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Multiplex PCR with SNP, STS and SSR Markers for Discriminating 114 Japanese Rice Cultivars

Hiroaki Tabuchi*1, Keiko Hayashi*2,
Ikuo Ashikawa*3 and Hitoshi Yoshida*4

Contents

Ⅰ. Introduction

Cultivar identification is important for the protection of the intellectual property rights of breeders and for the maintenance of specific cultivars used for breeding, without contamination from other cultivars [3, 5, 18, 23]. The identification of cultivars also helps farmers maintain premium prices for value-added 'brand cultivars' and helps consumers obtain reliable information on agricultural products [5, 31, 32]. In Japan, several polymerase chain reaction (PCR) methods that use DNA markers have been developed for the discrimination of agricultural products: for example, sweetpotato [40, 41], strawberry [16], bean [2, 33], shiitake [42], citrus [27], green tea [13], barley and wheat [4, 5, 15, 43], mat rush [11], laver [20], and chrysanthemum [10].

Because rice is the most important crop in Japan, several types of DNA markers have been examined and developed for rice cultivar discrimination: restriction fragment length polymorphism (RFLP) markers [6], random amplified polymorphic DNA (RAPD) markers [7, 24, 29], sequence tagged site (STS) markers [17, 28, 30, 36], simple sequence repeat (SSR) markers [1], and single nucleotide polymorphism (SNP) markers [14, 22, 26, 35]. To improve the convenience of cultivar discrimination, a multiplex PCR method was developed that involves simultaneous reactions with several markers. Discrimination by multiplex PCR with STS

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*1 Present address: NARO Kyushu Okinawa Agricultural Research Center, 6651-2 Yokoichicho, Miyakonojo, Miyazaki 885-0091, Japan
*2 NARO Agricultural Research Center, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan
*3 Previous address: NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan
*4 Present address: NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

markers using a standard thermal cycler and agarose gel electrophoresis has shown good reliability and has led to reductions in cost, time, and labor [30, 36].

In contrast, although SNPs are the most abundant polymorphisms for comparisons of the DNA of different cultivars [12], the popular detection method for SNPs that uses fluorescent dyes and analysis systems is expensive [14, 22, 26, 44]. When an SNP discrimination system is used with multiplex PCR, a rapid DNA preparation method, a standard thermal cycler and agarose gel electrophoresis, rice researchers can easily use SNP markers for the discrimination of breeding lines, the maintenance of cultivars without contamination, marker-assisted selection, and more. Hayashi et al. [9] reported that multiplex PCR with SNP markers could detect rice cultivars harboring rice blast disease-resistance genes. Suharyanto et al. [37] applied multiplex PCR with SNP markers to identify Japanese black pine. These studies demonstrated the possibility of discriminating rice cultivars using this technique.

In the present study, we developed DNA marker sets which can enable the easy and low-cost discrimination of rice cultivars with one grain or a piece of leaf. We developed 15 SNP markers and six STS markers, and our multiplex PCR with SNP markers was sufficiently reliable and as stable as using STS markers under optimized conditions. We constructed seven multiplex PCR marker sets composed of these 21 markers, and we applied these marker sets and one SSR marker (RM3120) for the discrimination of 114 Japanese rice cultivars and one foreign rice cultivar. Our technique discriminated all 115 cultivars with the use of a rapid DNA preparation method, a standard thermal cycler and agarose gel electrophoresis.

Ⅱ. Materials and Methods

1. Plant materials

A total of 114 Japanese rice cultivars and one foreign (Republic of India) *indica* rice cultivar, 'Kasalath' were analyzed. Table 1 provides the cultivar names and seed sources. All of the cultivars were seeds from breeders, except for 'Asahi,' 'Akebono,' 'Tokyofujikuramochi,' and 'Hinokemochi' (foundation seeds), and 'Norin 8,' 'Nihonmasari,' 'Raichoumochi,' 'Kaguramochi,' 'Kasalath,' and 'Tsukimimochi' which were stock seeds in our laboratory.

2. DNA preparation

Genomic DNA from each cultivar (several seedlings were used) was prepared essentially following the methods described by Murray et al. [25]. Cultivars were discriminated using one grain of hulled rice or a piece of fresh leaf (approx. 25 mm^2) using a modified version of the rapid DNA preparation method described by Monna et al. [21]. One grain or a piece of leaf was placed in a 2.0-ml microcentrifuge tube and homogenized with 0.4 ml of extraction buffer (100 mM Tris-Cl, 10 mM EDTA, 1 M KCl, and 2 % cetyltrimethylammonium bromide (CTAB), pH 8.0) using a Mixer Mill MM 300 (Qiagen, Tokyo) for 2 min at 30 rpm. The sample was centrifuged for 10 min at room temperature (RT), and the supernatant was transferred to a new 1.5-ml microcentrifuge tube. A 0.4 ml of chloroform/isoamylalcohol (24:1 v/v) was added and the contents were thoroughly mixed. The sample was centrifuged for 10 min at RT, and the aqueous layer was transferred to a new 1.5-ml microcentrifuge tube. A 0.4 ml of isopropanol was added, and the contents were mixed well. The sample was centrifuged for 10 min at RT. The pellet was rinsed with 70% ethanol and dried for 10 min. DNA was dissolved in 0.1 ml of 1/10 TE buffer (1 mM Tris-Cl and 0.1 mM EDTA, pH 8.0).

3. Designing primers of allele-specific PCR markers

We designed a primer for allele-specific PCR markers at a single SNP site based on the method of Hayashi et al. [8]. When the 3'-end of the primer corresponding to an SNP site was identified as the first nucleotide, the third nucleotide from the 3'-end was artificially substituted with a mismatched nucleotide. To discriminate multiple continuous SNPs, we designed the primer so that the continuous SNPs were located at its 3'-end and no

Table 1-1 Cultivar names and sources (institutes) from which seeds were provided.

Cultivar	No.[a]	Source
Kirara 397	5	Hokkaido Kamikawa
Hoshinoyume	17	Agricultural Experiment Station
Hoshitaro	18	
Akiho	19	Hokkaido Central Agricultural
Nanatsuboshi	20	Experiment Station
Tsugaruroman	21	Aomori Prefectural Agriculture
Mutsukaori	22	and Forestry Research Center
Mutsuhomare	23	
Yumeakari	24	
Masshigura	25	
Akihikari	11	Aomori Prefectural Agriculture
Fujisaka 5	26	and Forestry Research Center
Komanomai	27	Fujisaka Laboratory for Rice
		Research
Iwatekko	28	Iwate Agricultural Research
Kakehashi	29	Center
Hitomebore	2	Miyagi Prefectural Furukawa
Sasanishiki	8	Agricultural Experiment Station
Kokoromachi	30	
Manamusume	31	
Akitakomachi	4	Akita Prefectural Agriculture,
Takaneminori	32	Forestry and Fisheries Research
Menkoina	33	Center
Haenuki	7	Yamagata General Agricultural
		Research Center
Koigokoro	34	NARO Institute of Crop Science
Milky Queen	35	
Mochiminori	102	
Takanari	112	
Momiroman	113	
Yumehitachi	36	Plant Biotechnology Institute,
Tokyofujikuramochi	107	Ibaraki Agricultural Center
Yumenohatamochi	108	
Hinokemochi	110	
Goropikari	37	Gunma Agricultural Technology
		Center
Sainokagayaki	38	Saitama Prefectural Agriculture
Yumeminori	39	and Forestry Research Center
Fusaotome	40	Chiba Prefectural Agriculture
		and Forestry Research Center
Kinuhikari	6	NARO Agricultural Research
Dontokoi	10	Center, Hokuriku Research
Akinishiki	41	Center
Awaminori	42	
Shunyou	43	
Yumeaoba	44	
Kusayutaka	45	
Itadaki	101	
Hokuriku193	111	
Yukinosei	12	Niigata Agricultural Research
Koshiibuki	13	Institute
Gohyakumangoku	16	
Wasejiman	46	
Koganemochi	47	
Wataboushi	48	
Koshitanrei	49	
Hohohonoho	15	Ishikawa Agricultural Research
Ishikawa 43[c]	50	Center
Notohikari	51	
Koshihikari	1	Fukui Agricultural Experiment
Hanaechizen	14	Station
Yamahikari	52	
Sakihikari	53	
Ikuhikari	54	
Norin 22	55	Nagano Agricultural Experiment
Miyamanishiki	56	Station
Nipponbare	9	Aichi Agricultural Research
Nakateshinsenbon	57	Center
Hatsushimo	58	
Koganenishiki	59	
Akibare	60	
Hatsuboshi	61	
Mineasahi	62	
Akanezora	63	
Wakamizu	64	
Tsukinohikari	65	
Chiyonishiki	66	
Aichinokaori	67	
Asanohikari	68	
Aoinokaze	69	
Matsuribare	70	
Asahinoyume	71	
Akenohoshi	114	NARO Western Region
Kusanohoshi	115	Agricultural Research Center
Mienoemi	72	Mie Prefecture Agricultural
		Research Institute
Akinouta	73	Shiga Prefecture Agricultural
Yumeoumi	74	Technology Promotion Center
Yamadanishiki	75	Hyogo Prefectural Technology
		Center for Agriculture, Forestry
		and Fisheries
Omachikane	76	Tottori Agricultural Experiment
		Station
Asahi[b]	77	Agricultural Experiment Station,
Akebono[b]	78	Okayama Prefectural General
		Agriculture Center

Table 1-2 Cultivar names and sources (institutes) from which seeds were provided.

Cultivar	No.[a]	Source
Akiroman	79	Hiroshima Prefectural Technology Research Institute, Agricultural Technology
Haruru	80	Yamaguchi Prefectural Technology Center for Agriculture and Forestry
Oseto	81	Kagawa Prefectural Agricultural
Sanukiyoimai	105	Experiment Station
Matsuyamamii	82	Ehime Agricultural Experiment Station
Reihou	83	NARO Kyushu Okinawa
Yumehikari	84	Agricultural Research Center
Kurenaimochi	103	
Nikomaru	104	
Tachiaoba	106	
Yumetsukushi	85	Fukuoka Agricultural Research
Tsukushiwase	86	Center
Yumeshizuku	87	Saga Prefectural Agriculture Research Center
Morinokumasan	88	Kumamoto Prefectural Agricultural Research Center
Hinohikari	3	Miyazaki Agricultural Research
Koganemasari	89	Institute
Nishihomare	90	
Minamihikari	91	
Karinomai	92	
Akigeshiki	93	
Natsuhikari	94	Kagoshima Prefectural Institute
Hanasatsuma	95	for Agricultural Development
Norin 8	96	stock seeds in our laboratory
Nihonmasari	97	
Raichoumochi	98	
Kaguramochi	99	
Kasalath	100	
Tsukimimochi	109	

[a]Number corresponds to that in Fig. 4. [b]Foundation seeds. [c]A trade name of 'Ishikawa 43' is 'Yumemiduho'.

artificially substituted nucleotides were introduced. In this paper, we refer to these primers for sites with single and multiple continuous SNPs as 'SNP primers'.

We selected primers of STS markers from direct sequencing primers used in polymorphic DNA fragments of amplified fragment length polymorphism (AFLP) analysis among Japanese cultivars [38]. We also used the forward (RM3120-F) and reverse (RM3120-R) primers described by the International Rice Genome Sequencing Project [12] for SSR marker RM3120, which McCouch et al. [19] reported first. Table 2 indicates the markers used in this study. The 15 primers, 4P1-K-52, 17P1-K-51, 17P1-K-32, 26P1-K-5, 26P1-31, 6P1-N-522, 6P1-N-322, 34P3-51, 33P1-5, 34P1-A-3, 18P1-51, 18P1-31, 35P1-5, 34P2-5, and 34P2-3 are patented in Japan (patent no. 4344818). The two primers, WxMq497-5 and WxKo595-3 are also patented in Japan (patent no. 3569746). When using these patented primers for non-research purpose in Japan, please contact NARO Headquarters.

To determine the chromosomal position of the markers, a similarity search was performed using the DNA sequences of PCR products and rice genomic sequence data of 'Nipponbare' (IRGSP 1.0) with the BLAST software (http://www.shigen.nig.ac.jp/rice/oryzabase/). The first nucleotide positions of markers are indicated in Table 2.

4. PCR conditions

Standard PCR conditions were used: 32 cycles at 94°C (10 s), 60°C (10 s), and 72°C (30 s) with a Takara Thermal Cycler SP or a Takara Thermal Cycler Dice (Takara Bio, Kyoto, Japan) running in SP compatible mode. For the PCR with a single marker, the polymorphic region was amplified using 10 ng of genomic DNA from each cultivar as a template and 0.5 µM of each primer in 10 µl of reaction solution with TaKaRa Taq Hot Start Version (Takara Bio) following the manufacturer's protocol. For the multiplex PCR, the concentrations of primers in the 10 µl reaction mixtures are summarized in Table 2. Multiplex PCR was carried out using 1 µl of rapidly prepared DNA (from one grain or a piece of leaf) as a template.

The reliability of the different PCR markers for discrimination was examined by the following method. The PCR mixture was prepared in 50 µl with the same concentrations of DNA and primers described above. The PCR process was paused at the end of the extension step (72°C) at 25, 30, 35, and 40 cycles in the single marker

Table 2 Primer sequences and concentrations in multiplex PCR.

Mix. name	Marker[a] name	Chr. No.[b] Position	Marker Type	Primer name	Conc. (μM)	Length[c] (bp)	primer sequence [d]
Mix1	4P1	Chr.8	SNP	4P1-K-52	1.0	382	TTGTATCCAAATAAATCAGGACCACTCTG
		14,975,465		4P1-314	1.0	K	TGAAAGCAACTTAGACGAATAATAAACC
	17P1	Chr.8	SNP	17P1-K-51	0.5	254	TGACAACGTAGATTAGGTTTCTATCAATAATAC
		1,487,096		17P1-K-32	0.5	K	ACAATCCTGTTTGTCGCAGG
	26P1	Chr.1	SNP	26P1-K-5	0.5	201	TTGTTTAGACAAAGTAAAGGAGCAATGCT
		23,831,129		26P1-31	0.5	K	GATATCTTAGGGCCCGTTTGG
	6P1	Chr.11	SNP	6P1-N-522	0.5	131	CCAACTGCTGCAATAATGCC
		6,703,737		6P1-N-322	0.5	N	CGTAGCAACTCAATATAACATCAGCTAA
Mix2	34P3	Chr.1	STS	34P3-51	0.2	438	TCCCTCCTTCTCACGACTCC
		3,273,546		34P3-31-I	0.2	N	GACACCAGCTTCCCTTGGCC
	33P1	–	STS	33P1-5	0.2	260	AGTGCTCAAGAAATCATAGCTGA
				33P1-D3	0.2	K	AGTTTCCAGCACCTAGCC
	29P1	–	STS	29P1-D5	0.5	148	CATGAACTGTAAAGTAAACATTTTTTTCAC
				29P1-D3	0.5	A	GTGGCAGAAGGGTTTATTAGC
Mix3	31P1	Chr.7	SNP	31P1-AP-K-5[e]	1.5	410	ATGACACTGTTTTAGGAAAAACACAG
		1,558,197		31P1-311	1.5	K	ATCCCGAGGATGGAAGAAGC
	27P2	Chr.11	SNP	27P2-K-5	0.75	325	GGTAATCTTTCTTCTACATCGGAGC
		18,671,339		27P2-K-3[e]	0.75	K	CTTTGATTCATCCACTGGACATAAT
	19P1	Chr.1	SNP	19P1-S-5	0.5	230	GAGAGAGATTTTGTATCAGATTGCTGTCAA
		41,251,843		19P1-3H1	0.5	S	ACAAACAGCAGTTAGCTTGTGACC
Mix4	31P2	Chr.1	SNP	31P2-K-5	1.5	505	CATAAGAGAGTAGTTGATAAACAAATGGAAATAT
		23,956,428		31P2-31	1.5	K	AAAATGACCATTTAGCCCTTGG
	34P1	Chr.6	SNP	34P1-512	0.5	340	CGAGCAGATCCTCCTCGTC
		10,469,444		34P1-A-3[f]	0.5	A	CGGCAAGCACAGCGTTC
	38P1	Chr.11	SNP	38P1-S-5	0.4	193	CACCGAGATCATTGTTGTCTATC
		16,095,853		38P1-32	0.4	S	TTCTGCAGATTTTGATCCTTCG
Mix5	18P1	Chr.7	STS	18P1-51	1.5	593	GGCTGCTAGCGTCATCAGG
		25,059,632		18P1-31	1.5	N	TTCTCATCATAACATATTGATCTTGC
	35P1	Chr.11	STS	35NP1-52	1.5	208	ATATGTCCCGTGCTTTGTGC
		22,566,124		35P1-5	1.5	N	TCTGACACTCTTATCAACAATTACATGAC
	34P2	–	STS	34P2-5	0.5	91	ATTACTGCCAAACCGCTTGC
				34P2-3	0.5	K	TGTGATCCATTATTGTCCGTAACC
Mix6	aa06302p	Chr.6	SNP	aa06302p-5L	0.5	407	GTTAGTTGCTCATCAACCAGACAACC
		5,533,291		aa06302p-N3L	0.5	N	GCTGACATGTGGGCCCCATTTG
	aa09004p	Chr.9	SNP	aa09004p-5	0.75	328	AACACCGATTGGGAACCTTTTTG
		2,179,744		aa09004p-N3	0.75	N	TCTATTTATATTCAAGTCTATTTTCGCAGTCG
	WxMq	Chr.6	SNP	WxMq497-5	1.0	240	GAGGTTTTTCCATTGCTACAATCA
		1,767,590		WxKo595-3	1.0	M	AAACGCATCTGGTTGTCTTTATA
Mix7	aa02034p	Chr.2	SNP	aa02034p-5	0.5	296	CAGGTATACCATGCGGGTCAG
		2,153,544		aa02034p-K3	0.5	K	TAAAACAATTTAGTGGCAGTGCGCG
	dbsS10844p	Chr.2	SNP	dbsS10844p-H5	0.8	160	AGAATTCAGGATATGTCTTGATCTCCAAAA
		24,647,535		dbsS10844p-3	0.8	H	CTACGCCAGGTTCCAAGTCAC
	RM3120	Chr.8	SSR	RM3120-F[g]	1.0	192	GGAAGCTCTTTGCCATCATACTAGC
		27,820,246		RM3120-R[g]	1.0	N	GAATATAACACCCACTCCCTCTGTCC

[a]Arranged in decreasing order of product size. [b]The first nucleotide positions of markers in rice genomic sequence data (IRGSP 1.0) are indicated. –: These PCR fragments are absent in rice genomic sequence data. [c]The lengths of the PCR products for 'Koshihikari' (K), 'Nipponbare' (N), 'Sasanishiki' (S), 'Akitakomachi' (A), 'Hitomebore' (H), and 'Milkey Queen' (M). [d]Artificially substituted nucleotides are underlined. [e]Because two SNP sites are located at the first and third nucleotides from the 3'-end, these primers have no artificially substituted nucleotide at the third nucleotide from the 3'-end. [f]Because two SNP sites are located at the first and second nucleotides from the 3'-end, this primer has no artificially substituted nucleotide at the third nucleotide from the 3'-end. [g]These primers were described by the International Rice Genome Sequencing Project [12].

reactions, or 28, 30, 32, 34, and 36 cycles in the multiplex reactions; 5 µl of solution was sampled each time and the PCR was continued. The samples were applied to 2 % agarose gels (Agarose Type Ⅱ, Medium EEO, Sigma, Tokyo), separated by electrophoresis, and stained with ethidium bromide. Bands were detected under a UV transilluminator. Samples amplified with SSR marker RM3120 were analyzed by QIAxcel using QX DNA High Resolution Kit (Qiagen KK, Tokyo).

Ⅲ. Results

1. Development of SNP and STS markers

Based on the polymorphism information for genomic DNA sequences among 25 representative Japanese rice cultivars [38], 10 SNP markers (4P1, 6P1, 17P1, 19P1, 26P1, 27P2, 31P1, 31P2, 34P1, and 38P1) and six STS markers (18P1, 29P1, 33P1, 34P2, 34P3, and 35P1) were developed (Table 2). Allele-specific primers of SNP markers basically contain an artificial mismatch in the third nucleotide from the 3'-end to retain the highest specificity [12], while primers of STS markers don't contain mismatched nucleotides. However, even in case of STS markers, introduction of artificial mismatch is sometimes effective to adjust the intensity of signals of all markers to the same level in multiplex PCR by reducing the intensity of signal. We, therefore, chose 34P3-31-I primer which contained an artificial mismatch in the third nucleotide from the 3'-end.

Because these 16 markers were not enough to discriminate the 114 Japanese rice cultivars, we developed an SNP marker (WxMq), three SNP markers (aa02034p, aa06302p, and aa09004p) and an SNP marker (dbsS10844p) on the basis of SNP information reported by Sato et al. [34], Yamamoto et al. [44] and Sato et al. [35], respectively (Table 2). In spite of adding new SNP markers, it is still not possible to discriminate all 114 cultivars. Thereby we used SSR marker RM3120 described by International Rice Genome Sequencing Project [12] and McCouch et al. [19].

2. The reliability of the single and multiplex PCR with SNP and STS markers for the discrimination of rice cultivars

We performed the following reliability tests using four SNP (4P1, 6P1, 17P1, and 26P1) and three STS (29P1, 33P1, and 34P3) markers, combinations of which were designated as Mix1 and Mix2, respectively. We first analyzed the reliability of the PCR with the single SNP markers and agarose gel electrophoresis by changing the PCR cycles from 25 to 40 in increments of five, with positive and negative control DNA with which a PCR fragment is expected to be amplified and not amplified, respectively (Fig. 1). After 25 cycles, weak or no signals for PCR products were detected for four SNP markers (4P1, 6P1, 17P1, and 26P1) and three STS markers (29P1, 33P1, and 34P3) in the positive control. After 30 and 35 cycles with these six markers, PCR products were observed in the positive control but were not detected in the negative control. After 40 cycles in the negative control, very weak signals for PCR products were sometimes detected for three markers (6P1, 17P1, and

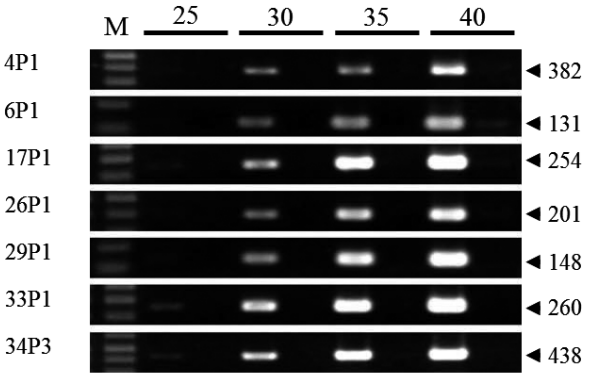

Fig. 1. Reliability of PCR using single SNP and STS markers.

PCR cycles are indicated above. The marker name is indicated at the left side. The length of the DNA fragment of each marker is indicated at the right side. Positive control DNA: 'Akitakomachi' in 4P1 - 26P1, and 'Hohohonoho' in 29P1 - 34P3. Negative control DNA: 'Gohyakumangoku' in 4P1 - 26P1, and 'Kirara 397' in 29P1 - 34P3. M: 100-bp ladder marker.

26P1). These results indicate that these seven markers could be reliable from 30 to 35 cycles.

Next, we used multiplex PCR with SNP markers for cultivar discrimination. Initially, the concentration of each primer in the reaction mixture was optimized for multiplex PCR with 32 cycles. The concentrations of primers in Mix1 and Mix2 are indicated in Table 2. The reliability of multiplex PCR with SNP and STS markers was tested by changing the number of PCR cycles from 28 to 36, in increments of two (Fig. 2). The PCR products for these markers were sometimes unclear after 28 cycles but were clear after 30 cycles in the positive control. A weak false-positive signal for the PCR product of marker 4P1 was sometimes detected after 36 cycles, but no other false-positive PCR products were observed. Weak extra PCR products of approx. 440, 300, 310, and 190 bp bands were sometimes detected above the 4P1, 17P1, 33P1, and 29P1 PCR products, respectively. However, these products had no influence on the discrimination results. These results show that the reliable ranges of PCR cycles of multiplex PCR with four SNP markers in Mix1 and three STS markers in Mix2 were from 30 to 34 cycles. We concluded that 32 or 34 cycles of PCR amplification produced the best results with our conditions.

We developed five other sets of multiplex PCR markers, Mix3 (three SNP markers: 31P1, 27P2, and 19P1), Mix4 (three SNP markers: 31P2, 34P1, and 38P1), Mix5 (three STS markers: 18P1, 35P1, and 34P2), Mix6 (three SNP markers: aa06302p, aa09004p, and WxMq), and Mix7 (two SNP markers: aa02034p, dbsS10844p). Although reliability tests of Mix3 - Mix6 were not carried out, the concentration of primers in these markers was adjusted to produce the best results after 32 or 34 cycles of PCR amplification (Table 2). Because the intensity of the signal tends to become weaker as the length of the PCR product becomes longer in a multiplex PCR, the concentration of primers for a longer PCR fragment was higher than that for the shorter PCR fragment in Mix1, Mix3, Mix4, and Mix5.

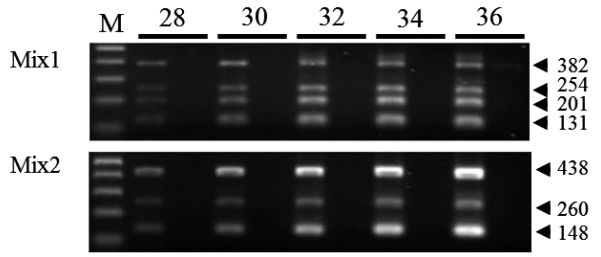

Fig. 2. Reliability of multiplex PCR with four SNPs (Mix1) and three STS markers (Mix2).

The PCR cycles are indicated at the top. The length of the DNA fragment of each marker is indicated at the right side. Positive control DNA: 'Akitakomachi' in Mix1 and 'Hohohonoho' in Mix2. Negative control DNA: 'Gohyakumangoku' in Mix1 and 'Kirara 397' in Mix2. The 100-bp ladder marker (M) indicates 100 bp, 200 bp, 300 bp, 400 bp, and 500 bp from the bottom to the top.

3. The discrimination of rice cultivars from one grain using multiplex PCR with SNP and STS markers

We applied these multiplex PCR markers with a rapid DNA preparation method from one grain to 114 Japanese cultivars including the 10 cultivars with the highest production volumes in Japan in 2014 and other major cultivars from each Japanese prefecture, and the foreign cultivar 'Kasalath' which has frequently been used for genetic analyses [26, 38].

Figure 3a shows typical results of 16 cultivars ('Koshihikari,' 'Hitomebore,' 'Hinohikari,' 'Akitakomachi,' 'Kirara 397,' 'Kinuhikari,' 'Haenuki,' 'Sasanishiki,' 'Nipponbare,' 'Dontokoi,' 'Akihikari,' 'Yukinosei,' 'Koshiibuki,' 'Hanaechizen,' 'Hohohonoho,' and 'Gohyakumangoku'). The genotypes of DNA fragments for the 115 cultivars are summarized in Figure 4. The lengths of the PCR fragments of 26P1 in 'Dontokoi,' and 18P1 in 'Hokuriku 193,' 'Takanari,' 'Akenohoshi,' and 'Kusanohoshi,' were observed to be larger than the sizes indicated in Figure 4 by about 10 bp and 30 bp, respectively. And the lengths of the PCR fragments of aa06302p in 'Momiroman' was observed to be shorter than the size indicated in Figure 4 by about 10 bp. Very weak (false-positive) signals were rarely detected for markers 4P1, 31P1, 34P1, and aa09004p, but they could be distinguished by the weakness of the signal. An example is shown in Figure 3c. In this case, distinct signal and no signal were detected for 'Hitomebore' and 'Nipponbare, ' respectively, but a very weak signal was

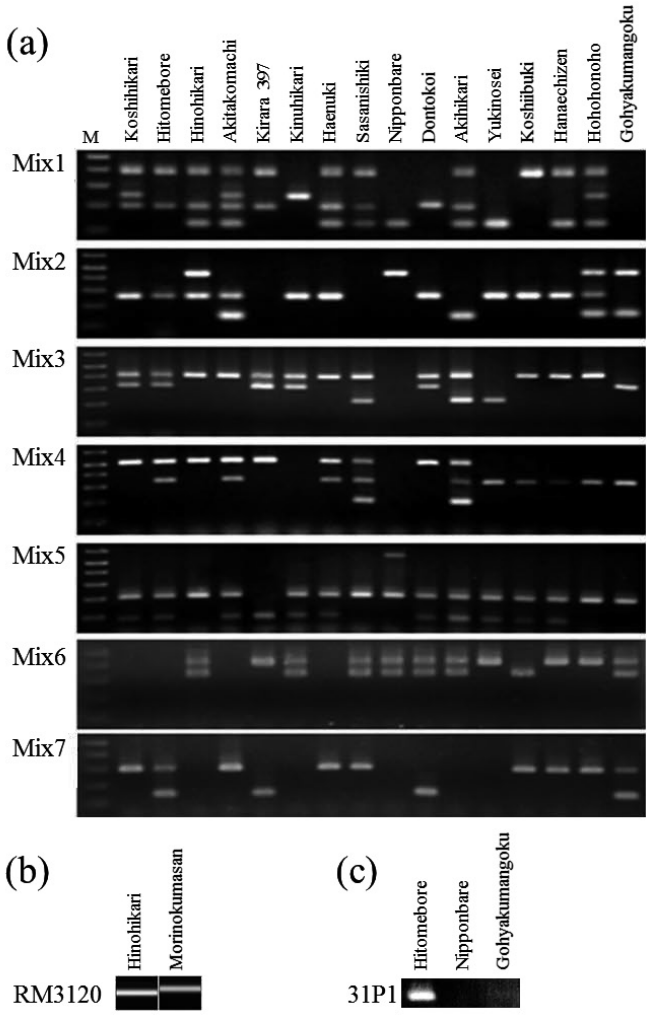

Fig. 3. DNA fragments amplified by seven marker sets of multiplex PCR with a standard thermal cycler and agarose gel electrophoresis using DNA from individual grains for 16 cultivars (a), DNA fragments amplified by RM3120 and detected by a capillary electrophoresis device QIAxcel for 'Hinohikari' and 'Morinokumasan' (b), an example of a very weak (false-positive) signal detected for markers 31P1 for 'Gohyakumangoku' (c).

Fragment lengths of 'Hinohikari' and 'Morinokumasan' amplified by RE3120 were caluculated as 196 and 198 bp, respectively. Cultivar names are indicated above. Mixture and marker names are indicated at the left side. 100-bp ladder marker (M) indicates 100 bp, 200 bp, 300 bp, 400 bp, and 500 bp from the bottom to the top.

detected for 'Gohyakumangoku'. Extra PCR products were sometimes detected, but they had no influence on the discrimination result, because the lengths of extra PCR products are different from those of other markers.

Finding genotypes for marker 33P1 in 'Yumeaoba' and 'Kasalath' and 31P1 in 'Yumenohatamochi' were difficult using multiplex PCR, and two PCR products were detected in 'Kasalath' by marker 38P1. However, single PCR with these markers for these cultivars enabled clear genotyping. 'Hinohikari' and 'Morinokumasan' showed the same genotype of these 21 markers. They could be discriminated by SSR marker RM3120. The length difference of DNA fragments of these two cultivars by RM3120 might not be detected by 2 % agarose gel electrophoresis but could be detected by a capillary electrophoresis device (QIAxcel) using the QX DNA High Resolution Kit, because the PCR fragment of 'Morinokumasan' was observed to be slightly larger than that of 'Hinohikari', probably by two base pairs (Fig. 3b, Table 2). Therefore, these seven sets of multiplex PCR

markers and one SSR marker discriminated all 115 of the cultivars. Similar results were obtained using DNA prepared from pieces of leaves (data not shown).

Ⅳ. Discussion

Our results showed that multiplex PCR with SNP markers developed in this study was sufficiently reliable and as stable as using STS markers to discriminate rice cultivars. We could easily discriminate 112 of 114 Japanese cultivars (the exceptions were 'Hinohikari' and 'Morinokumasan', which were discriminated with SSR marker RM3120) and one foreign rice cultivar 'Kasalath' using a rapid DNA preparation method, a standard

Marker			cultivar																																					
Mix Name	Marker Name	Length (bp)	1 Koshihikari	2 Hitomebore	3 Hinohikari	4 Akitakomachi	5 Kirara 397	6 Kinuhikari	7 Haenuki	8 Sasanishiki	9 Nipponbare	10 Dontokoi	11 Akihikari	12 Yukinosei	13 Koshibuki	14 Hanaechizen	15 Hohohonoho	16 Gohyakumangoku	17 Hoshinoyume	18 Hoshitaro	19 Akiho	20 Natsuisuboshi	21 Tsugaruroman	22 Mutsukaori	23 Mutsuhomare	24 Yumeakari	25 Masshigura	26 Fujisaka 5	27 Komanomai	28 Iwatteko	29 Kakehashi	30 Kokonomachi	31 Manamusume	32 Takanerminori	33 Menkoina	34 Koigokoro	35 Milky Queen	36 Yumehitachi	37 Gonpikari	38 Sanokagayaki	39 Yumeminori	40 Fusaotome
Mix1	4P1	382	1	1	1	1	1	0	1	1	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	1	0	1	1
	17P1	254	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
	26P1	201	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6P1	131	0	0	1	1	0	0	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0
Mix2	34P3	438	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
	33P1	260	1	1	1	1	0	1	1	0	0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	
	29P1	148	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mix3	31P1	410	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1			
	27P2	325	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	
	19P1	230	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	0	1	1	0	0	0	1	1	1	0	
Mix4	31P2	505	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	34P1	340	0	1	0	1	0	0	1	1	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1
	38P1	193	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Mix5	18P1	593	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0
	35P1	208	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1	
	34P2	91	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1
Mix6	aa06302p	407	0	0	1	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0	1	1	1	1	0
	aa09004p	328	0	0	1	0	0	1	0	1	1	1	1	0	1	0	0	1	0	1	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	0
	WxMq	240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Mix7	aa02034p	296	1	1	0	1	0	0	1	1	0	0	0	0	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	1	
	dbsS10844	160	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0	1	1	1	0	1
	RM3120	192	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Marker			cultivar																																					
Mix Name	Marker Name	Length (bp)	41 Akinishiki	42 Awaminori	43 Shunyou	44 Yumeaoba	45 Kusuyutaka	46 Wasejiman	47 Koganemochi	48 Wataboushi	49 Koshiianrei	50 Ishikawa 43	51 Notohikari	52 Yamahikari	53 Sakihikari	54 Ikuhikari	55 Norin 22	56 Miyamanishiki	57 Nakateshinsenbon	58 Hatsushimo	59 Koganenishiki	60 Akibare	61 Hatsuboshi	62 Mineasahi	63 Akanezora	64 Wakamizu	65 Tsukinohikari	66 Chiyonishiki	67 Aichinokaori	68 Asanohikari	69 Aonokaze	70 Matsuribare	71 Asahinoyume	72 Mienoemi	73 Akinouta	74 Yumeyouni	75 Yamadanishiki	76 Omachikarae	77 Asahi	78 Akebono	79 Akinoman	80 Haruru
Mix1	4P1	382	1	0	0	0	0	1	1	0	0	1	1	1	1	0	0	1	0	0	1	1	1	0	1	0	0	1	0	0	1	0	0	1	1	0	1	0	0	0	0	0	1
	17P1	254	1	0	0	0	1	1	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0
	26P1	201	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	1	1	1	0	0	0	1	0	1	0	1	
	6P1	131	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1	1	1	0	1	1	1	0	0	1	0	0	1	1	1
Mix2	34P3	438	0	1	1	0	1	0	0	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	1	0	1	1	1	0	0	
	33P1	260	1	0	0	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1		
	29P1	148	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Mix3	31P1	410	1	1	1	1	1	1	0	0	0	1	1	1	1	0	1	1	0	1	1	0	1	1	0	0	0	1	1	0	1	0	1	1	1	1	0	1	0	0	1	1	
	27P2	325	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	
	19P1	230	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	1	1	1	0
Mix4	31P2	505	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	1	1	1	0	0	0	1	0	1	0	1	
	34P1	340	0	0	1	1	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	
	38P1	193	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mix5	18P1	593	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	1	1	1	1	0	0	1	0	0	0	0	1	0	0
	35P1	208	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	
	34P2	91	1	0	0	0	0	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0	1	1	
Mix6	aa06302p	407	0	1	1	1	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1	
	aa09004p	328	1	1	1	1	1	0	1	1	1	0	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	0	1	1	
	WxMq	240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mix7	aa02034p	296	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	1	1	1	0	0	1	0	0	1	1			
	dbsS10844	160	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	0	0	1	1	1	0	1	1	1	0	1	0	0	0	0	1	0	
	RM3120	192	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 4-1. DNA fragments for 115 cultivars with 15 SNP, six STS and one SSR markers.

Marker			cultivar																																		
Mix Name	Marker Name	Length (bp)	81 Oseto	82 Matsuyamamii	83 Reihou	84 Yumehikari	85 Yumetsukushi	86 Tsukushiwase	87 Yumeshizuku	88 Morinokumasan	89 Koganemasari	90 Nishihomare	91 Minamihikari	92 Karinomai	93 Akigeshiki	94 Natsuhikari	95 Hanasatsuma	96 Norin 8	97 Nihonmasari	98 Raichoumochi	99 Kaguramochi	100 Kasalath	101 Itadaki	102 Mochiminori	103 Kurenaimochi	104 Nikomaru	105 Sanukiyoimai	106 Tachiaoba	107 Tokyofujikuramochi	108 Yumenohatamochi	109 Tsukiminochi	110 Hinokemochi	111 Hokuriku 193	112 Takanari	113 Momiroman	114 Akenohoshi	115 Kusanohoshi
Mix1	4P1	382	0	0	1	1	0	0	1	1	1	1	0	1	1	0	1	0	0	1	1	0	0	0	1	0	0	1	0	0	1	1	1	1	1	0	0
	17P1	254	0	0	0	1	1	1	0	0	0	0	0	1	1	1	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	1	1	0	1	1
	26P1	201	0	0	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1	0	0
	6P1	131	1	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0
Mix2	34P3	438	1	1	0	0	0	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
	33P1	260	0	0	1	1	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	1	0	0
	29P1	148	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0
Mix3	31P1	410	1	0	1	0	1	1	1	1	1	0	0	1	1	1	0	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1
	27P2	325	0	0	0	1	1	0	1	0	0	0	1	1	1	0	0	0	0	1	1	0	0	0	1	1	0	1	0	0	1	1	0	0	0	0	0
	19P1	230	1	1	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1	1	0	1	0	0	1	1	1
Mix4	31P2	505	0	0	0	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	0
	34P1	340	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0
	38P1	193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1
Mix5	18P1	593	1	1	0	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	1	0	0	0	1	1	1	1	1	0	0	1	1	1	1	1
	35P1	208	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	34P2	91	0	0	0	1	1	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0
Mix6	aa06302p	407	1	1	1	1	0	0	1	1	0	0	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	0
	aa09004p	328	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	WxMq	240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mix7	aa02034p	296	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	1	1	1	1	0	0	0	1	0
	dbsS10844	160	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	1	1	1	0	0	0
	RM3120	192	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 4-2. DNA fragments for 115 cultivars with 15 SNP, six STS and one SSR markers.

0: No DNA fragment is detected. 1: One DNA fragment is detected. 2: Two DNA fragments are detected. –: Not analyzed. Red cell: The lengths of the DNA fragment amplified by RM3120 of ‘Hinohikari’and ‘Morinokumasan’ were calculated as 196 and 198 bp, respectively. Green cell: The lengths of the PCR fragments of 26P1 in ‘Dontokoi,’ and 18P1 in ‘Hokuriku 193,’ ‘Takanari,’ ‘Akenohoshi,’ and ‘Kusanohoshi,’ were observed to be larger than the indicated sizes by about 10 bp and 30 bp, respectively. Orange cell: The lengths of the PCR fragments of aa06302p in ‘Momiroman’ was observed to be shorter than the indicated size by about 10 bp. Blue cell: Finding genotypes for these markers was difficult using multiplex PCR. We recommend single PCR with these markers and these cultivars. Yellow cell: A very weak (false positive) signal of the PCR fragment was rarely detected.

thermal cycler, agarose gel electrophoresis and seven multiplex PCR marker sets including 15 SNP markers as well as six STS markers, reducing the cost, time, and labor. Similar results were obtained for the identification of Japanese black pine [37]. Because SNPs are the most abundant polymorphisms between cultivars [12], it could be useful to conduct multiplex PCRs of SNP markers with a standard thermal cycler and agarose gel electrophoresis for many genotyping methods.

Although we found that multiplex PCR with SNP markers was useful, the optimization of PCR conditions may be necessary. We chose 32 cycles of PCR amplification for our markers, because weak false-positive signals for PCR products were sometimes detected after 40 or 36 cycles in a single or multiplex use of SNP markers of 4P1, 6P1, 17P1 and 26P1 (Figs. 1, 2). When the DNA polymerase or a thermal cycler is changed, the optimization of the PCR program may be also needed.

The seven marker sets we developed in this study can enable the easy and low-cost discrimination of rice cultivars with one grain or a piece of leaf from single cultivar, however, they are not adequate to detect mixed cultivars with bulk-prepared DNA from more than two cultivars. For example, these marker sets can’t identify ‘Haenuki’ when it is mixed with ‘Akitakomachi’. For the contamination test, a ‘negative kit’ was proposed, which was composed of individual or multiple STS DNA markers [30]. When using a negative kit, any PCR products were not detected for an uncontaminated sample, but one or more PCR products were detected if the sample

was contaminated with DNA of any other cultivars. A negative kit can be constructed with SNP markers more easily than with STS markers since we can select the cultivar for which a DNA fragment will be detected by choosing the 3'-end genotype of a SNP primer. Indeed, we have developed negative kits for each of the dominant 16 cultivars in Niigata prefecture, Japan, by using SNP markers as well as STS markers described in this study [39]. We expect other negative kits could be easily developed with SNP markers.

Ⅴ. Conclusion

We constructed seven multiplex PCR marker sets composed of 15 SNP and six STS markers to discriminate 114 Japanese rice cultivars including the 10 cultivars with the highest production volumes in Japan in 2014 and other major cultivars from each Japanese prefecture. The main findings of this study are summarized as follows;

① Single and multiplex PCR with SNP markers developed in this study were sufficiently reliable and as stable as the use of STS markers under optimized conditions.

② Seven multiplex PCR marker sets composed of 15 SNP and six STS markers could easily discriminate 112 of 114 Japanese cultivars (the exceptions were 'Hinohikari' and 'Morinokumasan') and one foreign rice cultivar.

③ 'Hinohikari' and 'Morinokumasan' were discriminated by an SSR marker, RM3120.

Ⅵ. Summary

The identification of cultivars is important for the protection of intellectual property rights of breeders, for the maintenance of crop prices for farmers, and for precise product information for consumers. In Japan, several PCR methods have been developed for crop discrimination. To distinguish rice cultivars in particular, several types of DNA markers have been used. Multiplex PCR methods were developed previously using STS markers to reduce labor, time, and cost. Although SNPs are the most abundant polymorphisms among cultivars, SNP marker sets for multiplex PCR have not been created for rice discrimination. The present study's results demonstrate that the agarose gel electrophoresis of multiplex PCR with SNP markers was as reliable as STS markers, and 114 Japanese rice cultivars including the 10 most produced cultivars in Japan in 2014 and other major cultivars from each Japanese prefecture were successfully discriminated using 15 SNP markers, six STS markers and one SSR marker.

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SNP・STS・SSRマーカーを用いた日本のイネ114品種識別用のマルチプレックスPCRマーカーセット

田淵宏朗*1・林敬子*2・芦川育夫*3・吉田均*4

摘　要

農業生産物における品種情報の担保は，育成者の権利を保護し，生産者にはブランド品種としての販売価格設定を可能にし，消費者には安心感を与えることに繋がることから，日本では様々な作物について品種識別技術が開発されている．これらの中で，マルチプレックス化されたSTSマーカーを用い，PCRで増幅したDNA断片をアガーロースゲル電気泳動で分離・検出してイネの品種識別を行う技術は信頼性も高く，高価な機材を使用しないため多くの研究施設で利用が可能である．本研究では，我々が開発したSNPマーカーによるマルチプレックスPCRが，STSマーカーと同様に安定性と信頼性が高いことを示した．さらに，15種類のSNPマーカーと6種類のSTSマーカーからなる7組のマルチプレックスPCRマーカーセットを作成し，1種類のSSRマーカーを併せて用いることで，2014年の日本での作付面積上位十位の品種および各都道府県での主要品種を含む日本のイネ114品種を識別可能であることを示した．

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＊1　現　農研機構九州沖縄農業研究センター
＊2　農研機構中央農業研究センター
＊3　元　農研機構作物研究所
＊4　現　農研機構作物研究所