Disclaimer: unless otherwise agreed by the Council of UPOV, only documents that have been adopted by the Council of UPOV and that have not been superseded can represent UPOV policies or guidance.

This document has been scanned from a paper copy and may have some discrepancies from the original document.

———

Avertissement: sauf si le Conseil de l'UPOV en décide autrement, seuls les documents adoptés par le Conseil de l'UPOV n'ayant pas été remplacés peuvent représenter les principes ou les orientations de l'UPOV.

Ce document a été numérisé à partir d'une copie papier et peut contenir des différences avec le document original.

———

Allgemeiner Haftungsausschluß: Sofern nicht anders vom Rat der UPOV vereinbart, geben nur Dokumente, die vom Rat der UPOV angenommen und nicht ersetzt wurden, Grundsätze oder eine Anleitung der UPOV wieder.

Dieses Dokument wurde von einer Papierkopie gescannt und könnte Abweichungen vom Originaldokument aufweisen.

———

Descargo de responsabilidad: salvo que el Consejo de la UPOV decida de otro modo, solo se considerarán documentos de políticas u orientaciones de la UPOV los que hayan sido aprobados por el Consejo de la UPOV y no hayan sido reemplazados.

Este documento ha sido escaneado a partir de una copia en papel y puede que existan divergencias en relación con el documento original.

E

BMT/5/10
ORIGINAL: English
DATE: August 27, 1998

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Fifth Session

Beltsville, United States of America, September 28 to 30, 1998

GENETIC IDENTIFICATION OF MORPHOLOGICAL MUTANTS OF STRAWBERRY CHARACTERIZED BY AFLP ANALYSIS

Document prepared by experts from Japan

GENETIC IDENTIFICATION OF MORPHOLOGICAL MUTANTS OF STRAWBERRY CHARACTERIZED BY AFLP ANALYSIS.

Kimura , T. and Y. BAN
National Center for Seeds and Seedlings. 2 - 2 Fujimoto Tsukuba Ibaraki 305 - 8605 Japan

Introduction

We have tried to discriminate mutant varieties from their original cultivars in some crop species by RAPD analysis. But it was difficult and needed enormous number of primers to discriminate them. Currently, AFLP (amplified fragments length polymorphism) analysis was developed. AFLP was one of the most efficient methods to discriminate cultivars, because of its superior efficiency and reproducibility.

In this study, we tried to identify genetic variation of mutant strawberry plants using fluorescence-aided AFLP analysis. Furthermore, we compared AFLP with RAPD analysis concerning the efficiency.

Materials and Methods

Five strawberry cultivars, 'Nyohou' , the mutant varieties of 'Nyohou' ('Kinuama' , 'No. 1' , 'No. 2') and F1 ('No. 3') crossed 'Nyohou' with the next progeny of 'Nyohou' , were used. Genomic DNA of each plant was extracted by CTAB method (Doyle & Doyle, 1987). AFLP analysis was performed according to the supplier's (Life Technologies) protocol. The amplified fragments of 5 strawberry cultivars were separated on 5 % denaturing polyacrylamide gels with 6M urea at 40W constant power using ALF DNA Sequencer II (Pharmacia Biotech). The efficiency of AFLP analysis was examined in comparison with RAPD analysis. RAPD analysis was tested with 204 primers (OPA01 - OPJ20, OPH02 - 20, OPQ07, 20, OPR07, OPU03, Operon Technologies, Alameda, CA, USA). DNA polymorphisms were evaluated between 'Nyohou' and 'Kinuama' .

Results and Discussion

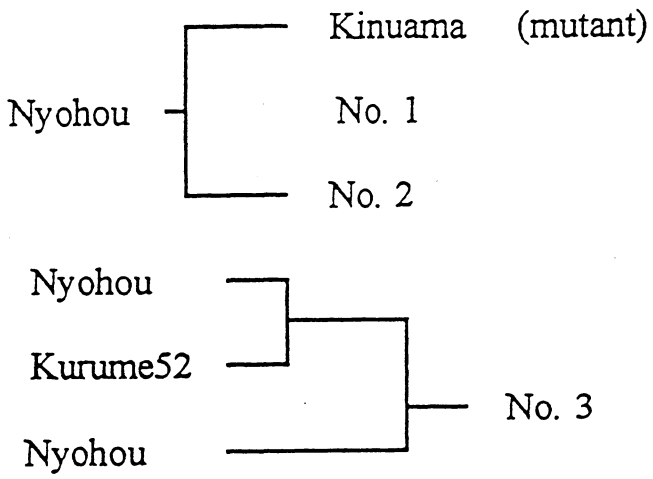
Reproducibility of AFLP fragments was examined by repeating PCR

experimemts with the same template DNA and primer combinations. Amplified bands ranging from 60 to 350bp were confirmed to be reproducible. A total of 247 amplified fragments were generated (30.9 bands per primer combination) using 7 combinations of selective primers and 26 bands (10.5%) showed polymorphisms. It was passible to discriminate all cultivars in this study at least 7 AFLPs. The frequency of DNA polymorphism depended on the primer combinations. Twelve polymorphic bands were obtained using primer combination of E-AGT and M-CTC. On the other hand, only one polymorphic band was detected from primer combinations of E-AGT/M-CAT, E-AGT/M-CTG and E-AGT/M-CTT.

In AFLP analysis, fourteen polymorphic bands were obtained between 'Nyohou' and 'Kinuama' from 7 primer combinations. 2.0 AFLPs per one primer combination were shown polymorphisms between mutant 'Kinuama' and its original cultivar. On the contrary, no polymorphic bands were obtained in RAPD analysis.

In conclusion, we could efficiently discriminate mutant varieties from their original cultivar by AFLP analysis. We considered that AFLP could be applicable to the genetic identification in strawberry.

Table 1. **Materials**



Characteristics	States	cultivaras
Earliness of Flowering	Earlier	No.1
Vigar of plant	Stronger	No.2, No.3

Table 2. **Sequences of selective primers**

Code	Sequences
*E - NNN 5' -	FITC-CTCGTAGACTGCGTACCAATTCNNN - 3'
	1)AGT
M - NNN 5' -	GATGAGTCCTGAGTAANNN - 3'
	1)CAC 2)CAG 3)CAT
	4)CTA 5)CTC 6)CTG 7)CTT

Asterisk indicated FITC-labelled primers
NNN indicated selective nucleotides

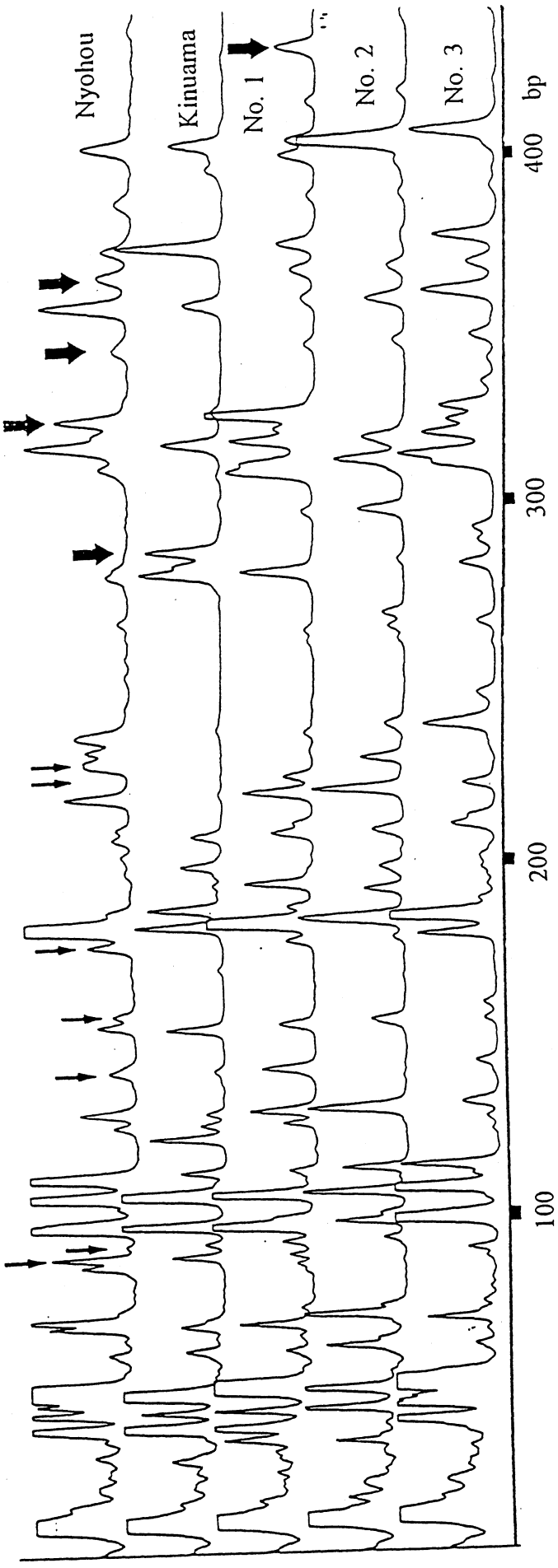


Figure 1. AFLP fingerprints of cultivars in strawberry. Fragments amang cultivars were obtained with E-AGT/M-CTCprimers . Arrows indicate polymorphic.

Table 3. AFLPs for 5 cultivars

E- \ M-	CAC a / b	CAG a / b	CAT a / b	CTA a / b	CTC a / b	CTG a / b	CTT a / b	TOTAL a / b
AGT	3 / 35	3 / 30	1 / 38	5 / 35	12/ 51	1 / 21	1 / 37	26 / 247

a indicates No. of polymorphic bands. b indicates No. of amplified bands. M - : Mse I primer , E - : Eco R I primer . AFLP reaction was performed with Mse I and FITC-labelled EcoR I primers.

Table 4. DNA fragments of strawberry cultivars by 4 polymorphic

Fragments	Nyohou	Kinuama	No.1	No.2	No.3
E-AGT/M-CTC-160	+	−	−	−	+
-180	+	−	+	+	+
-220	−	−	+	−	−
-230	+	−	−	+	−

+ : presence , - : absence .

Table 5. Polymorphic bands between each pair

	Nyohou	Kinuama	No.1	No.2	No.3
Nyohou		5.6 %	3.7	4.3	3.8
Kinuama	14		5.4	8.7	5.5
No.1	9	13		4.7	3.3
No.2	10	20	11		5.9
No.3	9	13	8	14	

Above diagonal means % of polymorphic bands.
Below diagonal means No. of polymorphic bands.

Table 6. **Comparison two methods**

Method of analysis	No. of used primers	No. of amplified bands	No. of polymorphic bands
AFLP	7	238 (34.0)	14 (2.0)
RAPD	204	886 (4.3)	0 (-)

Numbers in parentheses showed No. of bands perprimer pair on average.

[End of document]