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Full Length Research Paper

Molecular marker analysis of 'Shatangju' and 'Wuzishatangju' mandarin (*Citrus reticulata* Blanco)

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'Wuzishatangju'(*Citrus reticulata* Blanco) is an excellent cultivar derived from a bud sport of a seedy 'Shatangju' cultivar found in Guangdong Province in the 1980s. In this study, six molecular markers including random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), simple sequence repeat (SSR), sequence-related amplified polymorphism (SRAP), inter-retrotransposn amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) were used to study the genetic variations between 'Shatangju' and 'Wuzishatangju'. 1196 RAPD, seven SSR, 28 IRAP and 56 REMAP primers were used to detect the genetic variations between 'Shatangju' and 'Wuzishatangju'. However, no difference was observed between the two cultivars. These results indicate that there was a very close genetic relationship between 'Shatangju' and 'Wuzishatangju' and RAPD, SSR, IRAP and REMAP markers could not distinguish them. Two and 21 specific bands were obtained using 100 ISSR and 153 SRAP primers, respectively. The present research could be a valuable tool for identification of *Citrus* bud sport clones, which laid the foundations for the further study of the mechanisms of *Citrus* bud sports.

Key words: *Citrus reticulata* Blanco, random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), simple sequence repeat (SSR), sequence-related amplified polymorphism (SRAP), inter-retrotransposon amplified polymorphism (IRAP), retrotransposon-microsatellite amplified polymorphism (REMAP), identification.

INTRODUCTION

Citrus is one of the world's most important fruit crops which is widely grown in most areas with suitable climates between latitude 35°N to 35°S. With the rapid development of *Citrus* industry and taste for better quality, the demand for desirable cultivars has been increased to meet the challenge of process industry and particular consumer preferences. Cross hybridization as a genetic improvement strategy for citrus cultivar development have led to the production of a large number of improved cultivars.

Unfortunately, cross hybridization faces many serious impediments such as highly genetic heterozygosity, longer juvenility, nucellar embryo interference, sexual or incompatibility of many species. The fact that *Citrus* cultivars were maintained by vegetative propagation; the large number of cultivars originated from bud sport events. Therefore, bud sport selection is one of the most important breeding approaches in *Citrus*. In the past 20 years, the cultivars presently grown mainly originated from bud sport selection (Deng et al., 1996; Deng, 2005; Liu and Deng, 2007). However, most characteristics of a bud sport are identical to the original variety from which they are derived. Accurate characterization of bud sport and their original cultivar is crucial for the protection of future intellectual property rights over new cultivars.

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Traditional methods for identification of genetic variability based on morphological, physiological and agronomic traits are often laborious and time-consuming because these traits are not all available for sampling at a single time (Fang et al., 2001).

Furthermore, these methods are inherently weak since they are limited by environmental factors and subjectivity of observations. With the rapid development of modern biotechnology, the advent of molecular markers has made it possible to detect genetic difference between genotypes at DNA level. Molecular markers overcome some of these limitations and have been widely used in *Citrus* assisted-selection breeding, genetic diversity analysis, population genetics and molecular evolutionary genetics (Wang et al., 2000; Gong et al., 2008).

Molecular marker techniques are new types of genetic markers and have greatly promoted *Citrus* breeding as a whole. Currently, random amplified polymorphic DNA (RAPD) (Bretó et al., 2001; Luo et al., 2008; Qin et al., 2011), amplified fragment length polymorphism (AFLP) (Bretó et al., 2001; Liu et al., 2005), restriction fragment length polymorphism (RFLP) (Fang et al., 1997), inter-simple sequence repeat (ISSR) (Fang et al., 1997; Bretó et al., 2001), simple sequence repeat (SSR) (Liu et al., 2005; Barkley et al., 2006; Jannati et al., 2009; Ollitrault et al., 2010), and retrotransposon-based molecular markers (Zhang and Deng, 2006) have been successfully applied in identification of *Citrus* varieties.

'Wuzishatangju' (*Citrus reticulata* Blanco), derived from a bud sport of a seedy 'Shatangju' cultivar, is seedless, very tasty and easy-to-peel and has become one of the newly grown varieties during the last decade in China (Ye et al., 2006). Our previous studies showed that 'Wuzishatangju' results from gametophytic self-incompatibility which caused seedlessness by blocking fertilization in the ovary (Ye et al., 2009). Except for seed number, no difference was observed between 'Shatangju' and 'Wuzishatangju' cultivar in term of sprout appearance, growth habit, leaf type, flower color, fruit shape and size (Ye et al., 2006; 2009). Therefore, rapid and accurate identification of the new variety is of great significance for further extension and application.

In this study, RAPD, ISSR, SSR, SRAP, IRAP and retrotransposon-microsatellite amplified polymorphism (REMAP) markers were used to study the genetic variations between 'Shatangju' and 'Wuzishatangju'. The results presented herein aimed to determine whether it is possible to detect molecular markers that distinguish bud sport variety and its original cultivar in their early stage of development.

MATERIALS AND METHODS

Five-year-old trees of 'Wuzishatangju' (six trees) and 'Shatangju' mandarin (four trees) are planted in an orchard of South China Agricultural University.

Genomic DNA extraction

Genomic DNA was extracted from young leaves of 'Shatangju' and 'Wuzishatangju' using a cetyltrimethyl ammonium bromide (CTAB) method (Xiong et al., 2002). The quality and concentration of DNA were examined by ethidium bromide (EB)-staining 0.7% (w/v) agarose gel electrophoresis and spectrophotometer (Bio-RAD, USA) analysis. The working DNA solutions were prepared at 10 ng/µl.

RAPD analysis

1196 RAPD primers were used to detect the genetic variations between 'Shatangju' and 'Wuzishatangju' based on establishing an optimization of RAPD-polymerase chain reaction (PCR) reaction system and procedures (Qin et al., 2011). PCR products were examined by EB-staining 1.5% (w/v) agarose gel electrophoresis.

ISSR analysis

An orthogonal experimental design was used to optimize ISSR-PCR system (Table 1) using DNA from 'Shatangju' as template. 100 ISSR primers were synthesized according to the sequences from University of British Columbia and used to detect the genetic variations between 'Shatangju' and 'Wuzishatangju' based the optimized ISSR-PCR reaction system. The PCR parameters were followed by the method of Qiao et al. (2009). PCR products were examined by EB-staining 1.5% (w/v) agarose gel electrophoresis.

SSR analysis

Seven pairs of SSR primers were synthesized according to the sequences of Kijas et al. (1997) (Table 2). The 25.0 µl of PCR reaction volume contained 30 ng DNA, 2.0 mM Mg^{2+}, 0.2 mM dNTPs, 0.2 µM primers and 1.25 U r*Taq* DNA (5 U/µl). The PCR parameters were performed according to the procedure of Cao et al. (2007). PCR products were separated on EB-staining 2.0% (w/v) agarose gels.

SRAP analysis

Single factor test were used to optimize SRAP-PCR reaction system using DNA from 'Shatangju' as template. The 25.0 µl of reaction volume contained 1.5, 2.0, 2.5, 3.0 and 3.5 mM Mg^{2+}, 0.1, 0.15, 0.2, 0.25 and 0.30 mM dNTPs, 1.0, 1.25 and 1.5 U r*Taq* (5 U/µl). PCR amplification was carried out according to the procedure of Li and Quiros (2001). Differences in SSR fragments are often difficult to resolve on agarose gels and high resolutions can be achieved through the use of polyacrylamide gels in combination with $AgNO_3$ staining. Therefore, SRAP-PCR products were separated on both agarose gels (1.5%) and polyacrylamide gel electrophoresis (PAGE) (10%), respectively.

IRAP analysis

IRAP primers were synthesized according to the sequences of Wei (2007) (Table 2). An orthogonal experimental design was used to optimize IRAP-PCR system (Table 3) using IRAP5 primer and 'Shatangju' DNA as template. The PCR parameters were carried out according to the method of Kalendar et al. (1999) and Wei (2007).

Table 1. Orthogonal experimental design for ISSR.

S/N	Factor and level				
	DNA template (ng)	dNTP (mM)	r*Taq* DNA (U)	Primer (µM)	Mg^{2+} (mM)
1	10	0.2	0.5	0.2	3.0
2	10	0.3	2.5	0.4	1.5
3	10	0.4	1.5	0.8	2.0
4	10	0.5	2.0	0.6	3.5
5	15	0.1	0.5	0.6	2.0
6	15	0.2	2.5	1.0	2.5
7	15	0.3	1.0	1.0	3.5
8	15	0.5	1.0	0.4	2.5
9	20	0.1	2.5	0.2	3.5
10	20	0.2	1.5	0.4	2.0
11	20	0.3	2.0	0.8	2.5
12	20	0.5	0.5	1.0	1.5
13	25	0.1	2.0	0.6	1.5
14	25	0.2	1.0	0.8	2.5
15	25	0.4	1.5	0.4	3.0
16	25	0.5	2.0	0.2	2.5
17	30	0.1	1.5	1.0	3.0
18	30	0.3	1.0	0.2	1.5
19	30	0.4	0.5	0.6	3.5
20	30	0.4	2.5	0.8	2.0

REMAP analysis

REMAP primers were from eight SSR primers combined with a forward or reverse IRAP primer (Table 2). REMAP-PCR system was optimized as described by IRAP. The 25.0 µl of reaction volume contained 30 ng DNA, 2.0 mM Mg^{2+}, 0.2 mM dNTPs, 0.2 µM primers and 1.25 U r*Taq* (5 U/µl). The PCR parameters were: 94°C for 5 min then 35 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 1.5 min, with a final 72°C for 10 min.

RESULTS

RAPD analysis of 'Shatangju' and 'Wuzishatangju'

1196 RAPD primers were used to detect the genetic variations between 'Shatangju' and 'Wuzishatangju'. However, no specific band was obtained between 'Shatangju' and 'Wuzishatangju' (Figure 1). These results indicate that there was a very close genetic relationship between 'Shatangju' and 'Wuzishatangju' and RAPD could not distinguish them.

ISSR analysis of 'Shatangju' and 'Wuzishatangju'

A suitable ISSR reaction system for *Citrus* was established after screening various concentrations of *Taq* DNA polymerase, DNA template, Mg^{2+}, primers and dNTPs (Figure 2). The optimum PCR reaction system (25 µl) was 20 ng DNA template, 1.5 mM Mg^{2+}, 0.5 mM dNTPs, 1.0 µM primer and 0.5 U r*Taq.* The PCR parameters were: 94°C for 5 min then 35 cycles of 94°C for 1 min, 40 to 60°C for 1 min (different primers using different annealing temperature) and 72°C for 1.5 min, with a final 72°C for 10 min.

68 primers with clear bands were first screened from the 100 ISSR primers using DNA from 'Shatangju' and 'Wuzishatangju' as template. After further screening, two specific bands (>2000) were obtained using primers 808 (AGAGAGAGAGAGAGAGC) and 823 (TCTCTCTC-TCTCTCTCC), respectively (Figure 3).

SSR analysis of 'Shatangju' and 'Wuzishatangju'

Seven pairs of SSR primers were used to identify the genetic variation between 'Shatangju' and 'Wuzishatangju'. However, no difference was observed between the two cultivars (Figure 4).

SRAP analysis of 'Shatangju' and 'Wuzishatangju'

Abundant, stable and clear strips were obtained using 153 pairs of SRAP primers. Agarose gel electrophoresis and PAGE were used to detect the PCR products. As shown in Figure 5, agarose gel electrophoresis was well separated for fragment sizes ranging from 100 to 2000

Table 2. Primers used in this study.

Primer name	Primer sequence
SSR primers	
SSRF1	GACAACATCAACAACAGCAAGAGC
SSRR1	AAGAAGAAGAGCCCCCATTAGC
SSRF2	GAAAGGGTTACTTGACCAGGC
SSRR2	CTTCCCAGCTGCCACAAGC
SSRF3	GGATGAAAAATGCTCAAAATG
SSRR3	TAGTACCCACAGGGAAGAGAGC
SSRF4	GGTACTGATAGTACTGCGGCG
SSRR4	GCTAATCGCTACGTCTTGGC
SSRF5	GCACCTTTTATACCTGACTCGG
SSRR5	TTCAGCATTTGAGTTGGTTACG
SSRF6	GATCTTGACTGAACTTAAAG
SSRR6	ATGTATTGTGTTGATAACG
SSRF7	AATGCTGAAGATAATCCGCG
SSRR7	TGCCTTGCTCTCCACTCC
IRAP primers	
IRAP1	TCCGATGGCCATGATTTACTC
IRAP2	GGACCTATTTGCCAATGCT
IRAP3	CCAATTCCGGAAGGTTCTAGG
IRAP4	ATCTCCCATTTCCGACCACT
IRAP5	GGCTTGGATCGCTTGGAGGC
IRAP6	AGTACGTCATTGCCTGTCCG
IRAP7	AGTGTCGATCCCACGAGGAGG
REMAP primers	
SSR1	AGAGAAGAAACATTTGCGGAGC
SSR2	AATGCTGAAGATAATCCGCG
SSR3	GAAAGGGTTACTTGACCAGGC
SSR4	ACAACCTTCAACAAAACCTAGG
SSR5	ATCACAATTACTAGCAGCGCC
SSR6	GGTACTGATAGTACTGCGGCG
SSR7	AGAAGCCATCTCTCTGCTGC
SSR8	GCACCTTTTATACCTGACTCGG

bp. However, no specific band was obtained between ‘Shatangju’ and ‘Wuzishatangju’ (Figure 5). Compared to agarose gel electrophoresis, PAGE were effectively separated; the fragment size ranged from 80 to 800 bp and 21 specific bands (shown by arrows) were obtained (Figure 6). The results indicate that SRAP could distinguish ‘Shatangju’ and ‘Wuzishatangju’.

IRAP analysis of ‘Shatangju’ and ‘Wuzishatangju’

A suitable IRAP reaction system for citrus was established after screening various concentrations of DNA template, dNTP, Mg^{2+}, and *Taq* DNA polymerase (Figure 7). The optimum PCR reaction system (25 µl) was 1.5 mM Mg^{2+}, 0.3 mM dNTP, 0.2 µM primers, 1.25 U *Taq* DNA polymerase and 25 ng/µl DNA templates. Based on the optimized reaction systems, abundant, stable and clear strips were obtained (Figure 7) and 22 pairs of specific primers from 28 primer combinations with good repeatability and polymorphism were further screened out. However, no specific band was detected between ‘Shatangju’ and ‘Wuzishatangju’ (Figure 8).

REMAP analysis of ‘Shatangju’ and ‘Wuzishatangju’

56 pairs of REMAP primers were used to detect the genetic variations between ‘Shatangju’ and ‘Wuzishatangju’. 35 pairs of specific primers with good repeatability and polymorphism were further screened out. However, no specific band was detected between ‘Shatangju’ and ‘Wuzishatangju’ (Figure 9).

DISCUSSION

Bud sport selection has been widely used for creating novel cultivars in vegetatively propagated plants. The frequency of bud sports in *Citrus* is extremely high and many excellent *Citrus* cultivars such as ‘Navel orange’, ‘Satsuma mandarin’, ‘Grapefruit’, and ‘Clementine’ have been obtained through bud sport selection (Zhang and Deng, 2006). However, it is very difficult to accurately discriminate between bud sport varieties and their original cultivar since they show very little variability in all characteristics.

RAPD marker is a simple method to detect DNA polymorphism (Welsh and McClelland, 1990; Williams et al., 1990) and has been widely used for analysis of plant genetic diversities, cultivar identification and assisted-selection (Durham et al., 1992; Wang et al., 2000; Lei et al., 2009). Currently, RAPD marker has successfully been applied to identification of bud sport varieties in *Citrus* (Bretó et al., 2001; Luo et al., 2008; Qin et al., 2011), kiwifruit (Ning et al., 2003), pear (Gao et al., 2010) and grape (Wang et al., 2003). However, RAPD-PCR system is so sensitive that changes of any reaction component could significantly affect the results. In addition, bud sport is a kind of somatic mutations involved in chromosome number per cell, chromosome structure aberration and even a point mutation. Therefore, it is still controversial whether RAPD technology can be used to identify bud sport clones due to its limitation (Fang et al., 2001).

In this study, 1196 RAPD primers were used to detect the genetic variations between ‘Shatangju’ and ‘Wuzishatangju’ based on an optimized RAPD reaction system (Qin et al., 2011). However, no specific band was observed between ‘Shatangju’ and ‘Wuzishatangju’ (Figure 1) suggesting that RAPD marker could not distinguish the two cultivars.

SSR is an excellent molecular marker with the

Table 3. Orthogonal experimental design for IRAP.

S/N	Factor and level				
	DNA (ng)	dNTPs (mM)	Mg^{2+} (mM)	r*Taq* DNA (U)	Primer (μM)
1	10	0.1	1.0	0.15	0.80
2	10	0.2	1.5	0.20	0.90
3	10	0.3	2.0	0.25	1.0
4	10	0.4	2.5	0.30	1.1
5	15	0.2	2.0	0.25	1.1
6	15	0.1	1.5	0.30	1.0
7	15	0.4	2.0	0.15	0.9
8	15	0.3	2.5	0.20	0.8
9	20	0.3	1.0	0.30	0.9
10	20	0.4	1.5	0.25	0.8
11	20	0.1	2.0	0.20	1.1
12	20	0.2	2.5	0.15	1.0
13	25	0.4	1.0	0.20	1.0
14	25	0.1	2.5	0.25	1.1
15	25	0.2	2.0	0.30	0.8
16	25	0.3	1.5	0.15	0.9

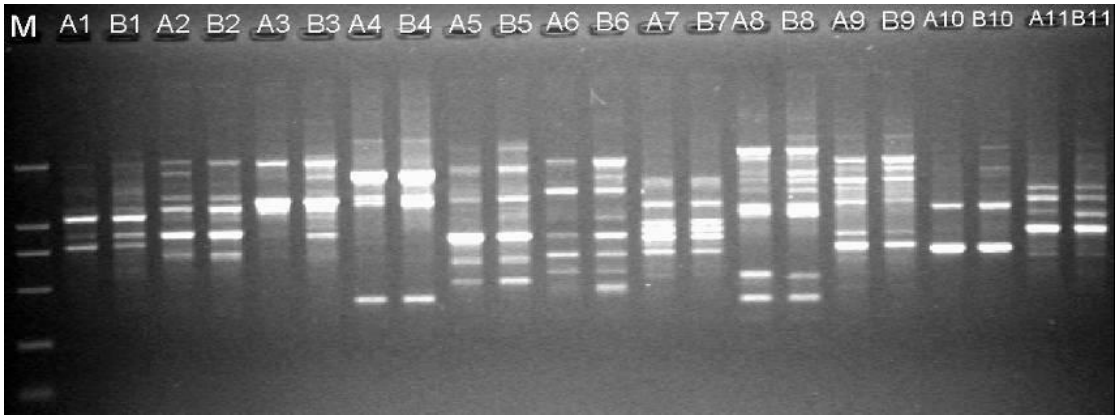

Figure 1. Partial RAPD results of 'Shatangju' (A1-11) and 'Wuzishatangju' (B1-11). M, DL2000 Marker; 1-11, PCR products using different RAPD primers.

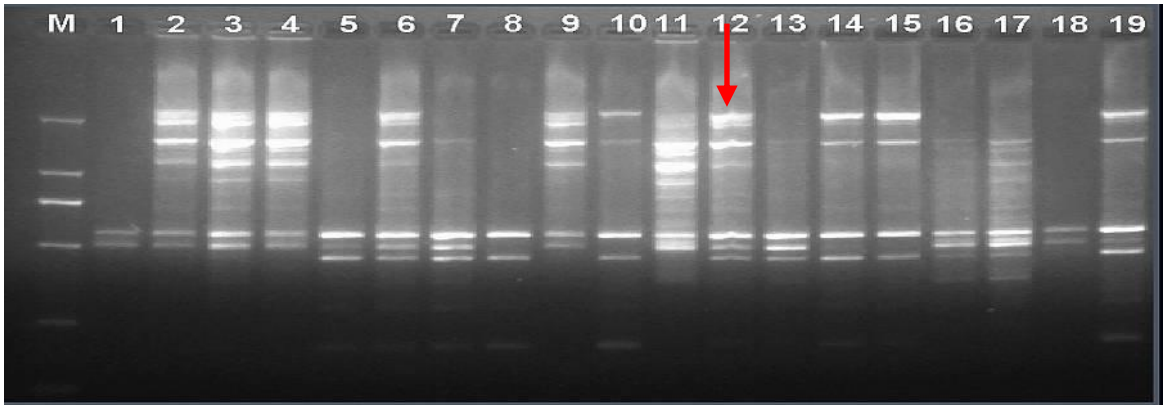

Figure 2. Optimization of ISSR reaction systems (optimum PCR reaction system was shown by an arrow).

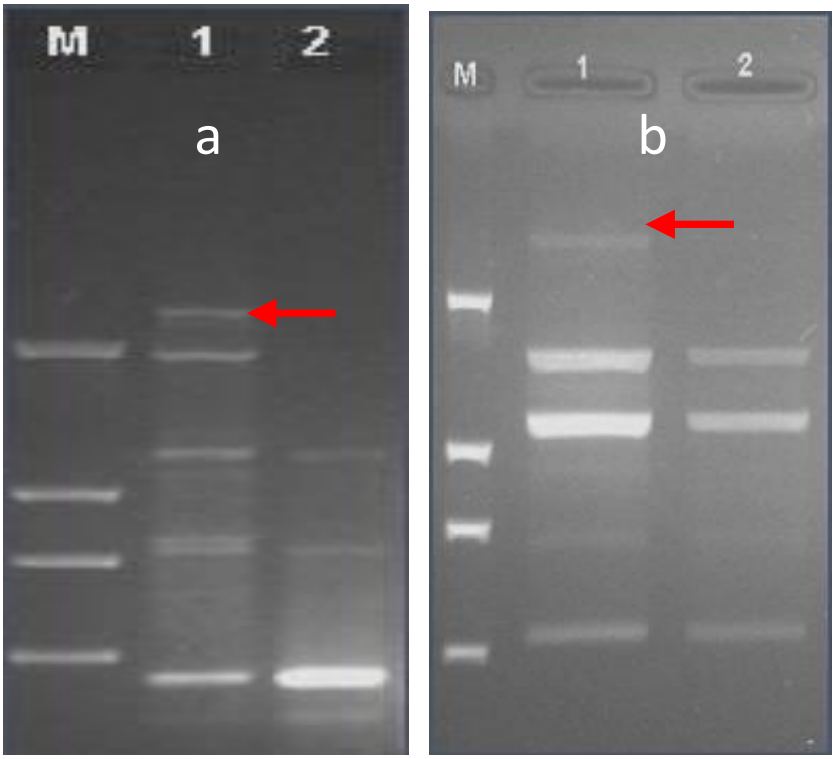

Figure 3. Two specific bands using ISSR primers 808 (a) and 823 (b). M, DL2000 Marker; 1, 'Shatangju'; 2, 'Wuzishatangju'.

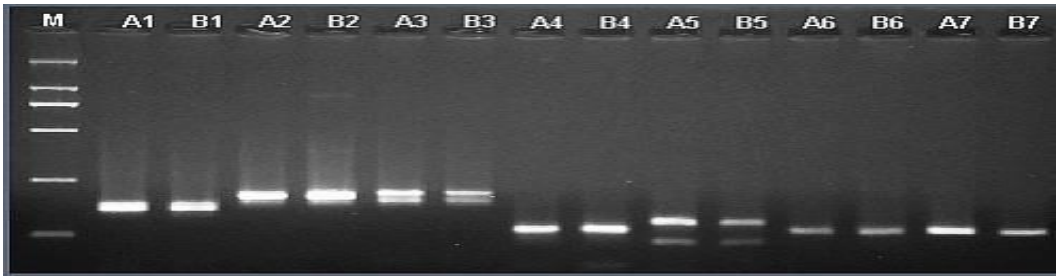

Figure 4. SSR results of 'Shatangju' (A1-7) and 'Wuzishatangju' (B1-7). M, DL2000 Marker; 1-7, 7 pairs of SSR primers.

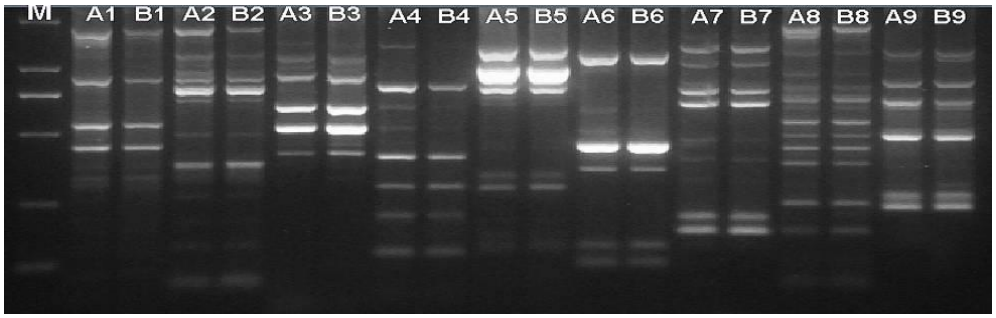

Figure 5. Partial SRAP results of 'Shatangju' (A1-9) and 'Wuzishatangju' (B1-9). M, DL2000 Marker; A,'Shatangju'; B, 'Wuzishatangju'; 1-9, 9 pairs of primers.

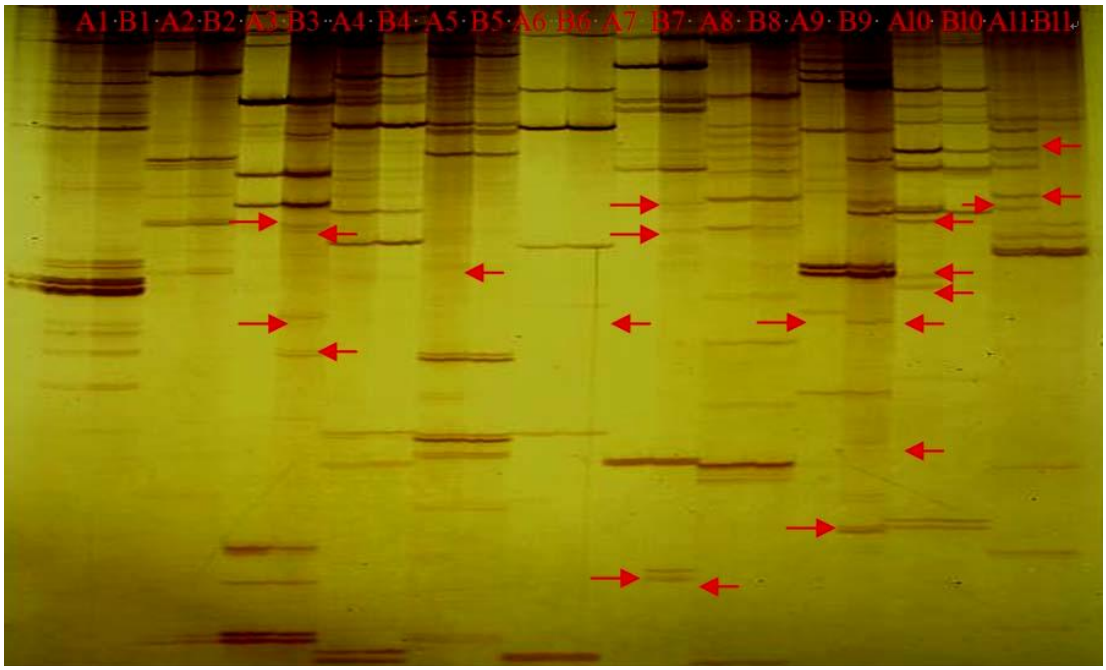

Figure 6. SRAP results of 'Shatangju' (A1-11) and 'Wuzishatangju' (B1-11).

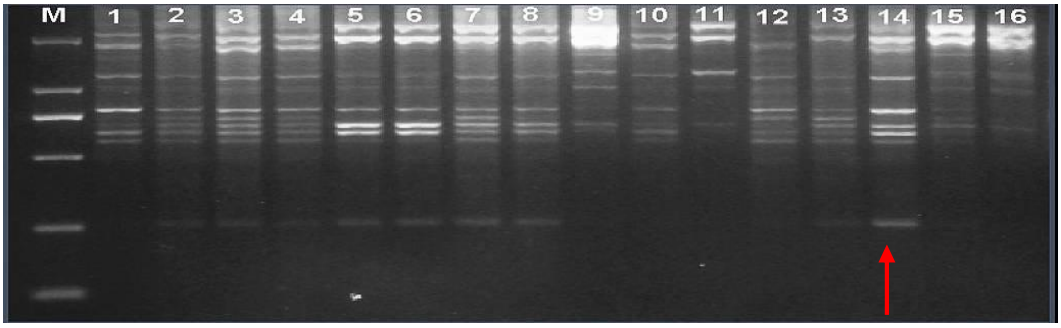

Figure 7. Optimization of IRAP reaction systems (optimum PCR reaction system was shown by an arrow). M, DL2000 Marker; 1-16, different combinations.

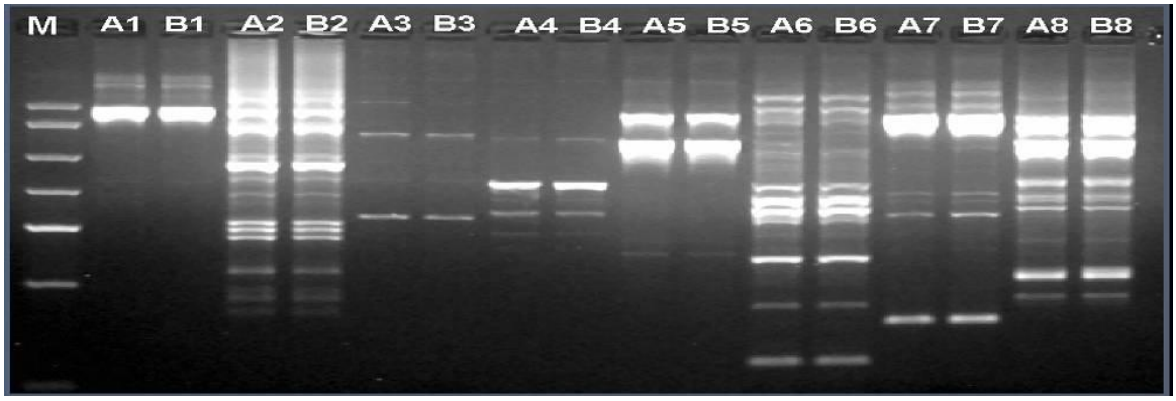

Figure 8. Partial IRAP results of 'Shatangju' (A1-8) and 'Wuzishatangju' (B1-8). M, DL2000 Marker; 1-8: 1, IRAP1; 2, IRAP5; 3, IRAP2-IRAP3; 4, IRAP2-IRAP4; 5, IRAP3-IRAP6; 6, IRAP4-IRAP5; 7, IRAP4-IRAP6; 8, IRAP5-IRAP6.

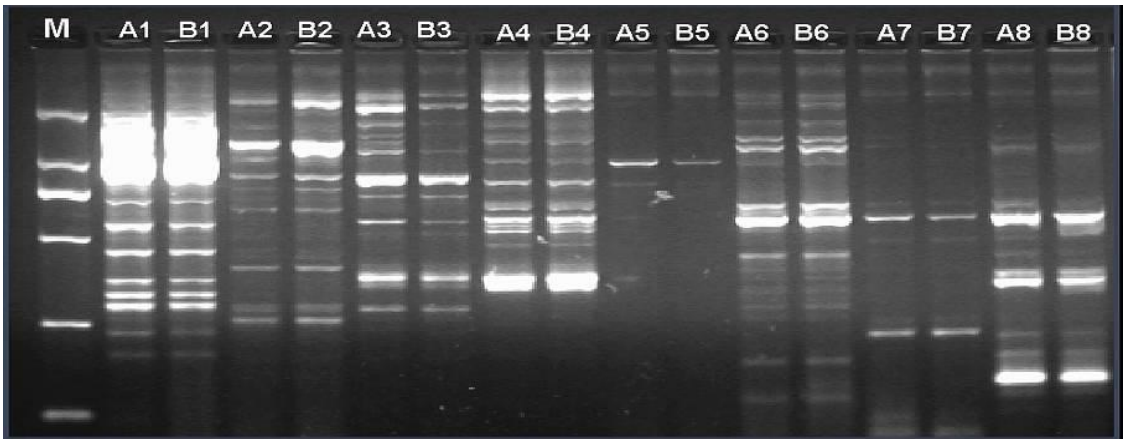

Figure 9. Partial REMAP results of 'Shatangju' (A1-8) and 'Wuzishatangju' (B1-8). M, DL2000 Marker; 1-8: 1, IRAP1-SSR3; 2, IRAP1-SSR4; 3, IRAP1-SSR5; 4, IRAP1-SSR7; 5, IRAP1-SSR8; 6, IRAP2-SSR2; 7, IRAP2-SSR3; 8, IRAP2-SSR5.

advantages of co-dominance, abundance, high reproducibility and simplicity. SSR has been considered one of the ideal molecular markers for diversity assessment of germplasm and marker-assisted selection in *Citrus* (Liu et al., 2005; Barkley et al., 2006; Jannati et al., 2009; Ollitrault et al., 2010). However, SSR analysis requires the construction of genomic library, the subse-quent hybridization with tandem repeated oligo-nucleotides and sequencing of the candidate clones to obtain working primers for a given study species. This is high cost, labor-intensive and time-consuming which has restricted its application in cultivar identification. In the present study, seven pairs of SSR primers were used to detect the genetic variations between 'Shatangju' and 'Wuzishatangju' and no difference was observed (Figure 4) which may be due to the insufficient SSR primers.

ISSR overcomes the limitations of SSR and it is now the marker of choice to identify different individuals as they are abundant, very reproducible, highly polymorphic, highly informative and quick to use. To date, ISSR marker has been successfully applied in germplasm characterization, genetic diversity and breeding in *Citrus* species (Fang et al., 1997; Bretó et al., 2001). In this study, 100 ISSR primers were used to detect genetic variation between 'Shatangju' and 'Wuzishatangju' and two specific bands (> 2000 bp) were obtained (Figure 3). However, further research needs to confirm whether the two fragments are the characteristics of 'Shatangju' and 'Wuzishatangju'.

SRAP is a newly developed molecular marker with the advantages of simplicity, low cost, co-dominant makers, highly reproducibility and easy assay. SRAP is a more preferred technique for revealing genetic diversity among closely related cultivars than RAPD, SSR and ISSR markers (Budak et al., 2004). Currently, SRAP has been successfully used to identify bud sport variation in many plant species (Han et al., 2008; Zhang et al., 2010). In this study, 153 pairs of SRAP primers were used to identify 'Shatangju' and 'Wuzishatangju' and 21 specific fragments were obtained (Figure 6). The results demonstrate that SRAP marker was an effective method to detect genetic variation between original parents and their bud sports.

The mobility of transposon elements can be responsible for changes in bud sport of some species (Yao et al., 2001; Kobayashi et al., 2004; Tao et al., 2006). IRAP and REMAP markers are two new retrotransposon-based DNA fingerprinting techniques with the advantages of highly reproducibility and stability. In our study, 100 retrotransposon primers were used to detect genetic differences between 'Shatangju' and 'Wuzishatangju' and no difference was observed (Figures 8 and 9). The results suggest that retrotransposon may not cause the bud sport of 'Wuzishatangju'.

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REFERENCES

Barkley NA, Roose ML, Krueger RR, Federici CT (2006). Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor. Appl. Genet. 112:1519-1531.

Bretó MP, Ruiz C, Pina JA, Asíns MJ (2001). The diversification of *Citrus clementina* Hort. ex Tan., a vegetatively propagated crop species. Mol. Phylogenet. Evol. 21:285-293.
Budak H, Shearman RC, Parmaksiz I, Dweikat I (2004). Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. Theor. Appl. Genet. 109:280-288.
Cao YF, Liu FZ, Gao Y, Jiang LJ, Wang K, Ma ZY, Zhang KC (2007). SSR analysis of genetic diversity of pear cultivars. Acta Hortic. Sin. 34:305-310.
Deng XX, Guo WW, Sun XH (1996). Advances in breeding and selection of seedless types of citrus in China. Acta Hortic. Sin. 23:235-240.
Deng XX (2005). Advances in worldwide citrus breeding. Acta Hortic. Sin. 32:1140-1146
Durham RE, Liou PC, Gmitter FG, Moore GA (1992). Linkage of restriction fragment length polymorphisms and isozymes in *Citrus*. Theor. Appl. Genet. 84:39-48.
Fang DQ, Roose ML, Krueger RR, Federici CT (1997). Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. Theor. Appl. Genet. 95:211-219.
Fang JG, Zhang Z, Zhou LH, Chen CS, Wang SH (2001). The possibility of using RAPD marker for the identification of fruit sport. J. Fruit Sci. 18:182-185.
Gao ZH, Pan HF, Xu YL, Zhang A, Shu B (2010). Random amplified polymorphic DNA (RAPD) analysis of 'Dangshansu Pear' sports '97-05-9'. Chin. Agric. Sci. Bull. 26:48-53.
Gong GZ, Hong QB, Peng ZC, Jiang D, Xiang SQ (2008). Genetic diversity of poncirus and its phylogenetic relationships with relatives as revealed by nuclear and chloroplast SSR. Acta Hortic. Sin. 35:1742-1750.
Han XY, Wang LS, Zheng AL, Jan DR, Shu QY (2008). Characterization of sequence-related amplified polymorphism markers analysis of tree peony bud sports. Sci. Hortic. 115:261-267.
Jannati M, Fotouhi R, Pourjan Abad A, Zivar S (2009). Genetic diversity analysis of Iranian citrus varieties using micro satellite (SSR) based markers. J. Hortic. Forest. 1:120-125.
Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999). IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor. Appl. Genet. 98:704-711.
Kijas JMH, Thomas MR, Fowler JCS, Roose ML (1997). Integration of trinucleotide microsatellites into a linkage map of *Citrus*. Theor. Appl. Genet. 94:701-706.
Kobayashi S, Goto-Yamamoto N, Hirochika H (2004). Retrotransposon-induced mutations in grape skin color. Science 304:982-982.
Lei TG, He YR, Wu X, Yao LX, Peng AH, Xu LZ, Liu XF, Chen SC (2009). Construction of DNA fingerprinting database of citrus cultivars (Lines). Sci. Agric. Sin. 42:2852-2861.
Li G, Quiros CF (2001). Sequence-related amplified polymorphisim (SRAP), a new maker system based on a simple RCR reaction: Its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103:455-461.
Liu Y, Sun ZH, Liu DC, Wu B, Tao JJ (2005). Assessment of the genetic diversity of pummelo germplasms using AFLP and SSR markers. Sci. Agric. Sin. 38:2308-2315.
Liu YZ, Deng XX (2007). Citrus breeding and genetics in China. Asian Australas. J. Plant Sci. Biotechnol. 1:23-28.
Luo J, Zhou XZ, Zhang YG (2008). Identification and genetic diversity analysis of 25 citrus resources and its sports strains by RAPD technique. Chin. Agric. Sci. Bull. 24:99-104.
Ning YY, Xiong QE, Zeng WG, Zeng GR (2003). Studies on red flesh sport from 'Red Sun' kiwifruit using RAPD marker. Acta Hortic. Sin. 30:511-513.
Ollitrault F, Terol J, Antonio Pina J, Navarro L, Talon M, Ollitrault P (2010). Development of SSR markers from *Citrus clementina* (Rutaceae) BAC end sequences and interspecific transferability in Citrus. Am. J. Bot. 97:e124-e129.
Qiao YC, Lin SQ, Yang XH (2009). Optimization of ISSR-PCR analysis and its application in germplasm of Loquat (*E. japonica* Lincll. cv) by uniform design. Genomics Appl. Biol. 28:123-126.
Qin YH, Hu HG, Ye ZX, Lin SQ, Miao HX, Zhang CY, Hu GB (2011). Identification of progenies derived from sexual hybridization of citrus using RAPD molecular marker. Acta Hortic. 894:123-132.
Tao NG, Wei J, Liu YZ, Cheng YJ, Deng XX (2006). Copia-like retrotransposons in a precocious mutant of trifoliate orange [*Poncirus trifoliata* (L.) Raf]. J. Hortic. Sci. Biotechnol. 81:1038-1042.
Wang SH, Cheng LG, Zhang Z, Fang JG (2000). Study on the identification of citrus cultivars with RAPD markers. J. Fruit Sci. 17:70-72.
Wang XP, Wang YJ, Zhang JX, Xu Y, Yang KQ (2003). Randomly amplified polymorphic DNA (RAPD) analysis of *Vitis* early-ripening mutant cultivar "Early Takasumi". Acta Bot. Bor-Occid. Sin. 23:473-476.
Wei J (2007). Characterization of retrotransposon elements and development of related molecular markers in citrus. PhD Dissertation of Huazhong Agricultural University.
Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18:7213-7218.
Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.
Xiong GM, Liang GL, Yan Y, Xiang SQ, Wu Q, Li XQ, Jiang D (2002). DNA extraction method for AFLP analysis in *Citrus*. J. Fruit Sci. 19:267-268.
Yao JL, Dong YH, Morris BAM (2001). Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADSbox transcription factor. Proc. Natl. Acad. Sci. USA. 98:1306-1311.
Ye WJ, Qin YH, Ye ZX, Teixeira da Silva JA, Zhang LX, Wu XY, Lin SQ, Hu GB (2009). Seedless mechanism of a new mandarin cultivar 'Wuzishatangju' (*Citrus reticulata* Blanco). Plant Sci. 177:19-27.
Ye ZX, Zeng T, Xu JK, Luo ZD, Hu GB, Zhang ZQ, Ji ZL, Chen YC, Chen GL, Chen LX, Lin SQ (2006). Wuzishatangju, a new mandarin cultivar. J. Fruit Sci. 23:149-150.
Zhang M, Deng XX (2006). Advances in research of citrus cultivars selected by bud mutation and the mechanism of formation of mutated characteristics. J. Fruit Sci. 23:871-876.
Zhang SP, Wang LJ, Lü ZW (2010). Optimization of SRAP system of pomegranate (*Punica granatum* L) and its application on identification of a white flower mutation. Acta Bot. Bor-Occid. Sin. 30:911-917.