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**IDENTIFICATION OF STRAWBERRY VARIETIES USING DNA MARKERS**

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## IDENTIFICATION OF STRAWBERRY VARIETIES USING DNA MARKERS.

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Abstract

In order to protect the plant breeders right of strawberry, the technical development in which the plants including fruit grown under the different environment conditions can be classified is required. Then, development of the classified technology using the Cleaved Amplified Polymorphic Sequence (CAPS) marker, which is one of the DNA polymorphisms, was tried. Three kinds of genes that have the difference DNA sequence between strawberry cultivars were utilized, and 7 kinds of markers were developed. By using these markers, it was possible to have identified 13 strawberry cultivars that are mainly cultivated and sold in Japan. 'Sachinoka' is one of the main strawberry cultivars and the cultivation is allowed only in Japan. Using this technique, we found 'Sachinoka' among imported strawberry fruits. The classification technology for rising cultivar newly is accelerated by development of new DNA markers.

Identification of Strawberry varieties using DNA markers

1. In recent years, fresh strawberries have been imported from other Asian countries at the harvest season of Japan; the amount, currently about 1000 t a year, is increasing. The main variety imported is 'Redparl'. However, there are strong suspicions that 'Sachinoka' and 'Tochiotome' might be mixed in with it, according to import merchants or strawberry breeders. The cultivation of 'Sachinoka' and 'Tochiotome' has never been licensed to any other country. Therefore, if the importation of these fruits is true, this indicates the infringement of breeders' rights. We assessed the use of DNA markers to identify strawberry fruits. We focused on the cleavage amplified polymorphism sequence (CAPS) method, which needs neither expensive equipment nor complicated procedures and displays simple and stable results from plants. Our study is the first development of CAPS markers for use in cultivated strawberries.

Designation of CAPS markers and Detection of Polymorphisms

2. We analyzed 3 loci, ascorbate peroxidase (APX), chalcone isomerase (CHI), and F3H, which are useful for identification of the main varieties distributed in Japan (Figure 1). Marker APX-*Mlu*I is part of the gene for APX, treated with *Mlu*I (Figure 1-I). This marker revealed 2 polymorphisms, fragments A and B. Therefore it divided strawberry varieties into 4 groups: having both A and B, either A or B, and neither. The A band was generated by *Mlu*I digestion. Varieties having polymorphic alleles with 1 *Mlu*I recognition site were polymorphic for A. The B band was detectable without endonuclease treatment. Sequence analysis showed that the B fragment had a 76-bp insertion. The middle intense band was derived from non-polymorphic alleles.

3. The gene for CHI was used for the CHI-*Pvu*II marker. The PCR product was digested with *Pvu*II. The gene for F3H displayed 4 polymorphisms when treated with different endonucleases (*Nco*I, *Hpa*II, *Acc*I or *Rsa*I). In these markers, the alleles which have (or don't

have) a certain endonuclease recognition site were detected as polymorphisms, as in the A band of APX-*Mlu*I. Varieties could be separated into 2 groups by each marker: presence or absence (+ or -) of polymorphic fragments. We successfully distinguished the 14 varieties by the use of at least 7 markers (Table 1).

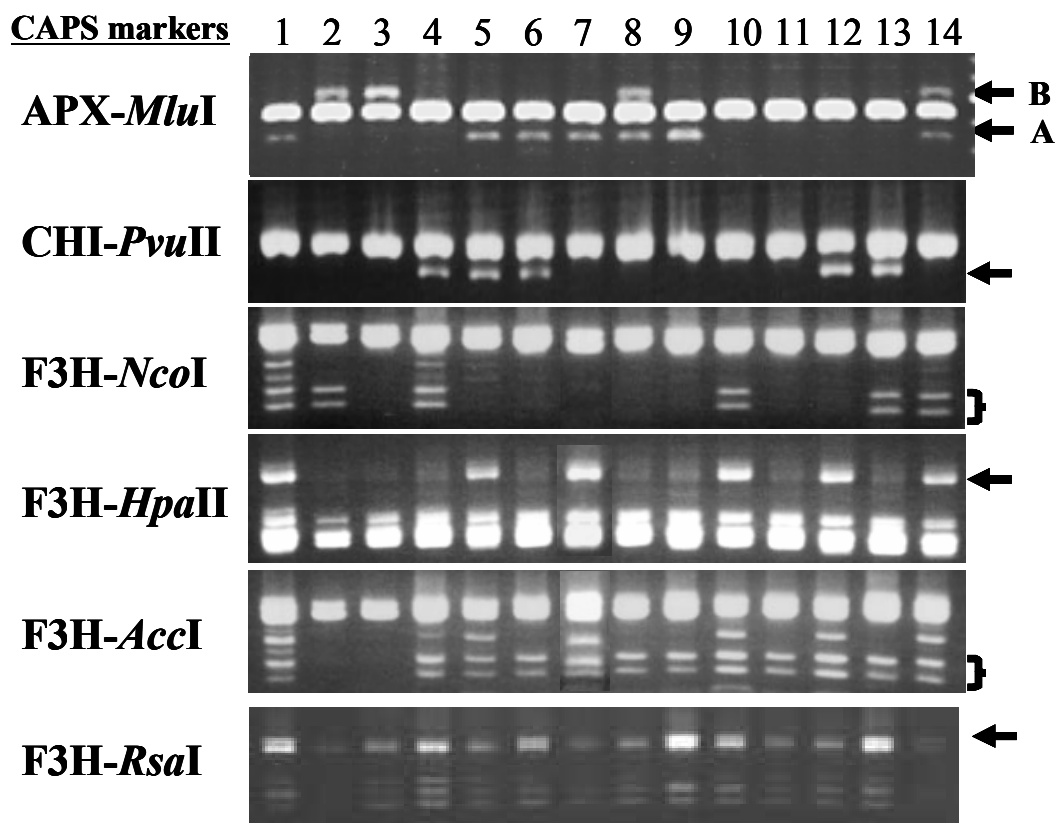


Figure 1 DNA polymorphisms among 14 strawberry varieties using CAPS markers.

- 1) Toyonoka 2) Nyoho 3) Tochiotome 4) Akihime 5) Sachinoka 6) Aiberry  
 7) Redparl 8) Nouhime 9) Sanchigo 10) Pisutoro 11) Aisutoro  
 12) Benihoppe 13) Keikiwase 14) Cesena

Table 1 Polymorphisms detected in 14 strawberry varieties using CAPS markers.

	APX-MluI	CH1-PvuII	F3H-NcoI	F3H-HpaII	F3H-AccI	F3H-RsaI
Toyonoka	A	-	+	+	+	+
Nyoho	B	-	+	-	-	-
Tochibrome	B	-	-	-	-	-
Akhome	-	+	+	-	+	-
Sachinoka	A	+	-	+	+	-
A berry	A	+	-	-	+	+
Redparl	A	-	-	+	+	+
Nouhime	AB	-	-	-	+	-
Sanchigo	A	-	-	-	+	+
Pisutoro	-	-	+	+	+	+
Aisutoro	-	-	-	-	+	-
Benhoppe	-	+	-	+	+	-
Ke kwase	-	+	+	-	+	+
Cesena	AB	-	+	+	+	+

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