Theor Appl Genet (2004) 109: 1519–1524
DOI 10.1007/s00122-004-1775-9

ORIGINAL PAPER

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Characterisation and transferability of apple SSRs to two European pear F_1 populations

Received: 23 April 2004 / Accepted: 13 July 2004 / Published online: 31 August 2004

Abstract European pear (*Pyrus communis* L.) is among the important fruit species for which only few genetic studies have been carried out. Available evidence indicates that simple sequence repeats (SSR) are very useful as molecular markers because they are codominant, highly polymorphic, abundant and reproducible. The present paper reports more than 100 apple SSR markers in two populations of European pear; a total of 41 SSR markers were then positioned on a genetic linkage map of the cross 'Passe Crassane' × 'Harrow Sweet' and 31 in the map 'Abbè Fétel' × 'Max Red Bartlett'. Syntenic relationships between pear and apple maps have been considered for the chromosomes carrying two or more SSR markers. The alignment among the two maps supports the colinearity of the two genomes with respect both to identification and to orientation of the linkage groups.

Introduction

Among molecular markers, microsatellites, or SSRs, are known for being highly polymorphic, codominantly inherited, abundant and evenly distributed in the genome. The most widespread technique for SSR detection is PCR with specific primers, which is simple, reproducible and suitable for automation (Gianfranceschi et al. 1998). However, SSRs have a higher initial cost when compared to other molecular markers, since they require the identification and sequencing of their DNA regions, the design of specific primers and the optimisation of the amplification conditions. Furthermore, A-T dinucleotides, which are the most abundant type of SSR in plants, are difficult to isolate from libraries (Rafalski et al. 1996).

In fruit tree species, microsatellites have been identified in *Vitis* (Di Gaspero et al. 2000), *Citrus* (Sankar and Moore 2001), *Olea* (Sefc et al. 2000), *Actinidia* (Huang et al. 1998), *Malus* (Guildford et al. 1997; Gianfranceschi et al. 1998; Hokanson et al. 1998; Liebhard et al. 2002), *Prunus persica* (Cipriani et al. 1999; Sosinski et al. 2000), *P. armeniaca* (Lopes et al. 2002) and recently in *Pyrus* (Yamamoto et al. 2002).

It is well known that SSRs isolated from one species can be transferred to other species in the same genus or family (Ellegren et al. 1997). Some apple SSRs have already been used to identify genetic diversity in pear (Yamamoto et al. 2001). Yamamoto et al. (2002) mapped ten apple SSRs (CH01B12, CH01E12, CH01H01, CH01H01, CH01H10, CH02B02b, CH02B10, CH02B12, CH02D11 and CH02F06; Gianfranceschi et al. 1998) in a pear population derived from the cross 'Bartlett' × 'Hosui'. These results enabled a first alignment between an apple and two pear genetic maps and confirmed the synteny between the two species (Yamamoto et al. 2001, 2002). However, matching these data with previously published pear maps (Weeden et al. 1994; Iketani et al. 2001) was not possible because the latter maps were based only on RAPD markers.

Here we report the screening and characterisation in four European pear cultivars of more than 100 apple SSRs available in the literature (Gianfranceschi et al. 1998; Liebhard et al. 2002; the VF series from B.A. Vinatzer et al., personal communication). The SSR screening was the basis for the construction and alignment of four linkage maps based on the segregation of the same markers in populations from the crosses 'Passe Crassane' (PC) × 'Harrow Sweet' (HS) and 'Abbé Fétel' (AF) × 'Max Red Bartlett' (MRB).

Communicated by H. Nybom

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Materials and methods

Plant material and DNA isolation

Two different populations of *Pyrus communis* L. were used for the construction of preliminary genetic linkage maps based on SSR and AFLP markers. The first population consists of 99 F_1 individuals resulting from the cross PC × HS and the second of 95 F_1 individuals from the cross AF × MRB. Both crosses were carried out at the DCA Experimental Station (Bologna, Italy), for breeding purposes. HS is a North American genotype derived from the cross 'Bartlett' × 'Purduet' (derived in turn from the cross 'Old Home' × 'Early Sweet'); the clone thus has half of its genome in common with MRB (a bud mutation of 'Bartlett'), as supported by the finding that all the SSRs tested in HS and MRB show a common allele. For each F_1 genotype, 4 g of fresh young leaves were ground in liquid nitrogen. DNA extraction was performed following a modified CTAB protocol (Maguire et al. 1994). DNA quality and quantity were tested in 1% agarose and measured by image analysis using the Molecular Analyst 1.4.1 software (BioRad, USA).

SSR markers

A total of 112 SSR apple markers (Guildford et al. 1997; Gianfranceschi et al. 1998; Hokanson et al. 1998; Liebhard et al. 2002; B.A. Vinatzer et al., personal communication) were tested for segregation in the two populations described above. PCR amplifications were performed in a PTC-200 MJ Thermal Cycler (Genenco) under the following conditions: 50 ng genomic DNA, 1 μM each primer, 100 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 1 U *Taq* polymerase. The first cycle of denaturation at 94°C for 2 min and 30 s was followed by 24 cycles of annealing (60°C for 45 s), extension (72°C for 1 min) and denaturation (94°C for 30 s); a final cycle of extension (72°C for 10 min) was added. In some cases, the amplification conditions were optimised for pear by adjusting the annealing temperature. Amplification products were separated on 5% denaturing polyacrylamide gels stained with the silver method and their weights determined by densitometric analysis using the Kodak Image System with a Gibco 100 bp ladder as the standard.

RGA, AFLP and AFLP-RGA markers

Resistance gene analogs were determined according to Dondini et al. (2004); AFLPs (Vos et al. 1995) and AFLP-RGAs (Hayes and Saghai Maroof 2000) by using the RGA primer T2E32F (5'-ATTTCCTTCCGGCTTCTTCAAG).

Map construction

JoinMap 3.0 (van Ooijen and Voorrips 2002) was used for the construction of parental linkage maps PC, HS, AF and MRB; Kosambi's function made it possible to convert recombination percentages into centiMorgan distances. For mapping, a LOD score of 5.0 was considered acceptable. As both populations were F_1, the markers that segregated as ab×ab (presence of the same SSR alleles in both parents of the F_1 population) or aa×bb (presence of the same homozygous allele in a parent of the cross and of a second homozygous allele in the second parent) were not considered. A single locus analysis was performed to identify markers showing a distortion from the expected segregation ratio. Skewed markers were discarded only if they introduced obvious discrepancies during the map construction.

Results and discussion

Transferability of SSR information from one species to a related second species can be defined as the probability of success in PCR amplification using heterologous primers designed from the first species. The transferability of apple SSR markers for fingerprinting and mapping analyses has been reported for a panel of 14 primer pairs (Guildford et al. 1997; Gianfranceschi et al. 1998) by Yamamoto et al. (2001, 2002) in a collection of Nashi and European pear varieties and in a population derived from the cross 'Bartlett' × 'Hosui'. Screening of 112 apple SSR primer pairs in two pear F_1 populations derived from the crosses PC × HS and AF × MRB (Tables 1 and 2) showed a large percentage of apple SSRs having a polymorphic segregation (78.5 and 79.4% respectively in the two populations; Table 2). This supports the conclusion that apple SSRs are useful markers for fingerprinting and map construction in pear. For map construction, 41 (37.5%, PC × HS) and 31 (27.7%, AF × MRB) SSRs were chosen from the whole population because of their co-dominance and because of their position on apple maps (Maliepaard et al. 1998; Liebhard et al. 2002). The numerous primer pairs in both populations that produced either an ab×ab or aa×bb (12.5% in PC × HS and 7.2% in AF × MRB) segregation were discarded (Tables 1 and 2), although they are potentially transferable to the analyses of other pear populations.

The SSR segregation data recorded in both populations are reported in Table 2, together with the allele size (in base pairs) as compared to that reported by Liebhard et al. (2002) in apple. In most cases the segregation patterns of the markers observed in both pear populations were very similar (Table 2). Some markers produced no amplification (CH01E09, CH01F09, CH02D08, CH02G09, CH03D01, CH03G04, CH04E02, CH05B06, CH05C04, CH05D02, CH05E04, VFM12), or showed faint amplification patterns (CH02A03, CH02A04, CH02D10, CH03C01, CH04D07, VFA23A, VFA23B). In a few cases (CH01G12, CH02B10, CH4D10, CH04F08,

Table 1 Transferability of apple SSRs in the PC × HS and AF × MRB pear populations. The presence of the same SSR alleles in both parents of the F_1 population is indicated by ab×ab segregation, while the presence of the same homozygous allele in one parent and of a second homozygous allele in the second parent is indicated by aa×bb segregation

Type of SSR loci in F_1 population	PC × HS		AF × MRB	
	No.	%	No.	%
Non-transferable	24	21.5	23	20.6
No amplification	15	13.4	14	12.5
Non-polymorphic or complex	9	8.1	9	8.1
Potentially transferable SSR	14	12.5	8	7.2
ab×ab segregation	13	11.6	8	7.2
aa×bb segregation	1	0.9		
SSR transferable for mapping	74	66.0	81	72.2
Codominant segregation	37	33.0	51	45.5
Dominant segregation	33	29.5	27	24.1
Multilocus	4	3.5	3	2.6
Total	112	100	112	100

CH5G03) the absence of amplification in one population corresponded to the absence of segregation or to a particularly faint or complex amplification pattern in the second population.

The high degree of similarity observed for the two pear maps depends at least in part on the relationships between HS and MRB (they have a common allele in all the amplification patterns analysed). It is however evident that a significant conservation of SSR loci is common to apple and pear. Even the allele size ranges are similar in the two species (except for CH2A04, CH02F06, CH03A04, CH3G07, CH04A06, CH04D08, CH04F08, CH05D11, MS14H03; Table 2).

The alignment of the species maps based on JoinMap analysis made it possible to verify the degree of synteny between the apple and pear genomes (Table 3). The SSR loci showed the same order and corresponding distances in the pear and in the apple maps (Fig. 1a, b and c), strongly suggesting the presence of highly conserved regions between the two genomes. The evidence for this conservation was clearer when linkage groups (LGs) with a high number of SSRs were constructed from the two pear populations: this is the case of LG 10 (CH01F07, CH02B03, CH02C11, CH03D11, CH04C06, CH04G09; Table 3 and Fig. 1a), LG 12 (CH01D09, CH01F02, CH04G04, CH05D04, CH05D11, CH05G07, CH04D02; Table 3 and Fig. 1b) and LG 14 (CH01G05, CH03G06, CH04C07, CH04F06, CH05D03, CH05G11; Table 3 and Fig. 1c).

For the PC × HS population, where the map work is denser, synteny can also be observed for LGs 2, 9 and 11. Apple and pear LGs 9, 10, 15 (Table 3), as indicated in the present work, correspond in the 'Bartlett' × 'Hosui' map (Yamamoto et al. 2002) to LGs 10, 5 and 6, respectively. A discrepancy with the map reported by Yamamoto et al. (2002) was observed for the position of the apple SSR

Table 2 Transferability of apple SSR primer pairs to pear. All the apple data are from Gianfranceschi et al. (1998) and Liebhard et al. (2002). The screening of the apple SSRs in the AF × MRB and PC × HS F_1 populations was performed by amplifying DNA from the two parents and eight F_1 individuals. All the PC × HS population data were confirmed by the analyses on the whole population. *Mw* Molecular weight, *ML* multilocus, *NC* molecular weight data not available, *NA* not amplified

SSR	Pear maps		Mw range (bp)	
	AF x MRB	PC x HS	Apple	Pear
CH01A09	ab×cd[a]	ab×ab[b]	198–384	180–190
CH01C06	ab×ab	ab×aa[c]	145–190	140–150
CH01D03	ab×cd	ab×ac[d]	135–160	120–150
CH01D08	ab×cd	ab×ac	240–290	250–300
CH01D09	ab×ac	ab×ac	135–172	140–160
CH01E01	ab×c0[e]	ab×ab	106–120	110–120
CH01F02	ab×cd	ML	174–206	160–180
CH01F03b	ab×aa	ab×ab	139–183	150–200
CH01F07	ab×cd	aa×bc[f]	174–206	180–200
CH01G05	ab×cd	ab×aa	140–188	140–150
CH01H01	aa×ab	ab×ab	114–134	100–110
CH01H02	ab×ac	ab×cd	236–256	230–250
CH01H10	a0×ab	a0×ab	94–114	100–125
CH02A10	aa×ab	ab×ab	143–177	150–160
CH02B03b	ab×c0	aa×ab	77–109	70–90
CH02B07	ab×aa	ab×cd	180–202	120–130
CH02B10	ab×cd	NA	121–159	90–100
CH02B11	ab×ac	ab×ac	114–158	70–100
CH02C02b	ab×ac	ab×cd	78–126	125–150
CH02C09	aa×ab	ab×ab	233–257	250–260
CH02C11	ab×cd	ab×ac	219–239	220–250
CH02D11	ab×cd	ab×aa	118–148	90–125
CH02D12	ab×cc	ab×ac	177–199	110–140
CH02F06	a0×bb	ab×ac	135–158	160–200
CH02G04	a0×ab	a0×b0	132–194	125–170
CH02H11a	ab×ab	ab×aa	104–132	125–140
CH03A03	ab×aa	ab×ab	154–182	110–150
CH03A04	aa×ab	aa×ab	92–124	180–190
CH03A09	a0×ab	aa×ab	125–143	100–125
CH03B06	a0×b0	a0×b0	111–131	80–110
CH03B10	ab×aa	ab×aa	99–121	80–90
CH03D02	ab×ac	ab×cd	201–223	175–225
CH05A04	ab×aa	ab×ac	159–189	170–180
CH05A05	ab×ac	ab×ab	198–230	200–210
CH05A09	ab×ab	ab×aa	152–200	190–210
CH05C06	ab×ab	ab×ac	104–126	90–110
CH05C07	ab×ac	ab×ac	111–149	110–150
CH05D03	ab×cd	ab×cd	152–187	160–200
CH05D04	ab×cd	ab×ac	174–214	160–230
CH05D08	ab×aa	ab×cc[f]	91–143	110–140
CH05D11	ab×c0	ab×cd	171–211	100–120
CH05E03	ab×a0	a0×b0	158–190	180–200
CH05E05	ab×aa	ab×ab	138–160	120–130
CH05E06	a0×bc	ab×cd	125–222	90–130
CH05F04	ab×cd	ab×ac	160–172	130–160
CH03D07	ab×ac	aa×b0	186–226	180–190
CH03D08	ab×cd	ab×aa	129–161	140–150

Table 2 (continued)

SSR	Pear maps		Mw range (bp)	
	AF x MRB	PC x HS	Apple	Pear
CH03D10	ab×ac	ab×cd	166–182	170–200
CH03D11	a0×bc	ab×cd	115–181	90–125
CH03D12	ab×ac	aa×ab	108–154	110–140
CH03E03	ab×ab	ab×aa	106–216	190–200
CH03G06	ab×ac	ab×cd	139–171	150–180
CH03G07	ab×ac	ab×ac	119–171	225–260
CH03G12	a0×b0	aa×b0	154–200	180–200
CH03H03	ML	ML	72–120	70–130
CH03H06	ab×cd	ab×ac	143–175	150–175
CH04A06	ab×ab	ab×aa	106–110	200–300
CH04A12	ab×c0	ab×cc	158–196	160–180
CH04B11	ab×cd	ab×aa	NC	90–110
CH04C06	ab×cd	ab×cd	155–186	180–210
CH04C07	ab×cd	aa×ab	98–135	120–150
CH04C10	aa×ab	ab×aa	133–180	125–150
CH04D02	aa×bc	ab×cd	118–146	130–150
CH04D08	ab×ac	ab×cd	116–142	180–230
CH04D12	ab×ac	ab×ac	143–163	110–170
CH04E03	ab×ac	ab×ab	179–222	175–200
CH04E05	aa×bc	ab×cd	174–227	160–190
CH04F03	ab×cd	ab×aa	175–191	170–190
CH04F04	ab×aa	ab×ab	144–166	100–140
CH04F06	ab×cd	ab×aa	159–179	170–180
CH04F10	a0×bc	ab×ab	144–254	200–240
CH04G04	ab×cc	ab×aa	170–186	170–190
CH04G07	ab×ac	ab×aa	149–211	130–175
CH04G09	ML	aa×bc	141–177	130–180
CH04H02	ab×aa	ML	162–262	170–225
CH05A02	ab×cc	ab×cc	111–135	110–120
CH05A03	ab×c0	ab×c0	182–220	190–210
CH05F06	aa×b0	ab×c0	166–184	160–175
CH05G01	a0×bc	a0×bc	236–276	160–210
CH05G07	ab×cd	aa×ab	149–197	150–220
CH05G08	ab×aa	ML	161–179	125–170
CH05G11	ab×ac	ab×cc	201–255	210–230
CH05H05	ab×ab	aa×ab	168–184	170–200
MS01A05	ab×ac	ab×aa	158–176	160–175
MS02A01	ab×ac	aa×bc	170–194	140–150
MS14H03	ML	aa×bc	230–292	100–130
NZ02B1	ab×cd	ab×ab	NC	260–300
VFC9	aa×bc	ab×ac	NC	130–170
VFE6	aa×ab	ab×ab	NC	140–160

[a]Four different SSR alleles in both parents of the F_1 population
[b]The same SSR alleles in both parents
[c]Only one segregating SSR alleles from one parent (dominant marker)
[d]Common SSR alleles in the two parents
[e]The null allele "0" (absence of an amplification product)
[f]A homozygous allele in one parent of the cross and two different alleles in the second parent

Table 3 Identification and denomination of pear LGs based on apple SSRs. All apple data are from Liebhard et al. (2002). The segregation data in AF × MRB and PC × HS populations were obtained by amplifying the DNA of all F_1 plants. *NC* Data not collected or not available

SSR	Linkage groups		
	Apple	AF x MRB	PC x HS
CH01A09	14	14	NC
CH01C06	8	NC	8
CH01D03	4/12	4	4
CH01D08	15	15	15
CH01D09	12	NC	12
CH01F02	12	12	12/4
CH01F07	10	10	10
CH01G05	14	14	14
CH02B03b	10	10	NC
CH02B07	10	NC	10
CH02C02b	4	NC	4
CH02C11	10	10	10
CH02F06	2	NC	2
CH02H11a	4	NC	4
CH03D02	11	11	11
CH03D10	2	2	2
CH03D11	10	10	10
CH03D12	6	NC	6
CH03G06	14	14	14
CH03G07	3	3	3
CH03H03	13	NC	2/13/14
CH04C06	10/17	10	10
CH04C07	14	14	14
CH04D02	12	NC	12
CH04E05	7	7	7
CH04F03	10	10	NC
CH04F06	14	14	14
CH04G04	12	12	12
CH04G07	11	11	NC
CH04G09	5/10	NC	10
CH04H02	11	NC	11
CH05A02	8/15	NC	8
CH05A03	9	9	9
CH05C06	16	NC	16
CH05C07	9	9	9
CH05D03	14	NC	14
CH05D04	12	12	12
CH05D11	12	NC	12
CH05E06	5	5	5
CH05F04	13	13	13
CH05F06	5	NC	5
CH05G01	11	11	11
CH05G07	12	12	NC
CH05G11	14	14	14
VFC9	1	NC	1

CH01F02: as reported in Table 3 and Fig 1, the marker in both pear populations characterised a locus on LG 12 (on LG 4 in HS), as in apple (Liebhard et al. 2002), while it was placed in LG 1 in the population Bartlett × Hosui (Yamamoto et al. 2002). The CH01D03 marker in apple also showed a similar behaviour: it can map either in the LG 4 or in LG 12 (Liebhard et al. 2002), suggesting that some chromosomal regions have been co-ordinately duplicated or translocated during evolution.

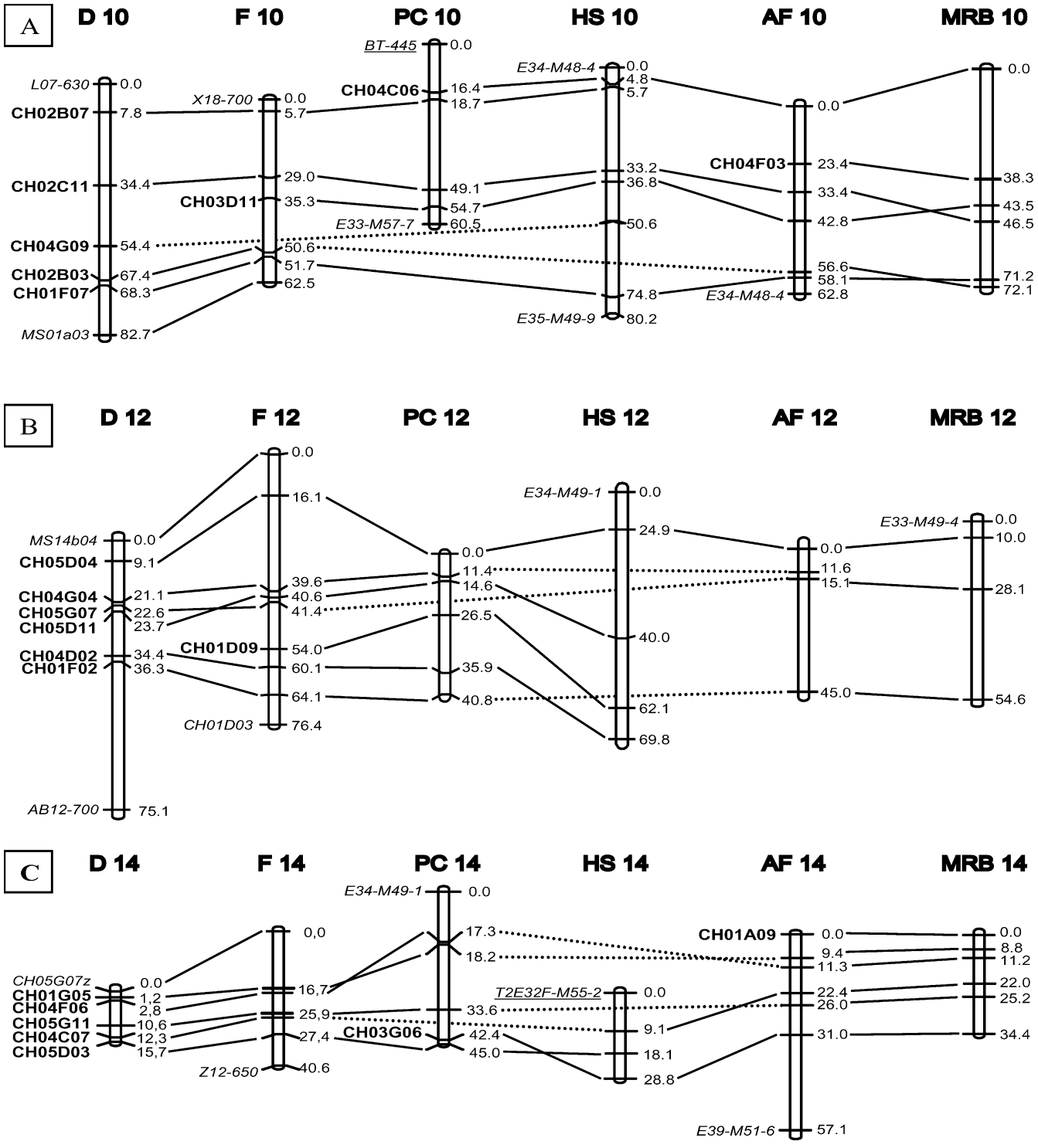

Fig. 1a–c Alignment of the LG 10 (**a**), 12 (**b**) and 14 (**c**) of the apple varieties Discovery (D) and Fiesta F (Liebhard et al. 2002) with the pear maps PC, HS, AF and MRB. RGAs and related markers are *underlined*. The markers at the *top* and the *bottom* of the LGs (if SSRs are not transferable) are indicated in *italics*. The synteny among chromosomes of the two species is demonstrated by the order and the relative distances of the positioned SSR markers

The similarity between two apple and pear linkage maps supports not only the study of the relationships between these related species, but also makes it possible to speed up the transfer of information from apple to pear since functional genes or QTLs are already mapped in the apple genome. This paper represents a further tool in this direction.

Acknowledgements We are grateful to Dr. Francesco Salamini for critical reviews of this manuscript and for all his suggestions. This research was supported with funds from MiPAF (Ministero delle Politiche Agricole e Forestali, Rome, Italy) and from the National Horticultural Research Institute (RDA), Suwon, Republic of Korea.

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