Genetic Diversity and Relationships between and within Kiwifruit (*Actinidia*) Wild Species and Cultivated Varieties Using SRAP Markers

Zhao Bin Jing¹, Ming Xu² and Yu Shan Lei²

¹College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, P. R. China.  
²Shaanxi Rural Science and Technology Development Center, Xi’an 710054, Shaanxi, P. R. China.

Authors’ contributions

This work was carried out in collaboration between all authors. Author ZBJ designed the study, wrote the protocol, performed experiments and statistical analysis with the assistant from author MX. Author YSL mainly contributed in this study for some materials. Author ZBJ managed the literature searches, wrote the first and final draft of the manuscript and edited the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Kiwifruit (*Actinidia*) is one of the most important fruits in the world. Genetic diversity may provide the raw materials for programmers of plant breeding and crop improvement.

Materials and Methods: The aim of this study was to reveal the genetic diversity and relationships of 30 kiwifruit genotypes belonging to twelve different species using the SRAP marker.

Results: 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 kiwifruit genotypes 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.

*Corresponding author: E-mail: 353813638@qq.com;
Conclusion: This study provided theoretical basis for the genetic diversity and further breeding programs of Actinidia.

Keywords: Chinese wild kiwifruit species; Actinidia; genetic diversity; SRAP.

1. INTRODUCTION

Kiwifruit (Actinidia) is one of the most important fruits in the world, and it is widely used in food production with a high nutritional values. Kiwifruit 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 \(2n=8x=232\) [1]. The most widespread commercially cultivated species in the world are A. chinensis and A. deliciosa, and both species are native to China [2]. Up to 2013, the annual production of kiwifruits reached about 170 tons in China, and this showed that the cultivated areas and yields of kiwifruits have actually increased [3]. 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) [3]. (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 [4]. 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 [5].

In this study, the aim 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 that including 12 wild kiwifruit species native to China were sampled from kiwifruit germplasm repository in Shaanxi kiwifruit experimental station, 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 [13]. 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 (Tables 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. [11], with some modification. A 20 µL volume, containing 40 ng of template DNA, 10×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/µL) (TaKaRa Biotechnology Dalian Co., Ltd., China). PCR was performed in the following conditions: 5 min of denaturation at 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 [14]. The pairwise genetic distances among all materials according to Nei [15] were calculated based on Jaccard’s similarity coefficient. Cluster analysis was performed using NTSYS-pc version 2.1e software [14]. 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. 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’, ‘Huate’ (A. eriantha), ‘Dazi-1’ (A. macrosperma) and ‘Shanlini-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
[16]. In tradition, the evaluation of wild resources is based on the phenotypic characteristics. However, some phenotypic characteristics were affected by temperature, altitude, rainfall and so on [17]. SRAP marker is a better molecular technique that could provide more polymorphisms information than ISSR, SSR, RAPD and AFLP [11,12]. 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

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

Fig. 2. Two-dimensional plot of the principal component of 30 kiwifruit genotypes based on 12 SRAP markers along first two principal axes

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. Shiquanhong, 14. Meiwei-HMZ, 15. Jinshuo, 16. Wangmai-1, 17. Wangmai-2, 18. Ruanzao-1, 19. Ruanzao-2, 20. Ruanzao-3, 21. Gezao-1, 22. Kuoye-1, 23. Gouzao-1, 24. Zhongyue-1, 25. Maohua-1, 26. Maohua-2, 27. Huate, 28. Dazi-1; 29. Sie-1, 30. Shanli-1
polymorphic bands (292), and 24.33 polymorphic bands per pairs of primers. Compared with previous studies, the wild kiwifruit species and cultivated varieties revealed a high level of genetic diversity. For example, Huang et al. [18] reported 12.4 polymorphic bands per locus in a 4 diploid and 6 tetraploid genotypes of *A. chinensis*. Zhen et al. [19] found that the average 23.7 alleles per locus by 9 microsatellite markers after analyzing 47 kiwifruit cultivars. In addition, Huang et al. [7] reported the 92% polymorphism for various *Actinidia* taxa using RAPD. Li et al. [20] 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 China [21]. Up to date, kiwifruit is an important and popular fruit in the world. Since the 1980s, lots of wild kiwifruit species were collected from different ecology areas in China. However, taxonomic fashions changes and it is still not to attempt formal descriptions of such infraspecific variation [22]. And the genetic 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 [5]. 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* [18,23,24]. Our findings from the clustering were similar with previous these results. Li et al. [21] claimed *A. eriantha* and *A. chinensis* had the close genetic relationship. However, other researchers deemed *A. eriantha* had relatively far relationship with *A. chinensis* [7,25,26]. However our results showed the same with Li et al. [21] 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. [25], Huang et al. [7] and Li et al. [26].

### Table 1. *Actinidia* materials used in this study

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

### 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 CAA ATT AAT |
| ME2         | TGA GTC CAA ACC GGAGC  | EM3         | GAC TGC GTA CAA ATT GAC |
| ME3         | TGA GTC CAA ACC GGAAAT | EM5         | GAC TGC GTA CAA ATT AAC |
| ME4         | TGA GTC CAA ACC GGACC  | EM6         | GAC TGC GTA CAA ATT GCA |
| ME5         | TGA GTC CAA ACC GGAGC  | EM8         | GAC TGC GTA CAA ATT CAC |
| ME6         | TGA GTC CAA ACC GGACA  | EM9         | GAC TGC GTA CAA ATT CAG |
| ME9         | TGA GTC CAA ACC GGAGG  | EM10        | GAC TGC GTA CAA ATT CAT |
| ME11        | TGA GTC CAA ACC GGAAC  | EM12        | GAC TGC GTA CAA ATT CTC |
| ME12        | TGA GTC CAA ACC GGAGA  | EM15        | GAC TGC GTA CAA ATT GAT |
|             |                        | EM16        | GAC TGC GTA CAA ATT GTC |
Table 3. The total and polymorphic loci generated in all genotypes by the selected twelve SRAP pairs of primes 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                               |

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 taxon, phylogenetic relationships, and genetic diversity of different germplasm could help to assist the future development of breeding strategies. For instance, Species *A. chinensis*, *A. delicosa*, 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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