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[摘要] 目的 ISSR-PCR  
方法 ISSR-PCR  
结果 ISSR-PCR 10 μl Premix Taq DNA  
0.3 μmol/L 10 ng DNA 20 μl 100 10  
结论 ISSR-PCR

[关键词]  
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### Establish and optimization of inter-simple sequence repeat PCR reaction system of *Gnaphalium affine*

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[Abstract] **Objective** To provide the experimental basis for the subsequent genetic diversity research through establishing and optimizing the inter-simple sequence repeat PCR (ISSR-PCR) reaction system of *Gnaphalium affine*. **Methods** The single-factor experimental method and full experimental method were used to optimize the ISSR-PCR reaction system of *Gnaphalium affine*. Under the optimal system, after screening primers and corresponding annealing temperatures, the systematic feasibility was verified. **Results** The optimal ISSR-PCR reaction system was consisted of 10 μl Premix Taq DNA polymerase, 0.3 μmol/L primer, 10 ng DNA template, and sterilized water added to 20 μl. Finally, 10 primers were screened from 100 universal primers, and verification results indicated the system had high stability, good reproducibility, and the selected primers had good polymorphism. **Conclusion** The ISSR-PCR amplification system of *Gnaphalium affine* was established for the first time and the primers with appropriate annealing temperatures were filtered out, which provided a reference for the subsequent genetic diversity research of *Gnaphalium affine*.

[Key words] *Gnaphalium affine* inter-simple sequence repeat PCR system optimization primer screening

*Gnaphalium affine* D.Don " "  
Compositae *Gnaphalium*

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13ZR1448800 [7]

[作者简介] Email jasmine\_jsg@163.com inter-simple sequence repeat,  
[通讯作者] Email sssnmr@163.com ISSR  
DNA

ISSR simple sequence DNA  
repeat, SSR ISSR SSR

[8]

ISSR

SSR

[9]

ISSR-PCR

## 1 材料与仪器

### 1.1 样品来源

2017

1

*Gnaphalium affine* D.Don

-80 °C

表 1 鼠曲草样品信息表

S1	2017-03-08	S5	2017-04-03
S2	2017-03-28	S6	2017-04-07
S3	2017-02-25	S7	2017-04-05
S4	2017-03-31	S8	2017-04-08

### 1.2 仪器与试剂

DNA

DK-8D

5418R

Eppendorf

NanoDrop 2000

Thermo

Mastercycler Pro

PCR

Eppendorf

JS-350

JS-Power 600

JS-2000

ISSR

Genewiz

Premix *Taq* DNA

6× Loading Buffer

DL15000 DNA Marker

DL2000

DNA Marker Takara Bio

ethidium

bromide, EB

## 2 实验方法

### 2.1 基因组 DNA 的提取及检测

DNA 0.8%

-20 °C

### 2.2 鼠曲草 ISSR-PCR 反应体系的建立

DNA

20 μl

PCR

DL2000 DNA Marker

EB

1.6%

" 55 V 90 min"

### 2.3 鼠曲草 ISSR-PCR 反应体系的优化

S7

DNA

CA<sub>8</sub>G

818

DNA

"

"

ISSR-PCR

### 2.4 引物筛选及退火温度的确定

$T_m$

$=4 \times G + C + 2 \times$

A + T

$T_m$

$T_m$

100

S1

S2

$T_m$

$T_m - 2 \sim$

$T_m + 4$

6

$T_c$

0

1

S1

$T_c - 2$

$T_c$

$T_c + 2$

3

"

"

### 2.5 鼠曲草 ISSR-PCR 反应体系的验证

8

DNA

ISSR

"

"

### 3 结果与分析

#### 3.1 基因组 DNA 的提取及检测

1

DNA  
OD<sub>260</sub>/OD<sub>280</sub> 1.7~1.9  
DNA

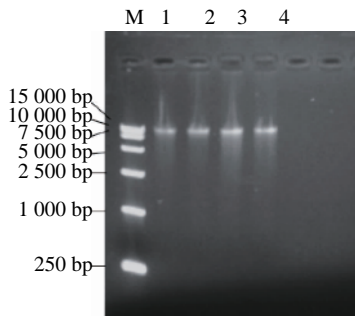


图 1 鼠曲草基因组 DNA 的电泳图谱

M.DL15000 DNA Marker 1~4. bp.DNA

#### 3.2 鼠曲草 ISSR-PCR 反应体系的建立

ISSR-PCR dNTPs Mg<sup>2+</sup> Taq  
DNA DNA  
DNA Taq DNA  
ISSR-PCR [10-12]  
dNTPs Mg<sup>2+</sup> Taq  
2 [12-14]  
Premix Taq DNA  
dNTPs Mg<sup>2+</sup>

表 2 预混酶的组成及浓度

TaKaRa Taq	1.25 U/25 μl
dNTPs	0.4 mmol/L
Tris-HCl, pH8.9	20 mmol/L
KCl	100 mmol/L
MgCl <sub>2</sub>	3 mmol/L

[10-14] ISSR-PCR  
10 μl Premix Taq 0.8 μmol/L  
30 ng DNA 20 μl PCR  
94 °C 5 min 94 °C 30 s  
30 s 72 °C 1 min

38 72 °C 10 min

#### 3.3 鼠曲草 ISSR-PCR 反应体系的优化

##### 3.3.1

DNA DNA  
2 0.3 μmol/L  
0.4 μmol/L

<0.3 μmol/L >0.4 μmol/L

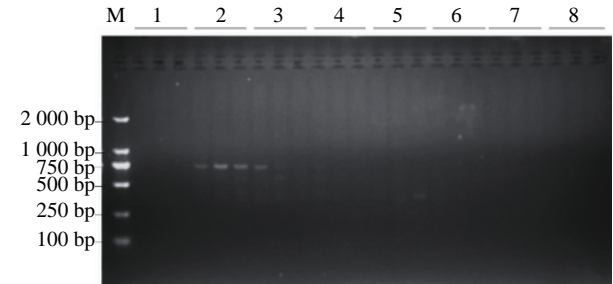


图 2 不同浓度引物对 ISSR-PCR 反应的影响

M.DL2000 DNA Marker 1~8. 0.2 0.3 0.4  
0.5 0.6 0.7 0.8 0.9 μmol/L bp.DNA

3 DNA DNA  
10~40 ng  
10 ng 2  
1 DNA >40 ng

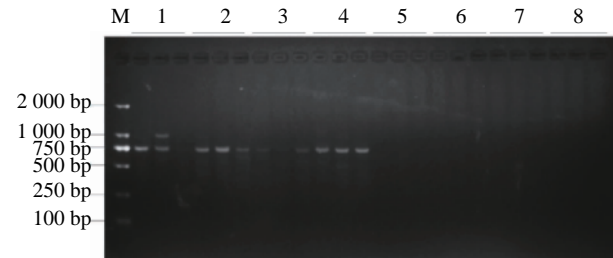


图 3 不同 DNA 模板量对 ISSR-PCR 反应的影响

M.DL2000 DNA Marker 1~8.DNA 10 20 30  
40 50 60 70 80 ng bp.DNA

##### 3.3.2

ISSR-PCR  
4  
4 DNA 16 3  
4 DNA 10  
ng  
DNA 15 ng 6 7  
DNA 20 ng  
DNA  
25 ng 14 10 ng DNA  
1~4  
0.30 μmol/L DNA

表 3 引物和 DNA 模板的全面实验考察

DNA	ng	$\mu\text{mol/L}$	DNA	ng	$\mu\text{mol/L}$
1	10	0.25	9	20	0.25
2	10	0.275	10	20	0.275
3	10	0.30	11	20	0.30
4	10	0.375	12	20	0.375
5	15	0.25	13	25	0.25
6	15	0.275	14	25	0.275
7	15	0.30	15	25	0.30
8	15	0.375	16	25	0.375

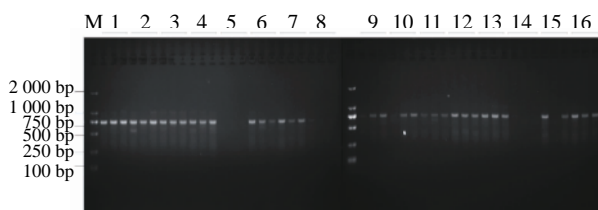


图 4 引物和 DNA 模板的全面实验扩增结果

M.DL2000 DNA Marker 1~16.  
bp.DNA

10 ng DNA

0.3  $\mu\text{mol/L}$

3.4 引物筛选及退火温度的确定

$T_m$  >57 °C  $T_m$  <48 °C

5

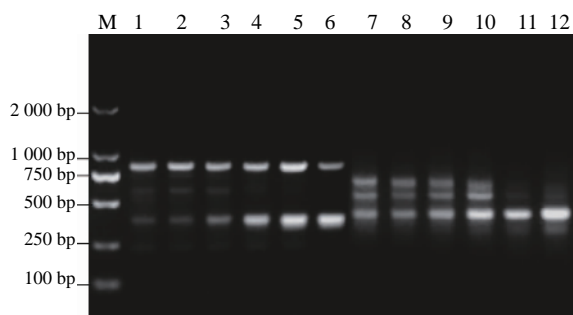


图 5 引物初筛的部分扩增结果

M.DL2000 DNA Marker 1~6. 851  
51.9 52.9 54.0 55.4 56.9 58.5 °C 7~12. 857  
51.9 52.9 54.0 55.4 56.9 58.5 °C  
bp.DNA

$T_c$

$T_c-2 T_c$

$T_c+2$  3

42

DNA

$T_c T_m$

6

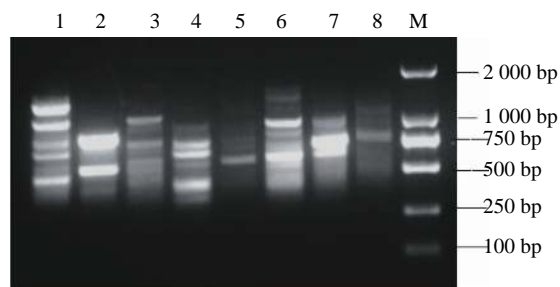


图 6 引物二筛的部分扩增结果

M.DL2000 DNA Marker 1~8.  $T_c=50.0$  °C 816  
825 807 810 812 817 819 821  
52.0 °C bp.DNA

25

17

4

3.5 鼠曲草 ISSR-PCR 反应体系的验证

810 816 834 846 847 848 857  
861 878 891 7

4 讨论

[15] ISSR

[16]

DNA

表 4 通过二筛引物的序列及最佳退火温度

	5' 3'	°C		5' 3'	°C
810	GA <sub>8</sub> T	52.0	848	CA <sub>8</sub> RG	56.0
816	CA <sub>8</sub> T	48.0	855	AC <sub>8</sub> YT	54.0
818	CA <sub>8</sub> G	54.0	856	AC <sub>8</sub> YA	50.0
825	AC <sub>8</sub> T	50.0	857	AC <sub>8</sub> YG	56.0
826	AC <sub>8</sub> C	54.0	861	ACC <sub>6</sub>	58.0
834	AG <sub>8</sub> YT	50.0	863	AGT <sub>6</sub>	46.0
842	GA <sub>8</sub> YG	54.0	878	GGAT <sub>4</sub>	50.0
846	CA <sub>8</sub> RT	50.0	891	HVH TG <sub>7</sub>	46.0
847	CA <sub>8</sub> RC	56.0			

R = A/G Y = C/T H = A/C/T V = A/C/G

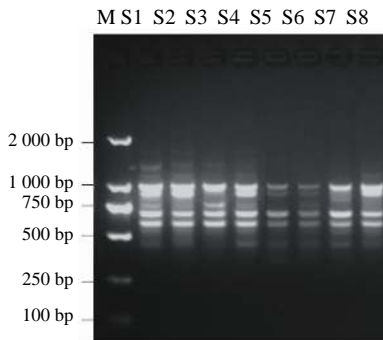


图 7 引物 891 对不同居群鼠曲草 DNA 模板的扩增结果  
M.DL2000 DNA Marker S1~S8. 1 bp.DNA

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DNA  
DNA  
10 ng  
DNA  
[13]  
 $T_c$   
3  
CA GA  
DNA  
GT CT  
[17]

[4] 0" E{enkp"F"hwpevkqp"kp"vw o qt" o ckpvgpcepeg [L]0"Ecpegt"Egmn 4234  
[L]0" .4239."58 ; 389/38;0 44 6 65:/6730  
[5] ." ." ." 0" [L]0" .4239."37 9 [36] OCUCO J C"E"R."DGPDTQQM"F"O"0"E{enkp"F3"fgi tcfwkqp"ku  
uwhhkekqpv"vq"kp fweg" I 3"egmn"e{eng"cttguv"fgurkvg"eqpukwkxg"gz/  
3388/338:0 [L]0" .4239."; 43 425/4270 [37] QIKPQ"U."PQUJQ"M."KTCJ CTC"P."gv"cn0"C"eqjqtv"uwvf{ "qh  
[6] ." 0" [L]0" .4239."; 43 425/4270 [37] E{enkp "F3"gzrtguukqp "cpf" rtqipquku "kp"824 "eqnqp "ecpegt  
ecugu[L]0"Enkp"Ecpegt"Tgu 422; 37 35 6653/665:0  
[7] 0" C76; [37] QIKPQ"U."PQUJQ"M."KTCJ CTC"P."gv"cn0"C"eqjqtv"uwvf{ "qh  
[L]0" .4237."56 6 477/47:0 [38] KUJ KMCY C"V."HWTKJ CVC"O."Q J VUWMK" [ . "gv"cn0"E{enkp"F3  
[8] ." ." ." 0" 52 [L]0" .4237. [38] qxgtxgzrtguukqp"tgncvgf"vq"tgvpqdncuvq o c"rtqvgkp"gzrtguukqp"cu"c  
4; 7 4;/540 [39] NK"J" [ ."ZIKCQ"Y"Y."OC"L"Y."gv"cn0"Fwcn"jki j"gzrtguukqp"qh  
[9] ." ." ." 0" Ngyku [L]0" .4239."47 6 5:8/ [39] UVCV5 "cpf"E{enkpF3"ku"cuuqekcvgf"ykvj"rqqt"rtqipquku"chvgt  
5;20 [39] ewtwkxg"tgugevkqp"qh"guqrjci gcn"uswo o qwu"egmn"ectekppq o c[L]0  
[:] ." ." ." 0" Ngyku [L]0" .4233."38 3 94/ [3:] . . . 0"E{enkpF3 E{enkpF5  
960 [3:] [L]0" .4238."44 : 837/8430  
[:;] ." . . \* +0" [3:] ." ." ." 0"E{enkpF3 XGIH  
]O\_0" < .422:0 [L]0" .4225."44 3  
[32] ." ." ." 0" [L]0" .4238."44 33 6;/760 :8;/20  
[33] ." ." ." 0"E{enkpF3."R38."Td [L]0" .4225."46 36 [42] ." ." ." 0"r38  
3493/34960 [L]0" .4239."59 6 7:7/7:;0 [L]0"  
[34] ." ." 0"E{enkpF3 [L]0" . [43] ." ." ." 0"R38 [L]0"  
422;."39 4 572/5750 [L]0" .4237."35 3 92/950 \* +.4237."35 3 92/950  
[35] E J QK" [ "L."NK"Z" [ ." J [ FDTKP I "R."gv"cn0"Vjg"tgswtg o gpv"ht [ [ ] 423:/34/46"" [ [ ] 423;/2;/44  
" [ [ ] ]



\* 68 +  
[33] ." ." ." 0" KUUT/RET [L]0" .4236."5; 34 4455/  
445:0 [L]0"  
[34] ." ." ." 0" KUUT/RET [L]0" .4235."66 7 832/8370  
[35] ." 0" KUUT [L]0" [37] EWK"E"L."NK" [ ."NKW" [ "N."gv"cn0"Fgvt o kpcvkqp"qh"i gpgvke" fk/  
xgtukv{ "c o qpi" Saccharina" igt o rncu o "wukpi "KUUT"cpf"TCRF  
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Ej kpc [L]0"Dkqnqi { \*Dcugn+ 423: : 3 G40  
[39] ." 0" 33 34 [L]0" .422;."4: 7 :96/: :40  
[ [ ] 423;/2;/34"" [ [ ] 423;/32/43  
[ [ ] ]