

# Genetic Relationships among *Pyrus pyrifolia* Cultivars from Southeastern China and Japan

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## Abstract

A total of 90 *Pyrus pyrifolia* cultivars from southeastern China and Japan were used to assess genetic diversity and overall similarity using eight Amplified Fragment Length Polymorphism (AFLP) primer combinations. Eighty-eight percent of the 429 bands produced were polymorphic. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis of these data support two major groups, mostly consistent with their geographic distribution. Most of the *P. pyrifolia* cultivars from southeastern China formed one group and most of the Japanese cultivars were in the other group. However, some cultivars from southeastern China and Japan clustered together. These results support our previous studies which indicated that some Japanese pear cultivars are genetically similar to those from Zhejiang and Fujian Provinces of China. Nevertheless, most Japanese pears are genetically distant from Chinese sand pears.

## INTRODUCTION

The genus *Pyrus* L. (pears), has 22 species according to Challice and Westwood (1973). *Pyrus pyrifolia* (sand pear) is one of most important commercial pear species in China. Prior randomly amplified polymorphic DNA (RAPD) results showed that *P. pyrifolia* accessions from Kochi Prefecture of Japan have a high genetic similarity with some Chinese sand pears and the former might originate from ancient China and Korea (Teng et al., 2002). Further, simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) analyses also showed similar results (Bao et al., 2007, 2008). This study estimates the genetic similarity of Chinese sand pears and Japanese pears in more detail by examining more accessions from these two areas than in previous studies. We examined Chinese sand pears from Fujian, Zhejiang and Jiangxi in southeastern China, and Japanese pears from Kochi, Chiba and Niigata using the AFLP technique.

## MATERIALS AND METHODS

### DNA Extraction

Fresh leaves of 90 *P. pyrifolia* accessions (Fig. 1) were collected from the China Pear Germplasm Repository in Xingcheng, Liaoning Province (CPGR); from the Chinese Sand Pear Germplasm Repository in Wuhan, Hubei Province; and from Tottori University, Tottori, Japan (TU) and stored at -70°C. Genomic DNA was extracted using the SDS method modified by Teng (2002). Due to the high content of phenol and polysaccharides in pear leaves, genomic DNA was then purified using a purification kit from SBS Genetech Co. Ltd., China. The final DNA concentration was measured by a fluorimeter (DU 800, Beckman, USA) and adjusted to 100 ng/μl.

### AFLP

AFLPs were generated as described by Vos et al. (1995) with modifications outlined by Bao et al. (2008). Primer combinations E-ACT/M-CAA, E-AAC/M-CAG, E-ACA/M-CAG, E-AGG/M-CAA, E-AGG/M-CTT, E-ACG/M-CTC, E-ACA/M-CAA

and E-AAC/M-CTA were screened over the plant material.

### **Electrophoresis**

Electrophoresis was carried out on the Bio-Rad Sequi-Gen GT system using a 6% denaturing polyacrylamide gel with 7 M urea, 5% acrylamide, and 1×TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.3). Gels were run for 3h at 45°C using a constant 75W. Bands were visualized with silver staining (Brant, 1991).

### **Data Analysis**

The bands were scored as 1 (presence) and 0 (absence), entered onto a Microsoft EXCEL spreadsheet, and transferred to Ntedit software (Rohlf, 1998). Similarity matrices were constructed using Dice's coefficient (Nei and Li, 1979) and a dendrogram was constructed using UPGMA by NTSYS-pc v. 2.02j (Rohlf, 1998).

## **RESULTS AND DISCUSSION**

### **Genetic Similarity**

A total of 429 bands were obtained using eight primer combinations, with E-ACA/M-CAA producing the largest number of bands. Eighty-eight percent of the bands were polymorphic (Table 1). Genetic similarity among the accessions ranged from 0.639 to 0.876.

### **Genetic Relationships among Cultivars from Japan and Southeastern China**

Cultivars from Japan and southeastern China were divided into five groups at the 0.756 level of genetic similarity. Group I and group II comprised of cultivars mostly from Japan and all from southeastern China, respectively, which was generally consistent with their geographic distributions.

Group I contained 95% of Japanese accessions and 5 accessions of the sand pears from Zhejiang and Fujian Provinces in China (Teng et al., 2002; Bao, 2007, 2008). Many accessions from Kochi Prefecture of Japan were scattered in this group, which have close genetic relationships with most Japanese pears. 'Yiwulizi', 'Qianxianshali' and 'Qiubaishali' from Zhejiang Province and 'Zongbaoli' from Fujian Province were in the same subgroup, while 'Huangqieli' from Zhejiang Province grouped with all the other Japanese pears in the other subgroup. Teng et al. (2002) showed that 'Yiwulizi' and 'Qianxianshali' were in the same clade as Japanese pears from Kochi Prefecture by using RAPD, later supported by SSR results (Bao et al., 2007). 'Umajrou' and 'Imamuraaki', cultivars from Kochi Prefecture, were in the same subgroup, as reported by Bao et al. (2007, 2008). Chinese sand pear 'Mandingxueli' clustered with Japanese pear 'Kamenashi' at the 0.740 level of genetic similarity which comprised the group IV. All these results support earlier results (Teng et al., 2002; Bao et al., 2007, 2008), suggesting that some pear cultivars in Japan have close genetic relationships with those from southeastern China.

Most of the sand pears from Zhejiang and Fujian Provinces in China clustered in the group II. All 'Puguali' accessions collected from different localities clustered together, suggesting they might have the same genetic backgrounds. Although 'Puguali' and 'Kuixingmake' were previously shown to have high similarity with Japanese pears (Bao et al., 2007, 2008), different results were obtained here possibly because all the samples were from *P. pyrifolia* rather than the greater range of species used in the earlier research. 'Zhenxiangli', 'Yunhexueli' and 'Xihuaxueli' from southern Zhejiang Province were in the same subgroup which was consistent with their morphology.

Group III was composed of 'Nuodaoli' and 'Jiuzhongli' which are native to Zhejiang Province.

'Ichiharawase' from Nara Prefecture, which is considered to be a primitive species in Japan, was genetically distant from all of the other samples analyzed.

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## Tables

Table 1. Primer combinations and percentage of polymorphic bands.

Primer combinations	Total number of bands	Percentage of polymorphic bands (%)
E-ACT/M-CAA	83	94.0
E-AAC/M-CAG	53	96.2
E-ACA/M-CAG	45	91.1
E-AGG/M-CAA	44	79.5
E-AGG/M-CTT	29	75.9
E-ACG/M-CTC	27	88.9
E-ACA/M-CAA	87	80.5
E-AAC/M-CTA	61	91.8
Total	429	88

**Figures**

- Japan
- ▲ Zhejiang, China
- Fujian, China
- ◆ Jiangxi, China

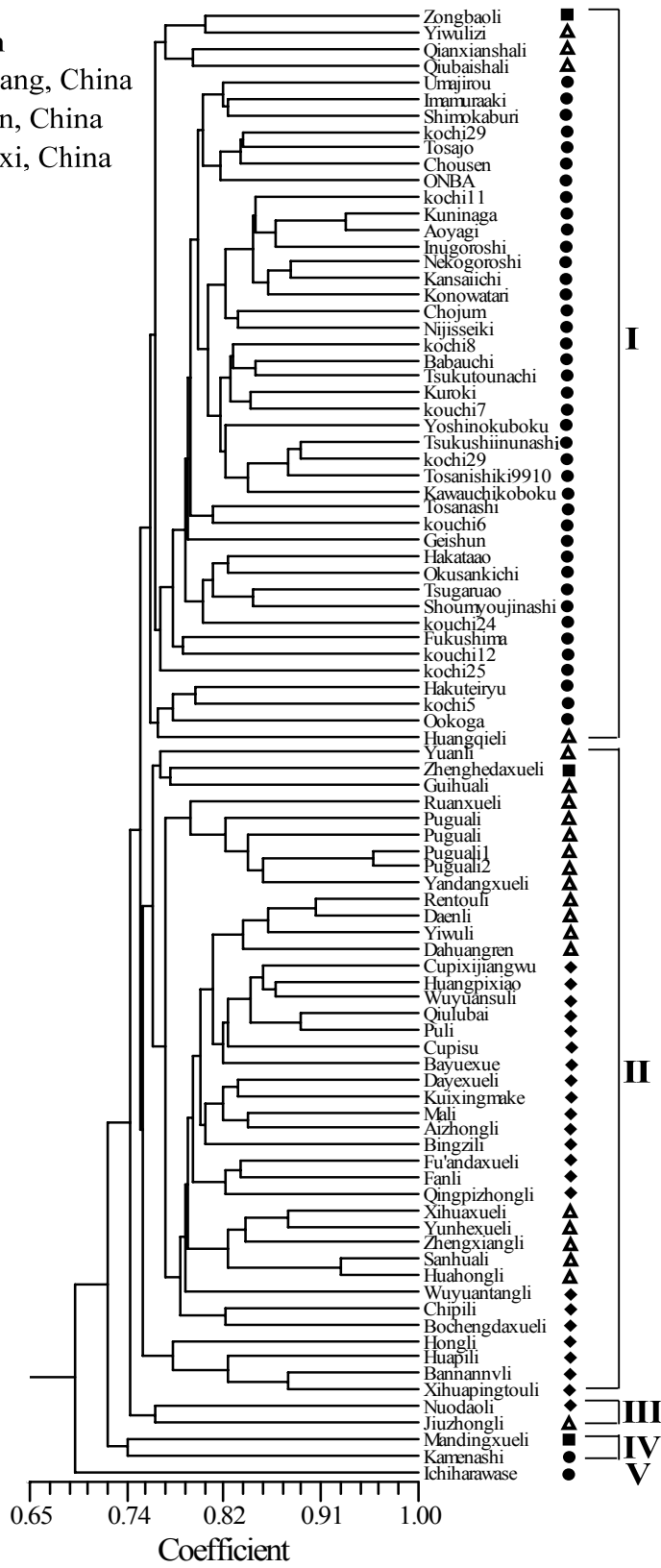


Fig. 1. Dendrogram of 90 pear cultivars resulting from UPGMA analysis based on Dice's similarity coefficient.