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Identification of the Kiwifruit Germplasms in Jiangxi Province by AFLP and Its Classification Significance

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Abstract The kiwifruit germplasms in Jiangxi province were identified by amplified fragment length polymorphism (AFLP) markers. Four primer pairs that had been selected from 64 ones had detected a total of 190 bands among 31 germplasms of kiwifruit, one hundred and seventy nine bands that representing 94.2 % of total bands were polymorphic. The identification rates of 31 germplasms were up to 100 %. The result suggests that applying AFLP markers to analyze the kiwifruit germplasms is feasible. Then Clustering analysis the AFLP result by UPGMA, the dendrogram indicated that the Dice similarity coefficients of 31 germplasms of kiwifruit ranged from 0.50 to 0.85, it suggested that their genetic relationships weren't near. Thirty one germplasms could divide into four groups according to the Dice similarity coefficient 0.56. *Sect. Leiocarpae* and *Sect. Maculatae* clustered into one group; *A. melliana* of *Sect. Strigosae* was one group; *A. chinensis var. rufopulpa*, *A. eriantha*, *A. fulvicoma var. lenata*, *A. styracifolia* and *A. jiangxiensis* in *Sect. Stellatae* clustered into the same group; but the two germplasms of *Sect. Stellatae*, *A. deliciosa* and *A. chinensis* clustered into the other group. Interestingly, the 'Ganmi-5' was in the same rank with *A. Chinensis* from the dendrogram, but it was the variety of *A. Chinensis* according to the traditional classification. It is necessary that the 'Ganmi-5' should be further researched in classification. The genetic relationships among the kiwifruit germplasms in Jiangxi province were identified and characterized by the molecular method, which was consistent with the traditional classification in certain degree and provided new evidences for the classification of the kiwifruit germplasms.

Key words kiwifruit; amplified fragment length polymorphism; fingerprinting pattern; clustering analysis; classification

应用 AFLP 标记对江西省猕猴桃种质资源的鉴别及其分类意义

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摘要 对江西省猕猴桃种质资源进行扩增片段长度多态性(AFLP)标记来鉴定分析. 首先从 64 对引物筛选出 4 对引物,对 31 份种质材料的 DNA 进行检测,得到 190 个扩增基因位点,其中多态性位点 179 个,多态性比例为 94.2 %,对 31 份种质材料的区分率达到 100 %. 然后,对扩增结果进行 UPGMA 聚类分析,谱系图显示,31 份种质材料之间的相似系数在 0.50～0.85 之间,表明猕猴桃种质之间遗传关系相对来说不是很近. 在相似系数 0.56 的水平上,可以将 31 份种质大致分为 4 个类

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群:净果组和斑果组为一类群;糙毛组为一类群;星毛组为一类群;中华猕猴桃和美味猕猴桃为一类群.从树状图中看,原为中华猕猴桃的一个变种的"赣猕5号",现与中华猕猴桃并列,有对其作进一步分类方面深入研究的必要.本研究从分子角度鉴定分析了江西省猕猴桃种质资源及其遗传关系,其结果在很大程度上与传统分类是一致的,同时也为江西省猕猴桃种质资源分类学研究提供了新的证据.

关键词 猕猴桃;扩增片段长度多态性分析;指纹图谱;聚类分析;分类

中图分类号 S 663.4

The classification of the kiwifruit germplasms went through four stages: morphology, palynology, biochemistry and molecular biology. Liang C. F. divided the Chinese kiwifruit germplasms into four sections: *Sect. Steliatae*, *Sect. Strigosae*, *Sect. Maculatae* and *Sect. Leiocarpae*. According to the characters of pith, he separated *Ser. Lamellatae* and *Ser. Solidae* from *Sect. Leiocarpae*, and divided the *Sect. Stellatae* into complete and incomplete *Sect. Stellatae*. Li *et al*. used branch analysis method to study the kiwifruit systematic development, they thought *Sect. Leiocarpae* could be divided into a single group, and suggested that it should establish two groups of *Sect. Maculatae* and *Sect. Leiocarpae*, and give them a subgenus position, *Sect. Maculatae* subgenus divided into three groups: *Sect. Maculatae*, *Sect. Stellatae* and *Sect. Strigosae*[1]. Huang *et al*[2] made polymorphic clustering analysis to the kiwifruit plants, their results didn't support the view of dividing kiwifruit plants into four groups, but they supported the division of *Sect. Stellatae*. He [3]observed leaf's skin micro-morphologic character of 35 representative Chinese kiwifruit plants and made branch analysis and UPGMA clustering analysis, which supported the group of *Sect. Stellatae* but didn't support three groups. The isoenzyme polymorphic research on kiwifruit did not support the traditional classification[4]. Li supported the most groups with the results of RAPD analysis, as *Sect. Leiocarpae*[5].

Although morphology markers and isoenzyme analysis were applied in germplasms identification and markers assistant selection of fruit trees[6~9], these methods had many shortages that limited their application, such as: limited remarkable quantities, low polymorphism level, etc. However, DNA molecular markers had advantages on these aspects[10,11]. The amplified fragment length polymorphism (AFLP) markers was the new molecular markers, which had high efficiency and stability. It was used to construct many the fingerprinting patterns of plants, for example: apple[12], grape[13] etc. So far, there were no reports that detecting the kiwifruit germplasm by the AFLP markers and others molecular markers[14,15]. On base of relating research[5, 16~19], this study used the AFLP markers to identify the 31 germplasms of kiwifruit that included 11 species, 5 varieties and 15 cultivars in Jiangxi, and researched their genetic relationship and classification at molecular level, which tried to provide new evidences for the kiwifruit germplasms classification.

1 Materials and Methods

1.1 Materials

The 31 germplasms of kiwifruit that included 11 species, 5 varieties and 15 cultivars were obtained from the orchard of Jiangxi Agricultural University Research Institution, Fengxin Fruit Institution Kiwifruit and Ruichang Agriculture Scientific Institution (Table 1).

1.2 Methods

1.2.1 AFLP Amplification

DNA was extracted and isolated by modified CTAB method[20].

AFLP amplification was made according to the reaction procedures which were provided by Beijing Dingguo company, the reaction procedures included template DNA preparation, enzyme digestion and ligation, pre-amplification, selective amplification and polyacrylamide gel electrophoresis[21].

1.2.2 Pattern recording and data analysis

1) Pattern record: Using fluorescent patterns which had been printed to record. The bands was scored as 1 for presence or 0 for absence from the fingerprinting patterns to generate the Table (0, 1).

2) Clustering analysis: All recorded data were input computer to calculate the frequency, coefficient of variation, coefficient of similarity and clustering analysis[22]. Using NTSYS-pc 2.10e data analysis software to calculate the Dice similarity coefficient of of the AFLP fingerprinting patterns ($Sij = 2Nij/Ni + Nj$, Nij is the number of all bands of i germplasm and j germplasm; Ni and Nj is the number of amplified bands of i germplasm and j germplasm, respectively), which gained the matrix of Dice's coefficient of similarity; Using UPGMA (unweighted pair-group method, arithmetic average) to clustering analysis, which gained the dendrogram of kiwifruit germplasms in Jiangxi province.

3) Polymorphic analysis: The polymorphic bands were the amplified bands that some materials had while the others didn't have. Polymorphic rates = (the number of all bands - the number of common bands)/the number of all bands ×100 %.

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2 Results

2.1 The effect of AFLP markers

Four primer pairs that had been selected from 64 ones (E-AAC + M-CAC; E-AAG + M- CTG; E-AAC + M-CA G; E-AAC + M-CTA) detected 31 germplasms of kiwifruit in Jiangxi province by AFLP In this study. The fingerprinting patterns of each primer pairs were clear and all germplasms were polymorphic (Fig. 1—4). The result suggests that applying AFLP markers to identify is feasible in this research.

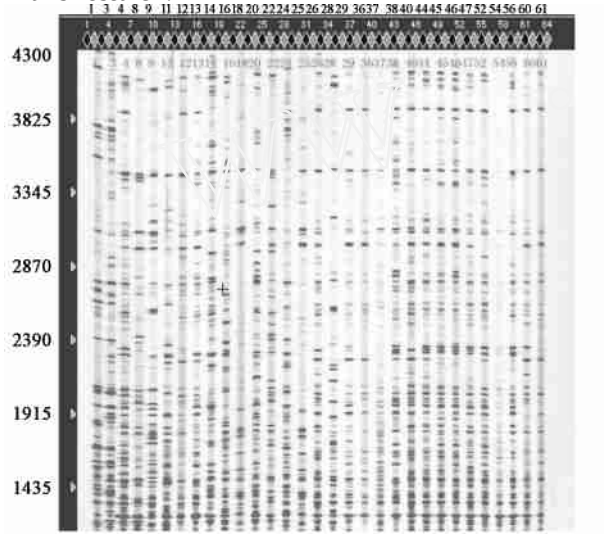

Fig. 1 Fingerprinting pattern of 31germplasms of kiwifruit in Jiangxi Province by AFLP and gel electrophoresis
(prime pairs: E-AAC + M-CAC)

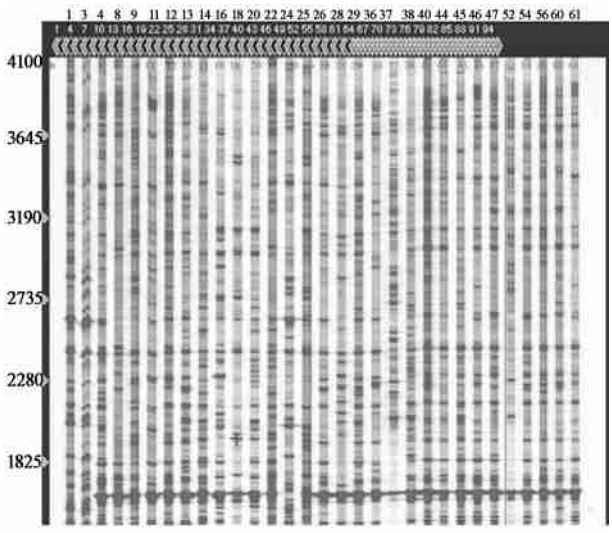

Fig. 2 Fingerprinting pattern of 31germplasms of kiwifruit in Jiangxi Province by AFLP and gel electrophoresis
(prime pairs: E-AAG + M-CTG)

2.2 The polymorphism of AFLP markers of samples

Using the AFLP to identify the kiwifruit germplasms, a total of 190 bands was detected by four primer pairs and 179 bands, representing 94.2 % of total bands, were

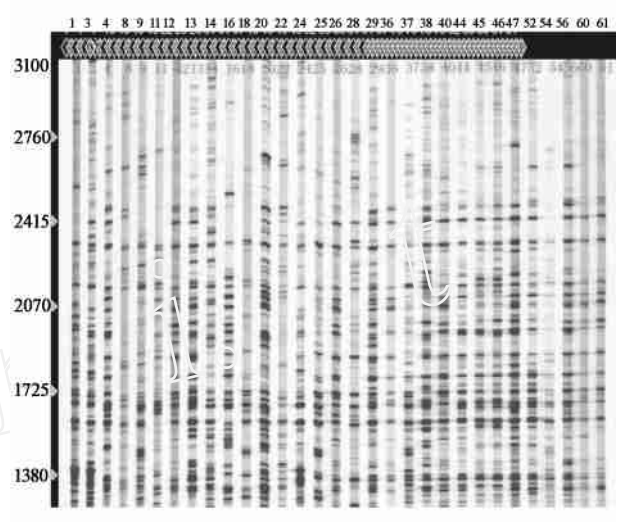

Fig. 3 Fingerprinting pattern of 31 germplasms of kiwifruit in Jiangxi Province by AFLP and gel electrophoresis
(prime pairs: E-AAC + M-CAG)

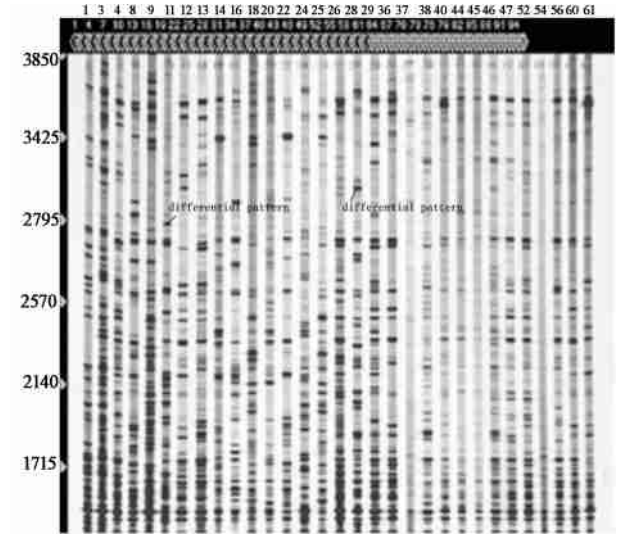

Fig. 4 Fingerprinting pattern of 31 germplasms of kiwifruit in Jiangxi Province by AFLP and gel electrophoresis
(prime pairs: E-AAC + M-CTA)

polymorphic. The identification rates of 31 germplasms of kiwifruit were up to 100 %. The number and proportion of the polymorphic bands of each germplasm were different. One hundred and eight polymorphic bands of *A. melanandra* was the most, and the proportion of the total was 60.3 %, which suggested that it had high heterozygous degree. Whereas Zaoxian had 54 polymorphic bands which was the least, and the proportion was 30.2 % (Table 1). Four primer pairs detected a total of 190 amplified bands, each primer pairs had an average of 47.5 amplified bands. From Table 2, the polymorphic bands of each primer pairs were very abundant. So we could absolutely distinguish 31

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Table 1 The number of polymorphic bands and proportion to total bands of 31 germplasms of kiwifruit

No.	Name	Section	Origin	Polymorphic bands	Polymorphic proportions(%)
1	*Actinidia arguta*	*Sect. Leiocarpae*	Wild	93	52.0
3	*A. melanandra*	*Sect. Leiocarpae*	Wild	108	60.3
4	*A. polygama*	*Sect. Leiocarpae*	Wild	103	57.5
22	*A. macrosperma*	*Sect. Leiocarpae*	Wild	71	39.7
24	*A. arguta var. purpurea*	*Sect. Leiocarpae*	Wild	66	36.9
25	*A. macrosperma var. mumoides*	*Sect. Leiocarpae*	Wild	64	35.8
8	*A. rubricaulis var. coriacea*	*Sect. Maculatae*	Wild	76	42.5
9	*A. sabiaefolia*	*Sect. Maculatae*	Wild	106	59.2
11	*A. melliana*	*Sect. Strigosae*	Wild	69	38.5
12	*A. chinensis*	*Sect. Stellatae*	Wild	87	48.6
13	*A. chinensis var. rufopulpa*	*Sect. Stellatae*	Wild	67	37.4
14	*A. eriantha*	*Sect. Stellatae*	Wild	84	46.9
16	*A. fulvicoma var. lenata*	*Sect. Stellatae*	Wild	66	36.9
18	*A. styracifolia*	*Sect. Stellatae*	Wild	70	39.1
20	*A. jiangxiensis*	*Sect. Stellatae*	Wild	77	43.0
37	*A. deliciosa*	*Sect. Stellatae*	Wild	64	35.8
26	Kuimi		Cultivar (Jiangxi)	82	45.8
28	Ganmi-5		Cultivar (Jiangxi)	74	41.3
29	Lushanxiang		Cultivar (Jiangxi)	89	49.7
36	Zaoxian		Cultivar (Jiangxi)	54	30.2
38	Jinfeng		Cultivar (Jiangxi)	78	43.6
40	Jinkui		Cultivar (Hubei)	92	51.4
44	Miliang-1		Cultivar (Hunan)	75	41.9
45	Alisen		Cultivar (New Zealand)	79	44.1
46	Wancui		Cultivar (Anhui)	91	50.8
47	Qinmei		Cultivar (Shanxi)	83	46.4
52	Jinkui-1 ♂		Cultivar (Hubei)	83	46.4
54	Jinkui-6 ♂		Cultivar (Jiangxi)	70	39.1
56	Zengbang-1 ♂		Cultivar (Hunan)	75	41.9
60	Fengxiong-1 ♂		Cultivar (Jiangxi)	70	39.1
61	Fengxiong-2 ♂		Cultivar (Jiangxi)	63	35.2

108 polymorphic bands of *A. melanandra* was the most and the proportion was 60.3%, whereas Zaoxian had 54 polymorphic bands which was the least and the proportion was 30.2%

germplasms of kiwifruit.

2.3 Clustering analysis the AFLP amplified bands

190 amplified bands were detected by the four primer pairs, which were recorded to 0 or 1. The Dice similarity coefficients matrix was built on the table(0,1). Clustering analysis of the Dice similarity coefficients by UPGMA, we gain the dendrogram (Fig. 5).

The Dice similarity coefficients of the 31 germplasms of kiwifruit ranged from 0.50 to 0.85. The result showed that the 31 germplasms could be divided into four groups according to the Dice similarity coefficient 0.56 and the genetic relationships among them weren't close. As follows:

Group Ⅰ: *Actinidia arguta*; *A. melanandra*; *A. polygama*; *A. rubricaulis var. coriacea*; *A. sabiaefolia*; *A. macrosperma*; *A. arguta var. purpurea*; *A. macrosperma var. mumoides*

Group Ⅱ: *A. chinensis*; Ganmi-5; Kuimi; Lushanxiang; Zaoxian; Jinfeng; *A. deliciosa*; Jinkui; Miliang-1; Alisen; Wancui; Qinmei; Jinkui-1 ♂; Jinkui-6 ♂; Zengbang-1 ♂; Fengxiong-1; Fengxiong-2

Group Ⅲ: *A. chinensis var. rufopulpa*; *A. eriantha*; *A. fulvicoma var. lenata*; *A. styracifolia*; *A. jiangxiensis* Group Ⅳ: *A. melliana*

Table 2 The polymorphic bands of each primer pairs

Prime pairs	Amplified bands	Polymorphic bands	Ratio of polymorphic bands(%)
E-AAC + M-CAC	51	49	96.1
E-AAG + M-CTG	49	47	95.9
E-AAC + M-CAG	51	49	96.1
E-AAC + M-CTA	39	34	87.2

Four primer pairs detected a total of 190 bands and 179 polymorphic bands was 94.2% of the total bands.

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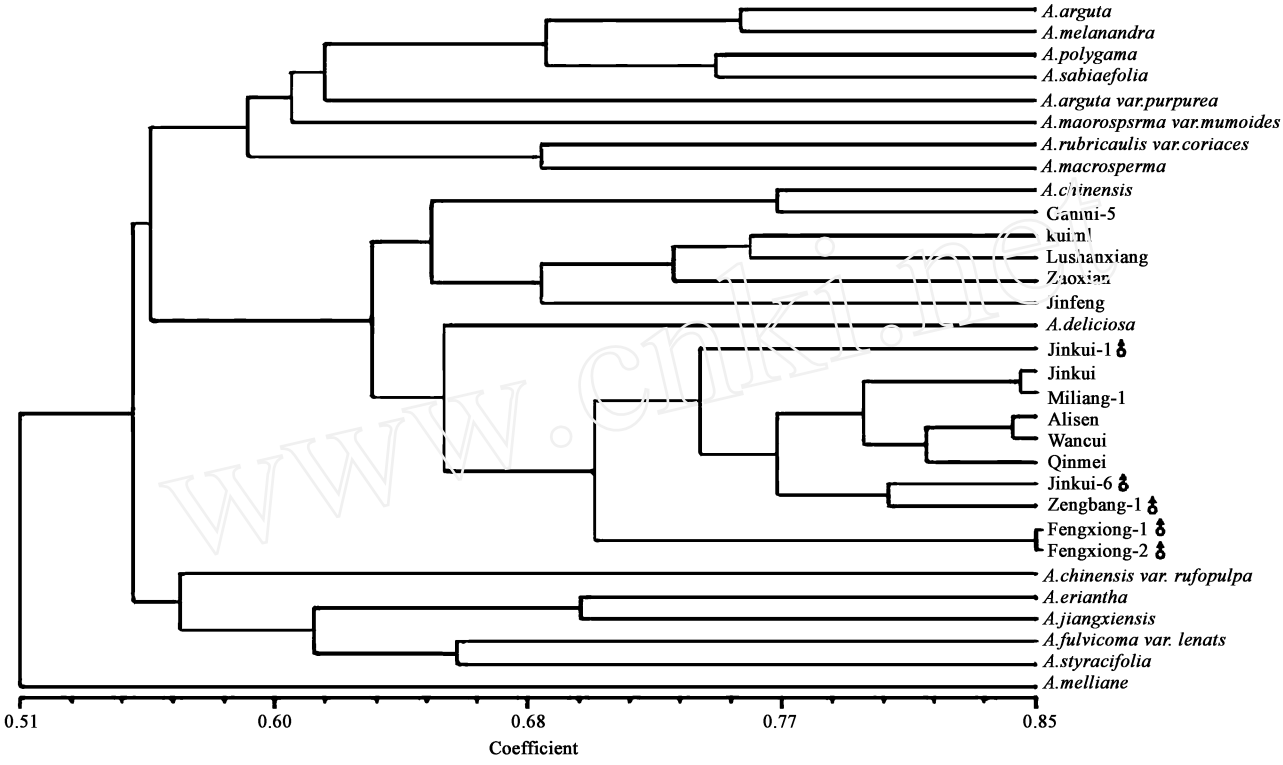

Fig. 5 The dendrogram of clustering analysis the Dice similarity coefficients by UPGMA
The 31 germplasms could be divided into four groups according to the Dice similarity coefficient 0.56

In general, the division result supported a majority of the traditional classification that created by Liang in this study. From the dendrogram, *Sect. Leiocarpae* and *Sect. Maculatae* clustered into the same group; *A. melliana* of *Sect. Strigosae* was one group, which had a far relationaship with the other germplasms. *A. chinensis var. rufopulpa*, *A. eriantha*, *A. fulvicoma var. lenata*, *A. styracifolia* and *A. jiangxiensis* of *Sect. Stellatae* clustered into one group, but two germplasms of *Sect. Stellatae*, *A. deliciosa* and *A. chinensis* were the other group. In this study, *Sect. Leiocarpae* and *Sect. Maculatae* cooperated into one group, although they had a little difference of the spot on the fruit surface and the beak on the top of he fruit according to the traditional classification, and there were no division of *Ser. Lamellatae* and *Ser. Solidae*. *A. melliana* of *Sect. Strigosae* was still one group, which consistented with the traditional classification. *Actinidia arguta* and *A. melanandra* had a near genetic relationship because their Dice similarity coefficient was 0.75. *A. Chinensie* and *A. deliciosa* separated from *Sect. Stellatae* and clustered into one group. Interestingly, the 'Ganmi-5' was in the same rank with *A. Chinensis* and their Dice similarity coefficient was 0.77 from the dendrogram, but it is the variety of *A. Chinensis* according to traditional classification. It is necessary that the 'Ganmi-5' should be further researched in classification. From the dot-diagram (Fig. 6), many dots accumulated to a column pattern at the coefficient of 0.56, but the dots were dispersive in the other position. The result of classifying the 31 germplasms by dot-diagram was consisted with the dendrogram.

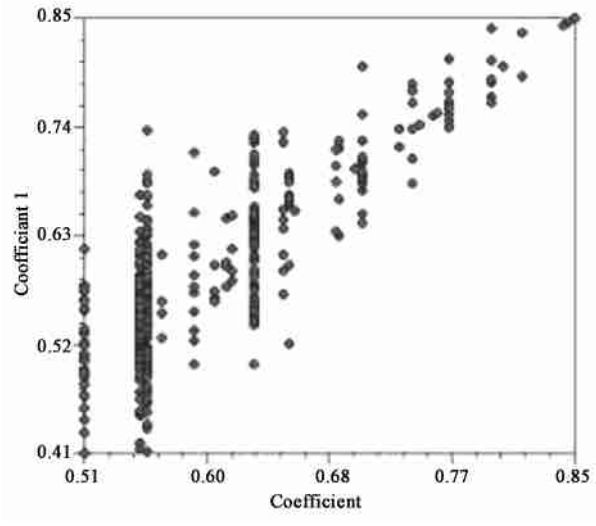

Fig. 6 The dot-diagram of clustering analysis the Dice similarity coefficients by UPGMA
The result of classifying the 31 germplasms by dot-diagram was consisted with the dendrogram

2.4 Identification of the genetic relationships of the kiwifruit germplasms in Jiangxi province

The dendrogram showed that the genetic relationships

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of 31 germplasms of kiwifruit in Jiangxi province. The result hadn't conflict with the traditional classification, but there were differential modification of some germplasms.

A. deliciosa that had been named *A. chinensis var. hispida* is the variety of *A. Chinensis*. In 1984, Liang C. F. and A. R. Ferguson had approved it as an independent species. The similarity coefficient was 0.70, which showed that it had a near genetic relationship with *A. Chinensis*. From the DNA fingerprinting patterns, they had some special bands respectively. *A. chinensis var. rufopulpa* is the variety of *A. Chinensis* which has the character of red pulp. They should have near genetic relationship, but their similarity coefficient was 0.63, which showed that *A. chinensis var. rufopulpa* became far from *A. Chinensis* during the process of evolution.

Wancui, the natural bud mutation of 'Haward', is the important cultivar of *A. deliciosa* in Jiangxi, which was breeded by Anhui Agriculture University. Its fruit shape is long column with flesh green pulp, the weigh is about 117.5 g, SSC is 16.5 % and Vc 71.5 mg per 100 g, it is ripe in the second ten days of October. The dendrogram and Dice similarity coefficient showed that it had near genetic relationship with Allison (the earliest immigrant species in New Zealand, it has long column fruit with thick and hard pericarp, green pulp and Vc 31.76 mg per 100 g), their similarity coefficient was 0.84. This might be related with their filiation, because Haward and Allison were immigrant from New Zealand and Wancui was the natural bud mutation of Haward.

There are two kinds of leaf shapes of *A. arguta var. perpurea*, one was big and thin and the other was thick and narrow, the later had nondense and small sawtooth. It was the new combination germplasms of *A. arguta*. Their similarity coefficient was 0.62, which showed that *A. arguta var. perpurea* and *A. arguta* had far genetic relationship. The reason might be the intensive differentiation of *A. arguta*, especially in the valley of the *Yangtse* Rive.

A. melliana of *Sect. Strigosae* was one group, which lost many common bands and had one special band in the DNA fingerprinting patterns (Fig. 4). This suggests that it had far genetic relationship with the other germplasms.

Zaoxian, Lushanxiang and Jinfeng were the cultivars in Jiangxi. From the dendrogram, the identification rates of them was up to 100 %. The similarity coefficient of Kuimi and Lushanxiang was 0.76, which indicated that they had near genetic relationship. In fact, they also had the same harvest time in September.

Jinkui-1 ♂ (introduced from Hubei Fruit Trees and Tea Institute) and Jinkui-6 ♂ (selected by Fengxin) were the male germplasms, which had no difference in morphology. Both of them were the pollination plants to Jinkui, but the pollination ability of the latter was more excellent than the former. They had far genetic relationship because their similarity coefficient was 0.69 and the DNA fingerprinting patterns was different. Zengbang-1 ♂ (the pollination plant to Miliang-1) had a little difference from Jinkui-6 ♂. Their similarity coefficient was 0.80, that is to say, their genetic relationships were near. Fengxiong-1 ♂ and Fengxiong-2 ♂ were very similar in morphology, but they had different pollination ability. The former could only pollinate to *A. Chinensis*, but the latter could pollinate not only to *A. Chinensis* but also to *A. deliciosa*. Their similarity coefficient was 0.85, which showed that they had only a little difference.

2.5 The analysis of the particular germplasms

The fruit of 'Qingmei' and 'Jinkui' were the big type fruit cultivars of *A. deliciosa*, their biggest fruits weighted 204 g and 203 g, respectively. Their genetic relationship was near from their Dice similarity coefficient of 0.77.

'Zaoxian' is a early ripe and flesh-eaten germplasm of *A. Chinedsis*, it is ripe in the middle of August.

The character of red pulp could enhance commercial value, especially in handicraft dish. There were some red pulp germplasms in China, such as *A. chinensis var. rufopulpa*. Its Dice similarity coefficient was 0.58 with *A. Chinesis* and was in another group, which indicated that it had far genetic relationship with the other germplasms.

'Ganmi-5' (Fig. 7) had short nodes, compact clusteres branches and small crown, which was good to culture without aid pole. Its fruit weights 85 g, the pulp is flesh green and tastes well with aroma, the SSC is 17.16 %, Vc is 83.9 mg per 100 g, the harvest time is the second ten days of October. From the dendrogram, it was in the same rank with *A. Chinensis*, the reason might be chronical variance and stable heredity under the natural environment. Comparing botanical characters between 'Ganmi-5' and *A. Chinensis* (Table 3),

Fig. 7 The plant of 'Ganmi-5'

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'Ganmi-5' had greater difference of *A. Chinensis*. It is necessary that the 'Ganmi-5' should be further researched in classification. As a special germplasm in Jiangxi, we should not only preserve it, but also make good use of it. The special bands (Fig. 4) in DNA fingerprinting patterns contrasting with *A. Chinesis* could be used to clone the shortening gene in breeding.

Table 3 The comparison of botanical character between A. Chinensis **and** 'Ganmi-5'

Botanical character	*A. Chinensis*	'Ganmi-5'
Plant height	30 m	1 m
Crown extent	≫1.5 m ×1.5 m	1.5 m ×1.5 m
Tress	2 m or so	1 m or so
Watershoot	6 m, even exceed 10 m	1.5 m
Nodes length	> 3.7 m	3.7 m
Twig	green、amaranth	blue-gray
A-year-branch	henna	henna
Top of tress	anti-clockwise twist	no-anti-clockwise twist
Lamina	roundness、short-roundness	near sphericity
Bud	obvious bud	obvious bud
Flower-bud	blend bud	blend bud
Petal	5—11	6
Germen station	epigyny	epigyny
Fruit shape	near sphericity, oblong	apple shape
Pericarp color	green, brown	sandy beige
Pulp color	green, Kelly, fleet red	emerald
Vc	100—400 mg/100 g or so	83.9 mg/100 g
Fruit autumn	middle ten days of Sep.	first ten days of Oct.

3 Discussion

3.1 The feasibility of applying AFLP to this research

From the results of the experiment, we could see that the AFLP markers had many merits. First of all, The AFLP markers had high amplified efficiency and abundance of information. Usually, each amplified band corresponded to a gene site of DNA in AFLP markers analysis. Four primer pairs detected a total of 190 amplified bands with various molecular mass of 31 germplasms of Kiwifruit in this study. That is, the genomic DNA of 31 germplasms of Kiwifruit were detected 190 genetic sites. The polymorphic amplified band suggested that one or more materials had mutated in this site. There were a total of 179 polymorphic bands which representing 94.2% of total bands. Four primer pairs (E-AAC + M-CAC; E-AAG + M-CTG; E-AAC + M-CAG; E-AAC + M-CAT) detected 49, 47, 49, 34 polymorphic bands, respectively. The precision and efficiency of this method were better than any other fingerprinting techniques. Furthermore, polyacrylamide gel electrophoresis was used to segregate amplified bands in AFLP analysis. The concentration of gel was 7%, which had high differentiation and could segregate the DNA molecules that had 0.2% length difference (1 bp in 500 bp). Finally, the results of AFLP analysis were stable and could be repeated well, because reasonable man-made ligation adapters reaction and pre-amplified reaction were used to purify selectively template after DNA double digestion. All together, using AFLP markers to identify the kiwifruit germplasms is feasible in this study.

3.2 The significance of classification about analysis the Kiwifruit germplasm in Jiangxi by AFLP

Using AFLP markers and clustering analysis to research the Kiwifruit germplasms, which gained the fingerprinting patterns and dendrogram of 31 germplasms of Kiwifruit in jiangxi. Thirty one germplasms of Kiwifruit in Jiangxi were divided into four groups: *Sect. Maculatae* and *Sect. Leiocarpae* clustered into one group; *A. melliana* of *Sect. Strigosae* was one group; *A. chinensis var. rufopulpa*, *A. eriantha*, *A. fulvicoma var. lenata*, *A. styracifolia and A. jiangxiensis* of *Sect. Stellatae* clustered into the same group, whereas the two germplasms of *Sect. Stellatae*, *A. deliciosa* and *A. chinensis*, clustered into the other group. The results didn't absolutely support traditional classification. It supported that *Sect. Strigosae* was one group. Although most germplasms of *Sect. Strigosae* was consistent with traditional classification, *A. deliciosa* and *A. chinensis* clustered into the other group from *Sect. Strigosae*. In general, This study made clear of genetic relationship between 31 germplasms of kiwifruit in jiangxi, which is consistent with the tradition classification in certain degree and provides new evidences for the classification of the kiwifruit germplasms. At the same time, the results of AFLP markers could also associate with morphology to expose the essence of genetic evolution mechanism and provide some evidences for establishing a reasonable classification of Kiwifruit germplasms.

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