Characterizing the genetic diversity of Indonesian soybean and groundnut landraces using microsatellite and SNP markers

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Why soybean is important

- Indonesia production
  - ☞National production in 2004 : 723.423 ton
  - ☞Average demand : 2.139.660 ton
  - ☞Import : 1.416.237 ton
  - ☞Prediction demand in 2010 : 2.800.000 ton
- Nutrition
  - ☞The main protein supply (tempeh, tofu, soy sauce)

Why groundnut is important

- Production
  - ☛National production in 2004 : 834.034 ton
  - ☛Average demand : 1.634.034 ton
  - ☛Import : 800.000 ton
- Nutrition
  - ☛The main lipid supply

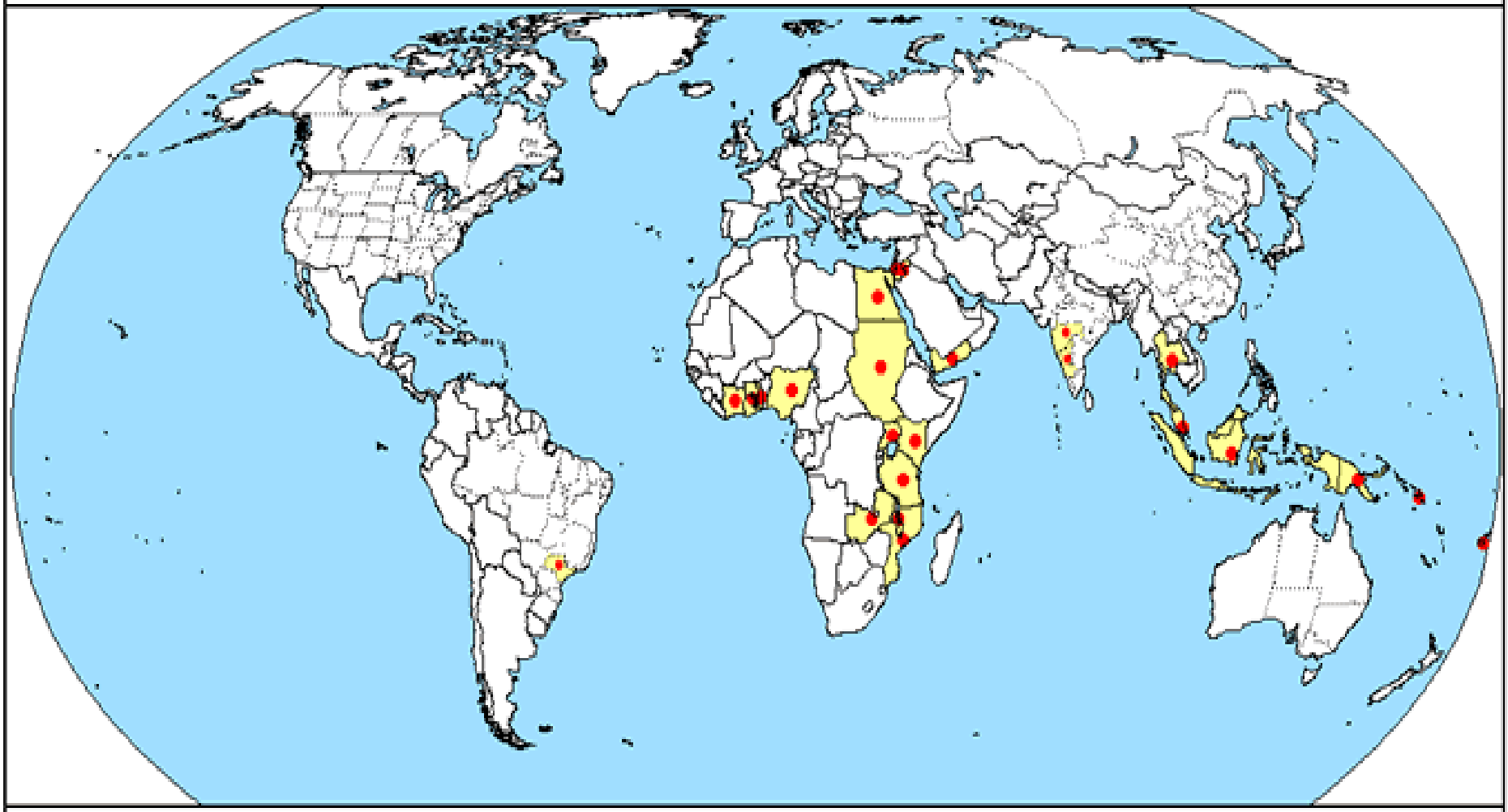
Problems of soybean and groundnut in Indonesia

	Diseases	Pests
Soybean	Virus (CMMV, ISDV), rust, *Phytophtora* sp. *Fusarium* sp.	Pod borer, *Etiela* sp., *Bruchid* sp., *Nezara viridula*
Groundnut	*Aspergillus flavus,* PStV, *Puccinia arachidis*	*Thrips, Aphids*

LOW INPUT TECHNOLOGY

Distribution Maps of Quarantine Pests for Europe

Cowpea mild mottle 'carlavirus'



National record | Subnational record

Present

Present only in some areas

EPPO 2003-06

AFLATOXIN

Indonesia is favourable conditions for aflatoxin

hot and humid climate

kernel moisture

- **insect damage**
- **poor fertilization**
- **drought**

decreasing host plant's immunity

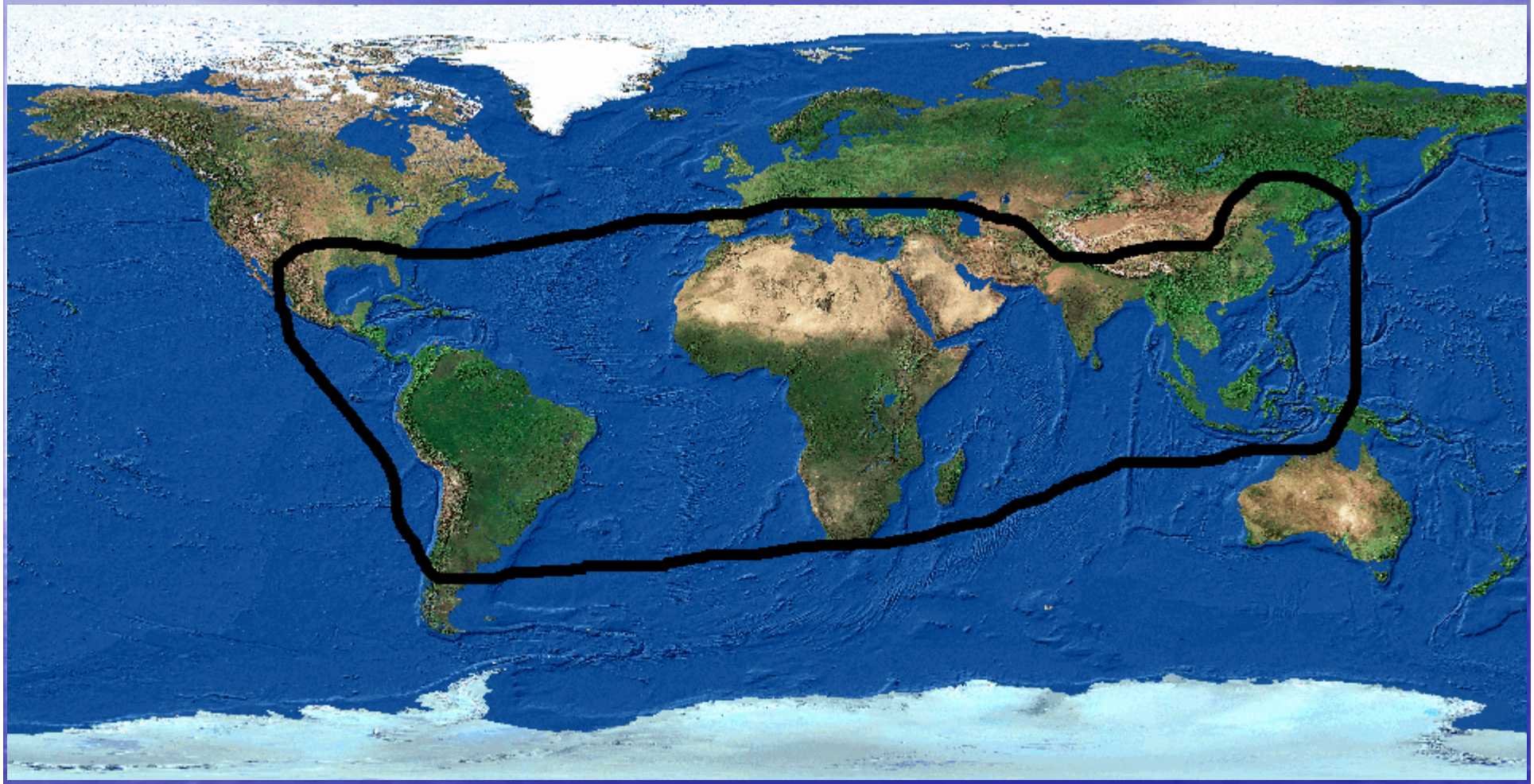
Invade the food supply at
- **production**
- **processing**
- **transport**
- **storage**

Impact for human health in Indonesia

Cancer due to aflatoxin
10 / 100000 pop. per annum

200 million population

20000 death / annum



Area of peanut intensive cultivation (solid line). (Weiss 2000)

Critical agricultural area (8 million ha)

- potential critical land (4.712 million ha)
- semi critical land (1.893 million ha)
- critical land (1.247 million ha)
- extremely critical land (224 000 ha)

Acid soil

- Ultisol (47.5 million ha)
- Oxisol (18 million ha)
- Histosol (20 million ha)

PROBLEMS SOIL

- Poorness
- Low knowledge of farmer

NON TECHNICAL PROBLEMS

Low input technology

NODULATION


Critical land

Crop Improvement

- Traditional Crops
  - well adaptation
    - abiotic: acid soil, drought, water lodging
    - biotic : pest and diseases
  - lower yield
- Modern breeding lines
  - lower adaptation
  - higher yield

COMBINATION FOR LOW INPUT TECHNOLOGY

CHARACTERIZING

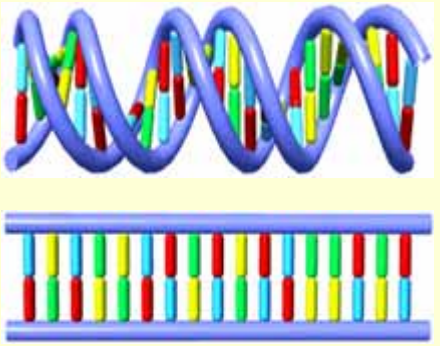
Morphology

- Relatively stable
- Low environmental effects

Agronomic

- Relatively labile
- Highly environmental effects

Molecular



- Stable
- No environmental effects

Molecular Characterizing

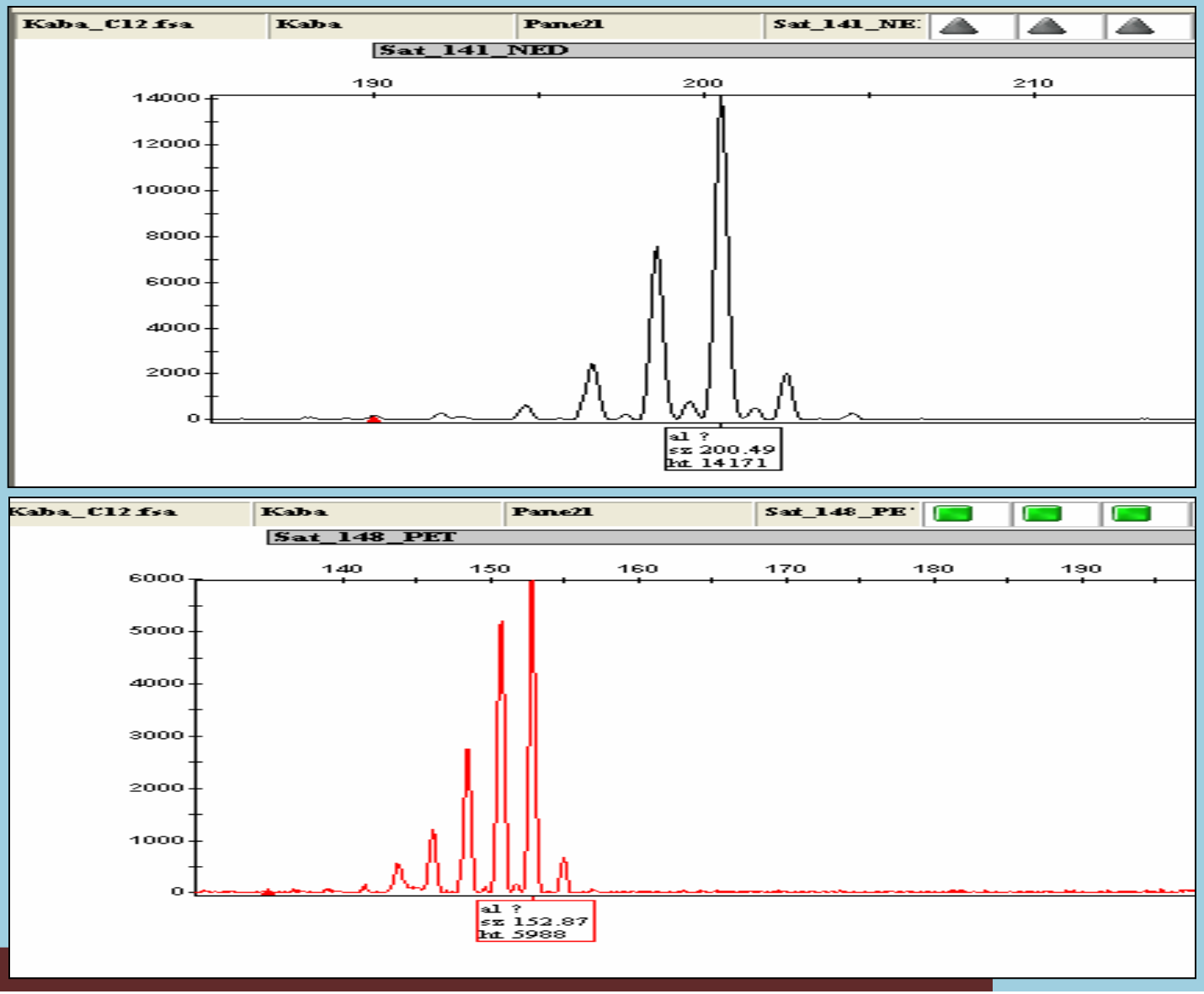
SSR

- Co-dominant
- Higher degree of polymorphism
- Suitable for evaluating genetic diversity among closely related cultivars

Sequencing

- SNP
- Insertion
- Deletion

Two different peaks showed by two different primers on variety of Kaba



GENOTYPING PROJECTS

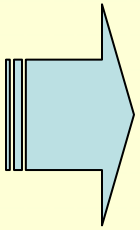
- **PROJECT 1:**
  - **Characterizing the genetic diversity of Indonesian soybean landraces using microsatellite marker**
  - **Characterizing the genetic diversity of Indonesian groundnut landraces using microsatellite marker**

- **PROJECT 2:**

  Characterizing the genetic diversity and SNP identification on nodulation related gene of Indonesian soybean accessions using DNA sequence variation

SSR Method

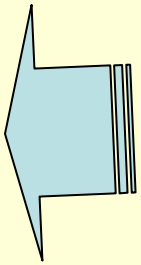
DNA Isolation



DNA Quantification

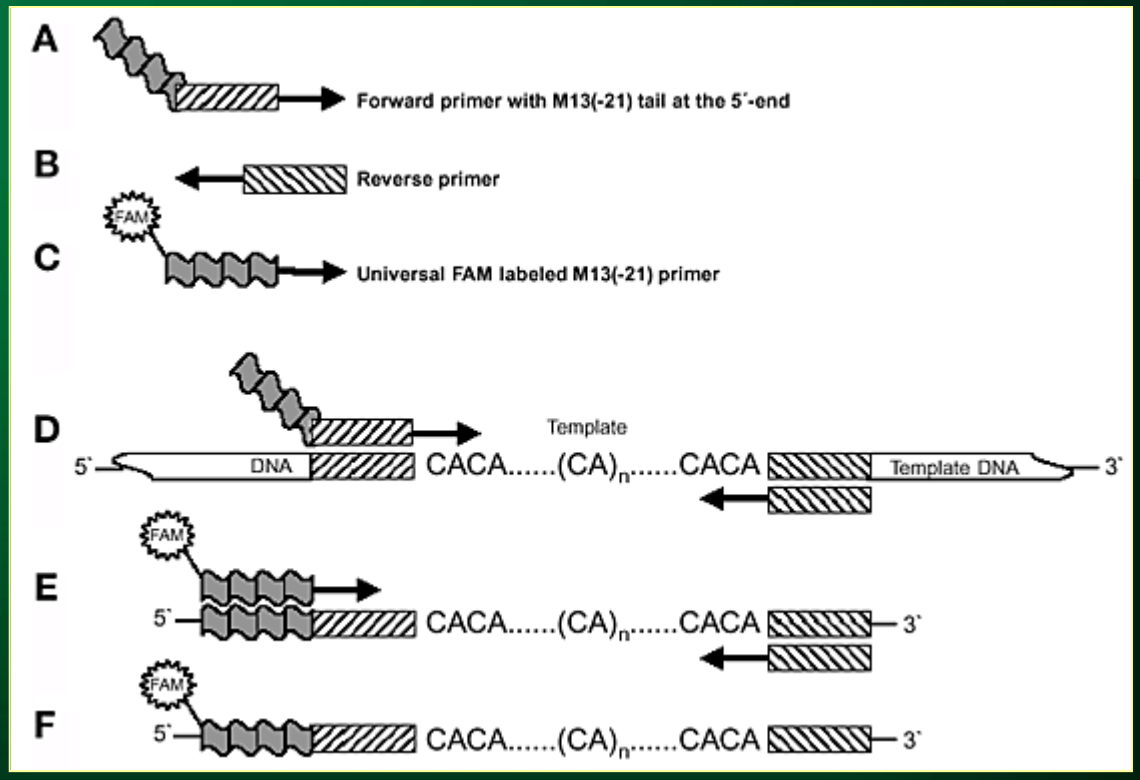
Hoerscht & picogreen methods

PCR



Fragment Analysis

ABI 310 Prism Genetic Analyzer



An economic method for the fluorescent labeling of PCR fragments (Schuelke, 2000)

Sequencing Method

PCR

Exo / SAP Digestion

PCR Precipitation

Sequencing

Clean Up of Sequencing Product

Sequence Running

PHENOTYPING PROJECTS

- **PROJECT 1:**

  Screening of Indonesian soybean accessions for highly nodulation

- **PROJECT 2:**

  Screening of Indonesian soybean accessions for CMMV resistant

- **PROJECT 3:**

  Screening of Indonesian groundnut accessions for *Aspergillus flavus* resistant

Screening of Indonesian soybean accessions for highly nodulation

- Factorial Design, 2 Replications
  - 1st factor : 96 soybean accessions
  - 2nd factor : Rhizobium aplication
- Parameter
  - Number of nodules
  - Number of active nodules
  - percentage of the root nodulated

Screening of Indonesian soybean accessions for CMMV resistance

- Factorial Design, 2 Replications
  - 1st factor : 96 soybean accessions
  - 2nd factor : Inoculation of CMMV
- Parameter
  - Disease score
  - Agronomic traits

Screening of Indonesian groundnut accessions for *Aspergillus flavus* resistance

- RCBD 10 replications
- Material
  - 96 soybean accessions
- Parameter
  - Aflatoxin

Preliminary Result

Table 1. SSR Primers for diversity study on soybean

No.	SSR locus	Annealing temperature (°C)	Linkage Group†*	cM Position in LG†*	Motif*
1	Sat_040	56	A2	118.64	(AT)23(GA)24
2	Sat_095	56	B1	81.31	(AT)48
3	Sat_127	57	H	28.80	(AT)28(CA)15
4	Sat_141	56	G	9.18	(AT)24(GA)11
5	Sat_148	56	M	63.93	(AT)28(GT)15
6	Sat_186	56	N	30.11	(AT)25(CT)18
7	Sat_370	56	J	37.40	(AT)39
8	Sat_399	56	K	44.66	(CATA)12(AT)25
9	Sat_404	58	C1	73.85	(GT)5(AT)25
10	Satt138	56	G	55.99	(ATT)47
11	Satt277	56	C2	107.59	(ATT)40
12	Satt424	56	A2	60.59	(ATT)52
13	Satt442	56	H	46.95	(ATT)35
14	Satt554	56	F	111.89	(ATT)33

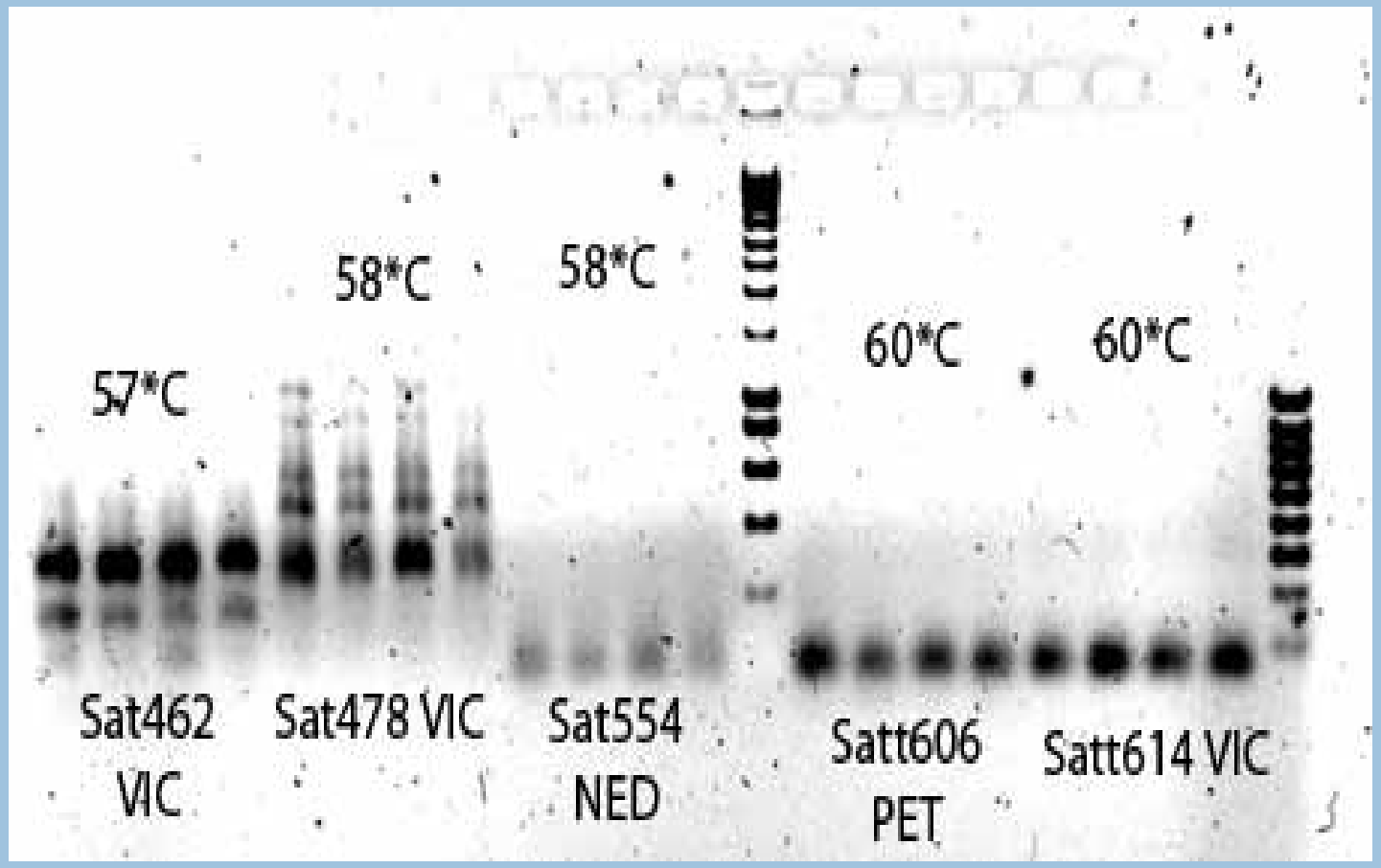
†Cregan et al. (1999), *(Soybean Genomics and Improvement Laboratory, 2005)

Table 1. SSR Primers for diversity study on soybean (continued)

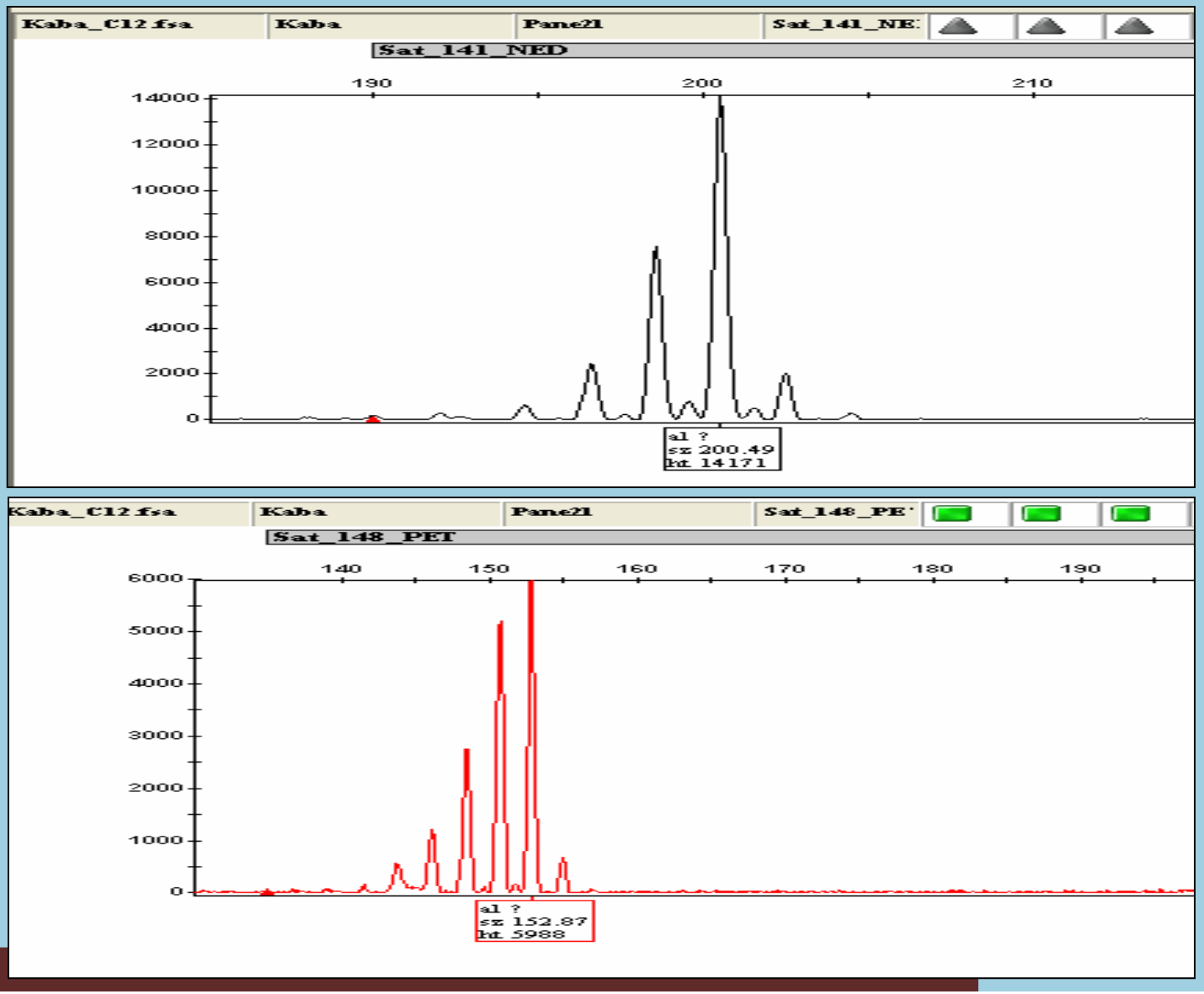
No.	SSR locus	Annealing temperature (°C)	Linkage Group†*	cM Position in LG†*	Motif*
15	Satt606	60	E	39.77	(ATT)26(AT)8
16	Satt614	60	I	31.94	(ATT)37
17	Sat_144	56	J	90.13	(AT)24(GA)11
18	Sat_401	56	H	66.21	(AT)36
19	Satt249	56	J	11.74	(ATT)20
20	Satt293	56	H	89.09	(AT)13(ATT)16
21	Satt635	56	H	4.88	(ATT)7CAA(TAA)3
22	Sat_180	56	H	104.37	(AT)23
23	Satt183	57	J	42.51	(ATT)13
24	Satt547	56	J	67.79	(ATT)18(ACT)3
25	Sat_155	58	I	98.06	(AT)19
26	Satt350	56	D1b	76.60	(ATT)17
27	Satt462	57	L	41.00	(ATT)20(AT)24
28	Satt478	58	O	71.10	(ATT)17

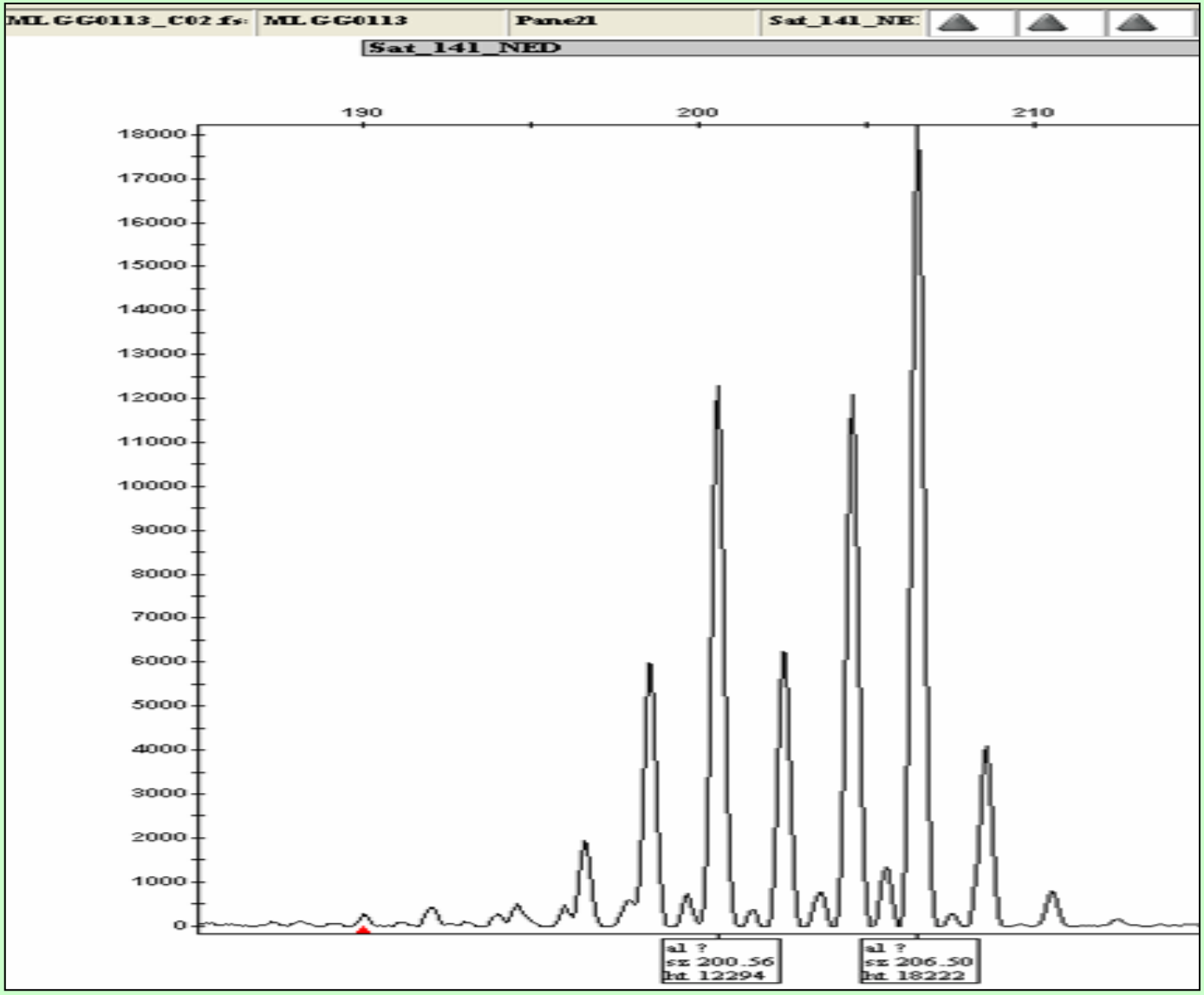
†Cregan et al. (1999), *(Soybean Genomics and Improvement Laboratory, 2005)

Different bands of different primers for four same samples



Two different peaks showed by two different primers on variety of Kaba





Two peaks showed by Sat_141 on MLGG0113

Two peaks showed by Sat_148 on MLGG0115

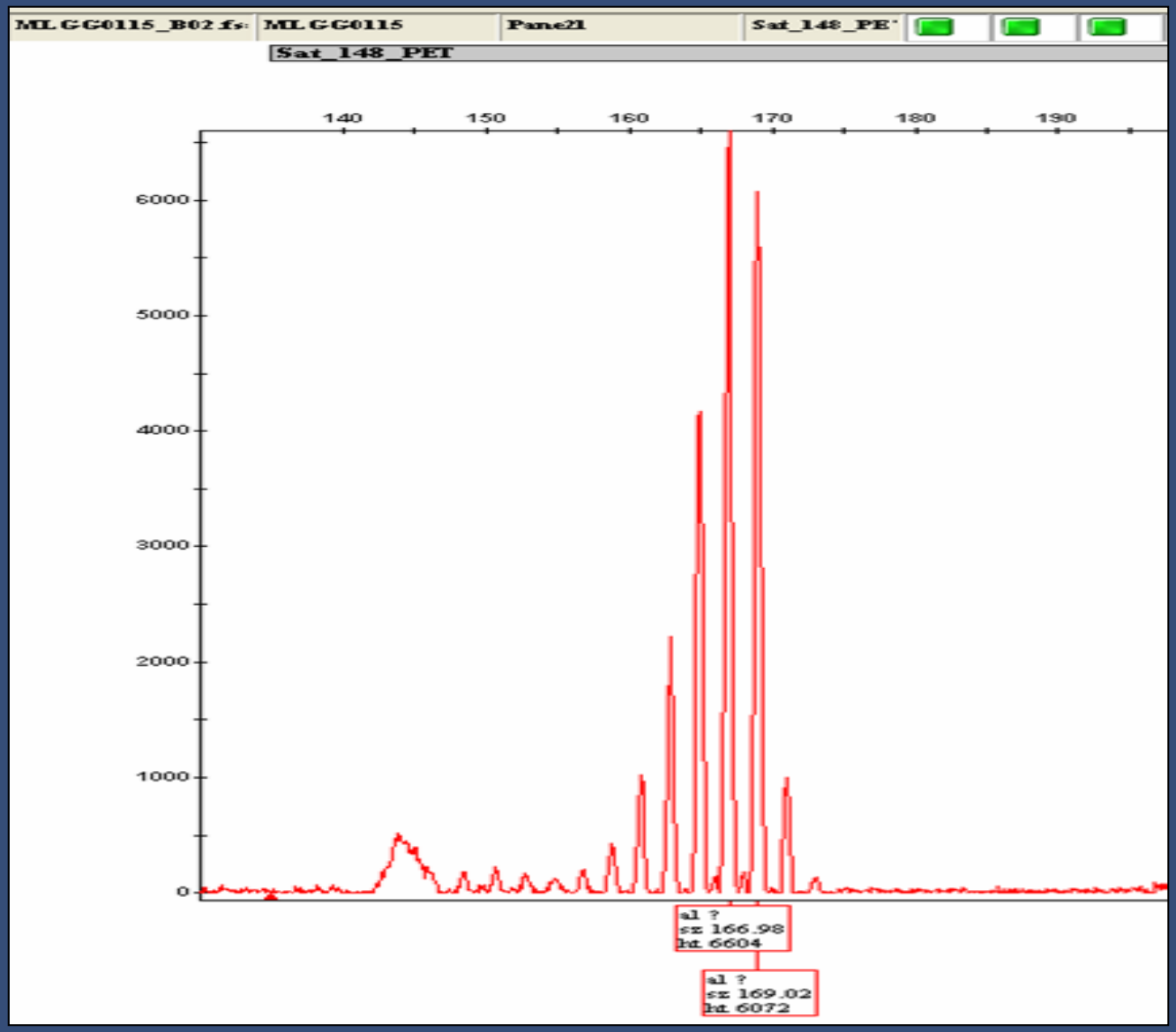


Table 2. Effectiveness of SSR primer to produce polymorphisms

SSR Locus	ΣReaction	ΣMissing Data	ΣPeak	Peak Range	Polymorphisms
Sat_040	22	74	1-2	246-277	Low
Sat_127	17	79	1	267	Low
Sat_186	96	0	1-2	171-178	Low
Sat_399	92	4	1-3	287-325	High
Sat_095	60	36	1-4	115-259	High
Sat_141	94	2	1-3	200,249	Low
Sat_148	94	2	1-3	139-164	High
Sat_370	67	29	1-2	198-200	High
Sat_404	96	0	1-4	51-137	Medium
Satt138	95	1	1-2	227-329	Medium
Satt277	90	6	1-4	126-250	High
Satt424	95	1	1.4	118-162	Low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Thank You