

## rDNA ITS

				1								
1.				318000								
2.				318000								
		15		<i>Rubus</i> L.				rDNA ITS				
		DNA		PCR				rDNA ITS				
15		ITS1	ITS2	5.8 S				255 258 208 211	164 bp	ITS1	ITS2	
138		41						5.8 S		4		
	15			0.139 0	0.008 1							15
		rDNA ITS										
		rDNA ITS										
	R282.12			A				0253 - 2670(2013)15 - 2143 - 07				

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### Cloning and analysis of rDNA ITS sequences from plants in *Rubus* L.

JIANG Ming<sup>1</sup>, LI Rong-rong<sup>2</sup>, GUAN Ming<sup>1</sup>, LI Jin-zhi<sup>1</sup>

1. College of Life Science, Taizhou University, Jiaojiang 318000, China

2. College of Pharmaceutical and Chemical Engineering, Taizhou University, Jiaojiang 318000, China

**Abstract: Objective** To provide the evidences for molecular identification as well as genetic diversity studies, sequencing and comparison of rDNA ITS sequences from 15 plants in *Rubus* L. were performed. **Methods** Full length rDNA ITS sequences were isolated from leaf genomic DNA by PCR method with universal primers, and these sequences was analyzed using bioinformatic softwares. **Results** The length of ITS1, ITS2, and 5.8 S sequences for the 15 plants in *Rubus* L. were 255 258, 208 211, and 164 bp, respectively. Total 138 variable sites were found in ITS1 and ITS2 sequences with 41 parsim

		ITS	
	inter-simple sequence repeat		
ISSR	DNA random amplified	<b>1</b>	
polymorphic DNA	RAPD	<b>1.1</b>	
	amplified fragment length polymorphism AFLP	15	
	simple sequence repeats SSR		
	internal transcribed spacer ITS		1
[13-18]	ITS		
	15	ddH <sub>2</sub> O	-80
	PCR		

1 15

Table 1 Sources of 15 plants in *Rubus L.*

				/ m
1	<i>Rubus amphidasys</i>	27°37'	119°06'	378
2	<i>R. buergeri</i>	28°41'	121°08'	312
3	<i>R. caudifolius</i>	27°43'	119°12'	1 300
4	<i>R. corchorifolius</i>	28°49'	120°58'	389
5	<i>R. coreanus</i>	29°15'	121°05'	843
6	<i>R. innominatus</i>	28°41'	118°53'	386
7	<i>R. irenaeus</i>	27°30'	118°59'	437
8	<i>R. lambertianus</i>	28°50'	121°05'	146
9	<i>R. parvifolius</i>	28°41'	121°08'	356
10	<i>R. pirifolius</i>	27°19'	120°27'	423
11	<i>R. reflexus</i>	29°15'	121°05'	697
12	<i>R. rosifolius</i>	28°50'	121°06'	86
13	<i>R. rufus</i>	27°31'	119°03'	651
14	<i>R. swinhoei</i>	28°40'	118°54'	248
15	<i>R. tsangii</i>	28°39'	118°54'	288

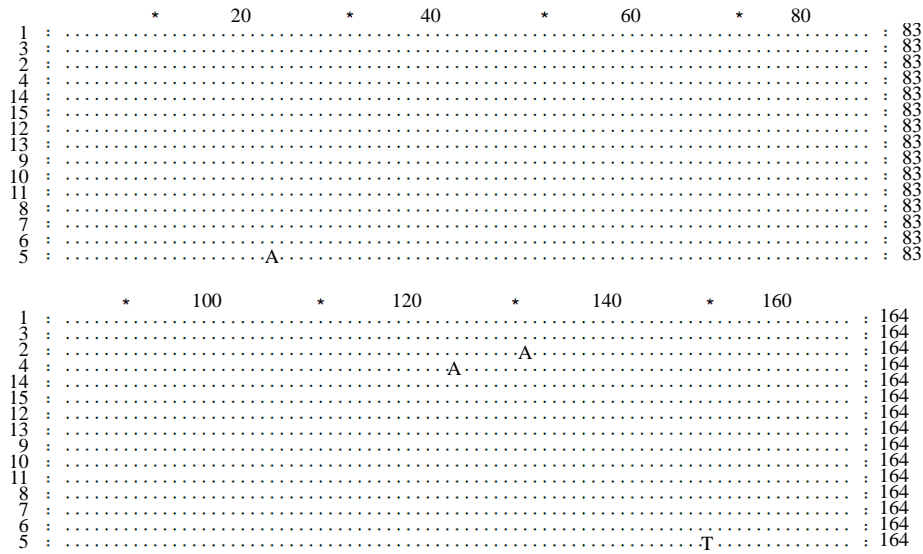
<b>1.2</b>			PCR	PCR	2 μL 10× PCR
	BIO-RAD C1000	PCR	BIO-RAD Gel Doc	20 mmol/L Mg <sup>2+</sup>	0.45 μL dNTPs
XR		SANYO MDF-382E	CN		0.45 μL Taq DNA
	BECKMAN Allegra	64R		0.5 μL	0.25 μmol/L
	DYY—12			30 ng DNA	ddH <sub>2</sub> O 20 μL
<b>2</b>				C1000 PCR	94 °C
<b>2.1</b>	<b>DNA</b>			5 min 94 °C	40 s 53.5 °C
	0.2 g	1.5 mL		75 s 33	72 °C 10 min
		DNA	DNA	<b>2.3 PCR</b>	
	SDS [19]			PCR	
<b>2.2 ITS</b>					DNA
	5'-TATGCTTAAAYTCAGCGGGT-3'		5'-		
	AACAAGGTTTCCGTAGGTGA-3'				2.5 μL
	DNA			p-GEM T-easy	Promega
					1 h

DH5α ITS 15 5.8 S  
 164 bp  
 PCR 3 ITS1 258 bp 256  
 2.4 bp 255 bp ITS2  
 ClustalX 1.81 [20] Excel ITS2 211 bp  
 LEN SUBSTITUTE GC 210 bp  
 MEGA 3.1 [21] ITS2 208 bp  
 PAUP 4.0 [22] MP maximum 15 5.8 S ITS1 ITS2  
 parsimony 1 000 GC 5.8 S  
 3 GC 54.3% 13 GC  
 3.1 ITS 54.9% ITS1 GC 52.7% 58.4%  
 DNA GC  
 15 ITS 2 56.1%  
 15 ITS 627 631 GC ITS2 GC 52.2% 60.6%  
 bp ITS GC 58.8%  
 630 bp GC 2

Table 2 ITS sequence full length and GC contents of plants in *Rubus L.*

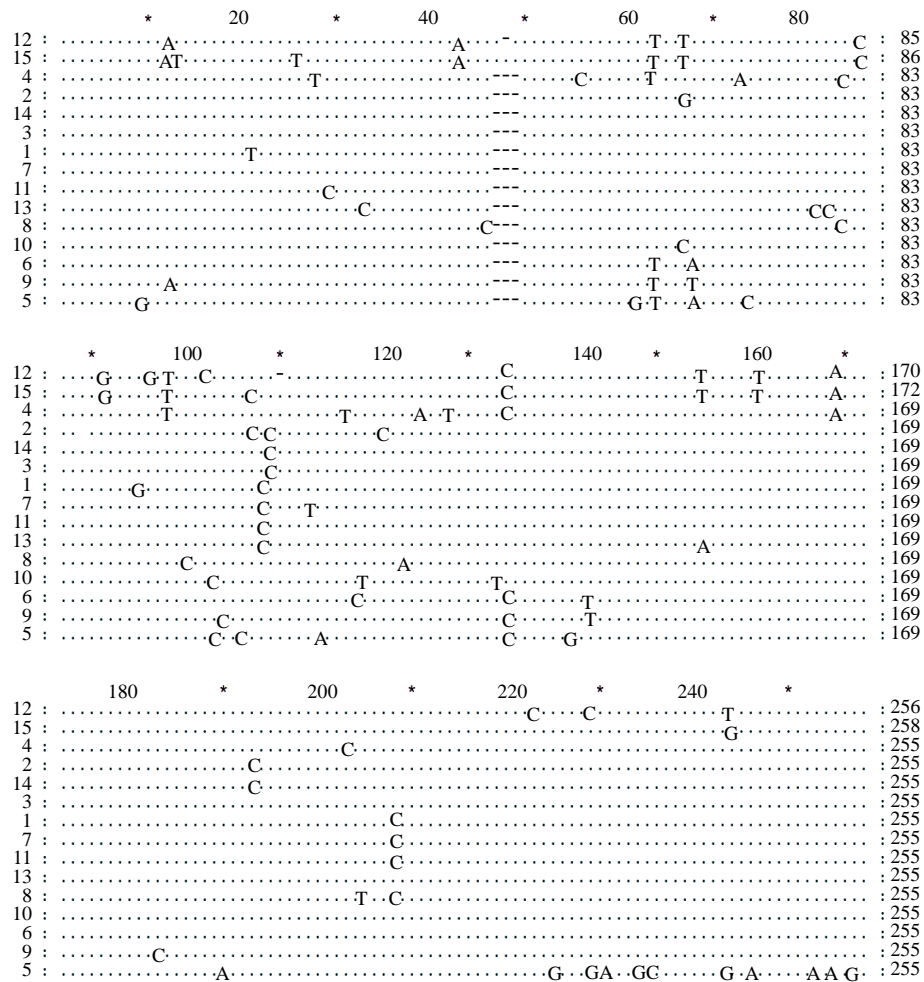
ITS	/ bp	5.8 S			ITS1			ITS2		
		/ bp	GC	/%	/ bp	GC	/%	/ bp	GC	/%
1	629	164	54.9		255	55.3		210	55.7	
2	628	164	54.9		255	55.7		209	57.9	
3	630	164	54.9		255	56.1		211	58.8	
4	628	164	54.3		255	53.3		209	55.0	
5	627	164	54.3		255	58.4		208	60.6	
6	627	164	54.9		255	54.5		208	57.2	
7	628	164	54.9		255	56.1		209	56.9	
8	628	164	54.9		255	56.1		209	57.4	
9	627	164	54.9		255	54.1		208	56.7	
10	628	164	54.9		255	56.1		209	56.5	
11	629	164	54.9		255	55.3		210	57.1	
12	628	164	54.9		256	55.1		208	52.4	
13	627	164	54.9		255	56.1		208	56.3	
14	628	164	54.9		255	55.7		209	57.9	
15	631	164	54.9		258	52.7		209	52.2	

3.2 5.8 S conserved site 160  
 15 5.8 S ClustalX variable site 4 parsim-info site  
 1 0  
 12 5.8 S 3.3 ITS1 ITS2  
 5.8 S 22 A 150 T 5.8 S ITS1  
 2 T G / 2  
 5.8 S 124 A 14 47 49 1 3  
 G 131 A 14 T 5.8 S / 1 /



1 15 5.8 S

Fig. 1 Comparison on 5.8 S sequences of 15 plants in *Rubus L.*



2 15 ITS1

Fig. 2 Comparison on ITS1 sequences of 15 plants in *Rubus L.*

Chinese Traditional and Herbal Drugs				43	15	2013	8	2147
ITS1	189			ITS2	1	3	/	
73.5%	68	26.4%	20	106	/			
	7.8%	ITS1	ITS2	1	15			ITS
	/			140	66.4%	70		
3	15	17	/	33.2%	21	10.0%		

3 15 ITS2  
**Fig. 3 Comparison on ITS2 sequences of 15**

3 15

Table 3 Genetic distances of 15 plants in *Rubus* L.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.029 4													
3	0.017 9	0.019 5												
4	0.044 7	0.055 0	0.041 3											
5	0.102 9	0.106 6	0.095 6	0.123 4										
6	0.026 1	0.029 4	0.019 5	0.044 7	0.092 0									
7	0.014 6	0.029 4	0.016 2	0.044 7	0.102 9	0.026 1								
8	0.022 8	0.031 1	0.017 9	0.046 4	0.102 9	0.026 1	0.021 1							
9	0.031 1	0.036 1	0.022 8	0.046 3	0.097 5	0.017 9	0.029 4	0.029 4						
10	0.024 5	0.024 4	0.016 2	0.044 7	0.097 4	0.026 1	0.022 8	0.024 5	0.027 8					
11	0.012 9	0.027 8	0.014 6	0.043 0	0.101 1	0.024 5	0.008 1	0.019 5	0.027 8	0.021 1				
12	0.067 9	0.074 8	0.062 5	0.067 8	0.137 0	0.058 9	0.062 3	0.069 6	0.062 3	0.067 7	0.060 6			
13	0.022 9	0.034 5	0.021 2	0.049 9	0.104 7	0.031 2	0.017 8	0.029 5	0.034 5	0.027 8	0.016 2	0.058 8		
14	0.019 5	0.019 5	0.008 1	0.043	0.097 4	0.021 1	0.017 8	0.019 5	0.024 4	0.017 8	0.016 2	0.064 3	0.022 8	
15	0.067 9	0.071 2	0.062 5	0.067 8	0.139 0	0.058 9	0.062 3	0.069 6	0.062 3	0.067 7	0.060 6	0.016 2	0.058 8	0.064 3

ITS1 255 258 bp ITS2 208 211 bp 5.8 S  
164 bp 5.8 S

5.8 S

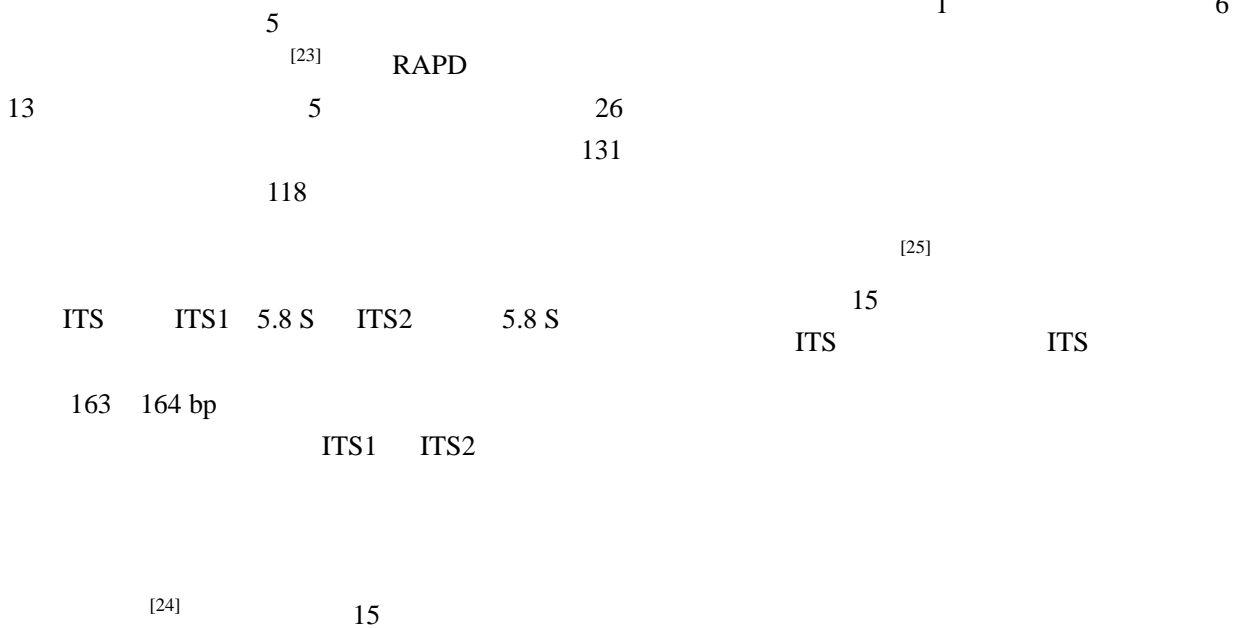
ITS1 ITS2 5.8 S

/

15

4 MP

Fig. 4 Phylogenetic tree constructed using MP method



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