Theor Appl Genet (1995) 90: 1138–1141

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Chloroplast DNA variation and parentage analysis in 55 apples

Received: 4 October 1994 / Accepted: 15 December 1994

Abstract The chloroplastic *atpB-rbc*L spacer and the first 53 codons of the *rbcL* coding sequence was sequenced for 40 apple cultivars and 15 wild species. This chloroplast DNA region is 904 base pairs long, and only five mutations sites were found among the tested samples. Although the cpDNA variation was low, some parentages are proposed based on the maternal inheritance of plastid DNA: the male and female parents are specified, or else suggested, for Worcester, Discovery, Starking, Starkrimson, Kidd's Orange Red, Priscilla, and Gloster, as well as for the putative wild origin for *Malus* × *domestica*.

Key words *Malus* · Non-coding DNA sequence · DNA polymorphism · *rbcL* · Parentages analysis

Introduction

There are several hundred apple cultivars. Their origins are often unknown, especially those of the old varieties which are of great value for their genetic and flavour diversity. Thus, biochemical and molecular investigations are currently proceeding to obtain genetic markers of cultivars and on accurate genealogy for further plant breeding work. For example, isozyme electrophoretic analysis has proved useful in apple cultivar identification (Weeden and Lamb 1985; Bournival and Korban 1987). Since the M13 phage repeat probe was able to detect DNA minisatellite-like sequences in plants (Rogstad and al. 1988), DNA 'fingerprints' have also been used for parentage analysis in some fruits, including apples (Nybom 1990; Nybom and Schaal 1990 a, b; Nybom et al. 1990). Additionally, RFLP (restriction fragment length polymorphism) has been applied to parentage analysis in apples (Alston and Batlle 1992; Ishikawa et al 1992; Simon and Weeden 1992; Ishikawa 1993). The recent RAPD method (randomly amplified polymorphic DNA) has also been used for apples (Harada et al 1993; Koller et al 1993). Here, we report the sequencing of the chloroplast *atpB-rbcL* spacer and the first 53 codons of the *rbcL* coding sequence (representing 904 base pairs), in 40 apple cultivars and 15 wild species. The aim of this analysis was to investigate: (1) the intra-specific DNA polymorphism of this cpDNA region in a crop plant, (2) the putative wild origin of the domestic apple, and (3) the parentages of some of the tested cultivars.

Materials and methods

Leaf samples of 55 apple cultivars and wild species were obtained from collections in Switzerland (Centre Horticole de Lullier, Arboretum d'Aubonne, Eidgenössische Forschungsanstalt für Obst-Wein- und Gartenbau-Wädenswil, Conservatoire et Jardin botaniques de Genève) and in Germany (Genbank Obst Dresden).

Total DNA was extracted by the method of Edwards et al. (1991), or by a modification of the method of Webb and Knapp (1990). A ±900-bp cpDNA region (the *atpB-rbcL* spacer and the first 53 codons of *rbcL*) was amplified by PCR using the oligonucleotides 5′GAAGTAGTAGGATTGATTCTC 3′ and 5′TACAGTTGTCCATGTACCAG 3′ as primers (Savolainen et al. 1994). The PCR products were run in a 1% agarose gel stained with ethidium bromid, cut off from the gel, purified with silica particles (Prep-A-Gene, Bio-Rad), and sequenced directly with the amplification primers and the additional primers 5′CCCTACAACTCATGAATTAAG 3′, 5′GTCTATCATTATAGACAATCCC 3′, 5′CATCATTATTGTATACTCTTTC 3′, and 5′GTAAATCCTAGATGTAAAA 3′ (Savolainen et al. 1994). The obvious cpDNA phylogenetic relationships (only five mutation sites found) were represented using the graphic facilities of the PAUP software (Swofford 1993). The *Rosa damascena* sequence (Savolainen et al. 1994) was used as an outgroup. DNA sequences of Idared, Discovery, and *R. damascena* were registrated in EMBL/GenBank Nucleotide Sequence databases under accession numbers X69749, X69750, and X69753, respectively.

Communicated by G. Wenzel

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TTTTTTGCGAAAATTACTGAAATCAAAAATAAATGTTCGATAGCAAAACA   50
AGTTAAGTTGATCGGTTAATTCAATAAGAAATAGGCCTTAGCGCTCGATT  100
TCGTTGGTACCATCCAACTGAATCCAATTCAATTGTTTACTTATTCAATT  150
TCAGTGAATTGAAAAATTCAACGAAAACCCATTTTCAAAACATCAAGTGT  200
TATGAATAAAAATTTTGATAAAGTCTTTTATTTGCCTATCATTATAGACA  250
ATACCTTCCATATTATCTATGGAATTCGAATCCGAACCCTATAAATTACG  300
ATTTCTTTTTTCTATCTCATTGGTCCTTATTTACGATATCAGCATATCGA  350
TTTACGCTTTAGCCTATTATTTTTAGCCTAAGTATTTTTCATGTTTATGG  400
ACGAATTCTGCATATTTTTATATTTAGGATTTACATATACAACATATATC  450
ACTGTCAAGAGCGAATTTCTTATTATTTAGATATTTCGATTCAAAAAAGT  500
AAGATATTAGAAACTTGAAAAACAAAGATTGGGTTGCGCCATACATATGA  550
AAGAGTATACAATAATGATGTATTTGGCGAATCAAATACCACGGTCTAAT  600
AACGAACCGTTCTGATTAGTTGATAATATTAGTTGATAGTTTTGTGAAAG  650
ATTCTGTGAAAGGTTTCATTAACTTCATAATTTATGTCGAGTAGACCTTG  700
TTCTTGTGAGAATTATTAATTGATTAGTTGTAGGGAGGGACTT ATG TCA  749
CCA CAA ACA GAG ACT AAA GCA AGT GTT GGA TTC AAA GCT  788
GGT GTT AAA GAT TAT AAA TTG ACT TAT TAT ACT CCT GAC  827
TAT GAA ACC AAA GAT ACT GAT ATT TTG GCA GCA TTT CGA  866
GTA ACT CCT CAA CCT GGA GTT CCA CCT GAG GAA GCA GG   904
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Fig. 1 DNA sequence of the *atpB-rbcL* spacer and the first 53 codons of *rbcL* in *Malus* × *domestica* cv Idared. The *rbcL* coding sequence begin at position 744. This sequence is identical in all apples, except for five mutation sites which are in *bold* characters: at position 17, C is replaced by T in Kidd's Orange Red, Starking, Priscilla, Starkrimson, Gloster, Yellow Transparent, Worcester, Discovery, and stock emla9b (=Paradis Jaune de Metz); at position 30, T is replaced by G in *M.trilobata*; at position 31, A is replaced by T in *M.ioensis*; at position 326, C is replaced by T in *M.pumila*, *M.baccata*, *M.halliana*, *M.floribunda*, *M.sieboldii*, *M.sargentii*, *M.toringoides*; at position 463, G is replaced by T in *M.kansuensis*

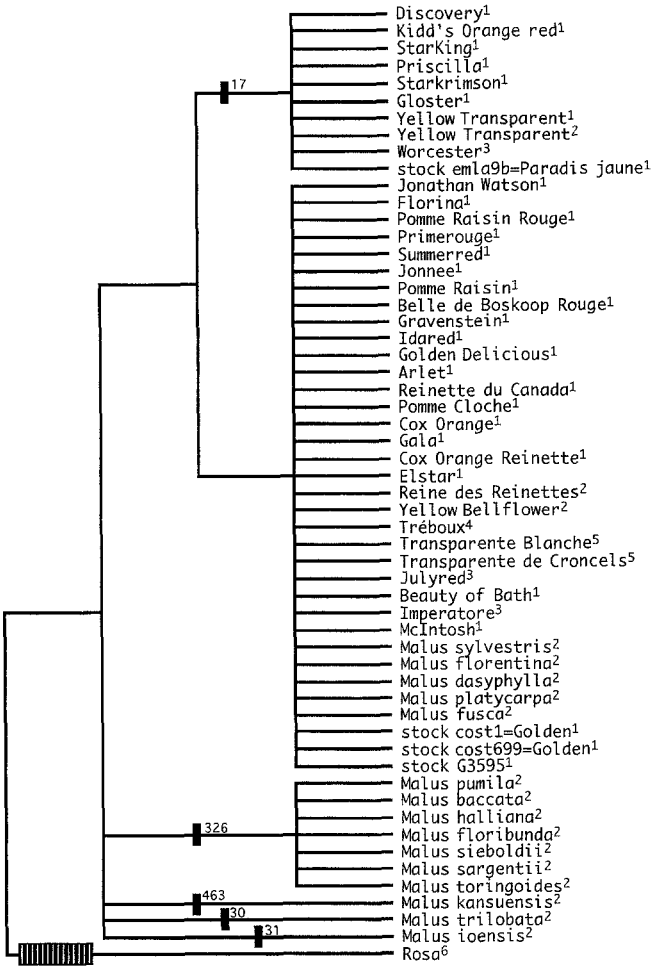

Fig. 2 Proposed phylogenetic relationships among the tested apples with an indication of the mutations and their position (see Fig. 1). Origin is indicated as: *1*, Centre Horticole de Lullier-CH; *2*, Genbank Obst Dresden-D; *3*, Eidgenössische Forschungsanstalt für Obst- Wein- und Garten-bau of Wädenswil (Swiss Federal Station for Fruit-Growing Viticulture and Horticulture)-CH; *4*, Arboretum d'Aubonne-CH; *5*, Personal collection of R.Corbaz-CH; *6*, Conservatoire et Jardin botaniques de Genève-CH

Results

From the 904-bp-long sequenced cpDNA region, only five mutation sites were found among the 55 apple samples (Fig. 1). *R. damascena* was used as an outgroup in the phylogenetic tree (Fig. 2). Its DNA sequence was aligned with the *Malus* sequences, yielding a 936-bp-long matrix with 62 variable sites (data not shown), which allows us to determine the probable plesiomorphic and apomorphic mutations. The first mutation is a transition at position 17; all the cultivars have a cytosine at this position except for Worcester, Gloster, Discovery, Kidd's Orange Red, Priscilla, stock Emla9b (=Paradis Jaune de Metz), Yellow Transparent 1 and 2 and the Red Delicious family (including Starking, Starkrimson), which have a thymine. The three other mutations are autapomorphic transversions, *Malus trilobata* having a guanine at position 30 instead of thymine, *Malus ioensis* having a thymine at position 31 instead of adenine, and *Malus kansuensis* having a thymine at position 463 instead of guanine. Finally, the last mutation is a synapomorphic transition for seven wild apples, *M.pumila*, *M.baccata*, *M.floribunda*, *M.sieboldii*, *M.sargentii*, and *M.toringoides*, which each have a thymine at position 326 instead of cytosine.

Discussion

cpDNA polymorphism of the *atpB-rbcL* spacer

According to the Harris and Ingram review (1991), intraspecific chloroplast DNA variation is relatively common and could have some effect on phylogenetic reconstruction. Thus, the first aim of the present study was to test the cpDNA variation of the *atpB-rbcL* spacer at the intra-specific level, because we have used this DNA region in phylogenetic analysis at higher taxonomic levels (Spichiger et al. 1993; Manen et al. 1994; Savolainen et al. 1994). In fact, we found a very low cpDNA diversity, as previously reported in other crop plants (e.g. Clegg et al. 1984; Ishii et al. 1986; Shoemaker et al. 1986), only one single mutation site being present in the DNA region sequenced among 40 cultivars (Fig. 1). This mutation (a transition at position 17, see Results) was found in ten samples and checked three times by repeating the procedures from extraction to sequencing. This demonstrates the high fidelity of the *Taq* polymerase and the PCR technique (Kwiatowski et al. 1991).

Putative wild origin of the domesticated apple *Malus* × *domestica*

The putative wild origin for *Malus* × *domestica* is hybrid and it has been suggested to be derived from *M.sylvestris*, *M.dasyphylla*, *M.pumila*, and some Asiatic species (Terpó 1968). We sequenced the *atpB-rbcL* spacer in 15 wild species, where four other mutation sites were found in addition to the mutation mentioned below (Figs. 1, 2). This still represents a low level of DNA variation, compared for example to *Galium* spp. (Manen et al. 1994), *Ilex* spp., or *Anemone* spp. (unpublished results), from which we sequenced the same DNA region. Despite this limited phylogenetic information, we can define some parentages. Taking into account maternal inheritance of cpDNA, the female parent and the resulting hybrid lineage must share the same DNA sequence. Thus, ten wild species are excluded from being the female parent of *Malus* × *domestica*. *M.kansuensis*, *M.trilobata*, and *M.ioensis*, which originated respectively from China, the Middle East, and North America (Schneider 1904; Bossard and Cuisance 1984), are excluded because of their characteristic autapomorphies (Figs. 1, 2). *M.pumila*, *M.baccata*, *M.halliana*, *M.floribunda*, *M.sieboldii*, *M.sargenti*, and *M.toringoides*, which originated from Eastern Asia (Hughes 1920; Bossard and Cuisance 1984), are also excluded because of the shared mutation at position 326 (Figs. 1, 2). Among the species analysed, only three European species, *M.sylvestris*, *M.florentina* and *M.dasyphylla*, (Terpó 1968), or two North American species, *M.platycarpa* and *M.fusca* (Schneider 1904; Sargent 1913), could have transmitted their plastid genome and thus be the potential female parent of *Malus* × *domestica*. Obviously, our results do not allow a precise determination of the hybrid nature of the cultivated apple which is probably of multiple origin with introgression. However, they indicate that several European and American wild species might be candidates for the female parent. Moreover, if the Asiatic wild species tested are involved, then these would be the male parent.

Parentages of some of the tested cultivars

Additionally, if we look at the proposed origins for the cultivars, Gala is presented as the hybrid of Kidd's Orange × Golden Delicious (Votteler 1993). Gala having a cytosine at position 17 (Fig. 1), the female parent is necessarily Golden Delicious and the male parent is Kidd's Orange.

Because the mutation at position 17 could only be transmitted maternally, some other parentages can be specified as presented in Fig. 3. For instance, Worcester has Beauty of Bath as male parent, and is the female parent of Discovery, but the male parent of Primerouge. The Red Delicious family (including Starking, Starkrimson) are the female parents of Kidd's Orange Red, Priscilla, and Gloster. Thus, our results are in disagreement with those of Aeppli et al. (1989) who concluded by following the 'ladies first' rule, that Red Delicious (=Richard Delicious) is the male parent. In fact, Kidd's Orange Red, Priscilla, and Gloster have Cox Orange, McIntosh, and Pomme Cloche as male parent, respectively. The Red Delicious family has been claimed to arise from a random seedling of Yellow Bellflower (Aeppli et al. 1989). Since this latter cultivar does not have the mutation found in Red Delicious it cannot be its direct ancestor. However, three explanations are possible: (1) the Red Delicious family is a mutated lineage of Yellow Bellflower, (2) it is a hybrid between Yellow Bellflower as male parent and an unknown lineage bearing the mutation as female parent, or (3) it is the direct descendant of an unknown lineage bearing the mutation. In order to resolve this situation, we have searched for this mutation in other old varieties. We found it in Yellow Transparent, which came from Russia and the Baltic states in the mid 19th century. Thus, based on our cpDNA sequencing, Yellow Transparent could be the female parent of the Red Delicious family and of Worcester. Other derived lineages can also now be followed by the presence of the mutation at position 17 (Fig. 1). Unfortunately, there is no available restriction enzyme which can be used as genetic marker for this position; therefore short sequencing runs remains the only alternative strategy.

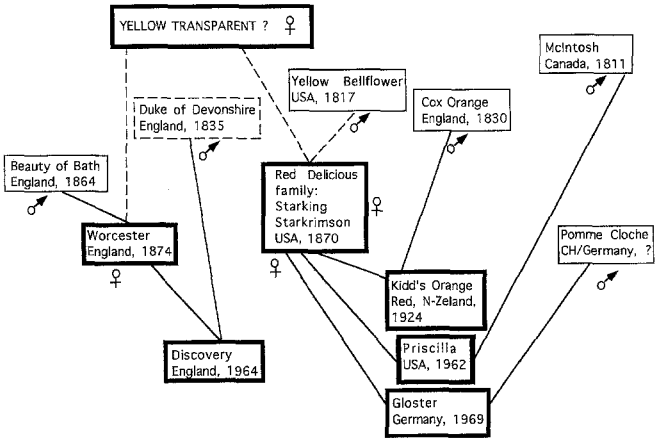

Fig. 3 Proposed parentages in the the Red Delicious family and other related cultivars (with place and time of origin) based on the maternal inheritance of chloroplast DNA. The cultivars whose DNA sequences have the mutation at position 17 (Fig. 1), i.e. the apomorphic thymine instead of the plesiomorphic cytosine, are framed in *bold*. Although Duke of Devonshine has not been sequenced, it is included in this figure according to the existing bibliography

Acknowledgements We thank the Genbank Obst of Dresden (G), the Arboretum of Aubonne (CH), and the Eidgenössische Forschungsanstalt für Obst-, Wein- und Garten-bau of Wädenswil (CH) for providing plant samples, and Sonia de Marchi for technical assistance. This work was partially funded by the Fonds National Suisse de la Recherche Scientifique (grant N. 31–28757.90).

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