Utilization of Stiff Culm Trait of Rice smos1 Mutant for Increased Lodging Resistance

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Abstract

Although the introduction of semi-dwarf trait into rice has led to improved lodging resistance making it capable of supporting high grain yield, lodging still remains a concern when attempting to further increase the grain yield of rice. However, improving the lodging resistance in rice by depending on the semi-dwarf trait alone is possible only up to a certain limit, beyond which other traits may be needed for reinforcement. To search for alternative traits relating to high lodging resistance, we identified 9 rice mutant lines possessing improved culm strength. To evaluate whether such lines can be useful for breeding lodging resistant rice, small organ size1 (smos1) mutant having increased lodging resistance but low tiller number and low grain yield, was chosen as a representative for a breeding trial. smos1 was crossed with ST-4 (from the Stock rice collection of Nagoya University Togo field #4), a cultivar with high tiller number and high grain yield, and from their progeny, LRC1 (lodging resistance candidate-1) was selected. Although the low tiller number trait of smos1 was not fully reversed in LRC1, this was compensated by an increase in grain weight per panicle, thereby resulting in high grain yield per plant. This important attribute of LRC1 was further enhanced by the improved lodging resistance trait inherited from smos1. Such improved lodging resistance in LRC1 and smos1 was revealed to be mainly due to increased culm diameter and culm thickness, which led to a high section modulus (SM) value, a parameter defining the physical strength of the culm. Since smos1 possesses high breaking-type lodging resistance which is different from semi-dwarf plants with high bending-type lodging resistance, an alternative approach of using thick culm lines for the creation of rice with increased lodging resistance is hereby proposed.

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Introduction

In the 1960’s, introduction of semi-dwarf trait into rice varieties combined with high nitrogen fertilizer input doubled rice grain yield, a success referred to as the “Green Revolution” [1–3]. Use of high amount of fertilizer allowed yield increase, while reducing plant height through the introduction of semi-dwarf gene increased lodging resistance, making rice capable of supporting grain-laden panicles. To avoid the future food crisis that may arise from human population explosion and climate change, it is urgent to increase rice grain yield per land area. In this context, lodging still remains a concern.

Lodging of cereal crops can be classified into three types [4]. Culm bending-type lodging is exhibited by plants when they cannot withstand the bending pressure, and is often observed in the upper internodes of rice during strong winds and rain. Culm breaking is another type of lodging that usually occurs at lower internodes (below the third internode counted from the top) as a result of excessive bending pressure at the higher internodes, and is determined primarily by the morphology and quality of the culm [5,6]. The third type is root lodging, which results from the inability of the roots to support the above-ground part [7]. Root lodging is infrequently observed in transplanted rice, as they possess well-established root system.

The widespread use of gibberellin (GA)-related semi-dwarf plants during the “Green Revolution” was due to their improved bending-type lodging resistance. Short-statured plants have lower “center of gravity” than normal plants, which improves their resistance against bending pressure [8]. However, attaining lodging resistance through the use of GA-related semi-dwarf trait has some limitations. First of all, further improving the bending resistance does not necessarily result in increased lodging resistance. This is because although semi-dwarf plants have increased bending resistance in the culm, their breaking resistance is significantly reduced [9]. In addition, GA dependent semi-dwarf plants have lower grain yield than their original cultivars [9].

From the reasons mentioned above, a rather different approach is required to further improve lodging resistance in rice. One possible way is to increase the physical strength of the culm to improve breaking-type lodging resistance. This should satisfy the requirement for supporting higher grain yield without depending on semi-dwarfism.

In this study, we searched for rice mutants with stiff culm and improved breaking resistance, and 9 such lines were identified...
from more than 3000 rice mutant accessions. To investigate whether these traits could be utilized in the breeding program for improved lodging resistance, *small organ size1* (*smos1*) was chosen from the mutant lines and used for a breeding trial. Just recently, molecular characterization of *smos1* mutant revealed that it contains a mutation in a gene coding for APETALA2 (AP2)-type transcription factor which acts as an auxin-dependent regulator of cell expansion [10]. Thus, the mutation results in decreased ability of the cells to expand, hence, reducing the cell size in various organs of *smos1*. Surprisingly, the mutant also exhibits an increase in cell numbers in several organs which could account for *smos1*’s high lodging resistance. Although the molecular mechanism leading to increased cell numbers is unknown, expression of DNA replication-related genes is altered in *smos1* mutant. From such observation, Aya et al. suggested one possibility that SMOS1 directly suppress cell proliferation through regulating these genes [10]. Unfortunately, the advantage of increased cell numbers of *smos1* is severely undermined by its poor number of tillers which translates into reduced grain yield per plant. Thus, as a crossing partner for *smos1*, we chose ST-4 (from the Stock rice collection of Nagoya University Togo field #4), a cultivar with high yield and large number of tillers. Through such a crossing combination, we succeeded to identify one progeny line possessing increased lodging resistance and grain yield. We hereby present that the stiff culm trait is yet another available option for breeding.

**Materials and Methods**

**Growth conditions**

Experiments were conducted at the Nagoya University Togo Field. Each rice line was planted in a 1 × 1 m plot with a planting density of 18 plants per square meter (15 cm horizontal and 30 cm vertical spacing between plants).

**Plant materials**

*smos1* is a mutant derived from T65. ST-4 originated from inbred japonica-indica multiple crossing lines, but the parents were undeterminable. Crossing *smos1* with ST-4 was conducted using the latter as pollen donor. Among the self-fertilized F5 population of *smos1*×ST4 lines, a line tentatively named LRC1 (lodging resistance candidate-1), was selected for investigation. In the F5 lines, we did not observe any obvious differences within individual lines, suggesting that most of their genome regions were already fixed.

**Analyses of morphological and agronomical traits**

For the measurement of each trait, plant samples were taken 40 days after heading, air-dried for 2 weeks, and analyzed.

**Analyses of lodging resistance**

Lodging resistance parameters were measured from rice plants 40 days after heading. Culm bending resistance was investigated by measuring the cLr value (lodging resistance factor), following the method of Grafius and Brown [11]. Bending moment at breaking was measured at a distance of 4 cm between two supporting points as described previously [12]. Physical parameters were calculated using the formula: \( M = \text{section modulus} \times \text{bending stress} \) [13]. \( M \) is the bending moment of the basal internode at breaking (g · cm). Section modulus \( SM = \frac{\Pi}{32} \times (a_1 \times b_1 - a_2 \times b_2) / a_1 \), where, \( a_1 \) is the outer diameter of the minor axis in an oval cross-section, \( b_1 \) is the outer diameter of the major axis in an oval cross-section, \( a_2 \) is the inner diameter of the minor axis in an oval cross-section and \( b_2 \) is the inner diameter of the major axis in an oval cross-section.

**Observation of the cross-section of the internode**

The uppermost down to the fourth internode of rice plants at the matured seed stage were sampled. The center of each internode was hand-sectioned and stained with toluidine blue.

**Confirmation of *smos1* mutation by PCR**

Genomic DNA of the F5 generation plants crossed with *smos1* and ST-4 were PCR amplified using the primers designed to amplify the *SMOS1* gene. Mutation was confirmed by sequencing the PCR amplified DNA.
Figure 2. Breaking-type lodging resistance and culm morphologies of selected lines. (A) Breaking-type lodging resistance evaluated in terms of bending moment at breaking. Data are means ± SD (n=3). The fourth internodes of MT-6 and MT-8 could not be evaluated due to short internode lengths. (B) Magnified view of the third internode cross-section of 7 lines showing high bending-moment-at-breaking value in comparison with original cultivars (T65, Nipponbare, and Kinmaze). Bar = 500 μm. (C) Relative culm thickness of each line. Thickness of the original cultivars is set as 1. Data are means ± SD (n=3). The uppermost to fourth internodes were measured. doi:10.1371/journal.pone.0096009.g002
Statistical analysis

Data on morphological and agronomical traits, and lodging resistance were examined using three or more individual plants for replication. Statistical analyses were carried out using R version 2.12.1 software. Significance test was conducted using the two-tailed Student’s t-test or the Tukey’s significant difference test.

Results

Screening for rice mutants with improved lodging resistance parameters

From among the >3000 rice mutant accessions of the Nagoya University Togo Field that we initially screened, we selected 14
lines showing improved cLr values (a measure for bending-type lodging resistance) for two seasons as compared to their respective original lines (T65, Nipponbare, and Kinmaze) (Figure 1A). Their gross morphologies are shown in Figure 1B. Next, we evaluated the bending moment at breaking (BMB, a measure for breaking-type lodging resistance) of the mutants (Figure 2A) using a high throughput handmade load-testing apparatus in the paddy field. Through these steps, we were able to classify the lines into two groups: 9 lines showed both increased cLr and BMB values, and 5 lines that only showed increased cLr values (MT-6 to -10).

Among the 9 mutants showing increased BMB, we further analyzed 7 lines, namely, smos1, MT-1, MT-2, MT-3, MN-1, MN-2, and MK-1 in terms of their culm morphology. The magnified view of the fourth internode of each culm is shown in Figure 2B. Almost all lines showed increased culm thickness in all the internodes tested as compared to original cultivars (Figure 2C).

Since our aim in this study is to find out whether there are other available options for breeding rice with improved lodging resistance other than the traditional use of semi-dwarf trait (which improves cLr), we decided to do a breeding trial using a rice line with improved BMB, while maintaining a good cLr value. For this purpose, we selected smos1 from among the candidate mutant lines since it showed the highest BMB (Figure 2A). Previously, we identified the causal gene for smos1 and found that it codes for an APETALA2 (AP2)-type transcription factor which controls organ size downstream of auxin signaling pathway [10]. Mutation in smos1 results in reduced cell elongation but is accompanied by an increased cell number in several organs.

We initially evaluated the global agronomical attributes of smos1 such as plant height, tiller number, grain weight per plant, grain weight per panicle, 1000-grain weight, grain number per panicle, primary branch number, and relative fertility in comparison to those of the original cultivar T65 (Figure 3). As a result, the plant height (Figure 3A), tiller number (Figure 3B), and total grain weight per plant (Figure 3C) of smos1 were reduced to 72.0%, 40.8% and 38.1% relative to that of T65, respectively. As for the remaining grain-related traits, those of smos1 did not vary significantly from that of T65, except for a slight 1000-grain weight and relative fertility reduction in smos1 (Figure 3E and 3H; 11% and 5.6% reduction, respectively). Overall, this shows that the low tiller number of smos1 is the main reason for the reduced grain weight per plant of this otherwise high yielding and lodging resistant line.

Morphological traits of smos1 x ST-4

For the breeding trial, we chose ST-4 as an ideal crossing partner to complement smos1’s unfavorable trait because of its high tiller number (14.5) and high grain yield (37.9 g of grain weight per plant). After crossing smos1 with ST-4 (Figure 4A), the resulting F1 plant was self-fertilized to proceed to the next generation (F2). Such procedure was conducted until the F5 generation, each time selecting for lines with high grain yield and thick culm through visual and manual checking in the field. In the F5 generation, there were six independent lines (#1 to #6) that possess smos1 mutant allele as confirmed by PCR, and showing similar or higher grain weight per plant relative to ST-4 (Figure 4B). Among them, we evaluated the BMB of the two highest yielding lines, #1 and #5, by using a precision load-testing machine (Tensilon RTM-25) for accurate measurement of BMB. Although both lines were...

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**Figure 4.** Selection of lines with high grain yield and improved lodging resistance from the F5 population of the smos1 and ST-4 cross. (A) Procedure to select for high grain yield and improved lodging resistance. (B) Grain weight per plant of lines from the F5 population of the smos1 and ST-4 cross. Data are means ± SD (n = 3). (C) Bending-moment-at-breaking values of line #1 and line #5 (n = 3).

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superior to T65, #5 showed a larger BMB than #1 line (Figure 4C). Thus, we renamed #5 line as LRC1 (lodging resistance candidate-1) and studied it in more detail.

Agronomical traits of LRC1

Figure 5A shows the gross morphology of LRC1 and its parentals smos1 and ST-4, together with T65. The plant height of LRC1 was slightly higher than that of smos1 and similar to that of ST-4, whereas T65 was significantly higher than the three plants (Figure 5A and 5B). As mentioned, the tiller number of smos1 was reduced compared to that of its original strain, T65 (Figure 5C). Compared to smos1, although LRC1 showed increased tillering with an average of 8.7, it was not fully restored to that of ST-4 (14.3) or T65 (13.0).

Next, we evaluated the panicle structure of LRC1 (Figure 6). The 1000-grain weight of LRC1 (26.2 g) was similar to that of smos1 (24.6 g) and T65 (27.6 g), whereas that of ST-4 was inferior (20.3 g; Figure 6B). On the other hand, the primary branch number (16.3) and grain number per panicle (264.7) of LRC1 were more similar to that of ST-4 (14.0 and 236.3, respectively) than to that of smos1 (12.3 and 169.7, respectively; Figure 6C and 6D). Relative fertility was similar between the four plants, although smos1 showed a slight decrease (Figure 6E). These data imply that LRC1 inherited the favorable 1000-grain weight trait from smos1 and the high grain number and primary branch number traits from ST-4, which led to a marked increase in grain weight per panicle (5.3 g; Figure 6F) compared to the two parentals and T65 (2.6 to 3.3 g). Thus, despite the relatively low tiller number of LRC1, it achieved a grain weight per plant (shown as grain yield per hectare in Figure 6G) that was 20.1% and 5.7% higher than that obtained from ST-4 and T65, respectively, though these values are not statistically significant since the standard deviation for each plant was relatively high.

Lodging resistance of LRC1

Next, we evaluated the lodging resistance of LRC1 in terms of cLr and BMB values. The cLr value of LRC1 was similar to that of smos1 and ST-4, but 1.9 times higher than that of T65 (Figure 7A). As for the BMB (Figure 7B), LRC1 had a value significantly higher than that of ST-4 and T65 in all the internodes analyzed, and smos1 showed a similar trend. Thus, the improved lodging resistance of smos1 was maintained in LRC1.

BMB can be further subdivided into two parameters. That is, the section modulus (SM), which is determined by the morphology

Figure 5. Morphology, plant height, and tiller numbers of T65, smos1, ST-4, and LRC1. (A) Gross morphology of plants at 30 days after heading. Bar = 10 cm. (B) Diagram for plant height. (C) Diagram for tiller numbers. Plant height and tiller numbers (n = 3) were measured at 40 days after heading. Tukey’s test was conducted for panels (B) and (C). doi:10.1371/journal.pone.0096009.g005

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Figure 6. Panicle structure and yield-related traits of T65, smos1, ST-4, and LRC1. (A) The gross panicle morphology of each line at 40 days after heading. Diagrams for (B) 1000-grain weight, (C) primary branch number, (D) grain number per panicle, (E) relative fertility, (F) grain weight per panicle, and (G) grain yield per hectare are also shown. Data are means ± SD (n = 3). Tukey’s test was conducted for panels (B) to (G).

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of the culm (thickness and diameter), and the bending stress (BS) coefficient, which is influenced by the quality of the culm such as the amount of cellulose and lignin within the cell wall [12]. To investigate the mechanism behind the improved culm stiffness of smos1 and LRC1, culm thickness, culm diameter, SM, and BS were measured at the fourth internodes of each line (Figure 8). Initially, we observed the transverse sections of the uppermost to the fourth internode of each plant was used for analysis. Data are means ± SD (n = 3). Tukey’s test was conducted for each panel.
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As was expected from their culm morphology, SMs of the fourth internodes of smos1 and LRC1 were 2.3 and 2.7 times higher than that of T65, respectively (Figure 8E) and although not significant, the SM of LRC1 was higher than that of smos1. This might be the reason why LRC1 showed a slightly higher BMB at the fourth internode relative to smos1 (Figure 7B).

As for the BS of each plant (Figure 8F), BS of smos1 (and LRC1) was much lower than that of T65 (Figure 8F). This suggests that there might be a trade-off between SM and BS, and that pronounced increase in culm thickness and diameter negatively affects culm quality that in turn decreases BS (yet to be proven).
Taken altogether, the increased breaking-type lodging resistance of smos1 and LRC1 results from the higher SM conferred by their thick and wide culms, which are lacking in T65.

Discussion

In the present study, we showed that some mutants with stiff culm have the potential to markedly increase breaking-type lodging resistance (Figure 2A). However, as far as we know, high BMB mutants have not been utilized in breeding programs up to now. This is probably due to the various unfavorable traits that are often associated with these lines, such as low tiller number, low grain yield, and some morphological defects. To assess the possibility of utilizing mutants with improved culm breaking resistance in breeding, we chose smos1 as a representative mutant line possessing increased lodging resistance but with reduced tillers and low grain yield. As a crossing partner, we chose ST-4, a high tillering and high grain-yielding line that has inferior breaking-type lodging resistance, to investigate the possibility of producing rice with high lodging resistance and high grain yield.

Selecting rice line with stiff culm and restored tiller number

The incomplete restoration of the tiller number of LRC1 to that similar to ST-4 suggests that it is difficult to separate traits related to culm thickness (or size) and tiller number, and that the two traits may be pleiotropic effects of the same mechanism(s). Such negative correlation between tiller number and culm size has also been reported. For instance, fine culm 1 (fc1) mutant shows high tiller number which results from diminished strigolactone signaling, has thin culms [14]. Similarly, high-tillering dwarf 3 mutant possesses small culm diameter although its mechanism is unknown [15]. These observations suggest that there is a trade-off between tiller number and culm size.

Even so, the significant improvement of smos1 in terms of lodging resistance is an attractive trait that can be harnessed in rice breeding programs. One possible way to overcome the negative effect of low tiller number is to increase grain weight per panicle. In Figure 6F, the observation that LRC1 had a significantly higher grain weight per panicle (5.3 g) than any of its parents (ST-4, 2.6 g; smos1, 3.1 g) or T65 (3.3 g) shows that provided a suitable crossing partner is used with smos1 and selection of best-performing progenies is done, good candidates with thick culm and a reasonable grain yield can be obtained.

Using lines with unfavorable traits— a paradigm shift in rice breeding

Although numerous researches have been devoted to increasing rice grain production, a substantial increase in yield has been slow in coming after the “Green Revolution” [3]. This calls for new strategies to overcome the current rice production bottleneck and possibly bring forth a Second Green Revolution. As shown in this study, a mutant line (smos1) that have been ruled out as a possible breeding line in the past due to its unfavorable trait surprisingly gave rise to a lodging resistant and high yielding progeny (LRC1) after crossing with ST-4. This means that even lines with unfavorable traits from a certain viewpoint can serve as important bioresources and that a paradigm shift in the way we look at them is important.

Author Contributions

Conceived and designed the experiments: KH HK TH MM. Performed the experiments: AO TH YS KA. Analyzed the data: KH AO TH YS KA MM. Contributed reagents/materials/analysis tools: HK AO TH YS KA. Wrote the paper: KH RO MM.

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