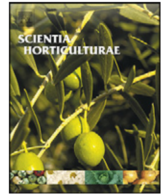
Scientia Horticulturae 123 (2010) 436–442

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Development of cleaved amplified polymorphic sequence (CAPS)-based markers for identification of sweetpotato cultivars

Masaru Tanaka [a,*], Yasuhiro Takahata [a], Hiroki Nakayama [a], Masaru Yoshinaga [a], Toru Kumagai [b], Makoto Nakatani [c]

[a] National Agricultural Research Center for Kyushu Okinawa Region, Miyakonojo, Miyazaki 885-0091, Japan
[b] National Institute of Crop Science, Tsukuba, Ibaraki 305-8518, Japan
[c] Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki 305-8686, Japan

ARTICLE INFO

Article history:
Received 4 August 2009
Received in revised form 9 November 2009
Accepted 11 November 2009

Keywords:
CAPS
Cultivar identification
DNA marker
Ipomoea batatas
Sweet potato

ABSTRACT

To develop a simple and reliable method to identify sweetpotato cultivars, we designed cleaved amplified polymorphic sequence (CAPS)-based markers and used them to perform genotyping of Japanese sweetpotato cultivars. In order to screen the CAPS-based markers, 13 primer pairs were designed from the exon sequences of 11 sweetpotato genes to amplify fragments containing an intron. By digesting the amplified products with 8 restriction enzymes having different recognition sites, a total of 27 polymorphic marker fragments were obtained. Genotyping of 60 Japanese sweetpotato cultivars using these markers suggested that the markers can effectively distinguish sweetpotato cultivars. Among the genes used for primer design, the gene encoding the dihydroflavonol 4-reductase (DFR) showed the largest degree of polymorphism. To our knowledge, this is the first report on the development of CAPS-based markers in sweetpotato.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Sweetpotato is one of the most important crops in tropical and subtropical regions. In Japan, sweetpotato is mainly cultivated in the middle to southern region of the country and utilized for both table and processing use. In many cases, different sweetpotato cultivars are utilized for the same purpose and show similar morphological characteristics, making them difficult to distinguish. This can lead to incorrect labeling of the cultivar names among the storage roots sold in markets, which can cause confusion for Japanese consumers.

Also, because sweetpotato is a vegetatively propagated plant, clones can be easily obtained from its edible portion, storage roots. Thus, it is possible that recently developed Japanese cultivars protected by breeder's right are brought to other countries in the form of storage roots and cultivated for use in processed products, such as flours, pastes, or food colorants, which are then illegally reimported to Japan. However, it is almost impossible to identify the original cultivars in these processed products based on their morphological characteristics. Thus, to protect the rights of breeders and to benefit the consumers, there is need of a reliable method for genetically identifying sweetpotato cultivars.

The cleaved amplified polymorphic sequence (CAPS) method is a reliable and simple technique to detect genetic polymorphism. In the CAPS method, partial sequences of genes are amplified by polymerase chain reaction (PCR) and digested with restriction enzymes to produce polymorphic fragments. Because the PCR primers used in the CAPS method are specific to exon sequences of the genes, amplification is highly stable among different cultivars and reproducible among different experiments. These PCR primers can be easily designed from genomic or EST sequences registered in public databases. In addition, marker fragments can be detected in agarose gels without using expensive facilities or equipments, such as DNA sequencers. The CAPS method has been successfully used for the cultivar identification of strawberry, which is a vegetatively propagated polyploid plant like the sweetpotato (Kunihisa et al., 2003, 2005). Identification of citrus cultivars by the CAPS method has also been reported (Omura, 2004).

In this study, to develop CAPS-based markers that could be used for the identification of sweetpotato cultivars, we first screened polymorphic fragments. Then, to test the ability of the selected markers to distinguish cultivars, we performed genotyping of Japanese sweetpotato cultivars.

* Corresponding author at: National Agricultural Research Center for Kyushu Okinawa Region, Yokoichi 6651-2, Miyakonojo, Miyazaki 885-0091, Japan. Tel.: +81 986 24 4273; fax: +81 986 24 4283.
E-mail address: mtanaka@affrc.go.jp (M. Tanaka).

0304-4238/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2009.11.011

2. Materials and methods

2.1. Plant materials and DNA extraction

For the screening of polymorphic markers, top leaves of 34 sweetpotato cultivars (Table 1), including the 21 cultivars shown in Fig. 1, were harvested from the nursery bed of the sweetpotato breeding laboratory at the National Agricultural Research Center for Kyushu Okinawa Region (KONARC) and dried at 50 °C overnight. The dried leaves were then ground into fine powder, and subjected to DNA extraction by the method described previously (Tanaka et al., 2007).

For the genotyping of Japanese sweetpotato cultivars, 60 of cultivars (Table 1) developed by the Ministry of Agriculture, Forestry, and Fisheries of Japan (MAFF) were analyzed. Two sets of leaf samples were collected independently from the nursery beds of the sweetpotato breeding laboratories in KONARC and the National Institute of Crop Science (NICS). DNA was extracted from these leaf samples as described above.

Table 1
List of the cultivars used in this study.

Cultivars used for marker screening		Japanese cultivars used for genotyping	
Cultivar name	Origin	Cultivar	Registered year
Kokei No. 14	Japan	Norin No. 1	1942
Beniazuma	Japan	Norin No. 2	1942
Sunny Red	Japan	Norin No. 3	1944
Yamagawamurasaki	Japan	Norin No. 4	1944
Kyushu No. 109	Japan	Norin No. 5	1945
Ayamurasaki	Japan	Norin No. 6	1945
Tanegashimamurasaki No. 1	Japan	Norin No. 7	1946
Tanegashimamurasaki No. 7	Japan	Norin No. 9	1948
Shin-eimurasaki	Japan	Norin No. 10	1950
Beni-aka	Japan	Kuroshirazu	1952
Choshu	Japan	Chihaya	1952
Yoshida	Japan	Shirosengan	1952
Genji	Japan	Okimasari	1952
Taihaku	Japan	Ajiyoshi	1952
Shichifuku	Japan	Fukuwase	1952
Genki	Japan	Nakamurasaki	1952
8205	Japan	Yakeshirazu	1954
Cuiollo	Uruguay	Benisengan	1955
Morado	Chile	Seto-aka	1955
Camote	Chile	Kurimasari	1960
Santa Catalina OP	Venezuela	Tamayutaka	1960
U.C.700	Venezuela	Beniwase	1961
Biscuit	Philippine	Gokokumasari	1961
66	Philippine	Satsuma-aka	1962
76(3)	Philippine	Ariake-imo	1962
76(4)	Philippine	Konasengan	1962
Ingahapon	Philippine	Tsukumo-aka	1962
94PH-75	Philippine	Beniyutaka	1966
Mup	Papua New Guinea	Koganesengan	1966
Nurilmum	Papua New Guinea	Naeshirazu	1974
Alotou-Sineada-1	Papua New Guinea	Benikomachi	1975
East Cape-1	Papua New Guinea	Minamiyutaka	1975
K-9	Papua New Guinea	Tsurusengan	1981
Nomad	Papua New Guinea	Beniazuma	1984
		Benihayato	1985
		Shiroyutaka	1985
		Shirosatsuma	1986
		Satsumahikari	1987
		Hi-Starch	1988
		Fusabeni	1989
		Beniotome	1990
		Hitachi Red	1993
		Satsuma Starch	1994
		Joy White	1994
		Ayamurasaki	1995
		Elegant Summer	1996
		J-Red	1997
		Harukogane	1998
		Sunny Red	1998
		Konahomare	2000
		Tamaotome	2001
		Murasakimasari	2001
		Benimasari	2001
		Purple Sweet Lord	2002
		Quick Sweet	2002
		Hamakomachi	2003
		Daichino-yume	2003
		Ayakomachi	2003
		Okikogane	2004
		Akemurasaki	2005

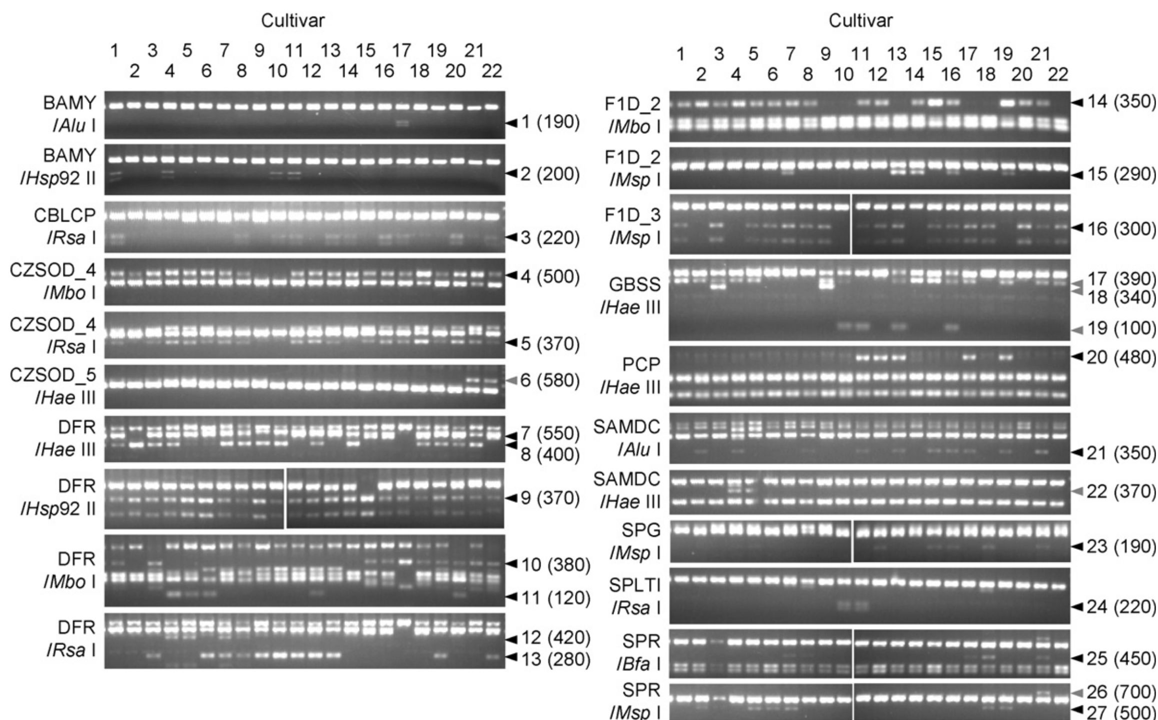


Fig. 1. Screening of polymorphic CAPS-based DNA markers. Arrowheads indicate the polymorphic fragments. Arrowheads in black indicate the CAPS markers, while those in grey indicate putative ALP markers. The approximate size of each fragment in base pairs (bp) is shown in the parentheses. The polymorphism of fragment No. 9 cannot be seen among the 21 cultivars shown here, but was detected among the other 13 cultivars used for the screening. The cultivar names are as follows: 1, Kokei No. 14; 2, Beniazuma; 3, Sunny Red; 4, Yamagawamurasaki; 5, Kyushu No. 109; 6, Ayamurasaki; 7, Tanegashimamursaki No. 1; 8, Tanegashimamurasaki No. 7; 9, Shin-eimurasaki; 10, Beni-aka; 11, Choshu; 12, Yoshida; 13, Genji; 14, Taihaku; 15, Shichifuku; 16, Genki; 17, 8205; 18, Cuiollo; 19, Morado; 20, Camote; 21, Santa Catalina OP; 22, U.C.700.

Table 2
Primer pairs used in the amplifications of CAPS-based markers.

Primer pair	Sequence (5′ → 3′)[a]	Expected product length (bp)	Target gene (accession no.)
BAMY	F: CCCGGTGTAATGCCAATTGGTAAC R: TGCTTCAGCTCATCTTCCACCTTC	310	β-Amylase (D12882)
CBLCP	F: AGTGGAACAGAAGCTGGGGCGATG R: TTCAATCCCGCACTCGTTTGTTCC	439	Cathepsin B-like cysteine proteinase (AF283476)
CZSOD_4	F: ATGGAGCTCCTGGAGACGATAACC R: CCTGCTTGTCAGTGATGGTGAATG	504	Cu/Zn superoxide dismutase (L36229)
CZSOD_5	F: CTGTTGTTGTTCATGGTGATCCCG R: CAGTGCTTTTGCTGAGCTCATGGC	508	Cu/Zn superoxide dismutase (L36229)
DFR	F: AGCTGCTGGAGTGATCTGGATTTC R: CCATGCTTCCTTCTCTGCCAGTAT	512	Dihydroflavonol 4-reductase (AB112545)
F1D_2	F: GGAACTATGCAACTGCCTCTGCTT R: GAAACCCCATACATTGTCAGAGGC	345	F1-ATP synthase delta subunit (AB026909)
F1D_3	F: GGATACCACCCAGAACTTCTTGCG R: GCGATCTATGTGTTTCAGCCTTCC	499	F1-ATP synthase delta subunit (AB026909)
GBSSI_2	F: TGGAGATGTTCTTGGAGGATTGCC R: ACTGATCATAACGGGGACACACTG	381	Granule bound-starch synthase (AB071976)
PCP	F: CCGTCAGATTTCGACTGGAGAGAC R: GAACCAGCTTCTTCAGGATCACAC	442	Papain-like cysteine proteinase (AF216783)
SAMDC	F: GCTCAAAGGCTTTGATAGAGGAGG R: CCCACCTTTAGACTCCATCAGTTC	737	S-adenosylmethionine decarboxylase (AF291761)
SPG	F: AAATTGCCTCACGACTGCTCTTCC R: GTCGCAGTCTACTAGCTCTTGCTC	331	Cysteine proteinase precursor (AY055589)
SPLTI	F: AACCACAAAAGAGAGGCAAACATC R: ATAATGATCTCCCACCTAGCCAAT	378	Leaf trypsin inhibitor (AF330702)
SPR	F: CTTGTCAAGTGCGTGTTGGATGAG R: CTCGGGAGAAAATGCTGGTGAGAA	586	Starch phosphorylase (L25626)

[a] F, sense primer; R, antisense primer.

2.2. Detection of CAPS-based markers and data analysis

PCR reaction was performed in a 20 μL mixture containing 1× PCR buffer, 1.5 mM dNTPs, 1 μM forward primer, 1 μM reverse primer, 0.5 U *TaKaRa rTaq* (TAKARA BIO, Ohtsu, Japan), and 20 ng DNA. The primers (Table 2) were designed using the GENETYX-MAC software (GENETYX Co., Tokyo, Japan). Amplification was performed using a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) under the following program: 94 °C for 3 min; followed by 30 cycles of 94 °C for 15 s, 64 °C for 30 s, 72 °C for 1 min; and a final step of 72 °C for 5 min. An aliquot of amplified product was then digested with 8 different restriction enzymes having four base pair recognition sites, namely AluI, BfaI, RsaI, Taq$^{\alpha}$I, MspI (New England BioLabs, Ipswich, MA), HaeIII, MboI (TAKARA BIO, Ohtsu, Japan), and Hsp92II (Promega, Madison, WI, USA). Digestion was performed at 37 °C for more than 2 h in a 10 μL mixture containing 1× restriction enzyme buffer, 1 U restriction enzyme, and 2 μL amplified product. The digested product was separated on a 2.0% agarose gel containing ethidium bromide and visualized under UV light. Each polymorphic fragment was scored as 1 (presence) or 0 (absence), and the resulting matrix of polymorphism data was analyzed Microsoft Excel spreadsheet-based macros. Calculation of Nei's genetic identity (Nei and Li, 1979) and cluster analysis by UPGMA method was performed using NTSYSpc software (Exeter Software, Setauket, NY, USA).

3. Results

3.1. Screening of CAPS-based markers for sweetpotato cultivar identification

To obtain polymorphic markers applicable to a wide range of cultivars, we screened polymorphisms using both Japanese and overseas cultivars. Nucleotide sequences of 11 sweetpotato genes were obtained from GenBank database (http://www.ncbi.nlm.nih.gov/) and used to design the 13 PCR primer pairs shown in Table 2. To effectively detect the polymorphism while maintaining the stability of amplification, primer pairs were designed in the exon sequences to amplify fragments containing an intron. Amplified fragments were digested with 8 restriction enzymes having different four base pair recognition sequences to produce polymorphic bands. Restriction enzymes with four base pair recognition sites were utilized because they were expected to detect sequence polymorphism with higher sensitivity than enzymes with six base pair recognition sites.

Consequently, all 13 primer pairs produced polymorphic fragments (Fig. 1). For some of these polymorphic markers, the same polymorphism was observed by different restriction enzyme treatments, suggesting that these polymorphisms resulted from differences in the length of the original amplification product. Such polymorphisms are sometimes called amplicon length polymorphisms (ALPs). Based on the clarity of polymorphism, we selected 27 markers, including both CAPS and putative ALP markers, to use in cultivar identification (Fig. 1, Table 3).

3.2. Genotyping of Japanese sweetpotato cultivars using selected markers

To verify the ability of the 27 markers to discriminate cultivars, we performed genotyping of 60 Japanese sweetpotato cultivars. For all of the 60 cultivars, detected genotypes were identical for the two DNA samples prepared from plants independently conserved at two different breeding sites (KONARC and NICS). Out of 27 markers tested, 24 markers showed polymorphism and successfully discriminated each of the 60 cultivars from the others (Table 4, Fig. 2). To further estimate the efficiency of discrimination, we performed pairwise comparisons of the detected genotypes between cultivars (a total of 1770 combinations). For 1764 combinations, differences in genotype were detected by more than one marker. For the other 6 combinations, namely 'Norin No. 2' vs 'Hi-Starch', 'Noron No. 6' vs 'Satsuma-aka', 'Kuroshirazu' vs 'Nakamurasaki', 'Chihaya' vs 'Benisengan', 'Chihaya' vs 'Satsuma-aka', and 'Seto-aka' vs 'Konahomare', the detected genotypes were different only at one marker. The average number of polymorphic markers between two cultivars was 6.56. The number of detected genotypes for one gene ranged from 2 to 13 (Table 5). An extremely large number of genotypes, 13, was observed for the dihydroflavonol 4-reductase (*DFR*) gene. For the other 10 genes, 2–7 genotypes were observed (Table 5).

4. Discussion

In this study, we developed CAPS-based DNA markers for use in identifying sweetpotato cultivars. To our knowledge, this is the first report on the development of CAPS markers in the sweetpotato. Ooe et al. (2004) reported the identification of sweetpotato cultivars used for production of hoshi-imo (steamed and dried storage root slices) using the markers based on the insertional polymorphism of retrotransposon *RTSP1*. This method is highly reliable and enables the detection of polymorphism even from highly degraded DNA. In this method, however, cloning and

Table 3
Number of selected polymorphic markers from each primer pair.

Primer pair	No. of polymorphic markers									
	AluI	BfaI	HaeIII	Hsp92II	MboI	MspI	RsaI	Taq$^{\alpha}$I	ALP	Total
BAMY	1			1						2
CBLCP							1			1
CZSOD_4					1		1			2
CZSOD_5									1	1
DFR			2	1	2		2			7
F1D_2					1	1				2
F1D_3						1				1
GBSS									3	3
PCP			1							1
SAMDC	1								1	2
SPG						1				1
SPLTI							1			1
SPR		1				1			1	3
Total										27

Table 4
Genotyping of the Japanese sweetpotato cultivars using the selected polymorphic markers.

Cultivar	Marker No.[a]																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Norin No. 1	–	–	–	+	+	–	+	–	+	–	–	–	+	+	–	+	–	–	–	–	–	–	+	–	–	–	–
Norin No. 2	–	–	+	+	–	–	+	–	+	–	–	–	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–
Norin No. 3	–	–	+	+	+	–	+	–	+	+	–	–	+	+	–	–	+	–	–	–	+	–	+	+	–	–	+
Norin No. 4	–	+	+	+	–	–	+	+	+	–	–	–	+	+	–	–	+	–	+	–	+	–	+	–	–	–	+
Norin No. 5	–	+	+	+	+	–	+	+	+	–	–	–	+	+	–	+	+	–	–	–	+	–	–	–	–	–	+
Norin No. 6	–	–	+	+	+	–	+	+	+	–	–	–	+	+	–	+	+	–	–	–	+	–	+	–	–	–	–
Norin No. 7	–	–	+	+	+	–	+	+	+	+	–	–	+	+	–	+	+	–	+	–	+	–	+	–	–	–	+
Norin No. 9	–	–	–	+	–	–	+	–	+	+	–	–	+	+	–	–	+	–	+	–	+	–	–	–	–	–	–
Norin No. 10	–	–	+	+	–	–	+	+	+	–	–	–	–	+	–	+	+	–	+	–	–	–	–	+	–	–	–
Kuroshirazu	–	–	–	+	+	–	+	–	+	+	–	–	+	+	–	+	+	–	+	+	+	–	–	–	–	–	–
Chihaya	–	–	+	+	–	–	+	+	+	–	–	–	+	+	–	+	+	–	–	–	+	–	–	–	–	–	–
Shirosengan	–	–	–	+	+	–	+	–	+	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Okimasari	–	–	–	+	+	–	+	–	+	+	–	–	–	+	–	+	+	–	+	+	+	–	–	+	–	–	–
Ajiyoshi	–	–	+	+	+	–	+	+	+	–	–	–	–	+	–	+	+	–	–	–	+	–	–	+	–	–	+
Fukuwase	–	–	+	–	–	–	+	–	+	+	–	–	+	+	–	–	+	–	+	+	–	–	+	–	–	–	–
Nakamurasaki	–	–	–	+	+	–	+	–	+	+	–	–	+	+	–	+	+	–	–	+	+	–	–	–	–	–	–
Yakeshirazu	–	+	+	–	–	–	+	–	+	–	–	–	+	–	–	+	+	–	–	+	–	–	–	+	–	–	+
Benisengan	–	–	+	+	–	–	+	+	+	–	–	–	–	+	–	+	+	–	–	–	+	–	–	–	–	–	–
Seto-aka	–	–	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	–	–	+	+	–	–	–	–	–	–
Kurimasari	–	–	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	–	+	–	+	–	+	–	–	–	–
Tamayutaka	–	–	+	+	+	–	+	–	+	+	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–
Beniwase	–	–	+	+	+	–	+	+	+	+	–	–	+	+	–	–	+	–	–	–	+	–	+	–	–	–	–
Gokokumasari	–	–	+	+	+	–	+	+	+	–	–	–	+	–	–	+	+	–	–	–	+	–	+	–	–	–	+
Satsuma-aka	–	–	+	+	+	–	+	+	+	–	–	–	+	+	–	+	+	–	–	–	+	–	–	–	–	–	–
Ariake-imo	–	–	–	+	+	–	+	–	+	–	–	–	+	–	–	+	+	–	–	+	+	–	–	–	–	–	–
Konasengan	–	–	+	+	–	–	+	–	+	–	–	–	+	+	–	+	+	–	–	–	+	–	+	–	–	–	–
Tsukumo-aka	–	–	–	+	+	–	+	+	+	–	–	–	+	–	+	+	+	–	–	+	+	–	–	–	–	–	–
Beniyutaka	–	–	+	+	+	–	+	+	+	–	–	–	+	–	–	+	+	–	+	–	+	–	–	–	–	–	+
Koganesengan	–	–	–	+	+	–	+	+	+	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	+
Naeshirazu	–	–	+	+	–	–	+	+	+	+	–	–	+	–	–	+	+	–	+	–	+	–	–	–	–	–	+
Benikomachi	–	–	+	+	+	–	+	+	+	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	+
Minamiyutaka	–	–	+	+	+	–	+	+	+	–	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	+	+
Tsurusengan	–	–	+	–	–	–	+	+	+	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–
Beniazuma	–	–	–	+	–	–	–	+	+	–	–	–	–	+	–	–	+	–	–	–	+	–	–	–	–	–	+
Benihayato	–	–	+	+	+	–	+	+	+	+	–	–	–	+	–	+	+	–	–	–	–	–	+	–	–	+	–
Shiroyutaka	–	–	+	+	–	–	+	–	+	–	–	–	+	+	–	–	+	–	+	–	–	–	–	–	–	–	+
Shirosatsuma	–	–	+	–	–	–	+	+	+	–	–	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–
Satsumahikari	–	+	+	+	–	–	+	+	+	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–
Hi-Starch	–	–	+	+	–	–	+	–	+	–	–	–	+	–	–	+	+	–	–	+	+	–	–	–	–	–	–
Fusabeni	–	–	+	+	+	–	+	+	+	–	–	–	+	+	+	+	+	–	–	–	+	–	–	+	–	–	–
Beniotome	–	–	+	+	+	–	+	+	+	+	–	–	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–
Hitachi Red	–	–	–	–	–	–	+	–	+	–	–	–	–	+	–	+	+	–	–	–	+	–	–	–	–	–	+
Satsuma Starch	–	–	+	+	+	–	+	+	+	+	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	+	+
Joy White	–	–	+	+	+	–	+	+	–	–	–	–	+	+	–	+	+	–	–	–	+	–	–	–	–	+	+
Ayamurasaki	–	–	–	+	+	–	+	+	+	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	+
Elegant Summer	–	–	+	+	–	–	–	+	+	–	–	–	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–
J-Red	–	–	+	+	–	+	+	–	+	–	–	–	+	+	–	–	+	–	+	–	+	–	–	–	–	–	+
Harukogane	–	–	+	–	–	–	+	+	+	–	–	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	+
Sunny Red	–	–	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	+	–	–	–	–	–	–	–	–	+
Konahomare	–	–	–	+	+	–	+	+	+	–	–	–	–	+	–	+	+	–	–	+	+	–	–	–	–	–	–
Tamaotome	–	–	+	+	+	–	+	+	+	+	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	+
Murasakimasari	–	–	+	+	–	–	+	+	+	–	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–
Benimasari	–	–	+	+	+	–	+	+	+	+	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–	+	–
Purple Sweet Lord	–	–	–	+	–	–	+	+	+	–	+	+	+	+	–	+	+	–	–	–	+	–	+	–	–	–	–
Quick Sweet	–	–	+	–	–	–	+	+	+	+	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	+	+
Hamakomachi	–	–	–	–	–	–	+	+	+	–	–	–	+	–	–	+	+	–	–	–	+	–	–	–	–	–	+
Daichino-yume	–	–	–	+	+	–	+	–	+	–	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–
Ayakomachi	–	–	–	+	+	–	+	+	+	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–
Okikogane	–	+	+	+	+	–	+	–	+	–	–	–	+	+	–	–	+	–	–	–	+	–	+	–	–	–	–
Akemurasaki	–	–	–	+	+	–	+	–	+	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–

[a] Marker numbers are shown in Fig. 1.

sequencing of the retrotransposon insertion sites are necessary before developing markers for each target cultivar. In other plants, SSR markers are widely used in cultivar identification. In the sweetpotato, although SSR markers have been developed (Buteler et al., 1999; Hu et al., 2004), they have not been applied to cultivar identification. Buteler et al. (1999) reported a relatively high frequency of insertion/deletion mutations in the flanking region of sweetpotato microsatellites, which seems unfavorable for developing markers applicable to a wide range of cultivars. In addition, the detection of polymorphisms using SSR markers requires the use of polyacrylamide gels or DNA sequencers. Considering these advantages and disadvantages of each marker system, it seems preferable to use different marker systems complementarily, depending on the purpose and target cultivars.

In cultivar identification, it is desirable that differences in genotype between cultivars can be detected by several different markers. Although the markers developed here effectively discriminated the majority of Japanese sweetpotato cultivars, a few cultivars showed the difference of genotype only at one marker. Thus, further screening of markers is necessary to clearly

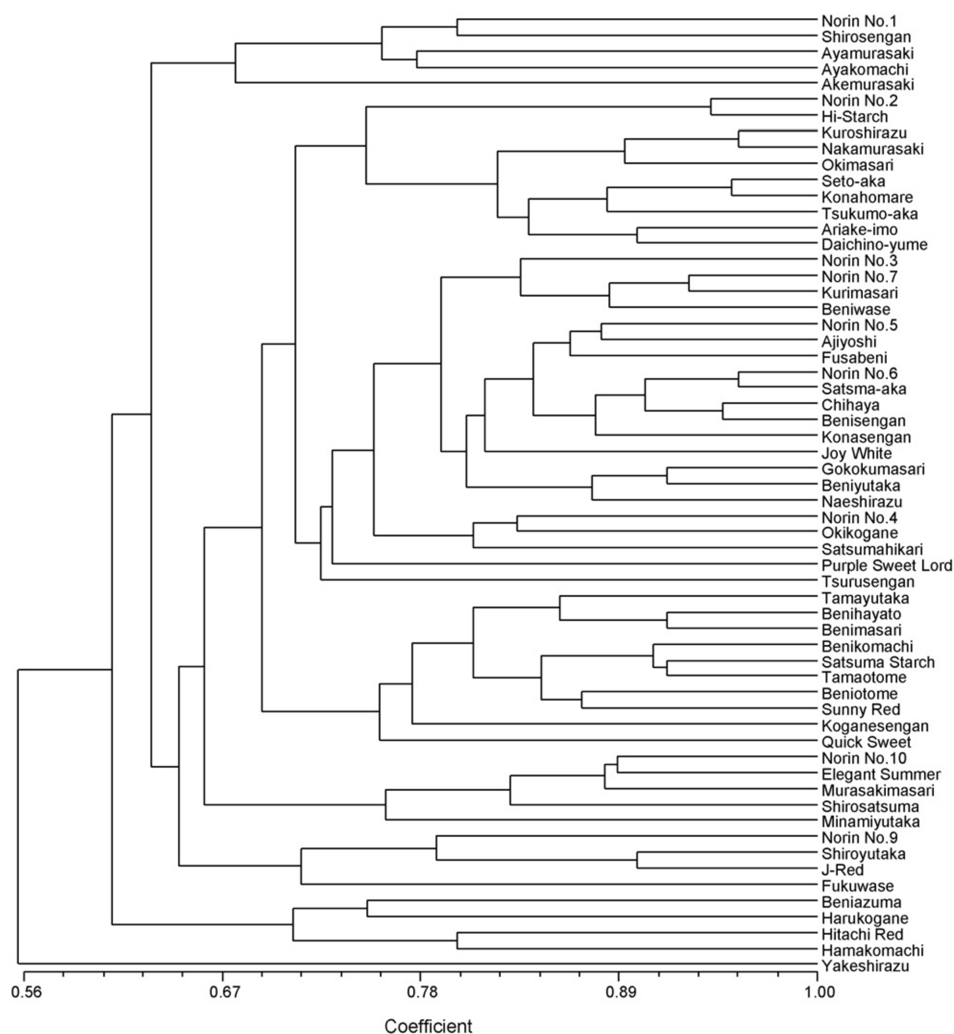


Fig. 2. Cluster analysis of 60 Japanese cultivars. The dendrogram was generated using UPGMA method based on Nei's genetic identity (Nei and Li, 1979) calculated from the genotyping data shown in Table 4.

distinguish these cultivars. The recent accumulation of nucleotide sequences of sweetpotato genes in public databases would facilitate the screening of novel CAPS markers. Among the genes used for primer design, the *DFR* gene showed the largest degree of polymorphism. Tanaka et al. (2004) suggested that at least three copies of *DFR* genes exist in the sweetpotato genome. Also, Kaundun and Matsumoto (2003) reported the detection of larger genetic variation in a multiple copy gene than in a single copy gene using CAPS markers. Thus, to develop CAPS markers with a better ability to detect of polymorphisms, it seems preferable to use multiple copy genes as targets of amplifications. In our results, polymorphism was often detected in relatively minor, low-intensity DNA fragments. A similar tendency was also reported for CAPS markers used for the identification of strawberry cultivars, and was thought to be caused by polyploidy of strawberries (Kunihisa et al., 2003). Kunihisa et al. (2005) reported that these problems can be overcome by the cluster-specific amplification method. In this technique, based on the nucleotide sequence of a CAPS fragment, primers are redesigned to specifically amplify the fragment containing the target polymorphism. This technique would be useful in improving the intensity of some of the markers developed here.

In this study, markers were designed from the genes encoding proteins involved in agriculturally and industrially important processes, such as starch or anthocyanin metabolism. The markers developed for these genes would be potentially useful for the genetic study of these important physiological functions, in addition to their use in cultivar identification.

Table 5
Number of genotypes detected for each gene.

Gene	No. of genotypes detected[a]
β-Amylase	2
Cathepsin B-like cysteine proteinase	2
Cu/Zn superoxide dismutase	4
Dihydroflavonol 4-reductase	13
F1-ATP synthase delta subunit	5
Granule bound-starch synthase	7
Papain-like cysteine proteinase	2
S-adenosylmethionine decarboxylase	2
Cysteine proteinase precursor	2
Leaf trypsin inhibitor	2
Starch phosphorylase	4

[a] Number of genotypes detected in the analysis shown in Table 4.

Acknowledgements

The authors thank Professor Makoto Tahara at Okayama University for his useful suggestions and critical reading of the manuscript. We also thank Ms. Rieko Gonbori and Ms. Masako Imamura (KONARC) for their assistance with the laboratory work.

References

Buteler, M.I., Jarret, R.L., Labonte, D.R., 1999. Sequence characterization of microsatellites in diploid and polyploid *Ipomoea*. Theor. Appl. Genet. 99, 123–132.

Hu, J., Nakatani, M., Mizuno, K., Fujimura, T., 2004. Development and characterization of microsatellite markers in sweetpotato. Breed Sci. 54, 177–188.

Kaundun, S.S., Matsumoto, S., 2003. Development of CAPS markers based on three key genes of the phenylpropanoid pathway in Tea, *Camellia sinensis* (L.) O. Kuntze, and differentiation between *assamica* and *sinensis* varieties. Theor. Appl. Genet. 106, 375–383.

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

Kunihisa, M., Fukino, N., Matsumoto, S., 2005. CAPS markers improved by cluster-specific amplification for identification of octoploid strawberry (*Fragaria* × *ananassa* Duch.) cultivars, and their disomic inheritance. Theor. Appl. Genet. 110, 1410–1418.

Nei, M., Li, H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76, 5269–5273.

Ooe, N., Tahara, M., Yamashita, H., Marutani, Y., Kuranouchi, T., 2004. Cultivar identification technique applied for hoshi-imo, steamed and dried sweetpotato slices, based on retrotransposon insertion polymorphism. Breed. Res. 6, 169–177 (in Japanese).

Omura, M., 2004. Agric. Hortic. 79, 142–145 (in Japanese, without English title).

Tanaka, M., Nakatani, M., Nakazawa, Y., Takahata, Y., 2004. Structural characterization of the dihydroflavonol 4-reductase B (*DFR-B*) gene in the sweet potato. DNA seq. 15, 277–282.

Tanaka, M., Nakayama, H., Takahata, Y., Jusuf Yakub, M., Hanarida Somantri, I., Minantyorini, Kurniawan, H., Nakatani, M., 2007. Molecular analysis of the genetic diversity in Indonesian sweetpotatoes. Acta Hortic. 760, 157–164.