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ORIGINAL PAPER

Development and cross-species/genera transferability of microsatellite markers discovered using 454 genome sequencing in chokecherry (*Prunus virginiana* L.)

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Abstract Chokecherry (*Prunus virginiana* L.) ($2n = 4x = 32$) is a unique *Prunus* species for both genetics and disease-resistance research due to its tetraploid nature and X-disease resistance. However, no genetic and genomic information on chokecherry is available. A partial chokecherry genome was sequenced using Roche 454 sequencing technology. A total of 145,094 reads covering 4.8 Mbp of the chokecherry genome were generated and 15,113 contigs were assembled, of which 11,675 contigs were larger than 100 bp in size. A total of 481 SSR loci were identified from 234 (out of 11,675) contigs and 246 polymerase chain reaction (PCR) primer pairs were designed. Of 246 primers, 212 (86.2 %) effectively produced amplification from the genomic DNA of chokecherry. All 212 amplifiable chokecherry primers were used to amplify genomic DNA from 11 other rosaceous species (sour cherry, sweet cherry, black cherry, peach, apricot, plum, apple, crabapple, pear, juneberry, and raspberry). Thus, chokecherry SSR primers can be transferable across *Prunus* species and other rosaceous species. An average of 63.2 and 58.7 % of amplifiable chokecherry primers amplified DNA from cherry and other *Prunus* species, respectively, while 47.2 % of amplifiable chokecherry primers amplified DNA from other rosaceous species. Using random genome sequence data generated from next-generation sequencing technology to identify microsatellite loci appears to be rapid and cost-efficient, particularly for species with no sequence information available. Sequence information and confirmed transferability of the identified chokecherry SSRs among species will be valuable for genetic research in *Prunus* and other rosaceous species.

Key message A total of 246 SSR primers were identified from chokecherry genome sequences. Of which, 212 were confirmed amplifiable both in chokecherry and other 11 other rosaceous species.

Keywords Chokecherry · Next-generation sequencing · Microsatellites · Marker transferability

Introduction

Chokecherry (*Prunus virginiana* L.) is a small tree or large shrub widely distributed across the United States and Canada. It is one of the native North American species (pincherry, cranberry, blueberry, etc.) used in small fruit production for beverages, jellies, dried fruit products, and wine. It is also used as an ornamental plant because of the beautiful white flowers in spring and colorful leaves and fruits in fall. Chokecherry belongs to the genus *Prunus* in the Rosaceae family. The genus *Prunus* consists of more than 200 species of trees and shrubs including many important stone fruit species, such as peach, sweet and sour cherry, nectarine, apricot, plum, almond, etc. Chokecherry has the same base chromosome number as other *Prunus* species ($x = 8$) (Rehder 1940); however, it is one of the few tetraploids, having 32 chromosomes ($2n = 4x = 32$) (Dai, unpublished).

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In the last two decades, advances in plant genetics and genomics facilitate the development of molecular markers that can greatly improve plant breeding efficiency through marker-assisted selection (MAS), gene mapping, and genetic transformation. Microsatellites or simple sequence repeats (SSRs) are repeating sequences of 1–6 base pairs of DNA. The DNA sequences flanking SSRs are conserved and can be used to design PCR primers that are used to amplify the intervening SSR. SSR markers are known to be co-dominant, reproducible, relatively abundant, and multi-allelic, and are widely used for genotype fingerprinting, genetic map construction, gene identification, and marker-assisted selection (Agarwal et al. 2008; Parida et al. 2009). SSRs can be discovered from SSR-enriched genomic libraries or random genomic sequences. A large number of SSR markers have been developed from expressed sequence tags (EST) data bases because EST-SSRs target the transcribed region of the genome and these SSRs appear to be more closely related to important agronomical traits (Qi et al. 2010). However, EST-SSR markers are less polymorphic than those from random genomic sequences and their development depends on the availability of sequence databases (Gupta et al. 2003). Recently, the application of next-generation sequencing (NGS) technology for genome sequencing leads to the discovery of a large number of genome-wide and gene-based microsatellites much more efficiently (Mardis 2008; Jun et al. 2011; Zalapa et al. 2012). Illumina and 454 sequencing technology are the two NGS technologies used for the discovery of SSRs in plants. The 454 sequencing method has one major NGS advantage: avoiding the SSR enrichment step because a great number of SSRs can be detected from a large DNA sequence. This method also produces longer reads (350–600 bp per read) that increases the likelihood of finding flanking DNA sequences that can be used to design SSR primers. To date, SSR markers have been developed using 454 genome sequences for more than 20 plants and this number is increasing rapidly (Zalapa et al. 2012).

It has been well recognized that some molecular markers including SSRs can be transferable from different genotypes within or between species or even between genera (Kalia et al. 2011). Such interspecific or intergeneric transferability makes SSRs a widely useful tool for genetic studies, such as fingerprinting, genetic map construction, and molecular marker identification. The transferability rate of SSRs depends on the genetic distance between the individual genotypes. The closer the genetic relationship between genotypes, the more transferable is the SSR (Luro et al. 2008). For instance, Singh et al. (2011) found that SSR markers derived from sugarcane had a high transferability rate within the *Saccharum* complex (98.0 %) and with cereal genomes (88.3 %).

Transferability of SSRs has been well applied to many aspects of genetic research in *Prunus* and other rosaceous species since the SSR markers were first developed in peach (Cipriani et al. 1999; Mnejja et al. 2010). Microsatellites have been discovered from many species in the family Rosaceae, particularly from most of the commercial species, such as peach (Aranzana et al. 2002), almond (Mnejja et al. 2005), apricot (Hagen et al. 2004), sweet cherry (Olmstead et al. 2008), sour cherry (Canli 2004), apple (Gasic et al. 2009), pear (Yamamoto et al. 2002), and strawberry (Lewers et al. 2005). Transferability of these SSRs in or between rosaceous genera has been evaluated. Decroocq et al. (2003) tested 10 apricot EST-SSR markers in a few *Prunus* and other rosaceous species (apple and pear) and found that only one marker was transferable across all tested species. Moreover, transferability of SSRs depends on the relationship between the species tested and the one from which the SSRs were identified. Mnejja et al. (2010) reported that SSR markers developed from peach and almond had a higher transferability rate in *Prunus* species than in three other non-*Prunus* rosaceous genera (apple, pear, and strawberry). Among *Prunus* species, a higher transferability rate was found from peach to plum than to cherry because the genetic distance between peach and plum is closer than the one between peach and cherry (Lee and Wen 2001). Wünsch (2009) reported that 13 out of 18 SSR markers developed from peach and cherry were transferable in 27 varieties of 10 *Prunus* species, but only two loci were polymorphic in all species.

The objectives of this study were to develop SSR markers from chokecherry genome sequences generated using Roche 454 sequencing technology and to evaluate transferability and polymorphism of the SSRs in other *Prunus* and rosaceous species. The SSR primer resource developed from this study will provide useful information and tools for genetic research in *Prunus* and other species in the Rosaceae family.

Materials and methods

Plant material and genomic DNA extraction

A total of 17 genotypes from seven *Prunus* and five other rosaceous species were used. The seven *Prunus* species were chokecherry (*Prunus virginiana*, three lines), sour cherry cultivars (*P. cerasus* L. 'Rheinische Schattenmorelle' (RS), 'Balaton', and 'North Star'), sweet cherry (*P. avium* L. 'Emperor Francis' (EF) and 'Schneider'), black cherry (*P. serotina* Ehrh.), peach (*P. persica* (L.) Batsch), apricot (*P. armeniaca* L.), and plum (*P. nigra* Aiton × *P. salicina* Lindl. 'Pembina'). Five other rosaceous species were apple (*Malus domestica* 'Haralson'),

crabapple (*Malus x* 'Dolgo'), pear (*Pyrus communis* L.), juneberry (*Amelanchier alnifolia* (Nutt.) Nutt. Ex M. Roem.), and red raspberry (*Rubus idaeus* L. 'Boyne').

Genomic DNA was extracted following the method of Lodhi et al. (1994) with some modifications that included the steps of washing ethanol-precipitated DNA with 70 % ethanol in a slow moving shaker for 3–5 h before being dissolved in TE buffer and the DNA was then digested with both RNase A (10 mg/ml) and Proteinase K (1 mg/ml) for another 60 min at 37 °C. The DNA concentration was determined using NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc.) and stored in a refrigerator (4 °C) until use.

NGS 454 genome sequencing and sequence assemblies

Approximately 20 μg of genomic DNA extracted from the root tissues of an X-disease resistant chokecherry was sent to the Center for Genetic, Proteomic, and Bioinformatic Research at University of Hawaii–Manoa for library preparation and sequencing using the 454 Genome Sequencer FLX Titanium (Roche Applied Science) following emulsion polymerase chain reaction (emPCR). Newbler was used to analyze the sequencer-generated SFF data and assemble the reads into contigs in FASTA format files via a command line (run Assembly),which was kindly provided by Dr. Zheng Jin Tu at the Supercomputing Institute for Advanced Computational Research at the University of Minnesota.

Identification of SSRs and design of SSR primers

All contigs or sequences longer than 100 bp were searched for microsatellites using the software Simple Sequence Repeat Identification Tool (SSRIT) available at http://www.gramene.org/db/markers/ssrtool. The minimum number of repeat motifs to be considered as a microsatellite was more than five repeats for 2- to 5-bp nucleotide motifs. Primers were then designed using the online software Primer3 (http://frodo.wi.mit.edu/primer3/).

Amplification of SSRs in chokecherry and other species

To validate the newly designed SSR primers and their transferability within the genus *Prunus* and among the species in other genera of Rosaceae, PCR amplification was conducted. Amplification reactions were carried out in 18 μl volumes containing 60 ng of template DNA, 2.0 mM $MgCl_2$, 10× buffer, 200 μM dNTP, 0.2 pmol of each primer, and 0.125 U *Taq* DNA polymerase. The amplification was performed under the condition of denaturing for 30 s at 94 °C (5 min for the first cycle), annealing for 30 s at 57 °C, and extension for 30 s at 72 °C (7 min for the final cycle) for 35 cycles. The PCRs were performed on a Programmable Thermal Controller PTC-100^{TM} or an Applied Biosystems 2720 Thermal Cycler.

PCR products were separated in a non-denaturing 6 % polyacrylamide gel (29:1 acrylamide:bis, J.T. Baker, Mallinckrodt Baker, Inc. NJ, USA). The gel was prepared as following. A mixture of 8.5 ml 10× TBE buffer, 25.5 ml acrylamide (29:1), 136 ml ddH_2O, 0.12 mg APS (Ammonium Persulfate), and 130 μl tetramethylenediamine (TEMED) was poured in between two glass plates for polymerization about 50 min and then pre-ran at 350 V in 0.5× TBE buffer for 1 h so that the ethidium bromide can migrate from buffer into the gel. Samples of PCR products were loaded into the gel and ran at 250 V for 3 h. The gel was visualized under UV lights and images were captured using software "Alphalmager".

Scoring of amplification

Gel images were scored based on the presence and absence of each band in the image. The number of bands amplified by each primer was recorded when the number was equal to or less than 5 and "M" was used when the number of amplified bands was more than 5.

Results

Analysis of 454 sequences of chokecherry

A total of 145,094 reads were generated from a half run of 454 sequencing of the chokecherry genome with a total length of 4,766,864 bp nucleotides. After assembling all reads, 15,113 contigs were obtained with an average contig length of 315.4 bp nucleotides, of which 3,438 of the contigs were smaller than 100 bp. Of 11,675 contigs that were larger than 100 bp, 9,651 (82.7 %) were in the range of 100–600 nucleotides in size. The other 17.3 % of contigs were larger than 600 bp including 49 scaffolds (0.42 %) with the length greater than 2,000 bp (Fig. 1).

Discovery and analysis of chokecherry microsatellites

Of all contigs (11,675) that were larger than 100 nucleotides, 405 contigs contained one or more microsatellites, and a total of 481 SSR loci were identified (Table 1). Among these SSR loci, 413 were dinucleotides (85.86 %), 62 trinucleotides (12.89 %), 4 tetranucleotides (0.84 %), and 2 pentanucleotides (0.42 %). The CT/GA repeat (24.32 %) was the most frequent repeat motif, followed by AT/TA (22.45 %) and AG/TC (21.83 %). A total of 246 SSRs (on 234 contigs) had flanking sequences suitable for PCR primer design and therefore, 246 primer pairs were

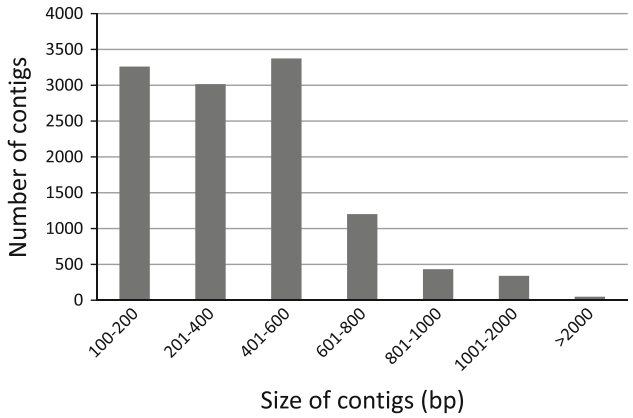

Fig. 1 Distribution of contigs with more than 100 nucleotides after assembly of sequence reads from a half run of 454 sequencing of the chokecherry genome

designed using the online software Primer3 (see Supplementary Material 1 for the information on all 212 amplifiable primer pairs). Eleven contigs contained more than one SSR locus. The primer C3280, C4274, and C13993 contained three SSR loci and C5595, C5948, C5956, C8681, C10447, C10749, C13713, and C15115 contained two SSR loci.

Amplification of new SSR primers in chokecherry and cross-species/genera

To verify the effectiveness of newly designed SSR primers, they were used to amplify genomic DNA of three chokecherry lines, six other *Prunus* species, and four other rosaceous species (Figs. 2, 3). Of all 246 new primers, 212 (86.2 %) effectively amplified DNA from chokecherries (see Supplementary Material 1). Tested in other cherry species, 176 (71.5 %), 160 (65.0 %), and 147 (59.8 %) of the chokecherry primers produced amplification in three sour cherry cultivars, 'RS', 'Balaton', and 'North Star', respectively, 155 (63.0 %) of black cherry, and 149 (60.6 %) and 152 (61.8 %) of sweet cherry cultivars 'EF' and 'Schneider'. For other *Prunus* species, 152 (61.8 %) of the primers amplified DNA of peach, followed by plum (142, 57.7 %) and apricot (139, 56.5 %). The amplification rate of these new chokecherry SSR primers in non-*Prunus* species in the Rosaceae family was 58.9 % in apple, 53.7 % in crabapple, 49.2 % in juneberry, 46.3 % in raspberry, and 28 % in pear.

A total of 76 (30.9 %) chokecherry primers produced amplicons in all twelve *Prunus* genotypes, and 26 (10.6 %) primers amplified DNA from both *Prunus* and all other rosaceous species. Nineteen (7.7 %) primers were considered to be chokecherry specific as they produced no amplicons in any other species tested. Two primers, C837 and C6387, can amplify DNA from all species except chokecherry line C_L (Cho1) and chokecherry line *c* (Cho2), respectively.

Table 1 Characterization of SSR loci discovered in 405 contigs of the chokecherry genome

Motif type	Repeat motif	No. of a given motif type	Frequency (%)
Dinucleotide	CT/GA	117	24.32
	AT/TA	108	22.45
	AG/TC	105	21.83
	AC/TG	41	8.52
	CA/GT	28	5.82
	CG/GC	14	2.91
Subtotal		413	85.86
Trinucleotide	CTC/GAG	8	1.66
	CCA/GGT	7	1.46
	AAC/TTG	6	1.25
	AAG/TTC	6	1.25
	CTT/GAA	6	1.25
	AGA/TCT	4	0.83
	AGT/TCA	3	0.62
	ATC/TAG	3	0.62
	AAT/TTA	3	0.62
	CCT/GGA	3	0.62
	ACT/TGA	2	0.42
	CAA/GTT	2	0.42
	CAC/GTG	2	0.42
	CTG/GAC	2	0.42
	ACA/TGT	1	0.21
	ACC/TGG	1	0.21
	ATA/TAT	1	0.21
	ATG/TAC	1	0.21
	CTA/GAT	1	0.21
Subtotal		62	12.89
Tetranucleotide	ACAA/TGTT	1	0.21
	GTGC/CACG	1	0.21
	TAAA/ATTT	1	0.21
	TTTG/AAAC	1	0.21
Subtotal		4	0.84
Pentanucleotide	CAACT/GTTGA	1	0.21
	TGGTT/ACCAA	1	0.21
Subtotal		2	0.42
Total		481	100

In this research, only sour cherry and sweet cherry had more than one cultivar. Most of the chokecherry SSR

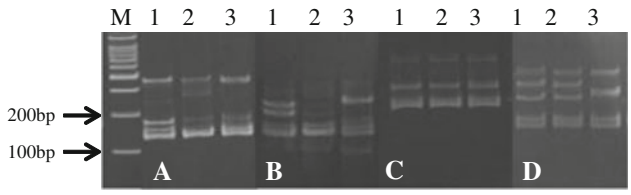

Fig. 2 A gel image showing amplification patterns from three chokecherry lines using four pairs of primers. **a** Primer C1476; **b** primer C1585; **c** primer C1795; **d** primer C7319. *M* = 100-bp DNA ladder; *1* chokecherry line C_L (Cho1); *2* chokecherry line *c* (Cho2); *3* chokecherry line *d* (Cho3)

primers produced polymorphism within three genotypes of sour cherry species (172, 69.9 %), while only 47 primers produced polymorphism between two sweet cherry genotypes.

Number of bands amplified in chokecherry and other species

The number of bands amplified by individual SSR primers varied with species and genotypes used (see Supplementary Material 2). Most primers produced more than two bands, especially in polyploid species (chokecherry and sour cherry) (Table 2). In average, each chokecherry SSR primer pair amplified 2.46 bands (excluding those that produced more than 5 bands). Chokecherry primers amplified an average of 3.33 and 2.52 bands from chokecherry DNA and sour cherry DNA, respectively. For other diploid *Prunus* species, the average number of bands amplified was 2.29 from apricot DNA, followed by wild cherry (2.22), plum (2.21), sweet cherry (2.18), and peach (1.86). For non-*Prunus* rosaceous species, the highest number of bands amplified was observed in apple (2.75), followed by crabapple (2.69), raspberry (2.26), juneberry (2.18), and pear (1.55).

Discussion

With the recent advances in DNA sequencing technology, particularly the application of NGS technology, a large amount of sequence data of plant species is being rapidly generated, providing a valuable resource for genetic research including molecular marker identification. Microsatellites (SSRs) have been discovered from NGS data for many plant species (Tangphatsornruang et al. 2009; Cavagnaro et al. 2010; Csencsics et al. 2010; Zhu et al. 2012; Zalapa et al. 2012). In this study, Roche 454 sequencing technology was applied to sequence the chokecherry (*Prunus virginiana*) genome. With a half sequencing run, a total of 145,094 reads covering 4.8 Mbp of the chokecherry genome was sequenced. Based on the published genome size of sour cherry (*Prunus cerasus*, $2n = 4x = 32$) (599 Mbp) and sweet cherry (*Prunus avium*, $2n = 2x = 16$) (338 Mbp) (http://icgr.caas.net.cn/973/%BB%F9%D2%F2%D7%E9%B4%F3%D0%A1.htm), the chokecherry ($2n = 4x = 32$) genome size can be estimated around 600 Mbp. Therefore, the obtained sequences in this study only covered 0.75 % of the chokecherry genome. Even with such a small coverage of the chokecherry genome, a total of 481 SSR loci were identified. The SSR frequency in the chokecherry genome was one per 10 kb (481 SSRs in 4.5 Mb), which is similar to that in poplar (1/4 kb) (Tuskan et al. 2004), *Arabidopsis* (1/6 kb) (Cardle et al. 2000), and sweet potato (1/7.1 kb) (Wang et al. 2011), lower than those in cucumber (1/1.8 kb) (Cavagnaro et al. 2010) and cranberry (1/2.5 kb) (Zhu et al. 2012), and much higher than that in rice (1/40 kb) (Temnykh et al. 2001), and mungbean (1/67 kb) (Tangphatsornruang et al. 2009). After assembling all 454 sequencing reads, the majority of contigs (80 %) are in the range of 100–600 nucleotides in size, which is optimal for SSR identification and primer design because the PCR products used for genotyping are usually 100–400 bp (Hayden and Sharp

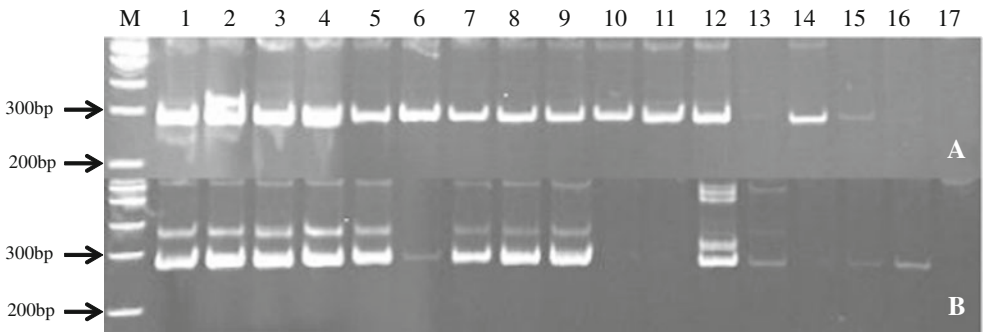

Fig. 3 A gel image showing amplification patterns from different species using primers C162 (**a**) and C1795 (**b**). *M* 100-bp DNA ladder; *1* chokecherry line C_L (Cho1); *2* chokecherry line *c* (Cho2); *3* chokecherry line *d* (Cho3); *4* sour cherry ('RS'); *5* sour cherry ('Balaton'); *6* sour cherry ('North Star'); *7* sweet cherry ('EF'); *8* sweet cherry ('Schneider'); *9* black cherry; *10* apricot; *11* peach; *12* plum; *13* apple; *14* crabapple; *15* pear; *16* raspberry; and *17* juneberry

Table 2 Performance of chokecherry SSR primers in *Prunus* and other rosaceous species

Alleles number	Cho1	Cho2	Cho3	RS	BA	NS	EF	Sch	Bch	Apr	Pch	Plu	App	Cra	Pea	Ras	Jbe
1	18	16	12	51	45	41	55	59	58	49	42	50	33	29	43	36	37
2	33	42	34	29	31	29	36	42	31	32	43	38	28	28	17	34	31
3	32	37	39	31	34	28	19	17	23	18	21	14	28	27	6	23	19
4	43	37	42	23	17	18	15	11	15	14	21	18	21	14	3	10	6
5	46	38	39	24	18	10	10	10	12	13	13	9	20	18	0	4	11
M	40	41	46	18	15	21	14	13	16	13	12	13	15	16	0	7	17
Subtotal	212	211	212	176	160	147	149	152	155	139	152	142	145	132	69	114	121
0	34	35	34	70	86	99	97	94	91	107	94	104	101	114	177	132	125
Total	246	246	246	246	246	246	246	246	246	246	246	246	246	246	246	246	246

Cho1 chokecherry line C_L, *Cho2* chokecherry line *c*, *Cho3* chokecherry *d*, *RS* sour cherry ('RS'), *BA* sour cherry ('Balaton'), *NS* sour cherry ('North Star'), *EF* sweet cherry ('EF'), *Sch* sweet cherry ('Schneider'), *Bch* black cherry, *Apr* apricot, *Pch* peach, *Plu* plum, *App* apple, *Cra* crabapple, *Pea* pear, *Ras* raspberry, *Jbe* juneberry, *M* the number of bands >5

2001). In this study, 234 of 481 contigs had satisfactory primer design sites and 246 primers were designed and validated in chokecherry. Of those, 212 (86.2 %) effectively amplified DNA from chokecherry, yielding more than 1,000 alleles that have potential to be used for genotyping populations. The efficiency of usable SSR identification (percentage of amplifiable SSRs in all obtained contigs) from 454 sequences of the chokecherry genome is 1.82 % (212/11,675).

It is not surprising that dinucleotide repeats were the most frequent motif type (85.9 %) followed by trinucleotide, tetranucleotide, and pentanucleotide repeat types in chokecherry genome SSRs (Table 1), as is often observed in other plant species (Tangphatsornruang et al. 2009; Yonemaru et al. 2009; Cavagnaro et al. 2010; Zhu et al. 2012). There is a significant difference in the relative abundance of a specific repeat motif between chokecherry and other species. In the chokecherry genome, CT/GA (24.3 %) was the most abundant SSR followed by AT/TA (22.5 %) and AG/TC (21.8 %), while CG/GC was the least frequent dinucleotide (Table 1). The other motif repeats appeared to be evenly distributed with a frequency lower than 1 % except CTC/GAG, CCA/GGT, AAC/TTG, AAG/TTC, and CTT/GAA in trinucleotides (Table 1). The motif repeat AG was the most frequent SSR motif (35 %) in cranberry (Zhu et al. 2012), while in poplar, sorghum, and mungbean sequences, AT-rich motifs accounted for the largest proportions at 31.4, 26.1, and 89.3 %, respectively (Tuskan et al. 2004; Tangphatsornruang et al. 2009; Yonemaru et al. 2009). However, the proportion of GC-rich motifs in this study was the smallest (2.91 %), which is in agreement with the results of genomic-SSR from rubber tree (Yu et al. 2011), cranberry (Zhu et al. 2012), and mungbean (Tangphatsornruang et al. 2009). Differences in SSR abundance in different studies from different species are often seen. However, some motifs, such as AT/TA and AG/TC, often showed a greater abundance in most species (Morgante et al. 2002; Tuskan et al. 2004; Song et al. 2010). Previous research suggested that SSRs with a greater number of motif repeats tend to be more polymorphic (Temnykh et al. 2001; Song et al. 2010); therefore, information on repeat motifs will help select candidate SSRs with a high potential of polymorphism, particularly from a large database with a large number of SSRs.

Interspecific and intergeneric transferability of SSRs make them useful for genetic research, such as fingerprinting and genetic map construction. Transferability of SSRs from one species to other species or genera has been reported in many plant groups, including cereals (Tang et al. 2006; Sim et al. 2009; Castillo et al. 2010; Ince et al. 2010), and woody species (Gasic et al. 2009; Park et al. 2010; Yu et al. 2011). Recent research showed that *Prunus* SSRs were transferable within *Prunus* or across species in the Rosaceae family (Mnejja et al. 2010). The rate of SSR transferability in different species is related to the genetic distance between the species from which the SSRs developed and other species. In this study, six *Prunus* species that belong to three subgenera: *Cerasus* (sour cherry, sweet cherry, and wild cherry), *Amygdalus* (peach), and *Prunophora* (apricot and Japanese plum), were compared for the transferability of chokecherry SSRs in these species. A similar transferability rate was observed within the same group. For example, an average of 65.4, 63.0, and 62.4 % of chokecherry SSR primers amplified bands from sour cherry, black cherry, and sweet cherry, respectively, while 56.5 and 57.7 % of chokecherry primers amplified bands from apricot and plum. Such a correlation between the genetic distance and SSR transferability rate was also found in other research studies. Mnejja et al. (2010) reported that a 100 % amplification rate was observed between peach and almond genomic SSRs, apricot and

Japanese plum or almond EST-SSRs, and European plum and Japanese plum genomic SSRs. They also found that peach DNA showed the highest amplification rate (91.6 %) and cherry DNA had the lowest (76.6 %) when the *Prunus* SSRs were used. A similar relationship is evident in this study where 61.8 % of chokecherry primers amplified in peach DNA, higher than in apricot (56.5 %) and plum (57.7 %). Relatively high percentages of amplification and polymorphism of SSRs were also observed in a separate research (data not shown), where 93 of 108 SSR primers (86.1 %) adopted from other *Prunus* species were transferable to chokecherry and 73 primers (67.6 %) showed polymorphisms in our populations. Further research confirmed that 70 of 234 chokecherry SSR sequences are homologous to the peach sequences in the NCBI database (Altschul et al. 1990). The results also showed that chokecherry SSR primers produced a high amplification rate (58.9 %) of apple DNA, which is supported by previous research that found a high degree of sequence similarity between *Prunus* and *Malus* (Gasic et al. 2009).

In this study, transferable chokecherry SSR primers were also tested for their ability to identify polymorphism in both sour and sweet cherry species. The result showed that 172 of 212 amplifiable chokecherry SSRs (81.1 %) were polymorphic in sour cherry species, while only 22.2 % (47 of 212 SSRs) were polymorphic in sweet cherry. The low polymorphism level in sweet cherry may be caused by the difference in ploidy level between chokecherry (4×) and sweet cherry (2×) and/or the low number of genotypes included in this research.

Although polymorphism of the chokecherry SSRs within species except cherries was not determined, variations of the amplification pattern were observed between chokecherry and other species. When using chokecherry primers that produced bands in chokecherry to amplify DNA of three other *Prunus* species, 66.5, 60.4, and 59.0 % of the amplification patterns in peach, plum, and apricot, respectively, were different from the ones in chokecherry. High variation in amplification pattern was also determined in other rosaceous species (63.7 % in apple, 60.8 % in crabapple, 54.7 % in juneberry, 51.9 % in raspberry, and 31.6 % in pear). Our result appears to not be consistent with the research of Gasic et al. (2009) in which only a few primer pairs of apple EST-SSRs amplified additional bands in other rosaceous species including pear, strawberry, rose, apricot, plum, almond, peach, sweet cherry, and sour cherry. It has been observed that the flanking regions of EST-SSRs derived from coding regions are more conserved (Holton et al. 2002; Wen et al. 2010), which could contribute to the lower variation of the amplification pattern across species or genera. In this study, SSRs developed from chokecherry genomic sequences are predominately dinucleic repeats. Luro et al. (2008) found that dinucleic repeats had a greater number of alleles per locus than trinucleic repeats. The higher number of alleles per locus for dinucleic SSRs and the tetraploid nature of chokecherry could be closely related to the high variation of the amplification pattern between chokecherry and other rosaceous species (Table 2, Supplementary Material 2).

In conclusion, a total of 246 SSR primers were identified from chokecherry genome sequences. Of which, 212 were confirmed amplifiable both in chokecherry and other 11 other rosaceous species. The high transferability rate of chokecherry SSRs to other rosaceous species will be particularly useful for the species from which genetic information is not available. This research demonstrated that the development of SSRs using random genome sequence data generated from Roche 454 sequencing was rapid and cost-efficient. These identified chokecherry SSRs will be useful for genetic mapping and quantitative trait loci analysis of important agronomic traits in chokecherry. Sequence information of the SSRs will also be a valuable resource for the research on molecular genetics and comparative genomic studies in *Prunus* and other rosaceous species.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep 27:617–631

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Aranzana MJ, Mas JG, Carbo J, Arús P (2002) Development and variability of microsatellite markers in peach. Plant Breed 121:87–92

Canli FA (2004) Development of a second generation genetic linkage map for sour cherry using SSR markers. Pak J Biol Sci 7:1676–1683

Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D, Waugh R (2000) Computational and experimental characterization of physically clustered simple sequence repeats in plants. Genetics 156:847–854

Castillo A, Budak H, Martin AC, Dorado G, Borner A, Roder M, Hernandez P (2010) Interspecies and intergenus transferability of barley and wheat D-genome microsatellite markers. Ann Appl Biol 156:347–356

Cavagnaro PF, Senalik DA, Yang L, Simon PW, Harkins TT, Kodria CD, Huang S (2010) Genome-wide characterization of simple sequence repeats in cucumber (*Cucumis sativus* L.). BMC Genomics 11:569–586

Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach

(*Prunus persica* (L.) Batsch): isolation, characterization and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

Csencsics D, Brodeck S, Holderegger R (2010) Cost-effective, species-specific microsatellite development for the endangered dwarf bulrush (*Typha minima*) using next-generation sequencing technology. J Hered 101:789–793

Decroocq V, Fave MG, Hagen L, Bordenave L, Decroocq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106: 912–922

Gasic K, Han Y, Kerbundit S, Shulaev V, Iezzoni AF, Stover EW, Bell RL, Wisniewski ME (2009) Characteristics and transferability of new apple EST-derived SSRs to other Rosaceae species. Mol Breed 23:397–411

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol Genet Genomics 270:315–323

Hagen LS, Chaib J, Fad B, Decrocq V, Bouchet P, Lambert P, Audergon JM (2004) Genomic and cDNA microsatellite from apricot (*Prunus armeniaca* L.). Mol Ecol Notes 4:432–434

Hayden MJ, Sharp PJ (2001) Sequence-tagged microsatellite profiling (STMP): a rapid technique for developing SSR markers. Nucleic Acids Res 29:e43

Holton TA, Christopher JT, McClure L, Harker N, Henry RJ (2002) Identification and mapping of polymorphic SSR markers from expressed gene sequences of barley and wheat. Mol Breed 9:63–71

Ince AG, Karaca M, Onus AN (2010) Polymorphic microsatellite markers transferable across *Capsicum* species. Plant Mol Biol Rep 28:285–291

Jun TH, Michel AP, Mian MAR (2011) Development of soybean aphid genomic SSR markers using next generation sequencing. Genome 54:360–367

Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK (2011) Microsatellite markers: an overview of the recent progress in plants. Euphytica 177:309–334

Lee S, Wen J (2001) A phylogenetic analysis of *Prunus* and the Amygdaloideae (Rosaceae) using ITS sequences of nuclear ribosomal DNA. Am J Bot 88:150–160

Lewers KS, Styan SMN, Hokanson SC (2005) Strawberry Gen-Bank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. J Am Soc Hort Sci 130:102–115

Lodhi MA, Ye GN, Weeden NF, Reisch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. Plant MolBiol Rep 12:6–13

Luro FL, Constantino G, Terol J, Argout X, Allario T, Wincker P, Talon M, Ollitrault P, Morillon R (2008) Transferability of the ESR-SSRs developed on Nules clementine (*Citrus clementine* Hort ex Tan) to other *Citrus* species and their effectiveness for genetic mapping. BMC Genomics 9:287

Mardis ER (2008) The impact of next-generation sequencing technology on genetics. Trends Genet 24:133–141

Mnejja M, Garcia-Mas J, Howad W, Arús P (2005) Development and transportability across *Prunus* species of 42 polymorphic almond microsatellites. Mol Ecol Notes 5:531–535

Mnejja M, Garcia-Mas J, Audergon JM, Arús P (2010) *Prunus* microsatellite marker transferability across rosaceous crops. Tree Gen Genomes 6:689–700

Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nat Genet 30:194–200

Olmstead JW, Sebolt AM, Cabrera A, Sooriyapathirana SS, Hammar S, Iriarte G, Wang D, Chen CY, van der Knaap E, Iezzoni AF (2008) Construction of an intra-specific sweet cherry (*Prunus avium* L.) genetic linkage map and syteny analysis with the *Prunus* reference map. Tree Genet Genomes 4:897–910

Parida SK, Kalia SK, Sunita K, Dalal V, Hemaprabha G, Selvi A, Pandit A, Singh A, Gaikwad K, Sharma TR, Srivastava PS, Singh NK, Mohapatra T (2009) Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. Theor Appl Genet 118:327–338

Park YH, Ahn SG, Choi YM, Oh HJ, Ahn DC, Kim JG, Kang JS, Choi YW, Jeong BR (2010) Rose (*Rosa hybrid* L.) EST-derived microsatellite markers and their transferability to strawberry (*Fragaria* spp.). Sci Hort 125:733–739

Qi X, Cui F, Yu L, Ding A, Li J, Chen G, Wang H (2010) Molecular tagging wheat powdery mildew resistance gene Pm21 by EST-SSR and STS markers. Molecular Plant Breeding 1(4). doi: 10.5376/mpb.2010.01.0004

Rehder A (1940) Manual of cultivated trees and shrubs, 2nd edn. McMillan, New York, pp 452–481

Sim SC, Yu JK, Jo YK, Sorrells ME, Jung G (2009) Transferability of cereal EST-SSR markers to ryegrass. Genome 52:431–437

Singh RK, Singh RB, Singh SP, Sharma ML (2011) Identification of sugarcane microsatellites associated to sugar content in sugarcane and transferability to other cereal genomes. Euphytica 182:335–354

Song Q, Jia G, Zhu Y, Grant D, Nelson RT, Hwang EY, Hyten DL, Cregan PB (2010) Abundance of SSR motifs and development of candidate polymorphic SSR makers (BARCSOYSSR_1.0) in soybean. Crop Sci 50:1950–1957

Tang J, Gao Y, Jia J (2006) Homologous analysis of SSR- ESTs and transferability of wheat SSR-EST markers across barley, rice, and maize. Euphytica 151:87–93

Tangphatsornruang S, Somta P, Uthaipaisanwong P, Chanprasert J, Sangsrakru D, Seehalk W, Sommanas W, Tragoonrung S, Srinives P (2009) Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). BMC Plant Biol 9:137–148

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations and genetic marker potential. Genome Res 11:1441–1452

Tuskan GA, Gunter LE, Yang ZK, Yin T, Sewell MM, DiFazio SP (2004) Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa*. Can J For Res 34:85–93

Wang Z, Li J, Luo Z, Huang L, Chen X, Fang B, Li Y, Chen J, Zhang X (2011) Characterization and development of EST-derived SSR markers in cultivated sweet potato (*Ipomoea batatas*). BMC Plant Biol 11:139

Wen M, Wang H, Xia Z, Zou M, Lu C, Wang W (2010) Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha curcas* L. BMC Res Notes 3:42

Wünsch A (2009) Cross-transferable polymorphic SSR loci in *Prunus* species. Sci Hort 120:348–352

Yamamoto T, Kimura T, Shoda M, Ban Y, Hayashi T, Matsuta N (2002) Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). MolEcol Notes 2:14–16

Yonemaru J, Ando T, Mizubayashi T, Kasugo S, Matsumoto T, Yano M (2009) Development of genome-wide simple sequence repeat markers using whole-genome shotgun sequences of sorghum (*Sorghum bicolor* (L.) Moench). DNA Res 16:187–193

Yu F, Wang BH, Feng SP, Wang JY, Li WG, Wu YT (2011) Development, characterization, and cross-species/genera transferability of SSR markers for rubber tree (*Hevea brasiliensis*). Plant Cell Rep 30:335–344

Zalapa JE, Cuevas H, Zhu H, Steffan S, Senalik D, Zeldin E, McCown B, Harbut R, Simon P (2012) Using next-generation

sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant science. Am J Bot 99:193–208

Zhu H, Senalik D, McCown BH, Zeldin EL, Speers J, Hyman J, Bassil N, Hummer K, Simon PW, Zalapa JE (2012) Mining and validation of pyrosequenced simple sequence repeats (SSRs) from American cranberry (*Vaccinium macrocarpon* Ait.). Theor Appl Genet 124:87–96