

Genetic diversity and relationships between and within kiwifruit

(*Actinidia*) wild species and cultivated varieties using SRAP markers

Abstract Kiwifruit (*Actinidia*) is one of the most important fruits in the world. The objectives of this study were to reveal the genetic diversity and relationships of 30 kiwifruit genotypes belonging to twelve different species by the SRAP markers. A total of 292 polymorphic bands were observed, with an average of 24.33 bands per pair of combined primers. The unweighted pair-group method of arithmetic average (UPGMA) analysis showed that the Jaccard's coefficient of similarity value varied from 0.15 to 0.77, indicating that abundant diversities exist among these wild species. The 30 genotypes of kiwifruit were divided into five groups using the cluster analysis and principal coordinate analysis. *A.rufa* and *A.arguta* had the far relationship, *A.chinensis*, *A.deliciosa*, and *A.eriantha* had the close genetic relationships. This study provided theoretical basis for the genetic diversity and further breeding programs of *Actinidia*.

Key words: Chinese wild kiwifruit species; *Actinidia*; genetic diversity; SRAP

1. Introduction

Kiwifruit (*Actinidia*) has become one of the most important fruits in the world since it has been started commercial in 1957. It mainly distributes in China and South Eastern Asia, which including a genus of 55 species and about 76 taxa native to China with ploidy levels ranging from diploid ($2n=2x=58$) to octoploid ($2n=8x=232$) (Li et al., 2009). The most widespread commercially cultivated species in the world are *A. chinensis* and *A.deliciosa*, and both species are native to China (Huang and Ferguson, 2007). Up to 2013, the annual production of kiwifruits reached 170 tons in China, and these data indicated that the cultivated areas and yields of kiwifruits have yet to actually increase due to breeders contributed a lot to breeding (Huang 2014). New advances in kiwifruits breeding of China may be concluded in two aspects: (1) since 1978, about 146 new varieties (lines) were select and released in the main planting area, and these varieties for extending cultivation were rare in large scale. The varieties that cultivation areas account for 5 percent of total areas in China only include Hongyang (*A. chinensis*), Xuxiang (*A. deliciosa*), Qinmei (*A.deliciosa*), and Jinkui (*A.deliciosa*) (Huang 2014). (2) The aim of breeding tends to be more diverse. For example, the green-fleshed kiwifruits were the main varieties in tradition. The yellow-fleshed and red-fleshed kiwifruit

have been breed due to its importance of meeting the demanding of markets and consumers. Although the kiwifruit cultivars were diverse, some problems still exist. Such as, most varieties were direct selections from the wild or seeding populations that showed only main traits are good without full scientific evaluation. Moreover, these varieties could have a narrow genetic basis (Datson and Ferguson 2011). The collection and evaluation of kiwifruit germplasm from wild is important to assist breeding programs as it will help to recover the useful traits through them to rich gene pool through the genetic diversity (Ferguson 2007).

Germplasm collection and genetic diversity could provide the raw materials for programmers of plant breeding and crop improvement (Ferguson 2007; Greene and Morris 2001). In recent years, in order to help to understand and evaluation kiwifruit germplasm different molecular markers have been explored to analyze their genetic diversity and relationships among kiwifruit, these include RAPD, SSR and SNP (Huang et al., 2002; Korkovelos et al., 2008; Zhou et al., 2011). Sequence-related amplified polymorphism (SRAP) is a frequently used as a molecular marker, due to its simple, reliable and effective (Jing et al., 2013a, 2013b). Some studies showed SRAP marker could provide more polymorphism information and the capacity of average discrimination in comparison to SSR, ISRR, RAPD and AFLP (Budak et al., 2004; Jing et al., 2013a, 2013b). So far, there is no report on evaluation of the genetic diversity and relationship among *A.chinensis*, *A.deliciosa*, *A.eriantha*, *A.macrosperma*, *A.rufa*, *A.arguta*, *A.polygama*, *A.tetramera*, *A.latifolia*, *A.kolomikta*, *A.indochinensis* and *A.reticulata* using SRAP marker.

In this study, the objective of this study was to assess the genetic diversity and genetic relationships of kiwifruits from different eco-geographic regions in China. For this purpose, our study may be providing theoretical basis for future germplasm conservation, utilization and kiwifruit breeding programs.

2. Materials and methods

2.1. Plant materials

A total of 30 kiwifruit genotypes including 12 wild kiwifruit species native to China were sampled from kiwifruit germplasm repository in Shaanxi rural science and technology development center at Xi'an, Shaanxi of China (Table 1). These genotypes included 6 *A.chinensis*, 9 *A.deliciosa*, and other 10 related species. And these different species of kiwifruit were collected from different ecogeographical locations in China. Fresh leaves were collected from plants and immediately stored in zip-lock bags with silica gel and brought back to laboratory for DNA extraction.

2.2. DNA extraction

All plant materials DNA were extracted from fresh leaves using a modification CTAB

method described by Doyle and Doyle (1987). The DNA quality was tested by 1.0 % (W/V) agarose gel electrophoresis. Genomic DNA concentration was measured using a Nanodrop ND-1000 (NanoDrop Technologies) spectrophotometer.

2.3. SRAP-PCR amplification

Twelve pairs of combined SRAP primers were employed (Table 2, 3). And these pairs of combined primers were selected from a total of 192 primer combinations based on their reproducibility, clarity, and highly polymorphic of the productive bands. PCR were carried out according to the previously established protocols by Jing et al. (2013b), with some modification. A 20 μ L volume, containing 40 ng of template DNA, 10 $\times$ PCR buffer (100Mm Tris-HC, pH 8.3; 500 mM KCl), 0.18 mM of each dNTP, 0.75 mM of each primer, 1.80 mM of MgCl₂, 0.75 units of Taq DNA polymerase (5 U/ μ L) (TaKaRa Biotechnology Dalian Co., Ltd., China). PCR was performed in the following conditions: 5 min of denaturation a 94 °C, 5 cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C and 2 min of elongation at 72 °C. In the following 30 cycles, the annealing temperature was increased to 50 °C, with a final elongation step of 5 min at 72 °C. PCR products were separated on 6% denatured polyacrylamide gels and detected by silver staining. The clear and reproducible bands were recorded and used for analysis. DL2000 DNA ladder (TIANGEN BIOTECH, Beijing Co., Ltd., China) was used as DNA markers.

2.4. Data analysis

Each SRAP locus was scored by present '1' or absent '0' for each of 30 genotypes, and generated a binary data matrix. A locus was considered polymorphic if more than one band at the same position was detected for all the samples. The binary data matrix was analyzed using NTSYS-pc version 2.1e software package (Rohlf 2000). The pairwise genetic distances among all materials according to Nei (1978) were calculated based on Jaccard's similarity coefficient. Cluster analysis was performed using the unweighted pair-group method with arithmetic average (UPGMA). The dendrogram was constructed using the SHAN program in NTSYS-pc version 2.10e software (Rohlf 2000). A Principal Coordinate Analysis (PCOA) was performed based on the variance covariance matrix of marker data.

3. Results

3.1. Polymorphism of the SRAP markers

In this study, 12 pairs of combined primers were selected from a total of 192 pairs of primers based on the clear and reproducible bands to evaluate their genetic diversities of the 30

kiwifruit genotypes. Results showed that a total of 292 bands were amplified in which their size of the bands range from 100 to 2000 bp (Table 3). Each pair of combined primers generated 15 (Me5+Em5) to 35 (Me9+Em12) bands with an average of 24.33 bands per pair of combined primers, of which 292 (100%) were polymorphic loci.

3.2. Cluster analysis

The genetic similarity value was calculated by Jaccard's coefficient to assess the genetic distance among 30 kiwifruit genotypes. And the Jaccard's coefficient of similarity value varied from 0.15 to 0.77. The UPGMA clustering method was used to construct the dendrogram based on the genetic distance of 30 kiwifruit genotypes. The dendrogram showed that all genotypes could be divided into five major clusters at the similarity level of 0.27 (Fig. 1). Cluster 1 was the largest cluster, and included 'Funiushan-1', 'Huayang-ZH1', 'Zhonghua-WS', 'Xixia-1', 'Yuhuang-2', and 'Hongyang', which belong to the *A. chinensis*, 'Lantian-MW1', 'Ningshanhong', 'Taibaihong', 'Taibailv', 'Huayang-MW1', 'Huyayang-MW2', 'Shiquanhong', 'Meiwei-HMZ' and 'Jinshuo', which belong to *A. deliciosa*. In addition, 'Maohua-1', 'Maohua-2', 'Huatae' (*A. eriantha*), 'Dazi-1' (*A. macrosperma*) and 'Shanli-1' (*A. rufa*), 'Wangmai-1', 'Wangmai-2' (*A. reticulata*) are also in this cluster. Cluster 2 consisted of 'Ruanzao-1', 'Ruanzao-2', 'Ruanzao-3', which belong to *A. arguta*. Cluster 3 is composed of only 'Gezao-1' (*A. polygama*). Cluster 4 included only species 'Sie-1' (*A. tetramera*). Cluster 5 included three species *A. latifolia* (Kuoye-1), *A. kolomikta* (Gouzao-1) and *A. indochinensis* (Zhongyue-1).

3.3. Principal component analysis

Principal coordinate analysis was performed based on the genetic similarity matrix to help understand the relationships between the genotypes (Fig.2). The distribution of different kiwifruit genotypes obtained from PCOA according to the two principal axes of variation that showed similar with dendrogram using UPGMA analysis.

4. Discussion

Evaluating diversity is the first step for future utilization and conservation of genetic resources (Naval et al., 2010). In tradition, the evaluation and identification of wild resources is based on the phenotypic characteristics. However, some phenotypic characteristics were affected by climate factors, such as temperature, altitude, rainfall and so on (Zhe et al. 2010). SRAP marker is a better molecular technique that could provide more polymorphisms information than ISSR, SSR, RAPD and AFLP (Budak et al., 2004; Jing et al., 2013b). Therefore, SRAP was used to analyze the genetic diversity and relationships among wild kiwifruit species and cultivated varieties. In this study, the twelve primer combinations generated high number of polymorphic bands (292), and 24.33 polymorphic bands per pairs of primers. Compared to previous studies, the wild kiwifruit species and cultivated varieties

revealed a high level of genetic diversity. For example, Huang et al. (1998) reported 12.4 polymorphic bands per locus in a 4 diploid and 6 tetraploid genotypes of *A. chinensis*. Zhen et al. (2004) found that the average 23.7 alleles per locus by 9 microsatellite markers after analyzing 47 kiwifruit cultivars. In addition, Huang et al. (2002) reported the 92% polymorphism for various *Actinidia* taxa using RAPD. Li et al. (2014) obtained an average of 96% polymorphic bands per pairs of primers in the 79 cultivars using AFLP marker. However, the polymorphic percentage was 100% for all pairs of primers in this study.

The genus *Actinidia* includes 55 species and about 76 taxa native to China (Li et al., 2009). Up to date, kiwifruit is an important and popular fruit in China. Since the 1980s, lots of wild kiwifruit species were collected in China. However, taxonomic fashions changes and it is still not to attempt formal descriptions of such infraspecific variation (McNeill 2004). And the genesis basis of kiwifruit and genetic relationships of some species have some controversy and confusion. For instance, the traditional green kiwifruit ‘Hayward’ has been variously treated over the past 100 years (Ferguson 2007). Therefore, it is necessary to analyze the genetic relationships of some kiwifruit species. In this study, the twelve species were clustered in five main groups. Some previous studies concluded that the close relationship existed between the *A. chinensis* and *A. chinensis* var. *deliciosa* (Testolin and Ferguson, 1997; Huang et al., 1997; Cipriani et al., 1998). Our findings from the clustering were similar with previous these results. Li et al. (2007) claimed *A. eriantha* and *A. chinensis* had the close genetic relationship. However, other researchers deemed *A. eriantha* had relatively far relationship with *A. chinensis* (Chat et al., 2004; Huang et al., 2002; Li et al., 2002). However our results showed the same with Li et al. (2007) that *A. chinensis* is very closely related to *A. eriantha*. In this study, we also found *A. rufa* and *A. arguta* had the far relationship. This result was the same as that obtained by Chat et al. (2004), Huang et al. (2002) and Li et al. (2002).

5. Conclusion

Our results suggested that SRAP marker is an efficient technique to evaluate the genetic relationships among wild kiwifruit species and cultivars. The studies of the taxa, phylogenetic relationships, and genetic diversity of different germplasm could help to assist the future development of breeding strategies. For instance, Species *A. chinensis*, *A. deliciosa*, and *A. eriantha* were found to be closely related. Therefore, some hybrids may be from crosses between *A. chinensis*, *A. chinensis* var. *deliciosa*, and *A. eriantha* based on the polyploidy levels. In addition, the results indicated that there was an abundant genetic diversity among wild kiwifruit species and cultivars, which provided theoretical basis for further breeding programs of *Actinidia*.

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Figure captions

Figure. 1

Dendrogram showing genetic relationships among 30 kiwifruit genotypes used through UPGMA analysis.

Note: 1. Funiushan-1, 2. Huayang-ZH1, 3. Zhonghua-WS, 4. Xixia-1, 5. Yuhuang-2, 6. Hongyang, 7. Lantian-MW1, 8. Ningshanhong, 9. Taibaihong, 10. Taibailv, 11. Huayang-MW1, 12. Huayang-MW2, 13.

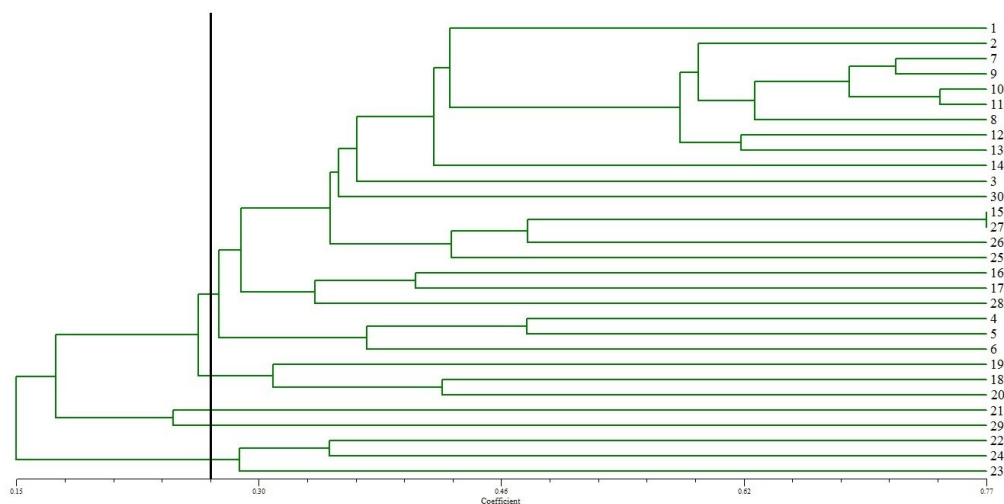
248 Shiquanhong, 14. Meiwei-HMZ, 15. Jinshuo, 16. Wangmai-1, 17. Wangmai-2, 18. Ruanzao-1, 19.
249 Ruanzao-2, 20. Ruanzao-3, 21. Gezao-1, 22. Kuoye-1, 23. Gouzao-1, 24. Zhongyue-1, 25. Maohua-1, 26.
250 Maohua-2, 27. Huate, 28. Dazi-1; 29. Sie-1, 30. Shanli-1.

251

252 Figure. 2

253 Two-dimensional plot of the principal component of 30 kiwifruit genotypes based on 12 SRAP
254 markers along first two principal axes.

255 Note: 1. Funiushan-1, 2. Huayang-ZH1, 3. Zhonghua-WS, 4. Xixia-1, 5. Yuhuang-2, 6. Hongyang, 7.
256 Lantian-MW1, 8. Ningshanhong, 9. Taibaihong, 10. Taibailv, 11. Huayang-MW1, 12. Huayang-MW2, 13.
257 Shiquanhong, 14. Meiwei-HMZ, 15. Jinshuo, 16. Wangmai-1, 17. Wangmai-2, 18. Ruanzao-1, 19.
258 Ruanzao-2, 20. Ruanzao-3, 21. Gezao-1, 22. Kuoye-1, 23. Gouzao-1, 24. Zhongyue-1, 25. Maohua-1, 26.
259 Maohua-2, 27. Huate, 28. Dazi-1; 29. Sie-1, 30. Shanli-1.



260

261 Figure 1

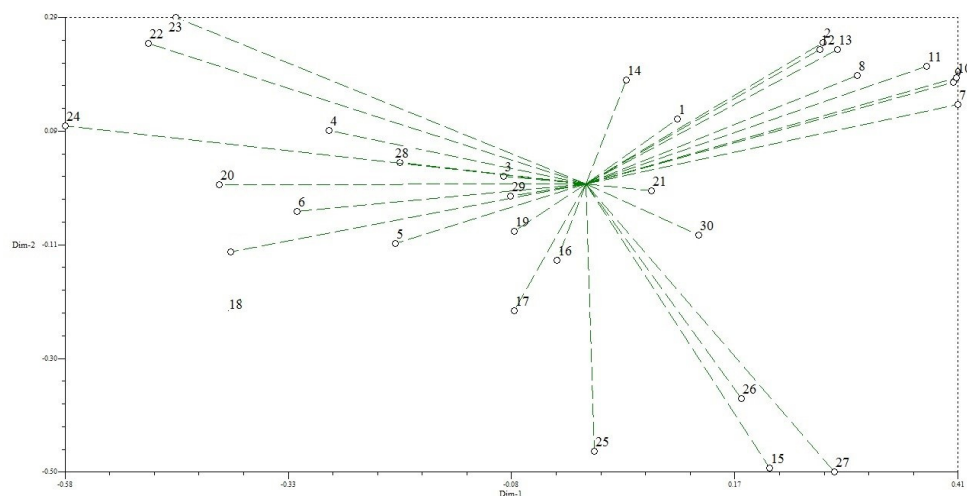


Figure 2

Table 1 *Actinidia* materials used in this study

Species	Category	Code no.	Genotype's name	Species	Category	Code no.	Genotype's name
<i>A.chinensis</i>	A	1	Funiushan-1	<i>A.cylindrica</i> var. <i>reticulata</i>	C	16	Wangmai-1
		2	Huayang-ZH1			17	Wangmai-2
		3	Zhonghua-WS			18	Ruanzao-1
		4	Xixia-1	<i>A.arguta</i>	D	19	Ruanzao-2
		5	Yuhuang-2			20	Ruanzao-3
		6	Hongyang			21	Gezao-1
<i>A.chinensis</i> var. <i>deliciosa</i>	B	7	Lantian-MW1	<i>A.polygama</i>	E	21	Gezao-1
		8	Ningshanhong	<i>A.latifolia</i>	F	22	Kuoye-1
		9	Taibaihong	<i>A.kolomikta</i>	G	23	Gouzao-1
		10	Taibailv	<i>A.indochinensis</i>	H	24	Zhongyue-1
		11	Huayang-MW1	<i>A.eriantha</i>	I	25	Maohua-1
		12	Huayang-MW2			26	Maohua-2
		13	Shiquanhong			27	Huate
		14	Meiwei-HMZ	<i>A.macrosperma</i>	J	28	Dazi-1
		15	Jinshuo	<i>A.tetramera</i>	K	29	Sie-1
				<i>A.rufa</i>	L	30	Shanli-1

268

Table 2 SRAP primer sequences used in this study

Primer code	Forward primer (5'–3')	Primer code	Reverse primer (5'–3')
ME1	TGA GTC CAA ACC GGATA	EM1	GAC TGC GTA CGA ATT AAT
ME2	TGA GTC CAA ACC GGAGC	EM3	GAC TGC GTA CGA ATT GAC
ME3	TGA GTC CAA ACC GGAAT	EM5	GAC TGC GTA CGA ATT AAC
ME4	TGA GTC CAA ACC GGACC	EM6	GAC TGC GTA CGA ATT GCA
ME5	TGA GTC CAA ACC GGAAG	EM8	GAC TGC GTA CGA ATT CAC
ME6	TGA GTC CAA ACC GGACA	EM9	GAC TGC GTA CGA ATT CAG
ME9	TGA GTC CAA ACC GGAGG	EM10	GAC TGC GTA CGA ATT CAT
ME11	TGA GTC CAA ACC GGAAC	EM12	GAC TGC GTA CGA ATT CTC
ME12	TGA GTC CAA ACC GGAGA	EM15	GAC TGC GTA CGA ATT GAT
		EM16	GAC TGC GTA CGA ATT GTC

269

270 **Table 3** The total and polymorphic loci generated in all genotypes by the selected twelve SRAP pairs of primes
 271 in this study

Primer combination	Number of total loci	Number of polymorphic loci	Percentage of polymorphic loci (%)
Me1+Em13	32	32	100
Me2+Em8	24	24	100
Me3+Em1	26	26	100
Me3+Em3	25	25	100
Me4+Em6	17	17	100
Me5+Em5	15	15	100
Me6+Em5	18	18	100
Me9+Em9	25	25	100
Me9+Em12	35	35	100
Me11+Em10	23	23	100
Me11+Em15	26	26	100
Me12+Em16	26	26	100
Total	292	292	100
Mean	24.33	24.33	100

272