RAPD Analysis of Peaches within Czech National Collection

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Abstract: The Random Amplified Polymorphic DNA (RAPD) technique was used to study the genetic diversity and relationships within the collection of the Czech National Plant Genetic Resources (PGR) of peaches (*Prunus persica* L.). The aim of the work was to elaborate a dendrogram of genetic similarity and to divide collection into clusters. 46 primers were applied to 6 cultivars differing in the place of origin, the fruit shape, the fruit colour, and in some other morphological characteristics. 12 primers were chosen which gave polymorphic repeatable strong and middle strong bands. They were subsequently used for the RAPD reactions within the whole collection of peaches. The selected RAPD primers distinguished 28 peach cultivars and RAPD data were used to group the accessions analysed. Almonds and peach × almond hybrids were clearly separated in the frame of the whole collection. The grouping corresponded to the botanical system, to the available information about pedigree, and to the cultivars description.

Keywords: *Prunus persica;* peach; RAPD primers; genetic resources

Analytical methods based on DNA amplification are very often used for the study of genetic diversity and genetic relationships within collections of plant genetic resources. Especially in the case of fruit trees are they very useful because the morphological character evaluation is very time-consuming and due to different expressions it must be evaluated in the course of several years. Thanks to the molecular markers, it would also be possible to choose promising genotypes for breeding programs very early, already in the stage of seedlings. DNA markers simply detect differences in genetic information, in other words, they are based on polymorphism in DNA sequences carried by two or more individuals (Samec 1993).

Molecular markers are used in different areas of genetics: in genetic mapping, in the studies of genome organisation, in the characterisation, description, and identification of agricultural cultivars. They are a very suitable tool for the characterisation of genotypes in gene banks. In this period, molecular markers also participate in marker-assisted selection which significantly influences the character of breeding and the creation of new genotypes.

In the case of the genetic resources collections, which are usually maintained *ex situ* in field gene banks, a number of problems exist associated with such maintainance. For example, the phenotypic character expression may be different under different conditions and, consequently, the identification of duplicate accessions may be very difficult. Duplicates occurring in gene banks are a waste of resources and a considerable pressure exist to find ways for their identification and elimination. They may be found to differ in molecular markers while their distinction by morphological characters is difficult or impossible (Hodgkin 1995). Several other reasons exist for the use of molecular genetic markers in the plant genetic resources. Some of

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them were summarised by Avise (1994), for example: (i) molecular data are genetic; (ii) molecular methods open the entire biological world for genetic scrutiny; (iii) molecular method access a nearly unlimited pool of genetic variability; (iv) molecular data can distinguish homology and analogy; (v) molecular data provide a common measure for assessing divergence; (vi) molecular approaches facilitate mechanistic appraisals of evolution; and (vii) molecular approaches are challenging and exciting.

Prunus persica is a well-explored species and it serves as well as the model genome for *Rosaceae* genomic (Abbott *et al*. 2002). Several strategies such as genetic mapping, molecular markers development, and plant diversity description have been used in the study of the peach genome recently. These molecular genetic approaches are usually based on RFLP, RAPD, SSR and AFLP methods.

Molecular genetic maps are usually constructed for the genome analysis. They were already constituted in several laboratories such as at Clemson University, USA (Eldredge *et al*. 1992; Rajapakse *et al*. 1995), at INRA – Centre de Bordeaux, France (Dirlewanger *et al*. 1998), at IRTA – Carretera de Cabrils (Barcelona), Spain (Aranzana *et al*. 2001b; Arús *et al*. 1994).

The using of DNA-based genetic markers is efficient and reliable also in the case of species with a low genetic variability such as peaches, nectarines, and almonds (Warburton, Bliss 1996; Rajapakse *et al*. 1995). The DNA markers discovered can be linked to interesting traits. For example, RAPD markers were mapped relative to the loci controlling flesh colour, adhesion, and texture (Warburton, Bliss 1996), and to the resistance against diseases (Dirlewanger *et al*. 1996).

Peach fingerprinting and variability analysis were carried out in the peach by means of isozymes (Messeguer *et al*. 1987) or by molecular methods such as RAPDs (Warburton, Bliss 1996), RAPDs and RFLPs (Quarta *et al*. 2001), AFLPs (Hagen *et al*. 2001) and AFLPs and SSRs (Aranzana *et al*. 2001a).

In this work, our aim was to distinguish the peach cultivars in the collection of the Czech National Plant Genetic Resources (PGR) of *Prunus* on the base of DNA differences. For this reason, we used RAPD approach which Williams *et al*. (1990) and others had recommended as a very suitable method for DNA-fingerprinting and the variability study. The goal of the work was to create the database of the characteristic RAPD profiles of individual varieties, and to create the similarity dendrogram of peaches, nectarines and almonds.

MATERIAL AND METHODS

Plant material. The total DNAs were extracted from young leaves of 28 cultivars included in the collection of the Czech National Plant Genetic Resources (PGR) maintained at the Experimental and Research Station Mendeleum in Lednice. The complete list of the cultivars observed is presented in Table 1.

DNA isolation. The DNA samples were obtained by the method of Bernatzky *et al*. (1986) with some modifications. Approximately 3.5 g of young fresh leaves were homogenised in liquid nitrogen and then suspended in the extraction buffer (0.35M sorbitol, 0.1M Tris-HCl, 25mM EDTA, 20mM sodium bisulphate; pH = 8). After centrifugation, the lytic buffer (200mM Tris-HCl, 2M NaCl, 50mM EDTA a 2% (w:v) CTAB; pH = 7.5) and 5% sarcosyl were added. After incubation in the water bath at 65°C chloroform-octanol (24:1) was added and the mixture was centrifuged. The DNA in the aqueous phase was precipitated with isopropanol, the DNA pellet was dissolved in TE and reprecipitated with cold 70% ethanol and washed with 90% ethanol. The DNA samples were purified by proteinase and RNA-ase and fenolated (to remove proteins, RNA, and polysaccharides). The DNA concentration was determined by means of a spectrophotometer or aliquots of the extract containing approximately 50–100 ng DNA were analysed on a low percentage agarose gel (0.8% agarose) alongside a range of uncut lambda DNA standards containing from 25 to 200 ng DNA.

RAPD. RAPD amplification was performed in volumes of 25 µl containing 1× PCR buffer, 1.5mM $MgCl_2$, 100µM each of dATP, dCTP, dGTT, dTTP (Promega), 0.4µM 10-mer primer, 20 ng of template DNA and 1 unite of *Taq* polymerase. Altogether 46 decamer primers (20 from OPE kit, 20 from OPM kit, 6 synthesised) were used to detect the fragment specific for cultivars in the population observed. The RAPD amplification was carried out in 96-well automated thermal cycler Biometra UNO II. The program consisted of: denaturation at 94°C for 3 min, followed by 40 cycles (denaturation at 94°C 20 s, annealing at 36°C for 1 min, extension at 72°C for 1 min), finishing at 72°C for 9 min. The amplified products were separated by elec-

Table 1. Observed collection of *Prunus* genotypes

No.	Genotype (origin)	Characteristic	No.	Genotype (origin)	Characteristic
1	Kando (CZ)	peach × almond	16	Armking (USA)	nectarine
2	MNVA-1 (CZ)	almond	17	IB3 (Macedonia)	almond
3	Favorita Moretini (Italy)	peach	18	Adriatica (Italy)	peach – cling
4	Cadaman (France)	peach × *P. davidiana*	19	Jance (China)	peach – cling
5	Barrier (Italy)	peach × *P. davidiana*	20	Ferragnes (France)	almond
6	GF-677 (France)	peach × almond	21	Balkonella*	nectarine – dwarf
7	Požár (CZ)	peach	22	Aurelie (France)	peach
8	Harbritte (Kanada)	peach	23	Duchesse d'Este (Italy)	peach
9	Envoy (USA)	peach	24	Ackerman Red Leaf*	peach × almond
11	*P. davidiana* (China)	botanical species	26	Maria Marta (Italy)	peach
12	Belmondo*	peach sandwich	27	Michelini (Italy)	peach
13	Pekova (CZ)	peach	28	Tendresse (Italy)	peach
14	Lesiberian (CZ)	peach (ssp. *mandsh.*)	29	Melodie (France)	peach
15	Manon (France)	peach	30	Queen Lady (USA)	peach

*origin of cultivar unknown

trophoresis in 1.5% agarose gel and visualised by ethidium bromid staining (Sambrook *et al*. 1996). The fragment size was determinated by 100 bp DNA ladder (New England Biolab).

Basic screening. 46 primers (40 primers of Operon – series E, M and 6 synthesised primers) were applied to 6 cultivars differing in the place of origin, the fruit shape, the fruit colour, and in some other morphological characteristics among the observed genotypes of peaches, nectarines and almonds as shown in Table 2. After the visual evaluation, out of the group of the primers screened those ones were chosen that revealed polymorphic repeatable strong and middle strong bands and distinguished the individual cultivars observed. These were subsequently used for RAPD reaction in all the collection.

Data analysis. RAPD data were registered as 1 for the presence of a band and 0 for its absence and were transformed into the binary matrix which was in the next step transferred into Popgen 1.32 software (Yeh & Boyle 1997). For the genetic identity matrix, Nei's distance parameter (Nei 1972) was used. The dendrogram was constructed on the basis of this matrix by UPGMA method and by Molecular Evolutionary Genetic Analyse (Sudhir *et al.* 2001).

Table 2. Cultivars included in basic screening

Variety	Number	Description
Kando	1/97	peach × almond, F_3 generation
Lesiberian	14/97	white flesh, *P. persica* ssp. *mandshurica*
Armking	16/97	yellow flesh, nectarine, bred in USA
Adriatica	18/97	yellow flesh, bred in Italy, cling
Anita	33/98	white flesh, bred in California
Maycreast	34/98	yellow flesh, bred in California

RESULTS

Basic screening

Out of 46 primers tested, 12 of them amplified non-polymorphic product and 34 primers amplified from 1 to 7 polymorphic bands ranged from 300 to 1500 bp. The number of polymorphic loci was 74 (41.81%) out of the total number 177 bands. The mean value of gene diversity (Nei 1973) was 0.1453. Consequently, 12 primers giving the highest rate of gene diversity were chosen for further work.

The characteristic electrophoretic records obtained during the basic screening of primers are presented in Figure 1. The list of the selected primers is shown in Table 3.

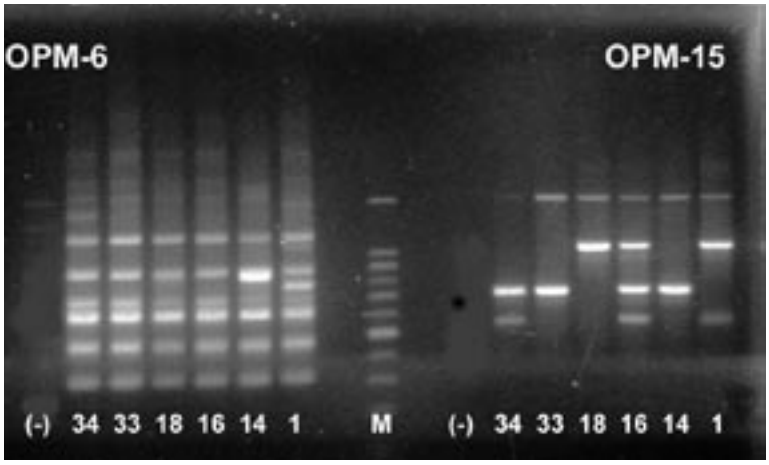


Figure 1. Characteristic electrophoretic records obtained during basic screening OPM-6, OPM-15 – polymorphic character

1/97, 14/97, 16/97, 18/97, 33/98, 34/98 = cultivars in Table 2
(-) = negative control, M = 100 bp DNA ladder

Table 3. Primers evaluated during basic screening

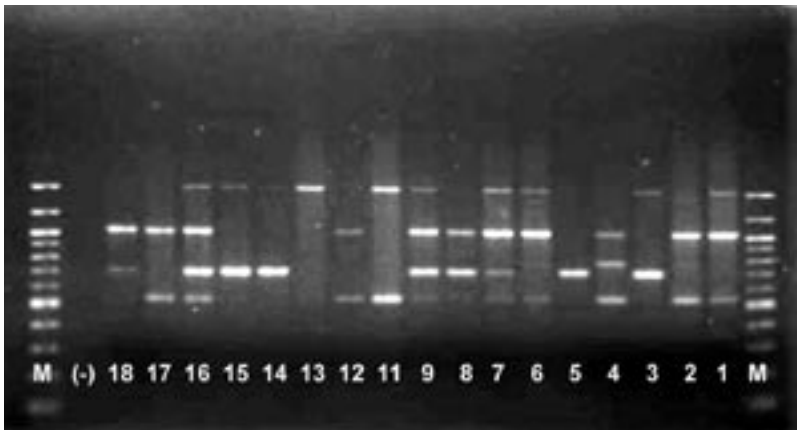
Primer		Primer	
S2	5'-CCTTGACGCA-3'	OPE-19	5'-ACGGCGTATG-3''
S4	5'-AAGACCCCTC-3'	OPE-20	5'-AACGGTGACC-3'
S5	5'-AGATGCAGCC-3'	OPM- 6	5'-CTGGGCAACT-3'
OPE-2	5'-GGTGCGGGAA-3'	OPM-16	5'-GTAACCAGCC-3'
OPE-14	5'-TGCGGCTGAG-3'	OPM-15	5'-GACCTACCAC-3'
OPE-17	5'-CTACTGCCGT-3'	OPM-18	5'-CACCATCCGT-3'

Collection data analysis

The group of RAPD primers selected during the basic screening distinguished all the analysed cultivars. An example of DNA spectrum of the selected primer M-15 is shown in Figures 2a, b.

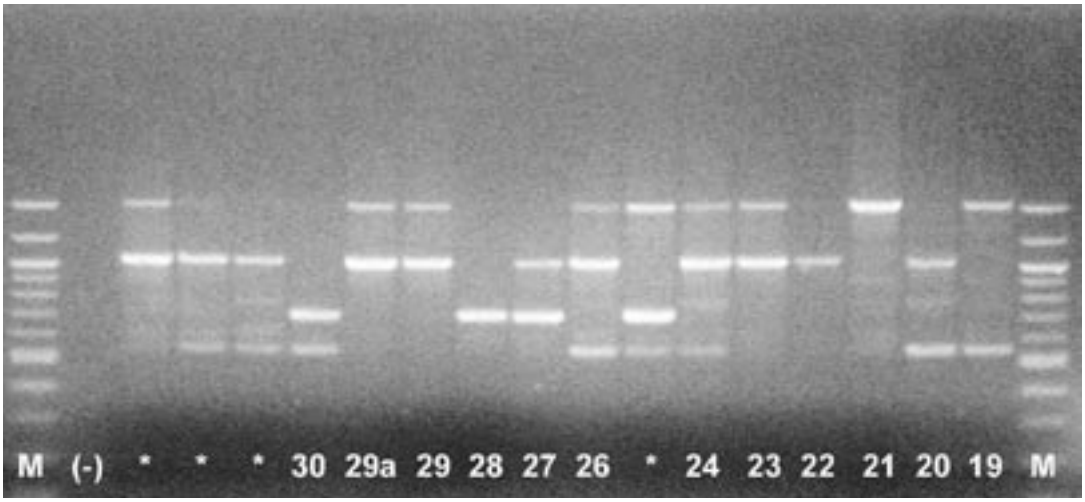
Altogether 28 cultivars of *Prunus* and 81 different polymorphic bands recorded across the collection of cultivars by means of 12 primers were used for the dendrogram construction (software MEGA v. 2.1; Sudhir *et al*. 2001) (Figure 3). The values of gene diversity (Nei 1973) of the individual bands ranged from 0.0465 to 0.4989 with the mean value of 0.2392.

Cluster analysis grouped the accessions as follows: cluster of almonds (MNVA-1, IB-3 and Ferragnes) which is clearly separated from the whole collection. The following cluster includes only peach × almond hybrid Kando. Peach × almond hybrids (Ackerman Red Leaf, GF-677) and hybrid between *P. persica* and *P. davidiana* (Cadaman) formed the next group. The remaining part of



1–18 = DNA samples from single cultivars, M = 100 bp DNA ladder, (-) = negative control

Figure 2a. Characteristic RAPD records of the part of collection analysed by means of primer M-15



19–30 = DNA samples from single cultivars, M = 100 bp DNA ladder, (-) = negative control, * = not evaluated in this work

Figure 2b. Characteristic RAPD records of the part of collection analysed by means of primer M-15

the samples were clustered together as a group of cultivars without any significant association with some simply manifested traits such as white or yellow flash distinguishing between nectarine and peach. This part included, for example, nectarine (Armking, Balkonella), *P. persica* ssp. *mandshurica* (Lesiberian), *P. persica* × *P. davidiana* (Barrier), botanic species *P. davidiana* (Davidiana) or cling cultivars (Adriatica, Jance). The genetic distance values of the clusters noticed were approximately similar (Figure 3).

DISCUSSION

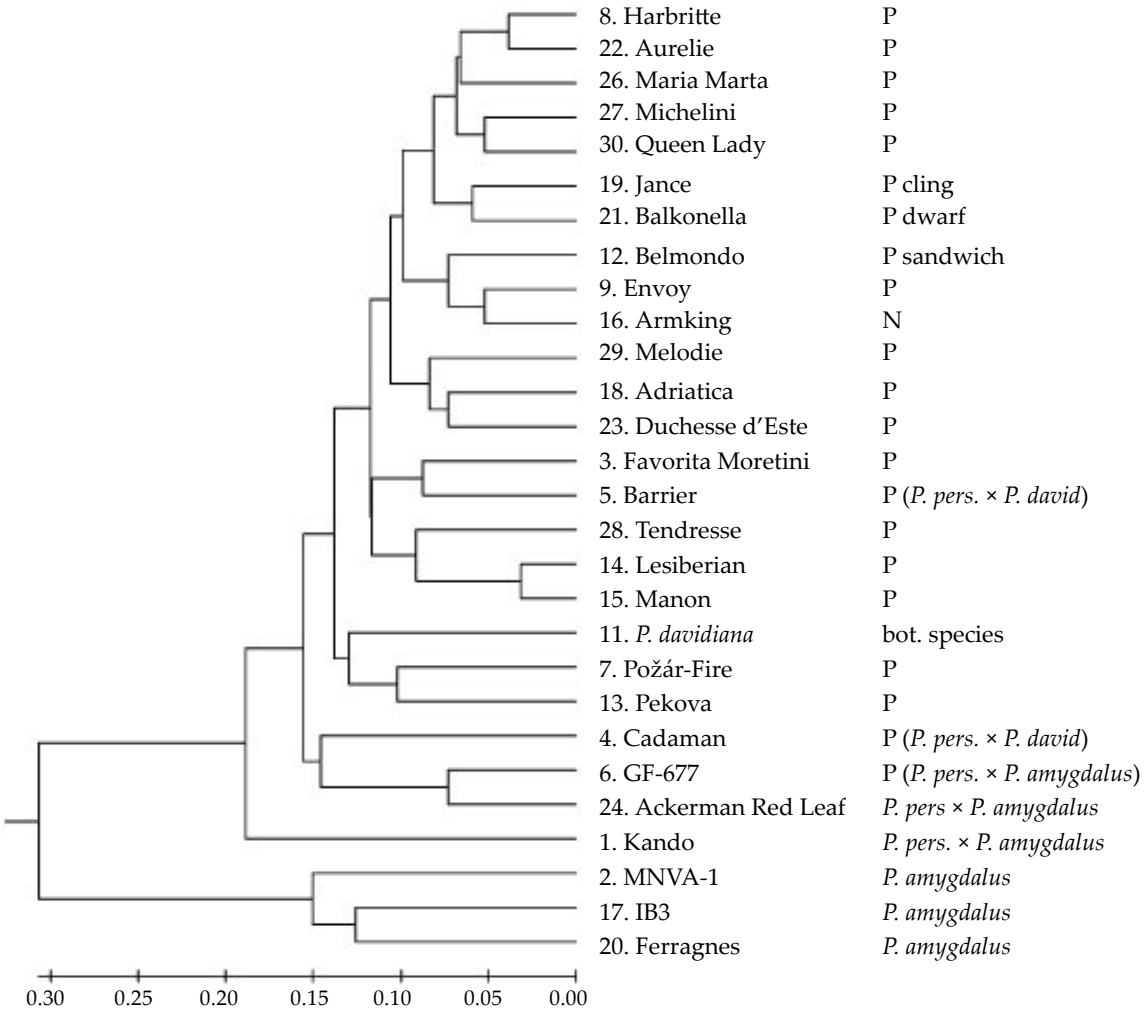
As already mentioned above, the group of almonds including cultivars Ferragnes, MNVA-1 and IB-3 is clearly separated by cluster analysis from the peach collection. Such arrangement is similar to that in the work by Quarta *et al*. (2001).

The locations of almonds, stand-alone peach × almond hybrid Kando and hybrids Ackerman Red Leaf, GF-677 and Cadaman in the dendrogram correspond with the botanical system and with the available information on cultivar pedigree (Okie 1998; Aranzana *et al*. 2002).

The remaining samples were differentiated and clustered together as a group of cultivars without any significant relation to some botanical or agricultural traits. The fact that RAPD analysis is unable to find any correlation between RAPD loci and other important traits and to create smaller groups characterised by some qualitative traits (for example yellow or white flash, peach or nectarine character, melting or non-melting pulp) corresponds with results of Quarta (2001). Better distinguishing but not absolutely distinguishing results were detected by means of other amplification methods in the works of Dierlewanger *et al*. (1998) – SSR, Aranzana *et al*. (2001a) – AFLP and SSR, Aranzana *et al*. (2002) – SSR.

On the other hand, the description of the genetic variability by means of RAPD markers which distinguish single genotypes included in the observed group by elctrophoretic records also presents a very important result. From the point of view of the genetic resources description, RAPD analysis was used in our genetic studies as the first step for the complex cultivar fingerprinting. The results can serve for the identification and description of *Prunus* cultivars within the genetic resource collections of peaches, nectarines, and almonds. In the near future, the RAPD method will be followed by some SSR loci analysis which is more exact and sensitive than the cheaper RAPD. On microsatellite a higher rate reproducibility was observed, as well (van Treuern 2000 – www.cgn.wageningen-ur.nl/PGR/research/molgen/). A good experience with the utilisation of SSR for the peach cultivar identification was confirmed by the work of Aranzana *et al*. (2002).

In the future, the collection of *Prunus* genotypes will be extended and used for further genetic studies.



P = peach (*Prunus persica*), P cling = clingstone peach, P sandwich = flat peach, *Prunus amygdalus* = almond, N = nectarine

Figure 3. Similarity dendrogram showing the genetic relationship within the analysed collection

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Abstrakt

Raddová J., Baránek M., Oukropec I., Vachůn M., Pidra M. (2003): **RAPD analýza broskvoní z Národních genových zdrojů ČR**. Czech J. Genet. Plant Breed., **39**: 113–119.

RAPD technika (Random Amplified Polymorphic DNA) byla použita ke studiu genetické diverzity a vzájemných vztahů v kolekci broskvoní (*Prunus persica* L.) Národních genových zdrojů ČR. Cílem práce bylo vytvořit dendrogram podobnosti a rozčlenit jednotlivé kultivary do klastrů. 46 primerů bylo aplikováno na 6 kultivarů, které se lišily místem původu, tvarem plodu, barvou dužniny a jinými morfologickými charakteristikami. Bylo vybráno 12 primerů dávajících polymorfní opakovatelné silné a středně silné pruhy. Tyto primery byly dále použity pro RAPD reakce ve zkoumaném souboru broskvoní. Vybrané RAPD primery rozlišily 28 kultivarů a RAPD data byla použita pro rozdělení jednotlivých vzorků do skupin. Mandloně a broskvomandloně byly jasně vyčleněny v rámci celého souboru. Rozdělení do skupin koresponduje s botanickým systémem, dostupnými informacemi o původu a popisu kultivarů.

Klíčová slova: *Prunus persica*; broskvoň; RAPD primer; genové zdroje

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