Molecular Characterization of Kiwifruit (*Actinidia*) Cultivars and Selections Using SSR Markers

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ABSTRACT. Forty-eight kiwifruit cultivars and selections, representing more than 90% of total world kiwifruit production, were investigated using nine SSR markers to establish genetic identities, and evaluate genetic diversity and relatedness. These nine SSRs were polymorphic and a total of 213 alleles were detected, resulting in a mean number of 23.7 alleles per locus, ranging from nine to 38 alleles. One hundred and thirty-three alleles were found to be common to both *A. chinensis* and *A. deliciosa*, while 33 and 36 were specific to *A. chinensis* and *A. deliciosa*, respectively. In addition, 34 alleles were specific to one single genotype and provided a set of valuable alleles for cultivar identification. A single SSR locus UDK 96-414 could differentiate all 48 genotypes except two presumable clones. Mean number of alleles per locus (A), percentage of polymorphic loci (P), and direct count heterozygosity (H_o) assessed for each genotype over all loci revealed considerable differences among these 48 genotypes. On average, $A = 2.6$, $P = 89.4\%$ and $H_o = 0.546$ were found in *A. chinensis* cultivars, while $A = 3.5$, $P = 97.0\%$ and $H_o = 0.671$ in *A. deliciosa* cultivars. Consensus fingerprint profiling using SSR markers is a useful and reliable method for establishing genetic identities of kiwifruit cultivars and selections. It also improves evaluation effectiveness of genetic diversity and relatedness compared to RAPD markers.

Commercial kiwifruit production is based on two *Actinidia* species, *A. deliciosa* C.F. Liang et A.R. Ferguson and *A. chinensis* Planch. The genus *Actinidia* Lindl. comprises 66 species and about 118 taxa with a remarkably wide natural range extending from the tropics (latitude 0°) to cold temperate regions (50°N). All these species occur naturally in China except four species that are native to neighboring countries: *A. strigosa* Hook & Thoms. in Nepal, *A. petelotii* Diels in Vietnam, *A. hypoleuca* Nakai and *A. rufa* (Sieb. et Zucc.) Planch ex Miq. in Japan (Huang et al., 2000). Since the first seed introduction of *A. deliciosa* was made from China into New Zealand in 1904, the economic potential of kiwifruit has been extensively exploited (Ferguson and Bollard 1990). Currently, the international kiwifruit industry has achieved more than 100,000 hectares with annual production of one million tons (Huang and Ferguson, 2001).

China has a long history of local consumption of the *Actinidia* as fresh fruits and Chinese herbal medicines for centuries (Cui, 1993). The successful development of kiwifruit industries, first in New Zealand and then in other countries, has stimulated China's interest in the potential of native *Actinidia* species for commercial production. A government organized nationwide selection program was initiated in China in 1977. This program had two missions: 1) to conduct a national germplasm survey and inventory of natural resources of *Actinidia*; 2) to select genotypes superior to the 'Hayward' cultivar developed in New Zealand, the single cultivar planted worldwide during the 1970s. As a result, more than 1400 genotypes were selected from wild populations of *A. chinensis*, *A. deliciosa*, and *A. arguta*. Most of

these selections were evaluated in experimental trials, regional tests, and replicated trials on a commercial scale, leading to the development of 57 cultivars and a large number of advanced selections. The outcome of the program made an unique situation for the Chinese kiwifruit industry because diverse varieties were planted for commercial production (Huang and Ferguson, 2001). Most of the cultivars in China are direct selections from wild populations of *A. chinensis* and *A. deliciosa*. These cultivars and advanced selections have many improved commercial traits such as large fruit size (average 80 to 100 g) with desirable appearance, high vitamin C content (250 to 350 mg/100g fresh flesh), high soluble solids content ($\approx 26\%$), and long storage life at 1 °C (4 months) or at room temperature (40 d). Some cultivars and selections are very precocious and set flowers in the first year after grafting, others are early producers (17 kg/plant of 3 years old), cold hardy, drought tolerant, and with wider adaptability. Some selections are particularly valuable because of different maturity, excellent tastes, various flesh colors, and special uses for processed products (Huang, 1999).

Three major *Actinidia* repositories have been established at Wuhan, Guilin, and Lushan in China as a result of increased attention to germplasm conservation. The national germplasm repository for *Actinidia* and the breeding program for kiwifruit cultivar improvement were established in 1978 at the Wuhan Institute of Botany (WIB) with the goals of conserving natural resources of *Actinidia* in China and developing superior cultivars. This repository is the largest for the conservation of *Actinidia* genetic resources. The living collection at WIB comprises 57 taxa of 36 species consisting of more than 200 accessions and 900 selections and lines of breeding progeny. However, for a plant species at an early stage of domestication (such as kiwifruit) to meet market demand for larger and sweeter-tasting fruit, new cultivar development from long-term breeding programs is required and characterization of germplasm collections is prerequisite for efficient maintenance and use of *Actinidia* genetic resources. Of

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great concern to kiwifruit breeders and germplasm custodians is the limited genetic diversity contained in commercially important cultivars and selections. Molecular fingerprinting can provide accurate genetic information for future breeding and germplasm collection efforts (Cantini et al., 2001; Hokanson et al., 1998; Lamboy and Alpha, 1998). Improving the integrity and efficiency of the *Actinidia* repository management is a part of our long-term

commitment to ex situ conservation and evaluation of *Actinidia* genetic germplasm.

Simple sequence repeats (SSRs) have recently been used successfully as genetic markers for identifying cultivars and germplasm accessions due to their co-dominant inheritance and hypervariability in fruit, such as grape (*Vitis* sp.) (Botta et al., 1995; Lamboy and Alpha, 1998), tea crabapples (*Malus hupehensis*).

Table 1. SSR variability measures for 48 kiwifruit cultivars and selections. Ploidy level for the cultivars and selections listed are based on data available from the computer database of *Actinidia* research center, WIB.

Cultivar or selection	Origin	Az	Pv	H _o ^x
<i>A. chinensis</i>				
Kuimi (4x)	Wild selection from Fengxin Co., Jiangxi province (Jx)	2.9	100	0.630
Zaoxian (4x)	Wild selection from Fengxin Co., Jiangxi province (Jx)	2.7	100	0.602
Jinfeng (4x)	Wild selection from Fengxin Co., Jiangxi province (Jx)	3.0	87.5	0.594
Yixiang (4x)	Wild selection from Xuishui Co., Jiangxi province (Jx)	2.7	88.9	0.556
Jiangyuan-1 (4x)	Wild selection from Jiangxi province (Jx)	3.1	100	0.659
Wuzhi-2 (4x)	Wild selection from Wuning Co., Jiangxi province (Jx)	3.1	100	0.676
Wuzhi-3 (4x)	Wild selection from Wuning Co., Jiangxi province (Jx)	3.0	100	0.639
Wuzhi-5 (4x)	Wild selection from Wuning Co., Jiangxi province (Jx)	3.0	100	0.639
Wuzhi-6 (4x)	Wild selection from Wuning Co., Jiangxi province (Jx)	2.9	88.9	0.630
Tongshan-5 (4x)	Wild selection from Tongshan Co., Hubei province (Hub)	3.0	100	0.648
Jinyang (4x)	Wild selection from Chongyang Co., Hubei province (Hub)	2.5	100	0.573
Jinnong-1 (2x)	Seedling selected from mixed seeds collected from wild at Fang Co., Hubei Province (Hub)	1.8	66.7	0.333
Jinshui-1-111 (2x)	Seedling selected from mixed seeds collected from wild at Fang Co., Hubei Province (Hub)	1.6	55.6	0.278
Luoyang-1 (4x)	Wild selection from Song Co., Henan Province (Hen)	3.1	100	0.676
Huaguang-3 (4x)	Wild selection from Funiu Mt., Henan Province (Hen)	2.7	88.9	0.521
Funiuhongrou (4x)	Wild selection from Funiu Mt., Henan Province (Hen)	2.8	77.8	0.509
Chuanmi-3	Wild selection from Henan Province (Hen)	1.9	85.7	0.429
Chuanmi-4	Wild selection from Henan Province (Hen)	2.3	100	0.546
Xiangma-6	Wild selection from Mayang Co., Hunan Province (Hun)	2.3	77.8	0.472
Jianke-1	Wild selection from Jianning Co., Fujian Province (Fj)	2.6	77.8	0.500
Xiaya-1 (4x)	Wild selection from Fujian Province (Fj)	2.8	100	0.615
Xiaya-15 (4x)	Wild selection from Fujian Province (Fj)	3.0	100	0.630
Guihai-4 (2x)	Wild selection from Longsheng Co., Guangxi Province (Gx)	1.8	66.7	0.333
Hongyang (2x)	Wild selection from Sichuan Province (Sc)	1.7	62.5	0.313
Qingyuanqiucui	Wild selection from Qingyuan Co., Zhejiang Province (Zj)	3.1	100	0.658

sis (Damp.) Rehd.) (Benson et al., 2001; Hokanson et al., 1998), *Citrus* (Gulsen and Roose, 2001), and sour cherry (*Prunus cerasus* L.) (Cantini et al., 2001). More than 40 SSRs from kiwifruit have been developed and about 20 SSRs were verified as single Mendelian loci in intra- and interspecific crossed families (Huang et al., 1998; Weising et al., 1996). The emergence of PCR-based molecular markers has provided the opportunity for a wide array of investigations in *Actinidia*, such as RAPD markers for cultivar fingerprinting and taxonomical relationships (Cipriani et al., 1996;

Huang et al., 2002), PCR-RFLPs of cpDNA and mtDNA for studying cytoplasmically genetic phylogeny (Chat et al., 1999; Cipriani et al., 1995; Testolin and Cipriani 1997), and combination of RAPD markers and SSRs for verifying somaclonal variation in tissue cultures (Palombi and Damiano 2002). However, SSRs for fingerprinting kiwifruit cultivars and germplasm accessions has not been explored probably because most newly developed cultivars and selections are restricted to China. Therefore, in the present study, we employed a set of nine genetically defined

Table 1 (continued). SSR variability measures for 48 kiwifruit cultivars and selections. Ploidy level for the cultivars and selections listed are based on data available from the computer database of *Actinidia* research center, WIB.

Cultivar or selection	Origin	Az	Pv	H _o ^x
Earligold (2x)	Seedling selection from a second generation of mixed open-pollinated seeds by HortResearch, New Zealand	1.9	88.9	0.444
Mean (<i>A. chinensis</i>)		2.6	89.4	0.546
<i>A. deliciosa</i> (all genotypes are 6x)				
Qinmei	Wild selection from Zhouzhi Co., Shaanxi Province (Shaanxi)	3.6	100	0.704
Yate	Wild selection from Hu Co., Shaanxi Province (Shaanxi)	3.7	100	0.709
Xixuan-1	Wild selection from Shaanxi Province (Shaanxi)	3.0	87.5	0.579
Changan-1	Wild selection from Changan Co., Shaanxi Province (Shaanxi)	3.3	77.8	0.520
Miliang-1	Wild selection from Fenghuang Co., Hunan Province (Hun)	4.1	100	0.752
Dongshanfeng	Wild selection from Dongshan Mt., Hunan Province (Hun)	3.4	100	0.669
Jinkui	Seedling selected from wild selection Zhuxi-4 at Zhuxi Co., Hubei Province (Hubei)	3.8	100	0.702
Qingcui	Wild selection from Dujiangyan Co., Sichuan Province (Sc)	2.9	77.8	0.502
Xinguan-2	Wild selection from Cangxi Co., Sichuan Province (Sc)	2.6	100	0.594
Lushanmei	Wild selection from Jiangxi Province (Jx)	3.8	100	0.719
Huamei-1	Wild selection from Funiu Mt., Henan Province (Hen)	3.9	100	0.721
Chuanmi-1	Wild selection from Henan Province (Hen)	2.8	100	0.621
Chuanmi-2	Wild selection from Henan Province (Hen)	3.2	100	0.667
Hayward	Seedling selection by New Zealand growers from mixed seeds collected at Yichang, Hubei	3.9	100	0.735
Bruno	Seedling selection by New Zealand growers from mixed seeds collected at Yichang, Hubei	4.0	100	0.720
Xianglv	Open-pollinated seedling of Hayward	4.1	100	0.748
Xuxiang	Open-pollinated seedling of Hayward	3.5	100	0.704
Xuguan	Open-pollinated seedling of Hayward	3.4	100	0.691
Shixuan-3	Open-pollinated seedling of Hayward	3.4	100	0.691
Mean (<i>A. deliciosa</i>)		3.5	97.0	0.671
Interspecific hybrid (<i>A. chinensis</i> x <i>A. eriantha</i>)				
Mantianxing	Wuzhi-3 x EA1998YN	2.8	100	0.630
Jiangshanjiao	Wuzhi-3 x EA1998YN	2.3	100	0.548
Mean (hybrids)		2.6	100	0.589
<i>A. arguta</i>				
Kuilv (2x)	Wild selection from Jian Co., Jilin province (Jl)	1.8	66.7	0.361

^xNumber of alleles per locus.

^yPercentage of polymorphic loci.

Table 2. Distribution of allelic frequencies and number of alleles in 48 kiwifruit genotypes.

Allelic frequency range	No. of alleles	Percentage
> 0.1000	11	5.2
0.1000–0.0749	15	7.0
0.0750–0.0499	34	16.0
0.0500–0.0249	72	33.8
0.0250–0.0099	47	22.0
< 0.0100	34	16.0

SSR markers developed at University of Udine (Huang et al., 1998) to fingerprint kiwifruit cultivars and selections and assess the genetic diversity and relatedness of cultivars and selections developed from two commercially important species, *A. deliciosa* and *A. chinensis*.

Materials and Methods

Genomic DNA was isolated from approximately 2 g leaf tissue from 48 cultivars and selections (Table 1) using a modified cetyltrimethylammonium bromide (CTAB)-based procedure outlined in Wagner et al. (1987). They included 26 cultivars and selections of *A. chinensis*, 19 of *A. deliciosa*, one of *A. arguta*, and two interspecific hybrids, which account for more than 90% of total world kiwifruit production (Huang and Ferguson, 2001). These cultivars and selections were maintained as field collections at the national germplasm repository for *Actinidia* at WIB, Wuhan, Hubei province, P.R. China. The RNA component of these individual extracts was removed by incubation in the presence of RNase A as described in Ausubel et al. (1987). DNA at 100 ng/ μ L was used as template for PCR amplification. Sixteen SSR primer pairs (Huang et al., 1998) were used for initial screening against 10 cultivars each of *A. deliciosa* and *A. chinensis* for reproducibility and intensity of DNA fragments. Nine SSR primer pairs were then chosen for genotyping the remainder of cultivars and selections. DNA amplifications were performed using a protocol described by Huang et al. (1998). The reaction consisted of the following in 25 μ L total volume: 100 ng of template DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 μ M of each primer, and 0.4 U of *Taq* DNA polymerase (Promega). The DNA samples were amplified using the following thermal profile: 5 min at 95 °C; followed by 35 cycles of 50 s at 94 °C, 50 s at 55 °C and 50 s at 72 °C; followed by 8 min at 72 °C. The amplification products were electrophoresed in 8% polyacrylamide denaturing gels using a Sequi-Gen GT/Power Pac 3000 Sequencer System (Bio-Rad) for approximately 1.5 h at 55 W, followed by silver staining (Promega, Madison, Wis.). Each sample/primer combination was amplified at least three times to verify reproducibility. The bands of amplified DNA fragments were manually scored and pBR322 DNA/*Msp*I ladder (Sino-American Biotechnology Company, China) was used to determine the size of amplified DNA fragments.

SSR markers were designated by the manufacturer primer code (referred to as locus) corresponding to the primer pair sequences responsible for their amplification, followed by a digital number indicating the product size in base pairs referred to as alleles. Number of alleles per locus (A), percentage of polymorphic loci (P), direct count heterozygosity (H_o) and genetic diversity were calculated according Weir (1996). When a cultivar showed one fragment (allele) at a locus, it was considered to be homozygous

at the locus. In cases when a cultivar was actually heterozygous for one allele and a null allele, the results reported herein underestimate the level of heterozygosity and the mean heterozygosity estimated may also include inter-locus heterozygosity because duplicate loci could occur in both *A. deliciosa* (hexaploid) and *A. chinensis* (includes diploid and tetraploid races), as well as in the interspecific hybrids (Caniti et al., 2001; Hokanson et al., 1998). The ploidy level of most cultivars and selections was previously determined (WIB *Actinidia* database, unpublished). The relative magnitude of genetic differentiation between *A. chinensis* and *A. deliciosa* cultivar groups ($G_{st} = D_{st}/H_T$, (Nei, 1978)) was calculated by different methods implemented in AMOVA (<http://acasun1.unige.ch/LGB/software/windoze/amova>) and Microsat (<http://hpg1.stanford.edu/projects/microsat/>) software.

For the purpose of examining the genetic relationships among the 48 cultivars and selections, allelic data were treated in binary format and scored as 1 (band present) or 0 (band absent), and were referred to as allelic phenotypes. Dice (1945) coefficients, which are equivalent to Nei and Li (1979) genetic identities, were calculated for all possible pairwise combinations of kiwifruit genotypes. A dendrogram was constructed based on the matrix of the Dice coefficients by unweighted pair-group mean analysis (UPGMA) using the software NTSYS-pc v.1.8 (Exeter Software, Setauket, N.Y.).

Results

SSR POLYMORPHISM AND GENETIC DIVERSITY. All nine SSR markers employed for characterization of the 48 kiwifruit genotypes were polymorphic. Six and four alleles were the maximum number of alleles observed in *A. deliciosa* ($2n = 6x$) and *A. chinensis* ($2n = 2x$ and $4x$) genotypes, respectively. Because the ploidy level of most cultivars and selections were previously known, this provided a crosscheck for accuracy of scoring alleles. In total, 213 alleles were detected, giving a mean number of 23.7 alleles per locus (A) ranging from nine alleles in UDK97-409 to 38 alleles in UDK96-026. Only 5.16% of the alleles had frequencies >0.1000 (Table 2). Nineteen homozygous null alleles were detected in a total of 432 combinations of 48 genotypes by nine loci (Table 3). Of the 213 alleles, two alleles were found to be specific to 'Kuilv' of *A. arguta* and 'Jiangshanjiao', an interspecific hybrid. Interestingly, 133 alleles were found to be common to both *A. chinensis* and *A. deliciosa*, while 33 and 36 alleles were specific to *A. chinensis* and *A. deliciosa*, respectively. In addition, 34 alleles were found to be specific to one single cultivar or selection and provided a valuable set of alleles to enhance the discrimination ability for cultivar identification (Table 3). Gene diversity measured for each locus ranged from 0.73 for UDK97-409 to 0.96 for both UDK96-026 and UDK96-414, with an average gene diversity value of 0.92 for all loci obtained after pooling all cultivars and selections from both *A. chinensis* and *A. deliciosa*. When gene diversity was calculated across all loci for comparison between *A. deliciosa* and *A. chinensis* cultivar groups, mean gene diversity of *A. deliciosa* was slightly higher than that of *A. chinensis* (0.91 vs. 0.89).

The mean number of alleles per locus (A), percentage of polymorphic loci (P) and direct count heterozygosity (H_o) assessed for each genotype over all loci revealed differences among these 48 cultivars and selections. On average, $A = 2.6$, $P = 89.4\%$ and $H_o = 0.546$ were found in *A. chinensis* genotypes, while $A = 3.5$, $P = 97.0\%$ and $H_o = 0.671$ in *A. deliciosa* genotypes. The highest direct count heterozygosity of 0.752 was observed in the *A.*

deliciosa cultivar, Miliang-1, and the lowest of 0.278 in a diploid *A. chinensis* selection 'Jinshui 1-111'. Similarly, the diploid *A. arguta* cultivar, Kuilv, was also found to be one of the lowest H_e (0.361) (Table 1).

SSR FINGERPRINTING KIWIFRUIT CULTIVARS AND SELECTIONS. All 48 kiwifruit genotypes were uniquely identified by one locus, UDK96-414, except two genotypes, 'Xiaya-1' and 'Xiaya-15', which were presumably selected from the same wild parent vine

(Zhang, personal communication) and showed the same allelic composition at eight loci (Table 3). However, 'Xiaya-1' revealed a homozygous null allele at locus UDK96-026-0/0/0/0 that was distinctly different from 'Xiaya-15', which revealed a tetra-allelic composition of UDK96-026-217/211/201/117 (Table 3). The UDK96-026 and UDK 96-414 are the most hypervariable loci, with 38 and 32 alleles detected, respectively. This suggests a possibility that somaclonal variability occurs and agrees with

Table 3. The SSR fingerprinting of kiwifruit cultivars and selections.

Cultivars	Locus code			
	UDK 96-001	UDK 96-015	UDK 96-016	UDK 96-030
Wuzhi-3	272/238	141/127	223/211/199	201/181/141/127
Wuzhi-5	286/276/272/256	133/131/127	211/199	165/157/141
Wuzhi-6	282/266/264	151/145/137/127	207/201	165/141/119
Miliang-1	282/276/264/246/238	145/141/137/133/127	253/223/201/197	165/141/135/115*
Tongshan-5	272/252	129/127	223/211/207/201	193/183/141
Jinnong-1	264	137/133	203	123/119/111
Wuzhi-2	282/276/238	167/141/127	215/207/201	181/165/141/119
Jinyang	272/256	141/127	223/203/199	205/145
Jinshui-1-111	280	141	207/203	147/123
Kuimi	282/280/264	133/127/121	223/215/207/203	145/131/123
Jinfeng	280/272/266/248	Null allele	223/207/201/195	157/149/131
Xinguan-2	282/268/264	137/129	221/215/201	161/147/139/131
Zaoxian	290/268/248	159/141/ 123	223/215/207/201	187*/157/149
Lushanmei	276/268/264/248	151/137/133/127	253/227/211/207/201	201/165/157/141
Huaguang-3	272/264	137/123	201/195	231/181/157/141
Shixuan-3	272/256/248	151/137/133/131/127	207/205/201	161/141/137
Funiuhongrou	280/272/266	145/127	213/201/195	151
Luoyang-1	282/280/260	133/127/123	227/215/207/203	145/131/123
Dongshanfeng	272/268/264	145/133/125	249*/227/211/203/191*	149/141/139
Huamei-1	286/270/248/242	163/151/141/133/121	253/227/213/207/203	201/161/133/123
Xianglv	270/248/238	151/145/141/127	253/227/213/207/203	183/165/123/113*/107
Hongyang	280	141	207/201	145/125*
Xiaya-15	276/268/246	163/141/127	205/201	155/119
Xixuan-1	276/260/252	141/127	229/223/211/209	153/141/139
Chang'an-1	290/282/280/260/242	141/135	213/195	205/141/131/123/119
Xiaya-1	276/268/246	163/141/127	205/201	155/119
Xiangma-6	296/286/282/276	145/141	203/199	153/137
Yate	290/280/272	145/141/127	207/203	157/155/147/135
Xuguan	272/256	151/135/131/127	257/207/201	231/147/135/119/111
Xuxiang	290/272/268/256	Null allele	229/207/195	217*/165/151/145/127
Chuanmi-1	272/268	159/145/131	251*/221/209	157/149/119
Bruno	268/248/238	151/145/141/131/127	257/229/207/205	231/155/145/131/123/107
Guihai-4	296	145/121	211/203	147
Chuanmi-2	280/270	151/141/127/115*	221/209/205/199	161/135/131/111
Chuanmi-3	286/260	175/145	205/199	183/111
Jinkui	Null allele	175/155*/151/135/129/123	209/205	161/141/131/105
Hayward	272/266/248/246	141/127	253/227/207/203	183/161/147/131
Qinmei	272/268/246/238	131/121	227/207/203	157/149/139/133
Qingyuanqiucui	282/272/252	129/127/121/109	209/203/199	165/157/139
Kuilv	272/260	Null allele	199	107/105
Yixiang	272/260	151/135	223/211/205/199	193/183/157/135
Jiangyuan-1	Null allele	159/131/129/121	205/197	161/141/139/119
Jianke-1	264	135/131	213/207/199	127/111
Qingcui	282/268	135/125	221/211/201/195	141
Chuanmi-4	272/264	167/145	209/201	181/173
Jiangshanjiao	Null allele	141/109	Null allele	173/141
Mantianxing	266/256/242	121/109	223/211/199	201/165/127
Earligold	282	131/123	211/205	183/131

*Allele specific to the cultivar.

results on detection of somaclonal variation by SSR markers (Palombi and Damiano, 2002).

GENETIC RELATEDNESS. The genetic identity, as measured by Dice coefficient, varies from 0.03 to 0.80 among the cultivars and selections, with the highest identity (0.80) observed between 'Xiaya-1' and 'Xiaya-15' and the smallest (0.03) observed between 'Kuily' of *A. arguta* and other cultivars of *A. chinensis* and *A. deliciosa*. The genetic relationships among the cultivars and advanced selections were examined by UPGMA cluster analysis, as

presented in Fig. 1. This dendrogram is a representation of apparent genetic similarity and it is not a pedigree or family tree. The high correlation coefficient ($r = 0.95$), as calculated by MIXCOMP program in NTSYS, indicated "the goodness of fit" of this SSR dendrogram. In order to examine genetic relationships among the taxa, a cut-off point ($D_1 = 0.09$) was determined according to Xu and Li's (1983) method of grouping taxa. The dendrogram, in general, separated 48 cultivars and selections into four clusters and one individual cultivar (Fig. 1). Cluster I consisted of mixed

Locus code				
UDK 96-034	UDK 96-040	UDK96-026	UDK97-409	UDK 97-414
202/188	190/180*/156/124	207/195/187/107	240/146/130	177/107/95
188/184	188/170/136/124	187/157/117/107	240/130	153/127/103
188/184	188/166/150/116	191/181/153/107	240/130	107
218/212/204/186	162/154/140	195/167/133/113	240/190/146/130	123/107/101*/95
202/194/188	170/156/154/144	205/193/181	240/146/130	153/133/107
216/212	128/116	213*/191	240/130	127/97
216/200/184	168/124/116	205/187/173*	240/132*/130	137/107/97
Null allele	166/116	209*/205/193/181	240/130	157/127/107
196/172	116	187/153	240	181/157
200/194/188/186	166/124	201/183	240/130	153/133/99
202/194/172	166/154	205/201/139/111	240	133/107/99
196/186	168/154/124	157/103	240/130	157/133/99
200/182	170/116	205/139/119	240/130	181/153
212/208/182/174*/168*	166/156/ 140/136	205/197/139/119	240/130	131/123/111
202/176	166/124	205/193/117	240	193*/165/107/93*
218/202	170/156/154/144	197/139/119	240/232/146/130	179/161/111/95
212/208/196/184	160/156/124	205/139/119	240	177/153/141
196/194/188/186	168/166/124	205/185/105	240/130	157/137/103
204/196	170/168/140/136	157/103	240/146/130	169/145/127/107/95
200/178	188/166/136/124	195/161*/111	Null allele	179/149/127/97
218/202/172	160/156/140/124	195/159*/111/109/103	240/232/226/130	169/157/113/97
196	144/136	193/185	Null allele	153/133
206/196/176	160/154/124/116	217*/211/201/117	240/130	183/121/99
206/180	170/166/150/124	Null allele	240/226/190/174/130	133
180	170/148/132/124	205	240/232/130	149/127/113
206/196/176	160/154/124/116	Null allele	240/130	183/121/99
186	162/150/140	195/167/113	240	147*/137/99
220*/206/200/186	156/150/144/124	205/181/139/119	240/232/174/146/130	169/149/133/103
216/204/182	156/154/128/116	203*/189/ 157	240/232/146/130	161/111/95
196/178/172	166/156/124	201/133/117	240/174/130	177/145/127/111
210/206	166/128	211/197/185	240/146/130	177/145/121/95
216/172	170/156/154/140/128	205/191/133/119/109	240/232/130	177/161/123
204/186	144/128	139/119	240	161/131
180/176	170/162/160/132	201/133/113	240/232/130	161/131/103
200/196	154	Null allele	Null allele	177/153
216/210/200	188/170/156/128	205/133/111	Null allele	169/145/103/97
202/190/180	170/160/156/154/124	205/187/133/113/103	240/226/146/130	177/153/123/103
196/186/184	170/160/154/124	Null allele	240/226/190/174/130	121/113/107/89*
202/176	188/170/136/124	201/185/113/103	240/130	153/123/99
190	148/132/110*	207/199	Null allele	Null allele
190/186	170/156/154	179*	240/146/130	145/123/103
Null allele	190/168/144	199/181/113/101*	240/174	145/123/99
210/200/190/184	170/168/160	193/153/123*/109	240	183/127/121
182	170/166/156/140/128	187/183/127*/113/109	240/174	177/141/121
190/160	154/140	189/185/105	240/130	185*/173*/141/123
184/172	166/150	191/173	240/168*/130	141/127/103
216/160	170/156/150	187/157/107	240/146/130	177/141/127
190/184	140/116	189/181	240/130	165/121

Fig. 1 SSR-based UPGMA dendrogram of Dice coefficients of genetic similarity among 48 kiwifruit cultivars or selections. All genotypes were separated into four major taxa groups labeled as I, II, III, and IV at the cut-off point ($D_1 = 0.09$), which was depicted as a vertical dashed line. Cluster I was further divided into four subclusters reflecting pedigree relationships and/or geographic origins of genotypes.

cultivars and selections from both *A. chinensis* and *A. deliciosa*, suggesting that natural hybridization occurs frequently between these two closely related species within sympatric distributed areas. Almost all the cultivars and selections in this cluster were selected from the wild, and in fact, *A. chinensis* and *A. deliciosa* were treated as two taxonomic varieties (var. *chinensis* and var. *hospita*) within single species *A. chinensis* until the late 1980s (Liang and Ferguson, 1986). Cluster II and cluster III included four and three *A. chinensis* cultivars, respectively, while cluster IV was comprised of two *A. deliciosa* cultivars. The cultivar, Kuilv, of *A. arguta* was separated from these clusters.

Cluster I could be further divided into four subclusters that appeared to reflect either known pedigree relationships or geographic origins of cultivars (Fig. 1). The first subcluster contained all *A. chinensis* cultivars except two interspecific hybrids and one *A. deliciosa* cultivar. The two interspecific hybrid cultivars, Mantianxing and Jiangshanjiao, were derived from an interspecific cross of the female *A. chinensis* parent 'Wuzhi-3' with a male parent of *A. eriantha* selection (EA1998YN). Most cultivars in this subcluster were selected from Hubei and the bordering areas between Hubei and Jiangxi provinces (Huang et al., 2001). The second subcluster was comprised mostly of *A. deliciosa* cultivars except two closely related *A. chinensis* selections ('Xiaya-1' and 'Xiaya-15'). This group appeared to be a representation of original gene pool of 'Hayward' and its derivatives because cultivars 'Shixuan-3', 'Xuguan' and 'Xianglv' were seedling selections from 'Hayward' and the old New Zealand cultivar, Bruno that was also derived from the original seeds introduced into New Zealand in 1904 (Ferguson and Bollard, 1990; Huang et al., 2001). The third subcluster included all *A. chinensis* cultivars selected from Hubei and Henan provinces, while the fourth subcluster had two cultivars of *A. deliciosa* selected from Sichuan province.

Discussion

SSR markers are hypervariable and can be used efficiently for identification of kiwifruit cultivars. The range of nine to 38 alleles per locus observed in this study is much higher than that of seven to 17 alleles per locus reported previously (Huang et al., 1998). The difference was simply due to a wider array of cultivars and selections, which had diverse genetic backgrounds and geographic origins, as well as different ploidy levels (Table 1). This result is also well reflected by low individual allelic frequencies, because 71.8% of the alleles had allelic frequency <0.05 (Table 2). The value of mean number of 23.7 alleles per locus observed in this study is high in comparison to the reported values of SSR studies conducted in other plant species with similar ecological and life-history characteristics of wide geographic range, outcrossing and long-lived woody perennials, such as olive (*Olea europaea* L.), 3.0 alleles per locus (Cipriani et al., 2002); *Citrus*, 5.5 (Kijas et al., 1995); tea crabapple, 9.4 (Benson et al., 2001); blueberry (*Vaccinium corymbosum* L.), 7.9 (Levi and Rowland, 1997); avocado (*Persea americana* M.), 9.5 (Lavi et al., 1994); *Pyrus*, 11.0 (Yamamoto et al., 2001); apple (*Malus domestica* Borkh), 12.1 (Hokanson et al., 1998); cherry (*Prunus* L. sp.), 10.7 (Cantini et al., 2001); black cherry (*Prunus serotina* L.), 13.5 (Downey and Iezzoni, 2000); and bur oak (*Quercus macrocarpa* M.), 14.3 (Dow et al., 1995). But it is similar to the value reported for grape cultivars, 27.6 alleles per locus (Lamboy and Alpha, 1998). The mean gene diversity of 0.92 across all loci in kiwifruit was also found to be higher than that in other plants such as olive, 0.55 (Cipriani et al., 2002); apple, 0.71 (Hokanson et al., 1998); tea

crabapple, 0.76 (Benson et al., 2001); and cherry, 0.81 (Cantini et al., 2001). The high ploidy level of *A. deliciosa* (hexaploid), different ploidy races (diploid and tetraploid) of *A. chinensis*, and ploidy origins (allohexaploid in *A. deliciosa* and autotetraploid in *A. chinensis*) (Huang et al., 1997; Testolin and Ferguson, 1997) might explain the high genetic variation of SSRs in kiwifruit. In addition, 19 null alleles provided supplemental genetic information to enhance the identification of kiwifruit cultivars. The inheritance of null alleles in *Actinidia* species has not yet been subjected to investigation and we believe this is the first case ever reported for SSR null alleles in *Actinidia* species. Genetic significance of SSR null allele segregation has proven to be of importance to human genetics (Callen et al., 1993) and should be worthy of further investigation in plant genetics.

Of 211 alleles detected in *A. chinensis* and *A. deliciosa*, 133 alleles were common to both *A. chinensis* and *A. deliciosa* (63.0%), with 33 and 36 alleles specific to *A. chinensis* (15.6%) and *A. deliciosa* (17.1%), respectively. This suggests that 75.6% of the alleles detected in *A. deliciosa* were from a shared gene pool of *A. chinensis*. Morphologically, the two species are very similar and a close phylogenetic relationship between *A. chinensis* and *A. deliciosa* has been demonstrated in previous investigations (Cipriani et al., 1996; Huang et al., 1997; Huang et al., 2002; Testolin and Ferguson, 1997). Our results agree with the hypothesis that *A. chinensis* is the sole progenitor of hexaploid *A. deliciosa* based on the observation of two species sharing 85% of allozymes (Testolin and Ferguson, 1997). Further partitioning the genetic variation of *A. chinensis* and *A. deliciosa* as a species complex revealed only 1.3% genetic variation between the species ($G_{st} = 0.013$) and provided additional evidence to support the hypothesis (data not shown).

One major application of molecular markers in germplasm repository management is marker aided conservation (MAC) for formulating appropriate collection policies. Therefore, information on genetic variability contained in individual cultivar is of great value to kiwifruit repository curators. Considerable differences of genetic variability among these 48 cultivars and selections were revealed by measuring mean number of alleles per locus (A), percentage of polymorphic loci (P) and direct count heterozygosity (H_o) for each cultivar and selections (Table 1). Within the *A. chinensis* cultivar group, tetraploids had higher values of A, P, and H_o than diploids ($A \geq 2.3$ in tetraploids vs. $A \leq 1.9$ in diploids; $P \geq 77.8\%$ vs. $P \leq 66.7\%$; $H_o \geq 0.50$ vs. $H_o \leq 0.33$), except for 'Earligold' with a high P (89.4%) and H_o (0.44). Similarly, all *A. deliciosa* genotypes were hexaploids and, in general, had even higher genetic variability than tetraploid *A. chinensis*. Thus, the *A. deliciosa* cultivar group showed higher A, P and H_o than the *A. chinensis* cultivar group (Table 1). In contrast with no relationship observed between ploidy and number of alleles of isozymes (Testolin and Ferguson, 1997), the positive correlation between genetic variability and ploidy levels found here suggests that polyploidy plays an important role in maintaining genetic diversity. This should supplement valuable information for the selection potential in kiwifruit breeding programs (Huang et al., 2000). SSRs provided unique identification for 48 kiwifruit cultivars and selections, which was a substantial improvement in discrimination effectiveness compared with a 77% partial discrimination of 13 kiwifruit cultivars using RAPD markers (Cipriani et al., 1996). In fact, a single SSR locus UDK 96-414 can generate sufficient numbers of 'allelic phenotypes' for as many as 47 kiwifruit genotypes. This result underlines the potential that informative markers can efficiently reduce the number of

SSR loci required for reliable identification of kiwifruit cultivars. Nevertheless, the SSR profile for each cultivar and selection in Table 3 furnishes the first benchmark of kiwifruit fingerprints informative to germplasm repository management and nurseries engaged in commercial kiwifruit propagation.

In conclusion, fingerprint profiling using SSR markers is a useful and reliable method for establishing genetic identities of kiwifruit cultivars and selections. SSRs also provide an improved discrimination tool for evaluating genetic diversity and relatedness compared to RAPD markers. SSRs, when genetically defined and properly used, should provide a useful reference for germplasm curators for developing sampling strategies and managing germplasm repositories and for breeders for parental selection.

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