The use of SSR markers in family Rosaceae

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Summary: The identification of plant species and study of their genetic relatedness is an important object of plant genetics. The Rosaceae family contains a lot of economically important fruit, ornamental, and wild plant species. The microsatellite markers have been proven to be an efficient tool for description of the genetic relatedness among varieties and species. Their evolutionary conserved regions enable them to differentiate among various accessions. This article intends to show proceeded identification and characterization projects on Rosaceae species by using SSR markers. The article presents sources of already published primer sequences. The use of already published primers can highly reduce the cost and duration of this kind of researches.

Key words: microsatellite, SSR, Rosaceae, molecular markers

Introduction

The identification of plant species and the study of their genetic relatedness has always been an important aspect for breeders. The detection of certain features in the plant's early stage of development is crucial for both the breeders and the growers. The concept of molecular markers has revolutionised the ability to differentiate among various taxons. Species with little genetic information available in the past now have hundreds of genetic markers. Until the early 60's morphological markers were the only tool for the scientist to identify plants. However these markers have a lot of unfavourable characteristics such as late expression, rare polymorphism, dominant recessive inheritance, dependence on the environment. The appearance of molecular markers has provided a more useful application for plant breeders. These markers don't depend on the environment, don't influence the plant's growth and its propagation, they are mostly codominant and their examinations are fast and cheap *(Hajósné,* 1999).

The first generation of molecular markers was isoenzymes markers which were useful for identifying cultivars, constructing molecular maps and studying gene expression. In spite of their widespread applicability they have some significant deficiencies: there are only a few mapped isoenzyme loci and not all of the variations at the DNA level can be traced with isoenzyme markers *(Hajósné,* 1999).

The polymorphism at the DNA level is much more frequent than that of in proteins. The DNA markers can be divided in two major groups: RFLP and PCR-based markers. The RFLP markers biggest advantage over isoenzymes is that they give us information from the whole genom and they can detect variation in the non-coding region as well. However this technique is costly, time consuming and the work with the radioactive ^{32}P isotope needs a special laboratory background *(Phillips,* 1994).

Fragments of genomic DNA suitable as genetic markers can be produced by PCR amplification. This can be done by synthesizing PCR primers to uniquely amplify portions of the sequence of known genes *(D'Oviedo* et al., 1990). No Southern blots, DNA hybridization are necessary. One the first PCR based DNA marker was the RAPD (Randomly Amplified Polymorphic DNA) The used primers usually consist of ten randomly arranged nucleotides. RAPDs are easy to develop, but they must be applied cautiously due to some repeatability problems, especially among different laboratories. The reliability of the PCR markers can be enhanced by using specific primers. There are some region of the genome such as microsatellites, that are significantly more polymorphic than other genomic regions. Assay these regions is a way of increasing the level of detectable polymorphism between individuals. The microsatellites or SSRs (Simple sequence repeat) generally contain 10-40 bp long tandem repeats. Unique sequences that flank the tandem repeats are evolutionary conserved. These conservative regions enable the same microsatellite markers to differentiate among accessions, cultivars or even related species by making special primers from them *(Huang* et al., 1998).

SSR markers used in investigation of the different species belonged to the family Rosaceae

This article review intends show proceeded identification and characterisation projects on Rosaceae species by using SSR markers. By taking advantages of these markers conservative features the use of already published primers

can help researchers to reduce the high cost of marker isolation and duration of their research.

The Rosaceae family contains a lot of economically important fruits (apple, peach, cherries, apricot) ornamentals (roses, hawthorn, sorb tree) and wild species. Considering SSRs as a new technique only a few Rosaceae species were studied previously. The most widespread investigation with SSR markers were undertaken on peach, apple and cherries.

Peach

Peach is a self-fertile and naturally self-pollinating species with very low genetic variability *(Hesse* et al., 1975). Its genetic base was dramatically narrowed by a cultivar selection. In spite of the great expectation of isoenzyme RFLP, and RAPD analysis revealed low polymorphism *(Dirlewanger* and *Bodo,* 1994) .

Thanks to these failures peach was the first stonefruit from which microsatellite markers had been isolated. The first step was to evaluate the potential of microsatellites for use in genetic studies of peach. The genomic and cDNA library screening indicated that CT repeats occurs every 100 kb, CA repeats in every 420 kb in peach genom. Microsatellite-containing clones were sequenced and specific primers were designed to amplify the microsatellite-containing region from genomic DNA. The level of polymorphism was high among the 28 peach cultivars and these markers tested well for their utility in cross species amplification within the Rosaceae *(Sosinski* et al., 2000).

Another study showed identical results. 17 primer pairs were designed from microsatellite loci cloned in two genomic libraries. Ten of these microsatellite loci were able to demonstrate Mendelien inheritance. Fifteen microsatellites were polymorphic in the ten examined peach genotypes. The study found ten primers that gave apparently correct amplification in all Prunus species (peach, nectarine, almond, apricot, plum, sweet and sour cherry) surveyed and 3 of them did the same on apple *(Cipriani* et al., 1999).

Microsatellite DNA was also used for testing the genetic origin of peach cultivars. The set of microsatellites discriminated all cultivars investigated, except several sport mutations. It was possible to analyze the paternity of several cultivars and in most cases, the parenthood was confirmed *(Testolin* et al., 2000).

These researches showed that SSR markers could reveal higher polymorphism than the preceding identifications (RAPD, RFLP) and the isolated microsatellite primers are capable of successful amplification in related species.

A research was undertaken weather isolated DNA from Peach fruit is equally suitable for SSR analysis as that of leaf tissue. The procedure described for DNA extraction yielded a sufficiently clean DNA *(Aranzana* et al., 2001).

Apple

Apple considering its economical importance has the most comprehensive SSR analysis. The examinations started in 1995 when microsatellites were used for disease diagnosis for apple propagation and nursery industry *(Sansavini,* 1995).

The abundance of microsatellites regions in apple genom and their ability to identify cultivars was tested in 1997. Screening of an apple genomic libraries with (GA)15 and (GT)15 probes demonstrated that these repeats are abundant, occurring about every 120 and 190 kb, respectively. Microsatellites isolated from the libraries contained numbers of repeats ranging from 7 to 39. Primers to these microsatellite loci were able to direct amplification in 21 different cultivars. As few as three microsatellite markers were sufficient to differentiate between all 21 cultivars *(Guilford* et al., 1997).

A similar examination carried out by using 66 apple accessions and eight SSR primers. The aim of this research was to determine genetic identities estimate genetic diversity, and to identify genetic relationships among these accessions. All eight primer pairs generated multiple fragments when used in amplification reactions with DNA from these accessions. High levels of variation were detected with a mean of 12.1 alleles per locus and a mean heterozygosity across all eight loci of 0.693. The eight primer pairs utilized in this study unambiguously differentiated all except seven accessions in this collection of 66 genotypes *(Hokanson* et al, 1998).

SSR markers were used to identify descedans from different apple cultivars and to check their supposed parents. Fifteen different microsatellite primer combination were used to established unique fingerprints of 28 apple cultivars and 42 promising genotypes of crosses between them. Fingerprints revealed that the supposed parent cultivars of certain genotypes were not always correct which indicates that uncontrolled crosspollination can occur or that even mixing up seed is possible. The progeny of other apple parents was analyzed with the same SSR primer combination to investigate if these fifteen microsatellites were sufficient to established unique fingerprints. The fact that all of the 80 genotypes of this progeny could be easily distinguished in combination and they are distributed over the whole apple genome gives strong evidence that with these mictosatellites, all the cultivars and their descendants can unambiguously be distinguished *(Kenis* et al., 2001.).

The utilization of SSR markers played an important role of constructing the European apple map in 1999. A total number of 202 Isoenzyme, RFLP, RAPD and 6 microsatellite marker were used in linkage analysis. The plant material was 'Prima' x 'fiesta' cross from 1998. Markers heterozygous in 'Prima' were arranged into 17 linkage groups covering 753 cM, markers heterozygous in 'Fiesta' into 19 linkage group covering 820 cM *(Maliepaard* et al., 1999).

Cherry

The sweet cherry cultivar identification with SSR markers were proceeded in 2000. Some 188 sweet cherry accessions were also analyzed using 13 microsatellite primers. In order to develop an identification key for each investigated sweet cherry accession, the data were analyzed separately for each microsatellite marker. The marker

showing the highest variation among accessions was chosen as 'first marker'. The remaining markers were used to separate the accessions in the groups created by the first marker until all accessions were clearly identified. Using this microsatellite based key was possible to create a powerful tool for sweet cherry cultivar identification, which could be the starting point for the development of a marker assisted cultivar identification database *(Boritzki* et al., 2000).

The most detailed sour cherry microsatellite fingerprint analysis with 50 accessions was completed in 1999. The used 10 primer pairs were designed on the basis of peach, sweet and sour cherry microsatellite loci. All ten primer pairs amplified fragments in sour cherry accessions tested. This research demonstrated that SSR primers can be used to differentiate among all but two of the 59 cherry accessions. The two accessions not differentiated are difficult to distinguish phenotipically, suggesting that they may be the same variety *(Cantani* et al., 2001).

Apricote

SSR markers were also proven to be useful in apricots. RAPD and microsatellite markers have been used to identify and characterize 50 apricot cultivars originating from different geographic areas. Thirteen of the 18 microsatellite primer pairs produced repeatable polymorphic patterns. All cultivars exept two, could be distinguished using microsatellite primers *(Hormaza,* 2001).

The most recently executed identification studies also used SSR markers as a tool for characterization in apricots.

An identification research completed on apricot with SSR markers. The aim of the study was to determine the genetic relatedness of Hungarian apricots to the southern European group. The twelve microsatellite primer originated from peach genom resulted polymorphic in the set of cultivars studied and allowed to distinguish all cultivars, except two sets of Hungarian cultivars. These two sets of presented the same combination of markers, according their pomological characters suggesting they are clones they could be the same cultivar *(Romero* et al., 2001).

Beside RFLP markers SSR markers are also used for constructing linkage map in apricot. The mapping studies have started on an almond x peach F2 population. However result suggest that the genetic information gathered on DNA sequence, gene position or gene cloning in one *Prunus* species is very likely to be useful in the others or even in other members of the Rosaceae *(Arus,* 2001).

Beyond these major projects there are some generally single efforts in other species (rose, almond, strawberry). It is very important economic interest to differentiate between rose varieties. Mini- and microsatellites fingerprints were used to identify genotypes and establish genetic distances between them. While genetic relationships within and between rose categories, based on known genetic history, it is possible to compare the DNA fingerprints. A perfect match was obtained after the comparative analysis *(Ben Meir* et al., 1997).

Microsatellite markers were successfully applied in almond (*Prunus dulcis*). Twelve SSR markers previously isolated from peach were tested on 28 accessions. All almond genotypes except one were identified and distinguished by combining SSR amplification (Pancaldi, 1999). Besides the RAPD, RFLP, and isoenzyme markers, six SSR markers were used for constructing genetic linkage map in almond. The estimated total size of the almond map was of 457 cM *(Joobeur* et al., 2000).

Random amplified SSR markers were used to examine *Phytophtora cactorum* infection on strawberry and apple. The analysis showed that leather rot of strawberry fruits and crown rot are not caused by genetically different strains of this species. Another results was that no clear genetic separation between isolates from different plants. This study revealed the fact that SSR markers are useful for pathogenetic purposes in Rosaceae species *(Hantula* et al., 2000).

The other species belong to this family haven't been studied yet. This doesn't mean that this kind of examination would be useless on them. Since this method is fairly new and costly this species haven't come on yet. This assumption is supported by the fact that other techniques (morphological, isoenzyme, RAPD -markers) were used among these species *(Table 1).*

Table 1 The utilization of genetic markers in those Rosaceae species where SRR analysis haven't been done yet (searched in database 1995–04.2001.)

Morphological markers	Isoenzyme markers	RAPD markers
Hawthorn *(Crataegus spp.)*	Pear *(Pyrus communis)*	Pear *(Pyrus communis)*
Strawberry *(fragaria X ananassa)*	Quince *(Cydonia oblonga)*	Strawberry *(fragaria X ananassa)*

Table 2 The list of those publications where primer sequences are available.

Name of the Authors	Date of publication	Number of primer pairs published
Cantini et al. (peach, sweet and sour cherry)	2001	10
Cipriani et al. (peach)	1999	17
Downey and Iezzoni (peach, sweet and sour cherry)	2000	5
Dirlewanger et al. (apple)	1998	8
Guilford et al. (apple)	1997	14
Sosinski et al. (peach)	2000	10
Hokanson et al. (apple)	1998	8

References

Aranzana, M.j., de Vicente, M.C. & Arús, P. (2001): Comparison of fruit and leaf DNA extracts for AFLP and SSR analysis in peach. Acta Horticulturae 546: 297–300.

Arús, P. (2001): Molecular markers for variability assessment and MAP comparison in *prunus* XII[th] International symposium on apricot culture and decline (abstracts)

Ben-Meir, H., Scovel, G., Ovadis, M., Vainstein, A., Altman, A. (ed.) & Ziv, M., (1997): Molecular markers in breeding of ornamentals. Acta Horticulturae, 447: 599–601.

Boritzki, M., Plieske, J., Struss, D., Geibel, M. (ed.), Fischer, M. (ed.) & Fischer, C. (2000): Cultivar identification in sweet cherry (Prunus avium L.) using AFLP and microsatellite markers. Acta-Horticulturae, 538 (2), 505–510

Botta, R., Akkak, A., Me G., Radicati, L., Casavecchia, V., Scannerini, S. (ed.), Baker, A. (ed.)., Charlwood, B. V. (ed.), Damiano, C. (ed.)., Franz, C. (ed.) & Gianinizzi, S., (1998): Identification of pear cultivars by molecular markers. Acta Horticulturae 457: 63–70.

Cantini, C., Iezzoni, A. F., Lamboy, W. F., Boritzki, M. & Darush, S. (2001): DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. Jour. Amer. Soc. Hort. Sci., 126 (2): 205–209.

Cipriani, G., Lot, G., Huang, W. G., Marrazzo, M. T., Peterlunger, E. & Testolin, R. (1999): AC/GT and AG/CT microsatellite repeats in peach [Prunus persica(L) Batsch]: isolation, characterisation and cross-species amplification in Prunus. Theor. Appl. Genet., 99: 65–72.

Dirlewanger, E. & Bodo, C. (1994): Molecular genetic mapping of peach. Progress in temperate fruit breeding. Kluwer, The Netherlands, 309–311

Dirlewanger, E., Pronier, V., Parvery, C., Rothan, C., Guye, A. & Monet, R. (1998): Genetic linkage map of peach (Prunus persica (L.) Batsch) using morphological and molecular markers. Theoretical and Applied Genetics, 97: (5–6), 888–895

D'Oviedo, R.,. Tanzarella, O. A. & Poceddu, E. (1990): Rapid and efficient detection of genetic polymorphism in wheat through polymerase chain reaction. Plant. Mol. Biol., 15: 169–171.

Downey, S. L. & Iezzoni, I. F. (2000): Polymorfic DNA Markers in Black Cherry (Prunus serotina) Are Identified Using Sequences from Sweet Cherry, Peach, and Sour Cherry. J. Amer. Soc. Hort. Sci., 125 (1): 76–80.

Guilford, P., Prakash, S., Zhu, J. M. Rikkerink, E., Gardiner, S., Bassett, H. & Forster, R. (1997): Microsatellites in Malus X domestica (apple): abundance, polymorphism and cultivar identification. Theoretical and Applied Genetics., 94: 2, 249–254.

Hajósné, M. (1999): Genetikai variabilitás a növénynemesítésben. Bp. Mezõgazda Kiadó.

Hantula, J., Lilja, A., Nuorteva, H., Parikka, P. & Werres, S. (2000): Pathogenicity, morphology and genetic variation of Phytophthora cactorum from strawberry, apple, rhododendron, and silver birch. Mycological-Research, 104: (9) 1062–1068.

Hesse, C. O., Janick, J. & Moore, J. N. (1975): Advances in fruit breeding. Purdue University Press, West Lafayette, Indiana, USA, pp 285–335

Hokanson, S. C., Szewc, McFadden A. K., Lamboy, W. F. & McFerson, J. R. (1998): Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus x domestica* borkh. subset collection. Theoretical Applied. Genetics, 97: 671–683.

Hormaza, I. J. (2001): Identification of apricot *(Prunus armeniaca* L.) genotypes using microsatellite and RAPD markers. Acta Horticulturae 546: 209–215.

Huang, W.G., Cipriani, G., Morgante, M. & Testolin, R. (1998): Microsatellite DNA in *Actinidia chinensis* isolation, characterization, and homology in related species. Theor. Appl. Genet., 97: 1269–1278.

Hudina, M., Stampar, F., Virscek, Marn, M. & Smole, J. (1996): Identification of pear (*Pyrus communis* L.) and quince (*Cydonia oblonga* Mill) based on isoenzyme variability in various tissues. Zbornik Biotehniske Fakultete Univerze v Ljubljani Kmetijstvo. 67: 173–182.

Hurtado, M. A., Badenes, M. L., Llacer, G., Karayiannis, I. (1999): Random amplified polymorphic DNA markers as a tool for apricot cultivar identification. Acta Horticulturae 488: 361–368.

Joobeur, T., Periam, N., Vicente, M. C., King G. J., Arus, P. & Vicente M. C. (2000): Development of a second generation linkage map for almond using RAPD and SSR markers. Genome, 43: (4) 649–655.

Kenis, K., Pauwels, E., Van Houtvinck, N. & Keulemans, J. (2001): The use of microsatellites to Established unique fingerprints for apple cultivars and some of their descendants. Acta Horticulturae 546: 427–431.

Maliepaard, C., Alston, F., Arkel, G. van, Brown, L., Chevreau, E., Dunemann, F., Evans, K., Gardiner, S., Guilford, P., Heusden, S. van, Janse, J., Laurens, F., Lynn, J., Manganaris, S., Nijs T. den, Periam, N., Rikkerink, E., Roche, P., Ryder, C., Sansavini, S., Schmidt, H., Tartarini, S., Verhaegh, J., Vrielink, R., King, G. van. Arkel G., van Heusden, S. den, Nijs, T., Tobutt, K. R. & Alston, F. H. (1999): The European apple map. Acta-Horticulturae, 484, 325–330.

Pancaldi, M., Kacar, Y., Kuden, A.B. & Sansavini, S. (1999): Using microsatellite sequences from peach for fingerprinting and genealogical analysis of almond. Ravista di Frutticoltura e di Ortofloricultura, 61: (11), 70–73.

Phillips, R. L. (1994): DNA-based markers in plants. Kluwer Academic Publishers Boston

Romero, C., Llácer, G., Badenes, M. L. & Pedryc, A. (2001): Relationship Among cultivars from Hungary and a European pool determined by SSR markers. XIIth International symposium on apricot culture and decline (abstracts)

Sansavini, S. (1995): Advanced technologies in fingerprinting and disease diagnostics for fruit, citrus, grape and olive propagation and nursery industries. Agro-Bio-Frut, Cesena, Italy, 6 May 1995, 154 pp.

Sosinski, B., Gannavarapu, M., Hager, L. D., Beck L. E., King, G. J., Ryder,C. D., Rajapakse, S., Baird, W. V., Ballard, R. E. & Abbott, A. G. (2000): Charactarization of microsatellite markers in peach (Prunus persica (L.) Batsch). Theoretical-and-Applied-Genetics, 101: (3), 421–428.

Testolin, R., Marrazzo, T., Cipriani, G., Quarta, R., Verde, I., Dettori, M. T., Pancaldi, M. & Sansavini, S. (2000): Microsatellite DNA in peach (Prunus persica L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. Genome, 43: (3), 512–520