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MOLECULAR CHARACTERISATION OF APPLE VARIETIES AND IDENTIFICATION OF RESISTANCE GENE ANALOGS

PHD THESIS

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BACKGROUND AND OBJECTIVES

Apple is one of the most widely grown fruits in the world. A total of 71.7 million tons of apple was produced on 4.957 million hectares in 2009. China was the leader of his own more than 2 million hectares and more than 31 million tons of crop quantity (FAOSTAT). Europe's apple production is approximately 16 million tons on 1.25 million hectares. A total of 575.368 tons apple was produced on 36.644 hectares of apples in Hungary in 2009. Based on this data Hungary was the 7th according to apple quantity, ahead Romania, Spain and Austria as well (FAOSTAT 2010). With this volume of cropped area only grape proceeded apple production, however, the harvest volume were ahead 25 thousand tons of the 550 thousand tons of grapes in 2009. Over the past year, Hungary produced 894 thousand tons of fruit (without grapes) from which more than 60% was the apple's rate (FAOSTAT 2010).

From geographical point of view, the most accepted gene center is Alma-Ata t (Father of Apples) in Kazakhstan. In the previous literature, however, many other regions are mentioned. According to others the gene center is in Western-Asia, Central-Asia, Middle-East or Siberia. In the formation of European cultivars: European paradise apple (*Malus paradisiaca*), European apple (*M. sylvestris*), Transylvanian crab (*M. dasyphylla*), Caucasian apple (*M. orientalis*) and the early crab (*M. praecox*) were possibly involved. Apple species are characterized by high heterozygosity and considerable genetic variability.

Since apple is one of the most cultivated fruit, to solve problems related to its cultivation is a primary task. In our study we deal with solutions for problems in apple production related to plant breeding. During our research we focused on three major problems which will be presented separately.

1.) RESISTANCE GENE ASSOCIATED MARKERS

In fruit production the diverse pathogens and pests can cause a significant loss of yield. The chemical control is important and indispensable in some cases, however, the need to reduce the environmental chemical exposure as well as support technologies which cause no danger on human health are increasingly set forth.

In these efforts resistance breeding has a priority role since its aims to develop diseases and pathogens resistant cultivars. Further important breeding objectives include self pollination, eco-tolerance, fertility, yield, transportability, storage life and to improve the quality of fruit.

In case of resistance the main objectives include development of resistance in cultivars against the two most important fungal disease powdery mildew (*Podosphaera leucotricha*) and scab (*Venturia inaequalis*) as well as the bacterial disease fire blight (*Erwinia amylovora*). The most recent molecular strategies, such as development and application of resistance gene analog (RGA) markers serve this purpose.

Resistance genes of various plants show a high degree of structural conservatism against pathogens, they are the so-called nucleotide-binding site (NBS) or leucine-rich repeat (LRR) sequences. Primers designed for these types of sequences allow us to identify markers which are linked to resistance genes in the genome.

According to the related literature and the aforementioned results it seemed appropriate to identify and test markers which are closely linked to resistance genes in order to ascertain the presence or absence of resistance gene(s).

2.) MOLECULAR IDENTIFICATION OF HUNGARIAN APPLE GENBANK GENOTYPES AND COMMERCIAL VARIETIES BY SSR MARKERS

Increasing number of apple varieties (more than 15 thousand types of the world) requires clear and precise identification of individual genotypes and their good severability from other clones, varieties, etc. For differentation two main groups of marker are used, morphological and molecular markers (now is increasingly common in use). In case of morphological markers the phenotypically appearing inherited properties are often not informative and reliably enough and they can be affected by the environment. In contrast, molecular markers are not influenced by environmental and epistatic effects and their number is unlimited since, in principle, all form of genome and their combinations are suitable for identification.

According to the related literature and the aforementioned results it seemed appropriate to develop metholody which is suitable to determine “molecular fingerprint” in case of each variety studied as well as the use to determine their origin (pedigree).

3.) MOLECULAR DIFFERENTATION OF BUD MUTANTS

Bud mutations can also be the starting materials of new varieties. These mutations result phenotypic changes, such as fruit color or shape, size and shape of the tree, branching tendency etc. Bud mutants are basically considered as clones of the basic variety that only differ in one or a few mutant variant allel.

According to the related literature and the aforementioned results it seemed appropriate to search and test molecular markers and techniques which could be successfully applied to detect the slight genetic differences existing among bud mutants. For this purpose AFLP (Amplified Fragment Length polymorphism), SSR (Simple Sequence Repeats) markers and a new S-SAP (Sequence-Specific Amplified polymorphism) technique have been involved in our study.

Presence of retrotransposons is often revealed in bud mutations. S-SAP approach essentially is polymorphism detection of retrotransposon bordering DNA sequences which may allow the separation of molecular clones of each budmutant groups.

OBJECTIVES

1. Our purpose was to detect, analyze and test linked RGAs which are suitable to detect the presence or absence of resistance gene(s) in case of the two most important fungal diseases, powdery mildew (*Podosphaera leucotricha*), scab (*Venturia inaequalis*) and the bacterial disease fireblight (*Erwinia amylovora*).
2. Our purpose was to find SSR markers which are effective to prepare and detect microsatellite fingerprint from genotypes of Apple Genbank Hungary (40 cultivars and varieties). Also our goal was to compare dendrograms resulted by our 6 primers with the 9 SSR markers of the European standard set in case of 37 examined genotypes focusing on their affinity.
3. Our purpose was to find and test such molecular markers and techniques which can successfully detect mutations, their locations in the genome and differentiate among bud mutants.

MATERIALS AND METHODS

Plant material:

Young apple leaves used for DNA purification were collected at the Research and Extension Centre for Fruit Growing, Újfehértó, Hungary. Since the experiments have three main areas the choice of plant material was also different.

- Resistance gene associated markers (3 resistant and 4 susceptible cultivars)
- Molecular identification of Hungarian apple genbank genotypes and commercial varieties by SSR markers (40 varieties and 66 commercial cultivars)
- Molecular differentiation of bud mutants (26 bud mutants from 3-3 bud mutant types and groups)

DNA extraction:

DNA was isolated from young apple leaves with DNeasy Plant mini kit (Qiagen, Biomarker Kft., Gödöllő) according to the manufacturer's protocol.

After DNA isolation the approaches differed each of them are discussed separately:

RESISTANCE GENE ASSOCIATED MARKERS

Design of degenerated primers

From NCBI (National Center for Biotechnology Information) database 10 known resistance gene sequences isolated from different plant species were collected. Amino acid and nucleotide sequences were aligned with BioEdit software (version 7.0.5.3) (Hall et al. 1999). The most conserved regions – showing only small differences among plant species in their amino acid sequences – were selected and degenerate primers were designed based on their nucleotide sequences. Designed oligonucleotides were synthesized by Csertex and BioScience Ltd.

PCR reaction with degenerate primers

The PCR (Polymerase Chain Reaction) reactions were performed with a Perkin-Elmer GeneAmp 9700 instrument in a final volume of 20 µl. The reaction mixture included the following components: 40 ng template DNA; 1 x PCR buffer; 3 µl dNTP; 1.5 µl $MgCl_2$; 0.75-0.75 µl each of the forward and reverse primers; 1.2 U Red-Taq DNA polimerase (Sigma). The cycling profile consisted of an initial denaturation step for 2 min followed by 35 cycles of 10 seconds at 94°C, 30 seconds at 59°C and 60 seconds at 72°C. The amplification process was finished with 5 min extension step at 72°C.

Cloning fragments into plasmid vector

PCR products were cloned into plasmid vectors by PGEM T-easy vector system (Promega). For ligation the following components were measured: 2 µl PCR product, 1 µl Teasy vector, 5 µl buffer, 1 µl T4 DNA ligase and 1 µl ligase buffer. Mixture was incubated overnight at 16°C.

Transformation of competent cells

To transform competent cells, Qiagen EZ competent cells (stored at -70°C) were used: 1.5 µl ligatures were added to 200 µl competent cells. After incubation on ice for one hour heat shock was used (90 seconds at 42°C) and 800 ml SOC medium was added. This was followed by culturing at 37°C for one hour then samples were centrifuged for 1 min. Cells were suspended in 100 ml of fresh SOC medium then solid LB medium containing ampicillin (50

µg/ml) and selection marker (10 µl X-gal (50 mg/ml); 40 µl (0,1M IPTG) were used for blue-white selection.

Testing of recombinant bacteria - Colony PCR

From white colonies growing on selective LB a small part was taken with sterile toothpicks and was suspended in 50 µl sterile water. Then it was placed into boiling water for 5 minutes followed by a 1 minute centrifugation step. 4 µl of the supernatant was used as template for PCR reaction. The reactions contained the following components: 4 µl supernatant; 1 x PCR buffer; 3 µl dNTP; 1.5 µl $MgCl_2$; 0.75-0.75 µl of each forward and reverse degenerated primers; 1.2 U Red-Taq DNA polimerase (AgBiotech). The cycling profile consisted of an initial denaturation step of 2 min at 94°C followed by 35 cycles of 20 seconds at 94°C, 30 seconds at 62°C and 60 seconds at 72°C. The amplification process was finished with a 5 min polymerisation at 72°C.

Plasmid isolation

Plasmid was isolated from positive samples of colony PCR by Plasmid Miniprep kit (BioRad). Exact DNA concentration was determined by NanoDrop ND-1000 spectrophotometer. For sequencing the concentration was adjusted to 100 ng/µl, from which 5 µl (500 ng) was loaded to the capillary electrophoresis device.

Sequencing and sequenogram analysis

Sequencing reactions were carried out in MBK (Agricultural Biotechnology Center) by ABI Prism 310 (Applied Biosystems) device with the necessary degenerate primers for amplification. Chromas (version: 2.31) computer program was applied for chromatograms. BLAST (Basic Local Alignment Search Tool) analysis was performed at the NCBI website for sequence analysis.

Designing and testing of SCAR markers

For individual amplification of the previously obtained fragments SCAR (Sequence Characterised Amplified Region) markers were designed by Primer 3 software. After primer synthesis PCR reaction was started with SCAR primers. The PCR reaction differed from the previously described colony PCR only in the primers applied.

MOLECULAR IDENTIFICATION OF HUNGARIAN APPLE GENBANK GENOTYPES AND COMMERCIAL VARIETIES BY SSR MARKERS

Microsatellite analysis:

The PCR reactions were performed in a final volume of 20 µl, the reaction mixture contained the following components: 40 ng template DNA; 1 x PCR buffer; 3 µl dNTP; 1.5 µl $MgCl_2$; 0.75-0.75 µl of the forward and reverse SSR primers; 1.2 U Red-Taq DNA polimerase (Sigma). The cycling profile consisted of an initial denaturation step of 2 min followed by 35 cycles of 20 seconds at 94°C, 30 seconds at 57°C and 60 seconds at 72°C. The amplification process was finished with 5 min at 72°C. Microsatellite analysis was performed according to Galli *et al*. (2005) and Garkava-Gustavsson *et al.* (2008).

Molecular markers used for the study:

For microsatellite analysis fluorescent labeled primers were used as follows: CH03g07, CH04g10, CH04e03, CH05c02, CH05d11, CH05e03, CH01d03, CH01h02, CH02c06, CH02c09, CH02c11, CH02d08, CH04c06, CH04e05, COL (Liebhard *et al.* 2002).

ALF-Automatic Laser Fluorescent analysis:

PCR products were separated on a 5% denaturing polyacrylamide gel (Reprogel, GE Healthcare Biosciences, AP Hungary Kft., Budapest). Allele sizes were determined with ALFexpress II DNS analyzer (Amersham Biosciences, AP Hungary Kft., Budapest) using ALFexpress^{TM} sizer as a molecular weight standard and Alwin Fragment analyzer software.

Evaluation of allele size data:

For cluster analysis, each detectable allele was scored as present (1) or absent (0) in all varieties. This resulted in a 40 X 71 matrix in case of the 6SSR marker and a 37 X 87 binary matrix in case of European markers. When commercial cultivars were involved into the comparison a 105 X 78 matrix was applied for similarity detection. All earlier described alleles (Galli *et al.* 2005) were present in case of varieties, however, some extra alleles were determined only in varieties. When the two set (6 and 8 SSR) were examined together a 37 X 158 matrix was applied. European markers were applied in case of bud mutants (27 X 107 matrix) as well. For further analysis unweighted pair group method using arithmetic means (UPGMA) was used and dendrogram was constructed based on Jaccard's similarity

coefficients (Jaccard 1908) by the statistic software package SPSS 17.0 for Windows (SPSS Inc., USA).

Based on the frequencies of observed microsatellite alleles, Polymorphism Information Content (PIC) was calculated by the formula: $PIC=1-\sum pi^2$, where 'pi' is the frequency of the i*th* allele of each SSR marker (Anderson *et al.* 1993). It is essentially the same as the index of heterozygosity. To find the optimal combination and minimum set of markers for identification, 40 different varieties (N=40) were analyzed according to Tessier *et al.* (1999). The probability of identity (PI) of obtaining the same SSR profile for two randomly chosen apple cultivars was calculated by the: $PI = \prod_{j=1}^{j} C_j$ formula.

MOLECULAR DIFFERENTATION OF BUD MUTANTS

Microsatellite analysis:

The 9 applied pairs of oligonucleotide primers belong to the "standard set" defined by the European working group on apple genetic resources. The PCR reaction and the evaluation of alleles are identical with the previously descibed approach under the heading "Molecular identification of hungarian apple genbank genotypes and commercial varieties by SSR markers"**.**

AFLP analysis:

By using AFLP technique it is possible to detect one base pair difference. Restriction endonucleases (adaptors: *EcoRI 33, EcoRI 36, EcoRI 37, EcoRI 44, MseI 48, MseI 55, MseI 60, MseI 61*) were applied in order to increase sensitivity. Detection was performed in polyacrylamide gels.

S-SAP analysis:

Altogether, three 'Ret-LTR' primers were designed (Ret-LTR1: 5' – AAA TGG AGT GAC AGA CGG GT – 3', Ret-LTR2: 5' – GGA GGG TTT TGA GGG ATG TG – 3', Ret-LTR3: 5' – CCT TCG GGA TGG GGT GTG TC – 3') for the long terminal repeat (LTR) regions of all available apple retrotransposon sequences (NCBI IDs: AJ291492, DQ898280, AM167520) until the start of this project. These primers were labeled by Cy-5 and were used in S-SAP

analysis with a combination of pne AFLP primer. PCR products were diluted to 20x and used for selective amplification where one labeled Ret-LTR primer was used in combination with an adaptorspecific primer that included three additional selective nucleotides at the 3' end (Mse-48: 5' – ~ CAC – 3' vagy Mse-55: 5' – ~ CGA – 3' vagy Mse-60: 5' – ~ CTC – 3' vagy Mse-61: 5' – ~ CTG – 3' vagy Eco-33: 5' – ~AAG – 3' vagy Eco-36: 5' – ~ACC – 3' vagy Eco-37: 5' – ~ACG – 3' vagy Eco-44: 5' – ~ATC – 3').

RESULTS

RESISTANCE GENE ASSOCIATED MARKERS

The applied molecular approaches: bioinformatic analysis of NCBI database, design of degenerate primers and PCR reaction with degenerate primers. Cloning fragments into plasmid vector, then transforming competent cells. Testing recombinant bacteria - colony PCR. Plasmid isolation, sequencing and evaluation of sequences. SCAR marker design and testing.

We were looking for sequences that are identical in different plant species or at least show a high rate of homology. We were seeking conserved regions of plant resistance genes in NCBI gene bank for designing degenerate primers. Bioedit software was applied for alignment of different conserved regions from different plants.

With these primers we were able to amplify fragments from both TIR-NBS-LRR and CC-NBS-LRR type resistance genes. After designing the primers PCR reactions were started using isolated DNA from resistant and susceptible genotypes as template. Since it is well-known that in plant genome hundreds of this kind of sequence exists we expected that we might obtain fragments which appear only in resistant genotypes. Unfortunately, we could not detect any size difference. Same size fragments were obtained in high copy number for disease resistant and susceptible genotypes as well.

Subsequently, amplified fragments with TIR1 F-and P-loop-R degenerate primers were cloned into pGEM T-easy vector. For ligation PCR products were used in order to clone all sequences which have the same size but differ in sequence. From white colonies regrown in selective medium masterplate was created.

PCR was started with the same degenerate primers from colonies which remained white after a few days. With these primers amplification of the fragments was performed. With this approach we have propagated the unique cloned sequences.

Purified plasmid DNA was isolated from 12 colonies which were positive with colony PCR. For fragment sequencing T7 and SP6 universal primers were used. Their nucleotide sequences were converted into amino acid sequences by Bioedit software then similarity was examined with other RGA candidates at amino acid level. With BLAST application protein sequences which probably have an important role in various resistances were compared to our amino acid sequences.

The evaluation revealed that 5 RGA candidates (NCBI ID: EF455013-EF455017) do not contain any sequence motifs essential in resistance development according previous publications. These motifs are present in each resistance gene and RGA. However, in case of other 9 RGAs, the alignment proved that resistance patterns (P-loop, RNSB-A, Tyrosine kinase-2, RNSB-B,-C RNSB, GLPL) are present and show high level of homology at amino acid level with previously descibed RGA sequences.

In order to identify these RGAs, these were converted to SCAR markers. In all cases (resistant, susceptible subjects) - except for one genotype - SCAR markers reproduced the same size of fragment. Since plant genome contains even hundreds of sequences which contain the conserved regions that we tested, it is possible that the isolated fragments are responsible for the general resistance of plants.

Only in case of Arga-4 primer a fragment without difference between resistant and susceptible subjects was received. There was no amplified fragment in case of 'Remo' variety. As in the rest of resistant subjects amplified fragments were detected in all case, we conclude that a mutation might have happened at the primer hybridization site in case of 'Remo' and this is why Arga-4 SCAR marker is not successful for fragment amplification. Further tests are necessary.

MOLECULAR IDENTIFICATION OF HUNGARIAN APPLE GENBANK GENOTYPES AND COMMERCIAL VARIETIES WITH SSR MARKERS

Applied molecular techniques: selection of SSR primers and start of PCR reaction. Determination of allele sizes on polyacrylamide gel, statistical evaluations and dendrogram preparation.

Each of the applied 6 SSR generated reliable microsatellite alleles in all 40 genotypes (cultivars, varieties). A total of 71 polymorphic alleles were amplified (average 11.8 allele / locus) and average PIC values (0.8) of markers proved to be high. The repeated PCR reactions showed the same results.

The probability of that two randomly chosen varieties display the same genotype at all investigated loci was calculated to be very low (2.53×10^{-5} which means 1 : 39525). This confirms the high potential of SSRs for differenetation of apple varieties.

In the next step the similarity matrices were calculated from 66 previously described commercial cultivars (Galli *et al.* 2005) in order to compare the results with that of the varieties. In case of commercial varieties PIC values were very high, especially in case of CH04e03, CH05d11 markers. However, according to PIC values the examined varieties (40) were more different from each other (average PIC: 0.8) than the commercial varieties (average PIC: 0.72). The comparison also showed that there are many more similarities among varieties than among the tested commercial cultivars. The comparison of PIC results also shows that the ancestors of commercial cultivars presumably with a much smaller plant material have been selected since the average PIC values showed a smaller value than in case of varieties.

In the next step, "European Standard" marker set was adopted for 37 varieties. A total of 84 polymorphic alleles were amplified (average 10.5 allele / locus) and average PIC values (0.72) of markers proved to be high. CH02c06 marker proved to be the best with its 15 alleles for distinction while CH04c06 proved to be the weakest with its 4 amplified alleles. Two markers (CH0406 and CH01h02) proved to be multi loci.

CH05d11 (0.86) and CH05e03 (0.84) markers showed high PIC values comparing with markers from “European Standard” set markers. In our opinion, it would be worth to replace multi loci markers (CH01h02 and CH04c06) to marker CH05d11 and CH05e03 and this would increase the suitability of the series (average PIC would increase from 0.78 to 0.84).

When comparing the dendrogram results of the two marker sets, only ‘Újvári őszi’ and ‘Nyári fontos’ show high degree of resemblance. In case of other varieties, however, very significant differences observed. Results of the two analyses were not overlapping, only the extremely high homology can be detected in both cases. The technique – because of the results of the two different series – is not considered to determinate the relationship between species, however, as the results show, identification and isolation of varieties specifically suited to the SSR technique.

With all of these data a webbased databank were established – Hungarian Apple Microsatellite Databank (MAMA: *Magyar Alma Mikroszatellit Adatbank*) – containing all SSR allele sizes of the examined cultivars (both commercial and cultivars from this study). Since the aforementioned markers showed enough power to discriminate the cultivars we recommend to employ these for determining the differences and phylogenetic distances of various apple cultivars. The webbased databank may be useful for breeders, breeding agencies and propagate institutes to determinate cultivars since their work could be done easier and more controllable.

MOLECULAR DIFFERENTATION OF BUD MUTANTS

Applied molecular techniques: SSR, AFLP and S-SAP.

Results of fingerprints of varieties proved the reliability to differentiate apple genotypes using microsatellite markers. Based on these results, we tried to discriminate bud mutants molecularly using already described SSR markers. After the 6 set series of SSR markers (Galli et al. 2005) proved to be unsuitable for this purpose the new European SSR set (9 SSR markers) was involved in our experiment. However, we were not able to detect even a single base-pair difference within bud mutants by the high-resolution ALF Express II machine.

Subsequently, AFLP technique was applied which can detect one base pair difference. Different restriction enzymes were used in order to increase the resolution. In some cases, we were able to detect fragments successfully which were missing in other sports. However, SCAR markers converted from polymorphic AFLP fragments did not appear to be suitable for the separation of sports. The detectable differences remain unique and have non genotype-specific nature. In this way they were able to detect differences between individuals – which was not included among our objectives – and not between bud mutants. In conclusion, AFLP technique was not adaptable to detect polymorphism between sports.

Since neither SSR nor AFLP techniques were suitable to achieve our goal we involved S-SAP technique for trial. With the development of S-SAP it became possible to detect displacements of retrotransposons within the genome. This technology combines the resolution ability of AFLP technique with selected primer sequence specificity (in this case, the target sequences for LTR elements in the genome). Altogether 24 primer combinations (3 Ret-LTR 8 adaptor specific primers) were carried out, their PCR products were visualized. 11-71 multiple bands were produced (altogether 4368 with the 24 primer combination) suggesting that the copy number of these retrotransposon elements is relatively high in apple genome. Ret-LTR3 + EcoRI-33 primer combination produced the most while Ret-LTR2 + MseI-55 primer combination the less multiple band. Surprisingly, only one combination (Eco-33 and Ret-LTR1) was appropriate for identifying polymorphism between the progenitor ‘Jonathan’ and its bud mutants. In other cases, the mutants were indistinguishable from each other; differences were not found even between different individuals within the same somatic mutants. Converting the found bands into a more reliable SCAR marker is under progress.

NEW SCIENTIFIC RESULTS

RESISTANCE GENE ASSOCIATED MARKERS

- 14 candidate RGA fragments were cloned and successfully uploaded to the NCBI database. Out of these, 9 sequences after sequencing demonstrated that they are conservative regions of known partially homologous or homologous resistance genes. From this we concluded that we cloned and tested RGA candidates that may be involved in the development of resistance.

- From the examined 9-RGA markers 8 SCAR markers amplified fragments which were the same in both susceptible and resistant varieties. From this we concluded that we isolated and tested SCAR markers which are available in both resistant and susceptible varieties. They can be specific fragments of genes involved in the response against infection of plants.
- Only in one case (Arga-4 primer) we were not able to amplify fragment in resistant 'Remo'. Since in case of other cultivars we were able to amlify fragments we suppose that 'Remo' cultivar suffered mutations in the primer link region hereby designed Arga-4 SCAR primer can not amplificate.

MOLECULAR IDENTIFICATION OF HUNGARIAN APPLE GENBANK GENOTYPES AND COMMERCIAL VARIETIES BY SSR MARKERS

- Molecular fingerprints of six loci in case of 40 genotypes (varieties, landraces, and clones) were determined from Hungarian apple gene bank maintained at Újfehértó. Applied primer-pairs for were able to distinguish all six genotypes from each other.
- Molecular fingerprints of eight loci (European Standard Set) in case of 37 genotypes (varieties, landraces, and clones) were determined from Hungarian apple gene bank maintained at Újfehértó. The results demonstrate that the primers used were useful in molecular characterization of each item.
- Results from 6 SSR standard and 8 SSR markers were compared and based on this, we proposed to substitute 2 multi-loci markers (CH01h02 and CH04c06) from the standard set to markers (CH05d11 CH05e03) which had given the best results in case of 6 SSR markers. These can further improve average PIC values (0.78 -> 0.84).
- 37 genotypes (varieties, landraces) from the Hungarian apple gene bank collection were analized with two different series of primers (6 and 8 SSR primers). Dendrograms demonstrated that SSR method is indeed capable of distinguishing among species, however, is unsuitable to determine genetic distance of species.
- We took part in the creation and expansion of Hungarian Apple Microsatellite Database.

MOLECULAR DIFFERENTATION OF SPORTS

Molecular technique (SSR, AFLP) failed to discriminate between different apple clones and their progenitors. This may lead to the conclusion that the testing methods of the genome non-coding part are unsuitable for the molecular detection of differences between sports.

- The increasing number of experimental results indicates that retrotransposons are involved in the molecular background of bud mutants. With the use of retrotransposon sequence-specific approach (S-SAP method) we were able to differentiate between 'Jonathan' basic varietie from and bud mutants.

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Publications

I. Publications related to the thesis:

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1. **Wichmann B**, Galli Z, Molnár S, Galbács Z, Kiss E, Szabó T, Heszky L (2007). Molecular identification of old Hungarian apple varieties. International Journal of Horticultural Science 13 (3) 37-43.

2. Galli Z, **Wichmann B,** Molnár S, Galbács Z, Kiss E, Szabó T, Heszky L (2008). Molecular fingerprinting of apple sport mutants. International Journal of Horticultural Science 14 (3): 7–10.

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1. **Wichmann B**, Galli Zs, Kiss E, Szabó T, Heszky L (2008). Régi magyar alma tájfajták genetikai elkülönítése SSR primerek segítségével. Agrár és Vidékfejlesztési Szemle 3:(2) pp. 175-183.

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