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ORIGINAL PAPER

Transferability of Newly Developed Pear SSR Markers to Other Rosaceae Species

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Abstract A set of 120 simple sequence repeats (SSRs) was developed from the newly assembled pear sequence and evaluated for polymorphisms in seven genotypes of pear from different genetic backgrounds. Of these, 67 (55.8 %) primer pairs produced polymorphic amplifications. Together, the 67 SSRs detected 277 alleles with an average of 4.13 per locus. Sequencing of the amplification products from randomly picked loci NAUPy31a and NAUpy53a verified the presence of the SSR loci. When the 67 primer pairs were tested on 96 individual members of eight species in the Rosaceae family, 61.2 % (41/67) of the tested SSRs successfully amplified a PCR product in at least one of the Rosaceae genera. The transferability from pear to different species varied from 58.2 % (apple) to 11.9 % (cherry). The ratio of transferability also reflected the closer relationships within Maloideae over Prunoideae. Two pear SSR markers, NAUpy43c and NAUpy55k, could distinguish the 20 different apple genotypes thoroughly, and UPGMA cluster analysis grouped them into three groups at the similarity level of 0.56. The high level of polymorphism and good transferability of pear SSRs to Rosaceae species indicate their promise for application to future molecular screening, map construction, and comparative genomic studies among pears and other Rosaceae species.

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Introduction

Pear (*Pyrus* spp.), one of the oldest fruit crops in the world, belongs to the genus *Pyrus*, subfamily Maloideae (Pomoideae), in the family Rosaceae. Economically, pear is the third most important temperate fruit species after grape and apple, and the recent genome sequencing of the diploid *P. bretschneideri Rehd*. cv. Dangshansuli (Wu et al. 2013) has allowed ready access to the DNA sequence of pear. Simple sequence repeats (SSRs) or microsatellite markers are widely used due to their multi-allelic nature, reproducibility, high polymorphism, and codominant inheritance (Campoy et al. 2011; Baraket et al. 2011; Swapna et al. 2011). Markers derived from the pear genome would be significant and useful in linkage map construction, genetic polymorphism evaluation, cultivar identification, traditional crossbreeding programs, and studies of genetic variability and the diversification process (Ferreira dos Santos et al. 2011).

Rosaceae is a large plant family containing more than 3,000 species, many of which are economically important fruit trees such as apple, apricot, cherry, peach, pear and plum as well as soft fruit crops like strawberry. In addition to pear, the whole genome sequences of both apple (Velasco et al. 2010) and strawberry (Shulaev et al. 2011) have been completed, making massive genomic data of pear, apple, and strawberry in terms of molecular marker maps, expressed sequence tags (EST), and gDNA sequences readily available. However, there is rather little genomic information available for other valuable fruit tree members of the big Rosaceae family. Due to their codominant and usually single-locus nature, SSR loci can be identified, and their alleles can be recognized in different genotypes of the same species and often in those of other close relatives. This means that a

specific set of SSRs can be used in different sets of genotypes or mapping populations, making them particularly useful for variability analysis, fingerprinting, molecular marker development, marker-assisted selection (MAS), map construction, and comparative studies (Mnejja et al. 2010). Therefore, it is highly valuable to investigate the transferability of SSR markers from pear, where they are easily developed and evaluated, to other Rosaceae species. Transferability to relatives contributes to the wide applicability of SSRs developed based on the pear genome. Recently, there have been several reports on the transferability of SSR markers in or across genera among Rosaceae fruit crops (Decroocq et al. 2003; Gasic et al. 2009; Gisbert et al. 2009; Sargent et al. 2009, 2007; Yamamoto et al. 2004; Yao et al. 2010; Wünsch 2009; Mnejja et al. 2010; He et al. 2011; Bouvier et al. 2012).

Comparative mapping within many plant families has been well studied, for example, in Brassicaceae, Leguminosae, Poaceae, or Solanaceae (Devos and Gale 2000; Doganlar et al. 2002; Kalo et al. 2004; Lukens et al. 2003) and allows the identification of a marker framework for map-based prediction of the location of candidate genes linked with agriculturally important traits within different species of the same family. Recently, efforts have been undertaken for genome comparisons within Rosaceae (Shulaev et al. 2008). Molecular markers have been widely applied to various aspects of research in apple and pear (Celton et al. 2009; Yamamoto et al. 2007), and SSR markers with good transferability can be applied to comparative mapping and genomic synteny between these two important fruits. Bouvier et al. (2012) discovered that a new pear scab-resistant gene, *Rvp1*, is located close to microsatellite marker CH02b10 from the European pear cultivar Navara maps in a genomic region syntenic to an apple scab-resistant gene cluster on linkage group 2. This genomic region is known to carry a cluster of scab-resistant genes in apple, indicating the first functional synteny for scab resistance between apple and pear. Molecular marker linkage maps of apple and *Prunus* have been shown to have a high level of macro-synteny (Dirlewanger et al. 2004).

The study of transferability of SSRs and EST-SSRs among Rosaceae is significant. He et al. (2011) evaluated 71 apple SSR markers distributed across 17 linkage groups and identified 39 SSRs transferable to loquats. Mnejja et al. (2010) studied a total of 145 microsatellite primer pairs from *Prunus* DNA sequences for transferability in a set of eight cultivars from nine rosaceous species (almond, peach, apricot, Japanese plum, European plum, cherry, apple, pear, and strawberry). Gasic et al. (2009) investigated the level of transferability of 68 apple EST-SSRs in 50 individual members of the Rosaceae family, representing three genera and 14 species. Wünsch (2009) studied 13 *Prunus* SSRs known to be transferable to certain *Prunus* species, tested them in 10 *Prunus*, and found that three were transferable to all and eight to all but one species. Apple EST-SSR primer pairs (94) were tested on four accessions of *Pyrus* to evaluate transferability, and 40 of 72 functional SSRs produced polymorphic amplicons. From these, eight SSR randomly selected loci were used to analyze genetic diversity and relationships among a collection of *Pyrus* and displayed reliable amplification and considerable polymorphism in both *Malus* and *Pyrus* (Yao et al. 2010). Despite this abundance of research, most previous studies about transferability are related to apple genomic SSRs or EST-SSRs, with little research on pear SSR transferability.

In this study, we present 150 pear SSRs developed from publicly available *Pyrus* genome sequences (Wu et al. 2013). In order to ensure the quality of the markers, all SSRs have been evaluated for polymorphisms in seven pear genotypes from different genetic backgrounds, and their transferability to 96 individual members of eight species of the Rosaceae family was studied.

Materials and Methods

Plant Material

The seven pear genotypes used in the primary evaluation of pear primers have broad genetic variation. They are Doyenne du Comice from France (*P. communis* L.); Housui from Japan (*P. pyrifolia* Nakai); Huobali from Yunnan province of China (*P. pyrifolia* Nakai), which is a semi-cultivated species; Kuerlexiangli from Xinjiang province of China (*P. sinkiangensis* Yü); Yali from Hebei province of China (*P. bretschneideri* Rehd.); Jingbaili from Beijing, China (*P. ussuriensis* Maxim.); and Bean Pear (*P. calleryana*) often used as rootstock. A total of 96 Rosaceae genotypes (Table 1) of eight species from seven genera and three subfamilies were used for transferability exploration; these cultivars were chosen largely based on their economic value. All the pear leaves were collected from trees located at the Pukou District pomology farm of Nanjing Agriculture University; apple, plum, peach, cherry, and apricot samples were collected from trees located at the production demonstration bases of Shangdong Institute of Pomology; strawberry leaves were picked in the greenhouse of Shangdong Agriculture University; loquat leaf tissues were collected in the loquat germplasm nursery of Suzhou Polytechnic Institute of Agriculture; and Japanese apricot leaves were collected in the Garden of Fujiabian Agricultural Technology Park in Nanjing of China.

DNA Extraction and Quality Control

Total genomic DNA of all the plants was extracted from young leaves based on the improved CTAB method (Pan et al. 2006). Subsequently, 5-μl DNA solution was loaded on 1.0 % agarose gel to check the quality.

Table 1 Plant material used for cross-species transferability in Rosaceae

Sample no.	Individuals tested	Origin	Sample no.	Individuals tested	Origin	Sample no.	Individuals tested	Origin
Prunoideae Japanese apricot (*Prunus mume*)			Prunoideae Cherry (*Cerasus pseudocerasus*)			Prunoideae Apricot (*Prunus armeniaca*)		
1	Bungo	Japan	33	Brooks	USA	64	Zhenzhuyouxing	China
2	Xiaoyezhugan	China	34	Nanyo	Japan	65	Hongguang	China
3	Gessekai	Japan	35	Van	Canada	66	Honghebao	China
4	Shirokaga	Japan	36	Hong Deng	China	67	Katy	USA
5	Xiaoqing	China	37	Ruby	USA	68	Mono	USA
6	Ruantiaohongmei	China	Maloideae Apple (*Malus x domestica*)			69	Jinkaite	China
7	Nankou	Japan	38	Sekaiichi	Japan	70	Sweet Production	Unkown
8	Xiyeqing	China	39	Huaguan	China	71	Red Mingtand	Unkown
9	Oushuku	Japan	40	Zaohong	China	72	Sungold	USA
10	Nanhong	China	41	Delicious	USA	73	Yubadan	China
Prunoideae Plum (*Prunus salicina*)			42	Braeburn	New Zealand	Prunoideae Peach (*Prunus persica*)		
11	Eldorda	Australia	43	Fuji	Japan	74	Beijing 9	China
12	Grandora	USA	44	Golden Delicious	USA	75	Datuanmilu	China
13	Black Amber	USA	45	Mikilifi	Japan	76	Kurakatawase	Japan
14	Bluebyrd	USA	46	Longfeng	China	77	Huayu	China
15	Methley	South Africa	47	Starking	USA	78	Hanmilu	China
16	Angeleno	USA	48	Dailv	China	79	Hakuto	Japan
17	Autumn Giant	Japan	49	Gala	New Zealand	80	Yumyong	Korea
18	Catalina	USA	50	Qinyang	China	81	Fudaotaowang	China
19	Weikeseng	China	51	HoKuto	Japan	82	Zhaoyanghong	China
20	Red Beauty	USA	52	Jonagold	USA	83	Shuguang	China
21	June Rosa	Uknown	53	Orin	Japan	84	Zaofengwang	China
22	Early Beauty	USA	54	Lujia 1	China	85	Hongjuhua	China
23	Bilvhongxin	China	55	Alps Otome	Japan	Maloideae Loquat (*Eriobotrya japonica*)		
24	Early Beauty	USA	56	Hanfu	China	86	Zhaozhong	China
Prunoideae Cherry (*Cerasus pseudocerasus*)			57	Xiushui	China	87	Qingzhong	China
25	Kosmytchekaja	Ukraine	Rosoideae Strawberry (*Fragaria×ananassa*)			88	Guanyu	China
26	Summit	Canada	58	Meiho	Japan	89	Gaoliangjiang	China
27	Druichba	Ukraine	59	Toyonoka	Japan	90	Jidanbai	China
28	Early Ruby	Ukraine	60	Nyoho	Japan	91	Tianzhong	China
29	Tieton	USA	61	Benihoppe	Japan	92	Qianxipeiyu	China
30	Rainier	USA	62	Gongben	Japan	93	Subai 1	China
31	Earty Rilbmond	China	63	Sweet Charlie	USA	94	Shiromogi	Japan
32	Lapins	Canada				95	Dahongpao	China
						96	Dazhong	China

SSR Markers of Pear Genome

A total of 120 primers (Supplemental Table 1) were developed from the pear genome sequence. All SSR markers were obtained by scanning the published pear whole genome sequencing scaffolds (Wu et al. 2013; http://peargenome.njau.edu.cn) and were assessed for their polymorphism levels in the seven pear genotypes mentioned. The SSR polymorphisms in pears were used to investigate transferability to 96 Rosaceae cultivars.

PCR and Electrophoresis

All polymerase chain reactions (PCRs) were performed in a total volume of 10 μl containing: 1 μl of 30 ng/μl genomic DNA template, 1 μl of 10×PCR buffer (without $MgCl_2$), 1 μl of 2.5 mM dNTP mixture, 0.6 μl of 25 mM $MgCl_2$, 0.1 μl each of forward and reverse primer (10 pmol/μl), and 0.1 μl of 5 U/μl Taq polymerase (Takara Biotechnology Company, Dalian). The reactions were performed with the following conditions: 94 °C for 3 min, then 39 cycles of 94 °C for

40 s, 55 °C for 50 s, and 72 °C for 1 min, and a final step at 72 °C for 10 min. PCR products (10 μl) were mixed with 2.5 μl formamide loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, 0.25 % xylenecyanol, pH 8.0), and 1.5 μl of each mixture and a molecular size marker of 100-bp ladder, Trans DNA 2 K Marker, or pBR322 DNA-MspI Digest (Beijing BLKW Biotechnology Co., Ltd) were loaded onto an 8 % non-denaturing polyacrylamide gel in 1× TBE buffer (Tris-borate, EDTA, pH 8.0), running under a voltage of 200 V and visualized using the silver-staining protocol described by Bassam et al. (1991).

Sequencing of Three SSR Loci in *Pyrus* Accessions

In order to verify the presence of SSR and prove the allelic variation of the SSR loci, amplification products from two randomly picked loci (NAUPy31a and NAUpy53a) were isolated with a DNA Gel Extraction kit AxyPrep™ (Axygen Inc.), and the fragments were cloned into the pMD18-T vector. The fragments were sequenced by Invitrogen, Shanghai, China.

Allele Data and Statistical Analysis

Allele sizes were estimated by comparison to a 100-bp ladder marker, Trans DNA 2 K Marker, and a pBR322 DNA-MspI Digest (Beijing BLKW Biotechnology Co., Ltd). SSRs were scored as dominant markers: presence of a band was recorded as "1" and absence as "0." All accessions were analyzed as a single population. The observed number of alleles (Na), effective number of alleles (Ne), number of alleles, Nei's gene diversity (*h*), and Shannon's information index (*I*) were calculated for each locus by the program POPGENE, version 1.31 (Yang and Yeh 1993). Only reproducible bands were used to calculate the Dice's similarity coefficients (Nei and Li 1979). The chi-square test for Hardy–Weinberg equilibrium (Phw) of primers was calculated using the POPGENE program (version 1.32), with 0.01 significance and 10,000 times simulation. An unweighted pair group method using arithmetic average (UPGMA) cluster analysis was performed based on the similarity matrix for 20 apple individuals using the NTSYS-pc program (Version 2.2j) (Rohlf 1998).

Results and Discussion

Polymorphism of SSR Markers

In total, 67 of 120 (55.8 %) SSRs produced amplifications in the seven pear genotypes (Doyenne du Comice, Housui, Huobali, Kuerlexiangli, Bean Pear, Yali, and Jingbaili). In general, the SSR markers produced high-quality banding patterns (Fig. 1), most of them giving product sizes ranging from 90 to 280 bp (Table 2). The 67 SSRs detected 294 alleles with an average of 4.1 per locus; the observed number of alleles (Na) ranged from 1.57 to 2.00, with an average value of 1.89; the effective number of alleles (Ne) ranged from 1.19 to 1.88, with a mean level of 1.51; Nei's gene diversity (*h*) ranged from 0.12 to 0.46, with a mean level of 0.31; and Shannon's information index (*I*) varied from 0.17 to 0.65, with an average value of 0.46. High-quality NTSYS analysis of the genetic distance among the seven genotypes according to the 67 SSRs gave distance values ranging from 0.2426 (Huobali and Kuerlexiangli) to 1.0119 (Jingbaili and Bean Pear), with an average of 0.69, indicating a high level of genetic diversity among the seven pear individuals, suggesting a good applicability of these markers to germplasm diversity research. Consequently, we picked these 67 pear SSRs for further transferability exploration across Rosaceae. Excepting their high quality, these 67 SSRs were chosen because almost all primers produced clear bands in all seven pear genotypes representing different pear classes, suggesting that primer sites in the genome are well conserved. This indicates the larger possibility of successful transferability to other Rosaceae species.

Sequencing Results of Two SSR Loci in *Pyrus* Accessions

The sequencing and alignment of SSR alleles derived from pears verified the presence of the SSR loci and revealed a high degree of conservation of SSR flanking regions in two loci (Fig. 2). The sequencing results showed a diversity of alleles in both loci. TA-repeats of allelic variation were detected for NAUPy31, and CT-repeats of allelic variation were detected for NAUpy53a (Fig. 2). The allelic diversity was mainly due to length variations of the microsatellite repeats, combined with point mutation events within the flanking regions. These variations may contribute to the formation of pear germplasm diversity.

Transferability to Other Species in Rosaceae

A set of 67 selected SSR (Table 2) polymorphisms in seven pear genotypes was evaluated using genomic DNA of 96 genotypes belonging to seven genera in Rosaceae, including *Prunus* (10 *Prunus mume* and 14 *P. salicina*), *Armeniaca* (8 accessions), *Cerasus* (13 accessions), *Amygdalus* (12 accessions), *Fragaria* (6 accessions), *Malus* (20 accessions), and *Eriobotrya* (11 accessions). *Prunus*, *Armeniaca*, *Cerasus*, and *Amygdalus* belong to Prunoideae, *Fragaria* belongs to Rosoideae, and *Malus* and *Eriobotrya* belong to Maloideae (Table 1). Overall, 61.2 % (41/67) of the tested SSRs successfully amplified at least one PCR product of the approximate size expected for a homologous gene in at least one of the Rosaceae genera screened (Table 3; Fig. 3). This result (61.2 %) was lower than the 75 % achieved from apple EST-SSRs to other Rosaceae (Gasic et al. 2009). The most likely

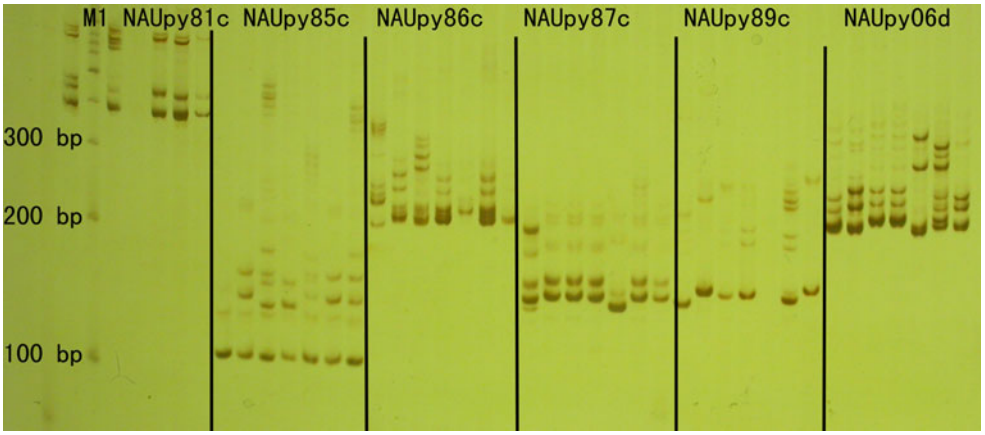

Fig. 1 Amplification of six SSRs, namely, NAUpy81c, NAUpy85c, NAUpy86c, NAUpy87c, NAUpy89c, and NAUpy06d, in seven pear genotypes. Lanes: M_1 100-bp ladder DNA marker, *1* Doyenne du Comice, *2* Housui, *3* Huobali, *4* Kuerlexiangli, *5* Bean Pear, *6* Yali, *7* Jingbaili

reason was that, compared to genomic SSRs, in theory, EST-SSRs have higher transferability because the EST-derived microsatellites are within transcribed regions of the DNA and are expected to be more conserved and less polymorphic than genomic SSR markers. However, the apple EST-SSRs to other Rosaceae had a lack of polymorphism, which is in agreement with the theory that genomic SSRs have a lower transferability but a better polymorphism. The good polymorphism characteristics of the transferable pear genomic SSRs in this study would be more valuable in application to Rosaceae genomic studies.

The highest transferability, 58.2 % (Table 3), was observed in the closely related apple (*Malus domestica*), in which the majority of pear SSRs are polymorphic (Supplemental Fig. 1) and had amplification patterns similar to those observed in pear. This indicates that primer binding sites between these two closely related rosaceous genera, *Malus* and *Pyrus*, are fairly well conserved (Table 3; Supplemental Fig. 1). This high level of transferability of SSRs was consistent with genome comparison of pear and apple (Wu et al. 2013) and was also similar to previous findings where apple SSRs have been reported capable of identifying polymorphism and detecting genetic diversity in pear (Yamamoto et al. 2001, 2004). Besides, Gasic et al. (2009) reported high transferability (59 %) of apple EST-SSRs to pear, and both Pieratoni et al. (2004) and Yamamoto et al. (2004) have reported amplification of apple SSRs in pear populations. Yao et al. (2010) tested 94 primer pairs on four accessions of *Pyrus* to evaluate the transferability of the markers, and 40 of 72 functional SSRs produced polymorphic amplicons. All of the above indicates that pear has close synteny with apple.

Two SSR loci, NAUpy43c and NAUpy55k, were then employed to analyze genetic diversity and relationship among a collection of 20 genotypes of apple. The two SSR loci could distinguish the 20 apple individuals and have detected 9 alleles (Table 4), and UPGMA cluster analysis grouped these individuals into three groups at the similarity level of 0.56 (Fig. 4). Based on the polymorphism revealed by the two SSR markers NAUpy43c and NAUpy55k, the cluster results largely agree with the traditional taxonomy based on pedigrees or geographic origins. The first group consisted of Longfeng and Xiushui: Longfeng was the progeny of Jinhong and Bailong, and Xiushui was a seedling of Ralls. The second group included Braeburn, Orin, Dailv, Qinyang, Lujia 1, Golden Delicious, and Starking, also called the Golden Delicious class. Among them, Dailv, a seedling of Golden Delicious, and Orin, whose female parent was Golden Delicious, were well known, and they belong to the Golden Delicious class. Lujia 1 has a close relationship with Starking, and Braeburn originated from New Zealand and was the progeny of Lady Hamilton and Granny Smith. Sekaiichi, Huaguan, Zaohong, Delicious, Fuji, Mikilifi, Gala, HoKuto, Jonagold, Alps Otome, and Hanfu formed the third group, also called the Delicious class. According to the pedigrees, Jonagold belongs to the Jonathan lineage, Mikilifi belongs to the Summer Pearmain lineage, and Sekaiichi, Huaguan, Zaohong, Fuji, Gala, HoKuto, Alps Otome, and Hanfu all have relationships with Delicious. In the third group, Fuji is the parent of HoKuto, Alps Otome, Hanfu, and Huaguan. Excepting Alps Otome, the other three cultivars grouped with Fuji together with Mikilifi with a similarity coefficient value of 0.78. These results were consistent with previous classifications (Zhang et al. 2012; Potts et al. 2012; Reim et al. 2012), indicating the good quality and usefulness of the two pear SSR loci in apple germplasm evaluation. In brief, SSR markers developed from pear showed high transferability in apple and can be valuable for application in germplasm evaluation and genetic relationship analysis of apple, as well as comparative mapping between pear and apple.

Loquat (*Eriobotrya japonica*), a subtropical evergreen fruit tree belonging to the Rosaceae subfamily Maloideae that also contains apple and pear, ranked in second place with a transferability of 32.8 %. The pedigrees of the majority of loquat cultivars are historically unknown, and the current system used for cultivar classification gives little information on genetic identity and variability, as it is mainly based on morphological traits linked to ecotype, flesh color, fruit shape, usage, and ripening time (Martínez-Calvo et al. 2008). Genetic diversity

Table 2 Parameter values of 67 SSR polymorphisms in seven pear genotypes

SSR locus	Size ranged in pears (bp)	Number of alleles	na	ne	h	I
NAUpy35c	130–160	6	1.86	1.37	0.25	0.40
NAUpy91b	130–160	4	1.86	1.67	0.36	0.53
NAUpy74v	110–115	5	1.29	1.20	0.12	0.17
NAUpy23f	147–230	4	2.00	1.44	0.29	0.46
NAUpy74f	110–125	3	2.00	1.44	0.28	0.44
NAUpy39h	100–110	5	2.00	1.19	0.16	0.30
NAUpy92a	125–190	4	2.00	1.70	0.40	0.59
NAUpy46k	125–190	5	2.00	1.19	0.16	0.30
NAUpy55k	150–166	3	2.00	1.51	0.32	0.50
NAUpy92e	200–210	5	2.00	1.31	0.23	0.38
NAUpy22c	210–280	2	2.00	1.80	0.43	0.62
NAUpy24c	180–230	5	2.00	1.83	0.45	0.64
NAUpy53a	180–200	3	1.86	1.45	0.28	0.43
NAUpy86n	220–260	4	2.00	1.44	0.29	0.46
NAUpy32c	150–180	4	2.00	1.63	0.37	0.56
NAUpy38c	120–160	5	2.00	1.41	0.27	0.44
NAUpy27c	100–200	4	1.86	1.37	0.25	0.40
NAUpy31a	180–240	4	1.71	1.34	0.22	0.34
NAUpy38e	90–120	2	1.86	1.38	0.25	0.39
NAUpy86c	200–210	2	2.00	1.66	0.37	0.55
NAUpy87c	150–160	5	2.00	1.50	0.33	0.51
NAUpy85d	140–200	4	1.71	1.51	0.28	0.41
NAUpy68n	110–160	3	2.00	1.81	0.44	0.63
NAUpy02z	160–190	3	1.57	1.40	0.24	0.35
NAUpy80k	120–200	4	2.00	1.63	0.38	0.57
NAUpy87d	190–210	5	1.71	1.37	0.23	0.35
NAUpy43c	140–160	5	1.43	1.41	0.21	0.29
NAUpy60e	100–110	6	1.57	1.40	0.24	0.35
NAUpy75t	95–180	4	2.00	1.73	0.41	0.60
NAUpy91t	110–150	4	2.00	1.69	0.40	0.59
NAUpy88x	120–200	2	1.86	1.62	0.35	0.50
NAUpy67t	90–120	3	2.00	1.52	0.32	0.50
NAUpy98x	100–150	3	2.00	1.88	0.46	0.65
NAUpy36t	100–150	3	1.71	1.46	0.27	0.40
NAUpy30u	180–260	4	1.86	1.59	0.34	0.50
NAUpy62u	180–220	4	2.00	1.49	0.33	0.51
NAUpy43w	90–110	4	1.71	1.61	0.32	0.46
NAUpy40c	220–250	4	2.00	1.66	0.37	0.55
NAUpy85c	110–170	4	2.00	1.70	0.39	0.57
NAUpy89c	150–160	4	1.86	1.37	0.26	0.41
NAUpy94x	160–180	3	1.86	1.53	0.32	0.47
NAUpy23d	160–180	2	1.86	1.53	0.32	0.47
NAUpy07u	180–210	4	2.00	1.59	0.36	0.54
NAUpy35v	130–150	4	2.00	1.48	0.31	0.48
NAUpy92x	110–120	4	1.71	1.39	0.24	0.37
NAUpy81m	140–150	4	1.57	1.40	0.24	0.35
NAUpy71v	120–135	4	2.00	1.63	0.37	0.56
NAUpy48t	130–160	4	2.00	1.50	0.33	0.51
NAUpy29u	130–160	4	1.57	1.47	0.25	0.36

Table 2 (continued)

SSR locus	Size ranged in pears (bp)	Number of alleles	na	ne	h	I
NAUpy41v	130–150	4	1.86	1.43	0.28	0.43
NAUpy33v	110–140	3	2.00	1.49	0.33	0.51
NAUpy13d	120–140	4	1.86	1.37	0.25	0.40
NAUpy85x	120–155	6	1.86	1.57	0.34	0.50
NAUpy98s	160–200	6	2.00	1.75	0.43	0.62
NAUpy28d	170–210	4	1.71	1.51	0.30	0.43
NAUpy31t	100–150	6	1.71	1.51	0.30	0.43
NAUpy66w	100–120	4	1.71	1.46	0.27	0.40
NAUpy30d	200–220	6	2.00	1.51	0.33	0.51
NAUpy90w	180–240	6	2.00	1.31	0.23	0.38
NAUpy73m	110–130	4	2.00	1.36	0.26	0.42
NAUpy35d	150–160	3	2.00	1.31	0.23	0.38
NAUpy30c	220–250	6	2.00	1.48	0.31	0.47
NAUpy06z	120–200	4	2.00	1.66	0.38	0.56
NAUpy06d	200–220	6	2.00	1.54	0.34	0.52
NAUpy58t	130–150	6	2.00	1.48	0.31	0.48
NAUpy45f	120–170	5	2.00	1.44	0.29	0.46
NAUpy56h	170–200	4	2.00	1.51	0.32	0.50
Average		4.14	1.89	1.51	0.31	0.46

Observed sizes—size range on 8 % high-resolution non-denaturing polyacrylamide gel. Number of alleles—total numbers observed in seven pear genotypes

na observed number of alleles, *ne* effective number of alleles [Kimura and Crow (1964)], *h* Nei's (1973) gene diversity, *I* Shannon's information index [Lewontin (1972)]

and the relationships among different cultivars of loquat are of great importance for the conservation of genetic resources, breeding initiatives, and national and international exchanges of materials (He et al. 2011). The loquat genome has not been sequenced, and only a small number of molecular markers of loquat are available, but this number can be increased by identifying pear SSRs that are transferable to loquat cultivars/accessions. In this study, 22 SSRs from pear were successfully transferred to loquat (Table 3; Fig. 3), which will provide new insight into the level of genetic diversity within loquat and synteny with pear. In previous research, apple SSRs had been transferred and applied in loquat. Soriano et al. (2005) used 30 SSRs from apple to assay the genetic relationships in loquat, 13 of which amplified polymorphic products and distinguished 34 of the 40 loquat accessions originating from different European countries. He et al. (2011) also identified 39 SSRs from apple that could be transferred to loquat. The pear SSRs transferred to loquat could be applied as new SSR markers in loquat for genetic research, such as map construction and comparison, resource assessment, and molecular breeding.

Transferability of pear SSRs to the Prunoideae members was at an analogous level: Japanese apricot 18.3 %, plum 16.9 %, cherry 15.5 %, apricot 12.7 %, and peach 14.1 %, indicating that these Prunoideae stone fruits have homology regions with the pear genome. In addition, the Prunoideae species shared most of the same amplified markers (Table 3), indicating that they have a close genetic relationship within themselves, which largely agrees with the traditional taxonomy. The SSRs transferred to Prunoideae successfully were polymorphic and produced special band patterns in the Prunoideae (Fig. 3), suggesting application of these SSRs as valuable tools for comparative mapping and genetic diversity analysis. Gasic et al. (2009) surveyed the transferability of apple EST-SSRs to Prunoideae and

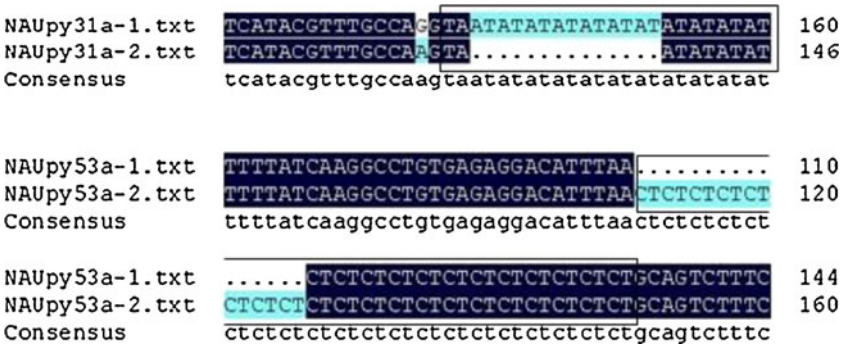

Fig. 2 DNAMAN alignment of sequences obtained from selected PCR bands amplified by primer pairs NAUPy31a and NAUpy53a in pears. *1–2* bands of different sizes; TA-repeat and CT-repeat differences within the fragments are shown highlighted in *blue*

Table 3 Transferability of pear SSRs to other Rosaceae fruit crops

SSR locus	Number of accessions in which an SSR was amplified								Total
	Japanese apricot	Plum	Cherry	Apple	Strawberry	Apricot	Peach	Loquat	
NAUpy22c	3/10	0/14	0/13	16/20	0/6	0/10	0/12	7/11	26/96
NAUpy24c	0/10	0/14	4/13	20/20	0/6	2/10	8/12	4/11	38/96
NAUpy32c	0/10	0/14	0/13	1/20	0/6	0/10	0/12	0/11	1/96
NAUpy38c	0/10	0/14	0/13	3/20	0/6	0/10	0/12	0/11	3/96
NAUpy40c	0/10	1/14	0/13	7/20	0/6	0/10	0/12	0/11	8/96
NAUpy43c	0/10	0/14	0/13	15/20	0/6	0/10	0/12	4/11	19/96
NAUpy86c	0/10	0/14	0/13	7/20	0/6	0/10	0/12	0/11	7/96
NAUpy87c	6/10	5/14	7/13	2/20	0/6	0/10	0/12	0/11	20/96
NAUpy89c	10/10	11/14	10/13	15/20	0/6	0/10	0/12	0/11	46/96
NAUpy27c	0/10	0/14	0/13	11/20	0/6	0/10	0/12	1/11	12/96
NAUpy91b	0/10	0/14	0/13	13/20	0/6	0/10	0/12	0/11	13/96
NAUpy85d	1/10	0/14	0/13	10/20	0/6	1/10	0/12	1/11	13/96
NAUpy87d	3/10	4/14	3/13	4/20	0/6	0/10	0/12	0/11	14/96
NAUpy46k	0/10	1/14	0/13	6/20	0/6	0/10	0/12	0/11	7/96
NAUpy55k	1/10	1/14	1/13	16/20	0/6	0/10	0/12	5/11	24/96
NAUpy80k	0/10	0/14	0/13	11/20	0/6	1/10	1/12	7/11	20/96
NAUpy81m	0/10	1/14	0/13	8/20	0/6	0/10	0/12	0/11	9/96
NAUpy86n	0/10	0/14	0/13	7/20	0/6	5/10	3/12	1/11	16/96
NAUpy98s	0/10	0/14	0/13	9/20	0/6	3/10	1/12	4/11	17/96
NAUpy36t	0/10	0/14	0/13	8/20	0/6	0/10	0/12	3/11	11/96
NAUpy48t	1/10	0/14	0/13	7/20	0/6	0/10	0/12	0/11	8/96
NAUpy58t	2/10	0/14	0/13	4/20	0/6	0/10	0/12	0/11	6/96
NAUpy67t	2/10	2/14	5/13	20/20	3/6	10/10	11/12	8/11	61/96
NAUpy91t	0/10	0/14	0/13	10/20	0/6	5/10	5/12	1/11	21/96
NAUpy07u	0/10	7/14	4/13	20/20	0/6	0/10	0/12	0/11	31/96
NAUpy30u	0/10	0/14	0/13	0/20	0/6	0/10	0/12	7/11	7/96
NAUpy62u	0/10	0/14	0/13	7/20	0/6	0/10	0/12	2/11	9/96
NAUpy33v	0/10	0/14	0/13	0/20	0/6	0/10	0/12	3/11	3/96
NAUpy35v	3/10	5/14	1/13	12/20	0/6	0/10	0/12	0/11	21/96
NAUpy41v	0/10	0/14	0/13	3/20	0/6	0/10	0/12	0/11	3/96
NAUpy74v	0/10	0/14	0/13	10/20	0/6	0/10	0/12	8/11	18/96
NAUpy43w	0/10	0/14	0/13	7/20	0/6	0/10	0/12	0/11	7/96
NAUpy94x	0/10	0/14	0/13	3/20	0/6	0/10	0/12	0/11	3/96
NAUpy92a	0/10	0/14	0/13	10/20	0/6	0/10	3/12	0/11	13/96
NAUpy31a	0/10	0/14	0/13	2/20	0/6	0/10	0/12	0/11	2/96
NAUpy74f	0/10	0/14	0/13	4/20	0/6	0/10	0/12	0/11	4/96
NAUpy23f	3/10	0/14	0/13	6/20	0/6	5/10	5/12	4/11	23/96
NAUpy45f	0/10	0/14	0/13	10/20	0/6	0/10	0/12	3/11	13/96
NAUpy39h	0/10	0/14	0/13	4/20	0/6	0/10	0/12	0/11	4/96
NAUpy56h	0/10	0/14	0/13	19/20	0/6	0/10	0/12	0/11	19/96
NAUpy38e	0/10	0/14	0/13	10/20	0/6	4/10	6/12	2/11	22/96
Number of transferable SSRs	11/67	10/67	8/67	39/67	1/67	9/67	9/67	22/67	41/67
Percentage (%)	16.4	14.9	11.9	58.2	1.5	13.4	13.4	32.8	61.2

Number of transferable SSRs—number of SSR markers that successfully amplified in at least one Rosaceae cultivar; the SSRs whose products amplified in Rosaceae crops ranged from 90 to 280 bp were considered to be transferable, and those beyond this range were not recorded in this table

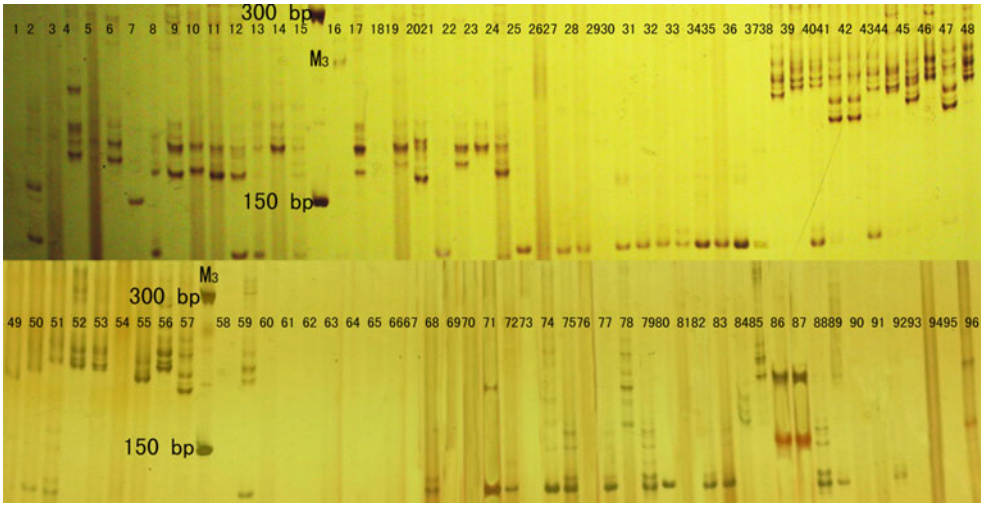

Fig. 3 Amplification of pear SSR marker NAUpy98s in Rosacea accessions; numbers in lanes are the same as Table 1; *1–10* Japanese apricot, *11–24* plum, *25–37* cherry, *38–57* apple, *58–63* strawberry, *64–73* apricot, *74–85* peach, *86–96* loquat, M_3 Trans DNA 2 K Marker

showed that the frequency of transferability ranged from 25 % in the subgenus *Armeniaca* to 38 % in the subgenus *Amygdalus*. Apple EST-SSRs were successfully amplified in 14 members of the subgenus *Prunophora*, represented by apricot and European and Japanese plums, with an average of 40 %, with the highest frequency of transferability (35 %) observed for Japanese plum. Substantial transferability of apple EST-SSR to peach, apricot, and European plum was also noted at 37, 25, and 29 %, respectively. The value of apple EST-SSR to Prunoideae members was much higher than that observed in this study, likely suggesting the higher conservation of EST regions used for marker development. Vendramin et al. (2007) studied a set of EST-SSRs isolated from the peach fruit transcriptome and their transportability across *Prunus* species. Apricot genomic SSRs showed considerable transferability, 20 %, in all *Prunus* species, but failed to amplify in apple (Messina et al. 2004). Mnejja et al. (2010) studied the transferability of 145 *Prunus* microsatellite markers across a set of eight cultivars from nine rosaceous species (almond, peach, apricot, Japanese plum, European plum, cherry, apple, pear, and strawberry). Of these, 16.6 % were transferable in pear, and the polymorphism of *Prunus* microsatellites was also detected at a low level. In this study, the transferability of pear SSRs to *Prunus*, 38 % (27/71) in total (Table 3), is higher than the above, and some displayed variable band types (Fig. 3); thus, they can play an important role in the genetic study of *Prunus* or related species. In contrast, Wünsch (2009) studied 13 *Prunus* SSRs known to be transferable to certain *Prunus* species, tested them in 10 *Prunus*, and found that three were transferable to all of them and eight to all but one species. *Prunus* SSRs (63.9 %) were transferable to other *Prunus* crops (Mnejja et al. 2010), overall showing a higher level of transferability to close species. Most pear SSRs transferred to *Prunus* detected only a single band and had a lack of polymorphism (Fig. 3), but their transferability indicates some extent of genomic collineation between pear and *Prunus*.

Only a few SSR primers were amplified (1.4 %) among six strawberry cultivars. Unlike other Rosaceae family crops such

Table 4 Data of the SSR fingerprint bands with two pear primers amplified among 20 apple genotypes

SSR locus	Band no.	Apple cultivars (sample no.)																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
NAUpy43c	1	1	1	1	1	1	1	0	1	0	1	0	1	0	0	1	1	0	1	1	0
	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	3	1	0	0	0	1	1	0	1	0	1	1	1	1	1	1	1	0	1	0	0
NAUpy55k	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0
	2	1	0	0	1	0	1	0	1	0	0	0	0	0	1	1	0	0	1	1	0
	3	0	0	0	0	1	1	1	1	0	1	1	0	1	1	0	1	1	0	1	0
	4	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1
	5	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	0	1	0
	6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1

1 presence of band, 0 absence of band; sample no.: *1* Sekaiichi, *2* Huaguan, *3* Zaohong, *4* Delicious, *5* Braeburn, *6* Fuji, *7* Golden Delicious, *8* Mikilifi, *9* Longfeng, *10* Starking, *11* Dailv, *12* Gala, *13* Qinyang, *14* HoKuto, *15* Jonagold, *16* Orin, *17* Lujia 1, *18* Alps Otome, *19* Hanfu, *20* Xiushui

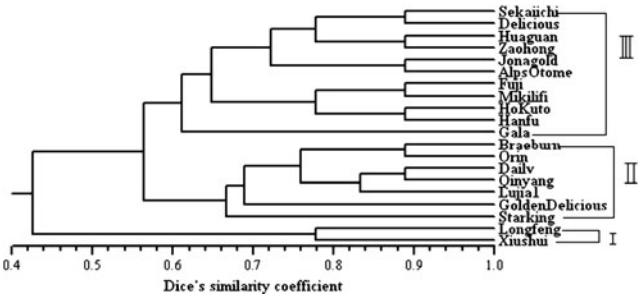

Fig. 4 UPGMA dendrogram of *Malus* accessions based on Dice's similarity coefficient (Nei and Li 1979) according to pear SSR markers NAUpy43c and NAUpy55k

as apple and peach, the strawberry is considered to be non-climacteric because the flesh does not ripen in response to ethylene. Genomically, strawberry genus has a small basic (x=7) genome size of ~240 Mb, while the cultivated *F.* ×*ananassa* is among the most complex of crop plants, harboring eight sets of chromosomes ($2n$=8x=56) derived from as many as four different diploid ancestors (Shulaev et al. 2011). Other research about transferability to strawberry found that only 12 of 145 Prunus SSR loci (8.3 %) tested were polymorphic in strawberry (Mnejja et al. 2010). This indicates that the strawberry has a comparatively more distant relationship with pear than other Rosaceae fruit trees, which is consistent with the divergent relationship of pear, apple, and strawberry based on whole genome comparison (Wu et al. 2013). However, Gasic et al. (2009) reported good transferability of EST-SSRs (49 %) from apple to strawberry. In addition, Zorrilla-Fontanesithe et al. (2011) investigated the transferability of 174 *Fragaria* SSRs to rose and raspberry, with the data ranging from 28.7 % for genic SSRs in rose to 16.1 % for genomic SSRs in raspberry. Collinearity analysis between pear and two other sequenced rosaceous species, apple and strawberry, has revealed that they share the same ancestor, but unlike pear and apple, strawberry did not have a whole genome duplication event (Wu et al. 2013). So, the higher transferability of EST-SSR over genomic SSRs within species also reveals the common ancestral origins with differential genome evolution. In addition, the level of polymorphism of an SSR marker seems to greatly depend on the germplasm on which it is tested. Thus, more exploration such as sequence comparison should be done to study the genetic relationship between pear and strawberry.

Conclusion

The SSR markers in this study displayed a high level of polymorphism in pears, suggesting valuable applications of these markers in future pear linkage map construction, genetic polymorphism evaluation, cultivar identification, and MAS. As useful PCR-based genetic markers, the pear SSRs can be applied to these various aspects of genetic research not only for *Pyrus*, but also for other members of Rosaceae. Our results reveal a relatively high level of transferability between pear and several other Rosaceae species, which means an increased number of SSR markers available for Rosaceae crops, which is particularly of value for those species with little genomic information. Most of the pear SSRs presented diversity when assessed in other Rosaceae species, implying that they will be significant for genetic research. Besides this, when mapped, these markers can be used for conducting macro-synteny studies among Rosaceae species to better understand genome organization and evolutionary relationships in this important fruit family. The transferability to each Rosaceae species was largely related to their consanguinity with pear, demonstrating the applicability of pear SSRs in phylogenetic and evolutionary studies of the Rosaceae family. Overall, these findings suggest that the high level of polymorphism and good transferability of pear SSRs result in their promise for wide use in QTL mapping, molecular breeding, investigation of population genetic diversity, comparative mapping, and evolutionary studies among pears and other Rosaceae species.

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