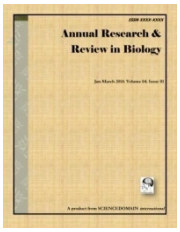

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Improved RAPD Analysis of *Canarium album* (Lour.) Raeusch from Sichuan Province along Yangtze River in China

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Authors' contributions

This work was carried out in collaboration between all authors. Author J Fu designed the project & experiments, wrote the first draft of the manuscript. Authors Z Mei, L Yang and T Zhang performed major experiments with equal contribution. Authors H Yu and L Gan managed the analyses of the study. Authors L Zhang and M Yang performed experimental assistance. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To build up the foundation of the classification and breeding of *Canarium album* cultivars in China.
Methodology: The improved RAPD analysis was performed in nine samples by prolonging the ramp time from annealing to extension by using ten RAPD primers, out of twelve, which were selected randomly to analyze the genetic characteristics.
Results: The polymorphism of the nine *C. album* was 86%, and the similarity coefficient ranged from 0.65 to 1.00. Among the similarity coefficients of the cultivars, the similarity coefficient of sample No. 2, 3 and 6 was highest (1.0), while the similarity coefficient of sample No. 5 and 7 was 0.98, which indicated that they are the same cultivars. The similarity coefficient of sample No. 9 and 4 was the lowest (0.65), which indicated that they are the different cultivars.
Conclusion: Our study provides a theoretical basis for the breeding and classification of *C. album* in Sichuan Province along Yangtze River of China. Also this study suggests that the improved RAPD amplification could be an effective technique to analyze the polymorphism and genetic relationship in different organisms, especially, *C. album*.

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Keywords: Canarium album; RAPD; ramp; clustering analysis; cultivar identification.

1. INTRODUCTION

Canarium album (Lour.) Raeusch is the native plant species of South-East China, particularly in the Guangdong, Hainan, Sichuan, and Fujian provinces. *C. album* has been introduced to other Asian tropical and semi-tropical regions, including Vietnam, Japan, Malaysia, etc. This plant species is well tolerated to poor soils. In China, *C. album* is basically used for oil, like European olive, hence it is also called Chinese Olive. The fruit, nut, seed, and root of *C. album* have a long history of usage as traditional medicines for the treatment of swollen and sore throat, excessive thirst, hematemesis due to cough, lacillary dysentery, epilepsy, puffer poisoning, and alcoholism [1]. The dried fruits are also reported to have anti-virus, anti-bacterial, anti-inflammation and detoxification activities [2]. Hejiang County of Luzhou City in Sichuan Province of China lies on the upstream of Yangtze River, where it is the largest production base of *C. album* in Sichuan. Dozens of cultivars of *C. album* are planted, and it is difficult to distinguish the different cultivars from the morphology. In this study, we focused on the genetic characterization of this fruity plant by employing the molecular technique, which might be useful for proper identification of this plant and distinguishing its different cultivars.

Since 1990s, a number of molecular marker techniques have been developed, including random amplified polymorphic DNA (RAPD) [3,4,5,6], inter-simple sequence repeats (ISSR) [7,8], The internal Transcribed Spaces [9], amplified fragment length polymorphism (AFLP) [10], which have been widely used in genetic identification of various plants and animals [11,12]. RAPD analysis is characterized by fewer requirements on template DNA, no pollution, and inexpensive, but it also has some disadvantages like poor reproducibility and low production. Fu et al. [13] reported that the resolution and production of RAPD are greatly increased by prolonging the ramp time from annealing to extension. To examine the effectiveness of RAPD method in *C. album*, an improved RAPD technique has been developed and applied by prolonging ramp time from 3ºC /s to 0.3ºC /s. Here, 3ºC/s RAMP time was the default RAMP time, which we called regular RAPD-PCR or regular PCR, while 0. 3ºC/s RAMP time was the reduced rate of RAMP time, which we called improved RAPD-PCR or RAMP-PCR. Our study distinguished the different cultivars of *C. album*, and established a genetic relationship between them by using improved RAMP PCR in Luzhou city of Sichuan Province along Yangtze River in China.

2. MATERIALS AND METHODS

2.1 Reagents

2XPCR Taq Mastermix was purchased from TianGen Biotech Co.LTD (Beijing). The RAPD primers were purchased from SBS Genetech Corporation. DNA Marker (DL2000) was purchased from SinoBio. Other reagents were analytical grade reagents, and used as in our previous experiments [13].

2.2 Plant Material

The DNA templates were collected from Hejiang County of Luzhou City, Sichuan Province in China, which is located at the upstream of Yangtze River. The name and accession location was as shown in Table 1. The fresh *C. album* leaves and fruits were carefully identified and

named according to the fruit size and weight, leaves size and colour (Table 1; some data not shown). The DNA material of fresh younger leaf samples for each accession was extracted with modified CTAB (Centrimonium bromide) method [14]. DNA quality was determined by electrophoresis on 1% agarose gels. DNA concentration was measured by spectrophotometry at 260 and 280 nm [15]. The final concentration of all DNA samples was adjusted to 10ng/μL for PCR analysis, and stored at -20ºC [16].

Table 1. Samples of *Canarium album* in this study

No.	Cultivars Name	Source	Cluster
1	Da Shuozi	Hejiang, LuZhou	I
2	Er Shuozi	Hejiang, LuZhou	II
3	Er Shuozi	Hejiang, LuZhou	II
4	Jin Xianggu	Hejiang, LuZhou	III
5	Er Shuozi	Hejiang, LuZhou	II
6	Er Shuozi	Hejiang, LuZhou	II
7	Er Shuozi	Hejiang, LuZhou	II
8	Cuipi	Hejiang, LuZhou	IV
9	Xiao Baiyuan	Hejiang, LuZhou	V

2.3 PCR Amplification

PCR amplification system with total volume of 20μl, including 2μl of 2.5 μmol/L primers, 3μl of DNA template of *C. album* (30 ng), 10μl of 2×PCR Taq Mastermix, and 5μl of deionized water. PCR amplification was performed as follows: initial denaturation at 95ºC for 90 s and followed by 40 cycles of reaction with the steps at 94ºC for 40 s, 36ºC for 90 s, 72ºC for 90 s, and final extension of 5 min at 72ºC. PCR of each accession were executed in a Mastercycler 5331 (Eppendorf, Germany). The ramp time from annealing to extension was adjusted from 3 to 0.3ºC/s to compare the resolution and production of the two methods. All the PCR reactions were repeated at least five times for nine samples.

The amplified PCR products were separated by electrophoresis on 1.5% agarose gel in 1×TAE buffer. Gels were visualized by 0.5μg/mL ethidium bromide staining and the images were documented using the ChemiDoc XRS （Bio-Rad, USA). Bands that were unambiguous and reproducible in successive amplifications were selected for scoring.

2.4 Statistical Analysis

Bands in the gel profiles were recorded as present (1) and absent (0). The similarity matrix (S.M) and the similarity index (S.I) were calculated using SM coefficient. The dendrogram based on UPGMA (unweighted pair group method with arithmetic mean) algorithm was generated using the SAHN module in NTSYS pc 2.1 packages [16,17].

3. RESULTS

3.1 Establishment of Improved RAPD Technique in Analysis of *C. Album*

To establish the condition of improved RAPD in *C. album*, SBS-A5 primer was used firstly and amplified with improved RAPD by adjusted RAMP time from annealing to extension with 3ºC/s to 0.3ºC/s in nine samples collected from Hejiang, Sichuan Province. The PCR

reaction for each sample was repeated for five times. As seen in the Fig. 2, all samples demonstrated similar repeatable fingerprints, in which each sample amplified two bands with the ramp time 3ºC /s (default time) (Fig. 1, left panel), while the ramp time was prolonged from 3ºC /s to 0.3ºC /s, the amount of PCR products (mainly large pieces of DNA) and number of DNA bands were obviously increased. The sample No. 3 only had 2 bands with regular PCR (Fig. 1, left), while the band number was increased to 7 with the improved RAPD technique (Fig. 1, right), indicating the production, resolution, and reproducibility of RAPD were significantly improved. Therefore we established the improved RAPD condition in *C. album*. As a consequence, the improved RAPD technique by prolonging the ramp time from annealing to extension 0.3ºC /s was applied to complete the subset of nine samples.

The PCR amplification difference in DNA quality and leaf ages were also compared (Supplementary Figure). All PCR products showed repeated band files, except for primer SBS-I1 in old leaves with higher efficiency in smaller size of bands (Supplementary Figure, left panel), whereas DNAs from the barks showed poor amplification unfortunately (data not shown).

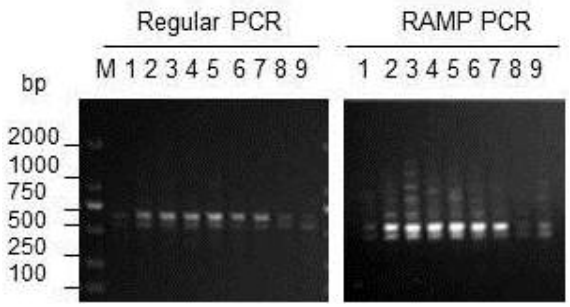


Fig. 1. Random amplified polymorphic DNA amplification results of DNA samples using SBS-A5 primer

Samples of number 1 to 9 represents DNA of different plant samples collected from Hejiang county (showed in Table 1). "M" indicates DL2000 DNA marker with molecular weight size (bp). Left panel indicates the results of regular RAPD amplification (regular PCR); right panel indicates the results of improved RAPD amplification (RAMP PCR).

3.2 Amplification Results of Improved RAPD Analysis

Twelve primers were used randomly to conduct improved RAPD analysis by prolonging the ramp time from annealing to extension 0.3ºC /s, and interestingly ten primers (Table 2) showed improved RAPD results. PCR reaction for each sample was repeated for five times, and generated reproducible polymorphic amplification bands. The characteristic bands and representative fingerprints in nine samples produced by these primers are shown in Fig. 2. The band sizes of PCR products ranged from 260 bp to 2000 bp (Fig. 2), and a total of 64 bands were obtained, among which polymorphic fragments accounted for 86%, indicating high genetic diversity among the nine accessions with an average number of amplification bands of 6.4 per primer. This result indicated that these 10 primers are useful in identifying DNA polymorphisms and performing genetic relationship analysis in *C. album* samples.

The amplification patterns with primers SBS-I1, SBS-I4, SBS-Q4 and SBS-Q19, showed more polymorphisms in nine *C. album* samples, whereas the amplification patterns with primer SBS-I2 and SBS-Q9 showed a few differences in each other (Fig. 2). The

amplification patterns of 1-8th samples are similar by using primers SBS- I2 and SBS-Q9 (Fig. 2), respectively, but different from 9th sample. The 9th sample missed a 1500 bp fragment which was present in other 8 samples, while using primers SBS- I1, SBS-I4, SBS-Q9 and SBS-Q19 (Fig. 2A).

Table 2. Sequences of RAPD primers

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
SBS-A5	AGGGGTCTTG	SBS-A15	TTCCGAACCC
SBS-I1	ACCTGGACAC	SBS-I2	GGAGGAGAGG
SBS-I4	CCGCCTAGTC	SBS-I10	ACAACGCGAG
SBS-Q4	AGTGCGCTGA	SBS-Q9	GGCTAACCGA
SBS-Q12	AGTAGGGCAC	SBS-Q19	CCCCCTATCA

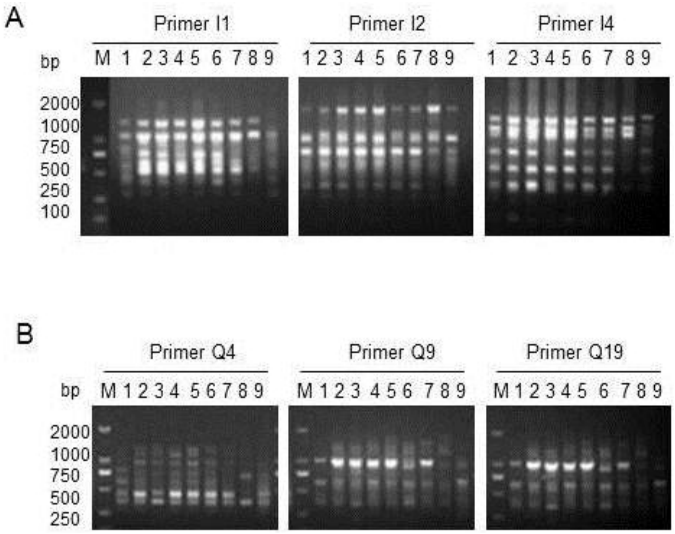


Fig. 2. The different RAPD banding patterns in *C. album* samples obtained with represented primers

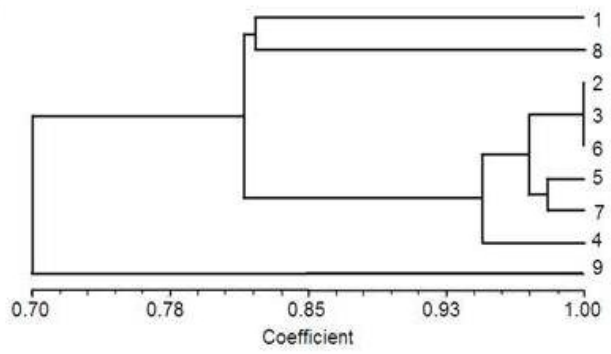
A. primer I set: SBS-I1, SBS-I2, and SBS-I4. B. primer Q set: SBS-Q4, SBS-Q9, and SBS-Q12. Sample number 1 to 9 represents DNA of different plant samples collected from Hejiang county (showed in Table 1). "M" indicates DL2000 DNA marker with molecular weight size (bp).

3.3 Genetic Distance and Cluster Analysis

The genetic relationship dendrogram was obtained by conducting cluster analysis to the similarity coefficient of above-mentioned nine samples (Fig. 3). The similarity coefficient of them ranged from 0.65 to 1.0, the similarity coefficient of sample No. 1 and samples No. 2, 3, 4, 5, 6, 7, 8, 9 were 0.82, 0.82, 0.80, 0.80, 0.82, 0.82, 0.82, 0.76, respectively (Fig. 3B); the similarity coefficients of sample No. 2 and samples No.3, 4, 5, 6, 7, 8, 9 were closer: 1, 0.94, 0.98, 1, 0.96, 0.80, 0.71, respectively (Fig. 3B). Among the similarity coefficients of the cultivars, the similarity coefficient of sample No. 2, 3 and 6 were highest (1.0), while the similarity coefficient of sample No. 5 and 7 was 0.98, which indicates that they are possibly the same species cultivars. The No. 2, 3, 6 and No. 5, 7 clades were clustered together with a 0.96 similarity index, then they clustered sample No.4 together with a 0.94 similarity index (Fig. 3A and B). The sample No.1 and No.8 clades were clustered together with a 0.82

similarity index, and they formed the sister to the former with a 0.80 similarity index (Fig. 3A). The 9th clade was in a basal polytomy with a 0.71 similarity index, which is consistent with the morphology (Fig. 3A and data not shown).

A



B

No.	1	2	3	4	5	6	7	8	9
1	1								
2	0.82	1							
3	0.82	1	1						
4	0.80	0.94	0.94	1					
5	0.80	0.98	0.98	0.96	1				
6	0.82	1	1	0.94	0.98	1			
7	0.82	0.96	0.96	0.94	0.98	0.96	1		
8	0.82	0.80	0.80	0.82	0.82	0.80	0.84	1	
9	0.76	0.71	0.71	0.65	0.69	0.71	0.71	0.71	1

Fig. 3. Dendrogram of nine *C. album* samples based on 64 PCR bands amplified by the 10 primers

A. Dendrogram of nine of *C. album* samples based on 64 PCR bands amplified by the 10 primers. Bar on the bottom indicates similarity index based on S.M. coefficient. B. Genetic distance dendrogram for *C. album* samples using improved RAPD. Sample number 1 to 9 represents different samples showed in Table 1.

4. DISCUSSION

RAPD technology is a molecular marker technique used to detect the DNA polymorphism developed in the late 1990s. Environmental impact might directly reflect the genomic information, and has been widely used to identify species in genetic relationship studies [18,19]. Although RAPD technique has been widely used in other fruit trees, still very few reports have devoted on *C. album*. In China, only Yang et al. [20] and Chen et al. [21] have analyzed and reported on *C. album* in Fujian Province or at the eastern of Guangdong Province with ISSR technology, and Nie [22] has analyzed and reported on *C. album* in

Fujian Province with RAPD technology. In their report, a total of 27 accessions of *C. album* genetic resources in Fujian Province were investigated and the traditional RAPD reaction system was established and optimized; the genetic relationship and genetic diversity of *C. album* L. germplasm in Fujian Province were also obtained. Although their study indicated the higher polymorphism, better reproducibility for cultivars identification, but Nie [22] selected 16 primers for RAPD analysis, of which 14 primers could be used for RAPD analysis and they got only 37 amplified bands, which is much less than ours. In our analysis, we obtained 64 bands in 10 primers from 12 randomly selected primers. As a result, numbers of amplified fragments across the tested accessions ranged from 2 (primer SBS-A5) to 7 (primer SBS-I1). The band sizes ranged from about 260 to 2000 bp. The 10 primers produced a total of 64 bands with an average of 6.4 bands per primer. 86% of the bands were found polymorphic. These results illustrate that DNA polymorphism could be detected among the nine *C. album* cultivars using our improved RAPD technique.

In determining the relationships between different populations, cluster analysis was performed to provide valuable data. Based on the scoring of 64 bands, the similarity matrix was used to analyze the genetic relationships. The genetic relationship dendrogram of *C. album* was constructed through cluster analysis. The similarity coefficients of the tested materials ranged from 0.65 and 1.0, revealing the genetic differences between different cultivars of *C. album*. The similarity coefficient of sample No. 2, 3, 6 and sample No. 5, 7 are the 1.0 and 0.98, and therefore clustered to group II (Table 1). The sample No. 1 and sample No. 8 clades are clustered together with a 0.82 similarity index, and they form the sister to the former with a 0.80 similarity index (Fig. 3). The 9^{th} clade is in a basal polytomy with a 0.71 similarity, which is consistent with the morphology (Fig. 3 and data not shown). The 9th Canarium accession had leaves and fruit that were obviously smaller in size than others and the color of 9^{th} *C. album* leaf are more yellowish than others, whereas the leaf of the 1^{th} is light green. Therefore DNA polymorphic analysis of different cultivars of *C. album* through improved RAPD markers could provide important information for the classification and breeding of *C. album*.

The reproducibility and resolution of RAPD are affected by many factors, such as the quality and concentration of template, cycles, temperature, primers, and some other unidentified factors [23]. However, our studies have shown that the results could be reproducible as long as reaction conditions are strictly controlled in *C. album* with five time repeats, and the amplification difference between DNA quality and leaf ages were also compared in Supplementary Figure. Unfortunately, DNAs extracted from the *C. album* barks showed poor amplification (data not shown), which suggested the DNA extraction from other methods, like proteinase K digestion and phenol/chloroform purification might get higher quality [12,15,24]. Therefore, another method is suggested for future experiment which was developed by Nie [22], a leaf DNA extraction method in avoiding of polyphenols, carbohydrate, and pigments combining with DNA to form insoluble compounds and inhibiting the activity of Taq DNA polymerase.

By using RAPD technique to analyze the fingerprints of *C. album* cultivars, it is proved that prolonging the ramp time can not only improve the production of RAPD but also increase the number of DNA amplified bands [13]. Specifically, in SBS-A5 amplification patterns, the band numbers and productions were enhanced obviously in sample No. 3 by RAMP-PCR from 2 to 6 (Fig. 1, right). The band numbers were increased from 2 to 3 in sample No.1 and No. 4. According to the abundant DNA polymorphisms, therefore, the *C. album* accessions are distinguishable using our improved RAPD technique. Thus, this method described here can amplify more specific DNA bands from using RAPD primers. The reason may be that

prolonged ramp time is beneficial to bind DNA template and primers to bind well. The slow heating speed increases the bond stability of primers with template DNA, preventing primers from detaching the template [12], and thus increased the number of bands and the PCR product yields.

5. CONCLUSION

Our study provides a theoretical basis for the breeding and classification of *C. album* from Luzhou city of Sichuan Province along Yangtze River in China. There was 86% polymorphism in nine *C. album* samples, and the similarity coefficient ranged from 0.65 to 1.00 by improved RAPD analysis. Among the similarity coefficients of the cultivars, the similarity coefficient of sample No. 2, 3 and 6 was highest (1.0), while the similarity coefficient of sample No. 5 and 7 was 0.98, which indicated that they are the same cultivars. The similarity coefficient of sample No. 9 and 4 was the lowest (0.65), which indicates that they are the different cultivars. This genetic characterization might also be beneficial for the preservation of genetic preservation of *C. album* and other species. Therefore, we found that this improved technique is more efficient than conventional RAPD technique, which suggests that improved RAPD amplification by prolonging ramp time could be an effective technique to analyze the polymorphism and genetic relationship in various organisms, including *C. album*.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

REFERENCES

1. Wang H, Song L, Tang H, He H, Dai C, Li X. Main chemical components for clearing heat and relieving sore throat in different germplasm of *Canarium album*. Zhongguo Zhong Yao Za Zhi. 2010;35(6):669-72. Chinese.
2. Zhang LL, Lin YM. Tannins from *Canarium album* with potent antioxidant activity. J Zhejiang Univ Sci B. 2008;9(5):407-15.
3. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18(22):6531–5.
4. Devaiah KM, Venkatas ubramanian P. Genetic characterization and authentication of *Embelia ribes* using RAPD-PCR and SCAR marker. Planta Med. 2008;74(2):194-6.
5. Ruzicka J, Lukas B, Merza L, Göhler I, Abel G, Popp M, Novak J. Identification of Verbena officinalis based on ITS sequence analysis and RAPD-derived molecular markers. Planta Med. 2009;75(11):1271-6.

6. Duttal SR, KarII PK, SrivastavaII AK, SinhaII MK, ShankarI A, Ghosh AK. Identification of RAPD and SCAR markers associated with yield traits in the Indian tropical tasar silkworm Antheraea mylitta drury. Genet Mol Biol. 2012;35(4):743-51.
7. Ganopoulos I, Merkouropoulos G, Pantazis S, Tsipouridis C, Tsaftaris A. Assessing molecular and morpho-agronomical diversity and identification of ISSR markers associated with fruit traits in quince (*Cydonia oblonga*). Genet Mol Res. 2011;10(4):2729-46.
8. Feofilov AV, Bardukov NV, Glazko VI. Gene pool differentiation between Altaic and trotting horse breeds inferred from ISSR-PCR marker data. Genetika. 2011;47(9):1230-5. Russian.
9. Varela ES, Lima JP, et al. Relationships in *subtribe Diocleinae* (Leguminosae; Papilionoideae) inferred from internal transcribed spacer sequences from nuclear ribosomal DNA. Phytochemistry. 2004;65(1):59-69.
10. Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 1995;23(21):4407–14.
11. Zhang GF, Guan JM, Lai XP, Lin J, Liu JM, Xu HH. RAPD fingerprint construction and genetic similarity of *Mesona chinensis* (Lamiaceae) in China. Genet Mol Res. 2012;11(4):3649-57.
12. Farkhari M, Naghavi MR, Pyghambari SA, Sabokdast. Genetic variation of jointed goatgrass (*Aegilops cylindrica* Host.) from Iran using RAPD-PCR and SDS-PAGE of seed proteins. Pak J Biol Sci. 2007;10(17):2868-73.
13. Fu JJ, Li LY, Xu X, Wang Z, Tang G, Yin CM, Lu GX. An improved method for increasing the efficiency of the technique of Random Amplified Polymorphic DNA (RAPD). Hereditas. 2000;22(4):251-2. Chinese.
14. Sharma P, Joshi N, Sharma A. Isolation of genomic DNA from medicinal plants without liquid nitrogen. Indian J Exp Biol. 2010;48(6):610-4.
15. Fu JJ. Short protocols in medical molecular biology. China Medical Science Press, Beijing; 2012. Chinese.
16. Mei Z, Fu S, Yu H, Yang L, Duan C, Liu X, Gong S, Fu J. Genetic characterization and authentication of *Dimocarpus longan* Lour. using an improved RAPD technique. Genet Mol Res. 2013; (In press).
17. Rohlf FJ. NTSYS-pc: numerical taxonomy system ver.2.1, Exeter Pub. Ltd., Setauket, New York, 2002.
    Available: http://www.exetersoftware.com/cat/ntsyspc/ntsyspc.html.
18. Shakeel M, Ilyas M, Kazi M. Evaluation of synthetic hexaploid wheats (derivative of durum wheats and Aegilops tauschii accessions) for studying genetic diversity using randomly amplified polymorphic DNA (RAPD) markers. Mol Biol Rep. 2013;40(1):21-6.
19. Vieira RF, Grayer RJ, Paton A, Simon JE. Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. Biochem Syst Ecol. 2001;29(3):287-304.
20. Yang PK, Zhuang DH, Ma RJ, Zhao Q, Du H. The ISSR Analysis of Genetic Diversity of *Canarium album* L. Germplasm Resourc in Eastern Guangdong. Chinese Agricultural Science Bulletin. 2011;27(24):86-92. Chinese.
21. Chen MX, Zhang QJ, Chen QX, Lin YF. ISSR Analysis of Main Cultivated and Fresh-eating *Canarium album* (Lour.) Raeusch in Fuzhou City. Modern Agricultural Science and Technology. 2011;21:129-30. Chinese.
22. Nie SZ. RAPD Analysis of Album (*Canarium album*) Genetic Resources in Fujian Province. Master's Degree Thesis of Fujian Agriculture and Forestry University, Fuzhou; 2005. Chinese.
23. Meunier JR, Grimont PA. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res Micobiol. 1993;144(5):373-9.

24. Fu JJ, Li LY, Lu GX. Relationship between microdeletion on Y chromosome and patients with idiopathic azoospermia and severe oligozoospermia in the Chinese. Chin Med J. 2002;115(1):72-75.

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