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

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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.

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.

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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