electrofusion.

Symmetric somatic hybridization is designed for equivalent combination of both parents. Protoplasts of both parents are mixed in a ratio 1:1 and fused. However, parts of genomes can be lost and asymmetry can occur. Parental genome doses can be variable in somatic hybrids, as described through the characterization of nuclear and cytoplasmic genome composition by Lovene et al. (2007) in somatic hybrids *S. bulbocastanum* + *S. tuberosum* haploids. Rokka et al. (2005) obtained different progenies from protoplast fusion of tetraploids *S. acaule* + *S. tuberosum*: both hexaploids with parental composition in a ratio 2:4 or 4:2 and tetraploids (2:2). Protoplast fusions of *S. bulbocastanum* + *S. tuberosum* dihaploid that were achieved by Boltowicz et al. (2005) offered hybrids. The aneuploidy of these hybrids was revealed and loss of *S. bulbocastanum* chromosome(s) was assumed. Trabelsi et al. (2005) obtained hybrid plants from fusion of *S. vernei* + *S. tuberosum* dihaploid. These hybrids were identified as asymmetric and elimination of a major part of the *S. vernei* parent was suspected. Liu et al. (2005) concluded that the spontaneous elimination of chromosomes were common and could occur for either or both fusion parents.

Asymmetry of somatic hybrids was also be induced by experimental factors. Protoplasts of cultural parent were affected by some metabolic agents (iodoacetic acid, iodoacetamide, rhodamine; Matibiri and Mantell 1994, Yarrow et al. 1999). Protoplasts of wild parent were affected by ionizing radiation (γ rays, α particles, UV light; Xu et al. 1993, Sidorov et al. 1994, Oberwalder et al. 1998, Oberwalder et al. 2000). Protoplasts treated in such a way create after fusion cytoplasmic hybrids - cybrids (Liu et al. 2005). Using mitotic spindle toxins (amiprophos-methyl, oryzalin or colchicine), microprotoplasts can be created. These microprotoplasts can be fused with the protoplast of cultural species enabling the transfer of only one or a few chromosomes of wild species (Ramulu et al. 1991, Yemets and Blume 2008).

Interspecific somatic hybrids with desired characteristics were obtained not so long ago. Extreme resistance to potato virus Y and *P. infestans* was discovered by Thieme et al. (2008) in their somatic hybrids *S. tarnii* + *S. tuberosum*. Gillen and Novy (2007) identified resistance to PLRV (potato leafroll virus) in hybrids of *S. etuberosum* + *S. tuberosum*. While transfer resistance to *P. infestans* into potato was not successful in protoplast fusion experiments of *S. bulbocastanum* + *S. tuberosum* dihaploid; resistance was absent in the obtained somatic hybrids (Boltowicz et al. 2005). Somatic hybrids between *S. pinnatisectum* and *S. tuberosum* dihaploid were also found to show no differences compared to potato parent in preliminary tests for resistance to *P. infestans* (Szczerbakowa et al. 2005). Fock et al. (2007) produced somatic hybrids of parents *S. stenotomum* and *S. tuberosum* with the aim to introduce resistance to bacterial wilt (caused by *Ralstonia solanacearum*). They confirmed both resistance transfer and its stability in long-term *in vitro* cultivation. Further, Kim-Lee et al. (2005) transferred bacterial wilt resistance into the progeny of *S. commersonii* + *S. tuberosum* haploid. Resistance against potato tuber moth larvae was found in *S. pinnatisectum*. Resistant somatic hybrids with intermediate levels of resistance to this pest were produced by Musmeci et al. (2005). Resistance to abiotic stress is very important too. The required tolerance to salinity was achieved through protoplast fusion of *S. berthaultii* + *S. tuberosum* by Bidani et al. (2007).

From the point of view of consumer health, the following reports became interesting. Savarese et al. (2009) found evidence for the creation of new glycoalkaloids in the hybrids of *S. bulbocastanum* + *S. tuberosum*. Before this finding, Vaananen et al. (2005) had analysed the somatic hybrids of *S. acaule* or *S. brevidens* with *S. tuberosum* and found that they contained none of the glycoalkaloids detected in the parental species.

Nouri-Ellouz et al. (2006), Przetakiewicz et al. (2007) and Nadolska-Orczyk et al. (2007) employed intraspecific somatic hybridisation for the production of tetraploid potato lines to create potential breeding materials. Some researchers focused on union of monoploid genotypes. They assumed to produce vigorous somatic hybrids. Johnson et al. (2001) predicted a reduced "genetic load" using this procedure. They achieved intermonoploid somatic hybrids. Later, Lightbourn et al. (2007) attained diverse somatic hybrids from monoploid protoplast fusion.

Somatic hybrid identification

Morphological characteristics were shown a good preliminary guideline for somatic hybrid identification. Differences in leaves, flowers, growth habit and other features served as identification markers. Somatic hybrids had a morphology intermediate between fusion parents and morphology near to one parent (Musmeci et al. 2005, Szczerbakowa et al. 2005, Trabelsi et al. 2005, Shi et al. 2006, Borgato et al. 2007, Fock et al. 2007, Przetakiewicz et al. 2007, Thieme et al. 2008, Greplová et al. 2008). The hybrid plants originating from the one fusion combination were different in some characteristics (Szczerbakowa et al. 2005, Przetakiewicz et al. 2007). Some somatic hybrids outperformed the cultivated parent (Nouri-Ellouz et al. 2006, Bidani et al. 2007). However, the symmetry of hybrids was impossible to predict from morphology type (Trabelsi et al. 2005).

Commonly used methods for hybrid identification became ploidy level determination using flow-cytometry (Oberwalder et al. 1997) or chromosome counting. Chromosome numbers of somatic hybrids were the sum of fused partners (Shi et al. 2006, Borgato et al. 2007) or less than the sum (Kim-Lee et al. 2005, Szczerbakowa et al. 2005). Thus for precise identification of hybrids, it was appropriate to complete with more exact methods. The magnetic cell sorter (MACS) technique was utilized for the selection of heterokaryons by Borgato et al. (2007) and then completed with DNA analysis. Cytogenetic identification of interspecific somatic hybrids was recently used (Tarwacka et al. 2009). To investigate the precise chromosomal composition in somatic hybrids, genomic *in situ* hybridization (GISH) was exerted (Escalanta et al. 1998, Dong et al. 2005, Lovene et al. 2007). This method can unambiguously confirm the origin of chromosomes in hybrid plants and the contribution of each parent (Horsman et al. 2001). GISH (Wolters et al. 1994) and FISH (fluorescence *in situ* hybridization; Srebniak et al. 2002) are able to show intra or inter-genomic translocation and chromosome rearrangements in somatic hybrids.

A biochemical analysis of isozymes (e.g. esterase, peroxidase, phosphatase) also served for identifying somatic hybrids (Trabelsi et al. 2005, Nouri-Ellouz et al. 2006, Toppino et al. 2008).

Molecular markers were exploited to verify the hybridity of *Solanum* genera since the nineteen nineties. Some of these were routinely applied, especially randomly amplified polymorphism DNA (RAPD; Rasmussen et al. 1997, Boltowicz et al. 2005, Szczerbakowa et al. 2005, Trabelsi et al. 2005, Shi et al. 2006, Przetakiewicz et al. 2007) or intersimple sequence repeat (ISSR; Trabelsi et al. 2005, Nouri-Ellouz et al. 2006, Bidani et al. 2007, Borgato et al. 2007, Toppino et al. 2008), simple sequence repeat (SSR; Milbourne et al. 1997, Matthews et al. 1999, Nouri-Ellouz et al. 2006, Thieme et al. 2008) and other techniques such as amplified fragment length polymorphism (AFLP; Thieme et al. 2008) or restriction fragment length polymorphism (RFLP; Novy and Helgeson 1994a, b; Williams et al. 1990, 1993; Oberwalder et al. 1997, 2000).

Identification of interspecific hybrids can be accomplished on the basis of identification of chloroplast and mitochondrial types. Lössl et al. (1999) presented five types of mitochondria (α, β, δ, γ and ε) and three types of chloroplasts (T, W and S). The origin of the plastom genomes in asymmetric somatic hybrids was identified by Rasmussen et al. (2000) using chloroplast (cp)- and mitochondrial (mt)-specific PCR markers. Scotti et al. (2004) used mtDNA gene probes on somatic hybrids and later (Scotti et al. 2007) assessed mtDNA variation in cultivated and wild relatives and correlation with ploidy and EBN. Przetakiewicz et al. (2007) analyzed preferentially T chloroplast and β mitochondria in somatic hybrids although the diploid parents line *S. tuberosum* had apart from these T, β organelles, W/S chloroplasts and α or ε mitochondria as well. Using chloroplast DNA analyses, Trabelsi et al. (2005) obtained recombinant hybrid plastomes from both parents.

Results of somatic hybrid backcrosses

The results of backcrossing somatic hybrids with potato demonstrate transfer of some desirable traits. Hexaploid somatic hybrids were more successful in backcrosses as shown by Rokka et al. (2005) for *S. acaule* + *S. tuberosum* somatic hybrids. Dong et al. (2005) obtained a series of backcross progenies from one hexaploid somatic hybrid *S. brevidens* + *S. tuberosum*. Using RAPD, GISH and FISH technique they identified all 12 *S. brevidens* chromosomes in the backcross progenies. Kim-Lee et al. (2005) observed segregation of resistance to bacterial wilt in the first backcross progenies of *S. commersonii* + *S. tuberosum* dihaploid somatic hybrids. Novy et al. (2007) verified transfer resistance to potato leafroll virus (PLRV) from somatic hybrids with *S. etuberosum* up to the third generation of backcrossing while resistance to virus Y (PVY) only to the second generation. Using analysis of *S. etuberosum* chromosomes in backcross of *S. etuberosum* somatic hybrid, Gillen and Novy (2007) revealed a recombination between the potato and *S. etuberosum* genomes and/or fragmentation of the *S. etuberosum* chromosomes. Transfer resistance to pink rot (*Phytophthora* *erythroseptica*) and leak (*Pythium ultimum*) from somatic hybrids *S. etuberosum* + *S. berthaultii* to backcross (BC) progeny was also reported (Thompson et al. 2007). High levels of resistance to PVY and to foliage blight was transferred from somatic hybrids to BC progeny by Thieme et al. (2008). The backcross was as well successful for somatic hybrids *S. pinnatisectum* + *S. tuberosum* obtained by Yermishin et al. (2006, 2008). These somatic hybrids had been first crossed with *S. phureja* IvP35 to reduce ploidy from tetraploid to diploid level.

Conclusion

Several techniques have been applied in efforts to incorporate desirable genes from noncrossable wild *Solanum* species into *S. tuberosum*. Different techniques are used and variously combined. Some researchers tried again to get sexual crosses in which they tested different accessions of wild species, bridge crosses, embryo rescue, application of phytohormones inter alia (Ramon and Hanneman 2002, Chen et al. 2004, Dinu et al. 2005, Hayes et al. 2005, Voronkova et al. 2007, Jansky and Hamernik 2009, Carputo et al. 2009). Another utilized method was ploidy level manipulation: ploidy decrease via *in vitro* anther cultures (Kopecký and Vagera 2006) or using *S. phureja* IvP35 as haploid producer (Yermishin et al. 2006, Yermishin et al. 2008, Rokka 2009); sexual increase of ploidy via 2n gametes (Carputo and Barone 2005); somatic ploidy increase via mitotic polyploidization using spindle toxins (Brown et al. 2004, Greplová et al. 2009).

Finally, the somatic hybridization enables transfer polygene traits, transfer cytoplasmic genes and their combination (Chen et al. 2004). The transfer of only part of the wild nuclear genome into recipient cultural species is allowed by the methods of asymmetric somatic hybridization or microprotoplast fusion (Orczyk et al. 2003). For similarity to sexual hybridization, somatic hybridization is more acceptable to the public than genetically transformed plants (Liu et al. 2005). In sum, somatic hybridization is currently proving to be a very promising technique for the incorporation of untapped genes.

References

Austin S, Baer MA, Helgeson JP (1985) Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *Solanum tuberosum* by somatic fusion. Plant Sci 39:75-82

Aversano R, Ercolano MR, Frusciante L, Monti L, Bradeen JM, Cristinzio G, Zoina A, Greco N, Vitale S, Carputo D (2007) Resistance traits and AFLP characterization of diploid primitive tuber-bearing potatoes. Genet Resour Crop Evol 54(8):1797-1806

Bamberg JB, Palta JP, Vega SE (2005) *Solanum commersonii* cytoplasm does not improve freezing tolerance in substitution backcross hybrids with frost-sensitive potato species. Am J Potato Res 82(3):251-254

Barsby TL, Shepard JF, Kemble RJ, Wong R (1984) Somatic hybridisation in the genus *Solanum*: *S. tuberosum* and *S. brevidens*. Plant Cell Rep 3:165-167

Bidani A, Nouri-Ellouz O, Lakhoua L, Sihachakr D, Cheniclet C, Mahjoub A, Drira N, Gargouri-Bouzid R (2007) Interspecific potato somatic hybrids between *Solanum berthaultii* and *Solanum tuberosum* L. showed recombinant plastome and improved tolerance to salinity. Plant Cell Tiss Org 91(3):179-189

Binding H, Nehls R, Schieder O, Sopory SK, Wenzel G (1978) Regeneration of mesophyll protplasts isolated from dihaploid clones of *Solanum tuberosum*. Physiol Plantarum 43:52-54

Binding H, Jain SM, Finger J, Mordhorst G, Nehls R, Gressel J (1982) Somatic hybridisation of an atrazine resistant biotype of *Solanum nigrum* with *Solanum tuberosum*. Part 1. Clonal variation in morphology and in agrazine sensitivity. Theor Appl Genet 63:273-277

Boltowicz D, Szczerbakowa A, Wielgat B (2005) RAPD analysis of the interspecific somatic hybrids *Solanum bulbocastanum* (+) *S. tuberosum*. Cell Mol Biol Lett 10(1):151-162

Borgato L, Conicella C, Pisani F, Furini A (2007) Production and characterization of arboreous and fertile *Solanum melongena* plus *Solanum marginatum* somatic hybrid plants. Planta 226(4):961-969

Bradeen JM, Mollov DS (2008) Herbicide tolerance in primitive diploid potato species comprising superseries Stellata: toward establishment of seedling cultivation conditions for field evaluations. Am J Potato Res 84(5):415-424

Brown CR, Mojtahedi H, Bamberg J (2004) Evaluation of *Solanum fendleri* as a source of resistance to *Meloidogyne chitwoodi*. Am J Potato Res 81(6):415-419

Brown CT, Mojtahedi H, James S, Novy RG, Love S (2006) Development and evaluation of potato breeding lines with introgressed resistance to Columbia root-knot nematode (Meloidogyne chitwoodi). Am J Potato Res 83(1):1-8

Camadro EL, Carputo D, Peloquin SJ (2004) Substitutes for genome differentiation in tuber-bearing Solanum: interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and endosperm. Theor Appl Genet 109(7):1369-1376

Carputo D, Cardi T, Palta JP, Sirianni P, Vega S, Frusciante L (2000) Tolerance to low temperatures and tuber soft rot in hybrids between *Solanum commersonii* and *Solanum tuberosum* obtained through manipulation of ploidy and endosperm balance munber (EBN). Plant Breeding 119(2):127-130

Carputo D, Barone A (2005) Ploidy level manipulations in potato through sexual hybridisation. Ann Appl Biol 146(1):71-79

Carputo D, Aversano R, Barone A, Di Matteo A, Iorizzo M, Sigillo L, Zoina A, Frusciante L (2009) Resistance to *Ralstonia solanacearum* of Sexual Hybrids Between *Solanum commersonii* and *S. tuberosum*. Am J Potato Res 86(3):196-202

Chen Q, Kawchuk L M, Lynch DR, Goettel MS, Fujimoto DK (2003) Identification of late blight, Colorado potato beetle, and blackleg resistance in three Mexican and two South American wild 2x (1EBN) Solanum species. Am J Potato Res 80(1):9-19

Chen SY, Liu SW, Xu CH, Chen YZ, Xia GM (2004) Heredity of chloroplast and nuclear genomes of asymmetric somatic hybrid lines between wheat and couch grass. Acta Botan Sin 46:110-115

Chen Q, Lynch D, Platt HW, Li HY, Shi Y, Li HJ, Beasley D, Rakosy-Tican L, Theme R (2004) Interspecific crossability and cytogenetic analysis of sexual progenies of Mexican wild diploid 1EBN species *Solanum pinnatisectum* and *S. cardiophyllum*. Am J Potato Res 81(2):159-169

Chen Q, Li HY, Shi YZ, Beasley D, Bizimungu B, Goettel MS (2008) Development of an effective protoplast fusion system for production of new potatoes with disease and insect resistance using Mexican wild potato species as gene pools. Can J Plant Sci 88(4):611-619

Colton LM, Groza HI, Wielgus SM, Jiang JM (2006) Marker-assisted selection for the broad-spectrum potato late blight resistance conferred by gene RB derived from a wild potato species. Crop Sci 46(2):589-594

Davey MR, Anthony P, Oower JB, Kowe KC (2005) Plant protoplast technology: Current status. Acta Physiol Plant 27(1):117-129

Deimling, S, Zitzisperger J, Wenzel G (1988) Somatic fusion for breeding of tetraploid potatoes. Plant Breeding 101:181-189

Dinu II, Hayes RJ, Kynast RG, Phillips RL, Thill CA (2005) Novel inter-series hybrids in Solanum, section Petota. Theor Appl Genet 110(3):403-415

Dong F, Tek AL, Frasca ABL, McGrath JM, Wielgus SM, Helgeson JP, Jiang J (2005) Development and characterization of potato-*Solanum brevidens* chromosomal addition/substitution lines. Cytogenet Genome Res 109(1-3):368-372

Escalanta A, Imanishi S, Hossain M, Fukui ON (1998) RFLP analysis and genomic *in situ* hybridisation (GISH) in somatic hybrids and their progeny between *Lycopersicon esculentum* and *Solanum lycopersicoides*. Theor Appl Genet 96:719-726

Fock I, Collonnier C, Lavergne D, Vaniet S, Ambroise A, Luisetti J, Kodja H, Sihachakr D (2007) Evaluation of somatic hybrids of potato with *Solanum stenotomum* after a long-term *in vitro* conservation. Plant Physiol Bioch 45(3-4):209-215

Greplová M, Polzerová H, Vlastníková H (2008) Electrofusion of protoplasts from *Solanum tuberosum*, *S. bulbocastanum* and *S. pinnatisectum*. Acta Physiol Plant 30:787-796

Greplová M, Polzerová H, Domkářová J (2009) Intra- and inter-specific crosses of *Solanum* materials after mitotic polyploidization *in vitro*. Plant Breeding 128:651-657

Gillen AM, Novy RG (2007) Molecular characterization of the progeny of *Solanum etuberosum* identifies a genomic region associated with resistance to potato leafroll virus. Euphytica 155(3):403-415

Gressel J, Cohen N, Binding H (1984) Somatic hybridisation of an atrazine resistant biotype of *Solanum nigrum* with *Solanum tuberosum*. 2. Segregation of plastomes. Theor Appl Genet 67:131-134

Hayes RJ, Dinu II, Thill CA (2005) Unilateral and bilateral hybridization barriers in inter-series crosses of 4x 2EBN *Solanum stoloniferum*, *S. pinnatisectum*, *S. cardiophyllum*, and 2x 2EBN *S. tuberosum* haploids and haploid-species hybrids. Sex Plant Reprod 17(6):303-311

Horsman K, Gavrilenko T, Bergervoet M, Huigen DJ, Joe ATW, Jacobsen E (2001) Alteration of the genomic composition of *Solanum nigrum* (+) potato backcross derivatives by somatic hybridization: selection of fusion hybrids by DNA measurements and GISH. Plant Breeding 120:201-207

Jansky S (2006) Overcoming hybridization barriers in potato. Plant Breeding 125(1):1-12

Jansky SH, Simon R, Spooner DM (2008) A test of taxonomic predictivity: Resistance to early blight in wild relatives of cultivated potato. Phytopathology 98(6):680-687

Jansky S, Hamernik A (2009) The introgression of 2x 1EBN *Solanum* species into the cultivated potato using *Solanum verrucosum* as a bridge. Genet Resour Crop Evol 56(8):1107-1115

Jansky SH, Simon R, Spooner DM (2009) A Test of Taxonomic Predictivity: Resistance to the Colorado Potato Beetle in wild relatives of cultivated potato. J Econ Entomol 102(1):422-431

Johnson AAT, Piovano SM, Ravichandran V, Veilleux RE (2001) Selection of monoploids for protoplast fusion and generation of intermonoploid somatic hybrids of potato. Am J Potato Res 78(1):19-29

Kim-Lee HY, Moon JS, Hong YJ, Kim MS, Cho HM (2005) Bacterial wilt resistance in the progenies of the fusion hybrids between haploid of potato and *Solanum commersonii*. Am J Potato Res 82(2):129-137

Kopecký D, Vagera J (2005) The use of mutagens to increase the efficiency of the androgenic progeny production in *Solanum nigrum*. Biol Plantarum 49(2):181-186

Kovacs K, Catana C, Pamfil D (2003) Somatic hybridization on potato. Bulletin of the University of Agricultural Sciences and Veterinary Medicine, Animal Husbandry and Technology, Book Series: Buletinul Universitatii de Stiinte Agricole si Medicina Veterinaria Cluj-Napoca Seria Zootehni si Biotehnologii Series 59, pp 279-283

Lebecka R (2008) Host–pathogen interaction between *Phytophthora infestans* and *Solanum nigrum* , *S. villosum* , and *S. scabrum*. *Eur J Plant Pathol* 120(3):233-240

Lightbourn GJ, Veilleux RE (2007) Production and evaluation of somatic hybrids derived from monoploid potato. Am J Potato Res 84(5):425-435

Lightbourn GJ, Jelesko JG, Veilleux RE (2007) Retrotransposon-based markers from potato monoploids used in somatic hybridization. Genome 50(5):492-501

Liu JH, Xu XY, Deng XX (2005) Intergeneric somatic hybridization and its application to crop genetic improvement. Plant Cell Tiss Org 82(1):19-44

Lovene M, Savarese S, Cardi T, Frusciante L, Scotti N, Simon PW, Carputo D (2007) Nuclear and cytoplasmic genome composition of *Solanum bulbocastanum* (+) *Solanum tuberosum* somatic hybrids. Genome 50(5):443-450

Lössl A, Adler N, Horn R, Frei U, Wenzel G (1999) Chondriome type characterization of potato: Mt a, b, g, d, e and novel plastid-mitochondrial configurations. Theor Appl Genet 99:1-10

Matibiri EA, Mantell SH (1994) Cybridization in *Nicotiana tabacum* L. using double inactivation of parental protoplasts and post-fusion selection based on nuclear-encoded and chloroplast-encoded marker genes. Theor Appl Genet 88(8):1017-1022

Matthews D, McNicoll J, Harding K, Milam S (1999) 5' – Anchored simple-sequence repeat primers are useful for analysing potato somatic hybrids. Plant Cell Report 19:210-212

Matveeva NA, Shakhovskii AM, Kuchuk NV (2008) Somatic hybrids between transgenic *Solanum tuberosum* potato plants and transplastome *Solanum rickii* plants. Cytology and Genetics 42(4):246-251

Milbourne D, Meyer R, Bradshew JE, Baird E, Bonar N, Provan J, Powell W, Waugh R (1997) Comparison of PCR-based for the analysis of genetic relationships in cultivated potato. Mol Breeding 3:127-136

Musmeci S, Gambino P, Innocenzi V, Arnone S, Lai A (2005) Eliciting of resistance against potato tuber moth larvae in tubers of *Solanum tuberosum* (+) *S. pinnatisectum* hybrids. (eds Casa R, Viola R) Proceedings of the Meeting of the Physiology Section of the European Association for Potato Research, Acta Hort 684:135-141

Nadolska-Orczyk A, Pietrusinska A, Binka-Wyrwa A, Kuc D, Orczyk W (2007) Diploid potato (*Solanum tuberosum* L.) as a model crop to study transgene expression. Cell Mol Biol Lett 12(2):206-219

Nouri-Ellouz O, Gargouri-Bouzid R, Sihachakr D, Triki MA, Ducreux G, Drira N, Lakhoua L (2006) Production of potato intraspecific somatic hybrids with improved tolerance to PVY and *Pythium aphanidermatum*. J Plant Physiol 163(12):1321-1332

Novy RG, Helgeson JP (1994a) Somatic hybrids between *Solanum etuberosum* and diploid, tuber bearing *Solanum* clones. Theor Appl Genet 89:775-782

Novy RG, Helgeson JP (1994b) Resistance to potato virus Y in somatic hybrids between

Solanum etuberosum and *S. tuberosum* × *S. berthaultii* hybrid. Theor Appl Genet 89:783-786

Novy RG, Gillen AM, Whitworth JL (2007) Characterization of the expression and inheritance of potato leafroll virus (PLRV) and potato virus Y (PVY) resistance in three generations of germplasm derived from *Solanum etuberosum*. Theor Appl Genet 114(7):1161-1172

Oberwalder B, Ruoss B, Schilde-Rentschler L, Hemleben V, Ninnemann H (1997) Asymmetric protoplast fusion between wild and cultivated species of potato (Solanum spp.) – detection asymmetric hybrids and genome elimination. Theor Appl Genet 94:1104-1112

Oberwalder B, Schilde-Rentschler L, Ruoss B, Wittemann S, Ninnemann H (1998) Asymmetric protoplast fusions between wild species and breeding lines of potato – effect of recipients and genome stability. Theor Appl Genet 97:1347-1354

Oberwalder B, Schilde-Rentschler L, Löffelhardt-Ruoss B, Ninnemann H (2000) Differences between hybrids of *Solanum tuberosum* and *Solanum circaeifolium* Bitt. obtained from symmetric and asymmetric fusion experiments. Potato Res 43:71-82

Orczyk W, Przetakiewicz J, Nadolska-Orczyk A (2003) Somatic hybrids of *Solanum tuberosum* – application to genetics and breeding. Plant Cell Tiss Org 74:1-13

Puite KL, Roest S, Pijnacker LP (1986) Somatic hybrid potato plants after eledtrofusion of diploid Solanum tuberosum and Solanum phureja. Plant Cell Rep 5:262-265

Rakosy-Tican E, Aurori A, Vesa S, Kovacs KM (2007) In vitro morphogenesis of sunflower (*Helianthus annuus*) hypocotyl protoplasts: the effects of protoplast density, haemoglobin and spermidine. Plant Ccell Tiss Org 90(1):55-62

Ramon M, Hanneman RE (2002) Introgression of resistance to late blight (*Phytophthora infestans*) from *Solanum pinnatisectum* into *S. tuberosum* using embryo rescue and double pollination. Euphytica 127(3):421-435

Ramulu KS, Verhoeven HA, Dijkhuis P (1991) Mitotic blocking, micronucleation and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. Protoplasma 160:65-71

Rasmussen JO, Waara S, Rasmussen OS (1997) Regeneration and analysis of interspecific asymmetric potato – *Solanum* spp., hybrid plants selected by micromanipulation or fluorescence-activated cell sorting (FACS). Theor Appl

Genet 95:41-49

Rasmussen JO, Lossl A, Rasmussen OS (2000) Analysis of the plastome and chondriome origin in plants regenerated after asymmetric *Solanum* ssp protoplast fusions. Theor Appl Genet 101(3):336-343

Rokka VM, Laurila J, Tauriainen A, Laakso I, Larkka J, Metzler M, Pietila L (2005) Glycoalkaloid aglycone accumulations associated with infection by *Clavibacter michiganensis* ssp *sepedonicus* in potato species *Solanum acaule* and *Solanum tuberosum* and their interspecific somatic hybrids. Plant Cell Rep 23(10-11):683-691

Rokka VM (2009) Potato Haploids and Breeding. (eds Touraev A, Forster BP, Jain SM) Advances in Haploid Production in Higher Plants, pp 199-208

Scotti N, Marechal-Drouard L, Cardi T (2004) The rpl5-rps14 mitochondrial region: a hot spot for DNA rearrangements in *Solanum* spp. somatic hybrids. Curr Genet 45(6):378-382

Scotti N, Cozzolino S, Cardi T (2007) Mitochondrial DNA variation in cultivated and wild potato species (*Solanum* spp.). Genome 50(8):706-713

Shepard JF, Totten RH (1977) Mesophyll cell protoplasts of potato: isolation, proliferation and plant regeneration. Plant Physiol 60:313-316

Shi YZ, Chen Q, Li HY, Beasley D, Lynch DR (2006) Somatic hybridization between *Solanum tuberosum* and *S. cardiophyllum*. Can J Plant Sci 86(2):539-545

Sidorov VA, Yevtushenko DP, Shakhovsky AM, Gleba YY (1994) Cybrid production based on mutagenenic innactivation of protoplasts and rescuing of mutant plastids in fusion products: potato with plastome from *S. bulbocastanum* and *S. pinnatisectum*. Theor Appl Genet 88:525-529

Srebniak M, Rasmussen O, Maluszyńska J (2002) Cytogenetic analysis of an asymmetric potato hybrid. J Appl Genet 43(1):19-31

Szczerbakowa A, Boltowicz D, Lebecka R, Radomski P, Wielgat B (2005) Characteristics of the interspecific somatic hybrids *Solanum pinnatisectum* (+) *S. tuberosum* H-8105. Acta Physiol Plant 27(3A):265-273

Tarwacka J, Szczerbakowa A, Wielgat B (2009) Cytogenetic characteristics of the interspecific somatic hybrids of *Solanum villosum* (+) *S. tuberosum*. Acta Biol Cracov Bot 51 Suppl:67-67

Thieme R, Rakosy-Tican E, Gavrilenko T, Antonova O, Schubert J, Nachtigall M, Heimbach U, Thieme T (2008) Novel somatic hybrids (*Solanum tuberosum* L.

plus *Solanum tarnii*) and their fertile BC1 progenies express extreme resistance to potato virus Y and late blight. Theor Appl Genet 116(5):691-700

Thompson AL, Taylor RJ, Pasche JS, Novy RG, Gudmestad NC (2007) Resistance to *Phytophthora erythroseptica* and *Pythium ultimum* in a potato clone derived from *S. berthaultii* and *S. etuberosum*. Am J Potato Res 84(2):149-160

Toppino L, Mennella G, Rizza F, D'Alessandro A, Sihachakr D, Rotino GL (2008) ISSR and isozyme characterization of androgenetic dihaploids reveals tetrasomic inheritance in tetraploid somatic hybrids between *Solanum melongena* and *Solanum aethiopicum* group Gilo. J Hered 99(3):304-315

Trabelsi S, Gargouri-Bouzid R, Vedel F, Nato A, Lakhoua L, Drira N (2005) Somatic hybrids between potato Solanum tuberosum and wild species Solanum vernei exhibit a recombination in the plastome. Plant Cell Tiss Org 83(1):1-11

Vaananen D, Ikonen T, Rokka VM, Kuronen P, Serimaa R, Ollilainen V (2005) Influence of incorporated wild *Solanum* genomes on potato properties in terms of starch nanostructure and glycoalkaloid content. J Agr Food Chem 53(13):5313-5325

Voronkova EV, Lisovskaja VM, Yermishin AP (2007) Diploid hybrids between allotetraploid wild potato species *Solanum acaule* Bitt., *S. stoloniferum* Schltdl. and dihaploids of *S. tuberosum* L. Russ J Genet+ 43(8):882-889

Williams CE, Hunt GJ, Helgeson JP (1990) Fertile somatic hybrids of *Solanum* species: RFLP analysis of a hybrid and its sexual progeny from crosses with potato. Theor Appl Genet 80:545-551

Williams CE, Wielgus SM, Haberlach GR, Guenther C, Kim-Lee H, Helgeson JP (1993) RFPL analysis of chromosomal segregation in progeny from an interspecific hexaploid somatic hybrid beween *Solanum brevidens* and *Solanum tuberosum*. Genetics 135:1167-1173

Wolters AMA, Jacobsen EO, Connell M, Bonnema G, Ramula KS, Jong H, Schoenmakers K, Wijbrandi J, Koomneef M (1994) Somatic hybridization as a tool for tomato breeding. Euphytica 79:265-277

Xu YS, Murto M, Dunckley R, Jones MGK, Pehu E (1993) Production of asymmetric hybrids between *Solanum tuberosum* and irradiated *S. brevidens*. Theor Appl Genet 85:729-734

Yarrow S (1999) Production of cybrids in rapeseed (*Brassica napus*). In: Hall RD (ed): Plant Cell Culture Protocols. Methods in Molecular Biology. Vol. 111.

Springer:211-224

Yemets AI, Blume YB (2008) Antimitotic Drugs from Microprotoplast-mediated Chromosome Transfer in Plant Genomics, Cell Engineering and Breeding. (eds Blume YB, Baird WV, Yemets AI, Breviario D) Plant Cytoskeleton: A Key Tool for Agro-biotechnology. Book Series: Nato Science for Peace and Security Series C - Environmental Security, pp 419-434

Yermishin AP, Makhan'ko OV, Voronkova EV (2006) Application of somatic hybrids between dihaploids of potato *Solanum tuberosum* L. and wild diploid species from Mexico in breeding: Generation and backcrossing of dihaploids of somatic hybrids. Russ J Genet+ 42(12):1414-1421

Yermishin AP, Makhan'ko OV, Voronkova EV (2008) Production of potato breeding material using somatic hybrids between *Solanum tuberosum* L. dihaploids and the wild diploid species *Solanum bulbocastanum* Dunal. from Mexico. Russ J Genet+ 44(5):559-566

Zlesak DC, Thill CA (2004) Foliar resistance to *Phytophthora infestans* (Mont.) de Bary (US-8) in 13 Mexican and South American *Solanum* species having EBNs of 1, 2, and 4 and implications for breeding. Am J Potato Res 81(6):421-429

3.2 Isolation, cultivation and fusion of protoplasts

3.2.1 Navrátilová B, Gajdová J, Skálová D, Greplová M, Vyvadilová M, Klíma M (2006) Electrofusion of protoplasts in selected vegetables of *Brassica*, *Cucumis* and *Solanum* genera. Acta Horticulturae (ISHS) 725:801-805

3.2.2 Greplová M, Polzerová H, Kreuz L (2007) Protoplast fusion by electric field at the model material *S. tuberosum* L. Scientific studies – Potato Research Institute Havlíčkův Brod 15:71-83

3.2.3 Greplová M, Polzerová H (2007) Shortening of time of the plant regeneration from protoplast-derived calli in genus *Solanum*. Scientific studies – Potato Research Institute Havlíčkův Brod 15:61-69

3.3 Creation of breeding materials through protoplast fusion of *S. tuberosum* and wild species *S. bulbocastanum* and *S. pinnatisectum*

3.3.1 Greplová M, Polzerová H, Vlastníková H (2008) Electrofusion of protoplasts from *Solanum tuberosum*, *S. bulbocastanum* and *S. pinnatisectum*. Acta Physiol Plant 30:787-796

3.4 Creation of breeding materials through asymmetric protoplast fusion of *S. tuberosum* and *S. verrucosum*

3.4.1 Polzerová H, Greplová M (2008) Introduction to cybridization between *Solanum verrucosum* and *Solanum tuberosum*. Scientific studies - Potato Research Institute Havlíčkův Brod 16:65-75

3.5 Evaluation of somatic hybrids in *in vitro* conditions

3.5.1 Polzerová H, Patzak J, Greplová M (2010) Early characterization of somatic hybrids from symmetric protoplast electrofusion of *Solanum pinnatisectum* Dun. and *Solanum tuberosum* L. Plant Cell Tiss Org DOI: 10.1007/s11240-010-9813-6

3.5.2 Greplová M, Polzerová H, Kreuz L (2009) Evaluation of *Solanum bulbocastanum* (+) *S. tuberosum* cv. Korneta somatic hybrids. Scientific studies - Potato Research Institute Havlíčkův Brod 17:47-53

3.6 Acclimatization and evaluation of somatic hybrids in *in vivo* conditions

3.6.1. Polzerová H, Greplová M (2009) Acclimatization of somatic hybrids and plantlets after chromosome doubling in *Solanum* genus. Acta Horticulturae (ISHS) 812:337-342

3.6.2. Greplová M, Polzerová H (2009) Analysis of the field performance of somatic hybrids. Scientific studies - Potato Research Institute Havlíčkův Brod 17:37-45

3.7 Characterization of new breeding materials in light of resistance to *Phytophthora infestans*

3.7.1 Polzerová H, Greplová M, Mazáková J (2009) Petri dish test for confirming *Phytophthora infestans* resistance compared with Laboratory assay. Scientific studies - Potato Research Institute Havlíčkův Brod 17:83-92

3.8 Creation of breeding materials through simultaneously solved biotechnological approach – *in vitro* polyploidization

3.8.1 Greplová M, Polzerová H, Domkářová J (2009) Intra- and inter-specific crosses of *Solanum* materials after mitotic polyploidization *in vitro*. Plant Breeding 128:651-657

4. CONCLUSIONS

The recapitulation of somatic hybridization and backcrossing of somatic hybrids is provided in the review. The particular information related to this biotechnological approach in the creation breeding materials can be found in the introductions of ten inserted articles. Although somatic hybridization is a method that has been known and used for nearly three decades for *Solanum* genera, it remains a biotechnological approach for overcoming sexual hybridization barriers and a tool for resistance transfer from wild relatives.

Protoplast isolation, cultivation and fusion using an electric field were the first part of this work. The best donor plants were obtained on media supplemented with $AgNO_3$ and Alar 85 (2,2-dimethylhydrazid kyseliny jantarové, Chemtura Europe B.V., Nizozemí). A single day cold and dark pre-treatment turned out to be advantageous due to unification of cell cycle. The protoplasts affected this way started cell wall regenerations earlier. The parameters of the electric fields were optimised with regard to a survival of fused protoplasts. The model fusion was designed for assurance that these parameters were suitable for efficient yield of hetero-fused protoplasts. The cultivation media were adopted and modified. Application of higher doses of zeatine proved advantageous to organogenesis.

The fusions between *S. tuberosum* and wild *Solanum* species (*S. pinnatisectum* and *S. bulbocastanum*) were carried out using optimised parameters. The obtained somatic hybrids were analysed by flow-cytometry and RAPD. Flow-cytometry proved a very good pre-selection system for regenerated plants. In spite of this finding analysis of DNA was essential.

The asymmetric somatic hybridization was included to prevent of transferring whole wild genome into somatic hybrids. UV light was employed to fragment donor nuclear genome (wild parent) and iodoacetic acid or iodoacetamide were used to eliminate organelles of recipient parent (cultured potato). Only hetero-fused protoplasts were able to regenerate cell walls and consequently undergo cell divisions and create calli. Four asymmetric somatic hybrids *S. tuberosum* + *S. verrucosum* were obtained from the described experiment.

The somatic hybrids *S. tuberosum* cv. Bintje + *S. pinnatisectum* 8166 were evaluated under *in vitro* conditions. They were analysed for chloroplast DNA and mitochondrial DNA composition and their hybridity was validated using SSR (simple sequence repeat) chromosome specific primers. The mitochondrial composition of somatic hybrids was a combination of both parents, while chloroplasts segregated and no recombination was recorded. The morphology of somatic hybrids was intermediate between parents but three morphological types were observed in the population. Nine of somatic hybrids were highly resistant to *Phytophthora infestans* in *in vitro* test. Another population of somatic hybrids was evaluated under *in vitro* conditions. The somatic hybrids *S. tuberosum* cv. Korneta + *S. bulbocastanum* PI 243345 showed abnormalities in growth habit. These somatic hybrids grew poorly and had no prospect.

A few somatic hybrids were grown under field conditions. Two hybrid plants *S. tuberosum* cv. Bintje + *S. pinnatisectum* 8166 offered good yield, good tuber quality and moderate resistance to *P. infestans*. Six somatic hybrids *S. tuberosum* dihaploid 243 + *S. bulbocastanum* 8003 were significantly inferior to cultural parent. The unconvincing results for these plants could be attributed to very small seed tubers.

To assess somatic hybrids in terms of resistance to the pathogen *P. infestans*, a laboratory test in a Petri dish was designed. This test was a modification of the laboratory test of Vleeshouwers et al. (1999). The results of these tests correlated significantly. The testing procedure showed high levels of resistance for genotypes *S. pinnatisectum* 8166, *S. pinnatisectum* PI320342 *and* three somatic hybrids *S. tuberosum* + *S. pinnatisectum* (both *S. pinnatisectum* 8166 and *S. pinnatisectum* PI320342).

The aim of this work was to establish a methodology for somatic hybridization of *Solanum* genera and achieve interspecific somatic hybrid with higher level of resistance to the pathogen *Phytophthora infestans*. The research succeeded in obtaining the somatic hybrids. Some hybrids have promising perspective for resistant breeding programme.

References

Vleeshouwers VGAA, Dooijeweert van W, Keizer LCP, Sijpkers L, Govers F, Colon LT (1999) A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. Eur J Plant Pathol 105:241-250

5. SOUHRN (SUMMARY, in Czech)

IZOLACE, KULTIVACE A FÚZE PROTOPLASTŮ RODU *SOLANUM*

Kulturní brambor *Solanum tuberosum* L. náleží do skupiny rodu *Solanum*, jejíž velký počet druhů je začleněn do 19 sérií (Spooner a Hijmans 2001). Plané druhy rodu *Solanum* se díky nutnosti adaptovat se na nejrůznější ekologické podmínky svého místa původu (zejména střední a jižní Amerika) staly rezervoárem genů rezistence k biotickým a abiotickým vlivům (Masuelli a kol. 2009, Camadro a kol. 2004, Jansky 2006). U druhů rodu *Solanum* bylo stanoveno hypotetické číslo endospermu (EBN, Endosperm Balance Number), které předpovídá úspěšnost daného planého druhu v sexuální hybridizaci. Plané druhy s číslem EBN 1 jsou sexuálně izolované a k využití jejich genů je třeba aplikovat nejrůznější postupy, jako je přemosťovací křížení (bridge crossing), manipulace ploidie, hormonální ošetření, včasná extirpace zárodku (embryo rescue), zprostředkované opylování (mentor pollination) nebo somatická hybridizace (Jansky 2006).

Brambor hlíznatý *S. tuberosum* zůstává stále významnou složkou potravy. Zemědělská výroba proto usiluje o ekonomickou výhodnost produkce a kvalitní surovinu. Velmi závažným patogenem, který snižuje kvalitu a zvyšuje náklady, je *Phytophthora infestans*. Cílem šlechtitelských programů u bramboru je vnesení genů rezistence k tomuto patogenu právě z planých druhů pocházejících zejména z Mexika. Oblast centrální vysočiny v Mexiku je považována za centrum genetické diversity jak pro brambory tak pro patogena *P. infestans*. Plané druhy *Solanum* pocházející z Mexika prodělaly koevoluci s patogenem *P. infestans* a už delší dobu jsou známy jako zdroje R-genů. Ukázalo se však, že jsou také zdrojem kvantitativní, rasově-nespecifické resistence, která je vysoce efektivní, stabilní a trvanlivá (Grunwald a Flier 2005). Přes všechny nové výzkumy se však ukazuje, že oomyceta *P. infestans* díky různým mechanismům biologie a patogenity zůstává vážným problémem (Fry 2008).

Cílem této práce bylo shromáždit poznatky o problematice, zvládnout a zavést metodu somatické hybridizace u rodu *Solanum* pro vytváření nových šlechtitelských materiálů a touto cestou využít cenné genové zdroje, které by klasickými postupy byly nevyužitelné. V této práci byly použity diploidní plané druhy s EBN 1 tj. *Solanum bulbocastanum* a *Solanum pinnatisectum* a diploidní planý druh s EBN 2 *Solanum* *verrucosum*.

Dílčí úkoly se týkaly shromáždění poznatků o somatické hybridizaci u rodu *Solanum*, studia podmínek izolace protoplastů, kultivace a regenerace, dále studia podmínek pro fúzi protoplastů elektrickým polem včetně ověření jejich vhodnosti pro tvorbu somatických hybridů a rovněž studia podmínek pro asymetrickou somatickou hybridizaci. Práce si kladla za cíl získat somatické hybridy, ověřit vhodnou metodu k identifikaci somatických hybridů, somatické hybridy charakterizovat v *in vitro* podmínkách a v polních podmínkách a zhodnotit u nich rezistenci k *P. infestans*.

Výsledky práce jsou shrnuty v 7 kapitolách (přehled o somatické hybridizaci a 10 publikovaných článků). Osmá kapitola je krátkým rozšířením o problematiku mitotické polyploidizace *in vitro*, jakožto metody, která má k somatické hybridizaci vztah v souvislosti s tvorbou tzv. mikroprotoplastů.

Úvodní část práce krátce seznamuje s historií somatické hybridizace a shrnuje přehled výsledků v dané oblasti u rodu *Solanum* zejména v posledních letech.

První experimentální část se zabývá jednak podmínkami kultivace donorových rostlin, podmínkami izolace protoplastů, jejich kultivace a regenerace, dále studiem vlivu růstových regulátorů na časnější nástup organogeneze u kalusů protoplastového původu a v neposlední řadě fúzí protoplastů elektrickým polem. K ověření vhodnosti parametrů elektrického pole bylo použito modelového materiálu *S. tuberosum* cv. Bintje a *S. tuberosum* dihaploid 243. Získané regeneranty byly prověřeny pomocí průtokové cytometrie z hlediska ploidie a byly vyřazeni aneuploidi. Potenciální somatické hybridy byly prověřeny z hlediska hybridnosti pomocí RAPD (random amplification of polymorphic DNA).

Fúze protoplastů *S. tuberosum* (dihaploid 243, dihaploid 299, cv. Bintje) s protoplasty mexických diploidních planých druhů *S. pinnatisectum* 8166 a *S. bulbocastanum* 8003 byla stěžejní prací. Mezidruhová somatická hybridizace vedla k získání somatických hybridů *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje a *S. bulbocastanum* 8003 + *S. tuberosum* dihaploid 243. V této práci byla porovnána efektivnost průtokové cytometrie a RAPD analýzy k prokázání hybridnosti získaných regenerantů. Při kultivaci somatických hybridů ve skleníku se ukázalo, že morfologické charakteristiky, které jsou typické už v *in vitro* podmínkách, zůstávají zachovány i při kultivaci *ex vitro*.

Asymetrická somatická hybridizace sleduje potřebu snížit množství přenášeného genetického jaderného materiálu z planého druhu za účelem omezení nadbytečných nežádoucích vlastností. Práce se tedy zabývala také optimalizací tohoto postupu. Jsou zde uvedeny podmínky pro inaktivaci jak semiautonomních organel kulturního druhu pomocí jodooctové kyseliny (IOAA) nebo jodoacetamidu (IOA), tak podmínky pro inaktivaci jader planého druhu UV zářením. Asymetrická somatická hybridizace poskytla čtyři asymetrické somatické hybridy – potenciální šlechtitelský materiál - *S. verrucosum* PI161173 (UV) + *S. tuberosum* cv. Karin (IOA).

Somatické hybridy *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje byly charakterizovány v *in vitro* podmínkách podle morfologie listů, vitálnosti a typu cytoplasmy. Hybridnost kromě RAPD potvrdila také SSR (simple sequence repeat) analýza pomocí chromosomově specifických primerů. *In vitro* test rezistence k *P. infestans* rozčlenil skupinu somatických hybridů na tři kategorie. Devět somatických hybridů připadlo do kategorie rezistentních, 1 do kategorie vnímavých a zbývajících 21 do kategorie intermediární. Statistické hodnocení potvrdilo vliv ploidie i typu cytoplasmy na morfologii (celkový vzhled, tvar listů), vliv na rezistenci k *P. infestans* však prokázán nebyl ani v jednom případě. Druhou skupinou hodnocenou v podmínkách *in vitro* byly hybridy *S. bulbocastanum* PI243345 + *S. tuberosum* cv. Korneta. Přes vysokou výtěžnost nebyl žádný hybrid perspektivní z hlediska dalšího možného využití.

Součástí práce byla aklimatizace somatických hybridů a hodnocení vybraných zástupců v polních podmínkách. V polním experimentu bylo hodnoceno celkem deset hybridů - dva hybridy *S. pinnatisectum* 8166 + *S. tuberosum* cv. Bintje a osm hybridů *S. bulbocastanum* 8003 + *S. tuberosum* dihaploid 243. Byly u nich sledovány agronomické vlastnosti, vývoj rostlin během vegetace, napadení patogenem *Phytophthora infestans* a *Leptinotarsa decemlineata*, výnos, počet hlíz a jejich celkový dojem. Somatické hybridy se *S. pinnatisectum* měly některé charakteristiky srovnatelné nebo i lepší oproti kulturnímu rodiči. Somatické hybridy se *S. bulbocastanum* se neosvědčily - zdaleka nedosahovaly parametrů kulturního rodiče.

Pro hodnocení získaných somatických hybridů - šlechtitelského materiálu získaného využitím mexických planých druhů s potenciálem rezistence k *P. infestans* -byla potřebná spolehlivá a rychlá metoda hodnocení přenosu této rezistence. Za tím účelem byl zaveden test na Petriho misce, který byl navržen jako modifikace laboratorního testu podle Vleeshouwers a kol. (1999). Oba testy navzájem dobře korelovaly. Testování ukázalo, že *S. pinnatisectum* 8166, *S. pinnatisectum* PI320342 a tři symetrické somatické hybridy měly vysokou úroveň resistence k *P. infestans* ve srovnání s kontrolou (*S. tuberosum* cv. Asterix).

Výsledkem práce je zavedení metody somatické hybridizace, získání originálních šlechtitelských materiálů a zavedení spolehlivého modifikovaného testu pro hodnocení somatických hybridů v laboratorních podmínkách. Zkušenosti získané při této práci spolu se zkušenostmi z polyploidizace *in vitro* jsou předpokladem dalšího využití těchto technik při tvorbě nových šlechtitelských materiálů.

Literatura

Camadro EL, Carputo D, Peloquin SJ (2004) Substitutes for genome differentiation in tuber-bearing *Solanum*: interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and endosperm. Theor Appl Genet 109(7):1369-1376

Fry W (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. Mol Plant Pathol 9(3):385-402

Grunwald NJ, Flier WG (2005) The biology of *Phytophthora infestans* at its center of origin. Annu Rev Phytopathol 43:171-190

Jansky S (2006) Overcoming hybridization barriers in potato. Plant Breeding 125(1):1-12

Masuelli RW, Camadro EL, Erazzu LE, Bedogni MC, Marfil CF (2009) Homoploid hybridization in the origin and evolution of wild diploid potato species. Plant Syst Evol 277(3-4):143-151

Spooner DM, Hijmans RJ (2001) Potato systematics and germplasm collecting, 1989 – 2000. Am J Potato Res 78:237-268

Vleeshouwers VGAA, Dooijeweert van W, Keizer LCP, Sijpkers L, Govers F, Colon LT (1999) A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. Eur J Plant Pathol 105:241-250

6. ANOTHER AUTHOR’S PUBLICATIONS

6.1 Selected abstracts presented in Symposia/Conference

Greplová M (2003) Potato mesophyll protoplast isolation and protoplast fusion using electric field. In: Materialy Meždunarodnoj jubilejnoj naučno-praktičeskoj konferencii, Naučnyje trudy, Minsk, Belorussia, July 7-10, 2003, p 337 – 340

Navrátilová B, **Greplová M**, Vyvadilová M, Klíma M, Gajdová J, Skálová D (2004) Electrofusion of protoplasts in selected vegetables of *Brassica, Cucumis* and *Solanum* genus. In: Fari M.G., Holb I. (eds): Book of Abstracts, 5th Symposium *In vitro* Culture and Horticultural Breeding, Debrecen, Hungary, 12-17 September, 2004, p 223

Greplová M, Navrátilová B, Vyvadilová M, Klíma M, Gajdová J, Skálová D (2005) The wild species od the genus *Brassica, Cucumis* and *Solanum* in somatic hybridization. In: Book of Abstracts, XVII International Botanical Congress, Vinna, Austria, 17-23 July, 2005, p 647

Greplová M, Polzerová H, Vlastníková H., Kopecký D, Švecová R , Domkářová J (2006) Development of breeding material using somatic hybridization and polyploidization within *Solanum* genus. International Plant Breeding Symposium (IPBS), Mexico City, 20-25 August, 2006, p 176

Greplová M, Navrátilová B, Klíma M, Polzerová H, Vyvadilová M, Kreuz L (2006) Study of inactivation of protoplasts usable in cybridizations. The Science of Selection: Potato Breeding Methodology for the 21st Century, Carlow, Ireland, 20-22 November, 2006, 36

Greplová M, Polzerová H (2007) A study of asymmetric somatic hybridization in genus *Solanum*. 7th symposium in the series „Recent Advances in Plant Biotechnology“ Plant Biotechnology: Impact on high quality plant production, Stará Lesná, Slovak Republic, 10-16 June, 2007, Book of abstracts, p 47

Greplová M, Polzerová H, Kopecký D, Suchánková P, Švecová R and Domkářová J (2007) Use of mitotic polyploidization and somatic hybridization for creating of new breeding materials in *Solanum* genus. 7th symposium in the series „Recent Advances in Plant Biotechnology“ Plant Biotechnology: Impact on High Quality Plant Production, Stará Lesná, Slovak Republic, 10-16 June, 2007, Book of abstracts, p 33

Polzerová H, **Greplová M** (2007) Acclimatization of the somatic hybrids at *Solanum* genus. 3th International Symposium on Acclimatization and establishment of micropropagated plants, Faro, Portugal, 11–16 September, 2007, Book of Abstracts, p 121

6.2 Original papers (not related to the topic of the PhD thesis)

Lebeda A, Doležalová I, Valentová K, Dziechciarková M, **Greplová M**, Opatová H, Ulrichová J (2003) Biologická a chemická variabilita maky a jakonu. Chem listy 97:548-556

Viehmannová I, Fernández Cusimamani E, Bechyne M, Vyvadilová M, **Greplová M** (2009) *In vitro* induction of polyploidy in yacon (*Smallanthus sonchifolius*). Plant Cell Tiss Org 97:21-25

Polzerová H, **Greplová M**, Frček J (2010) *In vitro* multiplication of *Lepidium meyenii* Walp. (J Appl Bot Food Qual, in press)

6.3 Protocols

Greplová M, Polzerová H, Domkářová J (2008) Metodika fúze protoplastů elektrickým polem (u rodu *Solanum*). In: Potato Research Institute Havlíčkův Brod, s.r.o. (ed), Metodické postupy využitelné ve šlechtění, praktické informace č. 19, 13-26, Tisk Hermann a spol. Havlíčkův Brod.

Greplová M, Polzerová H, Domkářová J (2008) Metodika mitotické polyploidizace *in vitro* (u rodu *Solanum*). In: Potato Research Institute Havlíčkův Brod, s.r.o. (ed), Metodické postupy využitelné ve šlechtění, praktické informace č. 19, 5-12, Tisk Hermann a spol. Havlíčkův Brod.

Polzerová H, **Greplová M**, Domkářová J (2010) Metodika testu rezistence k *Phytophthora infestans* na Petriho misce (u rodu *Solanum*) 496-4/KÚ2010. In: Výzkumný ústav bramborářský Havlíčkův Brod, s.r.o. (ed), Metodické postupy využitelné ve šlechtění II, Praktické informace č. 29, 3-11, Tisk Hermann a spol. Havlíčkův Brod.

Greplová M, Greplová E, Polzerová H, Domkářová J (2010) Metodika asymetrické somatické hybridizace (u rodu *Solanum*) 496-3/KÚ/2010. In: Výzkumný ústav bramborářský Havlíčkův Brod, s.r.o. (ed), Metodické postupy využitelné ve šlechtění II, Praktické informace č. 29, 13-27, Tisk Hermann a spol. Havlíčkův Brod.