Genomics and Applied Biology, 2011, Vol.2 No.5
http://gab.sophiapublisher.com

Research Report Open Access

Transcription of Rice Green Revolution Gene *sd1* is Clarified by Comparative RNA Diagnosis Using the Isogenic Background

Motonori Tomita [1] Shota Matsumoto [2]
1. Molecular Genetics Laboratory, Faculty of Agriculture, Tottori University, 101, Minami 4-chome, Koyama-cho, Tottori 680-8553, Japan
2. Molecular Genetics Laboratory, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
Corresponding author, tomita@muses.tottori-u.ac.jp; tomita206@gmail.com; Authors
Genomics and Applied Biology 2011, Vol.2 No.5 doi: 10.5376/gab.2011.02.0005
Received: 14 Nov., 2011
Accepted: 29 Nov., 2011
Published: 20 Dec., 2011

Preferred citation for this article:
Tomita et al., 2011, Transcription of Rice Green Revolution Gene *sd1* is Clarified by Comparative RNA Diagnosis Using the Isogenic Background, Genomics and Applied Biology, 2011, Vol.2 No.5 (doi: 10.3969/gab.2011.02.0005)

Abstract The *sd1* allele, on the long arm of chromosome 1, encoding a defective C20-oxidase in the gibberellin (GA) biosynthesis pathway (GA 20-oxidase, *OsGA20ox2*) confers the semidwarf phenotype that contributed to Green Revolution in rice. It has yet to be known whether *sd1* is transcribed. The two alleles at the *sd1/OsGA20ox2* locus of each line, the Japanese leading varieties Koshihikari and Hikarishinseiki having inherited over 99.8% of the Koshihikari genome, except for *sd1*, were successfully distinguished by RT-PCR amplification of the first exon followed by digestion with *Pma*CI. RNA extracted from the leaves and roots was employed as the template then the 779 bp fragment was clearly cleaved into the 613 bp and 166 bp fragments by *Pma*CI digestion in Hikarishinseiki, but not cleaved in Koshihkari. Therefore, the RNA diagnosis figured out clarified that *sd1* gene derived from Jukkoku was transcribed in Hikarishinseiki. It would be a first evidence of the transcription of *sd1*. Moreover, the taste and quality of Hikarishinseiki were in line with that of Koshihikari across Japan during two years, and it might be practically valuable as a brand rice description in the rice-producing districts of 13 prefectures in Japan.

Keywords Rice (*Oryza sativa* L); Semidwarf; Green Revolution; *sd1*; Transcription; RNA diagnosis; Trait performance; Lodging resistance

Background

The development of high-yielding semidwarf varieties of wheat and rice led to a rapid increase in the global production of cereal grains, which more than doubled from 1960 to 1990 (Khush, 1999). The semidwarf rice variety, IR8, was developed by using the Chinese landrace 'Dee-geo-woo-gen' (DGWG) and released by the International Rice Research Institute (IRRI). It was known as "miracle rice" that responds well to fertilizer and produces an increased yield without culm elongation. Widespread adoption of the miracle rice brought about a global "green revolution", particularly in the monsoonal regions of Asia, where typhoons frequently occur during the harvesting season (Athwal, 1971; Khush, 1999).

The semidwarf character is one of a very important agronomic trait in crop breeding. In other countries, many other short-culm cultivars were developed using an independent source of semidwarfism germplasms, such as the Japanese indigenous landrace 'Jukkoku' (Okada et al., 1967), or γ-ray-induced semidwarf cultivars such as 'Reimei' in Japan and 'Calrose 76' in the USA (Foster and Rutger, 1978). Although dwarf varieties of rice have contributed to the dramatic improvement and stabilization of yields worldwide, the dwarf stature of varieties derived from native or mutant maternal lines happens to be controlled by a single dwarf gene, *sd1* (Kikuchi and Futsuhara, 1997; Monna et al., 2002; Spielmeyer et al., 2002; Sasaki et al., 2002; Ashikari et al., 2002). The *sd1* allele is located on the long arm of chromosome 1 (Cho et al., 1994a; 1994b; Maeda et al., 1997), which confers the semidwarf phenotype without detrimental effects on grain yield (Hedden, 2003a; 2003b).

In Japan, Koshihikari suffers considerable lodging damage as a result of frequent powerful typhoons, and

thus, developing of lodging-resistant cultivar has been a longstanding challenge. The author developed a dwarf Koshihikari-type cultivar, 'Hikarishinseiki' (Tomita, 2009; rice cultivar No.12273, Ministry of Agriculture, Forestry and Fisheries of Japan) using a dwarf cultivar line with the heading time same as Koshihikari-type, which was selected by intercrossing the F_4 of Kanto No.79 (early-heading mutant line derived from Koshihikari) and Jukkoku (a cultivar with a semi-dwarf gene, *sd1*), as the maternal parent, and backcrossed it with Koshihikari 8 times. Having over 99.8% background of the Koshihikari genome, except for *sd1*, Hikarishinseiki would be the first cultivar to be registered as a Koshihikari-type dwarf with *sd1* in Japan (Tomita, 2009).

The dwarf feature of Hikarishinseiki is derived from the semidwarf gene *sd1*. *sd1* is a defective gene of *GA20ox−2*, which encodes a defective C20−oxidase in the gibberellin (GA) biosynthesis pathway (GA 20−oxidase, *OsGA20ox2*) (Monna et al., 2002; Spielmeyer et al., 2002; Sasaki et al., 2002; Ashikari et al., 2002). However, it has yet to be known whether the *sd1* gene possessed by Hikarishinseiki is transcribed. Subsequently, expression analysis was conducted by employing the RT-PCR method and *Pma*CI restriction enzyme digestion in order to clarify whether or not the *sd1* gene carried by Hikarishinseiki is transcribed.

An increasing number of private agricultural producers across Japan have spontaneously started to grow Hikarishinseiki as a typhoon-resistant, easy-to-grow cultivar instead of Koshihikari. As the recommended cultivars become widespread, there will become a growing need for information that rewards producers for their efforts. Here, we analyzed the results of 24 performance tests aimed at determining recommended cultivars, carried out over a period of two years from 2006 to 2007.

1 Results

1.1 Transcription of *sd1*, defective allele of *GA20ox-2*

We performed expression analysis of the *sd1* gene, which is introduced in the dwarf Koshihikari-type paddy rice variety 'Hikarishinseiki'. The two alleles at the *sd1/OsGA20ox2* locus on chromosome 1 of each line, Koshihikari and Hikarishinseiki, were distinguished by RT-PCR amplification of the first exon followed by digestion with *Pma*CI (Figure 1; Figure 2). A 779 bp fragment, employing one type of upper primer (F1) designed from the 1st exon and one type of downstream primer (R4) designed from the range of the 2nd to 3rd exon, contained only the exon. Moreover, 1,316 bp, 1,304 bp, 1,400 bp, and 1,366 bp fragments employing the four upper primers (F1~F4) designed from the 1st exon and the downstream primer (R6) designed from the 3rd exon, contained the 2nd intron. Lastly, 1,428 bp, 1,416 bp, and 1,478 bp fragments, obtained using three upstream primers (F1~F3) designed from the 1st exon and the RT primer (R5), contained both the 1st intron and 2nd intron. The target fragment was detected in these eight combinations of primers.

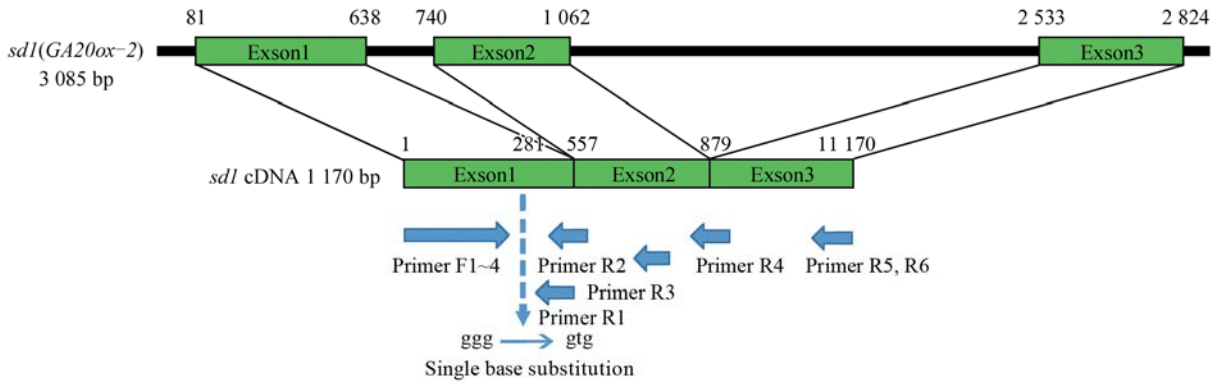

Figure 1 PCR primers designed for amplification of cDNA derived from *sd1* (*GA20ox−2*) transcript

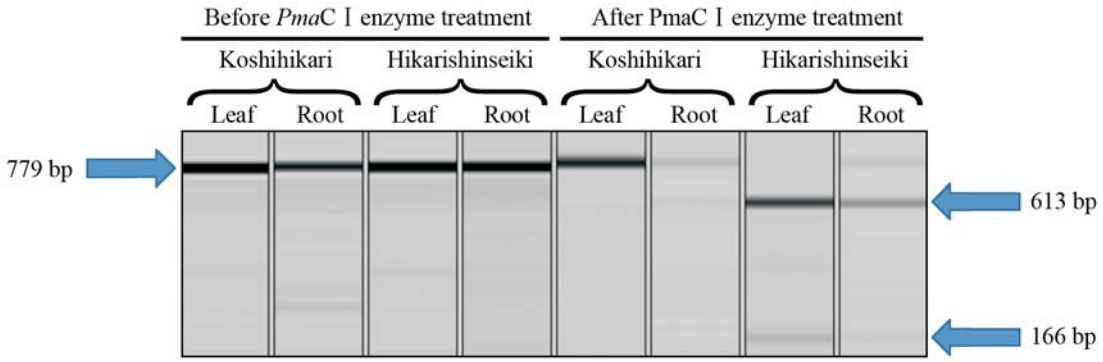

Figure 2 PCR amplification of cDNA from *sd1* (*GA20ox−2*) transcript and their *Pma*CI-digestion

Note: Left four lanes are PCR product by primers F1 and R4, and right four lanes show the fragments after PmaCI-digestion. Template RNAs ware extracted from leaf and root of Koshihikari and Hikarishinseiki, respectively. *Pma*CI split the PCR product from Hikarishinseiki into two fragments, while the PCR product from Kosihikari remained as a single fragment

RNA extracted from the root was used as the template then the 779 bp fragment by primers F1 and R4 was cleaved into the 613 bp and 166 bp fragments by *Pma*CI digestion in Hikarishinseiki (Figure 2). Accordingly, it can be said that *sd1* was transcribed in Hikarishinseiki.

1.2 Trait expression of *sd1* in the isogenic background through multiregional tests

We analyzed the data of performance tests conducted in 17 regions across Japan over a period of two years from 2006 to 2007 (Table 1; Table 2).

Heading: During the 2−year study period, Hikarishinseiki headed 'extremely early', on average 0.25 days earlier than Koshihikari.

Plant height: The average height of Hikarishinseiki is 72.2 cm (77% of Koshihikari, range 74%~81%), which is on average 20.6 cm (23%) shorter than Koshihikari. There was very little difference in the national average values for plant height and other characteristics during the 3–year study period, and as a result, shown values represent 3−year averages.

Degrees of lodging: The average degree of lodging was 0.2, which is between ±0~+3.8, indicating a significant improvement in lodging resistance.

Ear length: Ear length was 18.3 cm (98% that of Koshihikari), and the ratio to Koshihikari ear length ranged from 90% to 107%.

Number of ears: The average number of ears was 429/m^2 (109% that of Koshihikari), and the ratio to Koshihikari ear number ranged from 96% to 117%. In Ishikawa prefecture, the number of ears was 10% more than that of Koshihikari in 2 consecutive years, and numbers were higher than Koshihikari in 90% of prefectures. Having a large number of ears is one of the notable characteristics of Hikarishinseiki.

Brown rice yield: The average brown rice yield over the 2 years was 56.5 kg/a (101% that from Koshihikari), but the ratio to that from Koshihikari varied signify-cantly between regions from 86% to 124%. In some regions, average brown rice yield was ≥7% than that of Koshihikari in 2 consecutive years (Nagano: +13% in 2006, +8% in 2007, Ishikawa: +7% in 2006, +24% in 2007, Tottori: +8% in 2005, +9% in 2006), while in other areas (Toyama and Tokushima) they were ≤5%, indicating regional differences in yield. Moreover, the yield and number of ears did not necessarily correspond. In some areas, Hikarishinseiki showed a larger yield but similar number of ears compared to Koshihikari.

Grain weight of brown rice: The average grain weight of Hikarishinseiki brown rice was 22.0 g (101% that of Koshihikari, ranging from 96% to 105%).

Brown rice quality: The average brown rice quality was 4.6 and the difference with that of Koshihikari ranged from +1.0 to −1.5. This score indicates ‘medium’ quality, the same class quality given to Koshihikari.

Taste: Overall taste was on average −0.1 using

Koshihikari as the base cultivar. This score indicates a 'better than average' rating, equaling that of Koshihikari.

The difference between Hikarishinseiki and Koshihikari ranged from +0.25 to −0.64, and the average was −0.06 when Ishikawa prefecture (2005: −0.64, 2007: −0.59), where stricter taste standards are applied, was not included.

As shown above, although the characteristics of Hikarishinseiki as a dwarf variety of Koshihikari have now been clarified, significant regional differences in yield were revealed compared to Koshihikari. This may give us clues as to how yield can be further increased.

2 Discussion

Although Koshihikari is a dominant variety of rice, representing 40% of the rice cropping area in Japan, problem arises as a result of lodging during heavy rainstorms. In order to solve this problem, the dwarf Koshihikari-type paddy rice variety 'Hikarishinseiki', which shows stronger lodging resistance than that of Koshihikari and has 99.8% or more of the Koshihikari genome, was developed.

'Koshihikari sd1' was developed from first crossing 'Jukkoku' (*sd1sd1*) with 'Kanto No.79' (*Sd1Sd1*), an early-maturing mutant of 'Koshihikari' (Tomita, 2009). The pedigree method was conducted in breeding program during the later generations of 'Kanto 79' × 'Jikkoku', and then the dwarf *sd1* homozygous line ('Jikkoku'-type 'Koshihikari') whose heading date was the same as 'Koshihikari', was selected and fixed in the F_4 generation. The flanking substituted region adjacent to *sd1* had been restricted by eight recurrent backcrossing between the *Sd1sd1* descendants of 'Jikkoku'-type 'Koshihikari' short line and 'Koshihikari' (*Sd1Sd1*) as the recurrent parent. The semidwarf phenotype (*sd1sd1*) was done by the BC_8F_3 generation, therefore in which 'Koshihikari sd1' line carried ≥99.8% of 'Koshihikari' background in their genome.

In this study, the two alleles at the *sd1/OsGA20ox2* locus on chromosome 1 of each line, Koshihikari and Hikarishinseiki, were successfully distinguished by RT-PCR amplification of the first exon followed by digestion with *Pma*CI. RNA extracted from the root was employed as the template then the 779 bp fragment was clearly cleaved into the 613 bp and 166 bp fragments by *Pma*CI digestion in Hikarishinseiki, but not cleaved in Koshihkari. Therefore, it is concluded that *sd1* gene derived from Jukkoku was transcribed in Hikarishinseiki. This study would be a first evidence of the transcription of *sd1*, a defective gene of *GA20ox−2*, which contributed to Green Revolution in rice.

Hikarishinseiki would be considered to be an alternative to Koshihikari, being resistant to typhoon damage and easy to grow, and it was designated as a brand rice description in the rice-producing districts in Okayama, Tottori, Tokushima, Niigata, Kochi, Shiga, Mie, Kagawa, Hyogo, Kyoto, Hiroshima, Tochigi, and Kumamoto prefectures (by courtesy of Ministry of Agriculture, Forestry and Fisheries of Japan). In this study, the taste and quality of Hikarishinseiki were in line with Koshihikari across Japan during two years, and it would be practically valuable from the standpoint of production and distribution. Nowadays, preliminary tests to determine recommended cultivars have been carried out extensively across Japan, with three prefectures, Tottori, Wakayama, and Kanagawa, having proceeded to main tests.

3 Methods

3.1 Transcription analysis of *sd1*

Expression analysis of *sd1* was conducted by RT-PCR assay using RNA extracted from the leaves of Hikarishinseiki and Koshihikari grown since June 2009 at the farm of Tottori University and the roots of Hikarishinseiki and Koshihikari germinated on a Petri dish in the laboratory. After the reverse transcription reaction using an RT primer (R6: TCAGCTGGCCGC CTCGACCTGCGCCG) designed from the 3' end of *sd1* (Os01g0883800), RT-PCR was performed with 24 combinations of the following types of primers: four upstream primers (F1: GGAGCCCAAGATCCCGGA GCCATTCGTG, F2: CGACCTGAGGATGGAGCCC AAGATCCCG, F3: GACTCCACCGCCGGCTCTGG CATTGC, F4: CACGCCACCACAGCCGCACCAAC CAC designed from the 1st exon of *sd1*) and six downstream primers (R1: GAGGGTGCTGGAGAAG TAGTCGGCGAC designed from the 1st exon of *sd1*, R2: CACCCTCCCCATTGGCGCGAAGTCGG from the range of the 1st to 2nd exon, R3: TACCATGAAG GTGTCGCCGATGTTGATGACC from the 2nd exon, R4: CGTTCGACAGCGCCATGAAGGTGTCGCC from the range of the 2nd to 3rd exon, R5: TCAGCTG GCCGCCTCGACCTG from the 3rd exon, and R6 mentioned above designed from the 3' end). Primer

designing was based on the public sequence of Nipponbare (http://rgp.dna.affrc.go.jp). Since *sd1* confers *GA20ox−2* and shares homology with *GA20ox−1* (Os03g0856700), the primer was designed from the region showing low homology between *GA20ox−2* and *GA20ox−1*, allowing distinct labeling of *GA20ox−2* and *GA20ox−1*, respectively. PCR amplification took place in a 20 μL containing: 10 ng genomic DNA, 1 μM of each primer, 0.4 mM dNTPs, 1× GC Buffer Ⅰ, 2.5 mM $MgCl_2$, and 0.5 unit of *LA Taq* polymerase (Takara) in a total volume of 20 μL. and using following cycling conditions: 35 cycles, 94℃ 30 s, 58℃ 30 s, and 72℃ 1 min. PCR products were treated with the restriction enzyme *Pma*CI (CAC↓GTG), which can detect a single base substitution site of *sd1* in the 1st exon of *sd1* from Hikarishinseiki according to Tomita (2009). The transcription product of *sd1* was then analyzed.

3.2 Performance test of *sd1* in the isogenic background

Performance tests were carried out in a paddy field at Tottori University, Koyama, during 2006 and 2007. 'Hikarishinseiki' and 'Koshihikari' were sown on 20 April 2006 and on 21 April 2007, and 128 seedlings per plot were transplanted with two replications on 15 and 17 May, respectively. Similar performance tests were conducted at experimental stations in Miyagi, Ibaraki, Kanagawa, Nagano, Mie, Toyama, Ishikawa Kyoto, Wakayama, Hyogo, Shimane, Okayama, Tokushima, Ehime, Oita and Kumamoto, which are spread across Japan.

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

Author's contributions

MT conceived and designed the study and wrote the manuscript. MT and SM performed the experiments and analyzed the data. Both of authors read and approved the final manuscript.

Acknowledgements

The authors would like to express our gratitude to Japan Science and Technology Agency (JST) for the Grant-in-Aid for Adaptable and Seamless Technology Transfer Program through Target-driven R & D (No.08150094 and No.08001167) that supported this work to Motonori Tomita. We thank to all those who provided test data from prefectures across Japan.

References

Ashikari M., Sasaki A., Ueguchi-Tanaka M., Itoh H., Nishimura A., Datta S., Ishiyama K., Saito T., Kobayashi M., Khush G.S., Kitan H., and Matsuoka M., 2002, Loss-of-function of a rice gibberellin biosynthetic gene, GA20 oxidase (*GA20ox-2*), led to the rice 'green revolution', Breed. Sci., 52(2): 143-150 http://dx.doi.org/ 10.1270/jsbbs.52.143

Athwal D.S., 1971, Semidwarf rice and wheat in global food needs, Quart. Review Biol., 46(1): 1-34 http://dx.doi.org/10.1086/406754 PMid:4927378

Cho Y.G., Eun M.Y., Kim Y.K., Chung T.Y., and Chae Y.A., 1994a, The semidwarf gene, *sd-1*, of rice (*Oryza sativa* L). 1. Linkage with the esterase locus, *EstI-2*, Theor. Appl. Genet., 89(1): 49-53 http://dx.doi.org/10.1007/BF00226981

Cho Y.G., Eun M.Y., McCouch S.R., and Chae Y.A., 1994b, The semidwarf gene, *sd-1*, of rice (*Oryza sativa* L.). 2. Molecular mapping and marker-assisted selection, Theor. Appl. Genet., 89(1): 54-59 http://dx.doi.org/10.1007/BF00226982

Foster K.W. and Rutger J.N., 1978, Inheritance of semidwarfism in rice, *Oryza sativa* L., Genetics, 88(1): 559-574 PMid: 17248812; PMCid: 1224601

Hedden P., 2003a, Constructing dwarf rice, Nature Biotechnol., 21(8): 873-874 http://dx. doi.org/10.1038/nbt0803-873 PMid:12894201

Hedden P., 2003b, The genes of the Green Revolution, Trends Genet., 19(1): 5-9 http:// dx.doi.org/10.1016/S0168-9525(02)00009-4

Kashiwagi T., Togawa E., Hirots, N. and Ishimaru K., 2008, Improvement of lodging resistance with QTLs for stem diameter in rice (*Oryza sativa* L.), Theor. Appl. Genet., 117(5): 749-757 http://dx.doi.org/10.1007/s00122-008-0816-1 PMid:18575836

Khush G.S., 1999, Green revolution: preparing for the 21st century, Genome, 42(4): 646-655 http://dx.doi.org/10.1139/g99-044 PMid: 10464789

Kikuchi F., and Futsuhara Y., 1997, Inheritance of morphological characters. 2. Inheritance of semidwarf, In: Matsuo T., Shimizu S., Tsunoda S., Murata Y., Kumazawa K., Futsuhara Y., Hoshikawa K., Yamaguchi H. and Kikuchi F., editors, Science of the Rice Plant, Vol. 3, Tokyo: Tokyo Food and Agricultural Policy Research Center, pp.309-317

Maeda H., Ishii T., Mori H., Kuroda J., Horimoto M., Takamur, I., Kinoshita T., and Kamijima O., 1997, High density molecular map of semidwarfing gene, *sd-1*, in rice (*Oryza sativa* L.), Breed. Sci., 47(4): 317-320

Monna L., Kitazawa N., Yoshino R., Suzuki J., Masuda H., Maehar, Y., Tanji M., Sato M., Nas, S. and Minobe Y., 2002, Positional cloning of rice semidwarfing gene, *sd-1*: Rice "Green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis, DNA Res., 9(1): 11-17 http://dx.doi.org/10.1093/dnares/9.1.11 PMid:11939564

Okada M., Yamakawa Y., Fujii K., Nishiyama H., Motomura H., Kai S., and Imai T., 1967, On the new varieties of paddy rice, 'Hoyoku', 'Kokumasari' and 'Shiranui', Bull. Kyushu Agr. Exp. Sta., 12(3, 4): 187-224

Sasaki A., Ashikari M., Ueguchi-Tanaka M., Itoh H., Nishimura A., Swapan D., Ishiyama K., Saito T., Kobayashi M., Khush G.S., Kitano H., and Matsuoka M., 2002, Green revolution: a mutant gibberellin-synthesis gene in rice, Nature, 416(6882): 701-702 http://dx.doi.org/10.1038/416701a PMid:11961544

Spielmeyer W., Ellis M.H., and Chandler P.M., 2002, Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene, Proc. Natl. Acad. Sci., USA, 99(13): 9043-9048 http://dx.doi.org/10.1073/pnas.132266399 PMid:12077303 PMCid:124420

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(2): 173-181 http://dx.doi.org/10.1016/j.fcr.2009.05.004

Table 1 Comparison of agronomic characters of Koshihikari and Hikarishinseiki in 2006

Experimental locations	Cultivars	Heading date (m.d)	Maturity date (m.d)	Culm length (cm)	Panicle length (cm)	No. of Panicles (No./m^2)	Grain yield (kg/a)	1000-grain weight (g)	(1) Grain Quality	(2) Lodging degree	(3) Leaf blast score	(4) Panicle blast score	(5) Value of taste	(6) Eating quality	(7) Amylose content (%)	Protein content of brown rice (%)
Miyagi	Koshihikari	8.22	10.09	96.4	18.4	378	51.9	22.1	5.0	2.0			88.6			6.6
	Hikarishinseiki	8.22	10.10	75.5	17.7	406	46.6	22.0	5.0	0.0			86.3			6.8
Ibaraki	Koshihikari	8.06	9.15	88.4	21.0	374	60.4	21.8	5.0	0.5	1.3	0.8		0.00		6.9
	Hikarishinseiki	8.06	9.15	69.7	20.3	429	57.0	22.1	5.5	0.0	0.8	0.5		−0.53		7.2
Nagano	Kosdhihikari	8.09	9.21	96.0	18.7	496	67.5	20.2	4.0	5.0		0.0		0.00		
	Hikarishinseiki	8.09	9.25	76.0	18.3	500	76.2	20.9	5.0	1.0		0.0		−0.06		
Ishikawa	Koshihikari	8.05	9.10	91.8	18.0	360	56.6	21.9	3.0	3.0	0.0	0.0	84.6	0.00		6.7
	Hikarishinseiki	8.06	9.13	68.5	17.5	401	60.3	22.1	3.5	0.0	0.0	0.0	82.0	−0.64		7.2
Kyoto	Koshihikari	8.05	9.06	89.3	19.2	428	62.4	22.0	6.3	2.7				0.00		5.4
	Hikarishinseiki	8.04	9.08	71.2	19.1	427	68.1	22.4	8.0	0.0				−0.18		5.7
Hyogo	Koshihikari	8.11	9.14	87.9	18.1	416	53.8	22.4	4.0	1.0	0.5	0.0		0.00		
	Hikarishinseiki	8.11	9.13	78.2	19.2	435	51.1	21.8	5.0	0.0	0.0	0.0		−0.25		
Tottori	Koshihikari	8.08	9.19	91.0	19.5	326	54.4	23.9	4.0	2.9			60.0	0.00		
	Hikarishinseiki	8.08	9.19	71.0	18.0	369	59.1	24.1	4.5	0.1			59.0	0.05		
Shimane	Koshihikari	7.31	9.02	87.5	19.2	375	56.0	22.0	6.6	1.8	0.0	0.0	86.0	0.00		5.9
	Hikarishinseiki	7.30	8.29	67.3	17.3	415	54.3	22.1	7.0	0.0	0.0	0.0	85.0	0.06		5.6
Tokushima	Koshihikari	7.14	8.20	90.9	19.4	389	39.2	20.4	5.0	3.0	0.0	0.0				
	Hikarishinseiki	7.14	8.21	71.9	18.9	436	36.5	21.4	5.0	1.0	0.0	0.0				
Ehime	Koshihiksri	8.12	9.15	89.0	21.1	308	50.3	23.6	5.0	3.0			68.0	0.00	20.3	
	Hikarishinseiki	8.13	9.15	70.0	19.6	368	50.9	23.6	5.0	0.0			64.0	−0.13	20.2	
Kumamoto	Koshihikari	8.08	9.26	89.0	18.8	373	54.9	22.0	3.5	4.5	0.8	1.0		0.00		7.6
	Hikarishinseiki	8.08	9.24	69.0	18.8	381	56.4	22.7	3.8	1.0	0.8	1.0		0.22		8.1
Average	Koshihikari	8.06	9.14	90.7	19.2	383	55.2	22.1	4.7	2.7	0.4	0.3	77.4	0.00	20.3	6.5
	Hikarishinseiki	8.06	9.15	71.7	18.6	415	56.1	22.3	5.2	0.3	0.3	0.2	75.3	−0.16	20.2	6.8

Note: (1) Grain quality was classified into nine grade; 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) 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%; (4) 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%; (5) Value of taste was determined using a Taste-meter MA-90B (Tokyo Rice-producing Machine Factory, Japan); (6) Eating quality show the aggregate evaluation and classified into eleven degree; 5: excellent good to -5: especially bad.; (7) Amylose and protein content was measured by Near Infrared Spectrometer AN800 (Kett Electric Laboratory, Japan)

Table 2 Comparison of agronomic characters of Koshihikari and Hikarishinseiki in 2007

Experimental locations	Cultivars	Heading date (m.d)	Maturity date (m.d)	Culm length (cm)	Panicle length (cm)	No. of Panicles (No./ m^2)	Grain yield (kg/a)	1000-grain weight (g)	(1) Grain Quality	(2) Lodging degree	(3) Leaf blast score	(4) Panicle blast score	(5) Value of taste	(6) Eating quality	(7) Amylose content (%)	Protein content of brown rice (%)
Miyagi	Koshihikari	8.19	10.03	95.0	17.5	413	58.0	22.6	2.8	2.3	1.3	2.3		0.00		
	Hikarishinseiki	8.18	10.01	71.6	16.9	444	55.7	22.5	2.3	0.0	1.3	1.9		−0.43		
Ibaraki	Koshihikari	8.01	9.09	81.0	19.7	493	59.4	21.1	5.0	2.3	0.0	0.0		0.00		6.3
	Hikarishinseiki	7.31	9.09	65.2	18.9	520	57.3	21.1	5.0	0.0	0.0	0.0		0.12		6.7
Kanagawa	Koshihikari	8.12	9.20	89.6	17.2	334	36.1	20.6	5.5	4.0		0.0	68.0	0.00		
	Hikarishinseiki	8.11	9.20	76.7	17.2	379	44.3	21.9	3.5	1.0		0.0	69.0	0.07		
Nagano	Kosdhihikari	8.10	9.20	102.0	19.0	574	67.1	20.2	5.0	5.0	3.0	0.0		0.00		
	Hikarishinseiki	8.10	9.23	75.0	18.6	578	72.8	20.1	5.0	1.0	1.5	0.0		−0.20		
Mie	Koshihikari	7.23	8.25	85.3	19.8	420	58.3	21.5	5.0	1.9	0.2	0.3		0.00		6.2
	Hikarishinseiki	7.22	8.26	65.1	18.6	473	54.1	21.8	5.5	0.0	0.0	0.3		0.10		6.3
Toyama	Koshihikari	8.12	9.19	84.6	18.9	348	54.4	23.5	2.6	2.2	0.0	0.0	79.0	0.00		4.9
	Hikarishinseiki	8.12	9.18	65.2	17.4	405	51.4	22.2	3.9	0.0	0.5	0.0	77.8	0.00		5.1
Ishikawa	Koshihikari	8.09	9.14	99.5	18.6	413	51.8	21.5	3.0	3.8	0.0	0.0	75.0	0.00	17.2	6.5
	Hikarishinseiki	8.07	9.12	73.8	17.8	485	64.0	20.9	4.5	0.0	0.0	0.0	72.0	−0.59	16.5	7.0
Wakayama	Koshihikari	8.13	9.13	84.8	18.2	317	53.8	22.1	3.0	0.0	0.0	0.0				
	Hikarishinseiki	8.13	9.14	67.8	18.3	313	56.2	22.0	3.0	0.0	0.0	0.0				
Hyogo	Koshihikari	8.11	9.13	95.2	18.9	396	58.0	21.3	5.5	0.8	1.0	0.0		0.00		
	Hikarishinseiki	8.09	9.10	76.0	18.4	355	58.3	21.5	6.5	0.0	1.3	0.0		−0.21		
Okayama	Koshihiksri	8.11	9.12	95.9	18.5	309	55.7	21.6	5.0	1.5	0.0	0.0				5.6
	Hikarishinseiki	8.12	9.14	76.6	18.9	306	48.3	22.2	4.0	0.0	0.0	0.0				5.8
Tokushima	Koshihikari	7.15	8.19	87.0	17.5	443	53.0	20.4	4.5	3.5	0.0	0.0				
	Hikarishinseiki	7.15	8.18	67.9	17.1	479	49.1	21.4	5.0	0.0	0.0	0.0				
Ehime	Koshihikari	7.16	8.18	88.0	18.9	352	48.7	22.0	5.3	2.5						
	Hikarishinseiki	7.15	8.18	65.0	17.6	401	54.0	22.1	5.3	0.0						
Oita	Koshihikari	7.30	9.08	79.0	18.6	414	62.2	21.3	3.0	1.0	0.0	0.0	88.0	0.00		6.9
	Hikarishinseiki	7.30	9.11	58.0	18.0	438	61.9	21.5	2.5	0.0	0.0	0.0	85.0	−0.22		7.2
Average	Koshihikari	8.05	9.10	89.8	18.6	402	55.1	21.5	4.3	2.4	0.5	0.2	77.5	0.00	17.2	6.1
	Hikarishinseiki	8.04	9.10	69.5	18.0	429	56.0	21.6	4.3	0.2	0.4	0.2	76.0	−0.15	16.5	6.4
Average for 2 years	Koshihikari	8.03	9.09	91.1	18.9	399	56.2	21.8	4.3	2.5	0.3	0.3	76.5	0.00	17.9	6.5
	Hikarishinseiki	8.03	9.09	71.2	18.3	431	57.4	22.0	4.6	0.4	0.2	0.2	75.3	−0.07	17.6	6.7

Note: (1) Grain quality was classified into nine grade; 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) 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%; (4) 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%; (5) Value of taste was determined using a Taste-meter MA-90B (Tokyo Rice-producing Machine Factory, Japan); (6) Eating quality show the aggregate evaluation and classified into eleven degree; 5: excellent good to -5: especially bad.; (7) Amylose and protein content was measured by Near Infrared Spectrometer AN800 (Kett Electric Laboratory, Japan)