

Combining two semidwarfing genes *d60* and *sd1* for reduced height in ‘Minihikari’, a new rice germplasm in the ‘Koshihikari’ genetic background

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Summary

Dwarfing in rice has dramatically improved and stabilized rice yields worldwide, often controlled by a single dwarf gene, *sd1*. A novel semidwarf gene *d60* complements the gametic lethal gene *gal*, such that the F₁ between ‘Hokuriku 100’ (genotype *d60d60GalGal*, *Gal*: mutant non-lethal allele) and ‘Koshihikari’ (*D60D60galgal*, *D60*: tall allele) would show 25% sterility due to deterioration of gametes bearing both *gal* and *d60*. The F₂ would segregate as one semidwarf (1 *d60d60GalGal*):two tall and 25% sterile (2 *D60d60Galgal*):six tall (2 *D60d60GalGal*:1 *D60D60GalGal*:2 *D60D60Galgal*:1 *D60D60galgal*), skewed from a Mendelian segregation ratio of one semidwarf:three tall for a single recessive gene. To pyramid *d60* and *sd1*, into the Japanese super-variety ‘Koshihikari’, the F₁ (*D60d60Galgal*) of ‘Koshihikari’ × ‘Hokuriku 100’ was first backcrossed with ‘Koshihikari’, and the BCF₁ segregated into a ratio of one tall and 25% sterile (*D60d60Galgal*):two tall (1 *D60D60Galgal*:1 *D60D60galgal*). Tall, 25% sterile BC₁F₁ plants (*D60d60Galgal*) were then selected for pollen sterility and backcrossed with ‘Koshihikari’ as the recurrent parent. It is unnecessary to grow out and select a semidwarf from the BC_nF₂ if a pollen parent with ~70% pollen fertility is chosen from the BC_nF₁ to backcross with the recurrent parent. Semidwarfing genes *d60* and *sd1* were successfully pyramided into the ‘Koshihikari’ genome by crossing isogenic lines ‘Koshihikari *d60*’ and ‘Koshihikari *sd1*’, to produce ‘Minihikari’, a new parental source of both *d60* and *sd1*. ‘Minihikari’ displayed super-short stature due to the combination of *sd1* and *d60*, which are genetically and functionally independent.

1. Introduction

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

The semidwarf characteristic is a very important agronomic trait for crop breeding. In other countries, many other short-culm cultivars, such as the Japanese indigenous landrace ‘Jikkoku’ (Okada *et al.*, 1967), or γ -ray-induced semidwarf cultivars such as ‘Reimei’ in Japan and ‘Calrose 76’ in the USA (Foster & Rutger, 1978) were developed using independent sources of semidwarfism. Dwarf varieties of rice have contributed to the dramatic improvement and stabilization of yields worldwide, and although derived from several native or mutant maternal lines, dwarf stature is controlled by a single dwarf gene, *sd1* (Kikuchi & Futsuhara, 1997; Ashikari *et al.*, 2002; Monna *et al.*, 2002; Sasaki *et al.*, 2002; Spielmeier *et al.*, 2002). The *sd1* alleles on the long arm of chromosome 1 (Cho *et al.*, 1994a, b; Maeda *et al.*, 1997) encode a defective C20-oxidase in the gibberellin (GA) biosynthesis pathway (GA 20-oxidase, OsGA20ox2) (Ashikari *et al.*, 2002; Monna *et al.*, 2002; Sasaki *et al.*, 2002; Spielmeier *et al.*, 2002).

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The *sd1* gene confers the semidwarf phenotype with no detrimental effects on grain yield (Hedden, 2003a, b), but other options for dwarf breeding are limited.

A narrow genetic base, or monoculture, frequently leads to outbreaks of diseases or pests. The most dramatic recent pest epidemic to hit food crops because of genetic uniformity was the 1970 attack of southern corn leaf blight (SCLB) in the US maize (*Zea mays* L.) crop. Pathogen outbreaks also struck crops of potato in Ireland in the late 1840s; coffee in Sri Lanka in the 1870s; banana throughout the Caribbean; wheat in the USA in 1916; rice in Bengal and India in 1942; and oats in the USA in 1946 (Kaneko *et al.*, 2000). Therefore, a wider range of genetic diversity to cope with future environmental changes is essential. In Japan, 'Koshihikari', released in 1953, has been a leading cultivar since 1979. It was grown on 601 100 ha in 2009, accounting for over 37% of the total Japanese rice-growing area. 'Koshihikari' is also grown in various other countries (e.g. in the United States and Australia; available at: <http://www.tdb.maff.go.jp/toukei/a02stopframeset>). The demand for 'Koshihikari' is high because of its good nutritional quality, high resistance to pre-harvest sprouting, tolerance of cool temperatures at booting stage and wide adaptability. In contrast to its advantages, 'Koshihikari', with a culm length from 90 to 100 cm, has a serious disadvantage of poor lodging resistance (Kashiwagi *et al.*, 2008). Lodging caused by autumnal rains and typhoons hinders harvest and reduces yield and grain quality. Therefore, genetic improvement of 'Koshihikari' to resist lodging is critical.

To find a new dwarf gene to replace *sd1*, analyses focused on 'Hokuriku 100', a mutant line with culms ~15 cm shorter in length, close to 20% shorter than those of 'Koshihikari'. 'Hokuriku 100' is the most promising semidwarf mutant selected from ⁶⁰Co γ -irradiated 'Koshihikari' in a large-scale mutation breeding operation to enhance lodging resistance (Samoto & Kanai, 1975). The author discovered a novel dwarf gene, *d60*, together with a gametic lethal gene, *gal*, in a cross between semidwarf mutant 'Hokuriku 100' and its original tall variety 'Koshihikari' (Tomita *et al.*, 1989; Tanisaka *et al.*, 1990; Tomita, 1996). The gene *d60* was thought to have a unique mode of inheritance that complements the gametic lethal gene, *gal*. According to this hypothesis, the F₁ between 'Hokuriku 100' (genotype *d60d60GalGal*, *Gal*: non-lethal mutant allele) and 'Koshihikari' (*D60D60galgal*, *D60*: tall allele) should show 25% sterility because of the deterioration of gametes bearing both *gal* and *d60*. The F₂ progenies also segregated in a ratio of one semidwarf (1 *d60d60GalGal*):two tall and 25% sterile (2 *D60d60Galgal*):six tall (2 *D60d60GalGal*:1 *D60D60GalGal*:2 *D60D60Galgal*:1 *D60D60galgal*) (Tomita, 1996), which was skewed

from the expected Mendelian ratio for a single recessive gene of one semidwarf : three tall. The induced semidwarfing gene *d60* confers a fine plant phenotype with erect leaves, about 15 cm shorter than those of the original variety, and may be a promising alternative to the widespread gene, *sd1*. In this study, based on the genetic hypothesis mentioned earlier, BC_nF₁ plants (*D60d60Galgal*) selected for pollen sterility caused by *d60* and *gal* and used as pollen parents to allow introgression of *d60* by successive backcrosses. This study also aimed to pyramid both semidwarfing genes, *d60* and *sd1*, into 'Koshihikari'. Finally, a useful parental line 'Minihikari' was developed as a new genetic source for both *d60* and *sd1*. 'Minihikari' displays super-short stature through successful combination of *sd1* with *d60*, which are genetically and functionally independent.

2. Materials and methods

(i) Development of a isogenic lines in 'Koshihikari' genetic background

(a) Development of an *sd1* isogenic line

The author previously developed 'Koshihikari' *sd1*, which was released as a cultivar 'Hikarishinseiki' (Tomita, 2009; rice cultivar no. 12273, Ministry of Agriculture, Forestry and Fisheries of Japan). Short-culmed 'Koshihikari'-like lines, homozygous for *sd1* and with same heading dates as those of 'Koshihikari', were selected from 100 self-pollinated F₃ lines raised from 100 randomly selected F₂ plants of 'Kanto 79' (an early heading mutant line derived from 'Koshihikari') × 'Jikkoku' (a cultivar with a semi-dwarf gene, *sd1*) and fixed at the F₄ generation in 1989 (Fig. 2). The short-culmed 'Koshihikari'-like line and its descendants were backcrossed with 'Koshihikari' (*Sd1Sd1*) as the recurrent parent eight times between 1990 and 1997. *Sd1sd1*-heterozygous plants could be selected by their slightly erect leaves in the first generation of each backcross (BC_nF₁) and then backcrossed by 'Koshihikari' as the female parent.

(b) Development of a *d60* isogenic line

Semidwarfism in the rice cultivar 'Hokuriku 100' is controlled by the single semidwarfing gene, *d60*. However, *d60* was thought to pleiotropically activate the gametic lethal gene, *gal*. According to this hypothesis, the F₂ progenies of a cross between 'Hokuriku 100' (genotype *d60d60GalGal*, *Gal*: gametic non-lethal allele) and the original tall variety 'Koshihikari' (*D60D60galgal*, *D60*: is the tall allele) exhibit segregation distortion in a ratio of one semidwarf (1 *d60d60GalGal*):two tall and 25% sterile (2 *D60d60Galgal*):six tall (2 *D60d60GalGal*:1

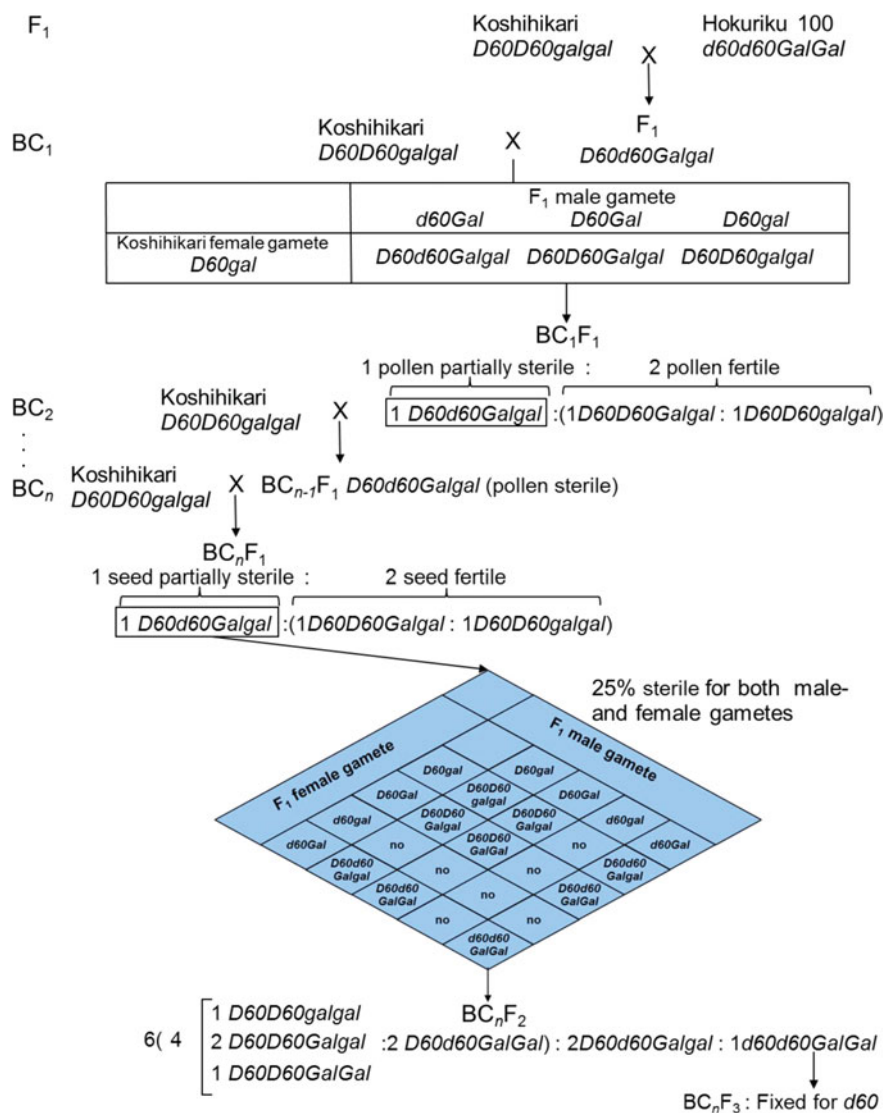


Fig. 1. Introgression of the *d60* dwarfing gene in relation to the gametic lethal-*gal* gene by backcross breeding.

D60D60GalGal : 2 *D60D60Galgal* : 1 *D60D60galgal*), because of the deterioration of the F₁ male and female gametes that carry both *gal* and *d60* (Fig. 1).

In this study, based on the genetic hypothesis mentioned earlier, BC_nF₁ plants (*D60d60Galgal*) were selected for pollen sterility caused by *d60* and *gal* and were used as pollen parents to introgress *d60* by successive backcrosses. Between 1987 and 1998, 'Koshihikari' was crossed with 'Hokuriku 100' (*d60d60GalGal*), and then backcrossed seven times to 'Koshihikari' (*D60D60galgal*) as the recurrent parent to minimize transmission of the flanking region of *d60* (Fig. 2). *D60d60Galgal*-heterozygous plants, recognized by pollen sterility prior to anthesis, were selected in the first generation of each backcross (BC_nF₁). The F₁ (*D60d60Galgal*) progenies of 'Koshihikari' × 'Hokuriku 100' were backcrossed to 'Koshihikari' (*D60D60galgal*) to produce 30 BC₁F₁ individuals that segregated in a ratio of one

tall and 25% sterile (*D60d60Galgal*):two tall (1 *D60D60Galgal* : 1 *D60D60galgal*). Tall and 25% sterile BC₁F₁ plants (*D60d60Galgal*) were then selected for pollen sterility prior to anthesis and backcrossed with 'Koshihikari' as female parent to produce 30 BC₂F₁ seeds. 'Koshihikari' was used as the female parent in each backcross generation. For each BC_nF₂ generation, 200–300 individuals were always grown out during the following year to confirm that short-culmed plants (*d60d60*) appeared in the BC_nF₂ generation, while backcrossing of 5–10 pollen sterile individuals from the BC_{n+1} F₁ was underway.

(c) Development of a line isogenic for *sd1* and *d60*

In 2000, 'Hikarishinseiki' ('Koshihikari *sd1*') was crossed with 'Koshihikari' *d60* and the double dwarf 'Koshihikari', containing both *sd1* and *d60*, was

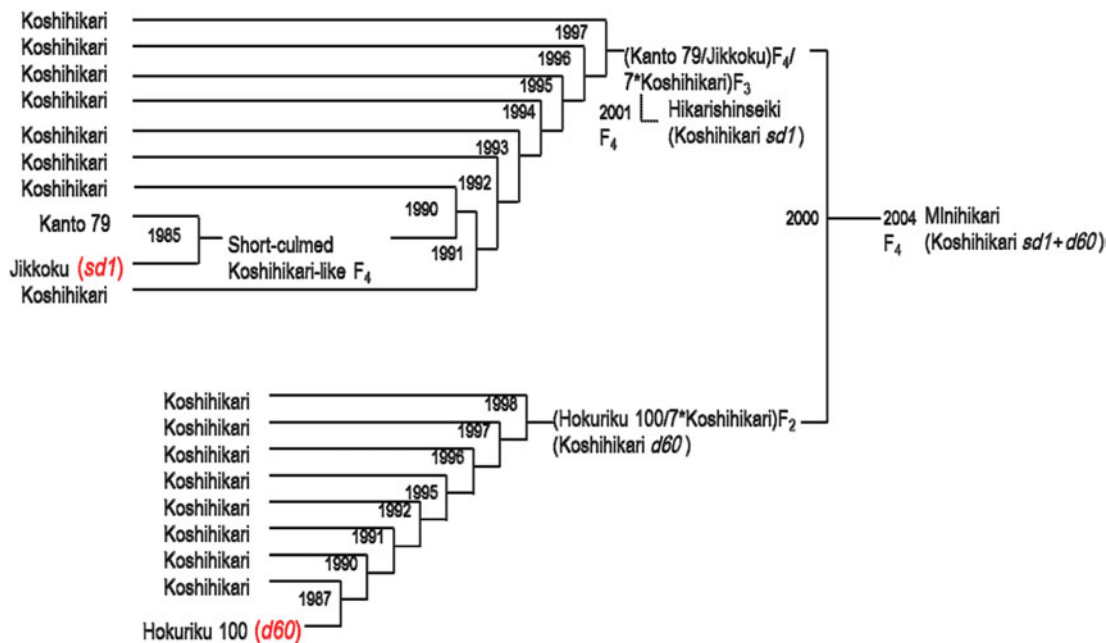


Fig. 2. Development of variety ‘Minihikari’, an isogenic line with *sd1* and *d60* dwarfing genes in the ‘Koshihikari’ genetic background.

selected from F_2 to F_4 generation and fixed in 2004 (Fig. 1). The super-short-culmed ‘Koshihikari’, designated as ‘Minihikari’, underwent varietal registration by the Ministry of Agriculture, Forestry and Fisheries (MAFF).

(d) Evaluation of *sd1* introgression

The two alleles at the *sd1/OsGA20ox2* locus on chromosome 1 of each line were distinguished by PCR amplification of the first exon of *sd1* followed by digestion of the PCR product with *PmaCI*. Primer design was based on the sequence reported for *sd1* from the rice variety ‘Nipponbare’ (<http://rgp.dna.affrc.go.jp>). Reaction mixtures contained 10 ng of template genomic DNA, 1 μ M each primer (F: 5'-GCTCGTCTTCTCCCCTGTTACAAATACCC-3'; R: 5'-ACCATGAAGGTGTCGCCGATGTTGATGACC-3'), 0.4 mM dNTPs, 1 \times GC buffer I, 2.5 mM $MgCl_2$, and 0.5 U *LA Taq* polymerase (Takara, Kyoto, Japan) in a total volume of 20 μ l. The PCR reaction program consisted of 35 cycles of 30 s at 94 $^{\circ}$ C, 30 s at 58 $^{\circ}$ C and 1 min at 72 $^{\circ}$ C. The ‘Jikkoku’ allele for *sd1* was detected by digestion of the PCR products with *PmaCI* (CACGTG) according to Tomita (2009).

(ii) Field evaluation of isogenic lines

Performance tests were conducted in a paddy at Tottori University, Koyama, during 2009 and 2010. ‘Minihikari’, ‘Koshihikari *d60*’, ‘Hikarishinseiki’ and

‘Koshihikari’ were sown on 18 April 2009 and 22 April 2010, and 128 plants per plot were transplanted with three replications on 14 and 18 May, respectively. In both years, planting density was 22.2 individual plants/m² (30 \times 15 cm), planted one by one. Fertilizers including N, P₂O₅ and K₂O were applied as dressings at 5, 13.6 and 7.2 kg/10 acre, respectively. Each replicate consisted of an area of 16 m² in each year, and plants were transplanted into three replications in a random order. The date at which 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 ten randomly selected plants in each plot. Based on the inclination angle between the base of the plant and the neck of panicles, plants were categorized into five groups by degree of lodging: standing (0), almost 70 (1), almost 50 (2), almost 30 (3), almost 10 (4), lodged (5). Thousand-grain weight, grain yield of brown rice, grain quality and eating quality were measured on bulks of 50 plants.

(iii) Data analyses

Data were analysed using analysis of variance (ANOVA) followed by *post hoc* tests to determine the statistical significance of the data, followed by multiple comparison analysis using the Holm method (Nagata & Yoshida, 2009), which is an improved version of the Bonferroni method.

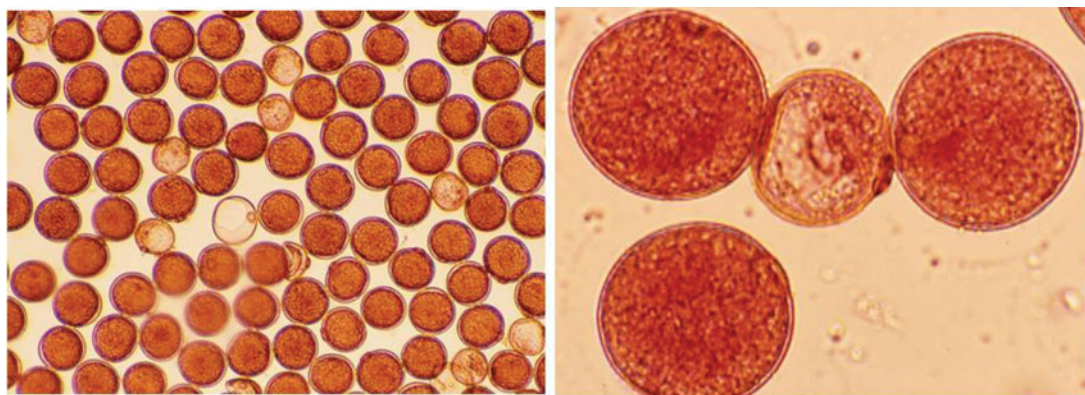


Fig. 3. Pollen observed in double heterozygous *D60* and *Gal* in BC_nF_1 plants.

3. Results

(i) Development of isogenic lines in 'Koshihikari' genetic background

(a) Development of an *sd1* isogenic line

The dwarf *sd1* homozygous strain ('Jikkoku'-type 'Koshihikari'), derived from the cross between Kanto 79' (*Sd1Sd1*) × 'Jikkoku' (*sd1sd1*) and with a heading date the same as 'Koshihikari', was selected by the pedigree breeding method and fixed in the F_4 generation (Tomita, 2009). After obtaining the 'Koshihikari' × ('Koshihikari' × 'Jikkoku'-type 'Koshihikari') BC_1F_1 (1 *Sd1Sd1* : 1 *sd1sd1*), continuous backcrosses were made between an *Sd1sd1* individual as the pollen parent in BC_nF_1 and the recurrent parent 'Koshihikari' until BC_8 (Fig. 2). The subsequent generation BC_nF_2 of pollen parent was developed simultaneously and a segregation of 3(1 *Sd1Sd1* : 2 *Sd1sd1*) : 1 *sd1sd1* was verified ($BC_8F_2 = 202 : 77$, $\chi^2 = 1.01$, $0.30 < P < 0.50$). The semidwarf phenotype 'Koshihikari *sd1*' (*sd1sd1*) was developed by the BC_8F_3 generation, at which point the plant genomes should be $\geq 99.8\%$ 'Koshihikari', based on theoretical expectations after eight recurrent backcrosses.

(b) Development of a *d60* isogenic line

The semidwarf gene *d60* from 'Hokuriku 100' has the potential to replace *sd1*, which is now widely distributed. In this study, semidwarf isogenic strains of 'Koshihikari' into which either *sd1* or *d60*, or both, had been introgressed, were developed to compare the effects of *sd1* and *d60* on aspects of the plant phenotype and on lodging resistance. The recessive *d60* mutation and the gametic lethal gene *gal*, which is present in 'Koshihikari' and other rice varieties, was thought to cause complementary lethality to both male and female gametes (Tomita, 1996). Accordingly, the pollen and ovule fertility of 'Koshihikari' (*D60D60galgal*) × 'Hokuriku 100' (*d60d60GalGal*) F_1 progeny is $\sim 70\%$, and the F_2 segregates six fertile,

long culm (4 *D60D60* : 2 *D60d60GalGal*): two partial sterile, long culm (F_1 type = (*D60d60Galgal*): one semidwarf (*d60d60GalGal*) (Fig. 1).

'Koshihikari' × ('Hokuriku 100' × 'Koshihikari') BC_1F_1 segregated 11 : 6, which approximately fit a ratio of two fertile, long culm (*D60D60Galgal* : *D60D60galgal*): one partial sterile long culm (*D60d60Galgal*) (Fig. 3). Partially sterile individuals were thought to be heterozygous *d60*, and the BC_1F_2 it segregated clearly into six fertile, long culm (4 *D60D60* : 2 *D60d60GalGal*): two partial sterile, long culm (*D60d60Galgal*): one semidwarf (*d60d60GalGal*) (154 : 51 : 26, $\chi^2 = 0.20$, $0.90 < P < 0.95$, Fig. 4). Subsequently, continued backcrosses were performed until the BC_7 with individuals selected in the BC_nF_1 for $\sim 70\%$ pollen fertility with 'Koshihikari' (Fig. 1). It is not necessary to grow out and select a semidwarf *d60* individual from BC_nF_2 if a pollen parent with $\sim 70\%$ pollen fertility (Fig. 3) is chosen from the BC_nF_1 to cross with the recurrent parent. The semidwarf phenotype (*d60d60GalGal*) was fixed by the BC_7F_3 generation, by which time plants should carry $\geq 99.6\%$ 'Koshihikari' genome.

(c) Development of the line isogenic for *sd1* and *d60*

In the F_2 of 'Koshihikari *d60*' (*Sd1Sd1d60d60GalGal*) × 'Koshihikari *sd1*' (*sd1sd1D60D60galgal*), the phenotypes (1 *Sd1Sd1* : 2 *Sd1sd1* : 1 *sd1sd1*) × 6 (4 *D60D60* : 2 *D60d60GalGal*) : 2 (*D60d60Galgal*) : 1 (*d60d60GalGal*) = 18 fertile long culm : six partial sterile, long culm : nine fertile, semidwarf : two partial sterile, semidwarf : one double dwarf segregated. Among these, the F_3 from the partial sterile, semidwarf (*sd1sd1D60d60Galgal*) segregated into six fertile, semidwarf (4 *sd1sd1D60D60* : 2 *sd1sd1D60d60*): two partial sterile, semidwarf (*sd1sd1D60d60Galgal*): one double dwarf (*sd1sd1d60d60GalGal*) (137 : 56 : 22, $\chi^2 = 1.84$, $0.30 < P < 0.50$, Fig. 4), and in the next generation (F_4) the double dwarf genotype (*sd1sd1d60d60GalGal*) was fixed (Fig. 5). This super-short-culmed 'Koshihikari', designated as variety

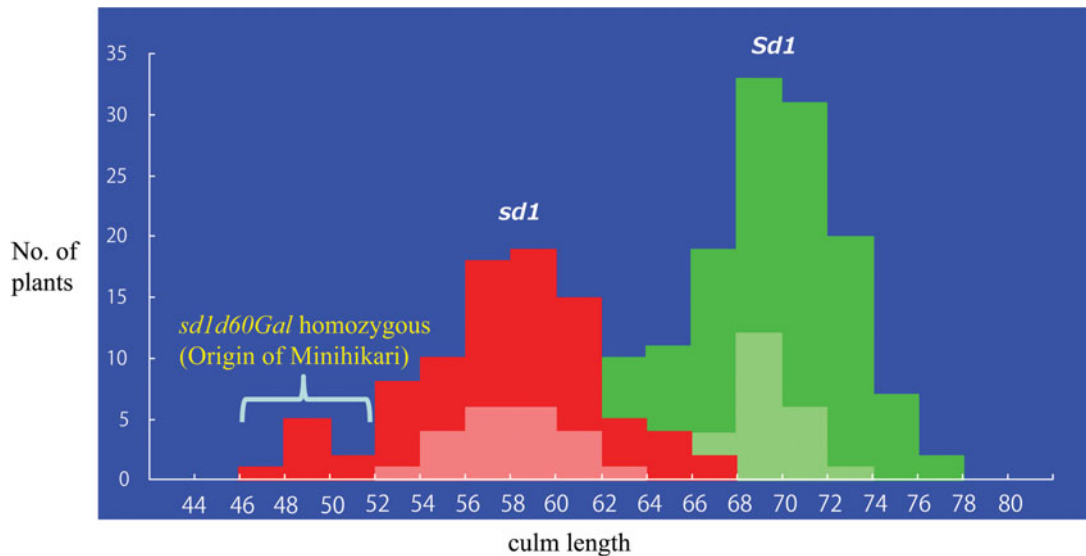


Fig. 4. Genotypic distribution for culm length in the F_3 (red) derived from the partial sterile, semidwarf ($sd1sd1D60d60Galgal$) following the cross between ‘Koshihikari $d60$ ’ ($Sd1Sd1d60d60GalGal$) and ‘Koshihikari $sd1$ ’ ($sd1sd1D60D60galgal$), and in the BC_1F_2 (green) derived from the partial sterile ($Sd1Sd1D60d60Galgal$) following the cross between ‘Koshihikari’ ($Sd1Sd1D60D60galgal$) and ‘Hokuriku 100’ ($Sd1Sd1d60d60GalGal$). Red shows $sd1$ homozygous and pale red shows partial sterility among them. Green shows $Sd1$ homozygous and pale green shows partial sterility among them.

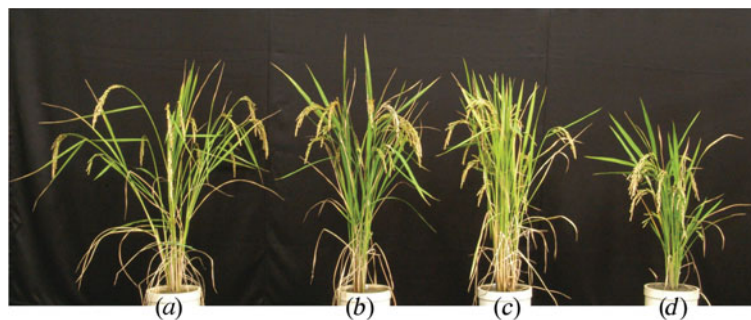


Fig. 5. Phenotype at maturity of ‘Koshihikari’ (a) and three isogenic lines in the ‘Koshihikari’ genetic background: $sd1$ (b), $d60$ (c), combination of $sd1$ and $d60$ (d).

‘Minihikari’, was registered by the Ministry of Agriculture, Forestry and Fisheries (MAFF 2010).

The $sd1$ allele from ‘Jikkoku’ has a G→T substitution in the first exon, which results in a single amino acid change from Gly to Val. *PmaCI* recognizes the substituted sequence (CACGTG) in the $sd1$ allele from ‘Jikkoku’. This revealed the $sd1$ allele in the double dwarf genotype (Minihikari) as two unique fragments, while the wild-type allele of ‘Koshihikari’ produced an undigested single fragment (Fig. 6). These results show that $d60$ and gal are inherited independently in the homozygous $sd1$ background.

(ii) Field evaluation of isogenic lines

The agronomic characteristics of ‘Minihikari’ and ‘Koshihikari’ are shown in Table 1. Isogenic lines created by backcrossing $d60$, $sd1$ or both, into ‘Koshihikari’, resulted in the $d60sd1$ line with an

additive, extreme dwarf phenotype (Fig. 5), which demonstrates that $d60$ and $sd1$ are functionally independent and not related to the GA_1 biosynthesis pathway. Relative to ‘Koshihikari’, the culm length of ‘Koshihikari $sd1$ ’ was 26.8% shorter, that of ‘Koshihikari $d60$ ’ was 26.0% shorter, and that of the double dwarf ‘Koshihikari $sd1d60$ ’, ‘Minihikari’, was 39.9% shorter (Fig. 5).

Both the early heading date and maturity dates of ‘Minihikari’ were the same as those of ‘Koshihikari’. The culm lengths of ‘Koshihikari $d60$ ’ (69.4 cm) and ‘Minihikari’ (56.4 cm) averaged 74 and 60% of the length of ‘Koshihikari’ (i.e. 26 and 40% shorter), respectively. ‘Minihikari’ and ‘Koshihikari $d60$ ’, had the highest degree of lodging, 0.0, which was clearly higher than that of ‘Koshihikari’, 4.4. The panicle lengths of ‘Koshihikari $d60$ ’ (17.1 cm) and ‘Minihikari’ (17.0 cm) averaged 91 and 90% of that of ‘Koshihikari’, respectively. The number of panicles

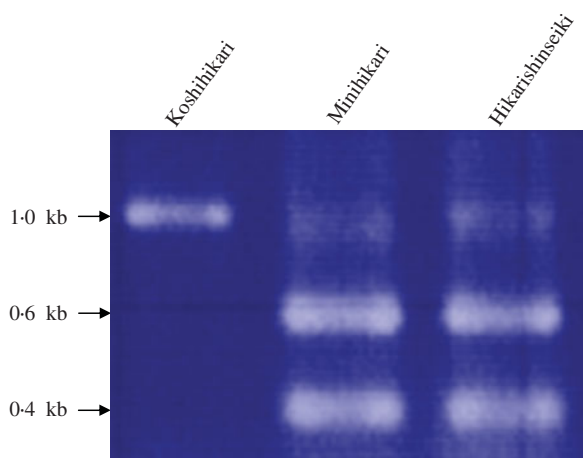


Fig. 6. DNA sequence analysis of *sd1* in the 'Minihikari' ('Koshihikari *sd1d60*') genome. The *sd1* allele from 'Jikkoku' has a G→T substitution in the first exon. *PmaCI* recognizes the substituted sequence (CACGTG) and digested the *sd1* allele from 'Minihikari' into two fragments, while the wild-type allele of 'Koshihikari' was not digested and remained as a single fragment.

of 'Koshihikari *d60*' (472/m²) and 'Minihikari' (515/m²) averaged 102 and 111% of that of 'Koshihikari', respectively. The thousand-grain weight of 'Koshihikari *d60*' (23.0 g) and 'Minihikari' (23.0 g) averaged 100% of that of 'Koshihikari', and the brown rice yield of 'Koshihikari *d60*' (62.9 kg/a) and 'Minihikari' (60.1 kg/a) averaged 96 and 92% of that of 'Koshihikari', respectively. The relatively greater number of panicles and sustainable yield may have resulted from the conversion of nutrients that were not directed to leaves and stems.

The leaves of 'Minihikari' averaged 18% wider than those of 'Koshihikari', were more erect, and retained a deep green colour longer (Fig. 5). The improved light-intercepting attitude of 'Minihikari' may thus improve its photosynthetic efficiency. Ripening period and grain quality (Table 1) were not significantly different among genotypes, according to ANOVA tests. Grain size and quality were also not significantly different. The brown rice quality of both varieties was ranked 'medium' (with a score of 4.3), and both scored 'minimum' for contents of 'white belly' and cracked grain. The flavour of 'Koshihikari *d60*' and 'Minihikari' scored an average of -0.17 and ±0.00 points against 'Koshihikari', respectively (Table 1), and 'Koshihikari *d60*' and 'Minihikari' ranked in the same 'upper medium' quality grade as 'Koshihikari'. Resistance to pre-harvest sprouting and grain shattering of both cultivars are both rated 'difficult' (Table 1). Flavour and quality of 'Koshihikari *d60*' and 'Minihikari' were rated as identical to those of 'Koshihikari'. Thus, the semi-dwarfing gene *d60* reduces lodging in 'Koshihikari *d60*' and 'Minihikari', while retaining the flavour and quality of 'Koshihikari'.

4. Discussion

'Koshihikari' remains a favourite rice cultivar in Japan. Production of the two major cultivars, 'Koshihikari' and 'Hitomebore', accounts for about 50% of the total rice production in Japan; so the genetic base of the rice crop in Japan is narrow. This raises the risk of crop loss due to disease and pests, and to lodging in 'Koshihikari' caused by frequent typhoons. Such major damage is a nationwide problem, and so the genetic improvement of lodging resistance in 'Koshihikari' has been a major rice breeding goal in Japan. This paper describes the development of semidwarf 'Minihikari', which was released in 2010.

To compare the effect of *d60* and *sd1* gene expression in the 'Koshihikari' genetic background, *d60* and *sd1* were introgressed into 'Koshihikari' by backcrossing each 7–8 times between 1985 and 2000 to 'Koshinishiki' as the recurrent parent. The short-culmed isogenic strains were generated, including 'Koshihikari *sd1*' (later named and registered as 'Hikarishinseiki') and 'Koshihikari *d60*'. 'Koshihikari *sd1*' and 'Koshihikari *d60*' were then crossed in 2001 to show that the two short-culm genes were not genetically identical, and to produce a new line carrying both *d60* and *sd1* at the F₄ generation. The culm length of this new line was very short, ~40 cm shorter than that of 'Koshihikari', showing the additive effect of both genes and demonstrating that *d60* was functionally independent from *sd1*. This extremely short-culmed 'Koshihikari', carrying the rice semi-dwarf genes *sd1* and *d60*, was named 'Minihikari'. Compared with 'Koshihikari', 'Minihikari' exhibited markedly high lodging resistance. We filed for registration of the cultivar with the Ministry of Agriculture, which was granted in October 2010 (varietal registration number 19985). 'Minihikari' proves that *d60* is independent from *sd1* and carries both genes in the genetic background of 'Koshihikari'.

'Minihikari' is the first short-culmed isogenic form of 'Koshihikari' carrying both an *sd1* and *d60* allele while retaining over 99.8% of the 'Koshihikari' genome. 'Minihikari' was registered to promote its use as a maternal parent containing both the useful semi-dwarfing genes *d60* and *sd1*, which will expand the gene pool for short-culm phenotypes. 'Minihikari' has remarkably enhanced lodging resistance over that of 'Koshihikari'. Moreover, its increased panicle number gave it an average of 3% increase in grain yield, combined with the highly prized flavour of 'Koshihikari'. The number of panicles of 'Koshihikari *d60*' (472/m²) and 'Minihikari' (515/m²) averaged 102 and 111% of that of 'Koshihikari', respectively. Murai *et al.* (2004) and Ogi *et al.* (1993) reported that an isogenic line carrying *sd1* derived from DGWG had more panicles

Table 1. Comparison of agronomic characters of 'Koshihikari' and isogenic 'Koshihikari' strains carrying semidwarfing genes *d60* or *sd1*

Cultivars	Genotypes	Heading date (m.d)	Maturity date (m.d)	Culm length (cm)	Panicle length (cm)	No. of panicles (no./m ²)	Grain yield (kg/acre)	1000-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 (%)
Koshihikari	Koshihikari	7-31	9-07	93-8	18-8	465	65-5	22-9	4-3	4-4	1-0	0-0	77-5	0-00	17-9	6-1
Hikarishinseiki	Koshihikari	7-31	9-07	68-7	17-3	507	68-0	23-2	4-3	0-6	0-5	0-0	76-0	0-22	17-6	6-4
Koshihikari	Koshihikari	7-31	9-07	69-4	17-1	472	62-9	23-0	4-3	0-0	0-0	0-0	76-5	-0-17	17-3	6-3
<i>d60</i>	<i>sd1</i>															
Mimihikari	Koshihikari	7-31	9-07	56-4	17-0	515	60-1	23-0	4-3	0-0	0-0	0-0	76-9	0-00	17-4	6-4
	<i>sd1d60</i>															

^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; and 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% and 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% and 100%.

^e Value of taste was determined using a Taste-meter MA-90B (Tokyo Rice-producing Machine Factory, Japan).

^f Eating quality shows the aggregate evaluation in 11 categories: 5, excellent through -5, especially poor.

^g Amylose and protein content were measured by Near Infrared Spectrometer AN800 (Kett Electric Laboratory, Japan).

than its parent cultivar 'Norin 29', while another *sd1* isogenic line had the same number of panicles in the 'Shiokari' genetic background. The semidwarfing gene *sd1* encodes a defective C20 oxidative enzyme, OsGA20ox2, which functions in the biosynthesis of gibberellin GA₁ (Sasaki *et al.*, 2002; Spielmeier *et al.*, 2002), and the defective enzyme results in a short culm. Hence, *sd1* appears to confer a pleiotropic effect of significantly increased panicle number in the 'Koshihikari *d60*' genetic background.

In this study, 'Koshihikari' × ('Hokuriku 100' × 'Koshihikari') BC₁F₁ segregated in a ratio of two fertile, long culm (*D60D60Galgal*:*D60D60galgal*): one partial sterile, long culm (*D60d60Galgal*). Moreover, the BC₁F₂ progeny segregation fit a six fertile, long culm (4*D60D60*:2*D60d60GalGal*): two partial sterile, long culm (*D60d60Galgal*): one semidwarf (*d60d60GalGal*) ratio. Subsequently, continuous backcrosses were successfully performed until the BC₇ generation using individuals selected in the BC_nF₁ for ~70% pollen fertility with 'Koshihikari'. This shows that *d60* and the gametic-lethal gene *gal* cause complementary lethality to both male and female gametes, a form of hybrid sterility. However, hybrid sterility caused by two loci, namely *d60* and *gal*, is quite different from the genetic systems of hybrid sterility previously discovered in rice, which consist of a single gene locus, such as *S5*, *S-a* or *S10* (Kitamura, 1962; Ikehashi & Araki, 1986; Sano, 1990; Zhang *et al.*, 2006; Chen *et al.*, 2008; Long *et al.*, 2008). Hybrid sterility between *O. sativa* and *O. glumaepatula* is only a rare case caused by duplicate genes, *S27* and *S28* (Yamagata *et al.*, 2010), but they have no dwarfing effects. In this study, BC_nF₁ plants (*D60d60Galgal*) were selected for pollen sterility caused by *d60* and *gal*, and were used as pollen parents to backcross *d60* in successive backcrosses. This method was shown to be effective in this study and can be used for other cultivars in addition to 'Koshihikari'.

If a gametic non-lethal allele *Gal* had not mutated simultaneously with the mutation from *D60* to *d60*, *d60* would have been eliminated due to lethality in M₁ gametes. Earlier studies in mutation breeding of semidwarf 'Koshihikari', conducted by several organizations prior to work done at Hokuriku National Agricultural Experiment Station, resulted in no introduction of new varieties. Subsequently, Samoto & Kanai (1975) increased the scale of mutation breeding by screening 200 000 M₁ plants. The semidwarf line 'Hokuriku 100' was then selected in M₅ plants derived from 298 short mutants selected from among 80 000 M₂ plants. Samoto & Kanai (1975) found that the rate of short-statured mutants they observed, 0.3%, was much lower than the 11.0% observed in wheat (Kaizuma *et al.*, 1967) and the 5.2% observed in barley (Toda *et al.*, 1972). The low rate of recovery

of short-statured mutants could be due to gametic lethality caused by interactions between *gal* and induced dwarf genes.

That is, the non-lethal allele *Gal* is essential to the transmission of *d60*. Thus, *d60* and *Gal* are rare and valuable mutant genes for the advancement of semi-dwarf breeding to replace *sd1*. The semidwarf gene *d60* from 'Hokuriku 100' has potential to replace the widespread *sd1*, and provides more options for dwarf breeding acquired through simultaneous mutation of two genes, one of which is essential for the heritability of the other, such as *Gal* is for *d60*. In this study, both semidwarfing genes *d60* and *sd1* were pyramided successfully in the 'Koshihikari' genome to develop a new and useful genetic resource for breeding dwarf rice.

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5. Declaration of Interest

None.

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