PhD theses

Application of molecular markers for the detection of grapevine seedlessness and for relationship studies of *Rosa* L. taxa

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1 Preliminaries of the work, objectives

1.1 Introduction

In the 50 years passed since the discovery of the structure and biological function of the DNA in the 1950's, nucleic acid based examinations have been spreading widely. Currently, these methods are quite common and are applied by researchers working on various fields of science. These days, DNA-based molecular marker techniques offer many possible applications with diverse approaches and techniques.

The versatility of the topic is also represented in this work, where answers for various questions – molecular taxonomy and the marker of a phenotypical property – were being searched. Questions of different nature require different methodological approaches, which necessitate various methods of evaluation and interpretation of the obtained data. As a consequence, the range of data analysis methods is vast, depending on the question and the applied marker technique.

Molecular markers can provide valuable information for the plant breeder on multiple levels. Clarification of the origin and relationships of genotypes that can be considered for breeding purposes – and in general, species and varieties of a given genus – facilitate the design of crosses and the following selection. However, by completing the crosses, the work of a plant breeder is not finished, what's more, the real work begins from this stage. The application of molecular marker techniques for some properties of particular importance in the breeding program can accelerate, facilitate the complicated process of selection. In some cases – e.g. in the case of pyramiding resistance genes of different origins – such form of genotype selection can be accessed via molecular markers, which could have been completed by phenotypical evaluation only in an extremely time-consuming and wearisome manner.

Current work introduces two fields of the application of molecular marker techniques. The first is searching relationships based on genetic fingertips of Rosa species, the second is the detection of a targeted gene and property of grapevine using a linked marker.

Diversity of the genus Rosa in Hungary

The great species diversity of *Rosa* L. – and many other – genus can be explained by the geographical peculiarities of the country. Three climatic factors interact in the Carpathian Basin, and the frequent interspecific hybridization of rose species along these introgressive collision lines results in a variability of complex pattern and in unique species (FACSAR, 2002).

Taxonomical research in botany can be best characterized with the following saying "when two taxonomists congregate, three different opinions will be formed". This is especially true for genera of such a complicated past as the *Rosa*. Extended past and recent hybridization and centuries-long, significant human impact plus the presence of wide range of poliploidy can significantly affect the taxonomy of the *genus*. Species are variable, easily forming crosses with each other, which renders their distinction quite difficult (FACSAR, 1993; WISSEMANN, 2003; FACSAR, 2004; KOOPMAN *et al.*, 2008). This is why the formation of a unified taxonomy and the taxonomical classification of roses are very difficult. The different taxonomical works reflect the contrast of locally precise works meticulously processing the local flora and the general works considering a number of uncertain taxa as a single species.

Due to the complexity of morphological variability – including the differences induced by environmental effects – the undisputable taxonomical interpretation of characters can often be a demanding task. By using molecular markers, many problems can be eliminated. However, we possess insufficient information on how the genetic pattern induced by extended hybridization can be explained by AFLP markers. By obtaining detailed knowledge on the effects introgression processes are exerting on markers, such a methodical complex can be set which may be suitable for answering some current questions e.g. considering the *Vitis sylvestris* (does the species even exist?).

Detection of seedlessness in grape (*Vitis vinifera* L.) with SCAR-CAPS marker

Breeding of seedless genotypes is an essential need in table grape breeding, since the decisive majority of table grape varieties marketed on the world market are seedless, with only a few exceptions (SPIEGEL-ROY *et al.*, 1990; BOUQUET and DANGLOT, 1996). During the breeding of a new variety, seedlessness is only one of the demanded properties, the variety has to fulfil many other requirements, so an offspring population with a large number of individuals is a key factor. In some cases however, – depending on the cross combination – only a minor part of the individuals of the hybrid families are completely seedless (ROYTCHEV, 1998), and it is very expensive to grow up the seedlings till they turn productive. Therefore, a molecular marker suitable for the prediction of seedlessness in seedling age can be an effective tool in the hands of a breeder.

However the genetic background of the stenospermocarpic seedlessness, which is desired in the table grape breeding, is not fully explained, it can be assumed on the basis of the consensus formed in recent years that it is controlled by a dominant inhibitor (*SdI*) gene (BOUQUET and DANGLOT, 1996), and linked markers are available to this gene (LAHOGUE *et al.*, 1998). These needs to be tested however on each parent combinations and it must be examined whether the marker is suitable for the detection of the property in the offspring generation of the given crosses.

1.2 Objectives

AFLP examination of some representatives of the genus Rosa in Hungary

In the first part of the work I deal with the question that in what extent AFLP technique can be applied for the examination of the extended hybridization processes determining the evolution and present diversity of the genus *Rosa*. To answer this question I set the following objectives:

- Examination of relationships between individual rose taxa and taxon groups of Hungary, including many hybrids, with AFLP markers,
- Finding those data evaluation methods which are suitable for the exploration of the information of the data structure regarding to hybrid taxa.
- As an addition to the AFLP examinations, I set as an objective the preliminary assessment of the ITS polymorphism of the *Pimpinellifoliae* section.

Detecting the seedlessness of grape with DNA-based molecular marker

In the second part of the work, the aim of the examinations was the assessment of the inheritance and applicability of the marker linked to the dominant *SdI* inhibitor gene responsible for the stenospermocarpic seedlessness of grape, and the detailed description of the *SCC8* marker locus. For these purposes I set the following objectives:

- Identification of *SCC8* genotypes of parent pairs of three hybrid families,
- Testing the applicability of the published SCC8 SCAR-CAPS marker linked to seedlessness, in the above families,
- Molecular description of the *SCC8* marker locus, and its localization on the 'Pinot noir' genome.
- I also set as an objective the potential development and fine-tuning of the SCC8 marker.

2 Materials and methods

2.1 Plant material

2.1.1 The examined rose taxa

Rose taxa examined in this work were collected from the gene bank maintained in the Soroksár Botanical Garden of the Corvinus University, Budapest. In a part of the examined taxa I possessed information on the chromosome number of the given individual, which has been of great help during the evaluation of the obtained results.

The greatest emphasis during the selection of the examined individuals has been laid – due to their significant role in introgressive processes – on the representatives of the section *Caninae*. The section *Synstylae* was represented by *R. arvensis* originally assessed as an outgroup; section *Pimpinellifoliae* had 3, while section *Rosa* had 5 individuals, and I also examined a *Cinnamomeae* rose (*R. blanda*). Among the subsections of the *Caninae* section, representatives of the *Rubigineae* are in greatest number in the sample set, and I also included in the examinations some representatives of the subsections *Vestitae*, *Trachyphyllae* and *Caninae*.

During the selection of the individuals, great emphasis was laid upon the inclusion of some evolutionary conserved (e.g. [50, 54] *R. jundzillii*), recent (e.g. [23] *R.* × *francofurtana* F_1, [108] *R. vetvičkae*), and culture (e.g. [22] *R.* × *francofurtana*, [26] *R. gallica*, [65] *R.* × *damascena*) hybrids as well.

The greatest proportion of the samples was collected from the area of the Carpathian Basin: Hungary, Slovakia, Czech Rebuplic, however – as controls – some rose individuals from Germany, Bulgaria, and also a Greek and Italian individual were included.

The *Rosa* taxa applied during the examination of ITS-polymorphism were also collected from the Soroksár Botanical Garden of the Corvinus University, Budapest. Among the representatives of *Pimpinellifoliae* section, the subspecies level varieties of *R. pimpinellifoliae*, and a *R. myriacantha* and two *R.* × *reversa* individuals were included in the examinations. For the sake of comparability of polymorphism, two *R. gallica* individuals differing on subspecies level were also included.

2.1.2 Applied families for the examination of grape seedlessness with markers

For the purposes of examining the marker linked to the stenospermocarpic seedlessness of grape, two hybrid generations from the crosses of SZ. NAGY LÁSZLÓ, conducted at the Department of Viticulture of the legal predecessor of the Corvinus University, Budapest, and an offspring generation from the seedless table grape breeding program of KOZMA PÁL, conducted at the Research Institute for Viticulture and Oenology currently belonging to the University of Pécs were studied.

In the first case we conducted the phenotypical and molecular analysis of the offspring generation originating from the cross of CsÉT159 ('Augusztusi muskotály' syn. 'Palatina') × 'Superior Seedless' variety (TII series). The second examined generation originated from the cross CsÉT164 × 'Flame Seedless' (SI series). CsÉT164 originates from the cross of 'Seyve Villard 12375' and 'Olimpia'.

In the second case, I also examined a family (VRH 3082-1-42 [*V. rotundifolia* × *V. vinifera*] BC_4 × 'Kismis moldavszkij') originating from the breeding program of KOZMA PÁL. This family carries resistance genes against powdery mildew and downy mildew beside seedlessness.

2.2 Applied methods and techniques

2.2.1 Sample collection and DNA extraction

Fresh rose leaf samples were collected in the late spring and cooled to -196 °C in liquid nitrogen on the spot of sample collection, and stored at -20 °Cuntil processing. Among the families used for grape seedlessness examinations, in the case of TI and SII generations, matured shoots were collected and stored at -20 °C. In the case of BC_4 × 'Kismis moldavszkij' family, elder leaves were collected in autumn, and stored frozen until processing.

In case of rose samples included in AFLP examinations, DNA was extracted from the leaves, and the DNA of TI and SII grape families was extracted from the phloem of the frozen shoots by using Qiagen DNEasy Plant Mini Kit system (Qiagen) as accordingto the manufacturers instructions.

For *Cel*I examinations from young rose leaves, and in the case of elderly, autumnal leaves of the BC_4 × 'Kismis moldavszkij' family, DNA was purified according to the method of XU *et al.* (2004) with slight modifications.

2.2.2 AFLP examination

AFLP process has been conducted according to the directions of VOS *et al.* (1995), starting from 50 ng DNA. During selective amplification, four combinations of primers with fluorescens dyes (FAM or

JOE) have been used: *Eco*RI+ACT, *Eco*RI+ATG, *Mse*I+CAG, *Mse*I+CTT. The amplified resctriction fragments were detected with caplillary electrophoresis (ABI Prism 3100, Applied Biosystems). Results of the runs were captured with the `Genescan` software (Applied Biosystems) and transformed to a pseudo gel image by using the `Genographer` software. The gel pattern was transformed to a binary data matrix (1: present fragment, 0: absent fragment).

2.2.3 *Cel*I polymorphism of rose ITS fragments

The ITS region was amplified with PCR reaction from the genomic DNA. The PCR reaction was conducted among standard circumstances with 50 °C primer binding temperature, 1 minute extension time, with ITS5 (`5′-GGAAGTAAAAGTCGTAACAAGG-3′`) and P2 (`5′-CTCGATGGAACACGGGATTCTGC-3′`) primers.

The *Cel*I enzyme used for detecting the polymorphism of ITS- sequences were provided by KISS GYÖRGY BOTOND (Agricultural Biotechnology Center, Gödöllő).

PCR products were denaturated in themselves or mixed with a reference sample in 1:1 ratio on 94 °C, and incubated for 1 hour with *Cel*I enzyme on 37 °C. The cleavage product was separated with denaturating polyacrylamide gel electrophoresis on 12 % denaturating gel.

2.2.4 SCAR-CAPS marker linked to the seedlessness of grape

For the detection of seedlessness, a SCAR-CAPS marker developed by LAHOGUE *et al.* (1998) were used. The SCC8 primer pair (SCC8F `5′-GGTGTCAAGTTGGAAGATGG-3′` and SCC8R `5′-TATGCCAAAAACATCCCC-3′`) amplifies the $scc8^{-}$ and $SCC8^{+}$ alleles linked to the seeded *sdI* and seedless *SdI* alleles on standard PCR conditions. PCR reactions wwew conducted at 20 μl final volume by applying 60 °C primer binding temperature and 90 seconds chain extension time.

To 8,5 μl PCR product, 5 U *Bgl*II restriction endonuclease (Fermentas) and 1 μl 10$\times$ enzyme buffer has been given, and the mixture was incubated for 1 hour at 37 °C. The digested PCR product was separated with agarose gel electrophoresis in 1.2 % 1$\times$ TBE gel.

For sequencing, PCR products were purified directly from the reaction mixture in case of homozygote individuals or from gel bands in case of heterozygote individuals, and then ligated into pGEM-T Easy (Promega) TA cloning vector. Based on the results of test digestions, positive clones were sequenced by Biomi Kft. (Gödöllő). The processing of the sequences was conducted with the `EMBOSS` software package and `CLC Sequence Viewer` software.

3 Results

3.1 AFLP analysis of *Rosa* species

The AFLP examination of 40 rose samples with 4 selective primer combinations resulted in 327 polymorph loci. In 27 % of the loci, below 10 % fragment frequency was found, however by excluding these loci, the neither the stress value of the ordination, nor the topology of the trees and the bootstrap support changed significantly, so these loci were also included during data analysis.

During the distance calculation at phenogram building, the three examined metrics (Jaccard, Dice and Ochiai) gave practically the same results. The application of Ochiai method resulted in slightly lower stress values and presented more isolated groups at the 2-dimension display of the ordination, so I relied on analyses based on the Ochiai index for the evaluation of the data. In every case – by applying various metrics, 2 and 3 dimension NMDS – the ordination showed isolated groups of sections *Pimpinellifoliae* and *Rosa*, and also isolated *R. arvensis* (*Synstylae* section) used later as an outgroup quite well. *R. blanda* representing the *Cinnamomeae* section was placed on the edge of the point cloud containing the representatives of the *Caninae* section.

The consensus tree built from the bootstrap replicates of binary data (Figure 1) is going to be introduced bottom up. The (2) *Rosa arvensis* used as an outgroup is followed by the well-segregated clade of section *Pimpinellifoliae* with the (9) *R. myriacantha* (*syn. R. pimpinellifolia var. myriacantha*), (6) *R. pimpinellifolia*, (7) *R. pimpinellifolia var. spinosissima* taxa. Also in this group, the (80) *R. heckeliana* can be found, which is classified into subsection *Vestitae* of section *Caninae*. The segregation of section *Pimpinellifoliae* with 72 %, and the segregation of the group of the tree containing the remaining samples with 88 % bootstrap support value could be observed in all further examinations, therefore this taxonomical group can be considered justified.

The next group consists of members of section *Rosa* ([65] *R.* × *damascena*, [63] *R.* × *speciosa*, [42] *R. gallica* and [26] *R. gallica*). *Rosa* × *damascena* is a thousands of years old culture taxon, one of its parents was *Rosa gallica*, while [63] *R.* × *speciosa* is the hybrid of *R. gallica* and *R. jundzillii*. This clade also contains the group consisting of (22) *R.* × *francofurtana*, (119) *R.* × *kmetiana* and (50) *R. jundzillii* with 68 % bootstrap support. On the evidence of its morphological features, *Rosa kmetiana* is

a taxon of uncertain origin and position, while one of its parents is undoubtedly *R. gallica* (FACSAR, verbal communication). *R. gallica* is also the pollen parent of the *R. jundzillii* hybrid taxon settled in the geohistorical past, which is currently classified into subsection *Trachyphyllae* of section *Caninae*, based on its asymmetric canina meiosis.

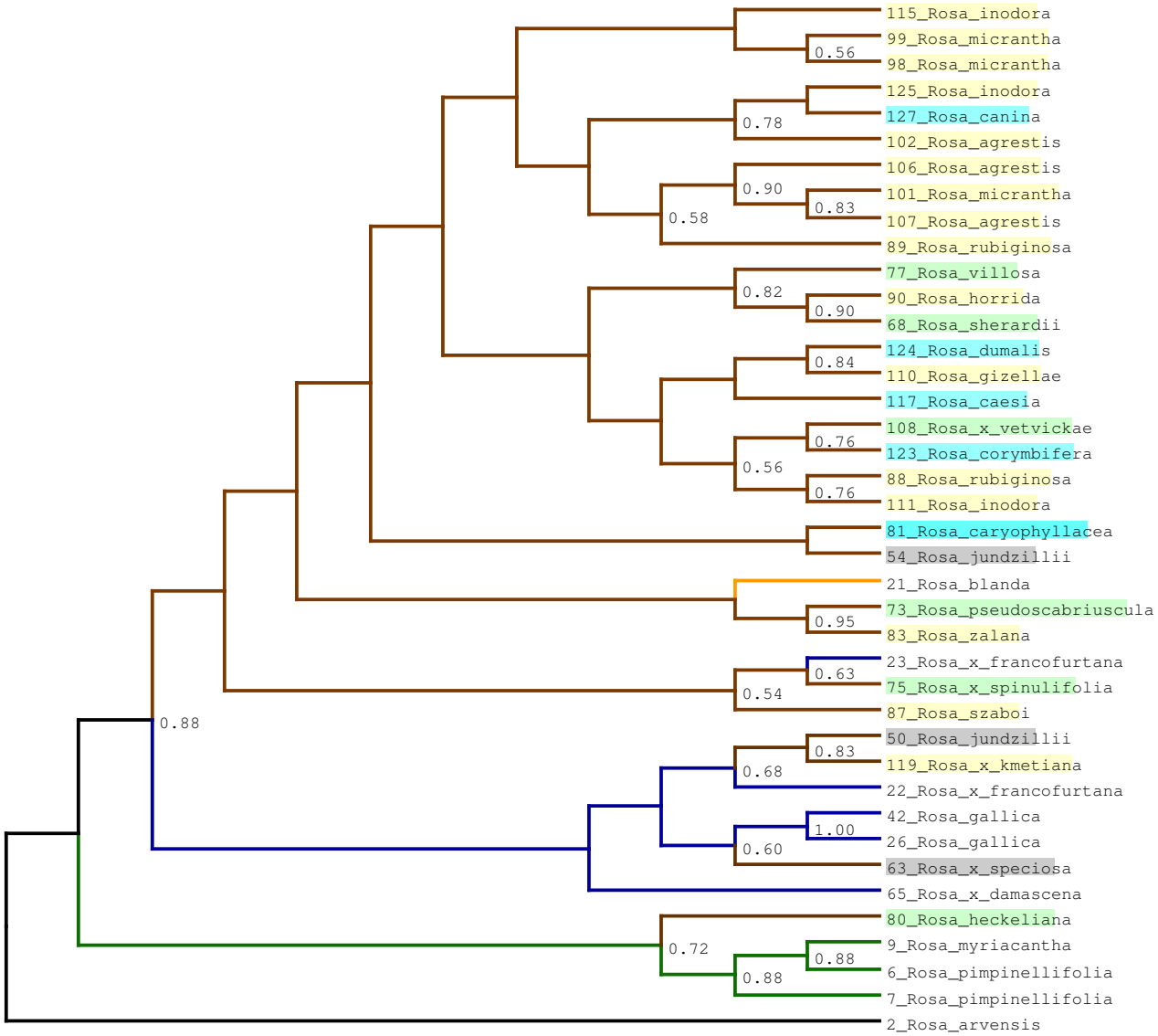

Figure 1: AFLP dendrogram showing the relationships of the 40 rosa taxa from the Carpathian Basin. The dendrogram shows the consensus of UPGMA trees calculated by Ochiai distance index from the bootstrap, 1000 times randomly reset AFLP data matrix. Supports of individual groups above 50 % are shown by bootstrap values displayed at the bifurcations. Colours of the branches show the section (black [*R. arvensis* outgroup]: *Sysntylae*, green: *Pimpinellifoliae*, blue: *Rosa*, orange: *Cinnamomeae*, brown: *Caninae*), and the colours of the background of taxon names indicate the subsections of the *Caninae* section (green: *Vestitae*, gray: *Trachyphyllae*, yellow: *Rubigineae*, blue: *Caninae*).

The greatest clade of the tree containing the remaining samples includes the members of the section called *Caninae* according to current nomenclature. The segregation inside the section does not show a clear image. Among the distinguished members of the recently joined *Caninae* section (POPEK, 1996, 2007), some taxa can be found with hardly interpretable positions. The bootstrap values of the greater groups are low, so far-reaching conclusions can not be drawn.

At the early branching clades of the tree, e.g. (87) *R. szabói* and (83) *R. zalana* can be found. These stand closer to subsection *Rubigineae*, however they were positioned on the tree beside taxa classified into subsection *Vestitae*. *R. szabói* was positioned to the first branch of section *Caninae* with 54 % bootstrap support, beside the open pollination seedling of (23) *R.* × *francofurtana* F_1 and (75) *R.* × *spinulifolia*. *R. zalana* shows a relationship of 95 % bootstrap support with a (73) *R. pseudoscabriuscula* sample. The only examined representative of *Cinnamomeae* section ([21] *R. blanda*) is also positioned in these early branching small groups. The (75) *R.* × *spinulifolia* is originated on one side from section *Cinnamomeae*.

The (81) *R. caryophyllacea*, (117) *R. caesia*, (123) *R. corymbifera*, (124) *R. dumalis* and (127) *R. canina* representing the *Caninae* subsection are positioned in the clade of the *Caninae* section in a scattered manner, the subsection shows a polyphyletic image.

The extraordinarily polyphyletic character of subsection *Vestitae* is an interesting phenomenon, however this can be caused by the hybridity of individual taxa belonging to this group. The (108) *R.* × *vetvickae*, which is supposedly a hybrid of *R. dumalis* and a taxon belonging to the *Vestitae* subsection forms a group of 76 % bootstrap support with the (123) *R. corymbifera* (= *R. dumalis* BECHST. pro parte) taxon. The (75) *R.* × *spinulifolia*, which is a hybrid of *R. tomentosa* and a taxon (*R. pendulina*) belonging to subsection *Cinnamomeae*, also shows a relationship with the groups of the supposed parent. The position of (73) *R. pseudoscabriuscula*, which is also a taxon of hybrid origin – but only one of the parents is known (*R. tomentosa* × ?) –, can be similarly interpreted.

The data set contained also such kind of individuals, which belong to the same species based on their morphologigal features, but their chromosome numbers are different. One of the taxa of such a kind is *R. gallica*: its individual with the number 26 is tetraploid ($2n = 4x = 28$), while individual number 42 is pentaploid ($2n = 5x = 35$). These two individuals were positioned very close to each other in every examinations and their bootstrap support is 100 % on the consensus tree. Another taxon of such a kind is *R. micrantha*: its individual number 98 is hexaploid ($2n = 6x = 42$) and supposedly a recent hybrid, while its individual number 99 is pentaploid ($2n = 5x = 35$), which is characteristic for the species. The similarity of these two individuals shows a lower bootstrap support (56 %), but they were classified together in all cases.

Due to their theoretical background, phenetic methods used for the evaluation of AFLP data are not capable of handling those groups which form a transition between two other groups, so “they can not deal with” the hybrid taxa, therefore I attempted to explore the structure of the dataset with other approaches. If a hybrid taxon simultaneously shows affinity towards both of its parent taxa, classic trees can not

display these relationships, because these trees classify every taxa hierarchically. During the bootstrap repetitions, the bootstrap support of the groups might be decreased because of such hybrids showing into two directions. Consensus tree networks however, can be highly suitable for the evaluation of such cases.

The minimal consensus network of the trees of bootstrap analysis is shown on Figure 2. The clearly segregating group of *Pimpinellifoliae* taxa can be observed here as well, the section functions practically as an outgroup. Section *Pimpinellifoliae* is followed on the tree by the segregating branches of *R. heckeliana* and *R. arvensis*, and the group containing the sections *Rosa* and *Caninae*. Section *Rosa* segregates quite well, similarly to the majority consensus analysis, and its role in the establishment

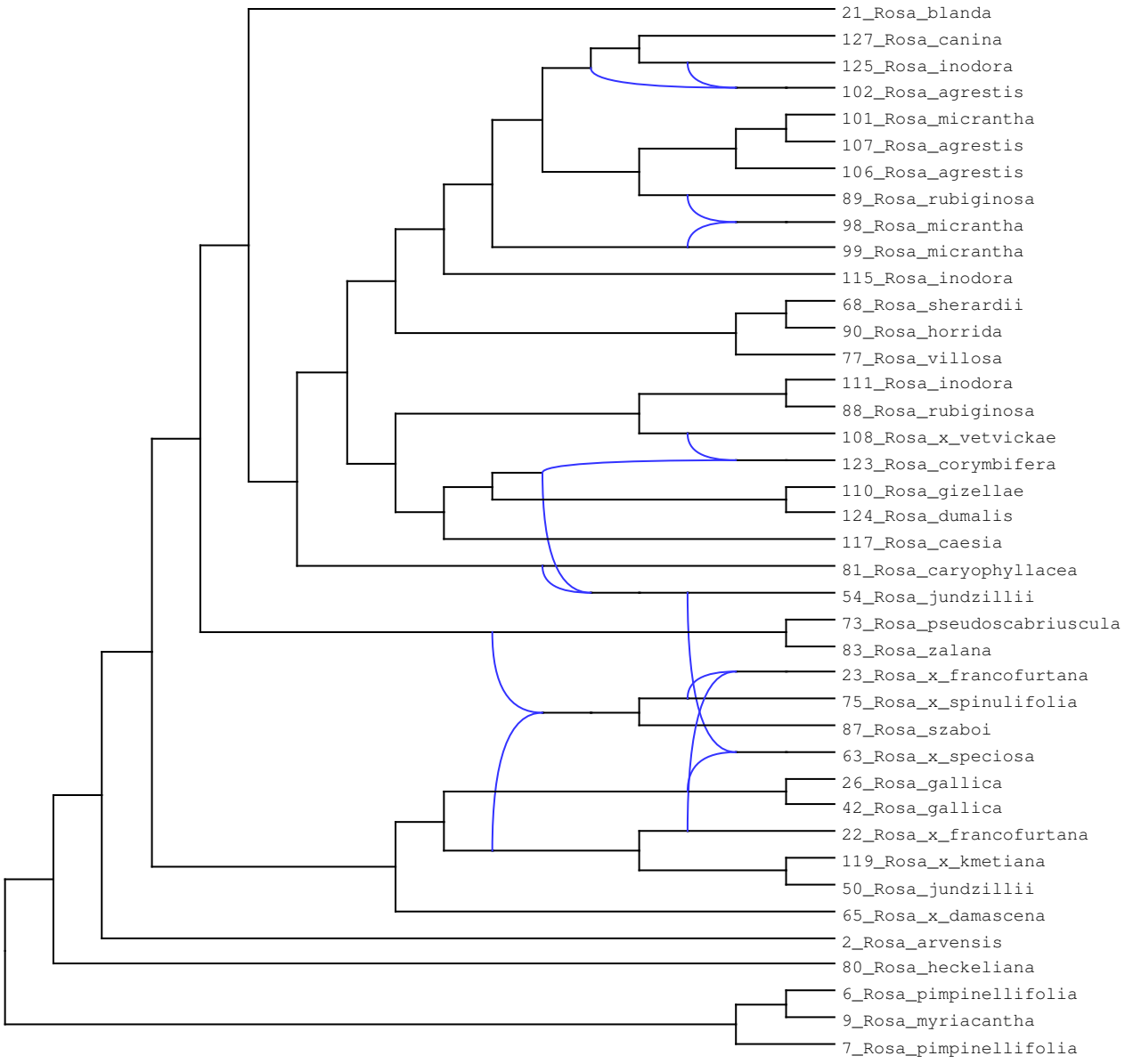

Figure 2: Consensus network of the bootstrap analysis. Minimal network consensus of UPGMA trees calculated from 1000 bootstrap resampling of AFLP data (with 20 % limit value).

of intersectional hybrids can be observed extremely well. Many hybrids, which were classified on the consensus tree in a "squeezed" manner, form a hybrid group between sections *Caninae* and *Rosa*. Such kind of hybrids are the (63) *R. speciosa*, the (87) *R. szabói*, (75) *R.* × *spinulifolia*, (23) *R.* × *francofurtana*; and the (83) *R. zalana*, (73) *R. pseudoscabriuscula*, (54) *R. jundzillii*, which are also actively participating in the hybridization processes according to the tree.

The (21) *R. blanda* (section *Cinnamomeae*) is also segregated form section *Caninae*. Members of subsection *Caninae*, ([81] *R. caryophyllacea*, [117] *R. caesia*, [124] *R. dumalis*) are more significantly segregated on the tree network than on the majority consensus tree. The (110) *R. gizellae* is positioned into this group, while (127) *R. canina* – which is not a typical *canina* rose – is being classified to section *Rubigineae*.

Based on the network consensus, it has been confirmed that a great proportion of the individuals in our *Vestitae* section sample set is of hybrid origin, therefore their polyphyletic character on the majority consensus tree can be explained by this feature. Subsection *Rubigineae* also shows a more uniform image.

The consensus network of bootstrap replicates supports the sections of classic rose systems and partly the segregation of the subsections of section *Caninae*.

Phylogenetic approach

Beside the phenetic evaluation of AFLP data, the examined samples were analyzed also from phylogenetic aspect. "Reticulate networks" (hybridization networks) are such a kind of networks, which are intended to display the role of hybridization in evolution. The hybridization network applies an evolution model for the building of the tree. In the hybridization network built according to the AFLP data (Figure 3) sections *Pimpinellifoliae* and *Rosa* are markedly segregated and a slight "improvement" can be observed at subsection *Caninae*, which turns to be monophyletic according the results of this analysis. Compared with earlier analyses, the position of subsection *Trachyphyllae* ([50] *R. jundzillii*, [54] *R. jundzillii* and [63] *R.* × *speciosa*) improved as well, however these do not form a unified clade.

Taxa representing the *Vestitae* section remain scattered on the phylogram, groups of hybrid origin do not show as clearly as on the network of phenetic trees (Figure 2).

The two *R. francofurtana* taxa (22 and 23) which were positioned segregated in phenetic analyzes show a common origin. The same phenomenon can be observed in the case of *R. micrantha* (98, 99, 101) and *R. agrestis* (102,106,107), and with the exception of some hybrid taxa ([119] *R. kmetiana*, [87] *R. zalana* and [83] *R. szabói*), subsection *Rubigineae* proved to be monophyletic.

The phylogenetic approach – and primarily the hybridization network – was capable to reveal many hereditary lines which could not be handled by the phenetic analysis (subsections *Rubigineae* and

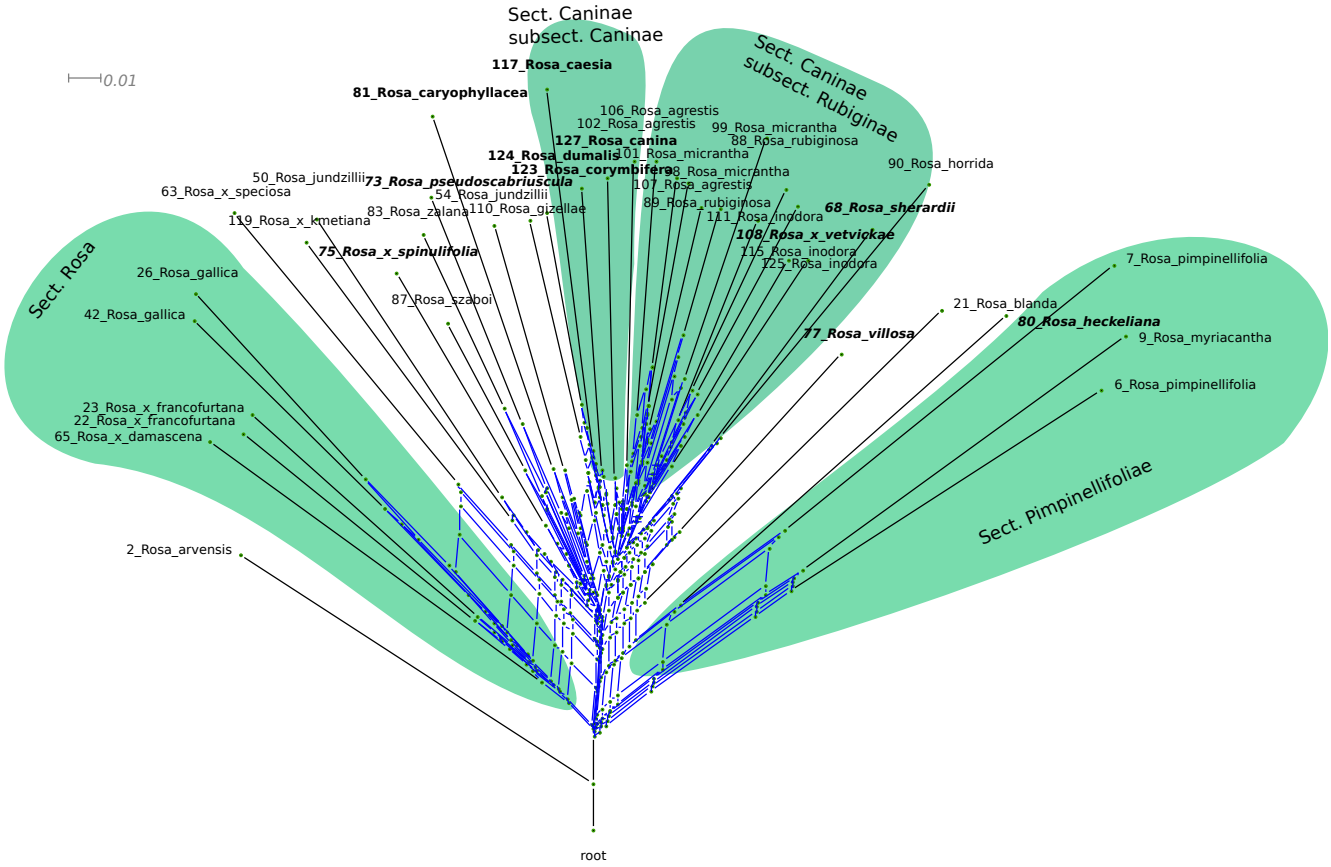

Figure 3: Evolution network of examined rose taxa built on the evoltution model based on hybridization. Members of subsection *Caninae* of section *Caninae* are displayed in bold, members of subsection *Vestitae* are displayed in bold and italic.

Canina), however the phenetic approach and the network consensus of UPGMA trees calculated from the bootstrap sampling highlighted taxa of hybrid origin extremely well.

3.2 Detection of grape seedlessness with SCAR-CAPS marker

3.2.1 SI and TII hybrid families

By the TII and SI hybrid generations originating from the crosses conducted by SZ. NAGY LÁSZLÓ, the *SCC8* SCAR-CAPS marker detected the phenotype of the examined individuals extremely well: when the marker detected seedlessness, the plants did not have seeds indeed, or just undeveloped seeds, while those plants which were detected to be seeded by the marker proved to be seeded indeed.

In the case of SI family, the seedless male line 'Flame Seedless' is described (ADAM-BLONDON *et al.*, 2001) as of $SCC8^{+}$/? genotype, so it carries beside the $SCC8^{+}$ allele either another dominant allele or a null-allele. The *SCC8* genotype of the CSÉT164 male line was formerly unknown, my examinations

show that it is of *scc8*$^{-}$/? genotype. The facts that individuals of *SCC8*$^{+}$/? genotype could be found in the offspring generation, and the large number of observed cases where the PCR step of the SCAR-CAPS marker was unsuccessful indicate that the CSÉT164 also contains a null-allele, so its *SCC8* genotype is *scc8*$^{-}$/0.

This assumption is supported by the fact that in the case of individuals with 0/0 genotype, PCR products were obtained by applying 50 °C primer binding temperature – therefore the DNA samples contained DNA that was suitable for PCR reaction –, these however contained only the cleaving sites at the end of the PCR product, and complete digestion could not be achieved in any of the cases. Based on these findings and the results obtained during the description of the *SCC8* locus (section 3.3), PCR products obtained with reduced primer binding temperature were amplified not from the 18. linkage group containing the *SdI* gene responsible for seedlessness, but supposedly from the 4. or 19. linkage groups.

By the TII family – in contrast with the SI family – it can be excluded that the male line carries a null-allele, since the genotype of 'Superior Seedless' *SCC8* is *SCC8*$^{+}$/*scc8*$^{-}$. However – similarly to the SI family – even in this hybrid family some samples were obtained that showed *SCC8*$^{+}$/? genotype on the gel, and some individuals could be observed, where the amplification of the *SCC8* locus was unsuccessful. The proportion of the types observed on the gel is closest to the basic situation where a 1:1 splitting ratio is obtained with the cross of a heterozygote and a recessive homozygote, but in this case the *SCC8*$^{+}$/? type should be considered as an artefact, and the 0/0 as a technical problem, even if the other families did not show any example of this phenomenon.

3.2.2 Seedlessness of the family VRH 3082-1-42 (BC_4) × 'Kismis moldavszkij'

In the multiresistant, seedless table grape breeding program conducted at the Research Institute for Viticulture and Oenology currently belonging to the University of Pécs, KOZMA PÁL uses the fourth backcross generation originating from *Vitis rotundifolia* as a source of resistance.

Since we did not possess preliminary information on the *SCC8* genotypes of the parents, the *SCC8* genotypes of varieties used most frequently in crosses (BC_4 resistance source, and 'Kismis vatkana', 'Kismis moldavszkij' seedlessness sources, further the 'Nimrang' variety) were examined.

The 'Sultanina' (syn. 'Thompson Seedless') variety – which is dominant homozygote to the *SdI* locus – was used as a control, it showed the genotype *SCC8*$^{+}$/*SCC8*$^{+}$ known from the literature. The 'Kismis vatkana' variety also proved to be homozygous, in this case however the possibility that this variety carries a null-allele beside the dominant *SCC8*$^{+}$ allele could not be excluded.

In case of the seeded 'Nimrang' variety, the *Bgl*II enzyme did not cleave the PCR product on the cleavage site linked to seededness. Since the size reduction of the PCR product, which is characteristic for the

other varieties – this is the result of the cleavage of the *Bgl*II enzyme in the 73. nucleotide position, which is also characteristic for the $SCC8^{+}$ and $scc8^{-}$ alleles – could not be observed, I assume that the PCR product does not originate from the appropriate locus (section 3.3). This assumption is supported by the fact that PCR products were obtained at the 'Nimrang' variety only by applying 50 °C primer binding temperature, in contrast with the 60 °C applied in other experiments.

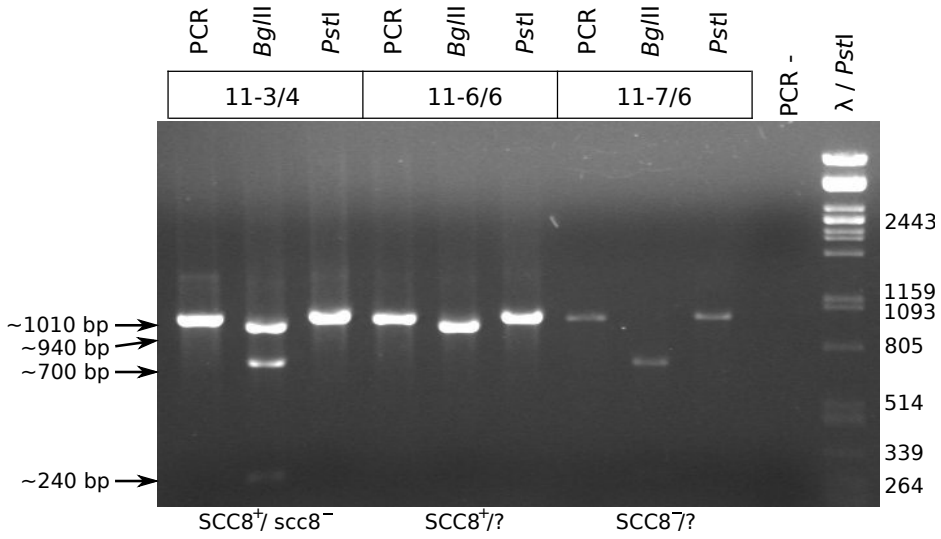

Figure 4: Typical examples of gel patterns observed in the BC_4 × 'Kismis moldavszkij' family with the SCC8 marker of seedlessness. PCR: PCR product without digestion; *Bgl*II and *PstI*: digested PCR product; PCR-: PCR null control; Size marker: *Pst*I-digested λ phage DNA.

Among the examined seedless varieties 'Kismis moldavszkij' was the only one which proved to be heterozygous to the *SCC8* locus with $SCC8^{+}/scc8^{-}$ genotype. BC_4 showed only the dominant allele of the locus. Since BC_4 is a seeded type, but its *SCC8* pattern shows a seedless image – therefore the variety is either a mutant for the cleavage site, or carries a recombinant *SCC8* allele –, the applicability of the marker in BC_4 crosses is limited. The marker can be valuably used only in those crosses, where the male line carrying seddlessness is heterozygous to the marker as well. Based on these preliminary findings, we started to examine the *SCC8* locus in the BC_4 × 'Kismis moldavszkij' family.

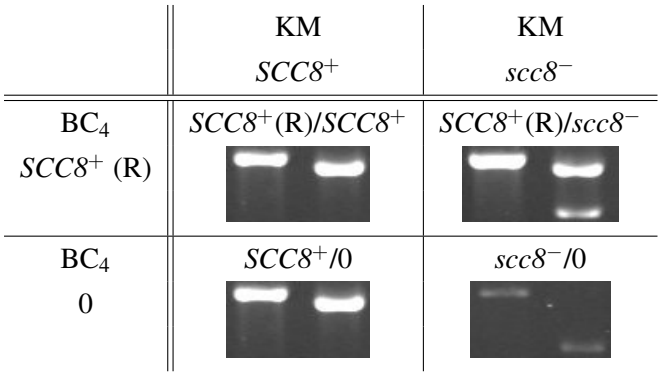

Figure 5: Punett square of the inheritance of *SCC8* locus in the BC_4 × 'Kismis moldavszkij' hybrid generation. KM: 'Kismis moldavszkij', $SCC8^{+}$ (R): the assumed recombinant or *Bgl*II cleavage site mutant allele of BC_4, 0: the assumed null-allele of BC_4. On the left of the gel sections the PCR product, and beside the result of *Bgl*II cleavage is displayed.

The gel pattern types observed in the BC_4 × 'Kismis moldavszkij' family are shown by Figure 4. Beside *SCC8*$^+$/*scc8*$^-$ heterozgotes (e.g. 11-3/4) and the *SCC8*$^+$/? "homozygotes" (e.g. 11-6/6) it was experienced in 13 cases, that the marker showed only the *scc8*$^-$ allele linked to the *sdI* allele responsible for seededness (e.g. 11-7/6, Figure 4) Since these individuals represented 17 % of the examined individuals of the family and proved to be seeded during phenotypical examination without exception, I assumed that the BC_4 was carrying a null-allele. According to the above-mentioned – assuming a "recombinant" and a null-allele in the BC_4 parent – a hypotethical Punett square was drawn (Figure 5). Beside the *SCC8*$^+$ and *scc8*$^-$ alleles of 'Kismis moldavszkij' which is heterozygous from the aspect of stenospermocarpic seedlessness, the recombinant (*SCC8*$^+$[R]) and the null-allele of BC_4 participate in crosses. According to the Punett square (Figure 5), individuals with gentotypes of *SCC8*$^+$/? (*SCC8*$^+$[R]/*SCC8*$^+$ and *SCC8*$^+$/0), *SCC8*$^+$/*scc8*$^-$ and *scc8*$^-$/0 should be obtained in a proportion of 2:1:1.

Seedlessness was inherited in the BC_4 × 'Kismis moldavszkij' generation in a 1:1 ratio, and the *SCC8* locus – in accordance with the drawn Punett square (Figure 5) – was inherited in a ratio of 2:1:1. Both segregations are supported by the χ^2 test (Table 1). The observed segregation of seedlessness and the marker in the BC_4 × 'Kismis moldavszkij' family supports the presence of a "recombinant" and a null-allele in the case of BC_4.

Table 1: Segregation ratios of stenospermocarpic seedlessness, and the proportion of genotypes observed at the *SCC8* locus in the BC_4 × 'Kismis moldavszkij' family.

	Seedless		Seeded		Total	Distribution	χ^2
	SCC8$^+$(R)/*SCC8*$^+$	*SCC8*$^+$/0	*SCC8*$^+$/*scc8*$^-$	*scc8*$^-$/0			
Phenotype	44		34		78	1:1	1.282
Genotype	44		18	13	75	2:1:1	2.919

3.3 Description of *SCC8* locus, marker development

For the sake of deeper understanding of the *SCC8* locus, first I localized it on the recently published (JAILLON *et al.*, 2007) "homozygous" 'Pinot Noir' genome. Allowing 15 % mismatch pairing, I amplified three loci from the 4., 18. and 19. linkage groups *in silico*, all of which were around 1 000 bp long (Figure 6). Based on the positions of the cleavage sites of *Bgl*II, the *SCC8* locus is located on the 18. linkage group. According to these the question arose, if the dominant marker (*SCC8*$^+$) of BC_4 was amplified from the appropriate locus, or from the 4. or 19. linkage group.

The locus on the 19. linkage group is not digested by the *Bgl*II enzyme, and since the size reduction of the PCR product in the BC_4 × 'Kismis moldavszkij' family was observed in every case (Figure 4),

I excluded the possibility that SCC8 primers amplify this locus in the family. However, probably this locus was amplified at 50 °C primer binding temperature in case of the 'Nimrang' variety (section 3.2).

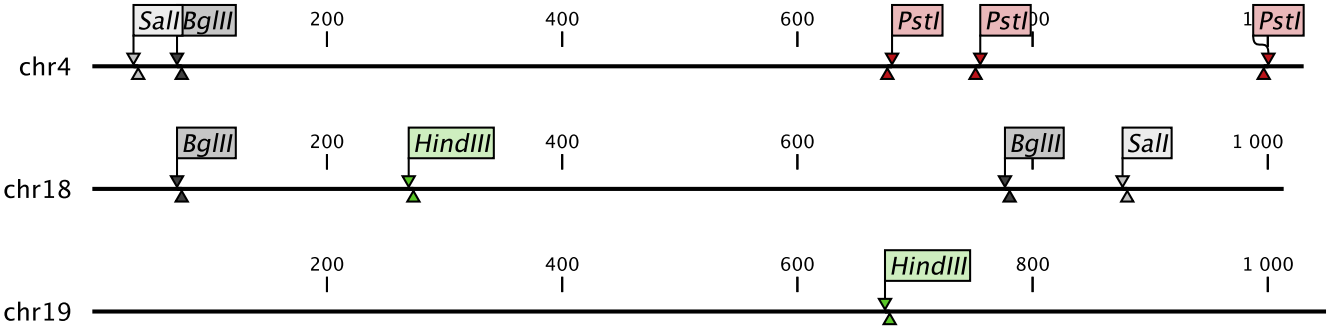

Figure 6: Sizes and *Sal*I, *Bgl*II, *Pst*I, *Hind*III cleavage sites of loci amplified with the SCC8 primers *in silico* from the genome-sequence of the 'Pinot Noir' PN40024 clone.

To verify whether the PCR product of BC_4 originates from the 4. or the 18. linkage group, the PCR product was digested with *Pst*I restriction endonuclease. Figure 4 shows that the *Pst*I enzyme has not digested the PCR product, therefore the PCR product of BC_4 may originate from the 18. linkage group.

Then the following question arose: what causes the dominant feature of the allele of the seeded BC_4 - point mutation on the cleavage site or recombination? Two answer this question, the *SCC8* loci of the characteristic types were sequenced. The PCR product of 'Kismis moldavszkij' – because the variety is heterozygous to the *SCC8* locus – was cloned, and a PCR product amplified from individuals with $SCC8^{+}/0$ and $scc8^{-}/0$ genotypes was direct sequenced. However, direct sequencing did not provide applicable results, so later the PCR products of these two types were cloned as well.

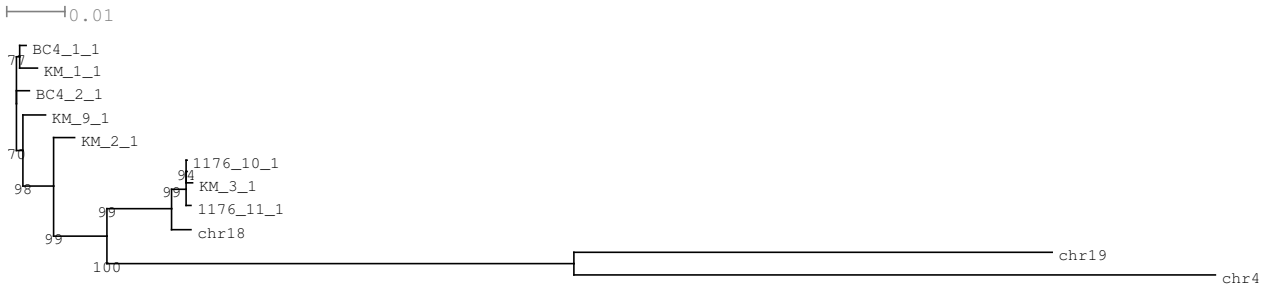

Figure 7: NJ tree of the sequenced *SCC8* loci. The values on the bifurcations display the bootstrap support calculated from 1 000 replicates in percentage. KM: 'Kismis moldavszkij'; chr4, chr 18, ch19: sections amplificable with SCC8 primers of the 4., 18., and 19. linkage groups of the 'Pinot noir' genome sequence.

During the control *Pst*I digestion of the cloned PCR products of 'Kismis moldavszkij' a band appeared which suggests that some fragments – even if in a small amount – can be found in the population of

the PCR products which were amplified not from the appropriate locus (in this case, from the 4. linkage group). This phenomenon gives the explanation for the fact that direct sequencing was not a feasible option in the case of genotypes theoretically carrying only one allele.

According to the tree (Figure 7) drawn from the multiple alignment of cloned *SCC8* alleles, the *SCC8* locus of BC_4 coincided with the point mutations of KM9 and KM2 (seedless alleles of 'Kismis moldavszkij') clones, which makes very probable that the allele of BC_4 does not carry an individual point mutation, but is recombinant between the *SdI* and *scc8*$^-$ loci.

Improved SCC8 marker

It has been proved during the analysis of the *SCC8* locus that in case of the application of primers suitable for the amplification of the *SCC8* locus published by LAHOGUE *et al.* (1998), there is a possibility that the primer pair also amplifies alleles from the 4. and 19. chromosomes which are not linked to seedlessness, therefore they can distort the obtained results.

Table 2: Sequences of primers designed for *SCC8* locus, excluding the 4. and 19. linkage groups.

Oligonucleotide	Sequence
scc-F6	5′-CAAGTTGGAAGATGGGGAGT-3′
scc-F61	5′-GCACCTGGGGAAGATCTCAT-3′
scc-R850	5′-CCAGGGGGTCTTTTAAAGTG-3′
scc-R914	5′-TCAAAAGAGGGTTGGCTCAC-3′

To eliminiate the possibility of such distortions, I designed new primers (Table 2). At the design of the new primers, the most basic aspect has been that these primers should not be complementer to the loci of the 4. and 19. linkage groups similar to *SCC8* locus of linkage group 18. The role of the new primers – beside the improvement of reliabilty – was to reveal whether the null allele of BC_4 is caused by the point mutations of the primer binding sites, or the reason is a realignment or differences of a greater scale.

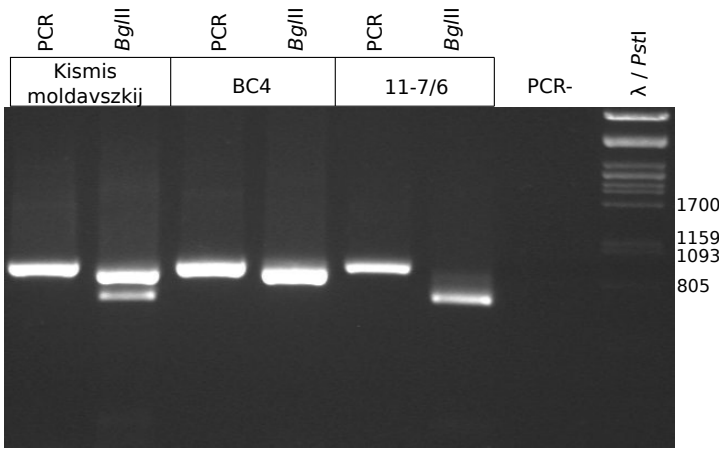

Figure 8: Detecting grape seedlessness with the scc-F6 and scc-R914 new SCC primers. Size marker: *Pst*I-digested λ phage DNA.

The *SCC8* locus was successfully amplified by every combination of the new primers, and according to the cleavage results, the marker is suitable for the detection of the dominant *SdI* locus responsible for seedlessness. None of the applied primer combinations resulted in the appeareance of the null allele of BC_4 (Figure 8).

4 Conclusions and recommendations

4.1 AFLP examination of *Rosa* species in Hungary

Effects of ploidity levels on the obtained results

An important consequence of the asymmetric meiosis of section *Caninae* is that the hybrids of the section are characterized by matrocline inheritance of both phenotypical features (WERLEMARK and NYBOM, 2001) and molecular markers (WERLEMARK *et al.*, 1999; NYBOM *et al.*, 2004, 2006). During fertilization for example, the pentaploid maternal partner provides 4*x* – in case of a tetraploid parent 3*x*, and in case of a hexaploid parent 5*x* – chromosomes to the genome of the zygote, while the viable pollens are haploid independently from the chromosome number of the parent, with 1*x* chromosomes. The other reason for the strong maternal effect frequently experienced in roses can be that they are liable for spontaneous apomixis (WERLEMARK, 2000).

Maternal inheritance however, does not prevail every time in case of phenotypical features. While the epicuticular waxes showed maternal inheritance (WISSEMANN *et al.*, 2007) during reciprocal crosses within the *Caninae* section, some features in the *Caninae* section are inherited through the paternal line (RITZ and WISSEMANN, 2003). Among the paternally inheriting features some can be found which are even important taxonomical characteristics. Such a feature is for example the falling down or remaining property of calyces during fruit bearing. In the cases of *R. canina* and *R. rubiginosa* maternal inheritance can be assumed due to their *Caninae* meiosis, but WISSEMANN and RITZ (2007) showed that during the reciprocal cross of the two species the falling of the calyx is inherited through the paternal line. This can cause disturbances at the taxonomic classification of some interspecific hybrids, because these taxa classified into one of the species based on paternal characteristics can be positioned closer to their other parent according to molecular markers.

As a result of the asymmetric meiosis, the potential genetic variability decreases, because only the seven bivalents are participating in the splitting of parental chromosomes during homologous recombination. This is balanced by the fact – due to the asymmetric meiosis as well – that individuals of the *Caninae* section form fertile hybrids even with members of other sections while maintaining their *Caninae* character.

Evaluation of AFLP data

The non-metric multidimensional scaling (NMDS) is not considered as a widely spread process for the evaluation of AFLP data, however, results of some studies are interpreted primarily by this method (O'HANLON *et al.*, 1999). The great advantage of this method is that it does no force the formation of groups, therefore taxa with doubtful position (e.g. hybrids) are not classified according to only one of their possible positions, which would cover their intermedier character.

One of the most widely used approach for interpreting AFLP data is the group formation (classification) process based on single dissimilarity indices calculated from a binary data matrix (BONIN *et al.*, 2007). The low bootstrap support of the UPGMA dendrogram can be attributed to the fact that hybrid taxa carry the markers of their parents in a mixed manner, therefore their position is uncertain and they disturb the group formation method. By the random modification of the data they will be classified to one or the other parent, and the strict group formation method results in very different trees, and their final consensus can not be considered reliable. If the bootstrap support of the tree is low because of the changing classification of the hybrids, then this set of UPGMA trees calculated from the bootstrap replicates carries exactly that kind of valuable information, which can not be displayed by traditional consensus trees.

The solution for this problem can be presented by the methodical group of tree networks, which can be considered a relatively new (HUSON *et al.*, 2009) process among tree building methods, which had been primarily applied on theoretical and model data matrices. In compliance with the expectations, the majority of the hybrids – depending on their origin – formed a well-segregated group and the explanation could be given for many branches of the tree which on the traditional consensus tree were hardly reconciliable with the classification based on morphological features and knowledge on these taxa. The positions of the hybrids met the expectations based on the former NMDS results, but the method of display is a way more transparent than that of applied in the case of NMDS.

Similarly to tree networks, hybridization networks also represent a relatively new methodical branch of phylogenetics (HUSON and KLOEPPER, 2005). Among the conducted analyses, the hybridization network gave the highest support for classic rose systems, primarily some individual subsections (*Caninae*, *Rubigineae*) of the *Caninae* section, while other sections (*Vestitae*, *Trachyphyllae*) did not show a definite segregation.

4.1.1 Taxonomical relationships of the examined rose taxa

In this part I attempt to give a short introduction and interpretation – within the frames determined by the sample set – of the taxonomical relationships of the examined taxa of the *Rosa* genus.

Section *Pimpinellifoliae* presented a well-defined clade in every analysis, without exception, which supports the segregation of the section within the genus *Rosa*. However, it must be noted that the three examined individuals are supposedly very close to each other genetically, so these can not represent the whole *Pimpinellifoliae* section. In many cases *R. heckeliana* was classified into this group, but the NMDS analysis points out that the taxon is segregated from the individuals of section *Caninae*. Classification into the same group can be caused by the fact that both section *Pimpinellifoliae* and *R. heckeliana* are markedly segregated from all the other individuals. The result of the hybridization network confirms the observations of (KOOPMAN *et al.*, 2008), whose bayesian analysis displayed the section as a base section of the genus *Rosa*.

Similarly to section *Pimpinellifoliae*, representatives of section *Rosa* formed a well-defined group despite the fact that many examined intersectional hybrids can be originated from one side from section *Rosa*, and some of these were positioned onto the *Rosa* clade ([119] *R.* × *kmetiana*, [50] *R. jundzillii*). The hybridization network positioned the (23) *R.* × *francofurtana* F_1 hybrid immediately beside the "classic" (22) *R. francofurtana*, while phenetic analyses did not show any relationship between the two taxa. The segregated character of section *Rosa* and its observed relationship with section *Caninae* corresponds with the results of former molecular genetic examinations (RITZ *et al.*, 2005; KOOPMAN *et al.*, 2008).

Section *Caninae* formed a well-defined group both during the phenetic and phylogenetic analyses, but regarding the subsection level, the situation is less explicit. The comparison of subsections with literature data is rendered more difficult by the fact that only a small number of studies deal with their analysis. Only the hybridization network validated the definite segregation of subsection *Rubigineae* within section *Caninae* observed formerly by KOOPMAN *et al.* (2008). The poliphyletic character of subsections *Vestitae* and *Trachyphyllae* in accordance with the conclusions of KOOPMAN *et al.* (2008) regarding these subsections, does not indicate their subsection level classification and segregation from subsection *Caninae* on the ground of AFLP data.

Based on my results, AFLP can be used extremely well for the examination of taxonomical groups with a complex past like the genus *Rosa*. The simultaneous application of the tree network drawn from the bootstrap repetitions of distances calculated from AFLP data and the hybridization network could provide the appropriate tool for the researcher. The former method explains and shows the origin of individual hybrids, while the latter is capable of relieving the disturbing effect exerted by taxa of hybrid origin on group formation.

These results can provide help for the interpretation of taxonomical relations of any other taxon group of which the establishment and formation is definitely affected by hybridization and introgression. Grapevine is one of these genera, and recently the following question is increasingly focused on: can the *V. sylvestris* interpreted as an independent species, does it even exist yet, and what role did it play in the culture evolution of *V. vinifera* (THIS *et al.*, 2006; BODOR *et al.*, 2010).

4.2 Detecting seedlessness of grape by molecular markers

4.2.1 Inheritance of the SCC8 marker in the examined families

The fact that the offspring generations of the crosses conducted by SZ. NAGY LÁSZLÓ – even if in small proportions – showed the presence of the *SCC8*$^{+}$/? type, allows us to conclude that the exact *SCC8* genotype of 'Superior Seedless' is *SCC8*$^{+}$/*scc8*$^{-}$, while that of 'Palatina' variety is *scc8*$^{-}$/0. Later, in the case of 'Palatina' variety, the presence of a null-allele was concluded by KORPÁS *et al.* (2009).

In the cross of BC_4 × 'Kismis moldavszkij', the BC_4 seeded maternal line is of *SCC8*$^{+}$/? genotype, therefore it acted as a "homozygote seedless". Based on the results of applying markers on the offspring generation it can be assumed that BC_4 carries a null-allele and a recombinant allele.

Two possible explanations can be given for the presence of *SCC8*$^{+}$ allele in BC_4: point mutation on the cleavage site, and recombination between the *SCC8* and *SdI* loci. However, based on the sequences of the *SCC8* allele of 'Kismis moldavszkij' and BC_4 it can be shown that the dominant *SCC8*$^{+}$ allele of BC_4 is a result of recombination and not that of a point mutation.

4.2.2 Description of the *SCC8* locus

It has been proven that the primers designed by LAHOGUE *et al.* (1998) may amplify a region of approx. 1 kb length not only from the 18. linkage group carrying the seed development inhibitor *SdI* locus, but also from the 4. and 19. linkage group of the grape. This is supported by the experiments conducted with reduced (50 °C) primer binding temperature, where PCR product was obtained in the PCR step even when amplification could not be observed on 60 °C primer binding temperature complying with the sequence of primers, and moreover, the fragments based on the cleavage of the PCR product originated from the 4. (in the case of 'Nimrang') or from the 19. ('Kismis moldavszkij') linkage group.

The presence of the three 'Pinot noir' loci of identical length, amplifiable with SCC8 primers, can be an additional proof for the hexaploid origin of grape (JAILLON *et al.*, 2007). The sequence of the loci are definitely different, however their similarities and nearly equivalent size support the hexaploidization taking place during the evolution of grape.

Improved SCC8 marker

The new primer pairs did not bind *in silico* to the 4. and 19. linkage groups of the genome sequence of 'Pinot noir'even when 30 % error (mismatch) was allowed, and find the complementer sequences on both alleles. They are prospectively suitable for the detection of seedlessness, the scc-F6 – sccR914 primer pair gave concluding results with the original SCC8 primer pair.

However, even with the application of the new primer pairs, the amplification of the null-allele was unsuccessful. If the primer pair could amplify the null-allele, it must have been shown on the gel. If the null-allele was dominant (*SCC8*$^{+}$), then it must have been be visible at the 11-7/6 individual of *scc8*$^{-}$/0 genotype, and if it was recessive (*scc8*$^{-}$), then heterozygote genotype must have been presented at the gel image of BC_4.

The observations above contradict to the presumption of KORPÁS *et al.* (2009), according to which – based on the recommendations of DAKIN and AVISE (2004) regarding to null-alleles observed in case of microsatellites – the presence of the null-allele is explained by point mutations taking place on the primer binding sites.

The reason why the null-allele observed both in the seeded and seedles grape varieties presumably acts as a null-allele is not the "imperfection" of the primers used in the PCR process, but larger chromosome realignment and mutations in these individuals.

4.2.3 MAS with the SCC8 marker

Beside the dominant inhibitor *SdI* gene responsible for seedlessness, the establishment of stenospermocarpic seedlessness is affected by further recessive genes (BOUQUET and DANGLOT, 1996). The presence of *SdI* allele is a necessary condition for the establishment of stenospermocarpy, but not sufficient for the formation of fruits which are considered to be seedles by the consumer. For this purpose, the presence of further recessive genes is required.

My results verify the applicability of the marker in the examined offspring generations, however thid should be tested in every case of different combinations, since the *SCC8* genotype of the parents used in the crosses can greatly affect applicability.

ADAM-BLONDON *et al.* (2001), and KORPÁS *et al.* (2009) concluded that the SCC8 marker can be applied better in seedless $\times$ seedless, than in seeded $\times$ seedless crosses. My results verify however, that if the *SCC8* genotype of the seedless parent was considered in the design of the breeding program and the parents were selected according to these, then the marker can be reliably applied for detecting seedlessness even in seeded $\times$ seedless crosses. The different judgements of the applicability of the marker may result from the different expectations. When our task is the detection by markers of complete stenospermocarpy, almost at the seedlessness level of 'Thompson Seedless', then higher effectiveness can be achieved indeed by applying seedless $\times$ seedless crosses, but this does not rely primarily on the applicability of the marker. The reason for the higher effectiveness is that other genetic factors affecting the level of seedlessness – including of course the *SdI* inhibitor gene as well – could be found as homozygotes in these crosses with greater probability.

In case of such a complex feature as the stenospermocarpic seddlessness of grape, the SCC8 marker is primarily suitable for pointing out the possibility of seedlessness. What level of seedlessness is shown by the individuals is the function of the inheritance of the other genes, therefore the forecast of the level of seedlessness can not be expected from the marker linked to the *SdI* gene.

4.3 New scientific results

AFLP examination of *Rosa* taxa in Hungary

1. Taxonomical relationships of some rose taxon groups in Hungary – including many hybrid taxa – was surveyed with AFLP markers. I verified the grounds of the establishment of sections *Pimpinellifoliae* and *Rosa*, and additionally I pointed out that based on the examined taxa the subsections *Vestitae* and *Trachyphyllae* of section *Caninae* proved to be polyphyletic, their segregation from subsection *Caninae* is not supported on the basis of my data.

2. The network of UPGMA trees calculated from the bootstrap replicates of phenetic analysis of AFLP data was capable of revealing and displaying the hybrid origin of individual *Rosa* taxa, while hybridization networks even with the inclusion of hybrid taxa supported the segregation of the examined sections and some subsections of the Rosa genus.

3. I pointed out that in the case of two *Rosa gallica* taxa with different ploidity levels ($2n = 5x = 35$, and $2n = 6x = 42$), the level of ploidity did not affect the classification using AFLP markers, they showed tight linkage despite different levels of ploidity.

Detecting seedlessness of grape with markers

4. I pointed out that both the 'Flame Seedless' and 'Palatina' varieties contain an *SCC8* null-allele, I described the exact *SCC8* genotype of the mentioned varieties: $SCC8^{+}/0$, and $scc8^{-}/0$ respectively. In the case of the multiresistant BC_4 genotype used frequently in the breeding program of KOZMA PÁL I proved the presence of a recombinant $SCC8^{+}$ allele and a null-allele.

5. I verified the applicability of the SCC8 marker for the prediction of stenospermocarpic seedlessness of grape on the SI, TII and VRH 3082-1-42 (BC_4) $\times$ 'Kismis moldavszkij' families.

6. I designed new primers for the improvement of the SCC8 marker which are selective for the chromosome section carrying the dominant *SdI* inhibitor gene of the stenospermocarpic seedlessness of grapevine on the 18. linkage group.

7. I pointed out that the null-allele of VRH 3082-1-42 (BC_4) is not a result of point mutations on the primer binding sites of the *SCC8* locus.

5 Publications in the topic of the theses

Journal articles

Journal articles with impact factor

DEÁK, T., S. HOFFMANN, P. BODOR, GY. D. BISZTRAY, P. KOZMA (2010): Marker assisted selection for seedlessness in a multiresistant table grape hybrid family. *Vitis*. (submitted paper)

Journal articles without impact factor

DEÁK, T., L. SZ. NAGY, GY. D. BISZTRAY, (2010): Molecular marker based detection of seedlessness in two powdery and downy mildew tolerant table grape families. *International Journal of Horticultural Science.* (accepted paper)

Conference papers

Hungarian conferences (proceedings)

DEÁK, T., G. FACSAR, GY. D. BISZTRAY, ZS. SASVÁRI, I. NAGY (2005): Magyarországi rózsafajok AFLP variabilitása (előzetes közlemény). In M. TÓTH (szerk.): *Válogatott teljes terjedelmű tudományos dolgozatok a Lippay János Tudományos Ülésszakon (2005. október 20-21.) bemutatott eredményekből*. Kertgazdaság különszám, Budapest. 265-270.

Hungarian conferences (abstract)

DEÁK, T., G. FACSAR, GY. D. BISZTRAY (2005): Magyarország rózsa fajainak (Rosa spp. L.) genetikai polimorfizmusa. *XI. Növénynemesítési Tudományos Napok, 2005. március 3-4., Budapest*. Összefoglalók. 53.

DEÁK TAMÁS, SZ. NAGY LÁSZLÓ, BALOGH ISTVÁN, BODOR PÉTER, BISZTRAY GYÖRGY DÉNES (2006): Szőlő magvatlanság kimutatása SCAR-RFLP markerekkel. *XII. Növénynemesítési Tudományos Napok, 2006. március 7-8.*. Budapest. Összefoglalók. 137.

DEÁK TAMÁS, BODOR PÉTER, BISZTRAY GYÖRGY DÉNES (2009): A szőlő magvatlanságáért felelős génjelöltek elemzése bioinformatikai módszerekkel. *Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. 2009 október 28-30., Budapest*. Összefoglalók. 260-261.

International conferences (proceedings)

DEÁK, T., G. FACSAR, GY. D. BISZTRAY (2005): Genetic Polymorphism of Rosa Genotypes Native to Hungary. *Acta Hort (ISHS)* 690. 57-62.

International conferences (abstracts)

DEÁK, T., S. HOFFMANN, P. BODOR, F. KERÉNYI, GY. D. BISZTRAY, P. KOZMA (2010): Usefulness of the SCC8 SCAR marker linked to seedlessness in Fungus resistant table grape breeding. *10^{th} International Conference on Grapevine Breeding and Genetics, 1-5. august 2010, Geneva, NY, USA*. Book of Abstracts. P-10.

Other publications

Journal articles

Journal articles with impact factor

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