THE UTILIZATION OF MOLECULAR MARKERS IN RECENT YEARS AT SELECTED INTERSPECIFIC HYBRIDS OF *SOLANUM* SPECIES

VYUŽITÍ MOLEKULÁRNÍCH MARKERŮ V POSLEDNÍCH LETECH U VYBRANÝCH MEZIDRUHOVÝCH HYBRIDŮ DRUHŮ *SOLANUM*

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Vědecké práce – Výzkumný ústav bramborářský Havlíčkův Brod, 2024, *30*: 9–18

The selected wild *Solanum* species were chosen for crossing by means of traditional breeding methods. These wild species, maintained in the genebank of Potato Research Institute in Havlíčkův Brod, are a good source of resistance to various biotic and abiotic factors. Hundreds of hybrids from many combinations, which were arisen by reciprocal crossing procedures (wild species × variety or dihaploid), and parental genotypes were tested using of PCR-based methods. We obtained the crossing materials with wild species (*S. acaule, S. berthaultii* and *S. demissum*) with potato varieties. The hybridity of progeny from interspecific hybridization was assessed visually through morphological traits and RAPD method. Hybridity was detected only in some of the crossing combinations. Genotypes from seven wild species (*S. acaule, S. berthaultii, S. bulbocastanum, S. brevidens, S. demissum, S. pinnatisectum* and *S. verrucosum*) were evaluated through PCR analysis. The plant material was initially cultured *in vitro*, transferred to greenhouse conditions, and subsequently subjected to DNA isolation using the GenElute Plant Genomic DNA Miniprep Kit (Sigma). Selected DNA markers were tested and applied for further analysis of both diploid and tetraploid potato collections. Wild species carrying resistance genes are actively used in breeding experiments. The availability of precise genomic sequences facilitates faster and more targeted breeding, enabling the identification of promising crosses and indicating where genetic material exchange between the parental lines ("father" and "mother") should ideally occur. This approach allows breeders to determine at an early stage whether a hybrid possesses desirable traits, such as disease resistance.

crossing; resistance; RAPD method; genebank

INTRODUCTION

There is an ongoing effort to expand the gene pool of the new potato gene resources for resistance to pests and diseases through distant hybridization. Cultivated potato *S. tuberosum* (2n = 4× = 48) is a vegetatively propagated species with tetrasomic inheritance and a high level of heterozygosity. Several important traits strongly depend on intralocus interactions and genotypes, multiallelic loci are more advantageous than monoallelic or diallelic ones. Unfortunately, because of the close relationship between varieties, the genetic base of modern varieties is relatively limited. Conversely, wild *Solanum* species, most of which are diploid (2n = 2× = 24), are a significantly better source of allelic variability comparing standard varieties. They have many valuable characteristics, including resistance to biotic and abiotic stresses (CARPUTO *et al.,* 1997).

Solanum species have different ploidy level and vary in their EBN – (Endosperm Balance Number). EBN represents their „effective ploidy" and does not always correlate with the current chromosomal number. Another factor influencing their evolution, besides EBN, is the production of so-called 2n gametes. These gametes have the same number of chromosomes as somatic cells and result from modified meiosis, which affects specific stages and micro- and megasporogenesis (CARPUTO *et al*., 2003). Mutually merging of 2n gametes leads to the formation of a tetraploid genotype (LAPTEVA, 1988).

An option to overcome barriers in interspecific crosses is the use of doubled haploids which arise from the reduction in chromosome number in crop varieties and tetraploid genotypes of 2n = 4× = 48 to 2n = 2× = 24. The formation of primary dihaploids by interspecific crossing of tetraploid *S. tuberosum* clones and diploid *S. phureja* species is the most commonly used method. This induces parthenogenesis (HORÁČKOVÁ, 1989). Distant hybridization presents many challenges that geneticists and breeders must consider. Hybrids typically exhibit low fertility or complete sterility, and the inheritance of traits in the progeny is often more complex (KOVÁČIK *et al*., 1983).

DNA markers can be used to determine differences in genome structure based on differences (polymorphisms) in DNA sequences between individuals. DNA polymorphism can be detected using various methods. The most common techniques include RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats) or RAPD (Random Amplification of Polymorphic DNA). The latter three are based on PCR (Polymerase Chain Reaction). Since its development PCR has become a very popular method, with numerous modifications.

RFLP analysis requires relatively large samples and is technically demanding and time consuming (GEBHARDT *et al*., 1989). Similarly, AFLP analysis is a relatively challenging technique. Another effective and advanced method is SSR analysis, which involves short repeated sequences with codominant inheritance and high abundance and polymorphism. However, this approach requires knowledge of the DNA sequence to design appropriate

primers. These limitations are overcome with the RAPD method, which amplifies genomic DNA using short, random oligonucleotides (typically decamers). This results in the formation of various amplification products, forming a characteristic pattern on electrophoresis gels, which can be analyzed. RAPD is capable of detecting polymorphism in regions where the sequence, function or chromosomal location is unknown, with dominant inheritance of the analyzed traits (PENNER, 1996). The advantages of RAPD include its simplicity, speed, and minimal technical requirements, and the variability it detects is relatively high. Consequently, RAPD is widely used in the identification and study of genomes in various cultivated species, including rice (MARTIN *et al*., 1997), tomatoes, wheat, beans (BAI *et al*., 1997), carrot (NAKAJIMA *et al*., 1997) and others.

Regarding the *S. tuberosum*, the RAPD method has been developed and applied for many years. Among the first successful uses of RAPD to identify varieties were the works of DEMEKE *et al.* (1993) and HOSAKA *et al.* (1994) can be classified. Soon after, „fingerprinting" became part of the overall characterization of newly introduced varieties (LYNCH *et al*., 1994).

The gene bank at the Potato Research Institute Havlíčkův Brod includes a subcollection of „dihaploids", largely produced by the PRI, and a subcollection of „wild species of the genus *Solanum*," which were obtained from international collections of genetic varieties of potatoes. These include *S. acaule, S. demissum, and S. berthaultii.* These wild species have long been kept for experimental purposes, and also serve as starting material for breeding (HORÁČKOVÁ, 2003).

The main aim of this study was to evaluate interspecific hybrids obtained from conventional crossbreeding of wild species or variety × dihaploids using molecular methods. There are 22 wild tuber-bearing species and one non-tuber-bearing species in our collection. The goal of this part of the study was to use molecular markers to assess the resistance of selected genotypes of wild species in PRI potato gene bank.

Potatoes are one of the most important food crops globally. Therefore, improving their traits can have a significant impact. However, sequencing the potato genome is challenging because common potatoes are tetraploid, i.e., they have four sets of chromosomes, which complicates the assembly of a reference genome. Recent research has focused on a diploid potato with only one genome, the so-called homozygote, which simplifies DNA sequencing and comparison. This plant, *Solyntus*, was produced as part of Solynta's hybrid breeding program.

A reference sequence, chromosome assembly version 4.04, is now available (HARDIGAN *et al*., 2016). The precise genomic sequence enables faster and more targeted breeding, making it easier to identify promising crosses and determine the ideal points of genetic material exchange between the „father" and „mother." This approach allows breeders to assess early on whether a potato possesses the desired traits, such as resistance to specific diseases.

MATERIALS AND METHODS

Solanum hybrids

90 hybrids from 23 reciprocal crossings (wild species × variety or *S. tuberosum* dihaploids) and 19 parental genotypes were tested. Hybrid seeds were sown in the greenhouse, resulting plantlets with good root system were put in pots. Four seedlings (8 week old) were taken for DNA isolation. Varieties and dihaploids were planted in pots (4l of soil) and grown in the greenhouse in a standard manner. Wild plants were transplanted from *in vitro* conditions to a greenhouse. For both types of parental materials leaves were also taken for DNA isolation.

„*Solanum* wild species"

In vitro materials used in the experiment are long kept in the gene bank PRI Havlíčkův Brod. Forty-eight genotypes were selected from seven wild species (*S. acaule, S. berthaultii, S. bulbocastanum, S. brevidens, S. demissum, S. pinnatisectum* and *S. verrucosum). In vitro* plantlets were transferred to greenhouse conditions (Figs 1–6).

DNA isolation

DNA was extracted from aerial parts of plants cultivated in a greenhouse using a commercial kit GenElute Plant Genomic DNA Isolation Kit (Sigma).

RAPD method

Amplification reactions were carried out in volumes of 20 µl and contained 200 ng template DNA, 10 µl FastStart PCR Master (Roche), 3.2 µM primers (IDT) and water.

Table 1: Primers used

116	TACGATGACG	SC 10-4	TACCGACACC
131	GAAACAGCGT	P71	GCATCTACGC
184	CAAACGGCAC	P72	CGGCCACTGT
308	AGCGGCTAGG	HEL3	GACGCTATCG

RAPD reactions performed in a thermocycler XP Cycler (Bioer), which has been programmed following profile: 3 min initial denaturation at 94 °C; followed by 40 cycles of 1 min at 94 °C, 1 min 40 s at 37.5 °C and 2 min when 72°C; final step was 10 min at 72° C and subsequent cooling 4 °C. Electrophoresis was performed in 1.5% agarose gel (TBE buffer) with ethidium bromide (0.2 $\mu g.ml^{-1}$).

PCR reactions for the detection of selected resistance genes were performed according to KASAI *et al*. (2000), GEBHARDT *et al.* (2006) and KAZUYUKI *et al*. (2011).

RESULTS AND DISCUSSION

I.

In the year 2012 seeds from 23 combinations (wild species × variety, dihaploids) crossed in the year 2011 were sown in the greenhouse. In addition, parents have been planted. The cultivation was conducted in greenhouse conditions in a standard way. The isolation and analysis of DNA was carried out in 19 genotypes of the parental material, and 90 samples from 23 combinations of hybrid material. Hybridity of individual clones (hybrids) was initially assessed by comparing the plant habit, colour of flowers etc. with similar characteristics of parental plants. For example in hybrids that arose from breeding combinations of dihaploids 314 *S. tuberosum* × *S. berthaultii 00259* violet colour of flowers was recorded, although 314 (dihaploid *S. tuberosum*) is white coloured and *S. berthaultii* (OCHOA, 1990) has purple flower crown or bright purple colour. From each cross combination were selected 4-8 for DNA hybrids, for subsequent comparison of the RAPD profiles of resulting hybrids and their parents. At the combination of *S. demissum 00236* × Sophie and *S. demissum 00245* × Romanze only one hybrid was used for testing, because one plant only grew in these combinations. Ninety hybrids and 19 parental genotypes were tested with eight primers (see Materials and Methods). Hybridity was found only in 4 breeding combinations (dihaploid 314 *S. tuberosum* × *S. berthaultii 00259*, *S. demissum 00236* × Sophie, *S. demissum 00245* × Romanze, *S. acaule 00029* × Sophie). Interspecific hybrids were verified using selected DNA, primers are listed (Table 1). Best results (relative to the entire file tested in the production of interspecific hybrids have been shown in crossing combination of wild species *S. tuberosum* × dihaploids at diploid level – from one test crossing combination one interspecific hybrid was confirmed. In contrast, the material obtained from the crossing combination of wild species × species on the tetraploid level on the test material by 23 combinations, we only confirmed three interspecific hybrids. DOMKÁŘOVÁ *et al.* (2007) also dealt with interspecific hybridization in their work. From the crossing, which took place between *S. tuberosum* × wild species at the Potato Research Institute in the years 2004–2006, combination of dihaploid *S. tuberosum* × *S. berthaultii* was the most successful one. The results prove that the barriers existing in classical interspecific hybridization can be appropriately overcome by crossing. The obtained hybrid materials are used next year for re-crossing in accordance with CARPUTO *et al.* (1997). The main reason is that although the wild species of *Solanum* genus carry a desired resistance, the table value is very low. Advanced materials from this work are transferred to the PRI gene bank.

II.

The whole set was tested using DNA markers and supplemented with positive controls adequate to the origin of the monitored resistance gene. The marker detecting the *Ryadg* gene was selected first. The RYSC3 marker was used for the *Ryadg* gene (KASAI *et al.,* 2000). In addition, three DNA markers were used to detect the presence of resistance genes (*H1, GroI* and *Sen1*) in the genome of the samples tested (GEBHARDT *et al*., 2006), (BARONE *et al*., 1995). Since 2014, additional primer sets have been used (KAZUYUKI *et al*., 2011), a primer set for the *H1* specific resistance gene, a primer set for the *R×1* gene (potato virus X), a primer set for the *R2* resistance gene (late blight) and a primer set for the marker resistance to potato Y virus (*Rychc*).

In 2013, DNA isolation was performed on 184 hybrids from 42 combinations resulting from reciprocal crossing and 40 parental genotypes. In addition, DNA isolation was performed on 45 hybrids obtained and partially tested in the previous year. The *Ryadg* gene was not detected in the tested hybrids, the *H1* gene (marker TG689) was detected in 8 hybrids, the *Gro1* gene (marker Gro1-4) in 93 hybrids and the *Sen1* gene (marker N127) in 51 hybrids.

In 2014, DNA isolation was performed on 40 hybrids from 9 combinations resulting from reciprocal crossing and 13 parental genotypes. Detection of the presence of resistance genes was performed only in 11 hybrids from 5 combinations. The *H1* gene using the TG689 marker was detected in 3 hybrids, using the N146 marker also in three, using the N195 marker in four. The *Gro1* gene (marker Gro1-4) in all 11 hybrids, the *Sen1* gene (marker N127) in 10 hybrids, the *R×1* gene (PVX marker) in three, the *R2* gene (marker R2-800) in four and the *Rychc* gene (marker Ry186) were not found in any hybrid.

In 2015, DNA isolation was performed on 152 hybrids from 41 combinations resulting from reciprocal crossing (wild species × variety, dihaploid *S. tuberosum*) and 38 parental genotypes. The *H1* gene using marker TG689 was detected in 14 combinations, the *Gro1* gene (marker Gro1-4) in 20 combinations and the *Sen1* gene (marker N127) in 17 combinations and the *Rysto* gene (marker GP122) in 29 combinations. The *R2* gene (marker R2-800), the *R×1* gene (PVX marker) and the *Rychc* gene (marker Ry186) were not detected in any combination.

In 2016, DNA isolation was performed on 36 hybrids from 11 combinations that resulted in reciprocal and 17 parental genotypes. The *H1* gene using the TG689 marker was detected in 3 hybrids, the *Gro1* gene (Gro1-4 marker) in 29 hybrids and the *Sen1* gene (marker N127) in 24 hybrids, the *R×1* gene (PVX marker) in none, the *R2* gene (marker R2-800) in three, the R*ysto* gene (marker GP122) in 27 and the *Rychc* gene (marker Ry186) were not detected in any hybrid.

In 2017, DNA isolation was performed on 111 hybrids from 30 combinations that resulted in reciprocal and 26 parental genotypes. The *H1* gene using marker TG689 was detected in 13 combinations, the *Gro1* gene (marker Gro1-4) in 21 combinations and the *Sen1* gene

(marker N127) in 16 combinations and the *Rysto* gene (marker GP122) in 27 combinations. The *R2* gene (marker R2-800), the *R×1* gene (PVX marker) and the *Rychc* gene (marker Ry186) were not detected in any combination.

In 2018, DNA hybrids were performed on 164 hybrids from 45 combinations resulting from reciprocal crossing and 33 parental genotypes. The *H1* gene using marker TG689 was detected in 13 combinations, the *Gro1* gene (marker Gro1-4) in 21 combinations and the *Sen1* gene (marker N127) in 16 combinations and the *Rysto* gene (marker GP122) in 27 combinations. The *R2* gene (marker R2-800), the *R×1* gene (PVX marker) and the *Rychc* gene (marker Ry186) were not detected in any combination.

In 2019, DNA hybrids were performed on 150 hybrids from 39 combinations resulting from reciprocal crossing and 27 parental genotypes. The *H1* gene using marker TG689 was detected in 13 combinations, the *Gro1* gene (marker Gro1-4) in 21 combinations and the *Sen1* gene (marker N127) in 16 combinations and the *Rysto* gene (marker GP122) in 27 combinations. The *R2* gene (marker R2-800), the *R×1* gene (PVX marker) and the *Rychc* gene (marker Ry186) were not detected in any combination.

In 2020, DNA isolation was performed on 93 hybrids from 24 combinations resulting from reciprocal crossing and 18 parental genotypes. The *H1* gene using marker TG689 was detected in 69 hybrids, the *Gro1* gene (marker Gro1-4) in 78 hybrids and the *Sen1* gene (marker N127) in 59 hybrids and the *Rysto* gene (marker GP122) in 70 hybrids. The *R2* gene (marker R2-800), the *R×1* gene (PVX marker) and the *Rychc* gene (Ry186 marker) were not detected in any of them.

In 2021, DNA isolation was performed on 41 hybrids from 12 cross-breeding combinations and 14 parental genotypes. The *H1* gene using marker TG689 was detected in 32 hybrids, the *Gro1* gene (marker Gro1-4) in 29 hybrids and the *Sen1* gene (marker N127) in 25 hybrids and the *Rysto* gene (marker GP122) in 29 hybrids. The *R2* gene (marker R2-800) was detected in 4 hybrids and the *R×1* gene (PVX marker) in 28 hybrids,

Hybrid materials were successfully obtained using wild species of the *Solanum* genus, which possess a high level of genetic diversity for economically important traits, such as resistance to potato late blight, and table quality. These materials will be tested again next year for specific traits, undergo morphological evaluation, and be used for further backcrossing. Selected promising materials have been incorporated into breeding programs with the goal of creating new potato genotypes.

As part of the characterization of the selected materials for their use for marker assisted selection (MAS), we applied appropriate DNA markers that can reliably identify the target gene. This approach provides an effective tool to replace provocation tests. Once the desirable traits have stabilized, the promising materials will be transferred to the gene bank at the Potato Research Institute in Havlíčkův Brod.

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Fig. 1: *Solanum. brevidens*

Fig. 2: *Solanum pinnatisectum*

Fig. 3: *Solanum verrucosum*

Fig. 4: *Solanum acaule*

Fig. 5: *Solanum berthaultii*

Fig. 6: *Solanum demissum*

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VYUŽITÍ MOLEKULÁRNÍCH MARKERŮ V POSLEDNÍCH LETECH U VYBRANÝCH MEZIDRUHOVÝCH HYBRIDŮ DRUHŮ *SOLANUM*

Vědecké práce – Výzkumný ústav bramborářský Havlíčkův Brod, 2024, *30*: 9–18

Vybrané plané druhy *Solanum* byly vybrány pro křížení pomocí tradičních šlechtitelských metod. Tyto planě rostoucí druhy, uchovávané v genové bance Výzkumného ústavu bramborářského v Havlíčkově Brodě, jsou dobrým zdrojem rezistence vůči různým biotickým i abiotickým faktorům. Metodami PCR byly testovány stovky hybridů z mnoha kombinací, které vznikly recipročním křížením (plané druhy × odrůda nebo dihaploid) a rodičovské genotypy. Získali jsme kříženec planých druhů (*S. acaule, S. berthaultii* a *S. demissum*) s odrůdami brambor. Hybridnost potomstva po mezidruhové hybridizaci byla vizuálně kontrolována morfologickými znaky a metodou RAPD. Hybridnost byla zjištěna pouze u některých hybridů z kombinací křížení. Pomocí PCR byly testovány genotypy ze sedmi planých druhů (*S. acaule, S. berthaultii, S. bulbocastanum, S. brevidens, S. demissum, S. pinnatisectum* a *S. verrucosum*). Rostlinný materiál byl převeden z podmínek *in vitro* do skleníku a tam pěstován. DNA byla izolována z těchto rostlin pomocí GenElute Plant Genomic DNA Miniprep Kit (Sigma). Byly vybrány a otestovány DNA markery. Vybrané markery byly použity pro následné testování diploidních a tetraploidních sbírek brambor. Pro experimenty s křížením se používají plané druhy nesoucí geny rezistence. Velmi přesná genomová sekvence umožňuje rychlejší a cílenější šlechtění, protože je snazší najít v DNA, která křížení s jinými odrůdami by mohla být zajímavá a kde by v ideálním případě měla probíhat výměna genetického materiálu mezi „otcem“ a „matkou“. To znamená, že šlechtitelé v rané fázi vědí, zda má genotyp požadované vlastnosti, jako je odolnost vůči konkrétním chorobám.

křížení; rezistence; metoda RAPD; genová banka

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