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Establishment of AFLP Technique and Assessment of Primer Combinations for Mei Flower

CHAODONG YANG[1,2], JUNWEI ZHANG[1], QIANG XU[3], CAIFENG XIONG[4] and MANZHU BAO[1,*]

[1]College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070 China; [2]Xiaogan University, Xiaogan 432100 China; [3]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070 China; [4]China Mei Flower Research Center, Wuhan 430074 China

Abstract. Mei flower is one of the most famous ornamental flowers in eastern Asia for its blossoming in early spring. Amplified fragment length polymorphism (AFLP) is one of the most frequently used techniques for analysis of genetic variation and is used herein for the first time in *Prunus mume*. This research provides a detailed and modified AFLP protocol for Mei genomic DNA digested with *Eco*RI/*Pst*I restriction endonuclease combinations. The 10 best primer pairs of high polymorphism were screened from 256 primer combinations that could reliably and repetitively distinguish 14 Mei samples and would be suitable for genetic analysis of more cultivars. Ten primer pairs produced up to a total of 524 AFLP bands and up to 233 polymorphic bands. The ratio of polymorphic bands scoped from 35.71% to 59.67%, and the average ratio was 44.46% in the 10 primers. AFLP is an effective, inexpensive, and timesaving technique for the genetic differentiation of the Mei cultivars, as evidenced in this study.

Full text[†]: This article, in detail, is available only in the electronic version of the *Plant Molecular Biology Reporter.*

Key words: amplified fragment length polymorphism, genetic markers, primers selection, *Prunus mume* Sieb. et Zucc.

Abbreviations: AFLP, amplified fragment length polymorphism; BSA, bovine serum albumin; PCR, polymerase chain reaction; RAPD, random amplification of polymorphic DNA; SDS, sodium dodecyl sulfate; SSR, simple sequence repeat; STS, sequence tagged site; *Taq*, *Thermus aquaticus*; TBE, tris-borate-EDTA; TE, tris EDTA.

Introduction

Mei flower (*Prunus mume*, 2n = 16) is one of the most famous ornamental flowers and fruit trees in China, Korea, and Japan and belongs to the Rosaceae family, subfamily Prunoidese, genus *Prunus* L. In 1976, Watkins postulated a center of

[*]Author for correspondence. e-mail: mzbao@mail.hzau.edu.cn; fax: +86 27 8728 2095; ph: +86 27 8728 2435.

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origin for *Prunus* in central Asia. Amplified fragment length polymorphism (AFLP) markers in apricot supported the historical dissemination from its center of origin in Asia (Hagen et al., 2002). The mountainous country in west China was considered to be the natural distribution and genetic diversity centre of Mei (Bao and Chen, 1994). Mei flower cultivars were classified into 3 branches, 6 groups, and 25 forms distinguished by its origin, branch gesture, petal color, and other morphological characters in China (Chen, 1996). Mei cultivars in Japan were classified into 7 groups examined by fruit size, petal color, and random amplification of polymorphic DNA (RAPD) markers (Shimada et al., 1994).

Most fruit crops in *Rosaceae* are woody perennials with a long intergeneration period due to their juvenile phase and large plant sizes, which make them poorly suited for classic genetic analysis. Diploid *Prunus* species have the smallest genome among the major cultivated plants, with a genome size of 0.3 pg/1C (Bennett and Leitch, 1995). A number of polymerase chain reaction (PCR)–based markers (RAPD, AFLP, sequence tagged site [STS], simple sequence repeat [SSR]) have been developed in recent years. Apricot diversity has been studied by using isozymes (Byrne and Littleton, 1989), RFLP (de Vicente et al., 1998), and AFLP (Hagen et al., 2002). Genetic linkage maps and comparative mapping of *Prunus* species have been published using molecular markers (Vilanova et al., 2003; Dirlewanger et al., 2004). AFLP markers of sharka resistance were examined in apricot (Hurtado et al., 2002). Thirteen self-incompatibility genes around the S-RNase gene have been investigated in *Prunus mume* (Entani et al., 2003). However, studies on Mei cultivar core collections; enforcement of cultivar patent rights; and analyses of genetic relationship, diversity, and phylogenetic data are very few at the DNA level. Knowledge of genetic variation among the available Mei flower germplasm is an important prerequisite for future breeding and improvement programs. Marker-assisted breeding offers great advantages for plant breeders for Mei cultivar improvement.

AFLP is frequently used for the identification of molecular markers because of certain advantages over other techniques, such as high level of identified polymorphism, high reproducibility, and relative technical simplicity. Because PCR primers are based on the sequences of the restriction enzyme and universal adapters to which they are ligated, the procedure requires no prior information about the nucleotide sequences under investigation. AFLP markers are highly polymorphic and reproducible and thus represent a powerful technique for DNA analysis that has improved fingerprinting and diversity studies (Vos et al., 1995). Modified AFLP methods have been developed for some species in recent years (Manubens et al., 1999; James et al., 2003; Kazachkova et al., 2004).

Mei has been domesticated for more than 2,000 y in China with abundant cultivars. However, the genetic relationships of Mei cultivars were unclear. To obtain Mei genetic variation information accurately and in detail, 14 Mei samples from the China Mei Flower Research Center employing the AFLP technique were investigated. The selected AFLP primers described below are especially useful when a large number of Mei cultivars is studied, as they can speed up the analysis and minimize expenses. In this study, an improved procedure was established, and optimal AFLP primer combinations in Mei were selected.

Materials and Methods

Plant materials

Thirteen Mei cultivars and a variety employed for screening AFLP primer combinations were collected from the China Mei Flower Research Center. These materials include Danhong Chuizhi, Xue Mei, Xiao Gongfen, Jiangnan Zhusha, Jiangsha Gongfen, Jiangnan Taige, Da Yu, Fenpi Gongfen, Duo-e Zhusha, Fuban Xiao Gongfen, Tiaoxue Chuizhi, Can Xue, Kinse-shidare, and *Prunus mume* var. *cernua*.

DNA extraction

Genomic DNA was isolated from leaf samples following the SDS method (Dellaporta et al., 1983) and diluted to 100 ng/μL for AFLP analysis. The quality of genomic DNA was examined by means of agarose gel (0.8%) electrophoresis, stained with ethidium bromide, and visualised under UV fluorescence. The quality and quantity of genomic DNA was accurately measured with spectrophotometric absorbency at 260 nm, 280 nm, and 220 nm, respectively.

AFLP adapters and primers

The AFLP reactions were referenced as described by Vos (1995). Adapters and primers were synthesized by Sunbiotech (China). Adapters were denaturated as follows: 65°C for 10 min, 37°C for 10 min, 25°C for 10 min, 4°C for 25 min, and stored at –20°C. The structure of the *Eco*RI adapter is:

5′-CTCGTAGACTGCGTACC
CATCTGACGCATGGTTAA-5′

The structure of the *Pst*I adapter is:

5′-ATGAGTCCTGAGGTGCA
GACGTACTCAGGACTCC-5′

Adapters are compatible with the restriction endonuclease combinations *Eco*RI/*Pst*I sites in this study. Primers sequences for the first round of PCR amplification included a core sequence and enzyme-specific sequences. Primers for the second round of PCR included the same core and enzyme sequences followed by 2 selective bases.

AFLP template preparation

Genomic DNA (200 ng) digested with *Eco*RI and *Pst*I, each restriction endonuclease with 6-bp recognition site. Digestion was performed at 37°C for 4 h with 3 U *Eco*RI, 3 U *Pst*I in 25 μL volume and 1× buffer H (90 mM Tris-HC1 [pH 7.5], 10 mM $MgCl_2$, 50 mM NaCl, and 50 ng/μL BSA). After digestion, the block was incubated at 65°C for 30 min.

The digestion block was added to a 25-μL mixture solution comprised of 50 ng *Eco*RI adapter, 50 ng *Pst*I adapter, 1 U T4 DNA ligase, and 1× ligase buffer (1 mM ATP, 30 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 10 mM MDTT, 50 ng/μL BSA) at 16°C for 4 h. This ligation mixture was diluted 10× with TE buffer, stored at –20°C, and used as template in the preamplification PCR reaction.

AFLP reactions

The preamplification volume contained 1 U *Taq* polymerase, 3 µL ligation mixture, 50 ng *Eco*RI primer, 50 ng *Pst*I primer, 0.2 mM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and sterile water to 25 µL. Preamplification primers had no selective base. This reaction was performed for 25 cycles with the following cycling profile: 1 min denaturation at 94°C, 30 s annealing at 56°C, and 90 s extension at 72°C. A final extension step incubated at 72°C for 5 min. After 30-fold dilution in TE buffer, this stock was used for all further selective amplifications.

Selective amplification volume contained 0.8 U *Taq* polymerase; 3 µL preamplification mixture; 50 ng *Eco*RI selective primer and 50 ng *Pst*I selective primer with 2 selective bases, respectively; 0.2 mM dNTPs; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; add sterile water to 20 µL. This reaction was performed for 35 cycles with the following cycling profile: 1 min denaturation at 94°C, 30 s annealing step (see below), and 90 s extension at 72°C. The annealing temperature in the first cycle was 65°C, and this was subsequently reduced each cycle by 1°C for the next 10 cycles and was continued at 56°C for the remaining 25 cycles. A final extension step incubated at 72°C for 5 min. All amplification reactions were performed in a PTC-100TM thermocycler (MJ Research, Inc.). In all blocks, 30 µL of mineral oil was added to prevent water evaporation.

Gel electrophoresis and silver staining

The quality and quantity of the amplification product was examined by means of agarose (0.8%) gel electrophoresis stained with ethidium bromide and visualised under UV fluorescence as a smear across bromophenol blue. We added 9 µL of deionized formamide, 1 µL of 5× TBE buffer, 0.05% (w/v) of bromophenol blue, and 0.05% (w/v) of xylene cyanol FF to the 10 µL PCR products, and the PCR products were denaturated for 3-5 min in boiling water. The blocks were immediately placed on ice for 10 min. The AFLP products were separated on 6.0% (w/v) denatured polyacrylamide gel. The gel was pre-electrophoresis run at 80 W (1700-1800 V) in TBE 1.0× on a vertical gel electrophoresis system about 30-40 min (Sequi-Gen@ GT, sequencing cell, BIO-RAD). After loading the denatured PCR products, electrophoresis was performed at 120 W for 2 h. Upon electrophoresis, gels were fixed and stained with silver nitrate referenced to manual of sequencing Kit Q4132 (Promega).

Results and Discussion

AFLP analysis - Primers selection with 1 sample

Extracted DNA of Mei was digested with *Eco*RI and *Pst*I restriction endonuclease combinations. The AFLP selective amplification was performed with selective extension using 2 bases: *Eco*RI + NN / *Pst*I + NN. Between 500 bp and the 67 bp bands from the markers (pUC mix, 8) were used as reference points, and the relative size of all bands were calculated. Banding patterns of Mei were analyzed by

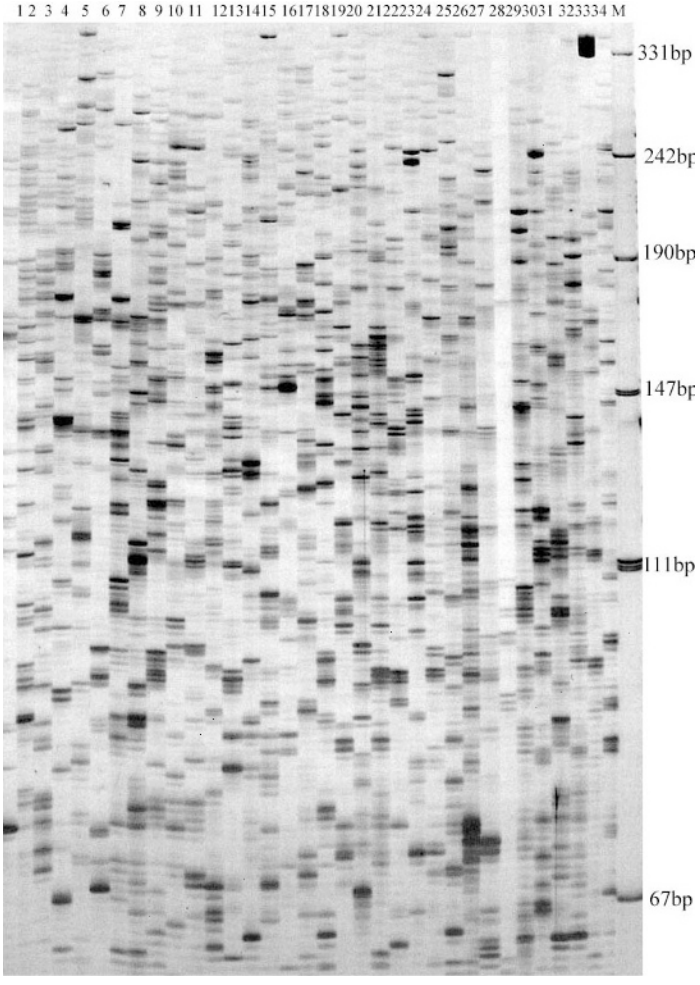

Figure 1. AFLP patterns by 34 different primer combinations in Xue Mei. Lane 1, E-AA/P-AG; lane 2, E-AA/P-TC; lane 3, E-AA/P-CA; lane 4, E-AT/P-AT; lane 5, E-AT/P-TG; lane 6, E-AC/P-AA; lane 7, E-AC/P-GT; lane 8, E-AG/P-GA; lane 9, E-AG/P-GC; lane 10, E-TA/P-AT; lane 11, E-TA/P-TA; lane 12, E-TA/P-TT; lane 13, E-TT/P-AC; lane 14, E-TT/P-CC; lane 15, E-TT/P-CG; lane 16, E-TC/P-AA; lane 17, E-TC/P-TC; lane 18, E-TG/P-AC; lane 19, E-TG/P-AG; lane 20, E-TG/P-TT; lane 21, E-TG/P-CG; lane 22, E-CA/P-AG; lane 23, E-CA/P-CC; lane 24, E-CT/P-AG; lane 25, E-CT/P-TT; lane 26, E-CT/P-CG; lane 27, E-CC/P-AG; lane 28, E-CC/P-GT; lane 29, E-CG/P-TG; lane 30, E-CG/P-GT; lane 31, E-GA/P-CA; lane 32, E-GA/P-GT; lane 33, E-GT/P-AG; lane 34, E-GT/P-TA and M, markers (pUC mix, 8).

scoring the relative migration of all major bands. Results of AFLP bands visualized by silver-stained polyacrylamide gel are presented for samples in figures.

In preliminary analysis, all 256 primer combinations were assayed by using Xue Mei to determine the number of AFLP bands produced by primers. The number of legible bands produced high variation and ranged from 6-74 bands. The AFLP band profiles of 34 primer combinations are shown in Figure 1. Fewer than 30 AFLP bands were seen in lanes 14, 15, 23, 25, 27, 28, and 30. Bands were not

well separated on 6% polyacrylamide gel for the similar size in the upper part of lane 33. More than 30 AFLP bands were seen in the other 26 lanes.

The numbers of all 256 primer combinations that produced AFLP bands in Xue Mei are shown in Table 1. Of 256 primer combinations, 135 produced more than 30 AFLP bands in Xue Mei (these numbers are bolded in Table 1); 121 primer combinations detected less than 30 AFLP bands (these numbers are italicized in Table 1). In these studies, we decided that AFLP bands of less than 30 produced by primers should be overlooked and that bands not well distinguished on 6% polyacrylamide gel should be ignored. In preliminary investigation, only 135 primer combinations produced more than 30 clear bands that were suitable for further selection of polymorphic bands in 5 Mei samples.

AFLP analysis - Primers selection with 5 samples

We selected *P. mume* var. *cernua* and 4 Mei cultivars, included Xue Mei, Duo-e Zhusha, Fuban Xiao Gongfen, and Can Xue, which had distinctive morphological characters described by Chen (1989, 1996). The AFLP patterns of 5 Mei samples are shown in Figure 2 with 8 primer combinations, where E-AT/P-AT, E-AC/P-AA, E-AG/P-GC, E-TA/P-CC, E-TT /P-GA, E-TC/P-AG, E-CA/P-GT, and E-CC/P-GC had polymorphic bands. Polymorphic bands were seen in 68 of 135 primer combinations; other primer combinations had identical AFLP patterns in 5 Mei samples. The primers revealing polymorphic bands are shown in Table 2 (numbers of polymorphic bands were not shown). To screen the best primers among the 68 preselected primers that could generate high polymorphic bands, more Mei cultivars were analyzed.

AFLP analysis - Primers selection with 14 samples

The accuracy information of Mei genetic variation is very important for further breeding and improvement programs. Selected AFLP primers should differentiate not only the parentage but also the Mei cultivars with unknown genetic relationship. *P. mume* var. *cernua*, Xue Mei, Duo-e Zhusha, Fuban Xiao Gongfen, and Can Xue were analyzed in the third step of AFLP analysis to keep the results consistent. Danhong Chuizhi, Da Yu, Fenpi Gongfen, Tiaoxue Chuizhi, and Kinseshidare with different morphological characters were included in this analysis. Jiangnan Taige and Jiangsha Gongfen were the hybrid progenies of Xiao Gongfen and Jiangnan Zhusha, which were attached here to optimize AFLP primer combinations for Mei.

In this research, primer combinations generating the AFLP bands and polymorphic bands were assessed in 3 steps with 1, 5, and 14 Mei samples, respectively. The last step evaluated 68 primers that generated higher polymorphic bands for Mei cultivars in this "narrow down" strategy. AFLP patterns of 14 Mei samples using E-TG/P-AG primer combination were shown in Figure 3, and the polymorphic bands differentiated these samples directly. Sixty-eight primers produced polymorphic bands ranging from 4-37 in 14 Mei samples. However, only 10 primer pairs detected polymorphic bands ranging from 15-37, and the results shown in Table 3 confirmed that they were the most suitable primers for the analysis of Mei cultivars. These 10 primer pairs produced up to a total of 524 AFLP

Table 1. Numbers of AFLP bands produced by all 256 primer combinations in Xue Mei.

	P-AA	P-AT	P-AC	P-AG	P-TA	P-TT	P-TC	P-TG	P-CA	P-CT	P-CC	P-CG	P-GA	P-GT	P-GC	P-GG
E-AA	**60**	**35**	**44**	**40**	**35**	**45**	**65**	**74**	68	**44**	**56**	**62**	**46**	**33**	**35**	**49**
E-AT	**40**	**51**	**37**	**38**	*21*	**41**	*27*	**49**	45	**38**	*23*	22	**34**	*25*	**38**	*29*
E-AC	**38**	*22*	22	18	**38**	**36**	**32**	**46**	48	**36**	**43**	**39**	**59**	**42**	**34**	**30**
E-AG	*21*	**36**	**40**	*13*	15	27	**32**	*23*	26	**46**	**34**	**48**	**44**	**33**	**48**	**34**
E-TA	**35**	**39**	**39**	**42**	**39**	**36**	**68**	**44**	32	**40**	**55**	**45**	**53**	**42**	**34**	*27*
E-TT	**50**	**44**	**32**	*27*	**35**	*23*	13	19	22	11	28	28	**52**	**35**	*27*	25
E-TC	**30**	**45**	*29*	**32**	**44**	*24*	**31**	*13*	22	24	25	**31**	**42**	*24*	**31**	*19*
E-TG	**32**	*24*	**34**	**31**	*29*	**37**	*17*	17	28	19	21	**34**	*16*	22	24	23
E-CA	*17*	12	22	**30**	*26*	**39**	*28*	17	22	23	22	29	**34**	**52**	**35**	*19*
E-CT	*20*	5	19	**32**	*7*	27	21	13	22	7	14	**36**	*21*	16	19	28
E-CC	*18*	20	20	**31**	**32**	**32**	*29*	**31**	30	**45**	*23*	23	**39**	*6*	**56**	*27*
E-CG	**35**	**47**	*18*	26	**44**	*23*	**33**	**31**	35	*26*	17	**33**	*16*	19	16	**35**
E-GA	**31**	*17*	**44**	*24*	**34**	**36**	*25*	**31**	31	*27*	27	26	25	**34**	*24*	28
E-GT	*26*	24	15	**44**	**31**	**33**	*25*	12	26	**30**	*19*	24	21	**34**	**35**	*13*
E-GC	**32**	*25*	**31**	*20*	29	**31**	*26*	12	**36**	**32**	**44**	**34**	**45**	**50**	**54**	**34**
E-GG	**42**	*23*	**31**	*11*	16	17	28	23	15	18	10	**32**	**39**	*15*	11	**30**

E indicates *Eco*RI adapter sequences; P indicates *Pst*I adapter sequences.

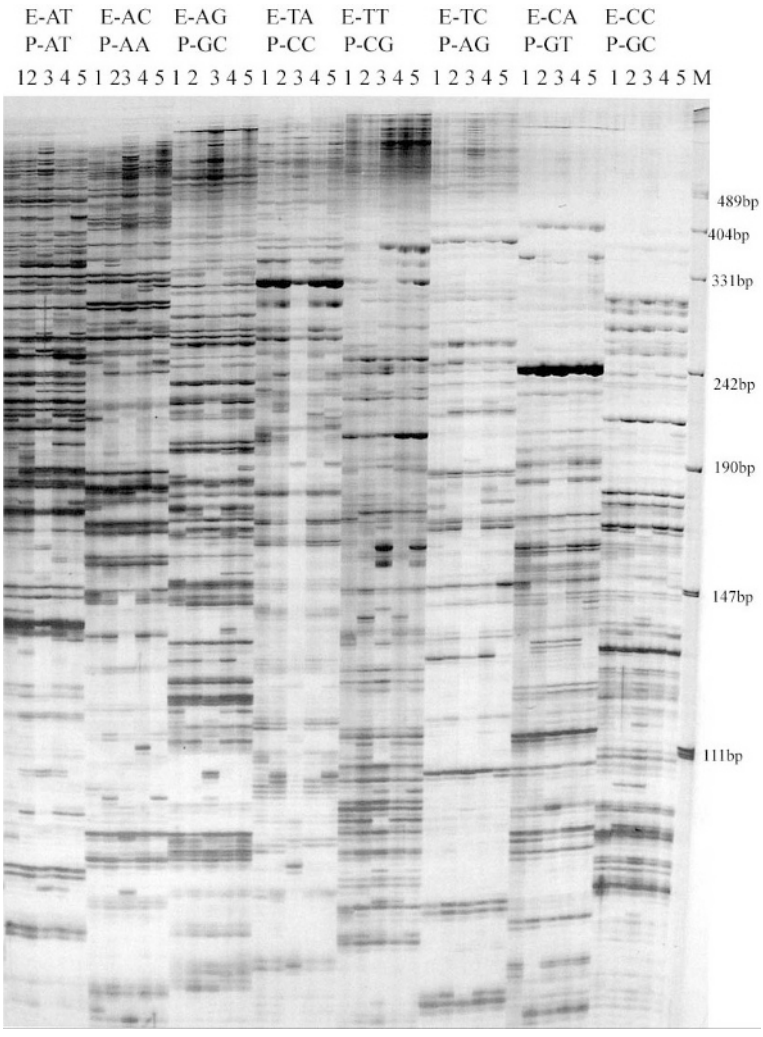

Figure 2. AFLP patterns of 5 Mei samples by 8 primer combinations: lane 1 *Prunus mume* var. *cernua*; lane 2, Xue Mei; lane 3, Duo-e Zhusha; lane 4, Fuban Xiao Gongfen; lane 5, Can Xue; and M, markers (pUC mix, 8).

bands and up to 233 polymorphic bands. The ratio of polymorphic bands for 10 primers scoped from 35.71% to 59.67%, and the average ratio was 44.46%.

In previous studies, rare-cutter (6-base cutter) and frequent-cutter (4-base cutter) restriction enzyme combinations generating high AFLP polymorphic bands were usually applied to investigate species genetic variation, such as *Eco*RI/*Mse*I, *Pst*I/*Mse*I, and so on. Moreover, isoschizomer of *Hpa* II and *Msp* I replaced *Mse* I to detect cytosine methylation in plant genome (Xu et al., 2000; Baurens et al., 2003). In the latter experiment, with the AFLP primers selected in this study, we can acquire more genetic information if frequent-cutter enzymes are used instead of the rare-cutter enzymes, such as *Eco*RI or *Pst*I. The genetic

Table 2. Polymorphic bands produced by all primer combinations in 5 Mei samples.

	P-AA	P-AT	P-AC	P-AG	P-TA	P-TT	P-TC	P-TG	P-CA	P-CT	P-CC	P-CG	P-GA	P-GT	P-GC	P-GG
E-AA				+		+	+	+	+		+	+			+	
E-AT		+		+						+						+
E-AC	+				+			+		+	+	+			+	+
E-AG							+								+	
E-TA	+	+								+	+	+				
E-TT	+	+	+	+									+	+		
E-TC	+	+		+	+								+			
E-TG	+		+	+		+										
E-CA				+							+			+		
E-CT						+										
E-CC				+									+		+	
E-CG	+	+			+											
E-GA	+		+		+				+							
E-GT				+	+											
E-GC			+			+	+			+						+
E-GG	+	+										+	+			+

E indicates *Eco*RI adapter sequences; P indicates *Pst*I adapter sequences.
+ indicates primer combinations produced polymorphic bands in 5 Mei samples.

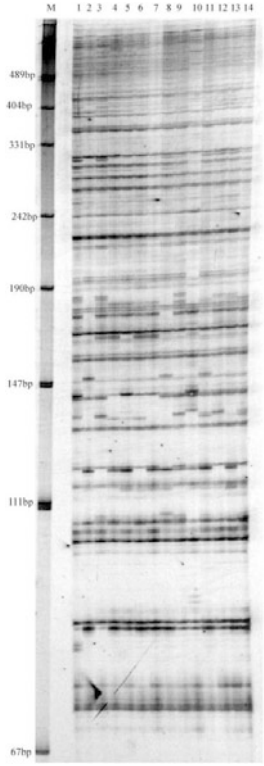

Figure 3. AFLP patterns of 14 Mei samples using E-AT/P-AT primer combination: M, markers (pUC mix, 8); lane 1, Danhong Chuizhi; lane 2, *Prunus mume* var. *cernua*; lane 3, Xue Mei; lane 4, Xiao Gongfen; lane 5, Jiangnan Zhusha; lane 6, Jiangsha Gongfen; lane 7, Jiangnan Taige; lane 8, Da Yu; lane 9, Fenpi Gongfen; lane 10, Duo-e Zhusha; lane 11, Fuban Xiao Gongfen; lane 12, Tiaoxue Chuizhi; lane 13, Can Xue; and lane 14, Kinse-shidare.

Table 3. Selected primers and level of polymorphism among 14 Mei samples from AFLP analysis.

Primer Combinations	No. of Amplified Bands	No. of Polymorphic Bands	Ratio of Polymorphic Bands, %
E-AT/P-AT	62	37	59.67
E-AC/P-TA	62	28	45.16
E-AG/P-GC	47	18	38.29
E-TA/P-AT	42	15	35.71
E-TC/P-AA	59	27	45.76
E-TG/P-AG	67	25	37.31
E-TG/P-TT	45	18	40.00
E-GT/P-AG	44	20	45.46
E-GT/P-TA	52	27	51.92
E-GC/P-CT	44	18	49.91
Total	524	233	44.46

E indicates *Eco*RI adapter sequences; P indicates *Pst*I adapter sequences.

variation of apricot (*Prunus armeniaca*) assessed by AFLP technique had been conducted in 3 different laboratories (Hurtado et al., 2002; Panaud et al., 2002; Ricciardi et al., 2002). The percentage of polymorphism generated by the primer E-AC was 45.16% in Mei, which is higher than the Hurtado (2002) result of 30.43% but lower than the Ricciardi (2002) result of 64.70% in apricot. The percentage of polymorphism generated by the primer E-AG was 38.29% in Mei, which is higher than the Hurtado (2002) result of 29.56% but lower than the Panaud (2002) result of 47.06% in apricot. AFLP fragments generated by E-AC and E-AG nuclear loci showed the higher numbers and percentage of polymorphism that indicated nucleotides variation colinearity in *Prunus,* which was concluded from these results.

Acknowledgments

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References

Bao M and Chen J (1994) Studies on the variation and distribution of *Prunus mume* Sieb. et Zucc. Acta Horticulture Sinica 21(1): 81-86.

Baurens FC, Bonot F, Bienvenu D, Causse S, and Legavre T (2003) Using SD-AFLP and MSAP to assess CCGG methylation in the banana genome. Plant Mol Biol Rep 21: 339-348.

Bennett MD and Leitch IJ (1995) Nuclear DNA amounts in angiosperms. Ann Bot 76: 113-176.

Byrne DH and Littleton TG (1989) Characterization of isozyme variability in apricots. J Am Soc Hortic Sci 114: 674-678.

Chen J (1989) Chinese Mei Flower cultivars, pp 32-107, China Forestry Publishing House, Beijing, China.

Chen J (1996) Chinese Mei Flowers, pp 100-151, Hainan Publishing House, Haikou, China.

de Vicente MC, Truco MJ, Egea J, Burgos L, and Arffls P (1998) RFLP variability in apricot [*Prunus armeniaca* (L.)]. Plant Breed 117: 153-158.

Dellaporta SL, Wood J, and Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1: 19-21.

Dirlewanger E, Graziano E, Joobeur T, Garriga-Caldere F, Cosson P, Howad F, and Aru P (2004) Comparative mapping and marker-assisted selection in *Rosaceae* fruit crops. Proc Natl Acad Sci 101: 9891-9896.

Entani T, Iwano M, Shiba H, Che FC, Isogai A, and Takayama S (2003) Comparative analysis of the self-incompatibility (S-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. Genes to Cells 8: 203-213.

Hagen LS, Khadari B, Lambert P, and Audergon JM (2002) Genetic diversity in apricot revealed by AFLP markers: species and cultivar comparisons. Theor Appl Genet 105: 298-305.

Hurtado MA, Westman A, Beck E, Abbott GA, Ll′acer G, and Badenes ML (2002) Genetic diversity in apricot cultivars based on AFLP markers. Euphytica 127: 297-301.

James CM, Lesemann SS, and Down GJ (2003) Modified AFLP analysis method for species with small genomes. Plant Mol Biol Rep 21: 303-307.

Kazachkova N, Fahleson J, and Meijer J (2004) Establishment of the amplified fragment length polymorphism (AFLP) technique for genotyping of pollen beetle (*Meligethes aeneus*) — a noxious insect pest on oilseed rape (*Brassica napus*). Mol Biol Rep 31: 37-42.

Manubens A, Lobos S, Jadue Y, Toro M, Messina R, Lladser M, and Seelenfreund D (1999) DNA isolation and AFLP fingerprinting of nectarine and peach varieties (*Prunus persica*). Plant Mol Biol Rep 17: 255-267.

Panaud O, Chaib A, and Sarr A (2002) Dynamic conservation of apricot *Prunus armeniaca* in saharian oases: use of AFLP markers to assess genetic diversity in traditional orchards. Euphytica 128: 301-305.

Ricciardi L, Giorgio V, de Giovanni C, Lotti C, Gallotta A, and Fanizza G (2002) The genetic diversity of Apulian apricot genotypes (*Prunus armeniaca* L.) assessed using AFLP markers. Cell Mol Biol Lett 7:431-436.

Shimada T, Haji T, Yamaguchi M, Takeda T, Nomura K, and Yoshida M (1994) Classification of mume (*Prunus mume* Sieb. et Zucc.) by RAPD assay. J Jpn Soc Hort Sci 63: 543-551.

Vilanova S, Romero C, Abbott AG, Llacer G, and Badenes ML (2003) An apricot (*Prunus armeniaca* L.) F2 progeny linkage map based on SSR and AFLP markers mapping plum pox virus resistance and self-incompatibility traits. Theor Appl Genet 107: 239-247.

Vos P, Hogers R, Bleeker M, Reijans, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407-4414.

Watkins R (1976) Cherry, plum, peach, apricot and almond. In: Simmonds NW (ed) Evolution of crop plants. pp 242-247. Longman, London New York.

Xu M, Li X, and Korban SS (2000) AFLP-based detection of DNA methylation. Plant Mol Biol Rep 18: 361-368.