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Genetic Relationship of Sweet Cherry (*Prunus avium* L.) Based on SSR Markers

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Abstract: Eleven cultivars of sweet cherry (*Prunus avium* L.), planted in Sichuan of China, were analyzed to assess the genetic relationship among them with SSR markers. Among 15 pairs of SSR primers, the result shows 10 of them could be amplified 3-7 alleles with an average of 5.2. Locus heterozygosity was 0.6584-0.8413 and the genetic diversity index was 0.7695. The genetic similarity coefficient among the 11 cultivars ranges from 0.5625-0.8636 with an average of 0.7339. Furthermore, according to the results of UPGMA cluster, the 11 sweet cherry cultivars can be divided into 3 groups. For the unknown parents of the sweet cherries, the Cv Summit is possibly the filial generation of Governor wood as female parent and Napoleon as male parent, the Black Tartarian might be related to Napoleon and the Mogami Nishiki might be related to Bing or Xiang Jiao. The direct cross and reciprocal cross will get different crossing progeny in sweet cherry. Thus, the SSR markers are the effective methods for the identification of sweet cherry parents especially, the female parent.

Key words: *Prunus avium* L., cultivar, genetic relationship, SSR, sweet cherry, China

INTRODUCTION

Sweet cherry originated in Europe, also known as the European sweet cherry (*Prunus avium* L.). It has been introduced to China for >100 years, at present the cultivated varieties basically fall into this type (Wang *et al*., 2005). As the feature fruits, in recent 10 years, in high altitude mountain areas from the North to Southwest China, it rapids develops as economic tree species of returning farming land to forestry and the ecological restoration and reconstruction and has gained high economic benefits. But also, some problems appeared, such as pollenizer is not or unreasonably disposed, resulting in the trees of 8 or 9 years old has flowered but not fruited; meanwhile varieties are too confused and some cultivars has unclear genetic relationship. It has serious impact on intensive management and breeding works. Thus, the study of the genetic relationship between cultivars is particularly necessary. In recent years, SSR marker with its high degree of polymorphism, reproducible, codominant, has been widely applied to the identification of genetic relationship among a variety of fruit trees (Struss *et al*., 2003; Rohrer *et al*., 2004; Sanchez-Perez *et al*., 2005; Bouhadida *et al*., 2007). Thus, we used SSR method to analyze 11 sweet cherry cultivars relationship mainly planted in Sichuan, so as to provide more information of species identification, matching hybrid combinations, disposing pollinated trees and genetic breeding.

MATERIALS AND METHODS

Plant materials and DNA extraction: Eleven sweet cherry varieties, obtained from the Maoxian sweet cherry production base in Aba Autonomous Prefecture of Sichuan (Table 1). Young leaves were collected from a single tree for each genotype, immediately frozen in liquid nitrogen and stored at -70°C.

Total genomic DNA was extracted by using CTAB method following Lv (2005) and we made some modification, took 0.5 g young leaves, add 4 mL CTAB extraction buffer, eventually resolve the DNA in 500 μL ddH_2O, stored at -20°C.

PCR amplification and electrophoresis: Fifteen pairs of SSR primers from sweet cherry (Struss *et al*., 2003; Clarke and Tobutt, 2003) and peach (Dirlewanger *et al*., 2002; Testolin *et al*., 2000), were synthesized by the Shanghai Biological Engineering Company.

The optimization of SSR reaction system was determined: Total 25 μL reaction system includes 50 ng template DNA 2 μL, 10× Buffer 2.5 μL, 2 5 mmol L^{-1} $MgCl_2$ 1.5 μL, 2.5 mmol L^{-1} dNTP 2.0 μL, 5 μmol L^{-1} SSR

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primer (forward primer and reverse primer) each 0.3 μL, 1.5 U Taq DNA polymerase 0.3 μL, ddH_2O 16.1 μL.

The PCR amplification program was devised: Pre-denaturation at 94°C for 3 min; denaturing at 94°C for 30 sec, annealing at 45°C for 30 sec, extension at 72°C for 30 sec, 30 cycles; at last extension at 72°C for 5 min. PCR products were separated by the electrophoresis on a 3% high-resolution agarose gel in 1× TBE buffer at 180 constant volts 1 h and visualized by staining with ethidium bromide under UV source and photographed using Bio-Rad Gel Doc 1000.

Data analysis: SSR fragments for each primer pair were scored as present 1 or absent 0, building database. Genetic similarity is based on Jaccard's coefficient.

$$GS_{(jk)} = a/a+b+c$$

a = The number of polymorphic bands present in both individuals j and k
b = The bands present in j but absent in k
c = The number o f bands present in k but absent in j

Gene heterozygosity is based on Nei's (1983) formula:

$$h = 1-\Sigma f_i^2$$

h = Heterozygosity
f_i = The i allele frequency

The average gene heterozygosity is based on the formula:

$$H = \Sigma h_i/r$$

r = The number of amplified locus

Clustering analysis was carried out according to Unweighted Pair Group Method Arithmetic averages (UPGMA) by using NTSYS-PC 2.1.

Table 1: Materials used in this study

Cultivar	Origin	Parents
Hong Deng	China	Napoleon x Governor wood
Ju Hong	China	Napoleon x Governor wood
Jia Hong	China	Bing x Xiang Jiao
Sato Nishiki	Japan	Governor Wood x Napoleon
Mogami Nishiki	Japan	Unknown
Nanyo	Japan	Natural crossing of Napoleon
CvSummit	Canada	Unknown
13-33	China	Napoleon x Governor Wood
Black Tartarian	Russia	Unknown
Van	Canada	Seedling of Empress Eugenie
Lapins	Canada	Van x Stella

RESULTS

Primer screening and SSR polymorphism: Use the screened ten primer pairs in preliminary experiment to amplify the eleven sweet cherry materials, the results are shown in the Fig. 1. Table 1 and 2, producing 52 fragments with an average of 5.2 alleles per locus. The number of alleles per locus ranged from 3-7. Locus heterozygosity was 0.6584-0.8413 and the genetic diversity index was 0.7695. The BPPCT026 had more polymorphism bands (alleles) and high heterozy-gosity, allele number was 7, heterozygosity was 0.8413.

Genetic similarity: GS values among 11 genotypes ranged from 0.5625-0.8636 (Table 3), with an average of 0.7339. No. 2 (Ju Hong) and No. 8 (13-33) had the highest value of GS (GS = 0.8636). The lowest genetic similarity is shown between genotype 9 (Black Tartarian) and 10 (Van) with GS value of 0.5625. The results show that the nearest genetic relationship was Ju Hong and 13-33 and the farthest was Black Tartarian and Van among these materials. Through, their parents, we can discern Ju Hong and 13-33 were bred by the Dalian Academy of Agricultural Sciences by using Napoleon and Governor Wood, whose genetic relationship is close. Moreover, Black Tartarian is an ancient species originated from Russia and its parents were unclear; Van was bred in Canada and selected from progeny of natural seedling, their genetic relationship was far. It indicated with the same parents and geographical origin, their genetic relationship was close.

Cluster analysis: Dendrogram is constructed according to SSR data (Fig. 2), 11 samples could be clustered into 3 groups based on genetic similarity coefficient (GS = 0.7317).

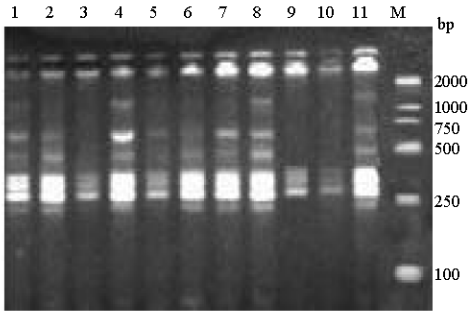

Fig. 1: Profile of amplification products from genomic DNAs of 11 sweet cherry using SSR primer BPPCT026

Table 2: Amplifed results of 11 sweet cherry materials with 10 SSR primers

Primers	Primer sequence (5'-3')	Repeat motif	References	No. of alleles	Locus heterozygosity
UCD-CH12	F: AGACAAAGGGATTGTGGGC				
	R: TTTCTGCCACAAACCTAATGG	(CA) 14	Struss *et al.* (2003)	6	0.7556
UCD-CH18	F: ATGGAAGGCCAAGGCAAC				
	R: AATGTTCCCGGTTATATGC	(CT) 23	Struss *et al.* (2003)	5	0.7676
BPPCT005	F: GCTAGCAGGGCACTTGATC				
	R: ACGCGTGTACGGTGGAT	(AG) 10	Dirlewanger *et al.* (2002)	6	0.8100
BPPCT026	F: ATACCTTTGCCACTTGCG				
	R: TGAGTTGGAAGAAAACGTAACA	(AG) 8 GG (AG) 6	Dirlewanger *et al.* (2002)	7	0.8413
BPPCT034	F: CTACCTGAAATAAGCAGAGCCAT				
	R: CAATGGAGAATGGGGTGC	(GA) 19	Dirlewanger *et al.* (2002)	5	0.8000
UDP98-022	F: CTAGTTGTGCACACTCACGC				
	R: GTCGCAGGAACAGTAAGCCT	(TG) 12 (AG) 24	Testolin *et al.* (2000)	3	0.6584
UDP97-402	F:TCCCATAACCAAAAAAAACACC				
	R:TGGAGAAGGGTGGGTACTTG	(AG) 17	Testolin *et al.* (2000)	6	0.8047
UDP97-403	F: CTGGCTTACAACTCGCAAGC				
	R: CGTCGACCAACTGAGACTCA	(AG) 22	Testolin *et al.* (2000)	5	0.7755
EMPA014	F: ATTTGCCTATTGGGTTCCTG				
	R: TGAATGATCACAGAACATCCAG	(AAC) 7GACG (AC) 8 (AG) 7	Clarke and Tobutt (2003)	4	0.7425
EMPA018	F: TCCAAGAACAAAGCCAAAATC				
	R: AATTTCAATGCATTCTGGATAG	(GA) 18	Clarke and Tobutt (2003)	5	0.7396
Average				5.2	0.7695

F = Forward primer; R= Reverse primer

Table 3: Genetic similarity among 11 cultivated sweet cherry

1	2	3	4	5	6	7	8	9	10	11
1.0000										
0.8158	1.0000									
0.5946	0.7692	1.0000								
0.7805	0.7209	0.5854	1.0000							
0.6944	0.7750	0.7429	0.6757	1.0000						
0.7500	0.8000	0.7381	0.7857	0.7317	1.0000					
0.7805	0.8043	0.6818	0.8571	0.7805	0.8298	1.0000				
0.7561	0.8636	0.7381	0.8333	0.7805	0.8085	0.8511	1.0000			
0.6190	0.7174	0.7317	0.6591	0.7500	0.8222	0.7500	0.6939	1.0000		
0.7568	0.7209	0.6667	0.6667	0.6667	0.6739	0.6458	0.6957	0.5625	1.0000	
0.7179	0.7907	0.7000	0.7143	0.7000	0.7391	0.7083	0.8000	0.5918	0.7805	1.0000

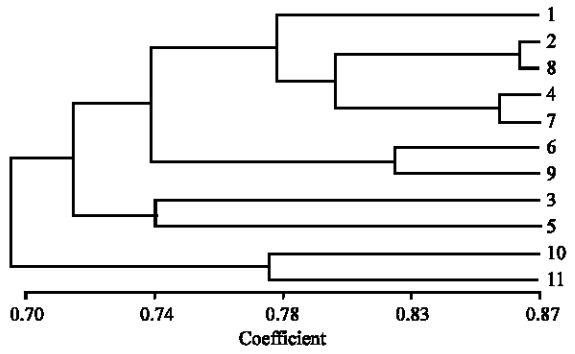

Fig. 2: Dendrogram showing the genetic relations among 11 cultivated sweet cherry genotypes

Group 1 consisting of 7 cultivars could be divided into 3 subgroups (GS = 0.7950), Hong Deng as the 1st subgroup; the 2nd subgroup included Ju Hong, 13-33, Sato Nishiki and Cv Summit; the 3rd subgroup consisted of Nanyo and Black Tartarian. In Group 1, Hong Deng, Ju Hong, Sato Nishiki, Nanyo and 13-33 were progeny of Napoleon, which shows Group 1 is associated with Napoleon. In the 2nd subgroup, the nearest genetic relationship was Ju Hong and 13-33, they were both hybrid progeny of Napoleon as female parent and Governor Wood as male parent. But Sato Nishiki (Governor Wood x Napoleon) and Cv Summit (parents unknown) clustered together, so the Cv Summit may be hybrid progeny of Governor Wood as female parent and Napoleon as male parent.

In addition, Hong Deng (Napoleon x Governor Wood) and Sato Nishiki (Governor Wood x Napoleon) were clustered in different subgroups, which shows direct cross and reciprocal cross will get different progeny of crossing between Napoleon and Governor Wood, the sweet cherry's direct cross and reciprocal cross will have a bigger genetic difference.

In the 3rd subgroup, Nanyo and Black Tartarian clustered together and have greater genetic similarity coefficient. The Nanyo is natural crossing of Napoleon, so Black Tartarian is related to Napoleon.

Group 2 included Jia Hong and Mogami Nishiki. Jia Hong is progeny of Bing as female parent and Xiang Jiao as male parent, their genetic similarity coefficient was 0.7492, so, the Mogami Nishiki was related to Bing or Xiang Jiao.

Group 3 consisted of Van and Lapins. Lapins was bred from Van x Stella by Canada Department of Agriculture Research Station in Summerland, Van and Lapins were grouped together. Thus, sweet cherry more inherited genes from the female parent.

DISCUSSION

The genetic relationship analysis is the basic research of origination and evolution, discovering new gene resources, improving current breeding materials. Obtaining relationship of crop genetic have many methods, such as morphological marker (Kang *et al*., 1997), biochemical marker (Beaver *et al*., 1995) and conventional cytogenetic methods. But the morphological marker is few and biochemical marker is also limited, special genetic materials difficultly are collected, as well as the cytology is a heavy workload, which limited the progress of genetic theory and application research (Zhou *et al*., 2001). However, the molecular marker has large information, high efficiency, no environmental restriction and influence, which generally covers the whole genome and can well reveals the materials genetic relationship. So, in resent years, using molecular marker studies has gradually increased to identify sweet cherry's cultivars (Wünsch and Hormaza, 2002; Struss *et al*., 2003; Kacar *et al*., 2005; Cai *et al*., 2006), to analyze genetic diversity (Wang *et al*., 2005; Ai *et al*., 2007) and genetic relationship (Cai *et al*., 2006; Chen *et al*., 2004). RAPD is mainly used in the genetic relationship study of sweet cherry (Wang *et al*., 2005; Cai *et al*., 2006; Chen *et al*., 2004) and SSR is relatively less.

On the relationship of the Hong Deng, Sato Nishiki, Van and Lapins. Cai *et al*. (2006) use the RAPD markers, take the Hong Deng and Sato Nishiki together, also the Lapins and Van. This result was consistent with our study; but different from Wang *et al*. (2005). Although, the RAPD method is simple and convenient, but its main problem is the amplified bands instability, repeatability and reliability obviously insufficient (Zhou *et al*., 2002).

Recently, Ai *et al*. (2007) used SSR markers to analyze Bohai Bay cherry varieties genetic diversity and genetic relationship, took the Van and Lapins in a subgroup, which also confirms our results, but the result of Hong Deng and Sato Nishiki is somewhat different from ours, they didn't cluster together according to their study. The cause may be collecting materials in different locations, one in Dalian of Liaoning, which has warm temperate sub-humid monsoon maritime climate, another in Maoxian of Sichuan, which mainly has warm temperate continental monsoon plateau climate (1900 m above sea level), different ecological conditions cause certain varieties to genetically mutate, so that clustering results have a certain difference.

This study shows to a certain extent the characteristics of species geographic origin by using SSR markers, which in many reports has been reflected (Kacar *et al*., 2005; Ai *et al*., 2007); but there were certain differences. Perhaps, the introduced species adapted to the local natural conditions and the hereditary material changed in species. Maoxian is located in Aba Autonomous prefecture, it is the arid valley of upper reaches of Min-jiang River area and has a unique climatic conditions, with an average annual temperature 12.1°C, annual rainfall is 480 mm. According to the survey, the quality of sweet cherry planted there is better than other sweet cherry-producing areas in China. Different habitats cause changes of the genetic material, which has to be studied further.

CONCLUSION

Using SSR markers to analyze genetic relationship of sweet cherry varieties in Maoxian, Sichuan, showed that among 15 pairs of SSR primers, 10 of them could be amplified 3-7 alleles with an average of 5.2. Locus heterozygosity was 0.6584-0.8413 and the genetic diversity index was 0.7695. The genetic similarity coefficient among the 11 cultivars ranges from 0.5625-0.8636 with an average of 0.7339. Furthermore, according to the results of UPGMA cluster the 11 sweet cherry cultivars were divided into 3 groups. Group 1 consisted of 7 cultivars, that were Hong Deng, Ju Hong, 13-33, Sato Nishiki, Cv Summit, Nanyo, Black Tartarian; the group 2 included Jia Hong and Mogami Nishiki; the group 3 consisted of Van and Lapins. The Cv Summit with unclear parents may be hybrid progeny of female parent Governor Wood and male parent Napoleon; the Black Tartarian may be related to the Napoleon; the Mogami Nishiki may be related to the Bing or Xiang Jiao. Positive cross and reciprocal cross will get different progeny. SSR is an effective method, which could be used to distinguish sweet cherry varieties parents, especially the female parent. The result was related to the parents and geographical distribution, which could reflect the genetic feature and regional characteristics of sweet cherry and has practical significance for selecting new variety and deploying pollination trees.

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REFERENCES

Ai, C.X., L. Xin, X.M. Yu, L.S. Zhang, H.R. Wei, K.J. Yuan, Q.R. Sun and Q.Z. Liu, 2007. Analysis of genetic diversity in Cerasus by SSR markers. Acta Horticult. Sinica, 34 (4): 871-876 (in Chinese). DOI: CNKI:SUN:YYXB.0.2007-04-012.

Beaver, J.A., A.F. Iezzoni and C.W. Ramm, 1995. Isozyme diversity in sour, sweet and ground cherry. TAG Theoretical. Applied Genet., 90 (6): 847-852. DOI: 10.1007/BF00222021.

Bouhadida, M., A.M. Casas, M.A. Moreno and Y. Gogorcena, 2007. Molecular characterization of Miraflores peach variety and relatives using SSRs. Scientia Horticult., 111: 140-145. DOI: 10.1016/j.scienta.2006.10.018.

Cai, Y.L., S. Li, D.W. Cao, Z.Q. Qian, G.F. Zhao and M.Y. Han, 2006. Use of amplified DNA sequences for the genetic analysis of the cherry germplasm. Acta Horticult. Sinica, 33 (2): 249-254 (in Chinese). DOI: cnki:ISSN:0513-353X.0.2006-02-005.

Chen, X.L., X.S. Chen, H.R. Shu and H. Xu, 2004. RAPD analysis of 15 cherry cultivars. J. Fruit Sci., 21 (6): 556-559 (in Chinese). DOI: cnki:ISSN:1001-7364.0.2004-06-011.

Clarke, J.B. and K.R. Tobutt, 2003. Development and characterization of polymorphic microsatellites from *Prunus avium* Napoleon. Molecul. Ecol. Notes, 3: 578-580. DOI: 10.1046/j.1471-8286.2003.00517.x.

Dirlewanger, E. P. Cosson, M. Tavaud, M.J. Aranzana, C. Poizat, A. Zanetto, P. Arús and F. Laigret, 2002. Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet., 105: 127-138. DOI: 10.1007/s00122-002-0867-7.

Kacar, Y.A., A. Iezzoni and S. Cetiner, 2005. Sweet cherry cultivar identification by using SSR markers. J. Biol. Sci., 5 (5): 616-619. DOI: 10.3923/jbs.2005.616.619.

Kang, S.H., M.Z. Bao, L.Q. Chen, Y.W. Huang and X.X. Liu, 1997. Classification of *Prunus mume* cultivars by pollen morphology. Acta Horticult. Sinica, 24 (2): 170-174 (in Chinese). DOI: cnki:ISSN: 0513- 353X.0.1997-02-014.

Nei, M., 1983. Molecular Population Genetics and Evolution. Translated by Wang, J.Y., Beijing: Agricultural Press, pp: 79-82 (in Chinese).

Rohrer, J.R., R. Ahmad, S.M. Southwick and D. Potter, 2004. Microsatellite analysis of relationships among North American plums (*Prunus sect. Prunocerasus*, Rosaceae). Plant Syst. Evol., 224: 69-75. DOI: 10.1007/s00606-003-0088-9.

Sánchez-Pérez, R., D. Ruiz, F. Dicenta, J. Egea and P. Martinez-Gomez, 2005. Application of Simple Sequence Repeat (SSR) markers in apricot breeding: molecular characterization, protection and genetic relationships. Scientia Horticult., 103: 305-315. DOI: 10.1016/j.scienta.2004.06.009.

Struss, D., R. Ahmad, S.M. Southwick and M. Boritzki, 2003. Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. J. Am. Soc. Horticult. Sci., 128 (6): 904-909. DOI: INIST:717, 35400011864957.0160.

Testolin, R., T. Marrazzo, G. Cipriani, R. Quarta, I. Verde, M.T. Dettori, M. Pancaldi and S. Sansavini, 2000. Microsatellite DNA in peach (*Prunus persica* L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. Genome, 43: 512-520. DOI: 10.1139/gen-43-3-512.

Xiu-Lan, L. 2005. Studies on biochemistry and molecular biology of genetic diversity of grape cultivar and resistant appraisal of downy mildew (D). Ya'an: Sichuan Agricultural University (in Chinese). DOI: CNKI:CDMD:1.2005.140782.

Wang, C.H., Y.K. Tian, J. Zhao, H.Y. Dai and D. Wang, 2005. RAPD Analysis of the genetic differences among several cherry varieties. Acta Botanica Boreali-Occidentalia Sinica, 25 (12): 2431-2435 (inChinese). DOI: cnki:ISSN:1000-4025.0.2005-12-011.

Wünsch, A. and J.I. Hormaza, 2002. Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach (*Prunus persica* (L.) Batsch) SSR sequences. Hered, 89 (1): 56-63. DOI: 10.1038/sj.hdy.6800101.

Zhou, Y.P., S. Ge and X.D. Wang, 2001. The molecular markers in systemic and evolution of plant. Beijing: Science Press, pp: 150-160 (in Chinese). ISBN: 7-03-008711-9/Q949.

Zhou, X.F., B.C. Zhuang, Y.M. Wang and H.K. Zhao, 2002. Population differentiation of wild soybean based on the RAPD and SSR analysis. Chinese J. Econ. Agric., 10 (4): 6-9 (in Chinese). DOI: cnki:ISSN: 1671-3990.0.2002-04-001.