# Genetic diversity of *Ustilago scitaminea* Syd. in Southern China revealed by combined ISSR and RAPD analysis

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# Why we did this research

- **Several studies on genetic diversity of smut fungi showed that the highest genetic diversity of this fungi populations was in Asia (Raboin et al., 2007; Braithwaite et al., 2004).**

- **According to research reported that Taiwan has 3 physiological races (Lee et al., 1999). But mainland China has 2 in 1990s, and no systemically investigated since then.**

# Why we did this research

- **Interestingly, the pathogenicity variations observed in Taiwan coincide with the genetic variation revealed by AFLP markers in these populations (Braithwaite et al., 2004)**

- **A range of sugarcane cultivars, viz. ROC series, cultivated in China were originated from Taiwan, and the currently dominant varieties planted in mainland China are ROC series (over 50%)**

# Why we did this research

- **Are there any special on their hereditary material for this fungi population? How about the race in current mainland China?**

# Molecular markers: useful tools for kinship and population studies

- **RAPD: Random amplified polymorphic DNA**
- **ISSR: Inter-simple sequence repeat**
- **advantages: high-efficiency, sharp sensibility and easy-detection.**

# smut strains analyzed

- **35 monosporidial mating-type isolates derived from 28 single-whips (sori) of sugarcane smut collected from Southern China were used.**

- **These fungal isolates were collected from 16 sugarcane cultivars including F134 that is resistant to the physiological race 1 but susceptible to the race 2, and NCo310 that is immune to both race 1 and race 2.**

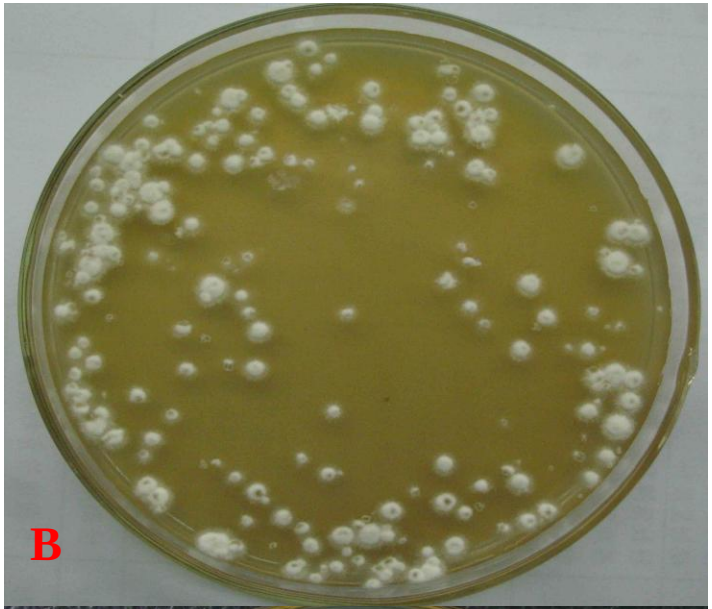
Table 1 **Smut strains analyzed**

| Isolate | Location | Host | Mating type |
|---|---|---|---|
| 1 | Guangzhou, Guangdong | F134 | + |
| 2 | Guangzhou, Guangdong | F134 | - |
| 3 | Guangzhou, Guangdong | YT 97-639 | + |
| 4 | Guangzhou, Guangdong | YT 97-639 | - |
| 5 | Guangzhou, Guangdong | ROC22 | + |
| 6 | Guangzhou, Guangdong | ROC22 | - |
| 7 | Zhanjiang, Guangdong | ROC10 | + |
| 8 | Zhanjiang, Guangdong | ROC10 | - |
| 9 | Guangzhou, Guangdong | CP94-1100 | + |
| 10 | Guangzhou, Guangdong | CP94-1100 | - |
| 11 | Zhanjiang, Guangdong | ROC22 | + |
| 12 | Zhanjiang, Guangdong | ROC22 | - |
| 13 | Zhanjiang, Guangdong | ROC16 | + |
| 14 | Zhanjiang, Guangdong | ROC16 | - |
| 15 | Shaoguan, Guangdong | ROC22 | - |
| 16 | Guangzhou, Guangdong | N:Co376 | - |

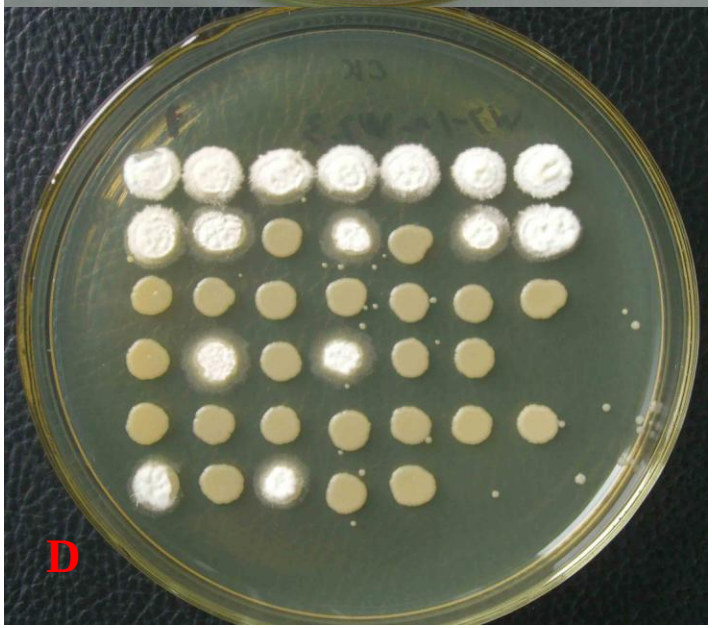
# Table 1 (continued)

| Isolate | Location | Host | Mating type |
|---|---|---|---|
| 17 | Honghe, Yunnan | YT 00-236 | - |
| 18 | Honghe, Yunnan | YT 00-236 | - |
| 19 | Honghe, Yunnan | ROC26 | - |
| 20 | Honghe, Yunnan | ROC10 | - |
| 21 | Honghe, Yunnan | ROC20 | - |
| 22 | Honghe, Yunnan | ROC16 | - |
| 23 | Honghe, Yunnan | ROC7 | - |
| 24 | Baise, Guangxi | ROC22 | - |
| 25 | Chongzuo, Guangxi | ROC22 | - |
| 26 | Chongzuo, Guangxi | ROC22 | - |
| 27 | Shaoguan, Guangdong | HoCP95-988 | - |
| 28 | Shaoguan, Guangdong | FN 28 | - |
| 29 | Zhanjiang, Guangdong | Yin 0518 | - |
| 30 | Zhanjiang, Guangdong | ROC22 | - |
| 31 | Zhanjiang, Guangdong | Taitang 1626 | - |
| 32 | Zhanjiang, Guangdong | ROC22 | - |
| 33 | Zhanjiang, Guangdong | ROC22 | - |
| 34 | Zhanjiang, Guangdong | YT 89-113 | - |
| 35 | Zhanjiang, Guangdong | ROC22 | - |

# Isolation procedure for the monosporidial mating-type strain





- **DNA exextraction: CTAB method**

- **Electrophoresis and polymorphism detection: 1.8% agarose gel**

# Data analysis

- **The markers were scored as 1(present) or 0 (absent)**

- **Jaccard similarity coefficient and UPGMA cluster analyses were conducted by using NTSYS-pc2.10 analytical software (Rohlf, 2000).**

# Result

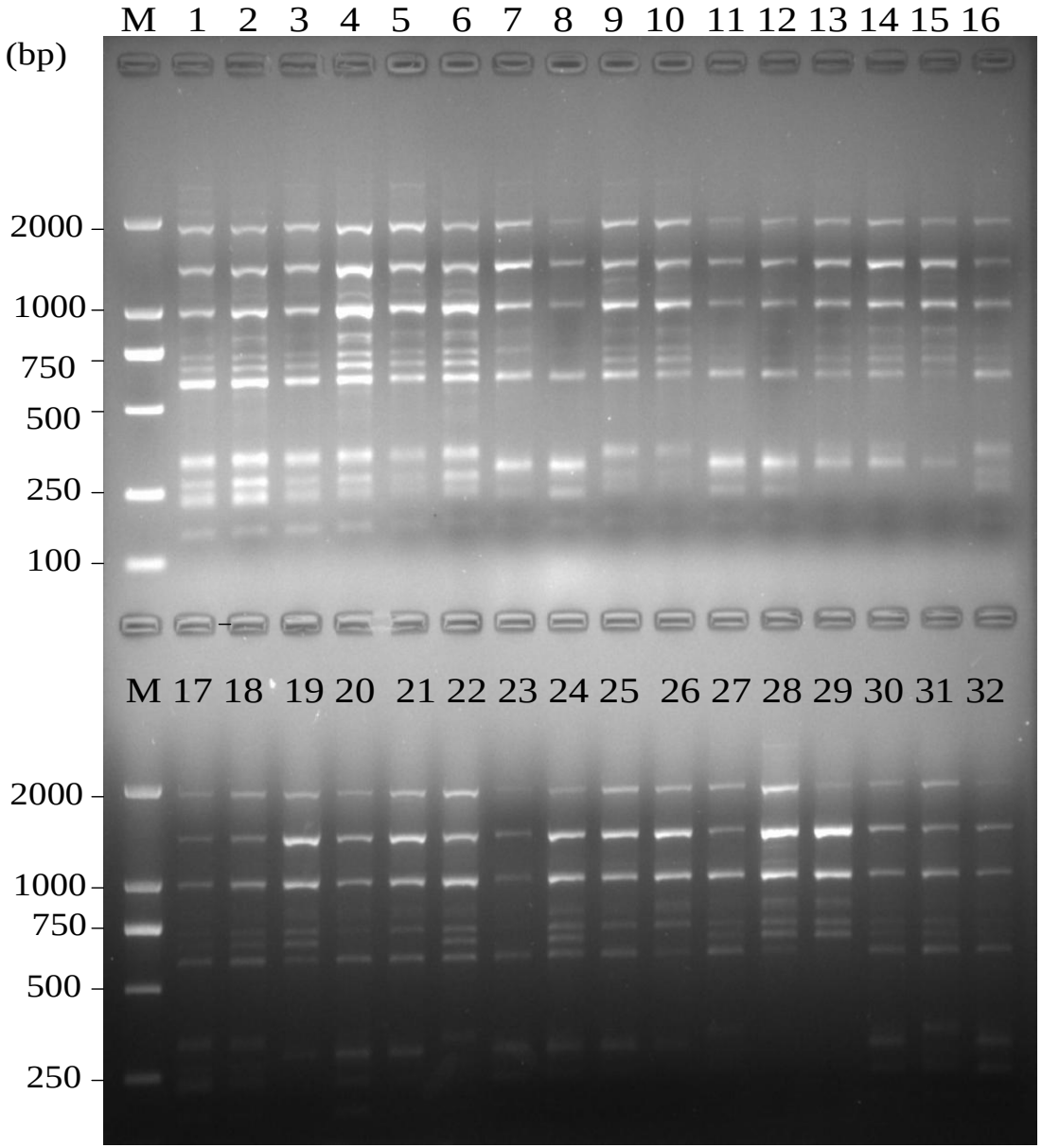
- **ISSR: 18 primer combinations**
- **RAPD: 18 primer combinations**

# Polymorphic allele analysis

# *U. scataminea* polymorphism determined by ISSR analysis

| primer code | Sequence | No. of amplified bands | No. of Polymorphic bands | Percentage of Polymorphic (%) |
|---|---|---|---|---|
| 104 | ATGATGATGATGATGATG | 8 | 6 | 75 |
| 113 | AGAGAGAGAGAGAGAGTC | 7 | 6 | 85.7 |
| 880 | GGAGAGGAGAGGAGA | 8 | 5 | 62.5 |
| 826 | ACACACACACACACACC | 7 | 4 | 57.1 |
| 827 | ACACACACACACACACG | 5 | 3 | 60 |
| 812 | GAGAGAGAGAGAGAGAA | 9 | 5 | 55.6 |
| 855 | ACACACACACACACACYT | 7 | 6 | 85.7 |
| 859 | TGTGTGTGTGTGTGTGRC | 7 | 6 | 85.7 |
| 815 | CTCTCTCTCTCTCTCTG | 4 | 4 | 100 |
| 811 | GAGAGAGAGAGAGAGAC | 7 | 4 | 57.1 |
| 835 | AGAGAGAGAGAGAGAGYC | 7 | 6 | 85.7 |
| 884 | HBHAGAGAGAGAGAGAG | 15 | 12 | 80 |
| 890 | VHVGTGTGTGTGTGTGT | 7 | 4 | 57.1 |
| 891 | HVHTGTGTGTGTGTGTG | 7 | 3 | 42.9 |
| 873 | GACAGACAGACAGACA | 6 | 4 | 66.7 |
| 117 | AGAAGAAGAAGAAGAAGAAGA | 6 | 6 | 100 |
| 857 | ACACACACACACACACYG | 6 | 4 | 66.7 |
| 876 | GATAGATAGACAGACA | 5 | 5 | 100 |
| Total | | 128 | 93 | |
| Average | | 7.1 | 5.2 | 72.7 |

Fig. 1



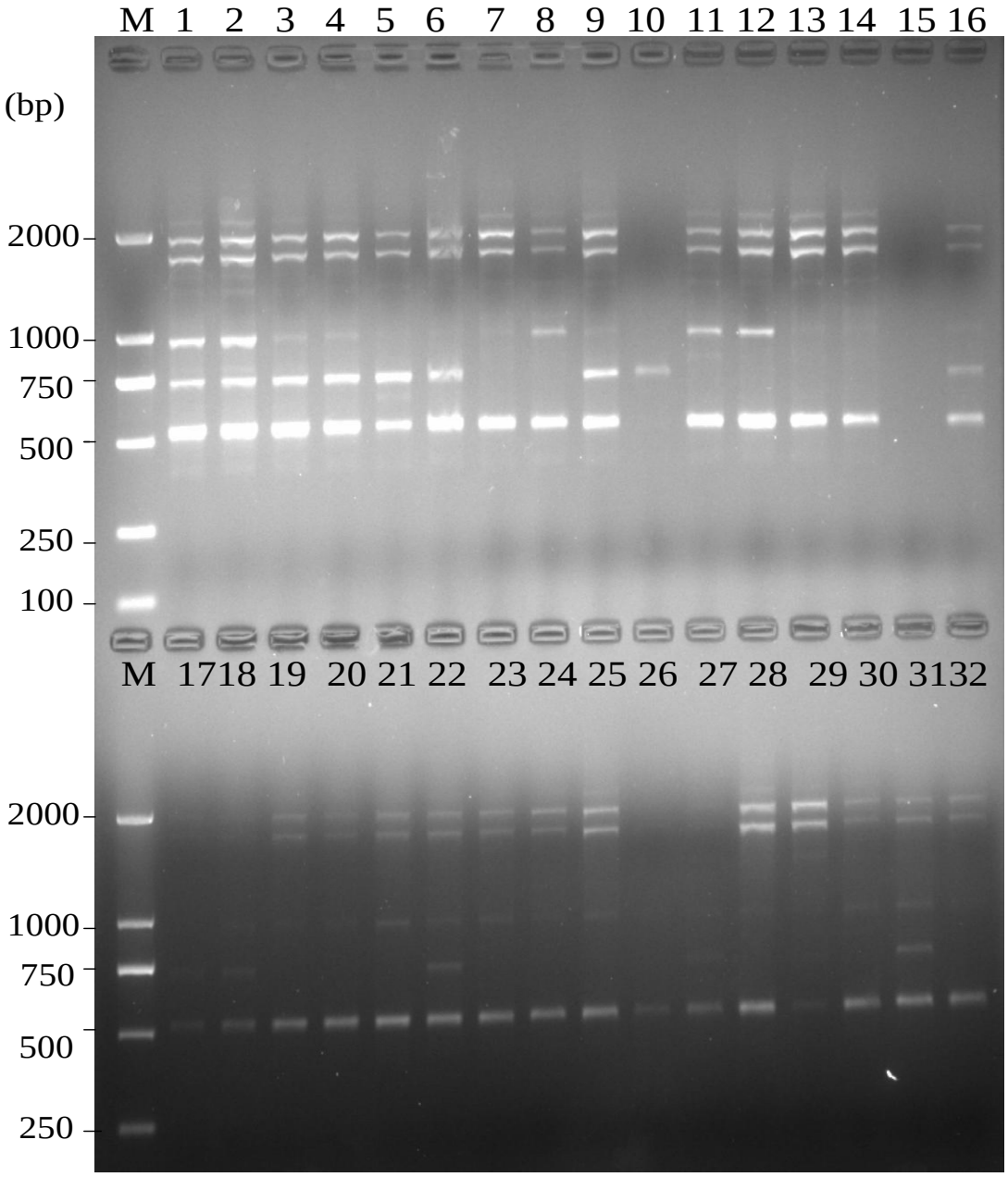

**ISSR primer 884**

- **the primer 884 generated the most number of bands with 12 polymorphic bands**

# RAPD-PCR analysis of *U. scataminea* polymorphism

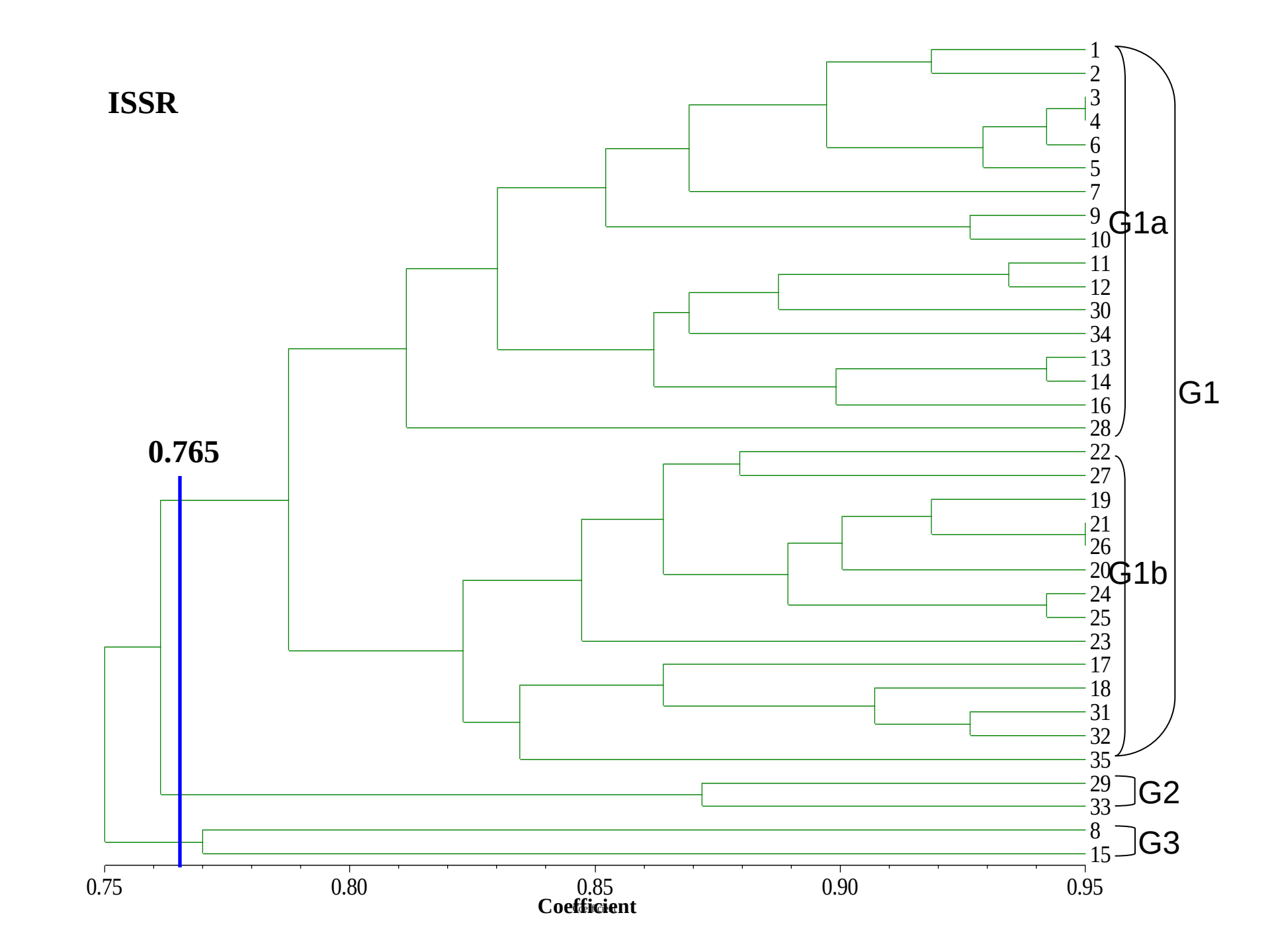
| Primer code | Sequence | No. of amplified bands | No. of polymorphic bands | percentage of polymorphic (%) |
|---|---|---|---|---|
| UBC203 | CACGGCGAGT | 7 | 4 | 57.14 |
| OPC08 | TGGACCGGTG | 7 | 7 | 100 |
| AA10 | TGGTCGGGTG | 7 | 5 | 71.43 |
| OPH19 | CTGACCAGCC | 4 | 2 | 50 |
| Z07 | CCAGGAGGAC | 6 | 4 | 66.67 |
| OPM13 | GGTGGTCAAG | 7 | 5 | 71.43 |
| S471 | AACGAGTCGG | 3 | 1 | 33.33 |
| UBC220 | GTCGATGTCG | 6 | 4 | 66.67 |
| UBC230 | CGTCGCCCAT | 6 | 4 | 66.67 |
| T5 | GGGTTTGGCA | 7 | 6 | 85.71 |
| OPR12 | ACAGGTGCGT | 8 | 6 | 75 |
| OPM14 | AGGGTCGTTC | 7 | 5 | 71.43 |
| AG13 | GGCTTGGCGA | 8 | 7 | 87.5 |
| S105 | AGTCGTCCCC | 4 | 2 | 50 |
| AB01 | CCGTCGGTAG | 6 | 5 | 83.33 |
| K7 | AGCGAGCAAG | 7 | 4 | 57.14 |
| S307 | GAGCGAGGCT | 7 | 6 | 85.71 |
| S104 | GGAAGTCGCC | 3 | 2 | 66.67 |
| Total | | 110 | 79 | |
| Average | | 6.1 | 4.4 | 71.8 |

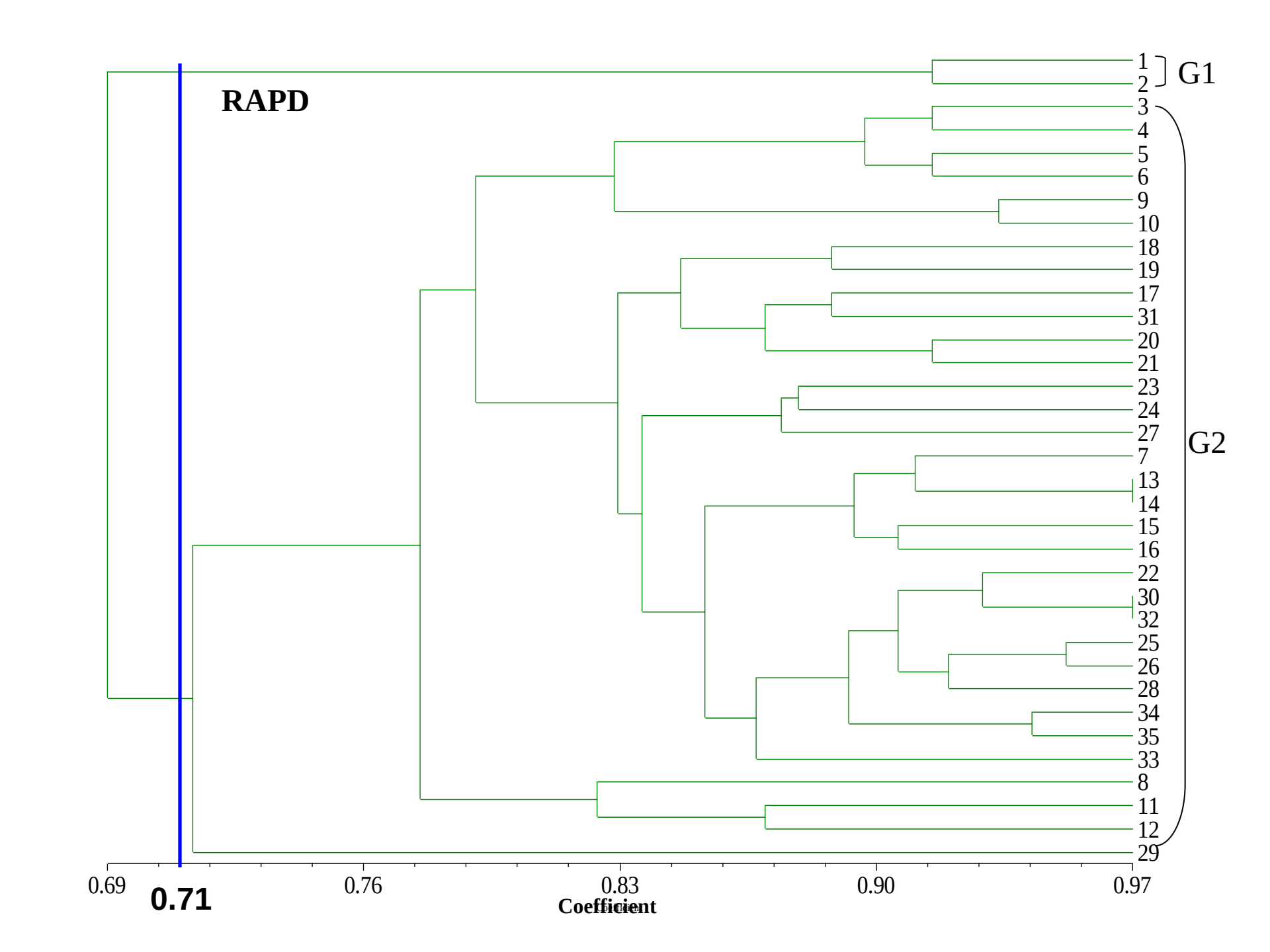
Fig. 2

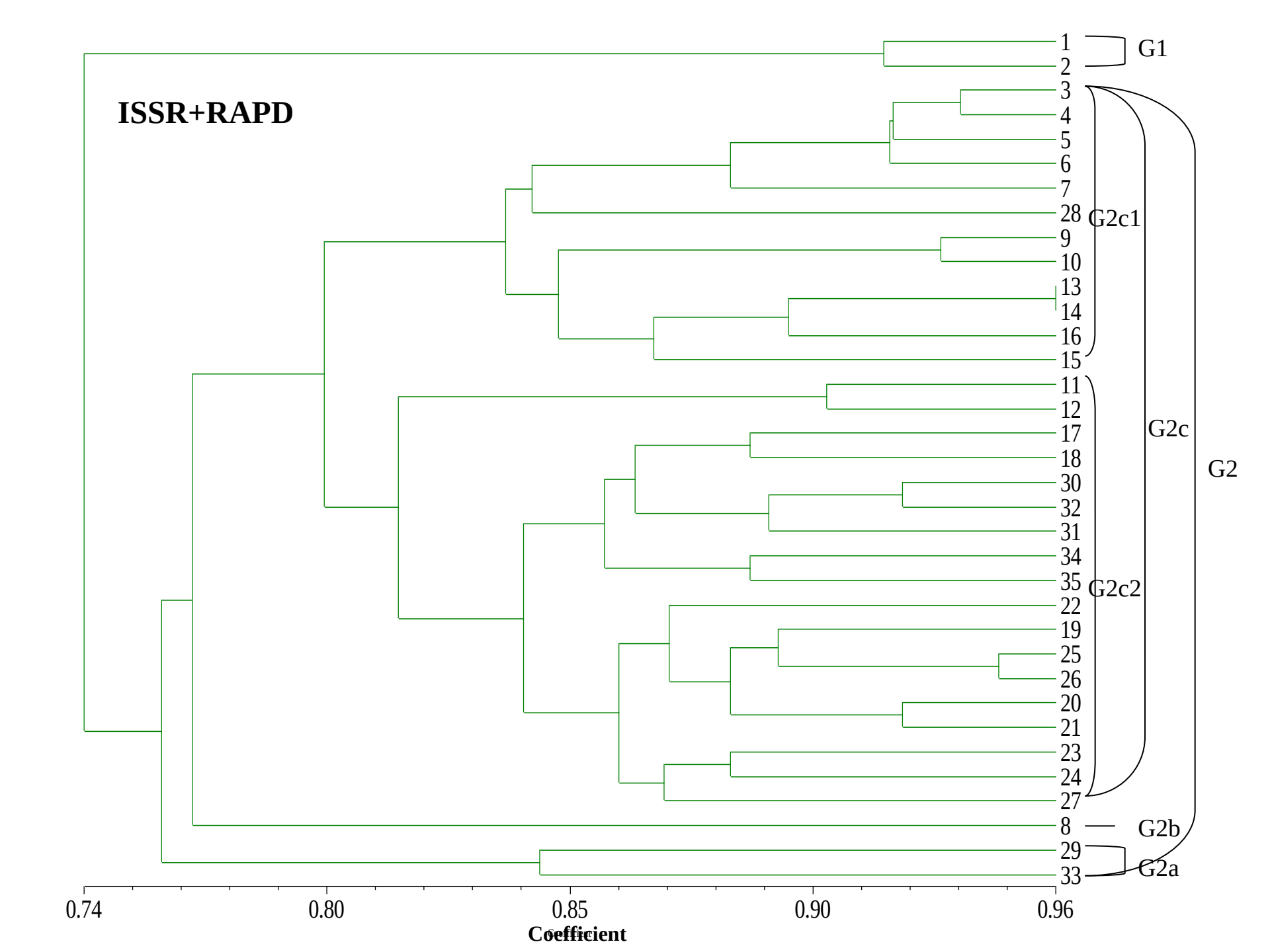



**RAPD primer Z07**

# Cluster analysis


ISSR
0.765
1
2
3
4
6
5
7
9
10
11
12
30
34
13
14
16
28
22
27
19
21
26
20
24
25
23
17
18
31
32
35
29
33
8
15
G1a
G1b
G1
G2
G3
0.75
0.80
0.85
0.90
0.95
Coefficient


RAPD
1
2
G1
3
4
5
6
9
10
18
19
17
31
20
21
23
24
27
7
13
14
15
16
22
30
32
25
26
28
34
35
33
8
11
12
29
G2
0.69
0.71
0.76
0.83
0.90
0.97
Coefficient


ISSR+RAPD
1
2
3
4
5
6
7
28
9
10
13
14
16
15
11
12
17
18
30
32
31
34
35
22
19
25
26
20
21
23
24
27
8
29
33
G1
G2c1
G2c2
G2c
G2
G2b
G2a
0.74
0.80
0.85
0.90
0.96
Coefficient

# Conclusions (1)

- **Combination of ISSR and RAPD markers were useful to estimate the genetic diversity for *U. scitaminea* population**

- **Our results showed that molecular diversity levels among 35 isolates of *U. scitaminea* collected from three main sugarcane-producing regions of Southern China were moderately diversified.**

# Conclusions (2)

- **plus and minus mating-type isolates from the same single-teliospore of *U. scataminea* had an extremely high genetic similarity coefficient**

- **The genetic diversity was associated in some degree with geographical origin, but not suitable to all isolates.**

# Presumable conclusion

- **isolates No. 1 and No. 2 clusters together and are likely to belong to race 2, as they infect host F134, while the remaining 33 isolates were clustered into the G2 group in which isolate No. 16 infects host NCo376. Since NCo376 is immune to race 1 and race 2, isolate No.16 may represent a new physiological race.**

- **it is possible that this new race may likely be the race 3 of *U. scitaminea* reported in Taiwan and this race was carried along with the sugarcane germplasms from Taiwan to the mainland of China.**


Thank you very much!!