GMR
The Original by FUNPEC-RP

Whole genome sequencing and trait investigation revealed a semidwarf isogenic rice variety with a genome and grain quality similar to the high-demand cultivar Koshihikari

M. Tomita

Research Institute of Green Science and Technology, Shizuoka University, Shizuoka, Japan

Corresponding author: M. Tomita
E-mail: tomita.motonori@shizuoka.ac.jp

Genet. Mol. Res. 23 (1): gmr19224
Received November 07, 2023
Accepted January 18, 2024
Published February 28, 2024
DOI http://dx.doi.org/10.4238/gmr19224

Abstract. The leading japanese rice cultivar, Koshihikari, is in high demand globally due to its superior food quality. However, Koshihikari has had a reduction in yield and a loss of quality due to lodging and immature chalky grains, which has been linked to global warming and the changing climate. The semidwarf gene *sd1* encoding a defective C20 oxidase in the gibberellin (GA) biosynthesis pathway (GA 20 oxidase, *OsGA20ox2*) was introduced into the Koshihikari genome through 14 backcrosses and the genome was surveyed with a next-generation sequencer. Trait investigation was conducted in six locations in japan for plant height, panicle numbers, yield, grain weight, quality, sensory taste, etc. The introgression of *sd1* resulted in a semidwarf phenotype designated as Hikarishinseiki, which is 21.4 cm shorter than Koshihikari. Hikarishinseiki has the same genome as Koshihikari, except for the *sd1* region. The taste and quality of Hikarishinseiki were equivalent to those of Koshihikari. Hikarishinseiki is registered under USDA Plant Variety Protection No. 201000072, as the first semidwarf isogenic Koshihikari using Jukkoku-derived *sd1* in areas other than Japan.

Key words: Rice; Semidwarf; Defective GA 20-oxidase gene; Hikarishinseiki

INTRODUCTION

The leading Japanese cultivar Koshihikari occupies 33% of the rice cultivation area in Japan. The demand for Koshihikari is also increasing in the US and other countries due to its good food quality as well as the growing popularity of sushi and sake (Nishiwaki et al., 2018). However, Koshihikari suffers considerable lodging damage as a result of strong hurricanes, typhoons, or heavy rainstorms, which have been increased by recent climate crises due to the global warming. Koshihikari has shown a reduction in yield and a loss of quality due to lodging and immature chalky grains. Control of plant height is one of the most important breeding subjects (Zeng et al., 2019). Thus, there is an urgent need for the development of lodging-resistant Koshihikari. Semidwarfing prevents plants from lodging at their fully ripe stage, makes them lodging-resistant, enhances their adaptability to heavy manuring, and improved the productivity of rice and wheat from 1960-1990, doubling rice yields and quadrupling wheat yields (Khush, 1999). The International Rice Research Institute (IRRI) developed a semidwarf rice variety IR8 in 1966 from Taiwanese native semidwarf variety Dee-geo-woo-gen (DGWG). IR8, named Miracle Rice, has been improved with lodging resistance and light reception attributes (Hergrove et al., 2016). The widespread adoption of miracle rice brought about a global “green revolution” (Hedden, 2003a; b; Gaur et al., 2020, Peng et al., 2021), particularly in the monsoon regions of Asia, where typhoons are common during the harvest season (Athwal, 1971; Khush, 1999). The dwarf feature of IR8 is conferred by the semidwarf gene *sd1* (first designated as *d47*, Kamijima et al., 1997) on the long arm of chromosome 1 (Cho et al., 1994a; b; Maeda et al., 1997) derived from DGWG. *sd1* encodes a defective C20-oxidase in the gibberellin (GA) biosynthesis pathway (GA 20-oxidase, *OsGA20ox2*) (Monna et al., 2002; Sasaki et al., 2002), in which 383 bp fragment between exon1 and exon2 was lost, and deletion probably resulted in a highly truncated inactive enzyme. On the contrary, to solve this problem, the author applied the different *sd1* allele, derived from the Japanese landrace ‘Jukkoku’ (Okada et al., 1967), to change Koshihikari into a semidwarf phenotype. The author developed a Koshihikari-type semidwarf cultivar designated as ‘Hikarishinseiki’ by eight backcrosses with Koshihikari (Tomita, 2009).

In this study, the author continued additional six backcrosses with Koshihikari. Furthermore, we used the high-throughput, long-read next-generation sequencer to prove the genomic identity of upgraded Hikarishinseiki with Koshihikari, except for the *sd1* region. As a result, Hikarishinseiki via the 14th backcross shows stronger lodging resistance than Koshihikari and has 99.9% or more of the Koshihikari genome. In addition, the investigation of traits showed no detrimental effects of *sd1* on grain characters.

MATERIAL AND METHODS

Development of Koshihikari *sd1*

First, crossing 'Jukkoku' (a cultivar with a semidwarf gene, *sd1* homozygous) with 'Kanto No. 79' (*Sd1* homozygous), leads to an early heading mutant induced by Koshihikari γ-irradiated (Tomita, 2009). Then the dwarf *sd1* homozygous line (Jukkoku-type Koshihikari), whose flowering date was the same as Koshihikari, was selected and fixed in the F_4 of Kanto 79 x Jukkoku. The backcross was performed 14 times between the *Sd1sd1* descendants of the Jukkoku-type Koshihikari short stem line in $BCnF_1$ with 'Koshihikari' (*Sd1Sd1*) as the maternal recurrent parent. Crossing Koshihikari with Jukkoku containing *sd1* leads to changes into a short culm. The offspring carrying the target *sd1* were selected in the $BCnF_1$ generation and were repeated 14 times backcrossed with Koshihikari.

Next-Generation Sequencing analysis

Whole genome sequencing was performed on both Koshihikari and Hikarishinseiki (Koshihikari sd1, $BC_{14}F_3$), which were integrated with the semidwarfing gene *sd1*, by 14 backcrosses with Koshihikari. The leaves were frozen in liquid nitrogen and powdered using a mortar and pestle. DNA was then extracted from each cultivar by the CTAB method. The DNA was fragmented and tagged so that the peak size of the fragments was approximately 500 bp using the Nextera® transposome (Illumina Inc., San Diego, CA). After purification of the transposome with DNA Clean & Concentrator™-5 (Zymo Research, Irvine, CA), adaptor sequences, including the sequence primers for fixation on the flow cell, were synthesized at both ends of each fragment using five cycles of PCR, comprising denaturation at 98°C for 10 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, and then the DNA fragments were subjected to size selection using AMPure XP magnetic beads (Beckman Coulter, Brea, CA). Finally, qualitative checks were performed using Fragment Analyzer™ (Advanced Analytical Technologies, Heidelberg, Germany), and quantitative measurements were performed using the Qubit® 2.0 Fluorometer (Life Technologies; Thermo Fisher Scientific, Inc., Waltham, MA) to prepare a DNA library for NGS. Sequencing was conducted in paired end 2 x 100 bp on a HiSeq 2500 next generation sequencer, according to the manufacturer's protocol (Illumina Inc., San Diego, CA). Illumina reads obtained were trimmed using Trimmomatic (version 0.39) (Bolger et al., 2014). Sequencing adapters and sequences with low quality scores at 3' ends (Phred score [Q]＜20) were cut. The raw Illumina WGS reads were quality checked by Quality Control with FastQC (version 0.11.9; Babraham Institute). The mapping of Koshihikari reads to the genome reference was performed with Burrows-Wheeler Aligner (BWA) software with the Nipponbare genome as a reference (version bwa-0.7.17.tar.bz2) (Li and Durbin, 2009). Duplicate reads were removed using Picard (version 2.25.5) (http://broadinstitute.github.io/picard) and secondary aligned reads removed by SAMtools (version 1.10.2) (Li et al., 2009), to construct the consensus sequence of the Koshihikari genome. The Hikarishinseiki reads were then mapped to the Koshihikari genome, and SNPs and Indels were detected using the SamTools software. To identify genetic variations among strains, single nucleotide variant (SNV) detection (variant

calling) and SNV matrix generation were performed using GATK< version 4.1.7.0 (McKenna et al., 2010).

Performance Test

Performance tests were carried out in five locations in Japan. Hikarishinseiki and Koshihikari were seeded in late April and seedlings were transplanted into a paddy field (120 m^2: 6.0 × 20.0 m) in late May with a density of 22.2 seedlings/m^2 (one seedling per 30 × 15 cm) prepared for each genetic line (250 plants for two replications). The paddy field was fertilized with 4.0 kg of basice fertilizer containing nitrogen, phosphorus and potassium (weight ratio, nitrogen: phosphorus: potassium = 2.6:3.2:2.6) at a rate with 4.3 g/m^2 nitrogen, 5.3 g/m^2 phosphorus, and 4.3 g/m^2 potassium evenly across the field. The heading date was recorded as the date on which 50% of all panicles had emerged from the flag leaf sheath. After ripening, 10 typical plants of each genotype were sampled twice. Culm length was measured as the length between the surface of the ground and the base of the panicle at the time of sampling. The sampled plants were air dried and measured for the following traits, panicle length, number of panicles, number of florets/panicles, proportion of fertile florets, and total panicle number: the number of panicles was counted by the sampled plants. Total number of florets: florets were counted to obtain the total number of florets. Floret number/panicles: the total number of florets (including both sterile and fertile florets) was divided by the total number of panicles. The weight of non-milled rice/1,000 grains, the eating taste, and the grain quality were measured using the bulk of 50 plants. The sensory taste test was conducted by evaluation of 20 panelists into 11 degrees; 5: excellent good to -5: especially bad against Koshihikari = 0. The grain quality was classified into nine grades; 1: excellent good to 9: especially bad low quality, by the panelists. The yield of unpolished rice was calculated using the following equation. The yield of not milled rice (g/m^2) = (number of panicles/m^2) × (number of florets/panicle) × (proportion of fertile florets) × (weight of not milled rice/grain). All statistical analyzes were performed with SPSS version 20.0 (IBM Japan, Tokyo, Japan).

RESULTS AND DISCUSSION

Genomic isogeneity of semidwarf rice Hikarishinseiki

Except for the target *sd1* gene region, the genome returned to Koshihikari every time the backcross was performed. The semidwarf phenotype (*sd1sd1*) was obtained in the $BC_{14}F_2$ generation, with the genome being ≥99.9% of the 'Koshihikari' background. The semidwarf Koshihikari (Koshihikari sd1) was designated as Hikarishinseiki.

Using the next-generation sequencer, we obtained a total read number of 66,093,171 with an average length of 124 bp and a total read number of 1,267,339,019 with an average length of 125 bp in Hikarishinseiki. By mapping the 99.91% reads of Koshihikari using the Nipponbare genome sequence as the reference, we obtained the consensus sequence of Koshihikari with a total length of 374,308,257 bp, with a mean

coverage of 35.68. Then the 99.88% reads of Hikarishinseiki were assigned to the consensus sequence of Koshihikari as reference. The mean coverage was 22.42. Furthermore, we prepared vcf files of entire genomes and compared the whole genome sequences of Hikarishinseiki by SNP calling with the consensus sequence of the Koshihikari genome. Figure 1 shows the frequency distribution of single nucleotide polymorphisms compared to Koshihikari per 0.1 Mb nucleotide sequence in the entire Hikarishinseiki genome. Except for the region around 38 Mb from the 5' end of chromosome 1 (red), the number of SNPs was less than 10 per 0.1 Mb. The results indicated that a large portion of the 12 rice chromosomes were substituted into the Koshihikari genome (Figure 1) after 14 consecutive backcrosses targeting the semidwarfing gene *sd1*. Jukkoku-derived SNPs together with *sd1* were found to be introgressed in the vicinity region around 38 Mb in the long arm of chromosome 1 (Figure 1). A single SNP from G to T was detected at 38,267,510 bp among the *Sd1/sd1* locus (Os01t0883800-01: 38,267,149-38,270,233 bp) at the distal end of the short arm of chromosome 1 in the Hikarishinseiki genome (Figure 2). The difference observed between the Koshihikari_*Sd1*/Jukkoku_*sd1* alleles was only one SNP from G to T in exon 1 of the GA20-oxidase gene, as reported by Sasaki et al. (2002). The range of SNP clusters derived from Jukkoku-derived SNP clusters accompanied by *sd1* ranged from 36,977,366 bp to 38,863,193 bp on chromosome 1, that is, the width was 1,685,857 bp, corresponding to 0.43% of the rice genome (Figure 2). Information on SNPs contributes to the genetic diagnosis for molecular breeding of rice (Ye at al., 2020). The other genomic sequences were almost replaced by the Koshihikari genome. Therefore, the Hikarishinseiki genome was almost homogeneous to Koshihikari except for the *sd1* region around 38 Mb of chromosome 1.

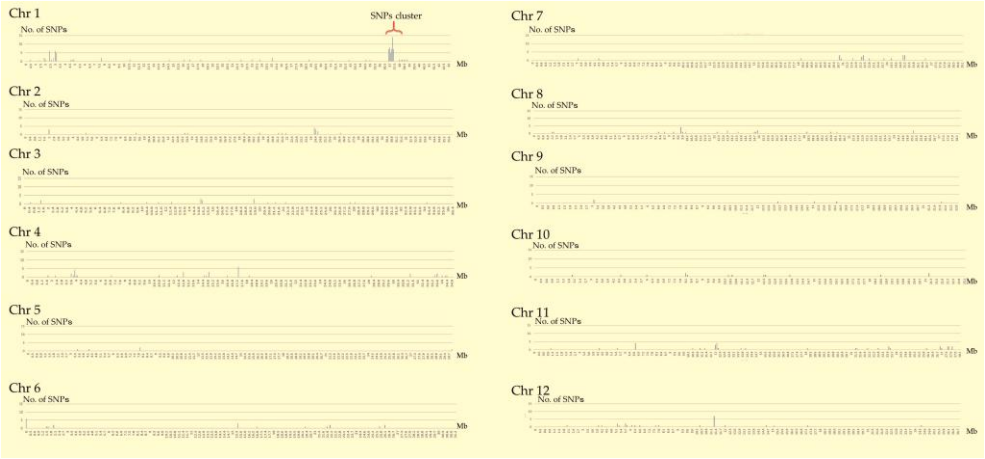

Figure 1. Frequency distribution of SNPs per 0.1 Mb detected by whole genome sequencing of Hikarishinseiki (Koshihikari sd1, $B_{14}F_3$) based on SNV calling against the reference genome of Koshihikari. There were at most less than 5 SNPs per 0.1 Mb over all genome, except for a SNP-cluster around the 37-38.5 Mb region of chromosome 1 (red), which contained 5 to 15 SNPs per 0.1 Mb. This SNP-concentrated region derived from Jukkoku. A large portion of the 12 rice chromosomes were substituted with the genome sequence of Koshihikari after 14 continuous backcrosses targeting the semidwarfing gene *sd1*.

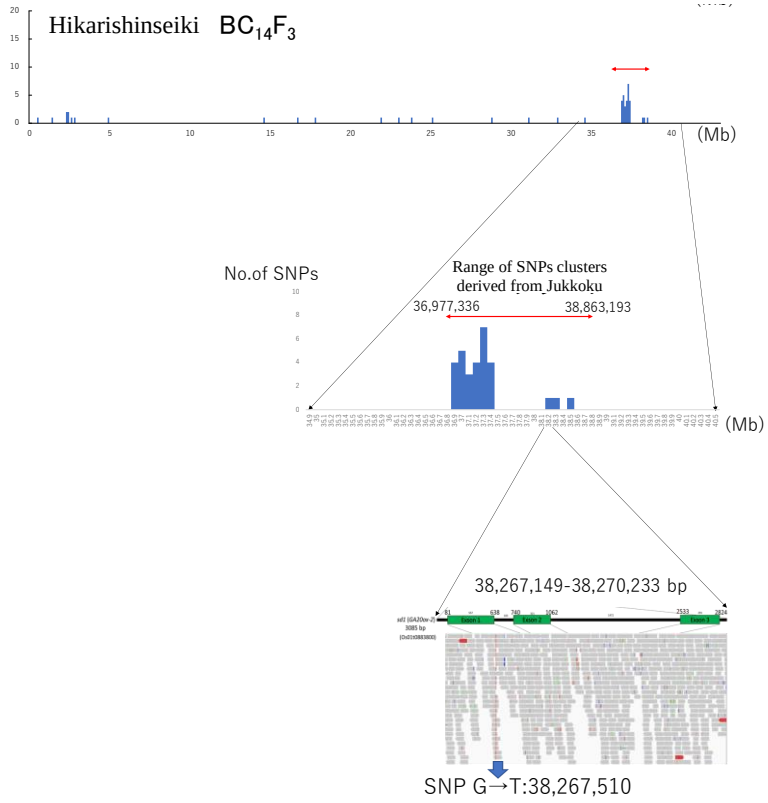

Figure 2. Genomic structure of *sd1=GA20ox-2* on chromosome 1 in the genome of Hikarishinseiki. A SNP from G to T in Hikarishinseiki located at 38,267,510 bp among the *Sd1/sd1* locus (Os01t0883800-01), which located at positions 38,267,149-38,270,233 from the end of the short arm of chromosome 1 in the Koshihikari genome. The range of SNPs clusters derived from Jukkoku accompanied with *sd1* was from 36,977,366 bp to 38,863,193 bp on chromosome 1, namely, the width was 1,685,857 bp corresponding to 0.43% of the rice genome.

Morphological characters of the Hikarishinseiki semidwarf rice

Hikarishinseiki headed an average of 1.0 days earlier than Koshihikari. The average culm length of Hikarishinseiki was 69.5 cm, which is on average 21.4 cm shorter than Koshihikari (90.9 cm) (Figure 3, Table 1). The introduction of the semidwarf gene Jukkoku_*sd1* resulted in a 23.5% reduction in culm length. The panicle length of Hikarishinseiki was 18.1 cm, averaging 96.3% of that of Koshihikari. The number of panicles (437/m^2) averaged 13.2% more than that of Koshihikari. The weight of 1,000 grains of Hikarishinseiki was 23.1 g (100.9% that of Koshihikari). The yield of not milled rice (57.3 kg/a) was 9.1% higher than that of Koshihikari. These yield data were improved compared to previous data (3% higher) in the eighth backcrossed isogenic background of Koshihikari (Tomita 2009). The average quality of brown rice was 5.2. This score indicates 'medium' quality, the same class of quality as Koshihikari. The eating quality was almost the same as that of Koshihikari. This score indicates a rating 'better than the average' equal to that of Koshihikari. The

Hikarishinseiki rice variety was registered as USDA Plant Variety Protection No. 201000072 on June 19, 2013.

Figure 3. Phenotypic alteration of *Oryza sativa* USDA Plant Variety Protection No. 201000072 "Hikarishinseiki", from original cultivar "Koshihikari". The average plant height of Hikarishinseiki is 69.5 cm, which is on average 21.4 cm (23.5%) shorter than Koshihikari (90.9 cm).

Table 1. Comparison of phenotypic characters of rice cultivars Koshihikari and Hikarishinseiki

Experimental locations	Cultivars	Heading date (m.d)	Culm length (cm)	Panicle length (cm)	No. of Panicles (No./㎡)	Grain yield (kg/a)	1000-grain weight (g)	(1) Grain Quality	(2) Lodging degree	(3) Eating quality
Tottori	Koshihikari	8.03	96.4	18.5	503	62.9	24.6	6.8	2.7	0.00
	Hikarishinseiki	8.02	71.3	17.6	517	67.6	24.1	7.3	0.1	0.10
Yonago	Koshihikari	8.06	87.8	19.3	317	49.7	22.8	4.6	3.0	0.00
	Hikarishinseiki	8.05	70.5	17.8	422	57.8	22.9	5.0	0.0	0.10
Miyagi	Koshihikari	8.19	99.3	17.7	467	43.0	21.8	4.5	3.8	0.00
	Hikarishinseiki	8.18	74.5	16.6	502	54.1	22.2	4.8	0.0	-0.43
Ehime	Koshihikari	8.12	89.0	21.1	308	50.3	23.6	5.0	3.0	0.00
	Hikarishinseiki	8.13	70.0	19.6	368	50.9	23.6	5.0	0.0	-0.22
Kumamoto	Koshihikari	8.09	82.0	17.4	335	56.4	21.7	3.0	3.0	0.00
	Hikarishinseiki	8.05	61.0	18.7	378	56.3	22.5	4.0	0.0	0.10
Average	Koshihikari	8.10	90.9	18.8	386	52.5	22.9	4.8	3.1	0.00
	Hikarishinseiki	8.09	69.5**	18.1	437**	57.3*	23.1	5.2	0.0**	-0.09

(1) Grain quality was classified into nine grades; 1: excellent good to 9: especially bad low quality.
(2) Lodging degree was determined based on the inclination angle of plant; 0: standing, 1: almost 70, 2: almost 50, 3: almost 30, 4: almost 10, 5: lodged.
(3) The sensory taste test was conducted by evaluation of 20 panelists into 11 degrees; 5: excellent good to -5: especially bad against Koshihikari = 0.
**: Statistically significant at the 1% level.
The average culm length of Hikarishinseiki was 69.5 cm, which is on average 21.4 cm shorter than Koshihikari (90.9 cm). Introgression of the semidwarf gene Jukkoku_*sd1* resulted in a 23.5% reduction in culm length. The panicle length of Hikarishinseiki was 18.1 cm, averaging 96.3% of that of Koshihikari. The number of panicles ($437/m^2$) averaged 13.2% more than that of Koshihikari. The thousand-grain weight of Hikarishinseiki was 23.1 g (100.9% that of Koshihikari). The yield of not milled rice (57.3 kg/a) averaged 9.1% higher than that of Koshihikari. The average brown rice quality was 5.2, indicating 'medium' quality, the same class of quality as Koshihikari. Eating quality was almost the same as that of Koshihikari.

Extension of the semidwarf rice Hikarishinseiki

The semidwarf gene *sd1* encoding a defective C20-oxidase in the gibberellin (GA) biosynthesis pathway (GA 20-oxidase, OsGA20ox2) was introduced into the genome of Koshihikari through 14 backcrosses to make the semidwarf phenotype, which is 21.4 cm shorter than Koshihikari. Whole genome sequencing by a next-generation sequencer enabled identification of the target DNA mutation point of Hikarishinseiki. Fourteen backcrosses were completed to narrow the target *sd1* DNA region. The *sd1* allele contains only a single amino acid substitution in the 1st exon of *OsGA20ox2*, which is different from Calrose 76 (Sasaki et al., 2002) , developed by Foster and Rutger (1978). In conclusion, the semidwarf rice designated Hikarishinseiki has a homogeneous genome of Koshihikari, except for the *sd1* region, by a next generation sequencing survey, and it has the equivalent taste and quality of Koshihikari.

The introgression of *sd1* into Koshihikari produced a good semidwarf phenotype of Hikarishinseiki that was nearly the same as Koshihikari without any detrimental effects on grain yield. The taste and quality of Hikarishinseiki were equivalent to those of Koshihikari. In the US, so far, semidwarf premium short-grain varieties, whose pedigrees include Koshihikari, were commercially grown, namely Calhikari-201 (McKenzie, 2003 PVP 9900310 USDA), and its sister line Calhikari-202 (McKenzie, 2013 PVP 201200460 USDA), etc. However, in these cultivars, for example, Calhikari-201 only went through two backcrosses with Koshihikari [=Koshihikari*2//(Koshihikari/S-101 with Calrose 76 *sd1*)] and both cultivars mature a week earlier than Koshihikari. Therefore, these are not completely semidwarf isogenes of Koshihikari. In addition, the semidwarf gene *sd1* was integrated into considerably many cultivars in the US, however, almost all were descent from Calrose 76 or IR8 (Kim et al., 2009). Therefore, Hikarishinseiki is the first semidwarf isogenic Koshihikari cultivar integrated with Jukkoku-derived *sd1*, which is registered as USDA Plant Variety Protection Certificate No. 201000072 (Tomita, 2013).

Hikarishinseiki may be considered an alternative to Koshihikari due to its resistance to hurricanes, typhoon damage, and ability to grow easily. It was designated as a brand rice description in Japanese rice producing districts in Okayama, Tottori, Tokushima, Niigata, Kochi, Shiga, Mie, Kagawa, Hyogo, Kyoto, Hiroshima, Tochigi, Kumamoto, Chiba and Wakayama prefectures (courtesy of the Ministry of Agriculture, Forestry, and Fisheries of Japan). Koshihikari is a premium quality rice that is also grown in the United States, and lodging is a limitation of this variety. Our work overcome the limitation by integrating the Jukkoku–derived semidwarf gene *sd1* into Koshihikari. The taste and quality of Hikarishinseiki comparable to Koshihikari would be valuable as a brand of rice on the world market. Crop damage is occurring on a global scale due to climate change and there is a desire for robust and stable cultivars. Our genomics-based breeding could lead to the rapid development of a cultivar that has the same genome of Koshihikari except for the integrated target gene, i.e., an isogenic Koshihikari substituted with the target gene. Furthermore, the author identified a new semidwarf gene *d60*, which is located at the chromosome 2 locus different from *sd1* (Tomita and Ishimoto, 2019; Tomita and Tanaka, 2019; Tomita and Tanisaka, 2019;

Tomita, 2019). Combining *sd1* and *d60* conferred double dwarfness (Tomita, 2012). As in this study, the author has applied to register 10 Super Koshihikari cultivars designated as the "Koshihikari Suruga" series, which were developed by smart genome breeding with NGS identification of relevant genes by backcrossing five times more to integrate robustness, increased biomass, larger grain size, and different ripening times into the Koshihikari genome, in addition to semidwarfism until now (Tomita et al., 2019; 2021; 2022; Tomita and Obara, 2021; Tomita and Tokuyama, 2022; Tomita, 2021 Plant Variety Registration No. 28385, 28386, 28387, 28388, 28685; Tomita, 2022 Plant Variety Registration No. 29367, 29368).

CONCLUSIONS

The semidwarf gene *sd1* encoding a defective C20-oxidase in the gibberellin (GA) biosynthesis pathway (GA 20-oxidase, OsGA20ox2) was introduced into the genome of Koshihikari through fourteen backcrosses to make the semidwarf phenotype, which is 21.4 cm shorter than Koshihikari. The semidwarf rice designated Hikarishinseiki has a homogeneous genome of Koshihikari, except for the *sd1* region, by a next-generation sequencing survey, and it has the same taste and quality of Koshihikari. Hikarishinseiki is registered under USDA Plant Variety Protection No. 201000072, as a first semidwarf Koshihikari integrated with Jukkoku-derived *sd1*.

ACKNOWLEDGMENTS

The author expresses gratitude to the Startup Supporting Program (funding agency: Bio-oriented Technology Research Advancement Institution (BRAIN)) under Grant Number JPJ010717.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Athwal DS (1971). Semidwarf rice and wheat in global food needs. *Q. Rev. Biol.* 46: 1-34.

Bolger AM, Lohse M and Usadel B (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114-2120.

Cho YG, Eun MY, Kim YK, Chung TY, et al. (1994a). The semidwarf gene, *sd-1*, of rice (*Oryza sativa* L). 1. Linkage with the esterase locus, *EstI-2*. *Theor. Appl. Genet.* 89: 49-53.

Cho YG, Eun MY, McCouch SR and Chae YA (1994b). The semidwarf gene, *sd-1*, of rice (*Oryza sativa* L.). 2. Molecular mapping and marker-assisted selection. *Theor. Appl. Genet.* 89: 54-59.

Foster KW and Rutger JN (1978). Inheritance of semidwarfism in rice, *Oryza sativa* L. *Genetics* 88: 559-574.

Gaur VS, Channappa G, Chakraborti M, Sharma TR. et al. (2020). 'Green revolution' dwarf gene *sd1* of rice has gigantic impact. *Brief. Funct. Genomics* 19: 390-409.

Hergrove T and Coffman WR (2016). Breeding History. In Rice that Changed the World: Cerebrating 50 Years of IR8. Rice Today: Laguna, Philippines, p. 6-10.

Hedden P (2003a). Constructing dwarf rice. *Nat. Biotechnol.* 21: 873-874.

Hedden P (2003b). The genes of the Green Revolution. *Trends Genet.* 19: 5-9.

Kamijima O, Tanisaka T and Kinoshita T (1997). Gene symbols for dwarfness. *Rice Genet. Newsletter* 13: 19-25.

Khush GS (1999). Green revolution: preparing for the 21st century. *Genome* 42: 646-655.

Kim SI, McKenzie KS and Tai TH (2009). A molecular survey of *sd1* alleles used in U.S. rice cultivars. *SABRAO J. Breed. Genet.* 41: 25-40.

Li H and Durbin R (2009). Fast and accurate short read alignment with Burrows-Wheeler Transform. *Bioinformatics* 25: 1754-1760.

Li H, Handsaker B, Wysoker A, Fennell T, et al.(2009). The sequence alignment/map (SAM) format and SAMtools. *Bioinformatics* 25: 2078-2079.

Maeda H, Ishii T, Mori H, Kuroda J, et al.(1997). High density molecular map of semidwarfing gene, *sd-1*, in rice (*Oryza sativa* L.). *Breed. Sci.* 47: 317-320.

McKenna A, Hanna M, Banks E, Sivachenko A, et al.(2010). The genome analysis toolkit: A MapReduce framework for analyzing nextgeneration DNA sequencing data. *Genome Res.* 20: 1297-1303.

McKenzie KS (2003). Rice Calhikari-201. Plant Variety Protection Certificate No. 009900310, United States Department of Agriculture

McKenzie KS (2013). Rice Calhikari-202. Plant Variety Protection Certificate No. 201200460, United States Department of Agriculture

Monna L, Kitazawa N, Yoshino R, Suzuki J, et al.(2002). Positional cloning of rice semidwarfing gene, *sd-1*: Rice "Green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. *DNA Res.* 9: 11-17.

Nagata Y and Yoshida M (2009). Statistical Principles for Multiple Comparisons Procedure. Scientist Press, Tokyo, 187p.

Nishiwaki M, Lii E, Mauromoustakos A and Wang Y (2018). Characterization of short-grain rice cultivars grown in Japan, California and Arkansas. *Stud. J. Dale Bumpers College Agri. Food Life Sci.* 19: 53-60.

Okada M, Yamakawa Y, Fujii K, Nishiyama H, et al.(1967). On the new varieties of paddy rice, 'Hoyoku', 'Kokumasari' and 'Shiranui'. *Bull. Kyushu Agric. Expt. Sta.* 12: 187-224.

Peng YL, Hu YG, Qian Q and Ren DY (2021). Progress and prospect of breeding utilization of green revolution gene *SD1* in rice. *Agriculture* 11: 611.

Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, et al.(2002). Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature* 416: 701-702.

Tomita M (2009). Introgression of Green Revolution *sd1* gene into isogenic genome of rice super cultivar Koshihikari to create novel semidwarf cultivar 'Hikarishinseiki' (Koshihikari-sd1). *Field Crops Res.* 114: 17––181.

Tomita M (2012). Combining two semidwarfing genes *d60* and *sd1* for reduced height in 'Minihikari', a new rice germplasm in the 'Koshihikari' genetic background. *Genet. Res. (Camb)* 94: 235–244.

Tomita M (2013). Rice Hikarishinseiki. Plant Variety Protection Certificate No. 201000072, United States Department of Agriculture

Tomita M (2019). Methodology to identify dwarfing gene *d60* that complements gamete lethal gene gal by Next-generation DNA sequencing analysis. *Med. Res. Arch.* 17: 1-9.

Tomita M (2021). *Oryza sativa* L. Koshihikari-Suruga Hd16. Plant Variety Protection Registration No. 28385 Official Gazette No. 391, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M (2021). *Oryza sativa* L. Koshihikari-Suruga d60Hd16. Plant Variety Protection Registration No. 28386 Official Gazette No. 391, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M (2021.) *Oryza sativa* L. Koshihikari-Suruga Gg. Plant Variety Protection Registration No. 28387 Official Gazette No. 391, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M (2021). *Oryza sativa* L. Koshihikari-Suruga d60Gg. Plant Variety Protection Registration No. 28388 Official Gazette No. 391, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M (2021). *Oryza sativa* L. Koshihikari-Suruga e1Gg. Plant Variety Protection Registration No. 28685 Official Gazette No. 1784, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M (2022). *Oryza sativa* L. Koshihikari-Suruga d60Bms. Plant Variety Protection Registration No. 29367 Official Gazette No. 1319, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M (2022). *Oryza sativa* L. Koshihikari-Suruga d65Bms. Plant Variety Protection Registration No. 29368 Official Gazette No. 1319, Ministry of Agriculture, Forestry and Fisheries, Japan

Tomita M, Ebata H and Nakayama K (2022). Large grain and semidwarf isogenic rice Koshihikari integrated with *GW2* and *sd1*. *Sustainability* 14: 11075.

Tomita M and Ishimoto K (2019). Rice novel semidwarfing gene *d60* can be as effective as Green Revolution gene *sd1*. *Plants* 8: 464.

Tomita M and Obara Y (2022). Year-round flowering gene *e1*, a mutation at the *E1* on rice chromosome 7 and its combination with green revolution gene *sd1* in an isogenic cell line. *Gene* 815: 146166.

Tomita M and Tanaka J (2019). Semidwarf gene *d60* affected by ubiquitous gamete lethal gene *gal* produced rare double dwarf with *d30* via recombination breaking repulsion-phase linkage on rice chromosome 2. *Genes* 10: 874.

Tomita M and Tanisaka T (2019). The gametic non-lethal gene *Gal* on chromosome 5 is Indispensable for the transmission of co-induced semidwarfing gene *d60* in rice. *Biology* 8: 94.

Tomita M and Tokuyama R (2022). Isogenic Japonica rice Koshihikari integrated with late flowering gene *Hd16* and semidwarfing gene *sd1* to prevent high temperature maturation and lodging by typhoon. *Life* 12: 1237.

Tomita M, Tokuyama R, Matsumoto S and Ishii K (2021). Whole genome sequencing revealed a late-maturing isogenic rice Koshihikari integrated with *Hd16* gene derived from an Ise Shrine mutant. *Int. J. Genomics* 2021: 4565977.

Tomita M, Yazawa S and Uenishi Y (2019). Identification of rice large grain gene *GW2* by whole-genome sequencing of a large grain-isogenic line integrated with japonica native gene and its linkage relationship with the co-integrated semidwarf gene *d60* on chromosome 2. *Int. J. Mol. Sci.* 20: 5442.

Ye CR, Luo HM, Deng W, Zhang JW, et al.(2020). Genome-wide association study on agronomic traits of temperate japonica rice (*Oryza sativa* L.). *Crop Breed. Appl. Technol.* 20: e22462011

Zeng YX, Chen Y, Ji ZJ, Liang Y, et al.(2019). Control of plant height by 24 alleles at 12 quantitative trait loci in rice. *Crop Breed. Appl. Technol.* 19: 200-207.