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Semidwarf Gene \textit{d60} Affected by Ubiquitous Gamete Lethal Gene \textit{gal} Produced Rare Double Dwarf with \textit{d30} via Recombination Breaking Repulsion-Phase Linkage on Rice Chromosome 2

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Abstract: The genotype of \textit{gal} and \textit{d60} were investigated in 33 rice varieties chosen from representative semidwarf and dwarf rice varieties. These were crossed with three tester lines, the \textit{d60Gal} line (genotype \textit{d60d60GalGal}), the \textit{D60gal} line (Koshihikari, \textit{D60D60galgal}), and the \textit{D60Gal} line (\textit{D60D60GalGal}). Each \textit{F1} plant was measured for culm length, and seed fertility. As a result, all \textit{F1} lines with the \textit{d60Gal} line showed tallness and partial sterility, reduced by 25\% in average from those with the \textit{D60gal} line (Koshihikari) and the \textit{D60Gal} line. These data indicated that the genotype of the 33 varieties is \textit{D60D60galgal} and that the \textit{d60} locus is not allelic to those of \textit{sd1}, \textit{d1}, \textit{d2}, \textit{d6}, \textit{d18k}, \textit{d29}, \textit{d30}, \textit{d35}, \textit{d49}, \textit{d50}, and \textit{qCL1} involved in the 33 varieties. In addition, the \textit{gal} gene is not complementarily activated with the semidwarf and dwarf genes described above, other than \textit{d60}. The \textit{Gal} gene will be ubiquitously distributed in rice. It is emphasized that \textit{Gal} is a rare and valuable mutant gene essential to the transmission of \textit{d60}. The double dwarf genotype of homozygous \textit{d30d60} was rarely gained in the \textit{F3} of the \textit{d30} line × \textit{d60} line by breaking their repulsion \textit{d60-D30} linkage on chromosome 2.

Keywords: rice; semidwarf gene; gamete lethal; non-Mendelian ratio; linkage; chromosome 2

1. Introduction

The breeding program that has made the greatest contribution in the history of mankind is the ‘green revolution’ in which the production of grain was dramatically increased in the 1960s with the development of dwarf varieties of rice and wheat [1]. Dwarfing prevents plants from lodging at their full-ripe stage, which makes them lodging-resistant to wind and rain, and has enhanced their adaptability for heavy maturing, which has dramatically improved (up to double) rice yields, and so has contributed to the stabilization of yields all over the world. Surprisingly, semidwarf rice varieties developed independently using different native varieties or artificially induced mutant lines as mother plants, which are controlled by a single dwarf gene \textit{sd1}. This is a defective C20-oxidase gene present in a late step in the gibberellin (GA) biosynthesis pathway [2], making options for dwarf breeding limited.

In order to find a novel dwarf gene to replace \textit{sd1}, the first author conducted gene analyses focusing on Hokuriku 100, a mutant line with culms approximately 15 cm shorter than those of the Koshihikari variety. A novel dwarf gene, \textit{d60}, was discovered, which gives rise to a good plant type with erect leaves by shortening culms by approximately 20\%. Furthermore, \textit{d60} complements the gametic lethal gene, \textit{gal}, to cause gametic lethality [3]. For example, in the \textit{F1} hybrid (genotype \textit{D60d60Galgal}) of Koshihikari (\textit{D60D60galgal}) × Hokuriku 100 (\textit{d60d60GalGal}), male and female gametes
having both gal and d60 become gametic lethal, and the pollen and seed fertility decrease to 75%. As a result, the F2 progeny show a unique mode of inheritance that is segregated into a ratio of 6 fertile long-culm (D60D60d60d60GalGal):2 partially fertile long-culm (D60d60Galgal = F1 type):1 dwarf (d60d60GalGal). Moreover, the isogenic line that was introduced with both d60 and sd1 derived from Jukkoku [4,5] into Koshihikari by backcrossing [3], viz. the d60sd1 line, and became the extreme-dwarf, indicating that d60 is functionally independent from sd1 and not related to the GA1 biosynthesis pathway [3]. Above all, d60 is expected to diversify semidwarf breeding as a novel alternative of sd1. However, in the process of cross breeding, d60 may cause gamete sterility if the counter parent has gal, and would result in an abnormal F2 segregation in an 8:1 ratio. Moreover, d60 may affect the segregation of linked genes in the process of heredity. In this study we show: (1) the distribution of gal and d60 were investigated in 33 representative semidwarf or dwarf varieties; and (2) double dwarfness of d60 and linked d30 was rarely gained from the F3 generation derived from the cross d30 and d60 line.

2. Materials and Methods

2.1. Test Crosses with Three Testers, d60Gal Line, D60gal Line, and D60Gal Line

In order to determine the genotype of gal and d60 in 33 varieties chosen from representative semidwarf and dwarf rice, namely dwarf varieties with d1 (Daikoku), d2 (Ebisu), d6 (Ebisumochi), d18 Kotaketamanishiki), d29 (Dwarf Kyushu 1), d30 (Waisei shirasasa), d35 (Tanginbozu), d50 (Fukei 71), semidwarf varieties with sd1 derived from Jukkoku (Jukkoku, Shiranui), semidwarf varieties with sd1 derived from IR8 (Kinuhikari, Taichung 65 d47), semidwarf varieties with mutant sd induced by γ-ray-irradiation (Reimei, M101, HS90), semidwarf varieties with gCL1 (Nipponbare), semidwarf varieties with unknown genes (Ishihikari, Koganebare, Nihonmasari), uncharacterized dwarf mutants induced by mPing (IM96, IM181, IM265), artificial mutant strains of Koshihikari (Kanto 79 (with early maturing gene e1), Hokuriku 100 (d60)), and several long-culm varieties (Koshishiki, Norin 1, Norin 22, Inochinoichi, Midoriyutaka, Ginbozu, Taichung 65, EG1) were used. These 33 varieties were crossed with the three tester lines, d60Gal line (d60d60GalGal), D60gal line (Koshihikari, D60D60galgal), and D60Gal line (D60D60GalGal). The d60Gal line was an isogenic Koshihikari having d60 and Gal, which was developed by seven times of continuous backcrossing with a recurrent parent Koshihikari and a non-recurrent parent of the d60 homozygous segregant in the F2 of Koshihikari × Hokuriku100 [3]. The D60Gal-homozygous line was developed from F1 progenies fixed in the genotype D60D60GalGal, which derived from fertile and tall heterozygous F2 plants (D60d60GalGal), and segregated in the F3 according to the Mendelian segregation ratio of 1 (semidwarf (d60d60GalGal)):2 (1 semidwarf:3 tall D60d60GalGal):1 (tall D60D60GalGal) [6]. For each test cross combination between the 3 tester lines and 31 varieties, 10 F2 plants were cultivated at the Field Science Center. Seedlings were individually transplanted into a paddy field with densities 22.2 seedlings/m² (one seedling per 30 × 15 cm). The paddy field was fertilized by 4.0 kg of basal fertilizer containing nitrogen, phosphorus, and potassium (weight ratio, nitrogen:phosphorus:potassium = 2.6:3.2:2.6) with 4.3 g/m² nitrogen, 5.3 g/m² phosphorus, and 4.3 g/m² potassium dispersed evenly across the field.

2.2. Genotyping Using the Test Crossed F1 Lines

Each F1 plant was measured for culm length and seed fertility. Three tester lines were isogenic lines in the genetic background of Koshishikari, which has a different single allele for D60d60 and Galgal loci, namely d60Gal, D60gal, and D60Gal. Taking into account the expectation that if the test subject has gal, F1 with the d60Gal line shows partial sterility, and both the F1 with the D60Gal line and the D60gal line shows fertility. On the other hand, if the test subject has a d60 allele, the F1 with the d60Gal line shows dwarfness, the F1 with the D60gal line shows partial sterility, and the F1 with the D60Gal line shows fertility. Each of the F1 plants were scored with heading time and culm length in the field. The length between the ground surface and the panicle base of the main culm was measured as the culm length for all plants. The time when the panicle of the first emerged from the flag leaf
sheath was recorded as the heading time for all plants. Three panicles were harvested from each F₁ plant, and the number of filled and unfilled spikelets was counted for each panicle. The percent of seed fertility was calculated as the number of filled spikelets divided by the total number of spikelets multiplied by 100. The genotype was determined by seed fertility. Aceto-carmine squash mounts to stain the pollens of several F₁ lines were conducted for Olympus BX40 microscopic examination.

2.3. Linkage Analysis for d60

Firstly, 318 F₂ plants of a marker gene line FL212 [7] that has d30 and gh2 on chromosome 2 (D60D60galgal) and the d60 line (d60d60GalGal) was used for segregation analysis for the marker genes and d60. The result showed that the segregation ratio of wild type to d30 homozygote at the d30 locus was 195:123, and wild type to gh2 homozygote at the gh2 locus was 218:100, and that both deviated significantly from 3:1 (Figure 1). When a recessive marker gene is fully linked to D60, the F₂ segregation ratio of wild type to recessive marker gene homozygotes will be 5:4 (Supplementary File 1). This was closer to the expected ratio of 5:4 for the cases where d30 is fully linked to D60. Then, F₃ lines (50 individuals/lines) from 56 gh2 homozygous F₂ individuals from the cross between FL212 (d30gh2) and the Koshihikari d60 line were developed, and the genotype of the F₂ was subsequently determined.

![Wild type: d30 homozygotes (A) Wild type: gh2 homozygotes (B)](image)

Figure 1. Excessive segregation of recessive homozygotes, according to the ratio of 5:4, considerably deviated from the Mendelian 3:1 ratio in the F₂ between the recessive marker gene line FL212 and the d60Gal line (d60d60GalGal). (A) Genotyping for the D30/d30 locus. d30 homozygous plants showed characteristic dwarf phenotypes with short panicles and small grains. According to the dwarf trait, we visually discriminated the d30 homozygote and wild type. As a result, the segregation ratio of wild type to d30 homozygote at the d30 locus was 195:123. In the correlation diagram with culm length and days to heading, red plots mean d30 homozygous plants, whereas vacant plots mean wild type plants. (B) Genotyping for the Gh2/gh2 locus. gh2 homozygous plants showed characteristic gold-coloring of unhulled grain. gh2 mutant is a lignin-deficient mutant, and Gh2 encodes a cinnamyl-alcohol dehydrogenase [8] According to the gold color of the matured hull, we visually discriminated the gh2 homozygote and wild type. As a result, the segregation ratio of wild type to gh2 homozygote at the gh2 locus was 218:100. In the diagram, green plots showed gh2 homozygous plants, whereas vacant plots mean wild type plants. Above all, the recessive morphological gene d30 and gh2 on the chromosome 2, segregated in the characteristic ratio of 5 wild type:4 recessive homozygotes, suggesting their linkage with D60 locus.
3. Results

3.1. Universal Distribution of Gal and D60 Except for the d60 Donor Hokuriku100

All F₁ lines when crossed with the d60Gal line showed tallness and partial sterility, being reduced by an average of 25% from those with the D60gal line (Koshihikari) and the D60Gal line (Table 1, Figure 2). Regarding the dwarf varieties with d1, d2, d6, d18, d29, d30, d35, and d50, F₁ lines with both of the D60gal line and the D60Gal line showed normal seed fertility over 90%, and there was statistically no significant difference between them. On the other hand, F₁ lines with the d60gal line showed partial seed sterilities in the lower 70% level, which were reduced by approximately 25% from the F₁s with the other two testers, namely the D60gal line and the D60Gal line, and the differences were statistically significant (5% level). Regarding culm length, each of the F₁ lines between the dwarf variety and the three testers showed almost the same normal length and there was statistically no significant differences between them. The above observations revealed that the all the dwarf varieties had gal, because the seed fertilities of F₁s with the d60Gal lines were significantly reduced by 25% in accordance to the frequency of the genotype d60gal gametes. Furthermore, all the dwarf varieties did not have d60, because the seed fertilities of F₁s with D60gal lines were at the normal 90% level, and culm length of the F₁s with the three testers showed statistically the same level. Therefore, the genotype of the representative dwarf varieties for D60Gal loci were determined as D60gal homozygous.

The results of dwarf varieties were also true to the other varieties. Namely, regarding the semidwarf varieties with sd1 derived from Jukkoku (Jukkoku, Shiranui), semidwarf varieties with sd1 derived from IR8 (Kimuhikari, Taichung 65 d47), semidwarf varieties with sd1 mutant induced by γ-ray-irradiation (Reimei, M101, HS90), semidwarf varieties with qCL1 (Nipponbare), semidwarf varieties with unknown genes (Isehikari, Koganebare, Nihonmasari), uncharacterized dwarf mutants induced by mPing [9] (IM96, IM181, IM265), artificial mutant strains of Koshihikari (Kanto 79 (with early maturing gene e1)), Hokuriku 100 (d60)], and several long-culm varieties (Koshihikari, Norin 1, Norin 22, Inochinoichi, Midoriyutaka, Ginbozu, Taichung 65, EG1), the seed fertilities of the F₁s with d60Gal lines were significantly reduced 25% from the lower 90% level of the F₁ seed fertility with the other two testers, D60gal line, and D60Gal line. In addition, regarding to culm length, each of the F₁ lines between these varieties and the three testers showed almost the same normal length, and there was statistically no significant difference between them. Therefore, the genotype of the semidwarf varieties with sd1 for the D60Gal loci were determined as D60gal homozygous.

These data gave the following facts. The genotype of the 33 varieties is D60D60galgalgal, and the d60 locus is not allelic to those of sd1, d1, d2, d6, d18, d29, d30, d35, d49, d50, qCL1, and unknown genes involved in the 33 varieties. In addition, the gal gene does not cause complementarily gamete lethality together with the semidwarf and dwarf genes other than the d60 described above. Based on the above facts it is suggested that the gal gene will likely be distributed universally in rice. Therefore, it is emphasized that the Gal is rare, and is a valuable mutant gene essential to the transmission of d60.
Table 1. Seed fertility (F) and culm length (C) of the F<sub>1</sub> lines by crossing each variety and three testers, the d60Gal line, the D60gal line (Koshihikari) and the D60Gal line.

| Variety              | a    | b       | c     | a and c | b and c | t-Value between a and c | t-Value between b and c |
|----------------------|------|---------|-------|---------|---------|-------------------------|-------------------------|
| Daikoku d1           | 73.3 | 101.0   | 95.4  | 104.8   | 95.6    | 105.2                  | 29.26 **                |
| Ebisu d2             | 72.4 | 94.2    | 95.1  | 98.3    | 94.8    | 98.2                   | 27.56 **                |
| Ebisumochi d6         | 72.6 | 93.2    | 96.5  | 93.5    | 96.4    | 94.0                   | 29.92 **                |
| Kottaketamanshiki d18| 72.8 | 102.6   | 96.6  | 104.6   | 96.6    | 106.3                  | 28.36 **                |
| Dwarf Kyushu 1 d29    | 74.6 | 96.6    | 98.7  | 97.9    | 98.5    | 98.1                   | 27.38 **                |
| Waisdiehsaraasa d30   | 73.9 | 82.3    | 95.9  | 83.7    | 95.6    | 84.1                   | 28.24 **                |
| d60Gal line           |      |         |       |         |         |                        |                         |
| D60gal line (Koshihikari) |    |         |       |         |         |                        |                         |
| D60Gal line           |      |         |       |         |         |                        |                         |
| t-Value between a and c |      |         |       |         |         |                        |                         |
| t-Value between b and c |      |         |       |         |         |                        |                         |
| Fuku 71 d50           | 73.8 | 83.6    | 95.6  | 88.6    | 94.9    | 87.4                   | 24.82 **                |
| Jukkou d2             | 72.8 | 84.7    | 94.2  | 88.5    | 96.6    | 87.8                   | 26.66 **                |
| Shiranui d1           | 72.4 | 83.2    | 95.6  | 86.3    | 95.2    | 86.5                   | 29.46 **                |
| M101 d1               | 73.4 | 74.3    | 95.4  | 79.4    | 95.6    | 78.6                   | 26.22 **                |
| Taichung 65 d47       | 73.8 | 82.5    | 95.5  | 85.1    | 95.7    | 85.8                   | 25.27 **                |
| Kiruhikari sd1        | 72.9 | 84.6    | 95.9  | 87.8    | 95.8    | 87.9                   | 25.13 **                |
| Reimei sd1            | 72.7 | 75.8    | 95.2  | 78.3    | 95.7    | 78.4                   | 25.46 **                |
| HS80 sd1              | 72.9 | 73.6    | 95.3  | 76.2    | 95.8    | 76.3                   | 25.37 **                |
| Isehikari unknown     | 73.2 | 86.1    | 95.8  | 88.3    | 94.7    | 89.0                   | 26.77 **                |
| Nipponbare qCL1       | 73.3 | 75.4    | 94.7  | 78.9    | 94.5    | 79.6                   | 27.82 **                |
| Koganebare unknown    | 74.2 | 80.0    | 95.4  | 88.7    | 94.7    | 85.8                   | 27.55 **                |
| Nihonmasari unknown   | 73.4 | 78.6    | 95.6  | 84.6    | 95.1    | 84.7                   | 26.82 **                |
| IM96 unknown          | 73.8 | 93.1    | 96.2  | 93.4    | 95.8    | 92.8                   | 25.67 **                |
| IM181 unknown         | 72.5 | 82.3    | 95.6  | 84.4    | 95.5    | 84.2                   | 25.46 **                |
| 1M265 unknown         | 73.8 | 81.9    | 96.0  | 82.7    | 95.7    | 82.9                   | 28.37 **                |
| Ginbozu               | 72.8 | 84.3    | 95.2  | 90.8    | 95.1    | 89.3                   | 26.26 **                |
| EG1                   | 72.4 | 88.6    | 95.3  | 87.2    | 95.6    | 86.8                   | 27.34 **                |
| Taichung 65           | 73.1 | 97.1    | 96.3  | 97.8    | 96.2    | 97.2                   | 25.66 **                |
| Inochinoichi          | 72.8 | 98.3    | 96.7  | 102.3   | 95.2    | 102.8                  | 29.25 **                |
| Mideriyutaka          | 73.4 | 113.6   | 93.9  | 114.2   | 94.6    | 114.3                  | 29.65 **                |
| Yutakakoshihikari     | 72.5 | 75.3    | 96.5  | 78.2    | 96.4    | 77.9                   | 25.86 **                |
| Kanto 79 c1           | 73.5 | 76.7    | 96.9  | 97.0    | 96.8    | 97.8                   | 27.25 **                |
| Norin 1               | 72.2 | 96.7    | 96.3  | 96.7    | 97.3    | 95.8                   | 30.64 **                |
| Norin 22              | 73.7 | 75.3    | 95.6  | 77.8    | 95.4    | 78.0                   | 28.39 **                |
| Koshihikari D60       | 73.6 | 72.7    | 96.2  | 76.6    | 96.6    | 76.4                   | 25.64 **                |
| Hokuriku 100 d60      | 95.6 | 62.8    | 73.4  | 72.8    | 96.5    | 72.6                   | 0.15                    |

* and **: Significant at 5% and 1% levels, respectively. All F<sub>1</sub> lines when crossed with the d60Gal line showed tallness and partial sterility, being reduced by an average of 25% from those with the D60gal line (Koshihikari) and the D60Gal line. These data gave the following facts. The genotype of the 33 varieties is D60D60galgal and the d60 locus is not allelic to those of d1, d2, d6, d18, d29, d30, d35, d49, d50, qCL1, and unknown genes involved in the 33 varieties. In addition, the gal gene does not cause complementarily gamete lethality together with the semidwarf and dwarf genes other than the d60 described above.
3.2. Double Dwarfness with d30 and d60 Broken by Their Repulsion Linkage on Chromosome 2

Each F1 line (50 individuals/line) was developed from 56 gh2 homozygous F2 plants in the cross between d30gh2 line and the Koshihikari d60 line, and determined F2s' genotypes (Table 2). First, 32 lines of gh2d30 homozygous F2 plants were classified into three genotypes (Figure 4). Thirty lines were homozygous of non-recombinant gametes d30-D60, because the F3 progenies were fixed in the d30 homozygous dwarf phenotype. The single line has the recombinant gametes d30-d60 and the non-recombinant gametes d30-D60 in the heterozygous plant, because d30d60 double recessive phenotypes appeared with approximately one fourth of the whole, namely, indicating a 3:1 segregation at the d60 locus in the d30 homozygous background. Only one single line was a d30d60 double recessive dwarf, having the recombinant gametes d30-d60 in the homozygous plant, due to its apparently shorter phenotype than the d30 homozygous plant (Figures 4 and 5).

Secondly, twenty lines having homozygous gh2 and heterozygous D30d30 were classified into three genotypes (Figure 3). Nine lines were heterozygous of the non-recombinant gametes D60-d30, d60-D30 and also for heterozygous Galgal, because these lines exhibited an excess segregation of the non-Mendelian 5:4 ratio at the d30 locus together with partial sterility, which is the same as in F2 (214:143, $\chi^2 = 2.786, 0.05 < p \leq 0.10$). On the other hand, 10 lines were heterozygous for the non-recombinant gametes D60-d30, d60-D30, and homozygous for Gal, because these lines segregated at the d30 locus in the Mendelian 3:1 ratio (301:87, $\chi^2 = 1.375, 0.10 < p \leq 0.90$). The single line has the recombinant gametes D60-D30 and the non-recombinant gametes D60-d30 in heterozygous, because the line segregated in a ratio of 3:1 at the d30 locus. Three lines were non-recombinant d60-D30 homozygous and one line was heterozygous for recombinant gametes d60-D30 and non-recombinant D60-D30 gametes, and also for heterozygous Galgal (Table 2). These results indicated that d60 is linked to d30 with the recombination value calculated as 3.57% (= 4 recombinant gametes/112 total gametes × 100) on chromosome 2.
Table 2. Genotyping of 56 F3 progenies derived from gh2 homozygous F2 plants.

| Genotype                                      | No. of F3 Lines |
|-----------------------------------------------|-----------------|
| gh2gh2d30d30D60D60Galgal non-recombinant + non-recombinant | 30              |
| gh2gh2d30d30D60D60GalGal non-recombinant + recombinant       | 0               |
| gh2gh2d30d30d60d60GalGal non-recombinant + recombinant       | 1               |
| gh2gh2d30d30d60d60GalGal recombinant + recombinant           | 1               |
| gh2gh2D30d30D60d60Galgal non-recombinant + non-recombinant   | 9               |
| gh2gh2D30d30D60d60GalGal non-recombinant + non-recombinant   | 10              |
| gh2gh2D30D60D60Galgal non-recombinant + recombinant          | 0               |
| gh2gh2D30D60d60GalGal non-recombinant + recombinant          | 0               |
| gh2gh2D30D60d60d60Galgal recombinant + recombinant           | 0               |
| gh2gh2D30D60d60D60GalGal recombinant + recombinant           | 0               |
| gh2gh2D30D60d60D60GalGal recombinant + recombinant           | 0               |
| gh2gh2D30D60d60D60GalGal recombinant + recombinant           | 0               |
| gh2gh2D30D60D60D60GalGal recombinant + recombinant           | 56              |

Recombinant value between d30 and d60 = 3.57%. According to the F3 genotyping as shown in Figures 3 and 5, F2’s genotypes of 56 F3 lines were identified. The green characters represent the recombinant gametes. These results indicated that d60 is linked to d30 with the recombination value calculated as 3.57% (= 4 recombinant gametes/112 total gametes × 100) on chromosome 2.
Figure 3. Genotyping of 20F3 lines derived from D30d30gh2gh2 F2 plants in the cross of the d30gh2 line FL212 and the Koshihikari d60Gal line. Twenty F3 lines having homozygous gh2 and heterozygous D30d30 were further classified into three genotypes. (A) Nine lines were heterozygous of the non-recombinant gametes D60-d30, d60-D30, and also for heterozygous Galgal, because these lines exhibited an excess segregation of the non-Mendelian 5:4 ratio at the d30 locus together with partial sterility (214 blue in histogram of culm length, yellow plot in the correlation diagram with culm length and days to heading):143 (red in histogram, blue plot in the diagram, $\chi^2 = 2.786, 0.05 \leq p \leq 0.10$). (B) Ten lines were heterozygous for the non-recombinant gametes D60-d30, d60-D30 and homozygous for Gal, because these lines segregated at the d30 locus in the Mendelian 3:1 ratio (301:87, $\chi^2 = 1.375, 0.10 \leq p \leq 0.90$). (C) The single line has the recombinant gametes D60-D30 and the non-recombinant gametes D60-d30 in the heterozygous plant, because the line is segregated in a ratio of 3:1 at the d30 locus. The green character represents the recombinant gametes.
Figure 4. Genotyping of 32F3 lines derived from d30gh2 homozygous F2 plants in the cross of d30gh2 line FL212 and Koshihikari d60Gal line. Each F3 line (50 individuals/line) was developed from 56 gh2 homozygous F2 plants in the cross between the gh2d30 line and Koshihikari d60 line, and determined F3's genotypes. Thirty-two lines of gh2d30 homozygous F2 plants were classified into three genotypes. (A) Thirty lines were homozygous of the non-recombinant gametes. (B) The single line has the recombinant gametes d30-D60 in the heterozygous plant, because of its apparently shorter phenotype than the d30 homozygous plant (Figure 5).

Figure 5. Phenotype of the D30D60 homozygous wild type (Koshihikari), and homozygous plant for d60D30, D60d30 and d30d60 (left to right).
4. Discussion

The threat of strong typhoons due to global warming is increasing [10]. This is a serious problem in rice production, because strong winds cause stem lodging and consequent yield losses and deterioration in crop quality [11]. Extensive damage from the lodging of rice due to frequent typhoons has become a social problem in recent years, and developing new varieties of typhoon-resistant rice by introducing dwarf genes is an imperative task. Hence, there is a pressing need to develop new short-culm rice cultivars resistant to strong winds [12]. So far, sd1 is the world’s only short-culm gene source in practical rice breeding. However, in the consideration of maintaining/expanding the genetic diversity of varieties, one should not rely only on sd1, which is a GA biosynthesis enzyme-defective gene, and should develop more new dwarf genes and promote their use in lodging-resistant breeding.

The excellent semidwarf quality of the rice mutant Hokuriku 100 is controlled by the single semidwarf gene d60 [3,6]. It is desirable to generate lodging-resistant rice cultivars that carry a novel short-culm gene, d60, as an alternative to sd1. However, d60 causes complementally gamete sterility, together with the gametic lethal gene gal. F2 progenies between d60Gal line (Hokuriku 100) and the original tall variety D60gal Line (Koshihikari) segregate distortedly into 1 semidwarf (d60d60GalGal):8 tall (2D60d60Galgal:2D60d60GalGal:4D60D60) ratio, because of the deterioration of the F1 male-and female-gametes having both gal and d60 [6] (Supplementary File 1). In this study, the author developed F1 lines between 33 representative dwarf or semidwarf lines and three isogenic tester lines, the d60Gal line instead of Hokuriku 100 [13], the D60Gal line, and the D60gal line. Three tester lines were isogenic lines in the genetic background of Koshishikari, which were different in only a single allele for the D60d60 and Gal/gal loci, namely d60Gal, D60gal, and D60Gal. Therefore, when the test subject has gal, F1 with the d60Gal line shows partial sterility, and both the F1 with the D60Gal line and the D60gal line show fertility. On the other hand, when the test subject has d60, F1 with the d60Gal line shows as a semidwarf, F1 with the D60gal line shows partial sterility, and F1 with the D60Gal line shows fertility. As a result, all F1 lines with the d60Gal line showed tallness and partial sterility lower than a 70% level, whereas the F1 lines with the D60gal line (Koshihikari) and the D60Gal line showed a 90% level. In conclusion, the genotype of the 33 varieties was determined as D60D60galgal, and d60 was different from sd1, d1, d2, d6, d18, d29, d30, d35, d49, d50, qCL1, and unknown genes involved in the 33 varieties. Moreover, there were no dwarf or semidwarf genes which were complementary with gal, except for d60.

The findings above suggest that the gal gene will likely be distributed universally in rice. Therefore, d60 is a dwarf gene that could not have been obtained by chance without Gal’s simultaneous mutation. The d60 gene could not have been transmitted without Gal. This means that the Gal gene is absolutely necessary to transmit d60, and d60 is very unique in that it always makes a pair with Gal and segregates according to an 8:1 ratio. In other words, d60 is a valuable gene because, without the gal to Gal mutation, d60 would not exist in a normal environment.

The d35 gene of Tanginbozu, which became the best rice breed in Japan between 1955 and 1964, was kaurenoic acid oxidase- or 3-β hydroxylase-defective in the same GA biosynthesis pathway [14]. The Daikoku type dwarf gene d1 in rice is defective in the α subunit of the heterotrimeric G protein, affecting GA signal transduction [15]. Both genes did not show complementary effects between d60 and gal.

A progeny test was conducted in the F3 of the cross between the Koshihikari d60 line and a line carrying a gene marker d30 on chromosome 2, which when segregated in a ratio of wild-type to d30 homozygote was 200:118, close to the theoretical segregation ratio of 5:4 at the d30 locus when completely linked to the D60 locus. This resulted in the genetic linkage between d30 and d60 loci on chromosome 2.

Here, we discuss the relationship between the complementary gamete sterility caused by gal, d60, and the previously reported hybrid gamete sterile genes in rice. Firstly, Oka [16] proposed that the duplicate S gene loci, which work as developmental factors in gametes, cause hybrid sterility when the F1 gametes receive both recessive S genes on each duplicate locus. For example, if parents A and
B have genotypes s1s1 +2/+2 and +1/+1s2s2, respectively, in which at least one + gene is necessary for normal development of the gamete, then 25% of their F1 hybrids will be sterile. This is because those gametes carrying the double recessive combination s1s2 deteriorate due to deficiencies during gamete development. This hybrid sterility is similar to that caused by gal and d60 in that two genes are responsible for both systems. However, gal and d60 cause both sex sterilities, whereas Oka [17] suggests that the duplicate S gene model can only explain male gamete sterility.

On the other hand, Kitamura [18] explained female sterility in indica/japonica hybrids by the one locus spor-o-gametophytic interaction hypothesis, that is, disharmony between one allele in the gamete and another in the surrounding sporophytic tissues. This model assumes parent genotypes of S/S and S/S, creating the hybrid S/S, in which the allele S present in the maternal tissue induces abortion of gametes carrying the opposite allele, Su. Thus, 50% of S/Sa plants are sterile and produce gametes carrying the S allele only; selfed progenies are all fertile. Ikehashi et al. [19–22] showed that this one locus model was a more likely explanation for indica/japonica hybrid sterility than the two loci model [16]. The allelic interaction model [22] has been accepted as the genetic basis of hybrid sterility and the allelic interaction S5 locus has been cloned [23].

In subsequent studies based on analyses of the fertility of a number of indica × japonica hybrids, over 30 female gametes sterility loci—including major genes—were identified and mapped [24–33], or male gametes sterility were identified [31]. So far, indica/japonica hybrid sterility loci were identified on chromosomes 4, 6, 7, 12, and 1 that lead to female gamete abortion through allelic interactions: S7 [24], S8 [25], S9 and S15 [27], and S16 [26], etc. Among them, the Sa locus has been successfully cloned [34]. One-locus allelic interactions for male sterility were also recognized in hybrids between two cultivated rice species Oryza sativa and Oryza glaberrima Steud. [35–37], O. sativa, and Oryza rufipogon [38], and O. sativa and Oryza. glumaepatula [39], and a series of S1 [37,40], S18 [40], S20 and S21 [35,36], S22A and S22B [39], were identified. Above all, hybrid sterilities in rice can be explained by a single locus allelic interaction. Therefore, hybrid sterility caused by the two genes d60 and gal is an extremely rare case in rice. Moreover, gamete breakdowns of both sexes, for gal and d60, are particularly rare and its ubiquities distribution is quite novel discovery.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/10/11/874/s1, Supplementary File 1. Identification of the chromosomal location of d60 by distorted segregation of morphological marker genes, which linked with D60. If the recessive morphological gene is tightly linked with D60, segregation ratio of wild-type/recessive homozygotes is deviated to 5:4 from the Mendelian 3:1 ratio.

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