Development of mini core collection of Japanese rice landrace

Kaworu Ebana^{*†1)}, Yoichiro Kojima^{†1,2)}, Shuichi Fukuoka¹⁾, Tsukasa Nagamine^{1,3)} and Makoto Kawase¹⁾

¹⁾ National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

²⁾ Toyama Agricultural Research Center, 1124-1 Yoshioka, Toyama 939-8153, Japan

³⁾ National Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

A mini core collection of Japanese rice landrace was developed based on a genome-wide SSR polymorphism survey of 236 accessions. The accessions used in the initial survey were selected based on the historical record and passport data from the collection maintained at the Genebank of the National Institute of Agrobiological Sciences (NIAS). These accessions were analyzed using 32 SSR markers. A total of 247 alleles were detected and the number of alleles per locus ranged from 3 to 15 (mean 7.7). Principal coordinate analysis using SSR data enabled classification of the accessions into three major groups, two Japonica groups and one Indica group. To develop a Japanese landrace mini core collection, the SSR data on the 236 accessions were subjected to cluster analysis and 50 groups were recognized at a similarity index of 0.88. A single accession from each of the 50 groups was selected. These 50 accessions retained 87.5% of the alleles detected in the original 236 accessions, and covered the variation of the initial set of accessions in terms of several agro-morphological traits.

Key Words: *Oryza sativa* L., Japanese landrace, mini core collection, genetic diversity, SSR.

Introduction

Crop landraces are valuable genetic resources, because they are considered to contain genetic diversity and therefore should complement and diversify the gene pool of advanced cultivars (Frankel and Soule 1981). Landraces of rice have extensive variation, but only a few have been introduced into elite lines (Yang 1994, Kobayashi *et al.* 2006, Fukuoka *et al.* 2006). The enhancement of such germplasm conserved in genebanks for further improvement of rice cultivars is important; however, it is difficult to evaluate many accessions at one time. The idea of a core collection was proposed by Frankel (1984) and is a small set of accessions (usually 10% of the original size) chosen to represent the genetic spectrum of the whole collection. Recently, a cultivar set smaller than a core collection has been developed as a “mini core collection” or “micro core collection” to evaluate their variation thoroughly (Upadhyaya and Ortiz 2001), because the size of a core collection is sometimes too large for users to handle. The authors developed a small core collection of world rice collection, the Rice Diversity Research Set of NIAS (Kojima *et al.* 2005). Only two Japanese accessions are included in this set. Japan is located at the edge of rice cultivation, and the genetic diversity of Japanese landraces seems smaller than the world-wide germplasm collection (Kojima *et al.* 2005); however, the local adapted cultivars

are expected to have a different genetic polymorphism to adapt to different environmental conditions. The set of Japanese landraces will be unique and important for understanding rice adaptation in the northern area. One of the objectives of this study was to develop a Japanese landrace mini core collection.

Another problem of using the germplasm is the “redundancy” of accessions. In the Genebank, some accessions with the same variety name have been stored and is difficult to know whether they are redundant or different accessions. Recently, molecular markers have been used for cultivar identification. It is important to identify redundant or different accessions with the same name.

In this study, the genetic variation of Japanese cultivars of landraces was estimated using SSR polymorphism and a representative set of accessions was developed from the Genebank of the National Institute of Agrobiological Sciences (NIAS).

Materials and Methods

Plant materials

Among the ca. 2000 Japanese accessions conserved in NIAS Genebank, 236 accessions, including 154 lowland and 82 upland landrace accessions mentioned in the historical records, were selected (Table 1) to cover the whole of Japan. We selected mainly landraces from Genebank accessions, which included 119 accessions with 12 variety names to clarify their “redundancy”. Accessions called “Akamai” are not included in the redundancy analysis because of their different morphology and passport data. Eighteen improved

Communicated by K. Okuno

Received March 27, 2008. Accepted June 13, 2008.

*Corresponding author (e-mail: ebana@affrc.go.jp)

† These authors contributed equally to this work

Table 1. List of accessions

JP No.	GB Acc. No. ^a	Name	landrace/improved	lowland/upland	Diversity Index	Identity ^b
14908		Aichiasahi	improved	lowland	19.8	
4982		Aikoku	landrace	upland	20.1	
5419		Aikoku	landrace	lowland	15.2	
6365		Aikoku	landrace	lowland	11.6	1
6369		Aikoku	landrace	lowland	12.6	
6730		Aikoku	landrace	lowland	11.6	1
6768		Aikoku	landrace	lowland	12.7	
9310		Aikoku	landrace	lowland	17.7	
9650		Aikoku	landrace	lowland	11.6	1
10993		Aikoku	landrace	lowland	12.2	
147444		Aikoku	landrace	lowland	11.6	1
5301		Akage	landrace	upland	26.7	
6729		Akage	landrace	lowland	15.2	
14994		Akage	landrace	upland	40.3	
34110		Akage	landrace	lowland	25.4	
219839		Akage	landrace	lowland	20.9	
4658		Akage	landrace	lowland	22.3	
4744		Akamai	landrace	upland	24.2	
4903		Akamai	landrace	upland	41.8	
4945		Akamai	landrace	upland	42.4	
7237		Akamai	landrace	lowland	44.0	
80563		Akamai	landrace	lowland	42.5	
9694		Akamai	landrace	lowland	26.3	
4663		Akayakan	landrace	upland	18.9	
6183		Akitakomachi	improved	lowland	14.9	
9707		Araki	landrace	lowland	13.6	
10736		Asahi	landrace	lowland	14.7	
14862		Asahi	landrace	lowland	19.9	
6746		Asahi	landrace	lowland	14.7	
6761		Asahi	landrace	lowland	17.4	
6812	00007503	Asahi	landrace	lowland	13.7	
6812	00007893	Asahi	landrace	lowland	13.8	
6812	00007957	Asahi	landrace	lowland	13.1	
6812	00016511	Asahi	landrace	lowland	14.1	
9328		Asahi	landrace	lowland	15.4	
9855		Asahi	landrace	lowland	15.2	
14996		Benkei	selection	lowland	15.0	
7199		Bouzu	landrace	lowland	17.3	
4950		Bouzu Mochi	landrace	upland	16.4	
6731		Bungo	landrace	lowland	15.3	
57655		Daikoku Wase	improved	lowland	14.5	
4860		Dango	landrace	upland	16.9	
4932	00004880	Dango Mochi	landrace	upland	18.2	
4932	00005449	Dango Mochi	landrace	upland	16.6	
4932	00005655	Dango Mochi	landrace	upland	16.8	
4919		Esojima Mochi	landrace	upland	16.8	
14924		Fukoku	improved	lowland	18.9	
7044		Fukubouzu	improved	lowland	17.1	
4887		Fundechangomi	landrace	upland	17.7	
14943		Gaisenmochi	landrace	upland	16.8	
4445		Gaisenmochi	landrace	upland	17.2	
4705		Gaisenmochi	landrace	upland	21.4	
4751		Gaisenmochi	landrace	upland	16.3	
4779		Gaisenmochi	landrace	upland	16.5	
4914		Gaisenmochi	landrace	upland	17.2	
5164		Gaisenmochi	landrace	upland	17.7	
6356		Ginbouzu	landrace	lowland	15.0	
15142		Gohyakumangoku	improved	lowland	12.7	

Table 1. (continued)

JP No.	GB Acc. No. ^a	Name	landrace/improved	lowland/upland	Diversity Index	Identity ^b
4819		Hakamuri	landrace	upland	19.4	
9663		Hakutou	landrace	lowland	20.2	
4904		Hassokuho	landrace	upland	24.7	
5769		Hattan	landrace	lowland	16.4	
6113		Himenomochi	improved	lowland	18.3	
4916		Hinode	landrace	upland	24.1	
7446		Hinode	landrace	lowland	12.9	
54242		Hinohikari	improved	lowland	13.2	
6363		Hinomaru	improved	lowland	13.9	
4535		Hirayama	landrace	upland	22.0	
88635		Hitomebore	improved	lowland	13.3	
5870		Hiyadachitou	landrace	lowland	18.2	
11289		Hiyokumochi	improved	lowland	15.5	
7638		HomanJinjaMai	landrace	lowland	17.1	
7313		Hosogara	landrace	lowland	15.4	
4763		Irimanishiki	landrace	upland	22.3	
7014		Ishijiro	landrace	lowland	17.8	
4859		Isikawa	landrace	upland	14.8	
9612		Iwai	landrace	lowland	13.1	
5906		Joushuu	landrace	lowland	16.9	
15126		Jukkoku	landrace	lowland	14.4	
9801		Kabashiko	landrace	lowland	17.0	
10643		Kabashiko	landrace	lowland	17.8	
10697		Kabashiko	landrace	lowland	14.2	
10698		Kabashiko	landrace	lowland	11.9	
10699		Kabashiko	landrace	lowland	19.9	
10703		Kabashiko	landrace	lowland	19.6	
10727		Kabashiko	landrace	lowland	43.8	
10741		Kabashiko	landrace	lowland	21.2	
10833		Kabashiko	landrace	lowland	21.6	
10875		Kabashiko	landrace	lowland	22.7	
10896		Kabashiko	landrace	lowland	16.3	
10897		Kabashiko	landrace	lowland	20.3	
10898		Kabashiko	landrace	lowland	11.6	
11211		Kabashiko	landrace	lowland	16.5	
11346		Kabashiko	landrace	lowland	18.2	
11385		Kabashiko	landrace	lowland	13.0	
105744		Kabashiko	landrace	lowland	18.8	
4719		Kahei	landrace	upland	19.1	
81255		Kairyuu 41 No.1-1	landrace	upland	23.6	
4798	00004940	Kameji	landrace	upland	20.6	
4798	00005309	Kameji	landrace	upland	16.9	
7350		Kameji	landrace	lowland	14.7	
7509		Kameji	landrace	lowland	13.8	
9329		Kameji	landrace	lowland	12.5	
9330		Kameji	landrace	lowland	17.1	
9671		Kameji	landrace	lowland	13.9	
9886		Kameji	landrace	lowland	20.2	
9895		Kameji	landrace	lowland	12.9	
9898		Kameji	landrace	lowland	12.4	
9905		Kameji	landrace	lowland	12.9	
14945		Kameji	landrace	lowland	13.8	
11485		Kamenoo	landrace	lowland	16.5	
206131		Kaneko	landrace	upland	21.9	
4403		Kaneko	landrace	upland	20.4	
4410		Kaneko	landrace	upland	31.7	
4754	00005507	Kaneko	landrace	upland	17.6	
4754	00005508	Kaneko	landrace	upland	16.9	

Table 1. (continued)

JP No.	GB Acc. No. ^a	Name	landrace/improved	lowland/upland	Diversity Index	Identity ^b
4817	00004937	Kaneko	landrace	upland	26.0	
4817	00005329	Kaneko	landrace	upland	22.0	
4929		Kaneko	landrace	upland	17.5	
4979		Kaneko	landrace	upland	20.2	
10788		Karahoushi	landrace	lowland	46.8	
4897		Kasanari Bouzu	landrace	upland	19.6	
4726		Ke Mochi	landrace	upland	18.5	
9622		Kinai Omachi 1	landrace	lowland	14.3	
9626		Kinai Omachi 2	landrace	lowland	17.0	
4748		Kirishima	landrace	upland	19.3	
7449		Kiryoyoshi	landrace	lowland	14.7	
6300		Koganemochi	improved	lowland	14.3	
7286		Kokuryoumiyako	landrace	lowland	15.5	
6460		Koshihikari	improved	lowland	13.1	
4791		Kurohige	landrace	upland	15.6	
4895		Kuroka	landrace	upland	22.2	
4482		Kurumi Wase	landrace	upland	21.8	
6735		Mansaku	landrace	lowland	17.8	
7405		Masaoka	landrace	lowland	11.1	
4923		Meguro Mochi	landrace	upland	18.1	
7448		Mikuni	landrace	lowland	13.9	
6876		Mitsui	improved	lowland	14.2	
7450		Miyako	landrace	lowland	13.1	
147541		Miyamanishiki	improved	lowland	11.7	
4828		Miyanishiki	landrace	upland	17.6	
7318		Moritawase	landrace	lowland	16.5	
14931		Mubouaikoku	landrace	lowland	11.4	
4653		Nagae Wase	landrace	upland	21.4	
7227		Nagoyashiro	landrace	lowland	17.8	
222430		Nipponbare	improved	lowland	13.1	
4659		Oiran	landrace	upland	22.2	
4951		Okabo	landrace	upland	16.8	
4783		Okatama Nishiki	landrace	upland	38.6	
4805		Okka Modoshi	landrace	upland	21.2	
4551		Omochi	landrace	upland	20.0	
7529		Omochi	landrace	lowland	13.8	
9872		Omochi	landrace	lowland	14.4	
14946		Omochi	landrace	lowland	17.1	
6319		Oobamochi	landrace	lowland	15.1	
4781		Oohata Wase	landrace	upland	20.2	
6906		Ouba	landrace	lowland	17.6	
4731		Owari Mochi	landrace	upland	17.8	
4833		Raiden	landrace	upland	18.0	
69789		Rikutou Rikuu 2	landrace	upland	17.8	
5416		Rikuu 132	improved	lowland	14.4	
7117		Sakai Kaneko	landrace	lowland	15.0	
15034		Sekitori	landrace	lowland	11.7	
4604	00005019	Sekiyama	landrace	upland	27.0	
4604	00005091	Sekiyama	landrace	upland	27.0	
5447		Sekiyama	landrace	lowland	22.2	
5448		Sekiyama	landrace	lowland	16.5	
5541		Sekiyama	landrace	lowland	22.2	
5542		Sekiyama	landrace	lowland	23.2	
5543	00006131	Sekiyama	landrace	lowland	16.8	
5543	00006133	Sekiyama	landrace	lowland	16.7	
5544		Sekiyama	landrace	lowland	17.4	
5546		Sekiyama	landrace	lowland	16.1	
5753		Sekiyama	landrace	lowland	16.6	

Table 1. (continued)

JP No.	GB Acc. No. ^a	Name	landrace/improved	lowland/upland	Diversity Index	Identity ^b
7041		Sekiyama	landrace	lowland	17.0	
7285		Sekiyama	landrace	lowland	16.5	
7537		Sekiyama	landrace	lowland	12.4	
11540		Sekiyama	landrace	lowland	17.8	
4674		Senbon	landrace	upland	21.7	
4418		Senbonmochi	landrace	upland	22.2	
7278		Senichi	landrace	lowland	15.6	
14334		Sensyou	landrace	upland	18.2	2
4385		Sensyou	landrace	upland	17.5	
4386		Sensyou	landrace	upland	18.6	3
4522		Sensyou	landrace	upland	17.9	4
4704		Sensyou	landrace	upland	17.9	4
4893		Sensyou	landrace	upland	18.6	3
4948		Sensyou	landrace	upland	18.2	2
5108		Sensyou	landrace	upland	18.4	
4958		Seridashi	landrace	upland	18.2	
4938		Shichimenchou Mochi	landrace	upland	23.7	
7494		Shikishima	landrace	lowland	11.8	
4747		Shindai Okoshi	landrace	upland	38.0	
4464	00004879	Shinriki	landrace	upland	23.1	
4464	00005729	Shinriki	landrace	upland	19.5	
6758		Shinriki	landrace	lowland	12.6	
7221		Shinriki	landrace	lowland	15.0	
7539		Shinriki	landrace	lowland	14.0	
9109		Shinriki	landrace	lowland	12.4	
9411		Shinriki	landrace	lowland	14.5	
10754		Shinriki	landrace	lowland	13.6	
11005		Shinriki	landrace	lowland	12.6	
9623		Shinyamadaho 1	landrace	lowland	13.1	
6962		Shinyamadaho 2	landrace	lowland	19.0	
7602		Shiratomamochi	landrace	lowland	16.6	
7104		Shirodama	landrace	lowland	11.4	
7280		Shirozasa	landrace	lowland	13.4	
4888		Shrohige	landrace	upland	19.5	
7334		Sinrikimochi	landrace	lowland	13.1	
7523		Sinrikimochi	landrace	lowland	16.9	
9416		Sinrikimochi	landrace	lowland	15.6	
9465		Sinrikimochi	landrace	lowland	17.9	
10891		Sinrikimochi	landrace	lowland	16.9	
10908		Sinrikimochi	landrace	lowland	17.5	
14937		Sinrikimochi	landrace	lowland	14.8	
34021		Sinrikimochi	landrace	lowland	15.5	
42863		Sinrikimochi	landrace	lowland	15.8	
7369		Sinsyuu	landrace	lowland	18.5	
7497		Sinsyuukaneko	landrace	lowland	16.9	
7293		Soutoku	landrace	lowland	17.9	
7297		Sugaippon	landrace	lowland	17.5	
14947		Takedawase	landrace	lowland	11.9	
7279		Takenari	landrace	lowland	14.3	
4869		Tamasari	landrace	upland	14.9	
10772		Touboshi	landrace	lowland	47.8	
6038		Tougou	landrace	lowland	15.6	
4700		Touzou Mochi	landrace	upland	20.4	
6912		Toyokuni	landrace	lowland	19.2	
4826		Urasan	landrace	upland	17.4	
100383		Wataribune	landrace	lowland	23.7	
6957		Yajouho	landrace	lowland	13.1	
4839		Yamada Bake	landrace	upland	23.3	

Table 1. (continued)

JP No.	GB Acc. No. ^a	Name	landrace/improved	lowland/upland	Diversity Index	Identity ^b
100487		Yamadaho	landrace	lowland	13.9	
6958		Yamadanishiki	improved	lowland	13.2	
7291		Yamatonishiki	landrace	lowland	17.2	
4666		Yorunoyuki	landrace	upland	17.6	

^a GB accession No. is added when JP No. is same as other accession.

^b Same number shows that their SSR profile is same and Diversity index is equal.

varieties were used as a control. Modern varieties from crosses were designated as ‘improved variety’, and varieties selected from landraces were included in ‘landrace’. Information on cultivation type, upland or lowland, followed the passport data of Genebank of NIAS.

DNA extraction and SSR analysis

Total DNA was extracted from young leaves of 10 plants in each accession with the CTAB method (Murray and Thompson 1980). When analyzing the genetic diversity of accessions, DNA is amplified in a final volume of 10 µl containing 10 mM Tris-HCl (pH 8.3) and 50 mM KCl (Perkin Elmer), 2.5 mM MgCl₂ and 2 mM dNTPs, 4 µM primer pairs, 5 ng total DNA and 0.5 unit of AmpliTaq Gold DNA Polymerase (Applied Biosystems). The amplification program was as follows: initial incubation at 95°C for 9 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. PCR products (1.5 µl) were denatured at 95°C for 2 minutes, and electrophoresed on 4.5% polyacrylamide gel containing 7 M urea in 1 × TBE buffer at 2,000 V for 80 min. Gels were stained with Vistra Green (Amersham Pharmacia Biotech) and detected by image scanner machine STORM 860 (Amersham Pharmacia Biotech). From the previous report on SSR analysis of rice (Akagi *et al.* 1997, Chen *et al.* 1997, Tenmykh *et al.* 2000, Tenmykh *et al.* 2001), 32 pairs of primers were selected for analysis (Table 2) to cover the genome with a small number of markers which could detect polymorphism in Japonica accessions.

Evaluation of genetic diversity

To estimate genetic difference among accessions, the diversity index of each accession was calculated as follows.

Diversity index = $-\log_{10} \prod_{i=1}^N p_i$, where p_i is the allele frequency of each SSR allele at locus i in the total accessions. This index shows the standard and outlier combination of alleles in the samples. This index was also calculated for each same variety name group to estimate “redundancy”.

Selection of mini core collection

Each of the SSR alleles was scored for presence (1) or absence (0). A similarity index was calculated using a simple matching coefficient of similarity (Sokal and Michener 1958) with these 1-0 data. Principal coordinate analysis using the coefficient was performed with NTSYS ver. 2.02

(Exeter Software). A dendrogram was generated from a pairwise distance matrix based on the unweighted pair-group method by arithmetic means (UPGMA) (Sneath and Sokal 1973) to reveal genetic relationships among accessions, using the software SPSS version 11.5 (SPSS Inc., USA). A total of 236 accessions, including “redundant” accessions, were used as a representative population. The number of accessions to be included in the collection was decided to retain nearly 90% of the alleles detected in 236 accessions. After constructing a dendrogram of 236 accessions, the accessions were divided according to the branching pattern of the dendrogram at a given coefficient level. One representative with highest diversity index at a branch was selected to retain as many alleles as possible. To decide the number of mini core collections, the percentage of alleles covered by the selected samples was counted and compared with the random sampling simulation. In the simulation, twenty trials were performed with each number of samples.

In order to evaluate the level of phenotypic diversity in the mini core collection, variation in the phenotype for 9 traits, including culm length, panicle length, number of panicles per plant, grain length, grain width, brown rice length, brown rice width, days to heading from seeding, and amylose content, was evaluated. These characters were evaluated in 2003, in Tsukuba, Ibaraki, Japan (N36° 03', E140° 10') under natural field conditions. One-month-old seedlings were transplanted to the paddy field in mid-May and ten plants were used for the evaluation. The mean, standard deviation, and the range of traits in the intensive collection and the original 236 accessions were compared.

Results

In the present study, 236 rice varieties were analyzed using 32 SSR markers. The number of alleles investigated ranged from 3 to 15 (mean 7.7) per locus and the total number of alleles detected was 247 (Table 2). The most variable locus was *RM539* on chromosome 6 with 15 alleles and the next was *RM259* on chromosome 1 with 14 allele. Most of the accessions showed different SSR profiles. Two accessions of Kameji, four accessions of Aikoku, and two accessions of Senshou showed the same SSR profiles (Table 1). The diversity index of each accession in all samples was from 11.1 to 47.8 (mean 18.2). Toboshi showed the highest value and “Akamai” accessions showed a rather high value. Masaoka showed the lowest value and “Aikoku” accessions

generally showed a low value (Table 1).

Redundant accessions

SSR profiles of 119 accessions of 12 variety name

groups were compared (Table 3). In the variety name groups of Sekiyama, Shinriki, Kabashiko and Akage, more than 90% of loci were polymorphic. Sekiyama accessions showed the highest variation (31/32 loci were polymorphic).

Table 2. Number of alleles detected at each SSR marker loci.

Marker Name	Repeat Motif	Chr.	Position (Mb)	Product Size	Number of alleles	Source
RM220	(CT)17	1	4.4	100–130	11	a
RM259	(CT)17	1	7.4	148–176	14	b
RM237	(CT)18	1	29.2	124–138	7	b
RM84	(TCT)10	1	31.5	118–124	4	b
RM236	(CT)18	2	2.1	190–196	4	b
RM208	(CT)17	2	35.2	164–176	4	a
RM207	(CT)25	2	35.4	110–132	10	b
RM213	(CT)17	2	35.5	126–150	6	a
RM571	(GT)11(AG)13	3	34.0	182–192	4	c
RM518	(TC)15	4	2.0	158–180	9	c
RM241	(CT)31	4	27.4	124–142	10	a
RM255	(AGG)5(AG)2-(GA)16	4	31.0	130–154	6	b
RM440	(CTT)22	5	19.8	161–217	13	c
RM480	(AC)30	5	27.5	199–221	12	c
RM217	(CT)20	6	4.2	114–144	11	b
RM539	(TAT)21	6	8.2	249–289	15	c
RM412	(GA)22	6	30.3	176–200	11	c
RM418	(ATT)21	7	18.8	245–290	11	c
RM560	(CT)12	7	20.2	224–240	8	c
RM25	(GA)18	8	4.4	120–124	3	b
RM25	(GA)18	8	4.4	146–164	6	b
RM515	(GA)11	8	20.4	211–219	5	c
RM219	(CT)17	9	8.5	184–204	9	b
RM201	(CT)17	9	20.8	136–150	4	b
RM215	(CT)16	9	22.0	146–160	7	b
RM216	(CT)18	10	5.0	132–154	5	b
RM228	(CA)6(GA)36	10	21.8	100–122	8	b
RM20B	(ATT)14	11	0.9	98–116	8	b
RM536	(CT)16	11	8.9	220–230	6	c
RM202	(CT)30	11	8.9	166–186	7	b
RM224	(AAG)8(AG)13	11	29.5	124–142	6	b
RM270	(GA)13	12	25.2	210–224	3	a

a Temnykh *et al.* 2000, b Chen *et al.* 1997, c Temnykh *et al.* 2001.

Table 3. Genetic variation among same named accessions

Classification	Number of accession	Polymorphic loci (%)	Number of allele per locus	Number of allele per locus per accession	Diversity Index AVE ($\pm$ SD)
Aikoku	10	78	2.1	0.21	5.5 $\pm$ 5.4
Akage	6	91	2.4	0.40	9.4 $\pm$ 3.2
Asahi ^a	10	84	2.0	0.20	5.7 $\pm$ 2.7
Gaisen-mochi	10	41	1.5	0.15	3.2 $\pm$ 2.1
Kabashiko	17	91	3.0	0.18	11.4 $\pm$ 1.5
Kameji	12	88	2.5	0.21	8.8 $\pm$ 4.1
Kaneko ^b	9	81	2.3	0.25	7.5 $\pm$ 4.6
Omachi	4	78	1.8	0.45	6.4 $\pm$ 4.7
Sekiyama	15	97	3.2	0.21	11.6 $\pm$ 5.0
Sensyou	8	16	1.2	0.14	1.2 $\pm$ 0.4
Shinriki	9	94	2.3	0.25	9.0 $\pm$ 4.2
Shinriki-mochi	9	81	2.3	0.26	9.0 $\pm$ 0.7

^a Asahi includes “Kyoto Asahi”.

^b Kaneko includes “Tokyo Kaneko”.

On the other hand, Senshou and Gaisenmochi showed only 16 and 41% polymorphic loci. Among accessions with the same variety name, Sekiyama, Shinriki, Kameji and Aikoku, upland accessions and lowland accessions showed a different diversity index, suggesting two types in each variety group (Fig. 1).

Fig. 1. Intragroup variation in the same name cultivar group. ○ lowland, ● upland accessions.

Genetic differentiation among Japanese rice landrace

PCA analysis of the 236 accessions showed two different types of variety groups in Japanese landraces, Japonica and Indica. The Japonica group was divided into two groups, Group I and II (Fig. 2). This grouping well matched the cultivation method, lowland and upland. Group I was a mixture of upland and lowland rice varieties and group II consisted only of upland rice. The major varieties in Japan are included in Group I. Markers which could signify Indica/Japonica were *RM 207* and *RM 208* on chr. 2, *RM 217* on chr. 6 and *RM 202* on chr.11. Markers which could signify lowland/upland were *RM236*, *RM207* on chr.2, *RM 440* on chr. 5, *RM 412* on chr.6, *RM 418* on chr.7, *RM216* on chr.10, *RM 536* and *RM20B* on chr.11. Improved varieties showed less genetic diversity (Table 4) and all of the alleles detected in the improved varieties were also detected in landraces (data not shown).

Selection of mini core collection

In a comparison of statistic and random sampling, the mini core collection of 50 samples was superior to cover the genetic diversity of Japanese landraces (Fig. 3). Fifty Japanese rice accessions, including 216 alleles of these 32 loci,

Fig. 2. Three groups of Japanese landrace accessions.

Table 4. Comparison of genetic diversity between improved varieties and landraces

Classification	Number of accession	Polymorphic loci (%)	Total number of alleles	Diversity Index
Improved variety ^a	18	87.5	60	14.8
(Leading variety) ^b	(4)	(31.3)	(42)	(13.5)
Landrace	218	100	247	18.4

^a "Improved variety" was defined as the result of crossbreeding.

^b "Leading variety" includes Koshihikari, Hitomebore, Akitakomachi and Hinohikari.

Fig. 3. Percentage of alleles contained in the accession set according to the sample size. ● Statistic sampling, ○ random sampling.

87.5% of the population, were selected as a representative set (Table 5). Comparison of the phenotypic variation in several agro-morphological traits between the collection and the original accessions showed that this collection included 75–100% of the range of variation (Table 6).

Discussion

The objective of the present study was to investigate the genetic diversity of Japanese landrace accessions and to develop a mini core collection. Fifty accessions were selected for the collection (Table 5), based on SSR data, and included 87.5% of the SSR alleles in the 236 accessions.

Among the 32 SSR loci investigated here, some loci showed higher levels of allelic diversity. The presence of highly diverse loci is attributed to the linkage with balanced selective genes (Ishimaru *et al.* 2001) or an allelic barrier (Harushima *et al.* 2002). The most variable locus was *RM539* on chromosome 6 with 15 alleles located at 8.2Mb of chromosome 6. *Hd1*, one of the major genes controlling the heading date is located in this chromosomal region. Japan is at the northern edge of rice cultivation and heading date seems a selective trait in Japan. The next polymorphic marker was *RM259* on chromosome 1 with 14 alleles and *Rf-3*, which is a restorer gene of CMS located in this region (Zhang *et al.* 1997). RFLP analysis of the world rice collection did not detect a relationship among polymorphic markers and genes (Kojima *et al.* 2005). This suggests that the association of markers and genes should be analyzed in genetically similar populations.

As for the Indica/Japonica marker, *Hd7*, which controls the heading date, is located near *RM207* and *RM208* on chr2. *Hd3a*, *RFT1* and *wx* are located near *RM217*. The heading date and waxiness of the grain (Olsen *et al.* 2006) are thought to be artificially selective traits. As QTLs in upland/lowland marker regions, QTL for phosphorous sensitivity (Wissuwa *et al.* 1998) near *RM207*, field resistance for rice blast near *RM418* (Tabien *et al.* 2002), spikelet number, amylose content (Cui *et al.* 2002) and allelopathy (Ebana *et*

Table 5. List of Japanese landrace mini core collection

ID	Variety Name	upland/lowland
JRC 01	Gaisen Mochi	upland
JRC 03	Hinode	upland
JRC 04	Senshou	upland
JRC 05	Yamada Bake	upland
JRC 06	Kaneko	upland
JRC 07	Irima Nishiki	upland
JRC 08	Okka Modoshi	upland
JRC 10	Hirayama	upland
JRC 11	Kahei	upland
JRC 12	Oiran	upland
JRC 13	Bouzu Mochi	upland
JRC 14	Meguro Mochi	upland
JRC 17	Akage	lowland
JRC 18	Hassokuho	upland
JRC 19	Wataribune	lowland
JRC 20	Hosogara	lowland
JRC 21	Akamai	lowland
JRC 22	Mansaku	lowland
JRC 23	Ishijiro	lowland
JRC 24	Joushuu	lowland
JRC 25	Dango	upland
JRC 26	Aikoku	lowland
JRC 27	Ginbouzu	lowland
JRC 28	Shinriki Mochi	lowland
JRC 29	Shichimenchou Mochi	upland
JRC 30	Morita Wase	lowland
JRC 31	Kameji	lowland
JRC 32	Omachi	lowland
JRC 33	Shinriki	lowland
JRC 34	Kyoutoasahi	lowland
JRC 35	Kabashiko	lowland
JRC 36	Sekiyama	lowland
JRC 37	Shinyamadaho 2	lowland
JRC 38	Nagoya Shiro	lowland
JRC 39	Shiroine (Kemomi)	upland
JRC 40	Akamai	upland
JRC 41	Akamai	lowland
JRC 42	Touboshi	lowland
JRC 43	Akamai	upland
JRC 44	Karahoushi	lowland
JRC 45	Hiyadachitou	lowland
JRC 46	Fukoku	lowland
JRC 47	Okabo	upland
JRC 48	Hakamuri (Yokoyama)	upland
JRC 49	Rikutou Rikuu 2	upland
JRC 50	Himenomochi	lowland
JRC 51	Shinshuu	lowland
JRC 52	Aichiasahi	lowland
JRC 53	Raiden	upland
JRC 54	Houmanshinden Ine	lowland

al. 2001) near *RM412*, UV-B resistance (Sato *et al.* 2003) near *RM516*, culm strength under upland conditions (Mu *et al.* 2004) near *RM440*, were reported. These QTLs seem related to the upland and lowland cultivation conditions or differences among the morphological differences of upland and lowland varieties. This result suggested an association

Table 6. Comparison of means and ranges for morphological descriptors between the core collection and the initial accessions

Descriptors	Mean $\pm$ SE ^a	Range	Retention ^b (%)
Culm length	102 $\pm$ 13	68–143	100
	68 $\pm$ 17	68–143	
Panicle length	21.3 $\pm$ 1.9	16.6–28.7	85
	22.2 $\pm$ 2.2	18.4–28.7	
Number of panicles per plant	10.7 $\pm$ 2.7	4.8–19.6	99
	11.5 $\pm$ 3.4	5.0–19.6	
Grain length	6.0 $\pm$ 0.4	5.2–7.0	92
	6.0 $\pm$ 0.4	5.3–7.0	
Grain width	3.4 $\pm$ 0.2	2.8–3.8	95
	3.4 $\pm$ 0.2	2.8–3.8	
Brown rice length	5.3 $\pm$ 0.4	4.6–6.4	97
	5.3 $\pm$ 0.4	4.6–6.4	
Brown rice width	2.9 $\pm$ 0.2	2.4–3.4	75
	2.9 $\pm$ 0.2	2.4–3.1	
Heading from sowing	116 $\pm$ 12	80–135	100
	115 $\pm$ 14	80–135	
Amylose content	17.5 $\pm$ 8.7	0.0–33.9	100
	17.2 $\pm$ 10.1	0.0–33.9	

^a Upper lines and lower lines represent the initial collection and the core collection, respectively.

^b Retention shows the percentage of the range preserved in the core collection.

among SSR markers and selective genes in Japanese rice landraces.

Comparison among accessions with the same variety name shows “redundant” accessions but they generally showed different SSR profiles (Table 1). In the two types of cultivation varieties included in the group, lowland and upland cultivated accessions were critically different (Fig. 1). The present study showed that upland and lowland types are genetically different even if they have the same variety name and should be considered not as redundant accessions but as different accessions. This result supports the importance of passport data and also the usefulness of molecular analysis of genetic diversity.

Genetic differentiation of Japanese rice landraces was estimated from the present result. Accessions were divided into three groups, two Japonica groups (Group I and II) and one Indica (Fig. 2). Group I includes both upland and lowland accessions, whereas Group II includes only upland accessions. Upland varieties in the Group II showed morphological characters such as thick culms, a small number of panicles and broad leaves, which suggested that they are tropical Japonica. The previous study of world-wide accessions with RFLP analysis did not clearly divide temperate and tropical Japonica (Kojima *et al.* 2005). SSR can divide these two variety groups and they are a better tool to identify

relative varieties than RFLP, because SSRs change rapidly (Charlsworth *et al.* 1994).

We selected a mini core collection of Japanese rice landraces (Table 5). This set includes 21 upland and 29 lowland accessions and covers 87.5% of the alleles. It also includes more than 90% of phenotypic variation in the original population except brown rice width (Table 6). This mini core collection of Japanese landraces is distributed by NIAS Genebank (core collections in NIAS Genebank: http://www.gene.affrc.go.jp/plant/core_collections.html; contact address: genebank@nias.affrc.go.jp). The SSR genotype and phenotype data are shown on the website. Accumulated data on the various traits and DNA polymorphism in the collection will enhance the efficient use of rice genetic resources.

Acknowledgements

The authors are grateful to the Genebank of the National Institute of Agrobiological Sciences for providing the germplasm. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genetic and molecular dissection of quantitative traits in rice, QT1001) and the Genebank Project of NIAS.

Literature Cited

- Akagi, H., Y. Yokozeki, A. Inagaki and T. Fujimura (1997) Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related varieties with these microsatellite loci. *Theor. Appl. Genet.* 94: 61–67.
- Chen, X., S. Temnykh, Y. Xu, Y. G. Cho, S. R. McCouch (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 95: 553–567.
- Charlesworth, B., P. Sniegowski and W. Stephean (1994) Evolutionary dynamics of repetitive DNA in Eukaryote. *Science* 371: 215–220.
- Cui, H., B. Peng, Z. Xing, G. Xu, B. Yu and Q. Zhang (2002) Molecular dissection of seedling-vigor and associated physiological traits in rice. *Theor. Appl. Genet.* 105: 745–753.
- Ebana, K., W. Yan, R. H. Dilday, H. Namai and K. Okuno (2001) Analysis of QTL associated with the allelopathic effect of rice using water-soluble extracts. *Breed. Sci.* 51: 47–51.
- Frankel, O. H. (1984) Genetic perspectives of germplasm conservation. In: Arber, W. K., K. Llimensee, W. J. Peacock and P. Stralinger (eds.) *Genetic Manipulation: Impact on Man and Society*, Cambridge University Press, Cambridge, pp. 161–170.
- Frankel, O. H. and M. E. Soule (1981) *Conservation and Evolution*. Cambridge Univ. Press. New York.
- Fukuoka, S., D. S. Tran, K. Ebana, T. N. Luu, T. Nagamine and K. Okuno (2006) Genetic organization of aromatic rice as revealed by RAPD markers: A case study in conserving crop genetic resources on farm. *Euphytica* 149: 61–71.
- Harushima, Y., M. Nakagahra, M. Yano, T. Sasaki and N. Kurata (2002) Diverse variation of reproductive barriers in three intraspecific rice crosses. *Genetics* 160: 313–322.
- Ishimaru, K., M. Yano, N. Aoki, K. Ono, T. Hirose, S. Y. Lin, L. Monna, T. Sasaki and R. Ohsugi (2001) Toward the mapping of physiological and agronomic characters on a rice function map: QTL

- analysis and comparison between QTLs and expressed sequence tags. *Theor. Appl. Genet.* 102: 793–800.
- Mu, P., C.Z.Li, C.P.Li., H.L.Zhang and X.K.Wang (2004) QTL analysis for lodging resistance in rice using a DH population under lowland and upland ecosystems. *Acta genetica Sinica* 31: 717–723.
- Murray, M.G. and W.F.Thompson (1980) Rapid isolation of high molecular weight plant DNA. *Nuc. Acids Res.* 8: 4321–4325.
- Kobayashi, A., K.Ebana, S.Fukuoka and T.Nagamine (2006) Microsatellite markers revealed the genetic diversity of an old Japanese rice landrace ‘Echizen’. *Genet. Res. Crop Evol.* 53: 499–506.
- Kojima, Y., K.Ebana, S.Fukuoka, T.Nagamine and M.Kawase (2005) Development of an RFLP-based rice diversity research set of germplasm. *Breed. Sci.* 55: 431–440.
- Olsen, K., A.Caicedo, N.Polato, A.McClung, S.McCouch and M.Purugganan (2006) Selection under domestication: evidence for a sweep in the rice waxy genomic region. *Genetics* 173: 975–983.
- Sato, T., T.Ueda, Y.Fukuta, T.Kumagai and M.Yano (2003) Mapping of quantitative trait loci associated with ultraviolet-B resistance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 107: 1003–1008.
- Sneath, P.H. and R.M.Sokal (1973) Numerical taxonomy. The principles and practice of numerical classification. Freeman and Co, San Francisco, 359 p.
- Sokal, R.R. and C. D.Michener (1958) A statistical method for evaluating systematic relationships. *Univ. Kansas. Bull.* 38: 1409–1438.
- Tabien, E., Z.Li, H.Paterson, A.Marchetti, W.Stansel and M.Pinson (2002) Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. *Theor. Appl. Genet.* 105: 313–324.
- Temnykh, S., W.Park, N.Ayres, S.Cattinhour, N.Hauch, L.Lipovich, Y.Cho, T.Ishii and S.McCouch (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 100: 697–712.
- Temnykh, S., G.DeClerck, A.Lukashova, L.Lipovich, S.Cartinhour and S.McCouch (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. *Genome Res.* 11: 1441–1452.
- Upadhyaya, H.D. and R.Ortiz (2001) A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theor. Appl. Genet.* 102: 1292–1298.
- Wissuwa, M., M.Yano and N.Ae (1998) Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 97: 777–783.
- Yang, G.P., M.A.SaghaiMaroof, C.G.Xu, Q.Zhang and R.M.Biyashev (1994) Comparative analysis of microsatellite DNA polymorphism in landraces and varieties of rice. *Mol. Gen. Genet.* 245: 187–194.
- Zhang, G., T.S.Bharaj, Y.Lu, S.S.Virmani and N.Huang (1997) Mapping of the Rf-3 nuclear fertility-restoring gene for ‘WA’ cytoplasmic male sterility in rice using RAPD and RFLP markers. *Theor. Appl. Genet.* 94: 27–33.