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Original scientific article

TRANSFERABILITY OF APPLE AND PEAR SSRS TO OTHER TEMPERATE POME FRUIT CROPS OF FAMILY ROSACEAE

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Extensive use of simple sequence repeat (SSR) is facilitated if loci would be transferable across species even in closely related genera to overcome high cost and efforts involved in their development as major constraints. In the present study, apple and pear genomic microsatellite primer pairs were used to amplify SSR loci in apple, pear, quince and loquat genotypes, respectively. Already reported SSRs were selected based on their polymorphic survey for successful amplification with at least one polymerase chain reaction (PCR) product of the approximate size expected for a homologous locus screened among apple and pear genotypes for further transferability exploration across other temperate pome fruit crops, respectively. Highest transferability of apple and pear SSR, 61.53 % and 73.33 % was observed in closely related quince and apple genotypes, respectively. This indicated that primer binding sites between these two closely related genera, *Malus* and *Pyrus*, are fairly well conserved. Maximum transferability rate was found to be 93.33 % and 80.00 % across all the subjected genotypes for primer CH05D11 and TSUenh016 in apple and pear, respectively. The transferability of markers is based on genomic similarity, and can reflect the relationship of genome collinearity and even evolution between species. This high level of transferability of apple and pear SSRs to other temperate pome fruit crops indicated their promise for application to future molecular screening, map construction, and comparative genomic studies, etc.

Keywords: apple, pear, SSR, temperate pome fruit crops, transferability, markers.

INTRODUCTION

Among the available molecular markers, microsatellites or SSRs are used as an ideal tool in a variety of applications due to many desirable features including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance and extensive

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genome coverage (VARSHNEY *et al.,* 2005). However, the cost and effort required for their development is one of the major constraints of using SSRs as a molecular marker (POWELL *et al.,* 1996). Thus, a more extensive use of SSRs in plants would be facilitated if such loci were transferable across species even in closely related genera. Many studies on comparative genetics have revealed that the gene content and order are highly conserved among closely related plant species. Sequence data obtained from many plants indicate the sufficient homology existing in the regions flanking the SSR loci of two or more closely related genera/species (KULEUNG *et al.,* 2004; GUTIERREZ *et al.,* 2005; KALIA *et al.,* 2011). Hence, primer pairs designed on the basis of the sequences obtained from one species could be used to detect microsatellites in related species even in multiple genera of same family. The cross transferability of SSRs is mainly useful in comparative genome mapping and phylogenetics. Furthermore, this method of microsatellite detection is especially useful in those crops where neither sequence information nor the genetic maps are available. Several studies have also demonstrated the cross transferability of SSR markers from one species to other species of the same genus and even to species of other genera (GASIC *et al.,* 2009; MNEJJA *et al.,* 2010; YU *et al.,* 2011). Thus, transferability of genomic SSR may be advantageous in fingerprinting or varietal identification of those plants for which DNA data base is not available. *Rosaceae* is a large plant family containing more than 3,000 species, many of which are economically important fruit trees of temperate region such as apple, pear, quince and loquat. However, there is rather little genomic information available for other valuable fruit tree members of the big *Rosaceae* family. Apple (*Malus* x *domestica* Borkh.) is one of the most widespread and popular fruit trees in the temperate regions of the world (JANICK *et al.,* 1996). About 59 species and 7500 cultivars were identified in all over the world. Pear (*Pyrus spp*.), one of the oldest fruit crops in the world, belongs to the genus *Pyrus*, subfamily *Maloideae* (*Pomoideae*). Economically, pear is the third most important temperate fruit species after apple, and the genome sequencing of the diploid *P. bretschneideri* Rehd. cv. 'Dangshansuli' has allowed ready access to the DNA sequences of pear (WU *et al.,* 2013). Some apple SSRs have already been used to identify genetic diversity in pear (YAMAMOTO *et al.,* 2001, 2002). There are several reports on the transferability of SSR markers in or across genera among *Rosaceae* fruit crops (GASIC *et al.,* 2009; GISBERT *et al.,* 2009; YAO *et al.,* 2010; HE *et al.,* 2011). Evaluation of genetic relatedness among temperate pome fruit crops of family Rosaceae using arbitrary oligonucleotide markers has been done in our previous findings (SHARMA *et al.,* 2011). In the present study, we reported cross-transferability of microsatellite markers developed in apple to pear, quince and loquat and pear to apple, quince and loquat, respectively.

MATERIALS AND METHODS

Plant materials and DNA isolation.

Young green leaves of each genotype of apple, pear, quince and loquat fruit were procured from the National Bureau of Plant Genetic Resources (NBPGR) Regional station, Phagli (HP) and other locations of Himachal Pradesh (India) for carrying out molecular marker studies (Table 1). Isolation of genomic DNA was done from the collected leaves of each subjected genotype by using CTAB method (DOYLE and DOYLE, 2011). RNA contaminants in all the samples were digested with 100µg/ml RNaseA for 30 minutes at 37˚C. DNA

concentration and purity were measured using UV/VIS spectrophotometer at 260 nm and 280 nm absorbance.

Table 1. Apple, pear, quince and loquat genotypes subjected to molecular characterization studies using SSR markers.

S.No.	Name of Genotype	Origin	S.No.	Name of Genotype	Origin
Apple			Pear		
1	Royal Delicious	USA	21	Kashmir Pear	India
2	Tydeman	UK	22	Chuger yongshiki	Japan
3	Well Spur	USA	23	Le conte	Japan
4	Silver Spur	USA	24	Tan-Yan Jhao	China
5	Red Baron	USA	25	Moodeung	Korea
6	Red Baldwin	USA	26	Keiffer	Japan
7	Gravenstein	Denmark	27	Nic-58127	India
8	Ingrid Marie	Denmark	28	Korean giant Pear	Korea
9	Gale Gala	New Zealand	29	Babugosha	India
10	Top Red	USA	30	IC-20092	India
11	Hardeman	USA	31	Hood	USA
12	Ambrich	India	32	King Pear	UK
13	Ambroyal	India	33	Doynee Bussarch	UK
14	Ambstarking	India	34	Baldwin Pear	USA
15	Margrate	UK	35	Stirling	USA
16	Wugenar	USA	36	Rakovslik	Hungry
17	Directeur *Van De Plassche*	Netherland	37	Wenatchee	USA
18	Survovets	USA	38	Nuggetz	Italy
19	Dessert of Isaac	UK	39	Harogen	USA
20	Summer Queen	USA	40	EC-566191	USA
Quince			Loquat		
41	Cydonia Quince	-	46	Nauni Gandal	India (HP)
42	Orange Quince	-	47	Nauni Kiwi	India (HP)
43	EC-024520	USA	48	Nauni DR	India (HP)
44	EC-024530	USSR	49	Palampur	India (HP)
45	Quince J/K	India (J&K)	50	Nauni Adm	India (HP)

* USA: United States of America, UK: United Kingdom, USSR: *Union of Soviet Socialist Republics,* J&K: Jammu and Kashmir, EC: Exotic Collection, IC: Indigenous Collection, HP: Himachal Pradesh

Microsatellite markers and PCR analysis

A total of 10 apple and 11 pear microsatellite primer pairs were selected depending upon their polymorphic information contents for their successful amplification with at least one PCR product of the approximate size expected for a homologous locus screened from earlier reports and were designed to amplify SSR loci in 20 genotypes of apple and pear, respectively (Table 2&3). DNA amplification reactions were performed in 15 µl volume containing 50-100

ng of template DNA, PCR buffer, 0.2 mM dNTP mix (GeNei, India), 2.5 mM $MgCl_2$and 1U Taq DNA polymerase (GeNei, India). Both forward and reverse microsatellite primers were added to a final concentration of 15 µl. Primers were synthesized as per the information available on the previously reported SSR markers (GIANFRANCESCHI *et al.,* 1998; LIEBHARD *et al.,* 2002; YAMAMOTO *et al.,* 2002; NISHITANI *et al.,* 2009). The samples were amplified in a thermal cycler (Applied Biosystems, USA) using following program: Initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at Ta°C (Table 2) for 1 min and extension at 72°C for 2 min with a final extension at 72°C for 8 min. PCR products were visualized on 3.5% (w/v) agarose gel stained with ethidium bromide (0.5 µg/ml), then DNA profile were visualized on a UV transilluminator and photographed by using Gel Documentation System (Syngene, UK). The allele sizes were calculated by comparing with 50 and 100 bp DNA ladder (GeNei, India). At least two independent PCR amplifications were performed for each primer.

Data analysis

The data on specific band position on electrophoresed gel was recorded by assigning '+' sign for the presence and '-' sign for the absence of band in all the subjected genotypes of temperate pome fruit crops. The percent transferability of each loci was observed to assess the transferability of apple and pear SSR markers in other related species and genera of temperate pome fruit crops, respectively.

RESULTS AND DISCUSSION

Amplification of genomic SSRs in apple

The good polymorphism characteristics of the transferable apple genomic SSRs in this study were more valuable in application to temperate pome fruit crops genomic studies. Total 10 apple genomic SSRs were amplified among 20 apple genotypes with expected size revealed in earlier reports (GIANFRANCESCHI *et al.,* 1998; LIEBHARD *et al.,* 2002). Consequently, these 10 apple SSRs (Tables 2 and 3) were picked for further transferability exploration across other temperate pome fruit crops belonging to three genera including pear (20 genotypes), quince (5 genotypes) and loquat (5 genotypes). Overall, 69.23% (9/13) 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 genera screened. Maximum transferability rate was found to be 93.33% across all the subjected genotypes for primer CH05D11 (Fig. 1) whereas, minimum was recorded for CH04G10 (0%) (Fig. 2, Tables 2&3). Highest transferability 61.53% was observed in the closely related quince genotypes, in which the majority of apple SSRs were polymorphic. This indicated that primer binding sites between these two closely related genera were fairly well conserved. The transferability rates to pear and loquat were 46.15% and 30.76%, respectively. Similarly, the highest transferability 58.20% was reported in the closely related apple (*Malus domestica*) in which the majority of pears SSRs were polymorphic (FAN *et al.,* 2013). This high level of transferability of SSRs was consistent with genome comparison of pear and apple in reported amplification of apple SSRs in pear populations (YAMAMOTO *et al.,* 2001; PIERATONI *et al.,* 2004; GASIC *et al.,* 2009; WU *et al.,* 2013).

Table 2. Transferability of apple SSRs to other temperate pome fruit crops

S.No.	Primer	Sequence (5' - 3')	Linkage group	Annealing Temp. (Ta °C)	Expected Size (bp)	Observed Size (bp)	Fruit crops			Total	Transferability (%)
							Pear	Quince	Loquat		
1	CH01E12(F)	AAACTGAAGCCATGAGGGC	8	55	243-248	250	0/20	4/5	0/5	4/30	13.33
	CH01E12(R)	TCAATTCACATGAGGCTG									
2	CH02C06(F)	TGACGAAATCCACTACTAATGCA	2	55	216-254	245	0/20	5/5	0/5	5/30	16.66
	CH02C06(R)	GATTGCGCGCTTTTTAACAT									
3	CH03D01(F)	CGCACCACAAATCCAACT									
	CH03D01(R)	AGAGTCAGAAGCACAGCCTC	2	58	95-115	100	13/20	4/5	2/5	19/30	63.33
4	CH03G07(F)	AATAAGCATTCAAAGCAATCCG									
	CH03G07(R)	TTTTTCCAAATCGAGTTTCGTT	3	55	119-181	170	1/20	4/5	3/5	8/30	26.66
5	CH04G10(F)	CAAAGATGTGGTGTGAAGAGGA									
	CH04G10(R)	GGAGGCAAAAAGAGTGAACCT	15	55	127-168	150	0/20	0/5	0/5	0/30	0
6	CH05C02(F)	TTAAACTGTCACCAAATCCACA									
	CH05C02(R)	GCGAAGCTTTAGAGAGACATC	11	60	168-200	165	9/20	3/5	0/5	12/30	40.00
7	CH05D11(F)	CACAACCTGATATCCGGGAC									
	CH05D11(R)	GAGAAGGTCGTACATTCCTCAA	12	55	171-211	200	18/20	5/5	5/5	28/30	93.33
8	CH05E03(F)	CGAATATTTTCACTCTGACTGGG									
	CH05E03(R)	CAAGTTGTTGTACTGCTCCGAC	2	55	158-190	160	0/20	2/5	0/5	2/30	6.66
9	CH02D08(F)	TCCAAAATGGCGTACCTCTC									
	CH02D08(R)	GCAGACACTCACTCACTACTATCTCTC	11	58	210-254	240	11/20	4/5	0/5	15/30	50.00
10	CH01B12(F)	CGCATGCTGACATGTTGAAT									
	CH01B12(R)	CGGTGAGCCCTCTAATGTGA	12	58	123-130	125	16/20	0/5	2/5	18/30	60.00
	Number of transferable SSRs						6/13	8/13	4/13	9/13	
	Percentage (%)						46.15	61.53	30.76	69.23	

Table 3. Amplification pattern of apple SSRs and their transferability to other temperate pome fruit crops

Amplification of apple SSR in apple (A1-A20)

S.No.	Primer	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20
1	CH01E12	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-
2	CH02C06	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-
3	CH03G07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	CH04G10	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
5	CH05D11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	CH05E03	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
7	CH03D01	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+
8	CH01B12	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+
9	CH05C02	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	CH02D08	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-

Transferability of apple SSR to pear (P1-P20)

S.No	Primer	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
1	CH01E12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	CH02C06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	CH03G07	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
4	CH04G10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	CH05D11	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-
6	CH05E03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	CH03D01	+	+		+	+	-	+	-	-	+	-	+	-	+	+	+	-	+	+	+
8	CH01B12	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	-	+
9	CH05C02	+	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-
10	CH02D08	+	+	+	+	-	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-

Transferability of apple SSR to quince(Q1-Q5) and loquat (L1-L5)

S.No	Primer	Q1	Q2	Q3	Q4	Q5	L1	L2	L3	L4	L5
1	CH01E12	+	-	+	+	+	-	-	-	-	-
2	CH02C06	+	+	+	+	+	-	-	-	-	-
3	CH03G07	+	+	+	+	-	+	-	-	+	+
4	CH04G10		-	-	-	-	-	-	-	-	-
5	CH05D11	+	+	+	+	+	+	+	+	+	+
6	CH05E03	-	+	+	-	-	-	-	-	-	-
7	CH03D01	+	+	+	+	-	+	+	-	-	-
8	CH01B12	-	-	-	-	-	+	+	-	-	-
9	CH05C02	+	+	+	-	-	-	-	-	-	-
10	CH02D08	-	+	+	+	+	-	-	-	-	-

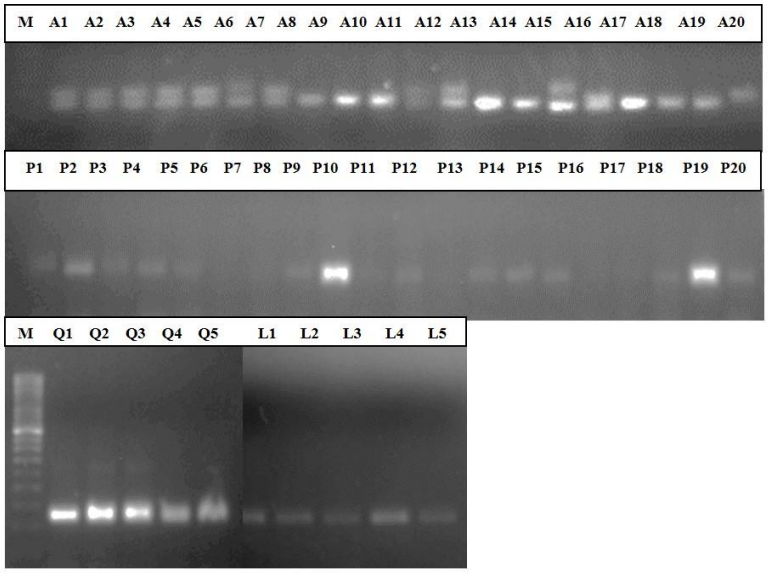

Fig 1 **Gel showing DNA banding profiles using apple SSR CH05D11**
Where; M: 50 bp ladder, (A1-A20) apple, (P1-P20) pear, (Q1-Q5) quince and (L1-L5) loquat genotypes

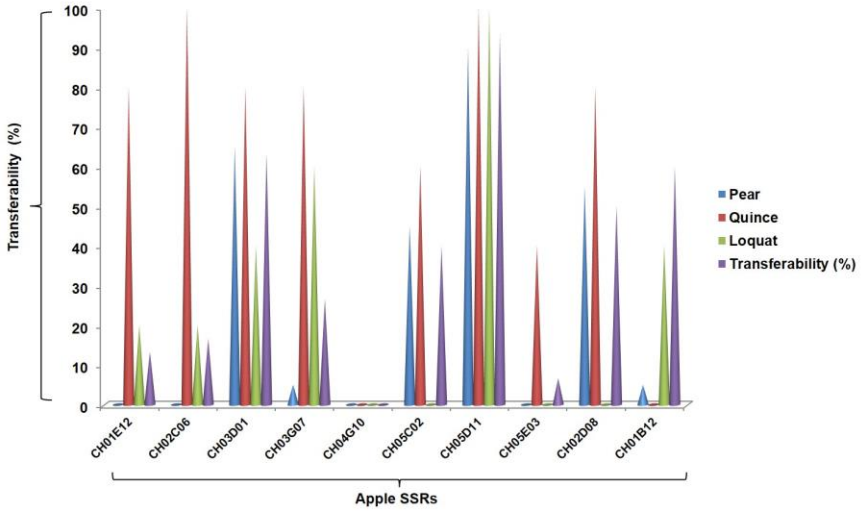

Fig 2 **Transferability of apple SSRs to other temperate pome fruit crops**

Amplification of genomic SSRs in pear

Total 11 pear genomic SSRs were selected among 20 pear genotypes for their successful amplification with at least one PCR product of the approximate size expected for a homologous locus screened in earlier reports (YAMAMOTO *et al.*, 2002; NISHITANI *et al.*, 2009). Maximum transferability rate was found to be 80.00% across all the subjected genotypes for primer TSUenh016 (Fig. 3) whereas, minimum was recorded for TSUenh046 (33.33%) (Tables 4 and 5). The good polymorphism characteristics of the transferable pear genomic SSRs in this study were more valuable in application to temperate pome fruit crops genomic studies. The highest transferability 73.33% was observed in the closely related apple genotypes, in which the majority of pear SSRs were polymorphic (Fig. 4, Tables 4 and 5). This indicated that primer binding sites between these two closely related genera, *Malus* and *Pyrus*, are fairly well conserved. The transferability rates to quince and loquat were 53.33% and 33.33%, respectively. The transferability of markers is based on genomic similarity and can reflect the relationship of genome collinearity and even evolution between species. Besides, high transferability (59.00 %) of apple SSRs to pear (GASIC *et al.*, 2009), and amplification of apple SSRs in pear populations have been reported (FAN *et al.*, 2013).

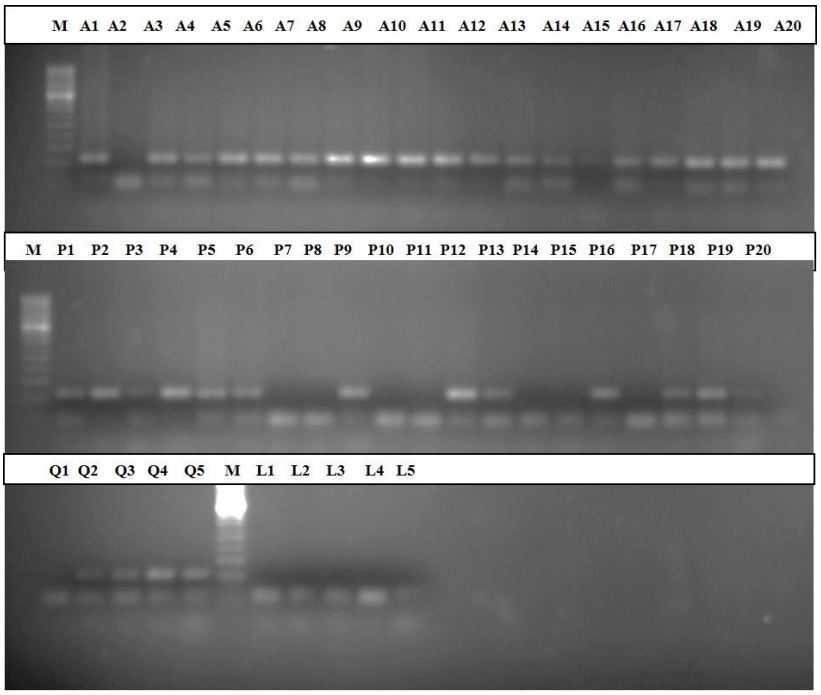

Fig 3 **Gel showing DNA banding profiles using pear SSR TSUenh016**
Where; M: 50 bp ladder, (A1-A20) apple, (P1-P20) pear, (Q1-Q5) quince and (L1-L5) loquat genotypes

Table 4. Transferability of pear SSRs to other temperate pome fruit crops

S.No.	Primer	Sequence (5'- 3')	Linkage group	Annealing Temp. (Ta°C)	Expected Size (bp)	Observed Size (bp)	Fruit crops			Total	Transferability (%)
							Apple	Quince	Loquat		
1	BGT23b(F)	CACATTCAAAGATTAAGAT	2	55	164-239	165	20/20	0/5	0/5	20/30	66.66
	BGT23b(R)	ACTCAGCCTTTTTTCCCAC									
2	NH011b(F)	GGTTCACATAGAGAGAGAG	4	60	163-215	200	16/20	3/5	2/5	21/30	70.00
	NH011b(R)	TTTGCCGTTGGACCGAGC									
3	TSUenh006(F)	ATCAGAGGCTACTCCAATGGTGA	7	57	112-140	115	19/20	4/5	0/5	23/30	76.66
	TSUenh006(R)	TGTTAAAGACCAGAAAGCCCTTG									
4	TSUenh025(F)	CACCTCCGTTAACCCCTCATAAT	13	57	195-260	250	17/20	4/5	2/5	23/30	76.66
	TSUenh025(R)	CTTCACCCCATCGAATCAAAAC									
5	TSUenh026(F)	GCGTTGAGTGACCTCTTTCATTT	17	55	132-200	150	13/20	5/5	0/5	18/30	60.00
	TSUenh026(R)	GGAAGTTGTGCTAGCAAAGAAGC									
6	TSUenh016(F)	TCATTTCATGGACTCTCAATCTCC	15	55	127-178	130	19/20	5/5	0/5	24/30	80.00
	TSUenh016(R)	CGAGGAGTCTGTCTGCGTCT									
7	TSUenh044(F)	GACAATTGGCTAAATACTCTTCTCG	11	55	145-172	160	18/20	0/5	1/5	19/30	63.33
	TSUenh044(R)	GGCGACGAAGTTGTGTTAGATTA									
8	TSUenh046(F)	GGTCATCACCCACTTAAAAACCA	6	55	142-156	150	9/20	0/5	1/5	10/30	33.33
	TSUenh046(R)	GTGCCCTGAAGTAATTGAGATGG									
9	NH030a(F)	TCCAAAGTTCAACACAGATCAAGAG	3	55	160-178	170	19/20	3/5	0/5	2/30	73.33
	NH030a(R)	TCCGGATTTTGTTGTCGGTTTTA									
10	NH036b(F)	TCCGGATTTTGTTGTCGGTTTTA	8	56	160-200	180	12/20	3/5	0/5	15/30	50.00
	NH036b(R)	ATTTCACTCTCTTCTCGCACCC									
11	TSUenh029(F)	GGAAGTTGTGCTAGCAAAGAAGC	10	55	175-240	200	13/20	2/5	0/5	15/30	50.00
	TSUenh029(R)	GCCTGTTTCCACTTATGCTCACT									
	Number of transferable SSRs						11/15	8/15	5/15	11/15	
	Percentage (%)						73.33	53.33	33.33	73.33	

Table 5. Amplification pattern of pear SSRs and their transferability to other temperate pome fruit crops

Amplification of pear SSR in pear (P1-P20)

S.No	Primer	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
1	TSUenh006	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	+	+
2	TSUenh025	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-
3	TSUenh026	+	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+
4	BGT23b	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+	-	-
5	NH011b	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+
6	TSUenh016	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
7	TSUenh044	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+
8	TSUenh046	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
9	NH030a	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+
10	NH036b	+	+	+	+	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+	-
11	TSUenh029	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	-

Transferability of pear SSR to apple (A1-A20)

S.No	Primer	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20
1	TSUenh006	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	TSUenh025	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+
3	TSUenh026	+	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+
4	BGT23b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	NH011b	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
6	TSUenh016	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	TSUenh044	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
8	TSUenh046	+	-	+	-	+	+	+	-	-	-	-	+	-	-	-	+	+	+	-	-
9	NH030a	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
10	NH036b	+	+	-	+	+	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-
11	TSUenh029	+	+	+	+	+	-	+	+	-	+	+	-	+	-	-	+	+	-	-	+

Transferability of pear SSR to quince (Q1-Q5) and loquat (L1-L5)

S.No	Primer	Q1	Q2	Q3	Q4	Q5	L1	L2	L3	L4	L5
1	TSUenh006	-	-	-	-	-	-	-	-	-	-
2	TSUenh025	+	+	+	-	+	-	-	-	+	+
3	TSUenh026	+	+	+	+	+	-	-	-	-	-
4	BGT23b	-	-	-	-	-	-	-	-	-	-
5	NH011b	-	-	+	+	+	+	+	-	-	-
6	TSUenh016	+	+	+	+	+	-	-	-	-	-
7	TSUenh044	-	-	-	-	-	-	-	+	-	-
8	TSUenh046	-	-	-	-	-	-	+	-	-	-
9	NH030a	-	-	+	+	+	-	-	-	-	-
10	NH036b	-	+	+	+	-	-	-	-	-	-
11	TSUenh029	-	-	-	+	+	-	-	-	-	-

Likewise, 94 primer pairs were tested on four accessions of *Pyrus* to evaluate the transferability of the markers, and 40 of 72 functional SSRs produced polymorphic amplicons (YAO *et al.,* 2010). All of the above interpretated results indicated that pear has close synteny with apple. Similarly, 30 SSRs from apple were used to assay the genetic relationships in loquat, 13 of which amplified polymorphic products and distinguished 34 of the 40 loquat accessions (SORIANO *et al.,* 2005) and also 39 identified SSRs from apple that could be transferred to loquat (HE *et al.,* 2011).

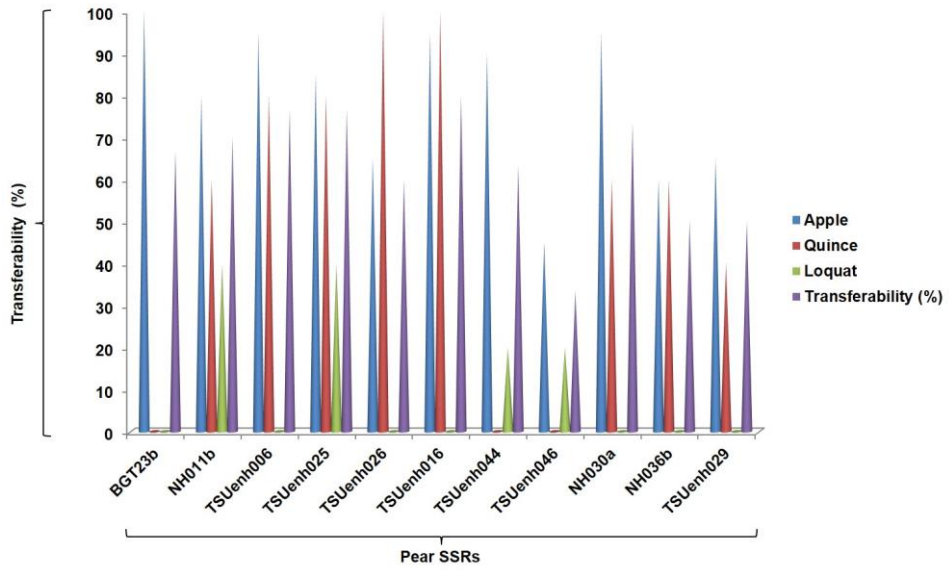

Fig 4 Transferability of pear SSRs to other temperate pome fruit crops

CONCLUSION

Results revealed a relatively high level of transferability of apple SSR to quince genotypes and pear SSR to apple genotypes, which means an increased number of SSR markers are available for temperate pome fruit crops, which is particularly of value for those species with little genomic information. Most of the apple and pear SSRs presented diversity when assessed in other temperate pome fruit crops, implying that they will be significant for genetic research. Besides this, when mapped, these markers can be used for conducting macro-synteny studies among temperate pome fruit crops for better understanding of genome organization and evolutionary relationships in this important fruit family.

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PRENOSIVOST SSR-ova JABUKE I KRUŠKE NA OSTALE VOĆNE VRSTE U PORODICI ROSACEAE

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Izvod

Opsežna upotreba SSR-ova olakšava se ako bi se lokusi mogli prenositi između vrsta, čak i u blisko povezanim rodovima, kako bi se prevazišli visoki troškovi i napori koji su uključeni u njihov razvoj kao glavna ograničenja. U ovoj studiji, genomski mikrosatelitni parovi prajmera jabuke i kruške korišćeni su za pojačavanje SSR lokusa u genotipovima jabuka, krušaka, dunja i japanske mušmule. Već prijavljeni SSR-ovi su izabrani na osnovu njihovog polimorfnog istraživanja za uspešnu amplifikaciju sa najmanje jednim proizvodom lančane reakcije polimeraze (PCR) približne veličine koja se očekuje za homologni lokus prikazan među genotipovima jabuka i krušaka za dalje istraživanje prenosivosti na druge vrste voća. Najveća prenosivost SSR-ova jabuka i krušaka, 61,53% i 73,33%, primećena je kod usko povezanih genotipova dunja i jabuke. To je ukazalo da su mesta vezivanja prajmera između ova dva blisko povezana roda, *Malus* i *Pyrus*, prilično dobro očuvana. Utvrđeno je da je maksimalna stopa prenosivosti 93,33% i 80,00% za sve ispitivane genotipove za prajmere CH05D11 i TSUenh016 u jabuci, odnosno kruški. Prenosivost markera se zasniva na genomskoj sličnosti i može odražavati odnos kolinearnosti genoma, pa čak i evoluciju između vrsta. Ovaj visok nivo prenosivosti SSR-ova jabuke i kruške na druge vrste voćaka ukazao je na njihovu mogućnost primene na buduće molekularne skrininge, izgradnju mapa i uporedne genomske studije, itd.

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