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ORIGINAL PAPER

Historical changes in population structure during rice breeding programs in the northern limits of rice cultivation

Hiroshi Shinada · Toshio Yamamoto · Eiji Yamamoto · Kiyosumi Hori · Junichi Yonemaru · Shuichi Matsuba · Kenji Fujino

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Abstract
Key message **The rice local population was clearly differentiated into six groups over the 100-year history of rice breeding programs in the northern limit of rice cultivation over the world.**
Abstract Genetic improvements in plant breeding programs in local regions have led to the development of new cultivars with specific agronomic traits under environmental conditions and generated the unique genetic structures of local populations. Understanding historical changes in genome structures and phenotypic characteristics within local populations may be useful for identifying profitable genes and/or genetic resources and the creation of new gene combinations in plant breeding programs. In the present study, historical changes were elucidated in genome structures and phenotypic characteristics during 100-year rice breeding programs in Hokkaido, the northern limit of rice cultivation in the world. We selected 63 rice cultivars to represent the historical diversity of this local population from landraces to the current breeding lines. The results of the phylogenetic analysis demonstrated that these cultivars clearly differentiated into six groups over the history of rice breeding programs. Significant differences among these groups were detected in five of the seven traits, indicating that the differentiation of the Hokkaido rice population into these groups was correlated with these phenotypic changes. These results demonstrated that breeding practices in Hokkaido have created new genetic structures for adaptability to specific environmental conditions and breeding objectives. They also provide a new strategy for rice breeding programs in which such unique genes in local populations in the world can explore the genetic potentials of the local populations.

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H. Shinada
Rice Breeding Group, Kamikawa Agricultural Experiment Station, Local Independent Administrative Agency Hokkaido Research Organization, Minami 1-5, Pippu, Hokkaido 078-0397, Japan

Present Address:
H. Shinada
Beans Breeding Group, Tokachi Agricultural Experiment Station, Local Independent Administrative Agency Hokkaido Research Organization, Shinsei Minami 9-2, Memuro, Hokkaido 082-0071, Japan

T. Yamamoto · E. Yamamoto · K. Hori · J. Yonemaru
Rice Applied Genomics Research Unit, National Institute of Agrobiological Sciences, Kannondai, Tsukuba, Ibaraki 305-8602, Japan

S. Matsuba · K. Fujino (✉)
NARO Hokkaido Agricultural Research Center, National Agricultural Research Organization, Sapporo, Hokkaido 062-8555, Japan
e-mail: kfujino@affrc.go.jp

Introduction

The genetic structure of domesticated species is influenced by the complexity of breeding practices exercised by humans. During the history of plant breeding programs in agriculture, hybridizations between parental cultivars with desirable traits and intensive artificial selection pressures have been essential for the development of new cultivars that are more adaptable, such as high yield, high product quality, and the ability to overcome abiotic and biotic

stresses. Although these selection pressures have restricted genetic variations among local populations (Yamamoto et al. 2010), more desirable genes and gene combinations have accumulated into the newest established cultivars exhibiting desirable phenotypes for important traits. Thus, detailed knowledge of the historical changes in genome compositions and phenotypic characteristics among local populations may be useful for identifying profitable gene and/or genetic resources and the creation of new gene combinations in plant breeding programs (Huang et al. 2012; Yadong et al. 2012).

Rice (*Oryza sativa* L.) is a staple food. Approximately, 50 % of the human population currently depends on rice as their main source of nutrition. Genetic improvements in breeding programs have enabled the stable production of rice over the world. Both the breeding system and domestication history have had a large influence on the differentiation of genetic structures in cultivated rice. Cultivated rice consists of five genetic groups: *indica*, *aus*, *aromatic*, *temperate japonica*, and *tropical japonica* (Garris et al. 2005). Many landraces among these groups constitute the bulk of genetic resources for rice breeding programs (Garris et al. 2005). The phenotypic variations involved in these landraces have been utilized to achieve the different objectives of rice breeding programs in each local region (Yamamoto et al. 2010). Such different breeding selections in local regions have created new genetic structures and generated genetic diversity between local rice populations (Nagasaki et al. 2010; Yamamoto et al. 2010; Yonemaru et al. 2012). However, which cultivar(s) or selective force(s) contributed to the differentiation of genetic groups among local rice populations remains unknown.

Hokkaido is the northern-most region of Japan and one of the northern limits of rice cultivation in the world. The climatic conditions of Hokkaido, such as a naturally long day-length and low temperatures, are considered to be unsuitable for rice cultivation. Rice cultivation began in the late 1800s in Hokkaido. Genetic improvements and the modernization of cultivation systems have enabled the stable production of rice in such marginal regions during the last century.

Intensive selection pressures during the history of rice breeding programs in Hokkaido from the late 1800s have focused on adaptability to specific local environmental conditions. For example, extremely early heading (short growth period) is required to avoid low-temperature stress at the maturing stage in autumn. Extremely early heading is achieved by selecting loss-of-function alleles in genes for photoperiod sensitivity. (Fujino and Sekiguchi 2005a, b, 2008; Nonoue et al. 2008). Tolerance to low temperatures at the fertilization stage has been improved (Shinada et al. 2013). Furthermore, improvements in eating quality have also become one of the most important objectives in not only Japan, but also Hokkaido for last three decades. These specific selection pressures may have generated the unique genetic structures of Hokkaido rice cultivars (Nagasaki et al. 2010; Yamamoto et al. 2010; Yonemaru et al. 2012).

Plant breeding programs have enabled the stable production of food in local regions. However, whether phenotypic selection contributes to shaping the genome structures of local populations during plant breeding programs has yet to be clarified. The aim of this study was to characterize historical changes in genome structures and phenotypic characteristics during 100-year rice breeding programs in Hokkaido. We discuss which cultivar(s) or selective force(s) contributed to the dynamics of genome structures and relationships between the dynamics of genome structures and phenotypic changes. The results of this study may provide a new strategy for rice breeding programs in local regions based on the whole genome structure.

Materials and methods

Plant material

Sixty-three cultivars composed of five landraces and 58 breeding lines were used to identify genetic and phenotypic differentiation among local rice populations from Hokkaido (Table 1). These cultivars were defined as the Hokkaido rice population in this study. In addition, Nipponbare was used as a control. Cultivars in the Hokkaido rice population were established between 1895 and 2008 and their pedigree was shown in Supplemental Fig. 1.

Seven traits were measured: heading date (HD), cold tolerance at the booting stage (CTB), number of spikelets per panicle (NS), number of panicles per plant (NP), 1,000-grain weight (TGW), amylose content (AC), and grain appearance (GA) as an index of grain quality. These cultivars were cultivated in an experimental paddy field at Kamikawa Agricultural Experiment Station, Pippu, Japan 43°51′N latitude in 2012. Sowing and transplanting were performed in late April and late May, respectively. All materials were planted with a 15.0 cm spacing between plants within each row and 30.0 cm spacing between rows. HD was the day when the earliest panicles had appeared in half of the individuals in each line. Three plants were harvested at the maturity stage, and the following four traits were measured: NS, NP, TGW, and GA. The degree of GA was determined by the rate of grain without chalkiness using RGQI 10B (SATAKE, Hiroshima, Japan). The CTB and AC were evaluated in the NARO Hokkaido Agricultural Research Center, Sapporo, Japan, 43°00′N latitude, in 2012. The CTB and AC were evaluated according to the method described by Kuroki et al. (2007) and Ando et al. (2010), respectively. The percentages of seed fertility obtained in the evaluation of CTB and the

Table 1 List of rice cultivars used in this study

Serial number	Variety	Established year	Cross combination/original line	Group
1	Akage	Landrace	Not applicable	I
2	Hokkaiwase	Landrace	Not applicable	I
3	Kuroge	Landrace	Not applicable	I
4	Minakuchiine	Landrace	Not applicable	I
6	Iburiwase	Landrace	Not applicable	I
5	Bouzu	1895	Akage (pure line selection)	I
7	Bouzu No. 6	1919	Bouzu (pure line selection)	II
8	Hashiribouzu	1924	Sakigake/Bouzu	I
9	Fukoku	1935	Nakateaikoku/Bouzu No. 6	IIIa
10	Wasebouzu	1936	Bouzu (pure line selection)	I
11	Wasefukoku	1936	Nakateaikoku/Bouzu No. 6	IIIa
12	Nourin No. 9	1937	Wasechinko/Hashiribouzu	I
13	Nourin No. 11	1937	Iburiwase/Wasebouzu	I
14	Nourin No. 15	1940	Ginbouzu/Hashiribouzu	IV
15	Nourin No. 19	1941	Hashiribouzu/Futafushi	I
16	Tomoenishiki	1941	Nourin No. 1 (pure line selection)	II
17	Oonocyuto	1941	Kinaiwase No. 22/Bouzu No. 6	II
18	Nourin No. 20	1941	Nourin No. l/Iburiwase	II
19	Ishikarishirage	1941	Sekiyama No. 8/Wasefukoku	IIIa
20	Eiko	1941	Tsurukame/Wasefukoku	IIIa
21	Kyowa	1941	Rikuu No. 132/Wasefukoku	IIIa
22	Nourin No. 34	1941	Ishikarishirage/Hokkai No. 84	IIIa
23	Nanei	1951	Tomoenishiki/Nourin No. 20	II
24	Shinei	1951	Tomoenishiki/Nourin No. 20	II
25	Hokkai No. 112	1951	Rikuu No. 132/Nourin No. 15	II
26	Tomoemasan	1951	Tohoku No. 14/Hokkai No. 87	II
27	Toyohikan	1953	Waseaikoku/Nourin No. 15	II
28	Hokuto	1953	Komochi/Kyowa	IIIa
29	Shinsetsu	1954	Kamedawase/Ishikarishirage	IIIa
30	Fukuyuki	1958	Hokkai No. l 12/Nourin No. 34	IV
31	Mimasari	1959	Tomoenishiki/Joiku No. 142//Oonotyuto/Nourin No. 34	IIIa
32	Sasahonami	1961	Fujisaka No. 5/Nourin No. 15	IV
33	Yukara	1962	Kanto No. 53/Eiko	IIIb
34	Shiokari	1963	Megurosakaemochi/Kyowa//Kyowa	IIIa
35	Sorachi	1967	Kuiku No. l2/Mimasari	IIIa
36	Hayayuki	1968	Shinei/Nourin No. 19	II
37	Matsumae	1970	Fukei No. 51/Hokkai No. 183	IIIb
38	Ishikari	1971	Hokkai No. 182/Kuiku No. 4	IV
39	Kitakogane	1973	Hokkai No. l 82/Joiku No. 230	IIIb
40	Kitahikan	1975	Shiokan/Yukara	IIIb
41	Hayakogane	1977	Hokuto/Joiku No. 272	IIIa
42	Tomoyutaka	1977	Hokkai No. 222/Dohoku No. 5	IV
43	Shimahikari	1981	Koshihomare/Sorachi	IIIb
44	Michikogane	1982	Kuiku No. 99/Hokkai No. 230	IV
45	Tomohikari	1983	Hokkai No. 230/Tomoemasan//Kuiku No. 99	IV
46	Kitaake	1983	Eikei7361/Dohoku No. 5	V
47	Yukihikari	1984	Hokkai No. 230/Tomoemasari//Kuiku No. 99	IV
48	Hayamasari	1986	Eikei75169 mutant line/Eikei76251	V

Table 1 continued

Serial number	Variety	Established year	Cross combination/original line	Group
49	Kirara397	1988	Torku No. 214/Dohoku No. 36	V
50	Hakutyoumochi	1989	Joiku mochi No. 381/Onnemochi	IIIb
51	Honoka224	1990	Torku No.214/Kuiku No. 110//Kuiku No. 114	IV
52	Hayakaze	1990	Hokuiku No. 74/Dohoku No. 36	V
53	Aya	1991	Eikei84271/Kitaake	–[a]
54	Kitaibuki	1993	Joiku No. 395/Jouiku No. 397	V
55	Akiho	1996	Joiku No. 394/Kuiku No. 133	V
56	Hoshinoyume	1996	Akitakomachi/Dohoku No. 48//Jouiku No. 397	V
57	Hokkai No. 287	1998	mutant line of Kirara397	V
58	Nanatsuboshi	2001	Hitomebore/Kukei90242A//Kuiku No. 150	V
59	Fukkurinko	2003	Kukei90242B/Joiku No. 418	V
60	Oborozuki	2003	Kuiku No. 150/Hokkai No. 287	V
61	Daichinohoshi	2003	Kuiku No. 151/Joiku No. 418	V
62	Hoshimaru	2005	Joiku No. 428/Kuiku No. 159	V
63	Yumepirika	2008	Satsukei96118/Joiku No. 427	V

[a] Aya belongs to no group

GA on each cultivar were transformed by an arcsine transformation before calculations for statistical analysis. The Tukey–Kramer HSD test was conducted using JMP (SAS Institute, Cary, NC, USA).

Four exotic cultivars, Cody (USA), Nakateaikoku (Honshu, Japan), Kanto No. 53 (Honshu, Japan), and Ginbouzu (Honshu, Japan), were used for genome-wide SNP typing to identify the patterns of pedigree haplotype blocks.

DNA analysis

Total DNA was isolated from young leaves using the CTAB method (Murray and Thompson 1980). Genotyping with SSR markers was performed as described by Fujino et al. (2004). Fragment sizes were determined using polyacrylamide gel (33 cm wide, 42 cm length) electrophoresis for the sequence grade.

A total of 134 SSR markers over the whole genome were used to prescreen the polymorphisms in six cultivars: Akage, Eiko, Yukara, Ishikari, Kitaibuki, and Hoshinoyume. Among them, 78 SSR markers showed clear polymorphisms. These were then used to genotype the Hokkaido rice population. Except for the markers exhibiting hyper multiple variations, the genotypes of 63 SSR markers were used for further analysis (Supplemental Table 1).

We used a 768-plex SNP set to identify the patterns of pedigree haplotype blocks (Nagasaki et al. 2010). SNPs were detected using the Illumina Bead Station 500G system (Illumina, San Diego, CA, USA). All experimental procedures for SNP typing followed the manufacturer's instructions. Haplotype blocks traced by pedigrees to an ancestral cultivar were defined as ancestral pedigree haplotype blocks for every 1 Mb using more than SNP and SSR markers (Additional file 1).

Data analysis

Phylogenetic analysis was performed with the PHYLIP software suite (Felsenstein 1989) using a total of 63 SSR markers. The dendrogram was constructed based on the *d* matrix using the unweighted pair group method with the arithmetic mean (UPGMA) method (Neighbor). Visualization and editing of the final tree were generated using Njplot v2.3. The reliability of inferred tree was tested by bootstrapping 1,000 times. Bootstrap values indicate the percentage of the number of times the partition of the genotypes into the two sets not separated by that branch occurred when the data were resampled 1,000 times.

The genetic structure of the Hokkaido rice population was estimated according to the method described by Nagasaki et al. (2010), using the STRUCTURE program ver. 2.3.2.1 (Pitchard et al. 2000).

The polymorphism information content (PIC) was calculated for each marker according to Nei (1973): $\mathrm{PIC} = 1 - \Sigma h_k^2$, where h_k is the frequency of the *k*th allele.

Results

Definition of genetic groups and population structures in the Hokkaido rice population

Phylogenetic analysis was conducted using the genotype of 63 SSR markers to understand the genetic relationship

of the Hokkaido rice population. An UPGMA dendrogram using Nei's distance showed that the Hokkaido rice population was clearly classified into six groups, except for Aya, which is the progeny of Nipponbare (Fig. 1). We then calculated the likelihood values for the inferred number of genetic clusters (K) with the STRUCTURE program to investigate the model of genetic structures in the Hokkaido rice population. No decrease in λn (k) implied that positive structures existed in the Hokkaido rice population (Supplemental Fig. 2). Regarding $K = 6$, the six populations corresponded well to the six groups obtained by phylogenetic analysis (Fig. 1).

This classification was clearly correlated with the history of rice breeding programs in Hokkaido (Table 1). Furthermore, the pedigree relationship of cultivars in each group showed that almost all cultivars in each group were the pedigree of key cultivars, except for group I (Supplemental Fig. 1). Group I consisted of all five landraces and six of their pedigrees established before 1941. The genetic distance suggested that group I was clearly distinct from the five other groups. The eight cultivars in group II were the pedigree of group I, Iburiwase and Bouzu, established between 1919 and 1953. Group III consisted of 19 cultivars. This group was divided into two sub-groups. Thirteen cultivars in group IIIa were mostly the pedigree of Wasefukoku established between 1935 and 1977. Group IIIb consisted of six cultivars established between 1962 and 1989. Four in this group were Yukara and its pedigrees. Eleven cultivars in group IV were mainly Nourin No. 15 and its pedigrees established between 1940 and 1990. Group V consisted of 13 cultivars established after 1983. These 13 cultivars were Kitaake and its pedigrees. These results suggested that Wasefukoku (group IIIa), Yukara (group IIIb), Nourin No. 15 (group IV), and Kitaake (group V) were causes of the differentiation of genetic groups among the Hokkaido rice population.

Admixtures of genetic structures between each group were frequently observed in all groups (Fig. 1), showing genetic and pedigree relationships among each group, from groups I to V. In group I, Iburiwase showed the admixture type of group I with II. This cultivar was found to be the progenitor of four cultivars in group II. In group II, Hayayuki and Bouzu No. 6, which are the pedigrees of cultivars included in group I, showed admixture between groups I and II. In group IIIa, five cultivars showed the admixture of group IIIa with II or IIIb. In group IIIb, four cultivars showed the admixture of group IIIb with IIIa and/or IV. Seven cultivars in group IV showed admixture between groups IV and IIIb. Three of them also showed admixture between groups IV and IIIa. Group V showed the admixture of group V with groups IIIb and IV.

Changes in genetic diversity among the Hokkaido rice population

Genetic diversity was represented as the allele number and PIC changed over the course of the rice breeding programs in Hokkaido. The overall average allele number was 4.46 per locus with a range of 2–9 and the PIC varied between 0.061 and 0.803, with an average value of 0.55 (Table 2).

Genetic diversity showed increases and decreases during rice breeding programs. The highest PIC and allele number of 2.98 and 0.42, respectively, were detected in group I. The allele number and PIC were higher at groups IIIa and IV than at groups II and IIIb, respectively.

Changes in traits among the Hokkaido rice population

Seven traits were compared between groups to understand the changes in traits during the history of rice breeding programs (Fig. 2; Supplemental Table 2). The significant differences between groups were identified by the Tukey–Kramer HSD test (Fig. 2; Supplemental Table 3). Significant changes were observed in five traits: CTB, NP, NS, AC, and GA, to the desirable phenotype for the objectives of rice breeding programs in Hokkaido.

A wide variation was observed in CTB levels among the Hokkaido rice population, with the average of 32.7 % ranging from 1.9 to 74.4 % (Fig. 2; Supplemental Table 2). The CTF level of 54.7 % in group V was the highest among the six groups and was significantly higher than those of groups I (23.4 %), II (20.8 %), IIIa (27.3 %), and IV (32.2 %). Furthermore, six cultivars in group V showed higher CTB levels, over 60 %. No cultivar showed this CTB level in other groups.

The NS varied between 43.5 and 126.5, with an average of 68.8 (Fig. 2; Supplemental Table 2). The NS of 53.2 in group V was the lowest among the six groups and was significantly lower than those of groups I (79.3), II (73.9), IIIa (73.4), and IV (69.0). In addition, variations in the NS in groups decreased.

The NP varied between 14.7 and 35.7, with an average of 25.3 (Fig. 2; Supplemental Table 2). The NP of 18.8 in group I was significantly lower than those of the other groups, 24.7–29.2.

The AC varied between 14.3 and 23.1 %, with an average of 20.4 %, except for the sticky rice, Hakuchomochi (Fig. 2; Supplemental Table 2). The AC of 18.5 % in group V was significantly lower than those of the other groups, 21.0–22.2 % (Fig. 2). The AC of three cultivars in group V, Hokkai No. 287, Yumepirika, and Oborozuki, was markedly lower, 14.9, 16.2, and 14.3 %, respectively. These three cultivars have the *Wx1-1* allele to decrease AC (Ando et al. 2010). Furthermore, the AC of 19.5 % in group V, except for these three cultivars,

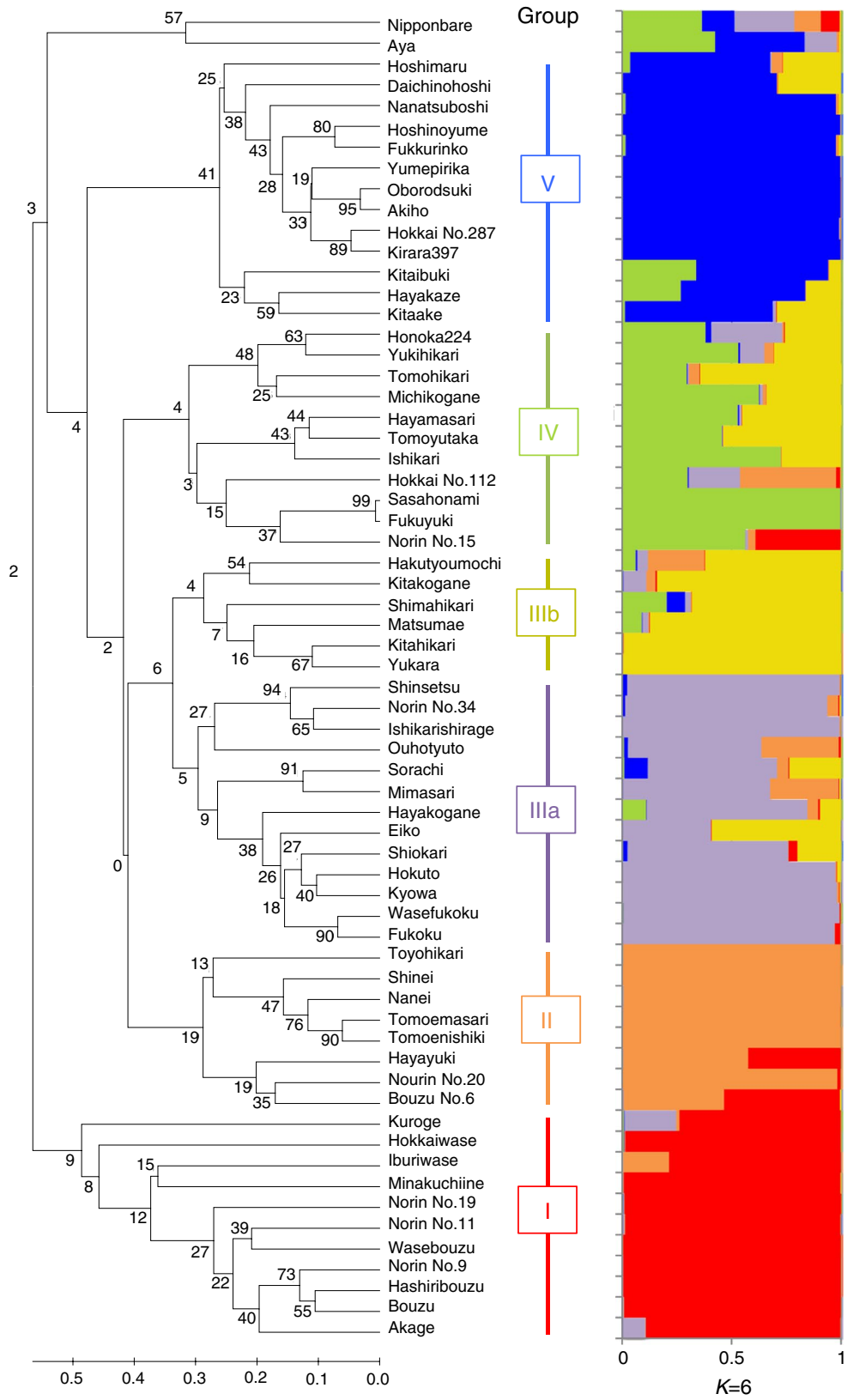

Fig. 1 Classification of the Hokkaido rice population by the UPGMA dendrogram and population structures ($K = 6$) using 63 SSR polymorphisms. The numbers in dendrogram indicate bootstrap values. The *color bars* showing the results of population structures indicate the different groupings into which cultivars were categorized

Table 2 Summary of polymorphism among the population

	No. of varieties	No. of alleles			PIC		
		Ave.	Min.	Max.	Ave.	Min.	Max.
All	63	4.46	2	9	0.55	0.061	0.803
Group I	11	2.98	1	7	0.42	0	0.843
Group II	8	2.06	1	5	0.33	0	0.719
Group IIIa	13	2.51	1	6	0.36	0	0.769
Group IIIb	6	2.02	1	4	0.34	0	0.722
Group IV	11	2.30	1	4	0.38	0	0.727
Group V	13	2.19	1	5	0.31	0	0.698

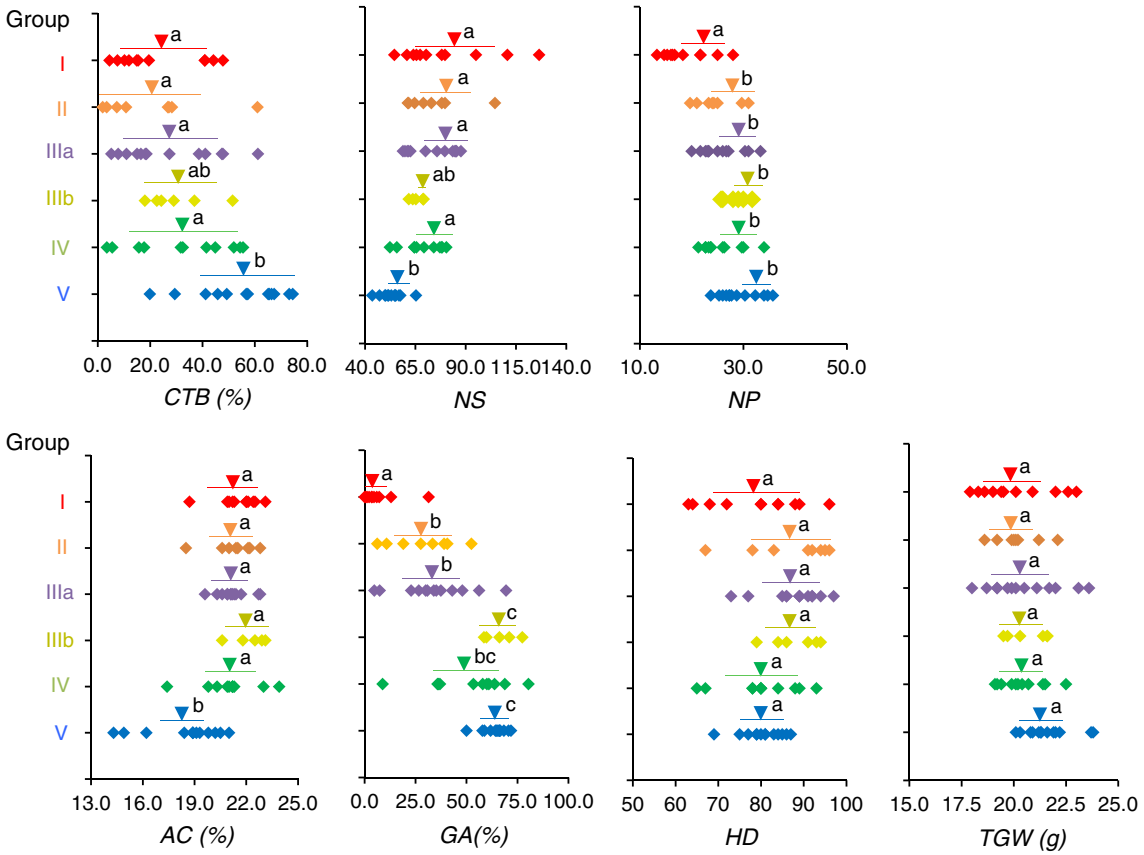

Fig. 2 Characteristics of the seven traits of each group in the Hokkaido rice population. *Arrowheads* indicate the mean value of each group. *Bars* indicate the standard deviation of each group. *Different letters* indicate significant differences at 5 % using the Tukey–Kramer HSD test. *HD* heading date, *CTB* cold tolerance at the booting stage, *NP* number of panicles, *NS* number of seeds per panicle, *TGW* 1,000 grain weight, *AC* amylose content, *GA* grain appearance

was significantly lower than those of groups I (21.5 %), II (21.3 %), IIIa (21.2 %), and IIIb (22.2 %) (Supplemental Table 3).

Large variations were observed in the GA, with the average of 40.5 % ranging from 0 to 80.4 %. The GA showed a continuous increase (Fig. 2; Supplemental Table 2). The GA of 6.7 % in group I was significantly lower than those of the other groups, while 66.6 and 64.0 % in groups IIIb and V were significantly higher than those in groups II and IIIa, 28.6 and 34.2 %, respectively. A wide variation in the GA was observed in group IV, ranging from 8.8 to 80.4 %. The GA of Nourin No. 15 was very low at 8.8 % (Supplemental Table 2).

No significant difference was observed in HD and TGW among the Hokkaido rice population, with the average of 83.2 ranging between 63 and 97 and the average of 20.6 g ranging between 17.9 and 21.6 g, respectively (Fig. 2; Supplemental Table 2).

Haplotype patterns of cultivars differentiating genetic groups among the Hokkaido rice population

Phylogenetic analysis and pedigree relationships suggested that the four cultivars, Wasefukoku (group IIIa), Yukara (group IIIb), Nourin No. 15 (group IV), and Kitaake (group V), caused genetic differentiation among the Hokkaido rice population. According to the pedigree information, Wasefukoku, Yukara, Nourin No. 15, and Kitaake were derived from crosses using germplasms grown outside of Hokkaido (exotic germplasms). Using the SNP typing array over the genome, the chromosomal regions introgressed from exotic germplasms were determined (Fig. 3). In all cases, a number of genomic regions from exotic germplasms were introduced into the Hokkaido rice population. Approximately 52 % of the whole Wasefukoku genome was introduced from Nakateaikoku at 21 genomic regions. Approximately, 35 % of the whole Yukara genome was introduced from Kanto No. 53 at 21 genomic regions. Approximately, 53 % of the whole Nourin No. 15 genome was introduced from Ginbouzu at 20 genomic regions. In contrast, only approximately 7 % of the whole Kitaake genome was introduced from Cody at 10 genomic regions.

Discussion

Genetic improvements in rice breeding programs have enabled the stable production of rice over the world. New genetic structures had been created during the diversification of cultivated rice, leading to adaptability to local specific environmental conditions. This study elucidated historical changes in genome structures and phenotypic characteristics during 100-year rice breeding programs in Hokkaido. These results may be useful for identifying profitable gene and/or genetic resources and the creation of new gene combinations in rice breeding programs.

Previous studies on genetic diversity and population structures were conducted using a wide sample of cultivated rice selected from various locations around the world, and demonstrated that a clear genetic structure originated from domestication events (Garris et al. 2005; Glaszmann 1987). Other studies focused on populations specific to a country. Groups found in populations from Argentina (Giarrocco et al. 2007), Thailand (Chakhonkaen et al. 2012), and USA (Lu et al. 2005) were correlated with genetic structures originated from domestication events. In contrast, groups found in populations from Europe (Courtois et al. 2012), Italy (Spada et al. 2004), and Japan (Yamamoto et al. 2010;

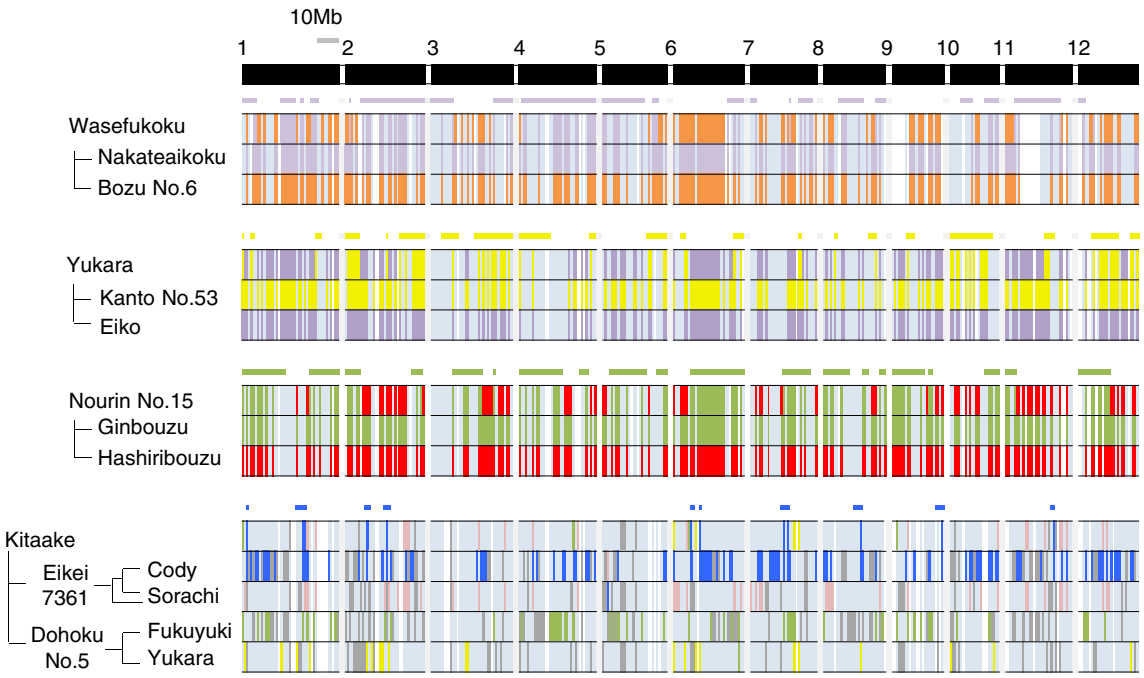

Fig. 3 Patterns of the pedigree haplotype blocks of Kitaake, Yukara, Wasefukoku, Nourin No. 15, and their related cultivars. Haplotype blocks longer than 1 Mb are shown. The *black bars* at the top indicate the range of the blocks in the 12 rice chromosomes. The same haplotype regions are indicated by the same color. *Blue horizontal lines* indicate the genomic region from Cody in the Kitaake genome. *Yellow horizontal lines* indicate the genomic region from Kanto No. 53 in the Yukara genome. *Purple horizontal lines* indicate the genomic region from Nakateaikoku in the Wasefukoku genome. *Green horizontal lines* indicate the genomic region from Ginbouzu in the Nourin No. 15 genome

Yonemaru et al. 2012) were correlated with the history and objectives of the breeding programs.

These studies showed the genetic diversity in each population and their genetic relationships between groups. However, the cause of the genetic differentiations in these populations remains unclear. In the present study, we used a population from a single local region with unique adaptability to specific environmental conditions. In addition, the pedigree of cultivars among the population was useful for elucidating the genetic relationships between each cultivar and group. These features have advantages for the identification of genetic cues for the genetic differentiation of populations, which may enhance genetic forces that shape local populations in plant breeding programs.

Phylogenetic analysis revealed that the Hokkaido rice population was clearly differentiated into six groups over the history of rice breeding programs in Hokkaido. Prominent admixture was shown to occur between groups (Fig. 1). Such admixtures between groups revealed that these crossings have been used for cultivar development to generate new groups. These results proposed a model of genetic differentiation in the Hokkaido rice population (Fig. 4). Landraces in group I were firstly cultivated in Hokkaido in the late 1800s. Group II was then generated by hybridization between landrace varieties or between landrace varieties and exotic germplasms as a breeding system, groups IIIa, IIIb, IV, and V were then generated using exotic germplasms. Phenotypic characteristics and genetic diversity were changed in this process.

Significant differences were observed in five traits between these groups (Fig. 2), which indicated that the differentiation of these groups in the Hokkaido rice population correlated with these phenotypic changes in the history of rice breeding programs. A lower AC and higher GA are preferred to achieve a good eating quality. An increase in the NP and decrease in the NS constitute the desirable plant type to increase yield under the environmental conditions in Hokkaido. A high CTB level is necessary for stable rice production. Genes introgressed from exotic germplasms or selections of favorable genes among the primary groups may have caused these phenotypic changes. Comparisons of haplotype patterns around the genes responsible for these traits may clarify the origins of the breeding force during rice breeding programs.

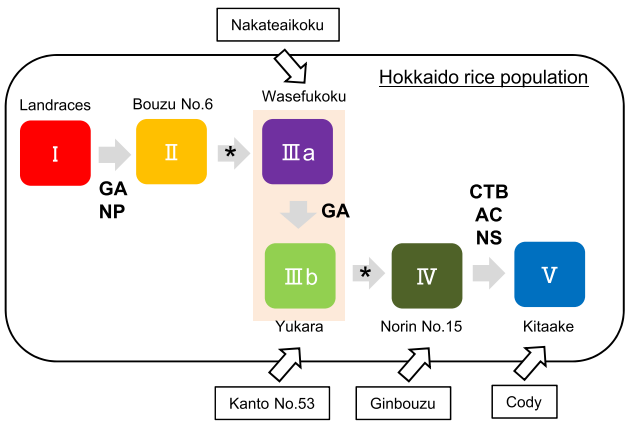

Fig. 4 Model of genetic differentiation among the Hokkaido rice population. *Asterisks* show the increase in genetic diversity. Traits with significant differences between groups were indicated. *Open arrows* indicate introgression from exotic germplasms (*box*)

In contrast, the selection of novel mutations generated in the field during rice breeding programs contributed to a desirable phenotype in breeding programs. The loss-of-function allele in *Hd5* was selected as a spontaneous mutation in various Bouzu (Fujino et al. 2013). This allele causes an early heading date in cultivars from Hokkaido and contributed to the expansion of rice cultivation to the north at that time (Nonoue et al. 2008; Fujino et al. 2013). *qLTG3-1*, which controls low temperature germinability, and *Hd16* controlling heading date were selected as spontaneous mutations during rice breeding programs (Fujino and Sekiguchi 2011; Hori et al. 2010, 2013). A marked change was also observed in the AC in this study, which was caused by *Wx1-1* derived from a somaclonal mutation (Ando et al. 2010). The AC is a major determinant of eating quality. This mutation and phenotype are desirable in current rice breeding programs.

Agronomic traits including yield, product quality, disease resistance, and stress tolerance are the results of interactions between QTLs or between QTLs and the particular genetic or environmental background (Wade et al. 2001). Local populations are useful for identifying genes under specific genetic backgrounds and environmental conditions. A relatively wide phenotypic variation and large allelic differences have been identified in Japanese cultivars in spite of their extremely low genetic diversity (Fujino and Sekiguchi 2011; Hori et al. 2010; Shinada et al. 2013; Takeuchi et al. 2008). These phenotypic variations may be derived from the introduction of exotic germplasms, selection of spontaneous mutations in their population, or continuous selection of pre-existing genes in the local population. These genes have the potential to exhibit a novel phenotype under different genetic backgrounds and environmental conditions. Such unique genes in local populations around the world can explore the genetic potential of the local populations.

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Conflict of interest The authors declare that they have no conflict of interest.

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