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Introgression of Green Revolution *sd1* gene into isogenic genome of rice super cultivar Koshihikari to create novel semidwarf cultivar 'Hikarishinseiki' (Koshihikari-sd1)

Motonori Tomita *

Molecular Genetics Laboratory, Faculty of Agriculture, Tottori University, 101, Minami 4-chome, Koyama-cho, Tottori 680-8553, Japan

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ABSTRACT

The rice super cultivar 'Koshihikari', grown on 37% of the total rice area in Japan, has been a leading cultivar since 1979. It is also grown in the USA and Australia. However, its tendency to lodge makes harvest difficult and reduces grain quality, and the regularity of large-scale typhoons compromises its production nationally. The genetic improvement of Koshihikari against lodging has been an extremely important theme for the past 30 years in Japan. This paper describes the development of a semidwarf form of Koshihikari by the fixing of an allele of the Green Revolution semidwarf gene *sd1* via eight recurrent backcrosses. The cultivar, with excellent flavor and high yield, was named 'Hikarishinseiki', which means "Bright New Century" (rice cultivar No. 12273; Ministry of Agriculture, Forestry and Fisheries of Japan: MAFF). Hikarishinseiki, with ≥99.8% of the Koshihikari genome, is about 20 cm shorter than Koshihikari. DNA monitoring of Hikarishinseiki confirmed its genomic substitution into Koshihikari except for *sd1* and a marker-based method to distinguish Koshihikari from Hikarishinseiki was developed. Its flavor (upper medium grade) and quality were the same as those of Koshihikari grown at 10 sites across Japan, but its yield was on average 3% higher (maximum 29% higher at Hyogo) than that of Koshihikari and it was highly resistant to lodging. Hikarishinseiki is the first semidwarf form of Koshihikari registered by MAFF. Some economical impact has been achieved by introducing Hikarishinseiki and it is already notified as a brand rice description in the rice-producing districts of Niigata, Shiga, Tottori, Okayama, Tokushima, and Kochi prefectures.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops, feeding half of the world's population (White, 1994), particularly those living in the monsoon areas of Asia. In Japan, 'Koshihikari', released in 1953, has been a leading cultivar since 1979. It was grown on 553,362 ha in 2007, accounting for over 37% of the Japanese total rice area, and over 60% in some prefectures. Koshihikari is also grown in various other countries (e.g., USA and Australia; http://www.tdb.maff.go.jp/toukei/a02stopframeset). The demand for Koshihikari is high because of its good eating quality, high resistance to pre-harvest sprouting, cool temperature tolerance at booting stage, and wide adaptability. Koshihikari and its descendants, which together account for 5 of the 10 leading cultivars, are grown on about 75% of the total paddy rice area in Japan. No cultivar with superior eating quality to that of Koshihikari has been bred so far.

However, the monoculture of Koshihikari and its close relatives increases the risk of loss to disease, pests, and typhoons. Therefore, it is essential to develop new cultivars to diversify the varietal gene pool in Japan. In contrast to its advantages, Koshihikari has the serious disadvantages of poor lodging resistance (Kashiwagi et al., 2008) and susceptibility to blast. In Japan, frequent autumnal rains and typhoons cause lodging at the late ripening stage. The culm length of Koshihikari, at 90–100 cm, is much longer than those of other cultivars. Lodging by the long and weak culm hinders harvest and lowers yield and grain quality. Frequent large typhoons cause large-scale damage to Koshihikari nationally. Therefore, the genetic improvement of Koshihikari against lodging is critical.

The recessive *semidwarf 1* (*sd1*) gene in rice confers a shortened culm and a greater harvest index, resulting in lodging resistance and responsiveness to fertilizer. *sd1* originated from the Chinese cultivar 'Dee-geo-woo-gen' (DGWG), and was used at the International Rice Research Institute in the Philippines during the early 1960s to produce the high-yielding semidwarf cultivar

* Tel.: +81 857 31 5351; fax: +81 857 31 5351.
E-mail addresses: tomita@muses.tottori-u.ac.jp, tomita206@gmail.com.

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IR8. This cultivar was called "miracle rice" because it dramatically improved the yield per unit area and averted the chronic food shortage that was anticipated after the rapid expansion of the Asian population in the 1960s. This remarkable achievement is known as the Green Revolution (Athwal, 1971; Khush, 1999). In Japan, many other short-culm cultivars, such as 'Hoyoku', 'Shiranui', and 'Kokumasari', were developed by using the Japanese indigenous landrace 'Jukkoku' as source of semidwarfism (Okada et al., 1967). Similarly, the γ-ray-induced semidwarf cultivars 'Reimei' in Japan and 'Calrose 76' in the USA (Foster and Rutger, 1978) have been widely used in rice breeding programs.

All of these semidwarf cultivars carry alleles at the *sd1* locus, in spite of their different parentage (Kikuchi and Futsuhara, 1997; Monna et al., 2002; Spielmeyer et al., 2002; Sasaki et al., 2002; Ashikari et al., 2002). The *sd1* alleles, on the long arm of chromosome 1 (Cho et al., 1994a,b; Maeda et al., 1997), encode loss-of-function mutations in GA 20-oxidase (*OsGA20ox2*), which regulates the synthesis of biologically active gibberellins (GAs), which catalyze three steps in the GA biosynthesis pathway (Monna et al., 2002; Spielmeyer et al., 2002; Sasaki et al., 2002; Ashikari et al., 2002). The *sd1* gene confers the semidwarf phenotype with no detrimental effects on grain yield (Hedden, 2003b).

The study described here developed a semidwarf form of Koshihikari by the transfer of an allele of *sd1*. This is the first such development in Japan. This cultivar, about 20 cm shorter than Koshihikari, was named 'Hikarishinseiki' (rice cultivar No. 12273; MAFF, 2004). Hikarishinseiki is the first semidwarf Koshihikari-type cultivar registered, and shares ≥99.8% of the Koshihikari genome. DNA monitoring of Hikarishinseiki confirmed its genomic substitution into Koshihikari except for *sd1*. Its lodging tolerance is much greater than that of Koshihikari, it yielded 3% more on average on account of an increased number of panicles, and it shares the good flavor of Koshihikari (upper medium grade). Its flavor and quality were identical to those of Koshihikari at 10 sites across Japan. Hikarishinseiki has been notified as a brand rice description in the rice-producing districts of Okayama, Tottori, Tokushima, Niigata, Shiga, and Kochi prefectures (MAFF, 2007, 2008, 2009). This paper describes the breeding, DNA monitoring, and field performance of Hikarishinseiki.

2. Materials and methods

2.1. Breeding of Hikarishinseiki

The semidwarfing gene *sd1* encodes a defective C20 oxidative enzyme, OsGA20ox, which functions in the biosynthesis of gibberellin GA_1 (Spielmeyer et al., 2002; Sasaki et al., 2002). The defective enzyme causes a short-culm. The *sd1* allele of DGWG lost a 383-bp region in *OsGA20ox*. The deletion introduced a stop codon and probably results in a highly truncated, inactive enzyme. On the other hand, the *sd1* allele of *japonica* landrace Jukkoku contains only a single amino acid substitution in the 1st exon of *OsGA20ox2* (Sasaki et al., 2002). This study used the *sd1* of Jukkoku.

Breeding of Hikarishinseiki began in 1985, when Jukkoku was crossed with 'Kanto No. 79', an early-maturing mutant of 'Koshihikari' (Figs. 1 and 2). Plants were selected from self-pollinated 100 F_3 lines raised from 100 randomly selected F_2 plants of Kanto 79 (long-culm) × Jukkoku (semidwarf). Fifty plants per line were investigated for culm length and days-to-heading. A short-culmed Koshihikari-like line with similar heading dates and homozygous for *sd1* was established at the F_4 generation in 1989.

Eight recurrent backcrosses were then performed to restrict the substituted region to *sd1*. The short-culmed Koshihikari-like line and its descendants were backcrossed with Koshihikari (*Sd1Sd1*) as the recurrent parent from 1990 to 2000. *Sd1sd1*-heterozygous plants could be recognized by their slightly erect leaves and were selected in the 1st generation of each backcross (BC_nF_1) (Fig. 3). Koshihikari was the female parent in each backcross generation except BC_2F_1. Each BC_nF_2 generation was always grown in the next year to confirm that short-culm plants (*sd1sd1*) appeared in the BC_nF_2 generation while backcrossing of BC_{n+1} was performed. The semidwarf phenotype (*sd1sd1*) was fixed by the BC_8F_3 generation, in which plants had ≥99.8% of the Koshihikari genome based on the theoretical expectation from 8 recurrent backcrosses.

2.2. Identification of sd1 introgression into Koshihikari

The two alleles at the *sd1*/*OsGA20ox2* locus on chromosome 1 of each line were distinguished by PCR amplification of the first exon followed by digestion with *Pma*CI. Primer design was based on the reported sequence of Nipponbare (http://rgp.dna.affrc.go.jp). Reaction mixtures contained 10 ng of template genomic DNA, 1 μM each primer (F: 5′-GCTCGTCTTCTCCCCTGTTACAAATACCCC-3′; R: 5′-ACCATGAAGGTGTCGCCGATGTTGATGACC-3′), 0.4 mM dNTPs, 1× GC buffer I, 2.5 mM $MgCl_2$, and 0.5 U *LA Taq* polymerase (Takara) in a total volume of 20 μL. The PCR reaction program consisted of 35 cycles of 30 s at 94 °C, 30 s at 58 °C, and 1 min at 72 °C. The PCR products were purified, ligated to the pGEM-T vector (Promega), and sequenced. The Jukkoku allele was detected by digestion with *Pma*CI (CAC ↓ GTG).

2.3. DNA monitoring and sequencing of sd1 flanking region

DNA monitoring of Hikarishinseiki was conducted by 51 SSR markers flanking *sd1* as shown in Table 1. The 5′ region flanking the 1st exon (1610 bp) of *OsGA20ox2* was amplified with specific primers designed on the basis of the reported sequence of Nipponbare (http://rgp.dna.affrc.go.jp/). Reaction mixtures contained 10 ng template genomic DNA, 1 μM each primer (5′-TGGCTCCGCCCTCTGCATCTCCTCATGGTC-3′, 5′-TCCGGGATCTTGGGCTCCATCCTCAGGTCG-3′), 0.4 mM dNTPs, 1× GC buffer I, and 0.5 U *LA Taq* polymerase (Takara) in a total volume of 20 μL. The PCR reaction program consisted of 35 cycles of 30 s at 94 °C, 30 s at 62 °C, and 2 min at 72 °C. The PCR products were purified, ligated to the pGEM-T vector (Promega), and sequenced.

2.4. Performance test of Hikarishinseiki

Performance tests were conducted in a paddy field at Tottori University, Koyama, during 2003 and 2004. Hikarishinseiki and Koshihikari were sown on 18 April 2003 and 22 April 2004, and 128 plants per plot were transplanted with two replications on 14 and 18 May, respectively. Similar performance tests were conducted at experimental stations in Chiba, Niigata, Fukui, Kyoto, Hyogo, Hiroshima, Yamaguchi, Tokushima, and Fukuoka, which are spread across Japan.

The date when 50% of all panicles had emerged from the flag leaf sheath was recorded as the heading date. Days-to-heading was the number of days from sowing to heading date. Culm length, panicle length, number of panicles, leaf length, and leaf width were measured on 10 randomly selected plants in each plot. Thousand-grain weight, grain yield of brown rice, grain quality, and eating quality were measured in bulks of 50 plants. Trait means were compared by the *t*-test.

3. Results

3.1. Breeding of Hikarishinseiki

Fig. 2 plots culm lengths and heading dates of 300 F_2 plants of Kanto 79 (early maturing and long-culm) × Jukkoku (late maturing

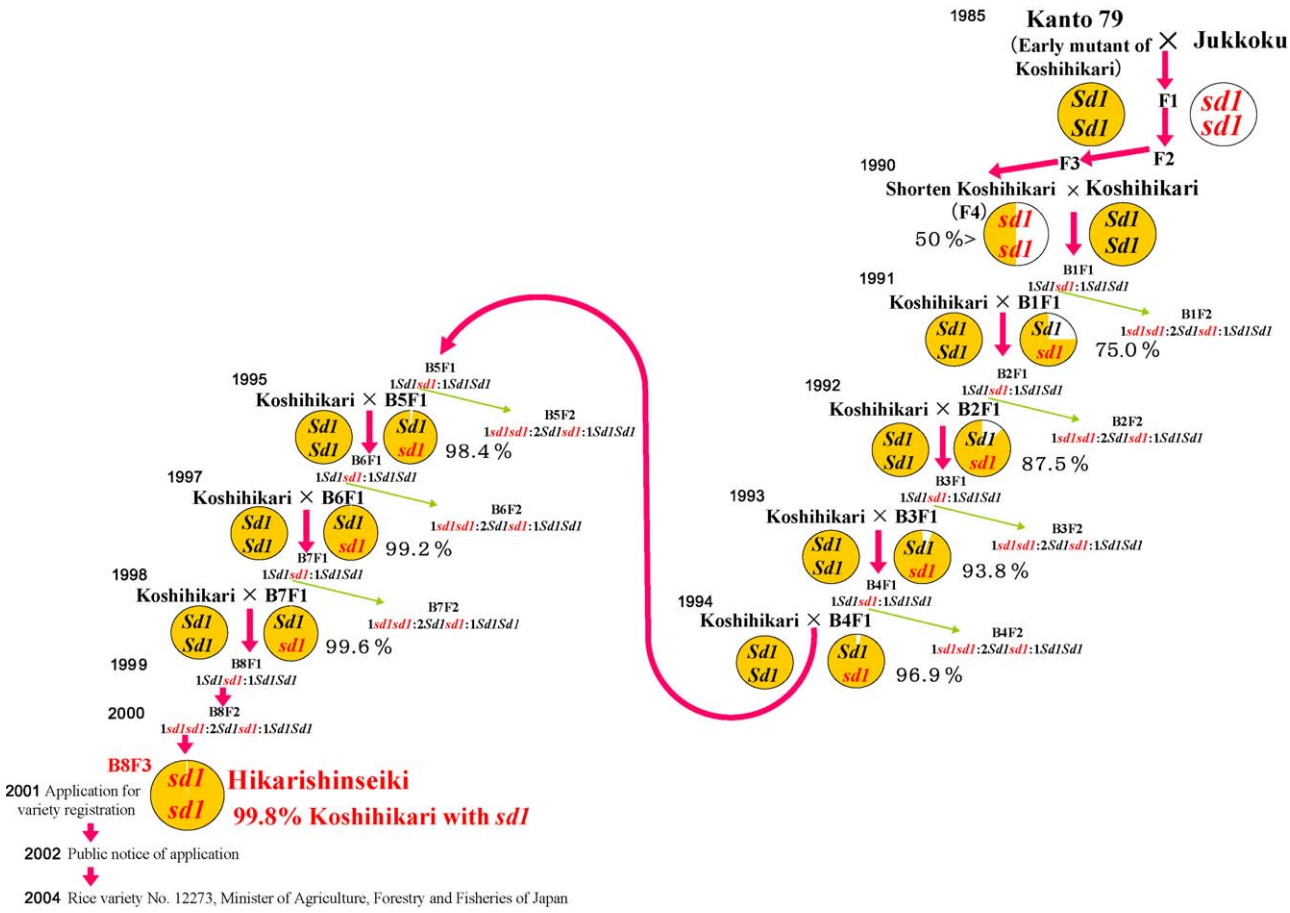

Fig. 1. Development of Hikarishinseiki (Koshihikari-sd1). To fix *sd1* in the Koshihikari background, 8 recurrent backcrosses with Koshihikari were performed. Yellow pie charts show the theoretical proportion of the Koshihikari genome.

and semidwarf). Since the parents' heading dates differ by about a month, individuals with the heading dates between that of the parents segregated in the F_2 generation (Fig. 2A). Because the most important hereditary character in respect of adaptability is heading date, short-culmed (*sd1sd1*) lines maturing at the same time as Koshihikari were selected by the pedigree breeding method from among the 100 F_3 lines. Twenty-eight lines had reduced height (20 cm shorter than Koshihikari), of which 2 had the same maturity date as Koshihikari. A representative line is shown in Fig. 2B. Thus, the short-culm Koshihikari-like lines, in which most characters were close to those of Koshihikari except for the short stature, were established by the F_4 generation in 1989. The F_4 lines thus carried a little more than half of the important genes of Koshihikari.

Eight recurrent backcrosses with Koshihikari from 1990 to 2000 increased the proportion of the Koshihikari genome (except for *sd1*) theoretically to ≥99.8% (Fig. 1). *Sd1sd1* heterozygotes recognizable by their slightly erect leaves were selected in the BC_nF_1 generation (Fig. 3A), and the decent of BC_nF_2 generation certainly segregated in the ratio of 3 tall:1 short (Fig. 3B). Because Koshihikari was always female-backcrossed except BC_2F_1, there was no risk of self-fertilized transmission of *sd1*. The short Koshihikari-like phenotype (*sd1sd1*) was fixed by the BC_8F_3 generation, and breeding was completed.

This short-culm cultivar was named Hikarishinseiki, which means "Bright New Century", in 2001, and was registered by MAFF in November 2004 (Fig. 4; rice cultivar No. 12273). The entire process took 19 years.

3.2. DNA diagnosis and genotype monitoring by SSR markers flanked sd1/OsGA20ox2 in Hikarishinseiki

Standard DNA-diagnosis analysis of brown rice with the Takara Bio Inc. PCR kit RR211A for rice identification revealed Hikarishinseiki to be identical to Koshihikari (Fig. 5A). So, 51 SSR markers flanking *sd1*, located from 32,331,665 bp to 38,432,536 bp on chromosome 1 (Table 1), were used for DNA monitoring of Hikarishinseiki. Monitoring confirmed that all SSR sites came from the Koshihikari background. The *sd1* allele from Jukkoku has a G → T substitution in the 1st exon, which results in alteration of a single amino acid from Gly to Val. *Pma*CI recognizes the substituted sequence (CAC ↓ GTG) in the *sd1* allele from Jukkoku. This revealed the *sd1* allele in Hikarishinseiki as 2 unique fragments, while the wild-type allele of Koshihikari remained as an undigested single fragment (Fig. 5B). This method can thus distinguish Koshihikari from Hikarishinseiki.

The 5′ region flanking the 1st exon (1610 bp) in each of Koshihikari (AB458234) and Hikarishinseiki (AB458233) was amplified and sequenced (Fig. 6). The DNA sequences were almost identical. However, a variable microsatellite repeat region $(AC)_n$ was found 177 bp upstream of the initial ATG of *sd1*/*OsGA20ox2*: $(AC)_6$ in Hikarishinseiki, $(AC)_7$ in Koshihikari and Nipponbare and $(AC)_8$ in DGWG. The microsatellite repeat region might be associated with regulatory expression of *sd1* or *OsGA20ox2*. Overall, the DNA analyses of Hikarishinseiki indicated its genomic identicalness to Koshihikari, except at *sd1*.

Table 1
DNA monitoring of Hikarishinseiki (Koshihikari-sd1) conducted by 51 SSR markers flanking *sd1*.

Locus	SSR motif	No. of repeats	Forward primer	Reverse primer	SSR start	SSR end	Expected size	Results Kohihikari-Hikarishinseiki
RM6648	ACG	8	CCTCTACCTCGCCCAACAGC	GAGGACCGACTCCCTGATCG	32,331,665	32,331,688	140	M
RM6387	ACG	8	CCAATCCTTTCCAAATCCAACG	GCCAAATATCTAGCCTCCTATTGAGC	32,542,477	32,542,500	159	M
RM3285	AG	13	AAGGAACGCGAGAGAAGAGAACC	ATTCTGAGCAGGAGAAGGGAAGG	33,044,063	33,044,088	297	M
RM8124	AT	44	TTGGAGATGATCAAATGGGTAGGG	CCGACAGATATTGCATGGTTGG	33,283,139	33,283,226	416	M
RM1003	AC	12	TTCCAGCTTTGCATGAACAACG	TGTACGCGACCCTGATGTCC	33,470,285	33,470,308	225	M
RM8061	AT	27	CGACGAACCTTCAATCTCAGACTTCG	ATGGGCCATCGTGCGTTTCC	34,119,563	34,119,616	198	M
RM6950	AAG	8	ACTTGTCTGTGTCACTAACCATGC	GATACATGGCGTCTCAACTACACC	34,502,555	34,502,578	179	M
RM6703	ACT	12	GCTTTCCTCTCCTCTCCTCTCC	CAAATCAGTGTCGTATGCAGTGG	34,508,480	34,508,515	200	M
RM5501	AG	27	GTTGGCGTACGTAGAGAGGAGTACG	CTTCATTGTCGCTGCCAGAGC	34,542,232	34,542,285	155	M
RM5811	AGG	10	ATGCACCACCAGCAGCTCAAACC	CGGATGAGGGAGTTGTAGCAGAAGG	34,629,779	34,629,808	299	M
RM6608	CCG	8	GCGGAGCTTTCCTCTACTCTTTCG	GTCATCCTCCTCGCCATCACC	34,683,328	34,683,351	118	M
RM6547	AGC	9	ATGACACACCACCACCACCAACG	GGAAGCGCAGCATCAACAAAGG	34,687,580	34,687,606	72	M
RM8085	AG	26	CCTCGTCCTCCTCGTCATCG	AGCAGCGAAATGGAAGGAACC	34,856,373	34,856,424	270	M
RM8084	AT	18	GCGCCCAATGCATGTAAATTCC	TGCCGATACCTGTGATCAAGTCC	34,895,399	34,895,434	289	M
RM3403	AG	17	CTGCCTCCTCCATTTCCCACTCC	CGAACGACTGCTCCCTCTTCAGC	34,990,000	34,990,033	166	M
RM5781	AAG	15	AGCGCGAAGGGAGAAACAGG	CTCCACGACCTTCTGCTTCTTGG	35,760,994	35,761,038	141	M
RM7250	AGAT	6	TTTGTAAAGGCGAATCCGAACACC	TGGTGGCTTGAGAACGTCTGTAGG	36,079,634	36,079,657	185	M
RM5407	AG	15	TTTCCGACTGAACAAGTACACAGC	GAGTGAGCAAGAACTGTCATCACC	36,376,256	36,376,285	164	M
RM3825	AG	23	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAGC	36,464,545	36,464,590	193	M
RM8278	AG	12	ACTGCGAACTACACTTTCACTGTACC	GACTGACTCGCATGCTTTCC	36,616,272	36,616,295	371	M
RM3738	AG	16	GTGATGGGAGAGCAGGAGAAGG	CGCAAGTGGACCTCTTATTGTGC	36,927,911	36,927,942	175	M
RM5448	AG	18	AATGTAATGGTGCTGCTACAGG	TCAATGTGCCATTCCATAGG	37,391,639	37,391,674	272	M
RM3290	AG	14	GGTGTTAGCATCGGCAGATAGGC	GTAACACGCGTGCGAAATGAGC	37,517,046	37,517,073	265	M
RM8104	AAG	10	GCCTTCCCTTTCCTCTCTTCTCC	CACTCTCACAAACCCGAAGTGC	37,529,252	37,529,281	186	M
RM6333	AAG	8	ACTCACTCACTCACCCACACAGC	TTGGAGAGGGAAGAGAAGACACG	38,002,989	38,003,012	184	M
RM8231	AG	22	GAGCAGCGTAAGATCTCCCTACC	CAAGCGAGCACATACATCAAAGC	38,191,237	38,191,280	166	M
RM8232	AG	19	TATCGTGGAAATCCTACCAACC	CCACCACGTGTGTACTCCTACC	38,196,824	38,196,861	99	M
RM6696	AAT	33	CACTTGACGAAGATGAAGGATGG	GAGTGAACTCCTTTGATGGTTTGC	38,215,044	38,215,142	299	M
RM8235	AC	20	GTTGACAGGGCTAAATGTAGATCG	ACTTTGCATGGACAGGTTATGG	38,432,497	38,432,536	399	M
Os01g66100	*sd1*				38,709,231	38,712,353	3122	
RM6504	CCG	8	ACCGCCTCATTCTTTCCGATCC	AGAGGCGGTAGTATGCTCGTTGC	38,979,977	38,980,000	98	M
RM1361	AG	25	ATGCTTGCAGACAATCGATGC	CTCTCCGCCTAAACAACTTGTGC	39,074,249	39,074,298	459	M
RM7246	ATCG	7	CATCACTGATCACTCAGTCACCTAGC	ATCTCCATTTCTCTCCGATGAGC	39,164,189	39,164,216	208	M
RM6292	AGC	12	TTCCTCGATTTAGACTTGCACAGC	GGGAAGGAGAAGTCCCAGAAGC	39,215,901	39,215,936	194	M
RM3482	AG	25	GCCGCTAATGTTGTTGTCAAGC	CGAAGCCAACGTAGTCCAATCC	39,713,330	39,713,379	173	M
RM3523	AG	32	TCCTGGCTGCTATTGTGGTTGG	AAGTCGTTCGTTGCATGAAGAGG	39,817,381	39,817,444	186	M
RM165	AG	11	AATCGGCGAGGTTTGCTAATGG	ATACGTGGTACGTGACGCTTTGC	40,100,318	40,100,339	100	M
RM1387	AG	44	GCTACCATGCACGAAACAGAGACG	ACCAAATGTGGCTGGCTGATCG	40,201,060	40,201,147	195	M
RM3520	AG	31	CTGCAAATGCACAGGAATCAGG	TCCTCTCGCCTTTCTTTCTCTCC	40,247,014	40,247,075	142	M
RM529	AG	12	TTCACCACAACGATAGAGACTTCTGG	GGGAAGAAGATGACAGAGCAAGC	40,664,014	40,664,037	249	M
RM414	ATGC	6	CAAGGAAGATCTTGTGGACCATGC	CTGCAGATGCAGAGGCAGAGG	40,749,589	40,749,612	159	M
RM8100	AT	21	GCGTGGAGATTCAGTTAAGTTTCACC	AATTCCGTGTCCGATTTCCTACC	41,012,244	41,012,285	384	M
RM5794	AGC	8	AATCCGTCAACCACCATGAACG	TTGCAGACTCATGGACACATGG	41,074,383	41,074,406	145	M
RM5536	AC	14	CACGTACCAGCCTTGATGAATCC	TGGGCTATACTAATCCCGTCATCC	41,160,287	41,160,314	175	M
RM8088	AT	35	AAAGGTTGACTGTTGTCAGTGG	CTAGCCCTTAGCAGTGATGC	41,685,447	41,685,516	309	M
RM6407	AGG	8	CAACCAGTTCCTCCTCCTATGACC	TTTGAAATGGTGGAGTCCAAGG	42,369,525	42,369,548	218	M
RM6237	ACG	9	TCTCCCATCTCCCATATTCCCATCC	GCGCATGACGTCGGAGATCG	42,560,324	42,560,350	300	M
RM6489	CCG	8	GGATACTCTATGTTCCTCTGCTGTGC	GTCTCCGCCGTCTCGTAAACC	42,607,610	42,607,633	148	M
RM3694	AG	15	GGCAGGAGAGGGAGAGAGAAAGG	GGAGAGCAGGACGCAGAAGAGG	42,648,733	42,648,762	169	M
RM6321	AAG	14	CCCAGGAATATCTCTATACACACAGC	CTCTACCTCGCTGTTGTCTCTGC	42,916,592	42,916,633	146	M
RM3362	AG	15	ATTGCGTGGGATATGGACTATGC	CCTCTTCAATTCCCTTCCTCTGC	43,036,403	43,036,432	78	M
RM6840	AAG	23	CGACTGGAAGAAGGGATCATGG	CACACTACCAAGACTCCGCTATGG	43,165,172	43,165,240	202	M

M: monomorphic.

3.3. Field performance of Hikarishinseiki

The agronomic characteristics of Hikarishinseiki and Koshihikari are shown in Table 2. Both heading date and maturity date (defined as "early") of Hikarishinseiki were the same as those of Koshihikari at 10 locations.

The culm length of Hikarishinseiki (72.4 cm) averaged 78% (range 72–83%) of the length of Koshihikari (i.e., 22% shorter). The lodging score of Hikarishinseiki averaged 0.6, against up to +1.7 in Koshihikari. The panicle length of Hikarishinseiki (18.3 cm) averaged 98% (range 93–109%) of that of Koshihikari. The number of panicles (449/m^2) averaged 9% (range 1–19%) more than that of Koshihikari. Murai et al. (2004) and Ogi et al. (1993) reported that an isogenic line carrying *sd1* derived from DGWG had more panicles than its parent cultivar 'Norin 29', yet another isogenic line carrying *sd1* had the same number of panicles in the 'Shiokari' genetic background. Hence, *sd1* appears to confer a pleiotropic effect of increasing panicle number very well in the Koshihikari genetic background. Thousand-grain weight (22.0 g) was the same (mean 101%, range 97–103% of Koshihikari), and brown rice yield (60.2 kg/a) averaged 3% (range −7% to +29%) higher than that of Koshihikari. The greater number of panicles and increased yield resulted from the conversion of nutrients that did not go to leaves and stems.

The leaves of Hikarishinseiki averaged 18% wider than those of Koshihikari, stood straighter, and retained a deep green color longer (Figs. 4 and 7). Hikarishinseiki's improved light-intercepting attitude might thus improve its photosynthetic efficiency. Bacterial leaf blight resistance and stem maggot resistance were the same by MAFF in varietal registration tests (MAFF, 2004). Field

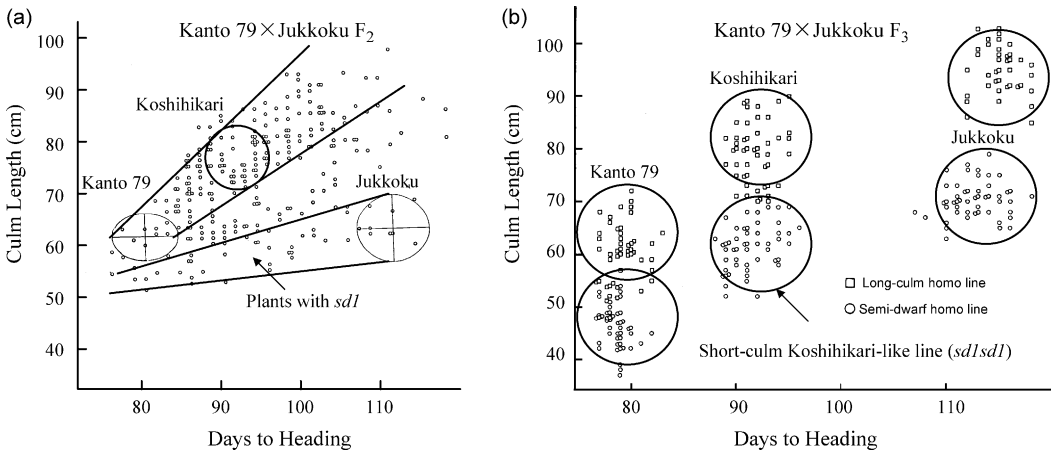

Fig. 2. Breeding of short-culm Koshihikari-like lines (*sd1sd1*). Development of Hikarishinseiki (Koshihikari-sd1) began with Jukkoku (*sd1sd1*) × Kanto No. 79, an early-maturing mutant of Koshihikari. Circles show the variation in parental or line values. (A) Relationship between heading date and culm length of individual plants in the F_2 generation. (B) Plant lines with the correct heading date same as Koshihikari were established at the F_4 generation.

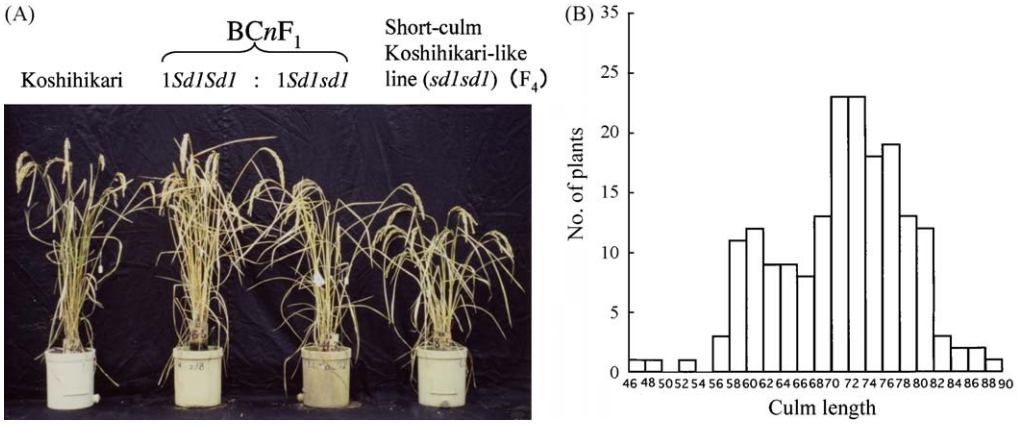

Fig. 3. The scheme for developing Koshihikari-sd1. (A) *Sd1sd1* heterozygotes were slightly more erect in the 1st generation of each recurrent backcross (BC_nF_1). Koshihikari was crossed as the female parent. (B) BC_nF_2 was always grown while BC_{n+1} backcrossing was in progress to confirm the segregation of short-culm plants (*sd1sd1*). *sd1sd1* homozygotes were achieved by the BC_8F_3 generation.

resistance against leaf blast was a little stronger in Hikarishinseiki. This can be attributed to good ventilation due to the erect leaves.

Ripening period and grain quality (Table 2) were not significantly different by *t*-test. Grain size and quality were equivalent (Fig. 8), as found by MAFF in varietal registration tests (MAFF, 2004). The brown rice quality of both was "medium" (score of 4.2), and both scored "minimum" for contents of white belly and cracked grain. The flavor of Hikarishinseiki scored against Koshihikari averaged +0.11 (Table 2), placing it in the same "upper medium grade" as Koshihikari. Pre-harvest sprouting and grain shattering habit of both cultivars are both rated "difficult" (MAFF, 2004). Amylose content, protein content, and flavor value were all also identical (Table 2).

Flavor and quality of Hikarishinseiki were rated as identical to those of Koshihikari by the Niigata National Research Institute of Agriculture, Chiba Agricultural Research Center, Kyoto National

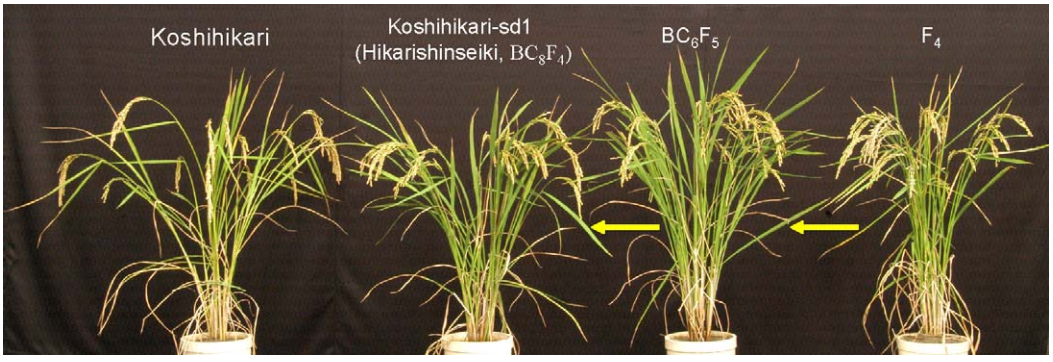

Fig. 4. Transition of Hikarishinseiki (Koshihikari-sd1) progenitors to Koshihikari phenotype. Hikarishinseiki has shorter and broader leaves and more panicles.

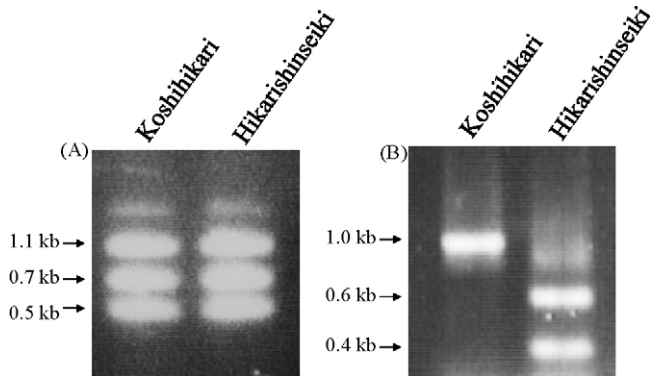

Fig. 5. DNA diagnosis of Hikarishinseiki (Koshihikari-sd1). (A) Standard DNA-diagnosis analysis of brown rice revealed Hikarishinseiki to be identical to Koshihikari. (B) DNA diagnosis at *sd1*. The *sd1* allele from Jukkoku has a G → T substitution in the 1st exon. *Pma*CI recognizes the substituted sequence (CAC ↓ GTG) and split the *sd1* allele in Hikarishinseiki into two fragments, while the wild-type allele of Koshihikari remained as a single fragment.

Research Institute of Agriculture, and Fukuoka Agricultural Examination Center. Thus, the semidwarfing gene *sd1* reduces lodging in Hikarishinseiki while retaining the flavor and quality of Koshihikari.

4. Discussion

4.1. First semidwarf form of Koshihikari

Koshihikari remains a favorite in Japan. Production of the two major cultivars, Koshihikari and 'Hitomebore', accounts for over 90% of total production in some prefectures in Japan, so genetic variation is limited. This raises the risk of crop losses to disease and insect pests, and to lodging in Koshihikari caused by frequent typhoons. Such major damage is a nationwide problem. The genetic improvement of lodging resistance in Koshihikari has been a major goal in Japan.

This paper describes the development of semidwarf Hikarishinseiki, which was released in 2004. Hikarishinseiki is the first short-culmed isogenic form of Koshihikari carrying an *sd1* allele while retaining ≥99.8% of the Koshihikari genome. This means that Hikarishinseiki is an essentially derived variety (EDV) of Koshihikari. Koshihikari, grown not only in Japan but also in the USA and Australia, is an internationally important cultivar, and its weak lodging resistance is a serious widespread problem. Under the EDV concept, a cultivar is deemed to be essentially derived from an initial cultivar if it is clearly distinguishable but conforms genetically to the initial cultivar. International rules administered by the Union for Protection of New Varieties of Plants cover registration and plant variety protection for EDVs. As Koshihikari was bred 56 years ago, its grant of protection has now expired. Therefore, the release and registration of Hikarishinseiki is globally acceptable. Hikarishinseiki has remarkably enhanced lodging resistance over that of Koshihikari. Moreover, its increased panicle number gave it an average 3% increase in grain yield (maximum 29% increase at Hyogo), with the highly prized flavor of Koshihikari. Thus, the lodging weakness of Koshihikari has been overcome by the development of Hikarishinseiki.

Hikarishinseiki can be grown in all the same regions as Koshihikari, which range in climate from cold through warm to hot. The performance of Hikarishinseiki has been tested since 2005 in 29 prefectures. Its flavor and quality were found to be the same as those of Koshihikari. Furthermore, since it is more compact than Koshihikari, it can be planted more densely and fertilized more without lodging, and so can yield more. Three prefectures carried it through to field examination in 2007. This is a first time a university-developed cultivar has been used in performance tests for recommendable cultivars in each of those prefectures in Japan.

Although many cultivars already carry *sd1* (Tabuchi et al., 2000), they do not have the desired characteristics of Koshihikari.

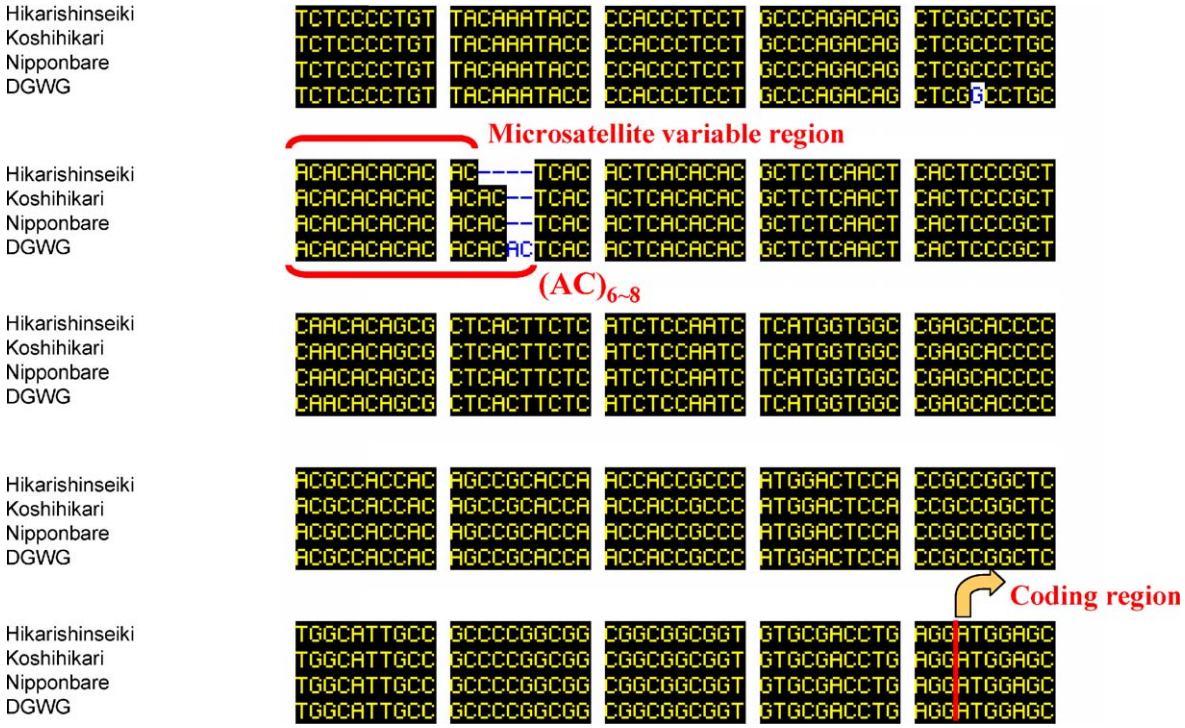

Fig. 6. Nucleotide sequences of 5′ region flanking the 1st exon (1610 bp) of *sd1*/*OsGA20ox2* of Koshihikari (AB458234) and Hikarishinseiki (AB458233). The DNA sequences were identical except for a variable microsatellite repeat region $(AC)_n$ 177 bp upstream of the ATG: $(AC)_6$ in Hikarishinseiki (Koshihikari-sd1), $(AC)_7$ in Koshihikari and Nipponbare, and $(AC)_8$ in DGWG.

Table 2
Comparison of agronomic characters of Koshihikari and Hikarishinseiki (Koshihikari-sd1) at 10 locations.

Experimental locations	Cultivars	Heading date (m.d.)	Maturity date (m.d.)	Culm length (cm)	Panicle length (cm)	No. of panicle (No./m^2)	Grain yield (kg/a)	1,000-grain weight (g)	Grain quality[a]	Lodging degree[b]	Leaf blast score[c]	Panicle blast score[d]	Value of taste[e]	Eating quality[f]	Amylose content (%)[g]	Protein content of brown rice (%)
Chiba	Koshihikari	8.03	9.08	93.0	18.0	416	62.2	21.5	5.0	3.5		3.5		0.00		
	Hikarishinseiki	8.03	9.08	73.0	18.2	461	62.2	21.6	5.0	2.0		2.0		0.17		
Niigata	Koshihikari	8.04	9.13	94.4	18.2	402	61.3	21.1	4.3	3.5	0.0	0.0	78.7	0.00	13.8	6.7
	Hikarishinseiki	8.04	9.13	72.8	17.7	449	62.6	21.6	4.3	0.5	0.0	0.0	78.3	−0.04	13.7	6.9
Fukui	Koshihikari	7.23	8.26	94.9	19.8	382	62.1	22.1	3.5	3.8			70.1			6.5
	Hikarishinseiki	7.23	8.26	68.5	19.3	428	63.8	22.6	3.8	0.5			70.9			6.2
Kyoto	Koshihikari	7.30	9.04	90.5	20.0	391	64.7	23.1	6.0	1.0				0.00		
	Hikarishinseiki	7.30	9.04	71.9	19.4	430	59.9	23.2	7.0	0.0				0.17		
Hyogo	Koshihikari	8.07	9.09	102.5	16.8	393	41.8	21.2	3.0	4.5	0.0	0.0				
	Hikarishinseiki	8.08	9.12	82.5	18.3	466	54.0	21.8	3.5	3.5	0.0	0.0				
Tottori	Koshihikari	8.05	9.16	88.8	16.2	397	66.5	22.1	4.5	2.0	0.0	0.0		0.00	18.6	6.3
	Hikarishinseiki	8.04	9.16	71.8	16.6	462	77.3	20.2	4.5	0.0	0.0	0.0		0.14	18.6	6.3
Hiroshima	Koshihikari	7.27	9.01	84.6	19.7	361	63.1	23.0	3.0	0.5	0.0	0.0		0.00		5.7
	Hikarishinseiki	7.26	9.01	68.8	18.3	376	60.4	23.1	3.0	0.0	0.0	0.0		0.25		6.1
Yamaguchi	Kosdhihikari	7.24	8.30	85.0	19.2	367	55.1	21.6	4.5	1.5						7.4
	Hikarishinseiki	7.23	8.29	67.3	17.9	385	51.4	21.4	4.3	0.0						7.4
Tokushima	Koshihikari	7.10	8.10	83.1	19.1	473	53.2	21.3	6.0	3.0	0.0	0.0		0.00		8.2
	Hikarishinseiki	7.10	8.09	68.7	18.2	480	52.6	21.3	7.0	0.0	0.0	0.0		0.25		8.6
Fukuoka	Koshihikari	8.04	9.07	78.0	19.1	392	59.3	22.2	2.0	0.0	0.0	0.0		0.00		
	Hikarishinseiki	8.04	9.07	67.0	18.2	427	57.6	22.3	3.0	0.0	0.0	0.5		−0.14		
Average	Koshihikari	7.29	9.03	92.8	18.8	411	58.3	21.9	4.0	2.3	0.0	0.5	74.5	0.00	16.2	6.9
	Hikarishinseiki	7.29	9.03	72.4	18.3	449	60.2	22.0	4.2	0.6	0.0	0.1	74.6	0.11	16.2	7.0

[a] Grain quality was classified into nine grade; 1: excellent good to 9: especially bad low quality.
[b] 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.
[c] Leaf blast score was determined based on the percentage of infected leaf area; 0:0%, 1:1%, 2: 2%, 3: 5%, 4: 10%, 5: 20%, 6: 40%, 7: 60%, 8: 80%, 9: 90%, 100%.
[d] Panicle blast score was determined based on the percentage of infected kernels; 0:0%, 1:1%, 2: 2%, 3: 5%, 4: 10%, 5: 20%, 6: 40%, 7: 60%, 8: 80%, 9: 90%, 100%.
[e] Value of taste was determined using a Taste-meter MA-90B (Tokyo Rice-producing Machine Factory, Japan).
[f] Eating quality show the aggregate evaluation and classified into eleven degree; 5: excellent good to −5: especially bad.
[g] Amylose and protein content was measured by Near Infrared Spectrometer AN800 (Kett Electric Laboratory, Japan).

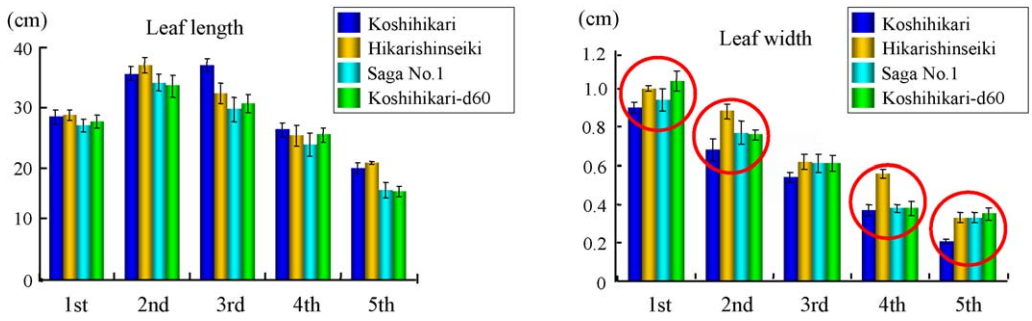

Fig. 7. Leaf length and width. Leaves of Hikarishinseiki (Koshihikari-sd1) averaged 18% wider than Koshihikari.

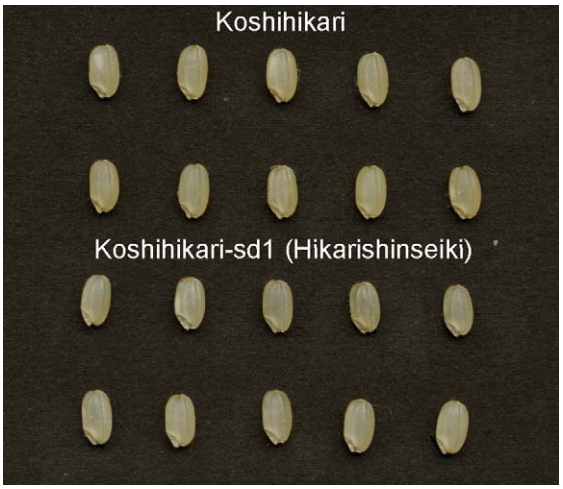

Fig. 8. The grain size and quality of Hikarishinseiki (Koshihikari-sd1) are equivalent to those of Koshihikari.

Since the registration of Hikarishinseiki, other semidwarf Koshihikari-type lines, such as 'Koshihikari Tsukuba SD No. 1' (Plant Genome Center, Tsukuba) and Koshihikari Eichi No. 4 (Honda Motor Co., Ltd., Nagoya, Ashikari et al., 2005), have been submitted for registration. However, MAFF will judge their novelty as new cultivars by comparison with Hikarishinseiki as the standard cultivar. The semidwarf Koshihikari-type lines bred by other institutions were backcrossed only 4–5 times, fewer than the 8 times of Hikarishinseiki, and they were inferior to Hikarishinseiki on account of a high rate of pre-harvest sprouting or fewer tillers. Performance tests showed that Hikarishinseiki is highly homogeneous to Koshihikari except in the short height and increased tiller number, making it easy to grow and high yielding. That is, Hikarishinseiki is now considered the first, and representative, "short-culm Koshihikari".

Moreover, short Koshihikari-type variety, such as Koshihikari Tsukuba SD No. 1 and Kinuhikari, using the *sd1* of indica origin (The DGWG allele has a 382-bp deletion in the 1st and 2nd exons), can make the application of DNA marker of *sd1* possible. Many isogenic lines of Koshihikari with indica genes are currently being developed. In contrast, the DNA markers could not be applied for Hikarishinseiki because *sd1* is of *japonica* origin (1 nucleotide substitution in the 1st exon). Therefore, selection based on the plant morphological genotype of *sd1* was required in this study. Finally, DNA monitoring of Hikarishinseiki confirmed its genomic identicalness to Koshihikari, except at *sd1*.

4.2. Production of Hikarishinseiki

Hikarishinseiki is now grown commercially in 21 prefectures, from Aomori in the north to Kagoshima in the south. In addition to its lodging resistance, other advantages have been reported anecdotally from farmers. For example, the erect growth habit improves pest and disease control and makes it easy to harvest by combine, thus making it suitable for cultivation in hilly and mountainous areas. It has achieved good grain yield (6.6 t/ha) under typical circumstances. In 2004, it achieved a good yield without lodging in spite of a direct hit of a typhoon in Tokushima prefecture (news report, Japan Broadcasting Corporation, 6 December 2004). Moreover, it did not lodge anywhere in response to typhoons 13 of 2004 and 14 of 2005. It was planted on 600 ha in 2008 and is expected to be planted on 1000 ha in 2009.

Usually, when a new cultivar is developed, each prefecture conducts performance tests in advance of the MAFF varietal registration examination, and distribution follows the completion of registration. However, since its official announcement, producers have spontaneously spread Hikarishinseiki, instead of awaiting the prefectural official recommendation. In response to requests by rice growers, Hikarishinseiki was declared as a brand rice description in Okayama and Tottori prefectures in 2007 (MAFF, 28 March 2007), in Tokushima prefecture in 2008 (MAFF, 27 March 2008), and in Niigata, Shiga and Kochi prefectures in 2009 (MAFF, 6 April 2009).

Isogenic Koshihikari-like lines with useful genes have been developed in many organizations (Ebitani et al., 2005; Ishizaki et al., 2005; Madoka et al., 2008; Takai et al., 2007; Takeuchi et al., 2006; Yamamoto et al., 2001). These lines can be reciprocally crossed to pyramid multiple useful genes. Hikarishinseiki can now contribute as an *sd1* donor in the Koshihikari background. RNAi gene silencing of *sd1* or *OsGA20ox2* is now available (Hedden, 2003a; Oikawa et al., 2004; Qiao et al., 2007). However, genetic modification by gene transformation is not publicly acceptable in many countries. In this study, Hikarishinseiki was developed by marker-assisted backcross breeding. Therefore, Hikarishinseiki is the first publicly acceptable, semidwarf form of the superior cultivar Koshihikari, whose flavor is globally appreciated, and can be used as a genetic donor of *sd1* alleles in the Koshihikari background.

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