Development of DNA markers identifying strawberry (*Fragaria* × *ananassa* Duch.) cultivars and detection 'Sachinoka' cultivar from imported strawberries.

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Introduction

In 1996 National Institute Vegetable and Tea Science (NIFTS) released a new strawberries (*Fragaria* × *ananassa* Duch.) cultivar, 'Sachinoka', which is high content vitamin C and is fit for the long transportation due to the tough flesh (Morishita et al.,1996). Cultivation of 'Sachinoka' increases gradually and it is one of favorite cultivars for consumer. In recent years, the strawberry production in Korea is also increasing rapidly and the quantity of production is almost equivalent to that of Japan. Fresh strawberries have been imported from other Asian countries including Korea at the same harvest season inJapan; the amount, currently about 1000 t a year, is increasing. However, there are suspicions that 'Sachinoka' might be mixed in with imported strawberries, according to import merchants or strawberry breeders. The cultivation of 'Sachinoka' has never been licensed to any other country. Therefore, if the importation of these fruits is true, this indicates the infringement ofbreeders' rights. On the other hand,the cultivar name is usually displayed in Japanese market, allowing Japanese consumers to choose their favorites. As the result the prices are different among the strawberries cultivars. We need the technique identifying the cultivars prevent from unjust display.

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 anddisplays simple and stable results from plants. Our study is the first development of CAPS markers for use in cultivated strawberries (Kunihisa et al.,2003).

1. Designation of CAPS markers and Detection of Polymorphisms

At first we analyzed 3 loci, ascorbate peroxidase (APX), chalcone isomerase (CHI), and flavanone 3-hydroxylase (F3H), which are useful for identification of the main cultivars distributed in Japan (Figure 1).

Marker APX-*Mlu*I is part of the gene for APX, treated with *Mlu*I (Figure 1). 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. Cultivars 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.

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-*MluI*. Cultivarscould 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).

Secondary, the same methodology was applied to generate new markers for identifying 64 strawberry cutivars including a new Korean cultivar, 'Myhang'. We tested 188 combinations of endonucleases and loci that potentially could generate polymorphisms. As a result, 15 combinations actually yielded discernable polymorphisms. Of the 21 markers (15 of newly acquired ones and 6 of mentioned above), 16 identified single polymorphisms, 4 each identified two independent polymorphisms, and the remaining one was derived from the chloroplast genome and presented as a single band with cytoplasmic inheritance.

2. Improvement of DNA markers

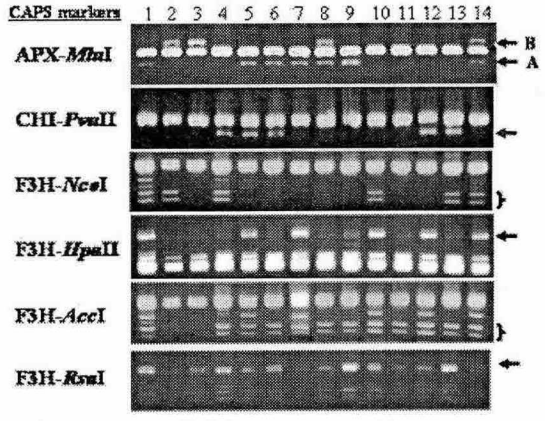

Figure 1 DNA polymorphisms among 14 strawberry barieties using CAPS markers.

1: 'Toyonoka', 2: 'Nyoho', 3: 'Tochiotome', 4: 'Akihime', 5: 'Sachinoka', 6: 'Aiberry', 7: 'Redparl', 8: 'Nohime', 9: 'Sanchigo', 10: 'Pistro', 11: 'Aistro', 12: 'Benihoppe', 13: 'Kekiwase', 14: 'Cssena

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

	APX-MluI	CHI-PvuII	F3H-NcoI	F3H-HpaII	F3H-AccI	F3H-PstI
Toyonoka	A	-	+	+	+	+
Nyoho	B	-	+	-	-	-
Tochiotome	B	-	-	-	-	-
Akihime	-	+	+	-	+	-
Sachinoka	A	+	-	+	+	-
Aiberry	A	+	-	-	+	+
Redparl	A	-	-	+	+	+
Nouhime	AB	-	-	-	+	-
Sanchigo	A	-	-	-	+	+
Pisutoro	-	-	+	+	+	+
Aisutoro	-	-	-	-	+	-
Benihoppe	-	+	-	+	+	-
Kekiwase	-	+	+	-	+	+
Cssena	AB	-	+	+	+	+

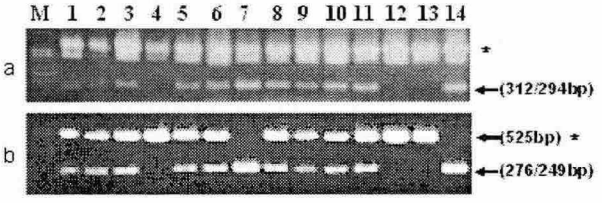

Figure 2 Comparison of electrophoretic patterns produced by original and improved MSR-AluI markers in strawberry cultivars.

(a) original MSR-AluI marker, (b) improved marker. Arrows indicate the polymorphic bands, and asterisks show the positions of amplified products before restriction digest. Fragment sizes were calculated based on sequence data. 1: 'Toyonoka', 2: 'Nyoho', 3: 'Tochiotome', 4: 'Akihime', 5: 'Sachinoka', 6: 'Aiberry', 7: 'Redparl', 8: 'Nohime', 9: 'Sanchigo', 10: 'Pistro', 11: 'Aistro', 12: 'Benihoppe', 13: 'Kekiwase', 14: 'Kurume IH-1 go', and M: size markers (lambda HindIII/HincII fragments)

Because the polymorphic alleles (except for the cytoplasmic marker) were minor among the alleles amplified in a sample, the polymorphic bands on the electrophoresis gels were relatively unclear compared with the non-polymorphic ones (Figure1). Therefore, these markers could function only as dominant markers in octaploid strawberry, unlike in diploidic plants. We then searched the target sequences amplifying only polymorphic portion derived from one genome and designated specific primer sets. As the results we succeeded to improvement the 20

CAPS markers by increasing the specificity of the primers for the polymorphic allele(s). MSR-*Alu*I (Figure 2) is a typical example of the improvement. With the improved version, extraneous bands disappeared, and the polymorphism became dramatically clearer. In fact, the band pattern now resembles that of a diploidic co-dominant marker, despite the octaploidy of the sample. Whereas the original marker divided cultivars into only 2 groups, the improved marker divided them into 3 groups. Therefore, the marker became more effective for identification. For instance, the improved marker clearly discriminated between the cultivars in lanes 6 and 7 (Figure 2, which were indistinguishable using the original marker).

3. Detection of 'Sachinoka' from improted strawberries.

We tried to identify the cultivars in 5 packages of fresh strawberry fruits imported from Korea and bought on the Japanese domestic market. For the identification we used 9 CAPS markers we had developed for strawberry. Our objective wasthrough cultivar identification of the fruits in each package, to check whether infringement of breeders' rights on 'Sachinoka'was occurring. We successfully identified the cultivars of most of all fruits, even when they were severely damaged. Twenty-seven of 71 fruits were 'Sachinoka' we therefore suspected patent infringement. All the fruits analyzed were either 'Redparl' or 'Sachinoka', and these 2 cultivars were mixed in 4 of the packages (Table2).

Table 2 Detection of 'Sachinoka' from imported strawberries.

Package No.	Sachinoka	Redparl	Total	Sachinoka ratio
1	1	19	20	0.05
2	0	4	4	0
3	2	13	15	0.13
4	11	6	17	0.65
5	13	2	15	0.87
Total	27	44	71	0.38

References

Kunihisa M, Fukino N & Matsumoto S (2003) Development of cleavage amplifid polymorphic sequence (CAPS) markers for identification of strawberry cultivars. Euphytica 134: 209-215

Morishita M, Mochizuki T., Noguchi Y., Sone K. & Yamakawa O.(1996) 'Sachinoka', a new strawberry variety for forcing culture. Bull.Natl.Res.Inst.Veg.,Ornam. Plants & Tea Japan 12:91-115

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