Molecular Markers for Fingerprinting among Cultivars of Oriental Pear (*Pyrus pyrifolia* (Burm.) Nak.)

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ABSTRACT

Molecular markers including simple sequence repeats (SSR), inter-simple sequence repeats (ISSR), and randomly amplified polymorphic DNA (RAPD) had used for identification within cultivars of Oriental pears. There were 21 polymorphic SSR locus screened from 52 primer pairs, and two of them (NH023, NH025) with fingerprinting ability. Additionally, 16 polymorphic primers of ISSR and 20 primers for RAPD were selected from one hundred primers by each, while 2 ISSR and 2 RAPD primers revealed fingerprinting ability. Furthermore, 24 specific amplification markers of SSR, 44 markers of ISSR, and 22 makers of RAPD were certainly obtained for identification between cultivars. This results indicate all of the SSR, ISSR and RAPD makers are useful for distinguishing among cultivars of Oriental pears.

INTRODUCTION

From the geographical point, pears are traditionally divided into two main groups: Occidental pears and Oriental pears. In Taiwan, the commercial cultivars mostly belonge to Oriental pears, and *Pyrus pyrifolia* (Burm.) Nak. is one of a important spices in Oriental type. According to the Yearly report of Taiwan's Agriculture in 2004, the growing area of pear was 8,456 ha and year' s output value was about 4 billion NT dollars. Since 1980, a breeding program had been undertaken by Agricultural Research Institute (ARI), and the objectives were to obtain high quality and low chilling cultivars. Nowadays, there are two new cultivars had been released for the cultivation in low land (Fig. 1). Regarding for the purpose of quickly identification between different genotypes and cultivars within our breeding program, this study is to establish the efficient molecular markers as a useful fingerprinting tools.

MATERIALS AND METHODS

•Plant Materials and DNA Extraction: Ten cultivars of the Oriental pears were tested (Table 1) , including a new cultivar 'Julip' selected by ARI. Genomic DNA was isolation from leaf samples of each cultivar and following by the DNeasy® Plant Kit (Qiagen).

•Primer Design :

(1)SSR: 52 flanking microsatellite sequences had been used. (Liebhard et al., 2002; Yamamoto et al., 2002; Guiford et al., 1997; Gianfranceschi et al., 1998).

(2)ISSR: 100 ISSR primers of the UBC Oligonucleotide Set100/9（University of British Columbia, Canada ）were tested.

(3)RAPD: A total of 100 UBC Oligonucleotide primers (Set/4) was tested.

•PCR Amplification and Fingerprinting Analysis: PCR was performed in a volume of 25 μl containing 1.25 u Taq polymerase (Roche-FastStart), 1xPCR buffer, 2.0 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, and 25 ~50 ng template DNA. Reactions were performed on a Gene Amp 9700® by an initial denaturation for 5 min at 95℃ followed by 35 cycles of 45 s at 94℃, 45 s at 56℃~58℃ (in SSR and ISSR analysis) or 38℃ (in RAPD analysis), 2 min at 72℃; and a final extension of 8 min at 72℃. Products were separated on a 1.5 % agarose gel (3% Metaphor agarose only for SSR) and stained with ethidium bromide to check and determine approximately size of amplification fragments.

RESULTS AND DISCUSSION

•The results indicated all of SSR, ISSR and RAPD makers are useful for fingerprinting among cultivars in *Pyrus pyrifolia* (Fig. 2).

•Comparison molecular markers between SSR, ISSR and RAPD, revealed ISSR with the higher polymorphism; several markers of ISSR presenting excellent discrimination among cultivars (Table 1).

•Summarizing the results from SSR and ISSR analysis, showed the di-nucleotide repeats of AG/CT are more enriching in *Pyrus*.

Fig. 1 New cultivar 'Julip' was selected by ARI.

Table 1 Markers of SSR, ISSR and RAPD for distinguishing among cultivars of *Pyrus pyrifolia* .

Cultivar	No. of distinguishable markers for each cultivar		
	SSR	ISSR	RAPD
Hengshan	3	1	4
Housui	1	10	4
Shinko	3	1	2
Julip	1	5	3
Shinseiki	1	3	0
Nijisseiki	0	0	1
Kousui	2	6	0
Akisui	4	4	4
Gold	1	7	1
Atago	8	7	3
Total	24	44	22

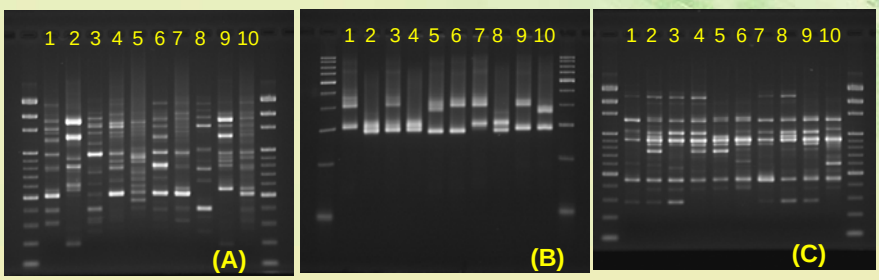

Fig. 2 Amplification profiles of *Pyrus pyrifolia* by analysis of ISSR(A: UBC873) SSR(B: NH023) and RAPD(C: UBC336) separately.
Cultivar: 1) Hengshan, 2) Housui, 3) Shinkou, 4) Julip, 5) Shinseiki, 6) Nijisseki, 7) Kousui, 8) Akisui, 9) Gold, 10) Atago

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