Development of two loquat (*Eriobotrya japonica* (Thunb.) Lindl.) linkage maps based on AFLPs and SSR markers from different Rosaceae species

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Abstract

Loquat (*Eriobotrya japonica* (Thunb.) Lindl.) is a Rosaceae fruit species of growing interest as an alternative to the main fruit crops. However, only a few genetic studies have been carried out on this species. This paper reports the construction of the first genetic maps of two loquat cultivars based on AFLP and microsatellite markers from *Malus*, *Eriobotrya, Pyrus and Prunus* genera. An F_1 population consisting of 81 individuals, derived from the cross between 'Algerie' and 'Zaozhong-6' cultivars, was used to construct both maps. A total of 111 scorable SSR loci resulted from the testing of 440 SSR primer pairs in the analyzed progeny and the SSR transferability to *Eriobotrya* was found to be 74% from apple, 58% from pear and 49% from *Prunus spp*. In addition, 183 AFLP polymorphic bands were produced using 42 primer combinations. The 'Algerie' map was organized in 17 linkage groups covering a

distance of 900cM and comprising 177 loci (83 SSRs and 94 AFLPs) with an average marker distance of 5.1 cM. Self-incompatibility trait was mapped at the distal part of the LG17 linkage group, as previously reported in *Malus* and *Pyrus*. The 'Zaozhong-6' map covered 870cM comprising 146 loci (64 SSRs and 82 AFLPs) with an average marker distance of 5.9cM. The 44 SSRs and the 48 AFLPs share in common by both maps were essentially collinear and, moreover, the order of the 75% of apple and pear SSRs mapped in *Eriobotrya* was shown to be consistent across the Maloideae subfamily. As a whole, these maps represent a useful tool to facilitate loquat breeding and an interesting framework for map comparison in the Rosaceae.

Introduction

The Dadu River Valley, in southwestern China, is considered the center of origin of the genus *Eriobotrya* (Zhang et al. 1990). Loquat (*Eriobotrya japonica* (Thunb.) Lindl.) was introduced from China to Japan in ancient times (s.XII) and to Europe much more later (s.XVIII) (Lin et al. 1999). The crop showed a successful adaptation to the Mediterranean climate and since the beginning of the 20th century was grown in regular orchards. Currently, Spain accounts for more than 50% of the total European production and some other countries, such as Italy and Israel, are also commercial producers.

Loquat is a subtropical evergreen fruit tree that blooms in fall and early winter (Lin et al. 1999). It belongs to the Maloideae subfamily of the Rosaceae beside apple or pear. The Maloideae, including loquat (Lu and Lin 1995), are functional diploids (2n=2x=34) for which an allopolyploid origin has been suggested (Chevreau et al. 1985). Most major loquat cultivars derive from chance seedlings (Huang et al. 1990) but breeding programs based on hybridization have also released some cultivars such as 'Zaozhong-6' in China or 'Nakasaki-wase' in Japan (Lin et al. 1999). The development of genetic maps, based on molecular markers, will be a useful tool to employ marker-assisted selection (MAS) within these programs, facilitating major advances in the future. Indeed, the analysis of quantitative traits in apple has progressed significantly since genetic linkage maps became available at mid-90s (Kenis and Keulemans 2005). First Maloideae maps were based on isoenzymes or restriction fragment length polymorphisms (RFLP) (Hemmat et al. 1994; Maliepaard et al. 1998), but more recently have been replaced by maps containing a backbone of Simple Sequence Repeats (SSRs) embedded in amplified fragment length polymorphism (AFLPs) markers (Liebhard et al. 2002; Kenis and Keulemans 2005).

In the last years, SSRs or microsatellites have become the marker of choice for multiple applications, proving to be particularly useful for integrating mapping results and assessing marker-gene associations (Silfverberg-Dilworth et al. 2006). The increasing number of SSRs available has been essential for the recent progresses in the genetic analysis of many plant species. In the Rosaceae, SSR markers have been shown to be extremely valuable not only for building integrated genetic maps but also for comparing maps from different genera exploiting their high transferability (Yamamoto et al. 2004; Pierantoni et al. 2004).

In this work, 440 SSRs derived from *Malus*, *Pyrus*, *Eriobotrya* and *Prunus* genera, were tested for their polymorphism in an *Eriobotrya japonica* (Thunb.) Lindl. intraspecific progeny from the cross 'Algerie' x 'Zaozhong-6' ('AxZ'). As a result, we reported the construction of the first two linkage maps of the *Eriobotrya* genus containing 103 SSR and 128 AFLP loci. The *S*-locus, that controls the self-incompatibility trait in Maloideae, was also positioned on this map on the basis of PCR products amplified using consensus primers developed from *Malus x domestica* and *Pyrus spp*. (Raspé and Kohn 2002).

'Algerie' is the main loquat cultivar in southeastern Spain, characterized by its precocity and quality and mostly grown in monoculture (Martínez-Calvo et al. 2000). In this context, the 'Instituto Valenciano de Investigaciones Agrarias' (IVIA), in collaboration with the growers association from Callosa d'En Sarriá (Alicante, Spain), began a breeding program in 2002 aimed at extending the crop season of 'Algerie'. (Llácer et al. 2008). One of the cultivars selected to be crossed with 'Algerie' was 'Zaozhong-6', a widespread cultivar in China which outstanding characteristics are also early ripening, good quality and a wide ecological adaptation (Lin et al. 1999). The objective of the 'AxZ' cross was, not only to construct a genetic linkage map, but also

to generate new variability for future crop breeding, an essential premise in the context of scarce genetic diversity of the available loquat germplasm (Soriano et al. 2005).

Material and methods

Plant material and DNA isolation

A loquat F_1 population, comprising 81 seedlings, derived from the intraspecific cross 'Algerie' x 'Zaozhong-6' made in 2003, was used for the construction of the linkage map. 'Algerie' is an Algerian cultivar of unknown origin and 'Zaozhong-6' is a Chinese cultivar originated from the cross 'Jiefangzhong' x 'Moriowase' in 1992 (Lin et al. 1999). All these trees are maintained at the germplasm collection of the *Instituto Valenciano de Investigaciones Agrarias (IVIA)* in Valencia (Spain). Isolation of high-quality DNA from loquat samples has been particularly difficult because of the coriaceous and pubescent nature of the leaves containing high levels of phenolic compounds. DNA was extracted from 200 mg of young leaves following the method of Doyle and Doyle (1987) with some modifications: reducing the ratio of fresh leaf tissue/CTAB buffer to 10 mg/ml and recovering nucleic acids by precipitation with 2 x volume of absolute ethanol and 0.5 x homogenate volume of 5M NaCl. DNA quantification was performed by comparison with lambda DNA (Promega, Madison, WI).

Microsatellites analysis

A total of 440 SSR primer pairs derived from *Malus* (249), *Prunus* (134), *Eriobotrya* (21) and *Pyrus* (36) genera have been tested for polymorphism in the 'AxZ' progeny (Table 1). SSR amplifications were performed in a GeneAmp®PCR System 9700 thermal cycler (Perkin-Elmer Corp., Freemont, CA) in a final volume of 10 μl containing 75 mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.8 μM of each primer, 20 ng of genomic DNA and 1 Unit of *Taq DNA*

polymerase (Invitrogen, Carlsbad, CA) using the following temperature profile: 95ºC for 2 min, then 35 cycles of 95ºC for 30 s, 50-71ºC for 1 min and 72ºC for 1 min and 30 s, finishing with 72ºC for 7 min. Initially, the screening was performed in a set comprising the two parents and six seedlings of the progeny, and those SSRs showing segregations suitable for mapping were tested in the whole population. PCR products were mixed with 10 µl of formamide loading buffer (98% formamide, 10 mM of EDTA, 0.1% bromophenol blue and xylene cyanol), heated at 95ºC for 3 min and immediately cooled on ice. Two microlitres of each sample were loaded on a 6% denaturing polyacrylamide gels (acrylamide-bisacrylamide 19:1) containing 7.5 M urea, in a Sequi-GenGT Sequencing Cell (BioRad, Hercules, Calif., USA) and electrophoresed at constant power of 80W for 1 h. Detection was performed by silver staining according to Bassam et al. (1991) and molecular sizes were determined by comparison with 10bp DNA ladder (Invitrogen, San Diego, Calif., USA).

AFLPs

DNA digestion, adaptor ligation and pre-selective and selective amplifications were carried out according to the manufacturer´s instructions (GIBCO BRL, Gathersburg, Md., USA) and standard procedures (Vos et al. 1995). Primer combinations including two or three selective bases for one primer and three for the other were selected. PCR products were dried and solved in 10 µl of formamide loading buffer, heated at 95º C for 3 min and immediately cooled on ice. Two microlitres of each sample were loaded on a 6% denaturing polyacrylamide gels (acrylamide-bisacrylamide 19:1) containing 7.5 M urea, in a cooled model S2001 sequencing gel electrophoresis apparatus (Life Technologies, Rockville, MD, USA). Electrophoresis was run at constant power of 90W for 1 h. Gels were silver stained according to Bassam et al. (1991).

Self-incompatibility trait

The *S*-allele fragments were amplified using the partial degenerated primers SC/C2-F [5'-GTT YAC BGT TCA CGG WTT GTG GCC-3´] and SC/R[5´- CGG CCA AAT WAT TTY CAA CTG-3´] designed from conserved regions of S-alleles sequences of *Malus x domestica* and *Pyrus spp*. (Raspé and Kohn 2002). PCRs were performed using a program of 35 cycles at 94º C for 30 s, 54 ºC for 45 s and 72ºC for 1 min and 15 s, with an initial denaturing of 94º C for 3 min and a final extension of 72 ºC for 10 min. The PCR reaction mixture contained 1 X PCR buffer (20 mM of Tris-HCl pH 8.4 and 50 mM of KCl), 2.0 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.30 µM of each primer, 30 ng of genomic DNA and 1 Unit of *Taq DNA polymerase* (Invitrogen, San Diego, Calif., USA). PCR products were electrophoresed and detected as described for SSRs.

Linkage analysis and map construction

The linkage analysis was carried out using Joinmap 3.0 software (Van Ooijen and Voorrips 2001) with the Kosambi mapping function (Kosambi 1944) used to convert recombination units into genetic distances.

In the 'AxZ' population two separated genetic linkage maps were constructed for each parent following the "two-way pseudo test-cross" model of analysis (Grattapaglia and Sederoff 1994) and setting a "cross-pollinator" data type. Linkage groups were established using as threshold a minimum logarithm of odds (LOD) of 6.0. In general, linkages considered for mapping were those with recombination frequency lower than 0.4 and LOD score larger than 3.0.

Results

Microsatellites and transferability

A total of 292 out of the 440 SSRs tested in the 'AxZ' progeny gave amplification. From these, 168 were monomorphic and 19 could not be scored because of their

complex band patterns. In the remaining 105 informative SSRs, 6 amplified two different loci resulting in a total of 111 scorable loci (Table 2). From these, 103 SSRs were finally mapped through the linkage analysis performed by JoinMap 3.0. Forty-two showed a co-dominant segregation being 74% of them fully informative (<ab×cd> and <ef×eg>) and the remaining 26% <hk×hk> segregating 1:2:1. Percentages of dominant markers did not vary among the different SSR sources, however SSRs mapped in 'Algerie' (42) almost doubled those mapped in 'Zaozhong-6' (27). Fourteen SSR loci showed distorted segregation at P<0.05 (14%) and 8 at P<0.01 (8%). Null alleles evidence were detected for 'Zaozhong-6', at least, in 6 loci and for 'Algerie' in 2 loci.

SSR transferability to *Eriobotrya* was found to be 74% from apple, 58% from pear and 49% from *Prunus spp*. No significant differences of transferability among series were detected within the same species. The percentage of polymorphic SSRs mapped is quite similar for all the tested species, overcoming the 85% (Table 3).

AFLPs

Additionally, a total of 183 AFLP polymorphic bands were produced using 42 *Eco*RI+2-3/*Mse*I+3 primer combinations (Table 4) and 128 of them were mapped (Fig. 1). According to the primer combination used the number of polymorphic markers ranged from 1 (E-AAC+M-CTG; E-ACA+M-CGC; E-ACC+M-CGC; E-ACG+M-CTC; E-ACT+M-CTA and E-AGG+M-CAG) to 12 (E-AGG+M-CTA). On average, 4.3 polymorphisms were scored per primer pair and 3.0 out of them were mapped. Segregation data were tested for deviation from the expected Mendelian ratios (3:1 and 1:1) using the χ^2 test. Considering all the polymorphic AFLP markers, 16 showed distorted segregation at P< 0.05 (9%) and 30 at P<0.01 (17%).

Self-incompatibility locus

The self-incompatibility trait was coded as a co-dominant marker (<ef×eg> as per JoinMap 3.0), based on the PCR-amplified fragments obtained with the SC/C2-F and SC/R primers (Raspé and Kohn 2002) from the parents and the progeny of the 'AxZ' cross. However, only two of the four expected genotypes were recovered (<eg> for 40 and <fg> for 36 individuals, respectively). This segregation deviates significantly (χ^2 = 76.4 at P<0.01) from the expected segregation ratio for a single co-dominant locus (1:1:1:1), but is in agreement with the *S*-locus segregation resulting from a half-compatible reaction involving one *S*-allele (<e>) shared by both parents (1:1). Self-incompatibility trait has been mapped at a LOD score of 6.0 at the distal part of the 'Algerie' LG17 linkage group (Fig. 1).

Genetic linkage maps

A total of 103 SSRs and 128 AFLPs were mapped in 'Algerie' and 'Zaozhong-6' maps (Tables 4 and 5). Eighty-three SSR loci were incorporated into 'Algerie' map and distributed throughout the genome, ranging from two markers in LG1, LG3, LG4 and LG13 to eight markers in LG5 (Fig. 1). Thirty-four of them segregated as dominant markers (<lm×ll> as for JoinMap 3.0) and therefore were only present in the 'Algerie' map. All the SSRs derived from *Eriobotrya* and *Prunus*, as well as three SSRs from *Malus* (CH01c08, CH04g12 and CH02e12) (Table 5) have been located in a Maloideae map for the first time. The 'Algerie' map was organized in 17 linkage groups, three of which split into two subgroups (LG3, LG10 and LG11), covering a distance of 900cM and comprising 177 loci: 83 SSRs and 94 AFLPs (Fig. 1). The average distance between adjacent markers was 5.1cM, ranging from 3.1cM in LG2 to 9.9cM in LG11a. Five linkage groups showed gaps over 20 cM (LG6, LG9, LG11, LG16 and LG17). A total of 44 markers (25%) showed distorted segregation, 22 at *P*<0.01 (6 SSR and 16

AFLP) and 22 at $P<0.05$ (13 SSR and 9 AFLP). Distorted markers are distributed throughout the maps but they locate mainly in several linkages groups (LG2, LG8, LG9, LG10, LG11, LG15 and LG17) (Fig. 1).

Sixty-four SSR loci were incorporated into the 16 linkage groups, four of which split into two subgroups (LG9, LG10, LG15 and LG17), of the 'Zaozhong-6' map, ranging from 1 marker in LG3 to 9 markers in LG5 (Fig. 1). Nineteen of them segregated as dominant markers (<nn×np> as for JoinMap 3.0) and therefore were only present in the 'Zaozhong-6' map. The 'Zaozhong-6' map covers a distance of 870cM and comprises 146 loci: 64 SSRs and 82 AFLPs (Fig. 1). The average distance between adjacent markers was 5.9cM, ranging from 3.8cM in LG2 to 13.1cM in LG6. Five linkage groups showed gaps over 20cM (LG5, LG6, LG11, LG15 and LG17). A total of 33 markers (23%) showed distorted segregation, 16 at $P<0.01$ (7 SSR and 9 AFLP) and 17 at $P<0.05$ (7 SSR and 10 AFLP). Distorted markers are distributed throughout the maps but they locate mainly in several linkages groups (LG2, LG5, LG8, LG9, LG10, LG15 and LG17) (Fig. 1).

More than 20 multilocus SSR were detected but most of them could not be scored because of their complex band pattern. In some cases, two loci were detected but only one could be reliably scored (*ssr*PaCITA16 and NH013a). At the end, a total of six multilocus SSR (CH04g09, CH02a08, Hi07e08 and Hi03e04 from *Malus*; *ssrEJ*46 from *Eriobotrya*; BPPCT14 from *Prunus*) were scored and mapped. CH04g09 and CH02a08 were located at the LG5 and LG10 groups in the *Eriobotrya* map, as previously reported in *Malus* by Liebhard et al. (2002). Hi07e08 was positioned at the LG7 and LG8 groups in *Eriobotrya*, whereas LG3 and LG8 were the reported positions in *Malus*, and Hi03e04 was situated on LG16 and LG17 groups, while in *Malus* it was described as a single locus located at LG13 (Silfverberg-Dilworth et al. 2006). Other SSRs located in

Eriobotrya groups differing from those previously assigned in *Malus* had been also described as multilocus by Liebhard et al. (2002), such as CH01b12 or CH04b10.

Colinearity

A total of 48 AFLPs and 44 SSRs, heterozygous in both parents, provided bridges between the two maps obtained, corresponding to the female ('Algerie') and male ('Zaozhong-6') parent. All the linkage groups showed marker bridges, except LG1 and LG13, ranging from one (LG4, LG11b) to twenty-one (LG16). These two maps were essentially collinear and only 4 minor marker inversions were found in linkage groups LG3, LG7, LG10 and LG15 (Fig. 1).

In addition, *Malus* and *Pyrus* SSRs allowed us to establish homologies with other *Maloideae* maps. According to the literature, 73 out of the 76 SSRs derived from apple and pear mapped in this work had been previously mapped in *Maloideae* species (Liebhard et al. 2002; Silfverberg-Dilworth et al. 2006; Yamamoto et al. 2004) and only 7 out of the 73 (10%) were positioned in *Eriobotrya* linkage groups differing from the original groups assigned in *Malus* and/or *Pyrus* (Table 5 and Fig. 1). Forty-three were held in common with the *Malus* reference map derived from the cross 'Fiesta' x 'Discovery' (Silfverberg-Dilworth et al. 2006), another 43 with the *Malus* linkage map developed by Fernández-Fernández et al. (2008) from the cross 'Fiesta' (*M. pumila*) x 'Totem' (*Malus* interspecific hybrid) and 20 with the *Pyrus* map developed by Yamamoto et al. (2004) from the cross 'Bartlett' (*P. communis*) x 'Housui' (*P. pyrifolia*).

Figure 2 shows the general colinearity among Maloideae genera maps by aligning *Eriobotrya*, *Malus* and *Pyrus* linkage groups sharing at least five linked SSRs (LG5, LG9, LG12 and LG16). Six additional groups (LG2, LG6, LG10, LG11, LG14 and LG17) show at least three linked markers previously located in other *Malus* and/or

Pyrus maps. The rest contain a maximum of two linked SSRs (LG1, LG3, LG4, LG7, LG8, LG13 and LG15). Only 6 out of the 46 SSRs comprised in the first 10 groups present conflict with the established order in the *Malus* and *Pyrus* maps (Fernández-Fernández et al. 2008; Silfverberg-Dilworth et al. 2006; Yamamoto et al. 2004). Two of these conflicts correspond to single genetic inversions, in LG5 (CH02b12-CH02a08(2)) (Fig. 2) and LG14 (CH01e01-CH04f06) (Fernández-Fernández et al. 2008). In other cases marker positions within the linkage group simply do not correspond with those previously reported (LG2, CH02b10; LG10a, CH05h12 and CH04c06; LG17, CH01h01) (Fernández-Fernández et al. 2008).

Discussion

Microsatellites and transferability

The percentage of amplified microsatellites in the analyzed *Eriobotrya* progeny depended on the source species and was roughly consistent with the generic relationships in the subtribe Pyrinae (formerly the Maloideae) (Campbell et al. 2007). In this work we have tested SSRs developed from two different subfamilies of the Rosacae: Maloideae and Prunoideae. Within the Maloideae, all SSRs developed from loquat (Gisbert et al. 2008) gave amplification, as expected, but only 74% of the *Malus* SSRs and 58% of the *Pyrus* SSRs amplified in loquat. In accordance to the phylogenetic distance from *Eriobotrya* this percentage decreased to 49% when we used *Prunus* SSRs (Campbell et al. 2007).

A high percentage of the total amplified SSRs (58%) were monomorphic. The degree of polymorphism was high for those SSRs derived from *Eriobotrya* (81%) but decreased significantly when they were from *Malus* (37%), *Pyrus* (38%) or *Prunus* (18%). In agreement with these results, Soriano et al. (2005) detected a mean value of 2.4 alleles per locus using *Malus* SSRs in a set of 40 loquat cultivars, while Gisbert et

al. (2008) amplified a mean value of 4.1 alleles per locus using *Eriobotrya* SSRs in 21 loquat cultivars. Similarly, using peach SSRs, Dirlewanger et al. (2002) found 4.2 alleles per locus in 27 peach cultivars and only 2.8 alleles per locus in 21 sweet cherry cultivars.

The degree of SSR polymorphism detected in *Malus* inter- and intraspecifc crosses has been usually significantly higher (over 90%) than this found in *Eriobotrya* (Liebhard et al. 2003; Fernández-Fernández et al. 2008; Silfverberg-Dilworth et al. 2006). A likely cause might be the origin of the SSRs tested. It has been often reported in the literature that polymorphism of SSRs originated from ESTs (Expressed Sequence Tags) is lower that found in the SSRs from genomic libraries (Ellis and Burke 2007). However, only 30 out of the 440 SSRs tested in this work derived from ESTs (Yamamoto et al. 2002d; Howad et al. 2005; Silfverberg-Dilworth et al. 2006). Thus, the main reason behind the low degree of polymorphism found in the 'AxZ' population seems to be a high genetic similarity between parents. Indeed, genetic diversity of loquat germplasm, assessed by SSRs and expressed as the mean number of alleles and expected heterozygosity, was showed to be low when compared with *Malus* or *Pyrus* (Guilford et al. 1997; Gianfraceschi et al. 1998; Yamamoto et al. 2001; Soriano et al. 2005). Similarity between parents was also confirmed by the low degree of variability found with AFLPs. On average, 4.3 polymorphisms per primer pair, rather less than the approximately 9.0 polymorphisms per primer pair found in apple by Kenis and Keulemans (2005). Several observations might support a relatively small genetic distance between some Chinese and European cultivars, the significant degree of self-compatibility present in loquat (Rodríguez 1983; Morton et al. 1987), the late introduction of loquat culture in Europe (Lin et al. 1999) and the substantial number of cultivars originated as sports (Martínez-Calvo et al. 2000).

The number of SSR dominant markers mapped in 'Algerie' (34) is significantly higher than that mapped in 'Zaozhong-6' (19). Moreover, only 6% of these markers showed evidence of null alleles in 'Algerie' but this percentage increases to 32% in 'Zaozhong-6'. In total, approximately 10% more alleles were detected in 'Algerie' indicating a slightly higher degree of heterozigosity for this cultivar.

As a whole, the 20% of the SSRs detected in this population showed distorted segregation, and this percentage is similar to that found in other *Malus* crosses, such as 'Fiesta' x 'Totem' with a 19% of distorted markers (Fernández-Fernández et al. 2008). Chance alone or technical causes, such as genotyping errors or missing values, might explain distortions in isolated markers. However, distorted markers often form clusters suggesting a biological background that might be the result of genetic load (Bratteler et al. 2006).

Eriobotrya maps and breeding

Thirty out of the 103 SSRs comprised in the 'Algerie' and 'Zaozhong-6' maps have been mapped for the first time in a Maloideae map: 16 from *Eriobotrya japonica*, 9 from *Prunus persica*, 3 from *Malus x domestica*, 1 from *P. armeniaca* and 1 from *P. salicina*. Three SSRs derived from *Malus* ESTs have also been mapped: two putatively associated with mRNA for MADS box proteins ($AJ320188_{SSR}$ and $U78949_{SSR}$) and one with an S-RNase gene ($AY187627_{SSR}$) (Silfverberg-Dilworth et al. 2006). This latter mapped on LG17 being tightly linked with the self-incompatibility phenotypic trait but not fully coincident. A few missing values and genotyping errors might explain this small mismatch. Moreover, CHVf1, a microsatellite marker isolated from a BAC clone of the *Vf* scab resistance region (Vinatzer et al. 2004) also segregated in the 'AxZ' population.

The seventeen linkage groups, corresponding to the basic chromosome number of the species, have been defined on the basis of SSRs held in common with *Malus* and *Pyrus* maps and named according to the 'Fiesta' x 'Discovery' *Malus* reference map (Liebhard et al. 2003). Five linkage groups are shown as split into two subgroups due to the low saturation of these genome regions (LG3, LG9, LG10, LG15 and LG17), and in a few cases no consistent linkage was found with their proper counterparts (A1, Z1, A11a, A13, Z15a and Z17a). In general, low saturated areas in *Eriobotrya* match roughly the longest gaps reported for 'Discovery' in the *Malus* reference map (Silfverberg-Dilworth et al. 2006). The relatively small number of recombinant events seems also to be behind the probably spurious excessive length of LG11. In spite of the inadequate coverage of some regions, SSRs were evenly distributed throughout the genome and each of the 17 LGs contains at least two of them (except LG1) allowing a reliable identification and orientation by alignment with the *Malus* reference map. The 'Algerie' and 'Zaozhong-6' maps showed a significant coverage of the *Eriobotrya* genome since their approximate 900 cM suppose between 61-79% of the total length of the close to completion *Malus* reference map (Silfverberg-Dilworth et al. 2006).

Silfverberg-Dilworth et al. (2006) proposed a set of 86 SSR primer pairs for the global coverage of the apple genome, though 16 additional chromosome segments were still uncovered. Interestingly, 72 of them were tested in this work and 23, present in 15 different LGs, were included in the 'Algerie' and 'Zaozhong-6' maps. Obviously, more SSRs are needed to increase saturation in both maps, nevertheless, these are the first loquat linkage maps available and represent a starting point to improve germplasm management and a useful tool for future assistance on *Eriobotrya spp*. breeding.

Colinearity within the Maloideae

The order of the 92 markers (48 AFLPs and 44 SSRs) share in common by the *Eriobotrya japonica* cultivars 'Algerie' and 'Zaozhong-6' is essentially collinear and the few inversions detected involve markers mapping relatively close together. In these cases, the calculated positions might be affected by missing values or by a low density of markers.

In general terms, the order of the 75% of apple and pear SSRs mapped in *Eriobotrya* is consistent across genera of the Maloideae subfamily. Part of the mis-allocations found might be attributed to the presumed multi-locus nature of some mapped SSRs such as Hi07e08, Hi03e04, CH04b10 or CH01b12 (Liebhard et al. 2002). On the other hand, inversions of the order between adjacent pair of markers, such as those found in LG5 and LG14, and other minor reordering involving more than two markers, found in LG2 in relation to CH03d10 and CH02b10, in LG10 involving CH02c06 and CH05h12, and in LG17 between CH01h01 and CH05g03, can be likely due to the low resolution of the maps. In fact, order disagreements in the middle part of LG2 are shared by different Maloideae maps (Silfverberg-Dilworth et al. 2006; Fernández-Fernández et al. 2008; Yamamoto et al. 2004). A more severe conflict was found between LG14 and LG15. According to previous *Malus* maps (Fernández-Fernández et al. 2008), CH01d08 should be mapped in the central part of LG15 and CH03g06 in the distal extreme of LG14. However, the analysis of the 'AxZ' population data shows the opposite, CH01d08 is grouped in LG14 and CH03g06 in LG15, suggesting a likely major rearrangement between these two LGs in *Eriobotrya*. More markers would be needed to discard mapping errors and to confirm this putative rearrangement

Some additional details reinforce the high degree of co-linearity observed between *Eriobotrya japonica* and other Maloideae species. On one side, the two multilocus SSRs CH04g09 and CH02a08 were located at the LG5 and LG10 groups in the *Eriobotrya*

map. This result supports the potential homeology between both groups in *Malus* previously suggested by different studies using isozymes and RFLPs (Malieppard et al. 1998), only RFLPs (Dirlewanger et al. 2004) or SSRs (Liebhard et al. 2002; Fernández-Fernández et al. 2008). On the other side, part of the SSR loci showing distorted segregation in the *Eriobotrya japonica* population grouped in clusters which location is coincident with that previously reported in *Malus*, for instance, at the top of LG2 and in a large section of LG15 (Fernández-Fernández et al. 2008). The origin of these clusters might be the selection occurred, at the pre- or post-zygotic level, against lethal or sublethal genes linked to the markers. Lastly, the self-incompatibility trait mapped at the distal part of LG17 linkage group as previously reported by Maliepaard et al. (1998) and Fernández-Fernández et al. (2008) in *Malus* and Yamamoto et al. (2004) in *Pyrus*.

Eleven *Prunus* SSRs, derived from peach, apricot and plum, were also mapped. Unfortunately, none of them had been mapped before in a Maloideae map and therefore co-linearity among them could not be checked directly. Moreover, none of the proposed relationships between *Malus* and *Prunus* linkage groups based on RFLPs (Dirlewanger et al. 2004) or SSRs (Yamamoto et al. 2002b; Fernández-Fernández et al. 2008) could be confirmed in this work. One reason behind this lack of co-linearity between *Malus* and *Prunus* might be that while SSRs have been shown to be useful for mapping alignment within species or subfamilies, their high mutation rate makes them not so proper to study synteny across highly divergent taxa. In any case, a higher number of transferable SSRs would be necessary to confirm their usefulness for comparative mapping studies across Rosaceae genera.

As a whole, on the basis of the substantial co-linearity observed, the maps constructed will be a valuable source of selected *Eriobotrya* SSRs for mapping and an interesting framework for map comparison in the Rosaceae.

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TABLES

Table 1.- Origin and sources of the SSR primers tested in the *Eriobotrya japonica* (Thunb.) Lindl 'AxZ' progeny

SSR origin and reference	Acronym	Group	SSRs	Total
Malus x domestica (Borkh.)				249
Liebhard et al. 2002	CH	Swiss Federal Institute of Technology, Zurich, Switzerland	140	
	MS	Horticultural Research International (HRI), Wellesbourne, UK	7	
Guilford et al. 1997	NZ	Horticultural and Food Research Institute of New Zealand, Auckland, NZ	14	
Hokanson et al. 1998	GD	USDA-ARS, Cornell University, Geneva, NY, USA	8	
Silfverberg-Dilworth et al. 2006	Hi/AF/AJ/AT/ AU/AY/CN/Z	Institute of Integrative Biology, Zurich, Switzerland	78	
Vinatzer et al. 2004	CH-Vf	Swiss Federal Institute of Technology, Zurich, Switzerland	2	
Pyrus spp.				36
Yamamoto et al. 2002a, b, c	NH/NB/KA/ BGT/HGA	National Institute of Fruit Tree Science, Tsukuba, Ibaraki, Japan	36	
Eriobotrya japonica (Thunb)Lindl				21
Gisbert et al. 2008	*ssrEJ*	IVIA, Valencia, Spain	21	
Prunus persica (L.) Batsch				96
Dirlewanger et al. 2002	BPPCT	INRA, Bordeaux, France	39	
Aranzana et al. 2002	CPPCT	CSIC-IRTA, Cabrils, Barcelona, Spain	36	
Yamamoto et al. 2002d	M/Ma	National Institute of Fruit Tree Science, Tsukuba, Ibaraki, Japan	10	
Howad et al. 2005	EPPCU	CSIC-IRTA, Cabrils, Barcelona, Spain	11	
Prunus armeniaca L.				21
Lopes et al. 2002	*ssr*PaCITA	Universidade dos Açores, Portugal	21	
Prunus domestica L.				6
Mnejja et al. 2004	CPSCT	CSIC-IRTA, Cabrils, Barcelona, Spain	6	
Prunus dulcis L.				5
Mnejja et al. 2005	CPDCT	CSIC-IRTA, Cabrils, Barcelona, Spain	5	
Prunus avium L.				6
Sosinski et al. 2000	ps	Clemson University, SC, USA	6	

Table 2.- Amplification and segregation genotypes of the SSRs tested in the 'AxZ' progeny as per JoinMap 3.0.

Species	Acronym	Tested	Amplified	Informative	Multi locus	Total	Mapped	abxcd (1:1:1:1)	efxeg (1:1:1:1)	hkxhk (1:2:1)	hkxhk (3:1)	lmxll (1:1)	nnxnp (1:1)
Apple	CH	140	104	49	2	51	49	1	14	5	2	16	11
	MS	7	5	1	0	1	1	0	0	0	1	0	0
	NZ	14	5	2	0	2	2	0	0	0	0	1	1
	GD	8	6	1	0	1	1	0	1	0	0	0	0
	Hi	63	50	11	2	13	12	1	2	5	1	2	1
	A-/CN/Z/U	15	13	3	0	3	3	0	1	0	0	2	0
	CH-Vf	2	1	1	0	1	1	0	0	0	0	0	1
Pear	NH	23	12	4	0	4	3	0	2	0	0	1	0
	NB	8	5	3	0	3	3	0	0	0	0	2	1
	KA/BGT/HGA	5	4	1	0	1	1	0	0	1	0	0	0
Loquat	*ssrEJ*	21	21	17	1	18	16	1	8	0	0	5	2
Peach	BPPCT	39	24	4	1	5	4	0	0	0	1	1	2
	CPPCT	36	15	2	0	2	2	0	0	0	2	0	0
	M/Ma	10	7	3	0	3	3	0	0	0	1	2	0
	EPPCU	11	3	0	0	0	0	0	0	0	0	0	0
Apricot	*ssr*PaCITA	21	11	2	0	2	1	0	0	0	0	1	0
Plum	CPSCT	6	4	1	0	1	1	0	0	0	0	1	0
Almond	CPDCT	5	1	0	0	0	0	0	0	0	0	0	0
Sweet Cherry	ps	6	1	0	0	0	0	0	0	0	0	0	0
	Total	440	292	105	6	111	103	3	28	11	8	34	19

Table 3.- Transferability, polymorphism and mapping summary of the *Malus*, *Pyrus,* *Eriobotrya* and *Prunus* microsatellites.

SSR origin	SSR tested	Transferability[a]				Polymorphism[b]			Mapping[c]		
		No Amp	Amplifications			Dom	Cod	Total Loci	A	Z	Total AxZ
			Comp	Mon	Info						
Malus x domestica	249	65 (26%)	18 (7%)	98 (39%)	68 (27%)	41	31	72	54	43	69 (95%)
Pyrus spp.	36	15 (42%)	1 (3%)	12 (33%)	8 (22%)	3	5	8	6	4	7 (88%)
Eriobotrya japonica	21	0 (0%)	0 (0%)	4 (19%)	17 (81%)	7	11	18	14	11	16 (89%)
Prunus spp.	134	68 (51%)	0 (0%)	54 (40%)	12 (9%)	10	3	13	9	6	11 (85%)
Total	440	148 (34%)	19 (4%)	168 (38%)	105 (24%)	61	50	111	83	64	103 (93%)

[a] Transferability: No Amp (no amplification), Comp (complex pattern), Mon (monomorphic) and Info (informative SSR)
[b] Polymorphism: Dom (dominant loci), Cod (codominant loci) and Total loci (including multi-locus SSR)
[c] Mapping: A ('Algerie' map), Z ('Zaozhong-6' map) and Total AxZ (both maps)

Table 4.- Summary of the AFLP markers segregating in the 'AxZ' population

Primer combination	Marker code	Total polymorphic bands[b]	Polymorphic bands mapped in Algerie	Polymorphic bands mapped in Zaozhong-6	Polymorphic bands mapped in both maps
EcoRI-AA / MseI-CAC	E-AA + M-CAC(x)[a]	8	4	3	4
EcoRI-AA / MseI-CAT	E-AA + M-CAT(x)	5	3	4	5
EcoRI-AA / MseI-CTA	E-AA + M-CTA(x)	2	0	1	1
EcoRI-AA / MseI-CTG	E-AA + M-CTG(x)	5	0	1	1
EcoRI-AAC / MseI-CAC	E-AAC + M-CAC(x)	9	6	6	8
EcoRI-AAC / MseI-CAT	E-AAC + M-CAT(x)	9	1	2	2
EcoRI-AAC / MseI-CGC	E-AAC + M-CGC(x)	6	3	2	4
EcoRI-AAC / MseI-CTA	E-AAC + M-CTA(x)	2	1	1	2
EcoRI-AAC / MseI-CTG	E-AAC + M-CTG	1	0	0	0
EcoRI-AAG / MseI-CTA	E-AAG + M-CTA(x)	4	2	1	3
EcoRI-AAG / MseI-CTC	E-AAG + M-CTC	4	0	1	1
EcoRI-ACA / MseI-CAC	E-ACA + M-CAC(x)	8	4	5	5
EcoRI-ACA / MseI-CAT	E-ACA + M-CAT(x)	4	3	1	3
EcoRI-ACA / MseI-CGC	E-ACA + M-CGC	1	0	1	1
EcoRI-ACA / MseI-CTA	E-ACA + M-CTA(x)	3	3	0	3
EcoRI-ACA / MseI-CTG	E-ACA + M-CTG(x)	2	0	1	1
EcoRI-ACC / MseI-CAC	E-ACC + M-CAC(x)	7	4	5	6
EcoRI-ACC / MseI-CAT	E-ACC + M-CAT(x)	7	6	3	6
EcoRI-ACC / MseI-CGC	E-ACC + M-CGC	1	1	0	1
EcoRI-ACC / MseI-CTA	E-ACC + M-CTA(x)	4	2	4	4
EcoRI-ACC / MseI-CTG	E-ACC + M-CTG(x)	5	3	2	4
EcoRI-ACG / MseI-CAT	E-ACG + M-CAT(x)	4	1	1	2
EcoRI-ACG / MseI-CTC	E-ACG + M-CTC	1	1	0	1
EcoRI-ACT / MseI-CAT	E-ACT + M-CAT(x)	4	0	0	0
EcoRI-ACT / MseI-CTA	E-ACT + M-CTA	1	1	1	1
EcoRI-ACT / MseI-CTG	E-ACT + M-CTG(x)	3	1	2	2
EcoRI-AGG / MseI-CAC	E-AGG + M-CAC(x)	2	1	1	2
EcoRI-AGG / MseI-CAG	E-AGG + M-CAG	1	0	0	0
EcoRI-AGG / MseI-CAT	E-AGG + M-CAT(x)	7	3	4	5
EcoRI-AGG / MseI-CGC	E-AGG + M-CGC(x)	3	2	1	2
EcoRI-AGG / MseI-CTA	E-AGG + M-CTA(x)	12	6	8	9
EcoRI-AGG / MseI-CTG	E-AGG + M-CTG(x)	4	1	2	2
EcoRI-AAT / MseI-CTA	E-AAT + M-CTA(x)	8	6	4	7
EcoRI-AAT / MseI-CTC	E-AAT + M-CTC(x)	6	3	4	5
EcoRI-ACA / MseI-CGG	E-ACA + M-CGG(x)	4	2	2	3
EcoRI-ACG / MseI-CGT	E-ACG + M-CGT(x)	2	2	2	2

EcoRI-AGA / MseI-CGG	E-AGA + M-CGG(x)	8	5	2	6
EcoRI-AGA / MseI-CTG	E-AGA + M-CTG(x)	5	5	0	5
EcoRI-AGC / MseI-CGG	E-AGC + M-CGG(x)	2	2	1	2
EcoRI-AGG / MseI-CGA	E-AGG + M-CGA(x)	2	2	1	2
EcoRI-AGG / MseI-CGG	E-AGG + M-CGG(x)	2	1	0	1
EcoRI-AGT / MseI-CGT	E-AGT + M-CGT(x)	5	3	2	4
Total		183	94	82	128

[a](x) identifies the polymorphic bands numbered according to their sizes (the bigger the size the smaller the number)
[b] 'Total polymorphic bands' includes mapped and not mapped AFLP bands

Table 5.- Summary of SSRs mapped in the 'Algerie' (A) and 'Zaozhong-6' (Z) maps indicating linkage group (LG) and segregation type (ST)

***Malus* SSR**	**LG**	**A**	**Z**	**ST**	***Malus* SSR**	**LG**	**A**	**Z**	**ST**
Hi02c07	LG1	0.5	-	lm×ll	CH02c09*	LG15	4.1	-	lm×ll
CHVf1	LG1	n.a.[d]	0.2	nn×np	CH05g05	LG15	0.6[a]	0.6	hk×hk
CH03d10	LG2	3.1	3.1	hk×hk	NZ02b01	LG15	-	1.1	nn×np
CH03b01	LG2	0.0	-	lm×ll	CH02e12[b]	LG15	-	0.8	nn×np
CH02b10	LG2	0.6	-	lm×ll	CH03g06	LG15[c]	2.4	2.4	hk×hk
Hi15h12	LG3	4.2	4.2[a]	hk×hk	CH02d10a**	LG16	13.4	13.4	ef×eg
CH01c08[b]	LG3	-	0.1	nn×np	CH05a09	LG16	2.7	2.7	ef×eg
Hi23d11b	LG4	1.0	1.0	hk×hk	Hi04e04	LG16	0.5	0.5	hk×hk
CH04g09(1)	LG5	3.6	3.6	ef×eg	CH02a03	LG16	-	0.0	nn×np
CH02b12	LG5	5.9	5.9	ef×eg	Ch04b10	LG16[c]	0.2	0.2	hk×hk
CH04e03	LG5	2.4	2.4	ef×eg	Hi03e04(1)	LG16[c]	2.7	2.7	hk×hk
CH03a09	LG5	1.8	-	lm×ll	CH05g03**	LG17	29.7	29.7	ef×eg
CH02a08(2)	LG5	0.9	n.a.	lm×ll	CH04c10	LG17	0.3	-	lm×ll
U78949-SSR**	LG6	13.2	13.2	ef×eg	AY187627	LG17	1.5	n.a.	lm×ll
Hi03a03**	LG6	33.4	33.4	ef×eg	CH01h01	LG17	-	3.8	nn×np
CH03d12	LG6	-	1.4	nn×np	Hi03e04(2)	LG17[c]	0.0	-	lm×ll
MS06c09	LG7	0.4	0.4	hk×hk	***Eriobotrya* SSR**	**LG**	**A**	**Z**	**ST**
CH04e05	LG7	-	0.9	nn×np	ssrEJ014[b]	LG1	1.6	-	lm×ll
Hi07e08(2)	LG7[c]	1.3	1.3	ef×eg	ssrEJ86*[b]	LG2	7.8	7.8	ef×eg
CH01h10	LG8	7.3	7.3	ef×eg	ssrEJ88[b]	LG3	1.6	-	lm×ll
Hi07e08(1)*	LG8	6.7	6.7	hk×hk	ssrEJ46(2)[b]	LG4	n.a.	0.8	nn×np
CH04g12*[b]	LG8	8.5	8.5	ef×eg	ssrEJ282[b]	LG5	5.3	5.3	ef×eg
GD142	LG9	2.1	2.1[a]	ef×eg	ssrEJ61[b]	LG5	7.1	7.1	ef×eg
AJ320188-SSR	LG9	1.3	-	lm×ll	ssrEJ324[b]	LG5	6.0	6.0	ab×cd
NZ04f3	LG9	0.8	-	lm×ll	ssrEJ49**[b]	LG5	-	8.3	nn×np
CH01f03b	LG9	0.1	-	lm×ll	ssrEJ104*[b]	LG7	9.6	9.6	ef×eg
CH05a03	LG9	-	0.1	nn×np	ssrEJ12[b]	LG8	6.0	6.0	ef×eg
Hi04a05	LG9	-	0.1	nn×np	ssrEJ271	LG8	5.0	5.0	ef×eg
CH04c06	LG10	3.7	3.7	hk×hk	ssrEJ329b*[b]	LG10	3.8	n.a.	lm×ll
CH1f07a**	LG10	27.0	27.0	ef×eg	ssrEJ42*[b]	LG11	5.0	-	lm×ll
CH02b03b**	LG10	11.9	11.9	hk×hk	ssrEJ66[b]	LG14	2.2	2.2	ef×eg
CH04f03*	LG10	4.8	-	lm×ll	ssrEJ37*[b]	LG14	8.7	8.7	ef×eg
CH04g09(2)	LG10	0.6	-	lm×ll	ssrEJ56[b]	LG17	1.5	n.a.	lm×ll
CH02a08(1)*	LG10	5.4	-	lm×ll	***Pyrus* SSR**	**LG**	**A**	**Z**	**ST**
CH01b12	LG10[c]	3.1	3.1	ef×eg	NH033b	LG2	7.1	7.1	ef×eg
CH05h12	LG10	-	0.1	nn×np	NB103a	LG5	-	3.5	nn×np
Hi04g11*	LG11	10.6	10.6	ab×cd	NB106a	LG9	1.7	-	lm×ll
CH04g07	LG11	2.3	n.a.	lm×ll	NH024b*	LG11	3.9	-	lm×ll
CH04d07	LG11	-	0.8	nn×np	NB105a*	LG11	3.9	-	lm×ll

CH02d12	LG11	2.9	2.9[a]	ef×eg	KA16	LG12	1.1	1.1	hk×hk
CH04h02	LG11	0.5	0.5[a]	hk×hk	NH026a	LG16	3.3	3.3	ef×eg
CH04g04	LG12	1.3	1.3	ef×eg	***Prunus* SSR**[b]	**LG**	**A**	**Z**	**ST**
CH02h11b	LG12	6.1	6.1	ef×eg	BPPCT-30[b]	LG1	-	0.1	nn×np
CH03c02	LG12	1.5	-	lm×ll	BPPCT-6[b]	LG4	0.3	-	lm×ll
CH05d11	LG12	-	0.3	nn×np	BPPCT-8[b]	LG5	-	1.5	nn×np
Hi04g05	LG13	1.1	1.1[a]	hk×hk	BPPCT-14[b]	LG6	2.5	2.5	hk×hk
CH03a08	LG13	0.2	n.a.	lm×ll	M06a[b]	LG7	1.3	-	lm×ll
CH04f06	LG14	5.3	5.3	ef×eg	Ma062[b]	LG11	0.1	-	lm×ll
CH05g07	LG14	1.0	-	lm×ll	Ma36a[b]	LG12	2.6	2.6	hk×hk
CH01e01	LG14	1.3	-	lm×ll	CPPCT-10[b]	LG12	2.2	2.2	hk×hk
CH04c07*	LG14	-	6.2	nn×np	CPSCT-26[b]	LG14	1.5	-	lm×ll
CH01d08	LG14[c]	1.3	-	lm×ll	CPPCT-28[b]	LG16	0.2	0.2	hk×hk
CH03h06**	LG15	36.9	36.9	ab×cd	ssrPaCITA16[b]	LG17	0.6	-	lm×ll

Values shown correspond to the chi-square of the goodness of fit for the segregations 1:1 (lm×ll or nn×np), 3:1 and 1:2:1 (hk×hk) or 1:1:1:1 (ef×eg or ab×cd)

[a] SSRs not linked to any marker in one of the two maps

[b] SSRs previously not assigned to any linkage group in Maloideae

[c] SSRs mapped in linkage groups different from the original assignments in Maloideae

[d] n.a. indicates detection of null allele

* SSRs showing distorted segregation at P<0.05

** SSRs showing distorted segregation at P<0.01

FIGURE LEGENDS

Fig. 1 Molecular linkage maps of the 'Algerie' and 'Zaozhong-6' cultivars obtained from the 'AxZ' population. Groups were numbered according to Liebhard et al (2002). All SSRs are in *bold*. Newly mapped SSRs are *underlined* and *dashed lines* indicate polymorphic but not linked SSRs. Self-incompatibility (SI) trait is *blackboxed*. *Solid circles* indicate anchor markers with other *Malus* and *Pyrus* maps. SSRs mapped in linkage groups different from the original assignments in Maloideae are indicated by *grey boxes*. The CM distances are shown on the left in 'Algerie' and on the right in 'Zaozhong-6'. *Asterisks* indicate markers with distorted segregation by the *P* significance level of the χ^2 test: * at $P<0.05$ and ** at $P<0.01$

Fig. 2 Alignment of *Eriobotrya*, *Malus* and *Pyrus* linkage groups sharing at least five linked SSRs, (**a**) LG5, (**b**) LG9, (**c**) LG12 and (**d**) LG16 from different SSR-based maps: 'Fiesta' (F) x 'Discovery' (D) map of *Malus* (Silfverberg-Dilworth et al. 2006), 'Fiesta' x 'Totem' (FxT) interspecific map of *Malus* (Fernández-Fernández et al. 2008),

‘Bartlett’ (B) x ‘Housui’ (H) interspecific map of *Pyrus* (Yamamoto et al. 2004) and ‘Algerie’ (A) x ‘Zaozhong-6’ (Z) map of *Eriobotrya* from this work

Fig. 1

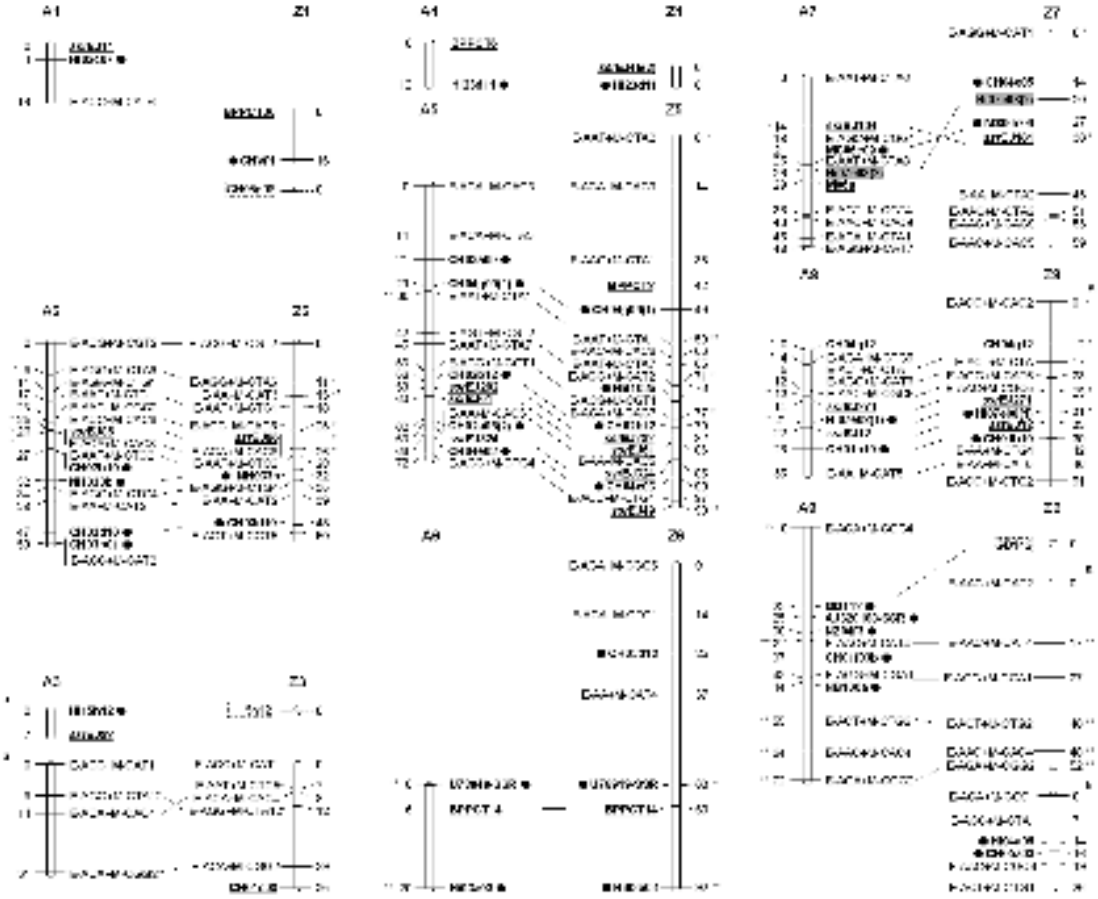

Fig. 1 continued

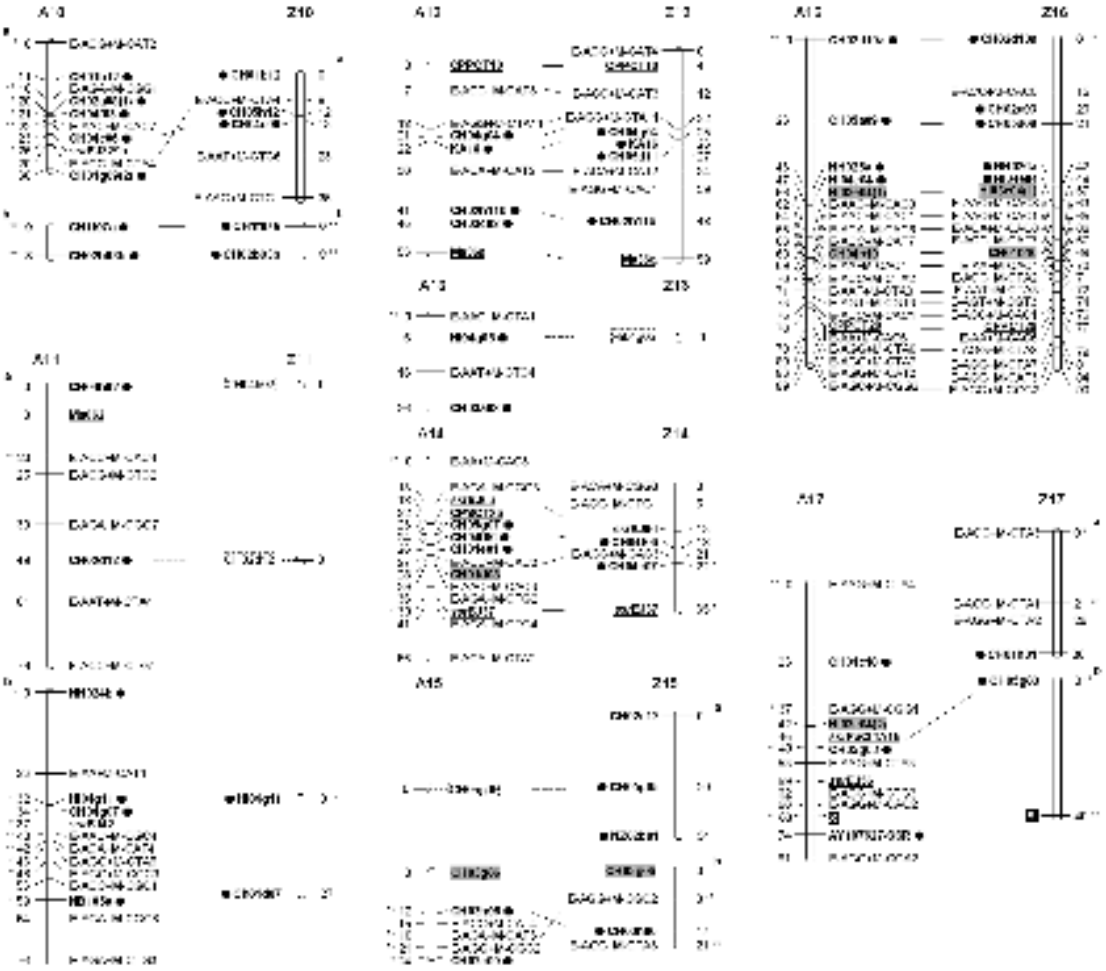

Fig. 2