Lignin is a complex phenylpropanoid polymer, and is biosynthesized via oxidative coupling of p-hydroxycinnamyl alcohols (monolignols) and related compounds that are formed in the cinnamate/monolignol pathway (Umezawa 2010). Lignin fills the spaces between cell wall polysaccharides and confers mechanical strength and imperviousness to the cell wall (Boerjan et al. 2003). Therefore, lignin biosynthesis is closely related to the evolution of land plants.

Lignin has several properties that present obstacles to chemical pulping, forage digestion, and enzymatic hydrolysis of plant cell wall polysaccharides for biofueling. For these processes, it would be beneficial for plant materials to either have less lignin, or to have lignin that is easier to remove. Mutant plants in which genes encoding lignin biosynthetic enzymes are down-regulated are generally expected to have lower lignin content and higher enzymatic saccharification efficiency. For these reasons, lignin biosynthesis is an area of great interest (Chiang 2006; Dixon and Reddy 2003; Vanholme et al. 2008; Weng et al. 2008).

Several brown midrib (bm) mutants have so far been isolated from the C4 grasses, maize, sorghum and pearl millet, but have not been detected in C3 grasses including rice (Oryza sativa). In the present study we characterized the cad2 (cinnamyl alcohol dehydrogenase 2) null mutant isolated from retrotransposon Tos17 insertion lines of Oryza sativa L. ssp. japonica cv. Nipponbare. This mutant exhibited brown-colored midribs in addition to hulls and internodes, clearly indicating both bm and gold hull and internode (gh) phenotypes. The enzymatic saccharification efficiency in the culm of cad2 null mutant was increased by 16.1% than that of the control plants. The lignin content of the cad2 null mutant was 14.6% lower than that of the control plants. Thioacidolysis of the cad2 null mutant indicated the presence of cinnamaldehyde structures in the lignin. Taken together, our results show that deficiency of OsCAD2 causes the bm phenotype in addition to gh, and that the coloration is probably due to the accumulation of cinnamaldehyde-related structures in the lignin. Additionally, this cad2 null mutant was useful to silage purposes and biofuel production.

**Key words:** Brown midrib, lignin, cinnamyl alcohol dehydrogenase, gold hull and internode, rice.

Abbreviations: 4CL, 4-hydroxyphenylpyruvate CoA ligase; bm, brown midrib; CAD, cinnamyl alcohol dehydrogenase; CAOMT, cinnamyl CoA-O-methyltransferase; CCoAOMT, caffeoyl CoA O-methyltransferase; CCR, cinnamoyl CoA reductase; gh, gold hull and internode; H/N, p-hydroxybenzaldehyde/vanillin; S/V, syringaldehyde/vanillin; WT, wild type; TLC, thin-layer chromatography

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Published online September 5, 2013
It was concluded that \( bm1 \) is not a null mutation of \( ZmCAD2 \), but affects its expression, possibly through alteration in upstream or downstream non-coding regions (Halpin et al. 1998). Later, Guillaumie et al. reported that \( bm1 \) was probably in a gene that regulates the expression of the \( ZmCAD \) gene family (Guillaumie et al. 2007). On the other hand, the \( bm3 \) mutation was found to occur in the gene encoding caffeic acid \( O\)-methyltransferase (\( ZmCAOMT \)) (Morrow et al. 1997; Vignols et al. 1995). In addition, downregulation of \( CAOMT \) in maize using sorghum \( CAOMT \) resulted in a \( bm \) phenotype (He et al. 2003).

In sorghum, at least four independent \( brown \) \( midrib \) loci were established, which were represented by \( bm2, bm6, bm12, \) and \( bm19 \) (Saballos et al. 2008). The abbreviation \( bm \) was adopted to distinguish it from \( bm \), already in use for the sorghum \( bloomless \) mutants (Saballos et al. 2009). \( SbCAD2 \) was found to be responsible for the phenotype of the \( bm6 \) mutants (Saballos et al. 2009; Sattler et al. 2009), while the \( bm12 \) mutation was found to be located in the gene encoding \( SbCAOMT \) (Bout and Vermerris 2003). Similar coloration was observed in a gymnosperm tree; a mutant pine (\( Pinus taeda \)) deficient in \( CAD \) showed brown coloration in the wood (MacKay et al. 1997).

In addition to \( bm \) mutants, some transgenic plants in which genes encoding enzymes in the cinnamate/monolignol pathway were downregulated showed unusual red, brown or orange coloration, which was not observed in the corresponding wild-type plants. For example, \( CAOMT \) downregulation in poplar (\( Populus tremula \times Populus alba \)) (Van Doorselaere et al. 1995) and in aspen (\( Populus tremuloides \)) (Tsai et al. 1998) resulted in xylem tissues with pale rose and reddish brown coloration, respectively. \( CAD \) downregulation in alfalfa (\( Medicago sativa \)) (Baucher et al. 1999), tobacco (\( Nicotiana tabacum \)) (Chabannes et al. 2001; Halpin et al. 1994; Hibino et al. 1995), and poplar (\( P. tremula\times P. alba \)) (Baucher et al. 1996) resulted in various red, brown and pink colorations of the xylem. With \( CCR \) downregulation, brown (Chabannes et al. 2001) and orange-brown (Piquemal et al. 1998) coloration was observed in the xylem of transgenic tobacco plants (\( N. tabacum \)). In addition, brown coloration in the xylem of \( 4CL \)-downregulated tobacco (\( N. tabacum \)) (Kajita et al. 1996) and red coloration of xylem of \( CaCAOMT \)-downregulated poplar (\( P. tremula\times P. alba \)) (Zhong et al. 2000) were reported.

The mechanisms for this coloration in the mutants have not been fully elucidated. However, the red-purple coloration of the \( CAD \)-downregulated tobacco has been attributed to the incorporation of hydroxyxycinnamaldehydes into lignin (Hibino et al. 1995), because a synthetic lignin, a dehydrogenation polymer from coniferaldehyde, exhibited red-purple, wine-red like coloration (Higuchi et al. 1994), and because hydroxyxycinnamaldehyde contents in the mutants are significantly elevated compared with the corresponding wild-type plants (Barrière et al. 2004; Sattler et al. 2009). Similarly, the red-brown coloration of transgenic aspen (\( P. tremuloides \)) in which \( CAOMT \) was downregulated was also ascribed to a higher amount of coniferaldehyde residues in the transgenic line (Tsai et al. 1998).

Interestingly, no \( bm \) mutants have been identified or described for the \( C3 \) grasses including rice (\( Oryza sativa \)) which is another important Gramineae crop (Sattler et al. 2010), although \( CAD \)-deficient rice mutants (Oookawa et al. 2008; Zhang et al. 2006) and \( CAD \)-downregulated rice (Shiba et al. 2007) were described. It has been suggested for reasons yet to be determined that \( C3 \) grasses do not accumulate the characteristic light-brown pigment in the midribs of their leaf blades (Sattler et al. 2010). A point mutation of \( OsCAD2 \) gene in \( O. sativa \) \( L. ssp. indica \) cv. Zhefu802 (hereafter referred to as Zhefu802) exhibited an obvious reddish-brown pigment in the internode, and basal leaf sheath at the heading stage and golden yellow coloration of the hull, and designated as the \( gold \ hull \) and \( internode \) (\( gh \)) 2 mutant (Zhang et al. 2006). Another \( gh2 \) mutant of an unknown background showed lower \( OsCAD2 \) gene expression and lignin content compared with a control rice plant (Ookawa et al. 2008). In addition, Oryzabase (http://www.shigen.nig.ac.jp/rice/oryzabaseV4/) shows a number of rice \( gh \) mutants (\( gh1, gh2, \) and \( gh3 \)). For example, a \( gh2 \) mutant of \( O. sativa \) \( L. \ ssp. japonica \) cv. Miyazaki No.1 was reported by Iwata and Omura (1971), while recently a \( gh1 \) mutant was found to be due to a mutation of a chalcone isomerase gene (\( OsCHI; Os03g0819600 \)) involved in the flavonoid biosynthesis (Hong et al. 2012). Again, however, none of the \( gh \) mutants were reported to show the \( bm \) phenotype.

Rice straw, together with other inedible lignocellulosic biomass products such as corn stover, sugarcane bagasse, and wheat straw, is expected to be a promising feedstock as an industrial fermentation substrate (Park et al. 2011). In Japan, annual domestic production of rice straw accounts for about 9.6 Mt. However, more than 60% of rice straw is deposited on rice fields (Park et al. 2011). In addition, rice is an important model plant for large-sized Gramineae bioenergy plants, such as \( Erianthus \), napier grass, and switch grass (Yamamura et al. 2013). Hence, many research projects for bioconversion of rice straw to fermentable carbohydrates are on-going in Japan (Park et al. 2011). In this bioconversion, enzymatic saccharification of lignocellulosic materials is the key step, and is affected largely by the amount and structure of lignins. In this context, we characterized a rice mutant which had a \( Tos17 \) insertion into \( CAD2 \) gene and found that the mutant exhibits higher enzymatic saccharification efficiency and the \( bm \) phenotype as well as the \( gh2 \) phenotype.
Materials and methods

Plants
The gh2 mutant line (NE4246) was identified among the Tos17 insertion mutant population derived from O. sativa L. ssp. japonica cv. Nipponbare. Mutagenesis with Tos17 and polymerase chain reaction (PCR) screening of mutants were performed as described by Kumar and Hirochika (2001) and Miyao et al. (2003). The homozygous gh2 mutant and GH2 sibling plants and the wild type were cultivated in both the test field of the National Institute of Agrobiological Science and the green house facility of the Research Institute for Sustainable Humanosphere, Kyoto University.

Tos17 insertion at the GH2 gene locus in the gh2/gh2 mutant and no insertion in the GH2/GH2 plant were confirmed by genomic PCR analysis (Figure 1). Total DNA was extracted from leaf blades with a DNeasy plant mini kit (Qiagen, Hilden, Germany), and PCR was done with total DNA as the template and three primers [OsCAD2 1st intron fwd (P1): 5’-TGGTATGCAATTCTGTGGCCATGCGT-3’, OsCAD2 2nd exon rev (P2): 5’-TTCCGCTGTGGTGGATTAGTCAGTCA-3’, P3: 5’-CAGCAAGTGATGTTGTCTCAGGC-3’]. The PCR products were analyzed on a 1% (w/v) agarose gel and were visualized with ethidium bromide. An OsCAD2 (Os02g0187800) RNA-interference (RNAi) knock-down plant was prepared previously (Hattori et al. 2012) using the specific primer set: CAD2_RNAi_f, 5’-CACGCAAGATGTTGTCTCAGGC-3’ and CAD2_RNAi_r, 5’-CGGATGATCAGTCA-3’. Specific primers (UBQ5 fwd, 5’-ACCCTCGACTGCCACTACT-3’ and UBQ5 rev, 5’-ACGCTAAGCGTGTGT-3’) as an internal control. The ΔΔCt method was adopted for calculation of gene expression (User Bulletin #2, Applied Biosystems).

qRT-PCR analysis
Total RNA was extracted from each part of rice plants with an RNeasy plant mini kit (Qiagen). First-strand cDNA was synthesized in a 20 µl reaction mixture containing 1 µl of SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA), 1 µg of total RNA, 50 pmol Oligo(dT)20 primer (Invitrogen), 10 nmol dNTPs, 0.2 µM DTT, and 1 µl of RNase OUT (Invitrogen) at 42°C for 50 min. The reaction mixture was diluted 8-fold with TE buffer (pH 8.0).

Quantitative real-time RT-PCR (qRT-PCR) was conducted using an Applied Biosystems 7300 Real-time PCR System. For each reaction, the 20 µl mixture contained 1 µl of first-strand cDNA, 12.5 µl of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), and 2 pmol each of the forward and reverse primers: CAD2_fwd, 5’-GGTATTGGAGCTCTGGCAGCCTGG-3’ and CAD2_rev, 5’-CATATACTGTGGAGTGGCAGATGGTAT-3’. The amplification program was as follows: 50°C for 2 min, 95°C for 10 min and 40 cycles at 95°C for 15 s and 60°C for 1 min (Suzuki et al. 2006). Single fluorescence reading was performed at each cycle immediately following the elongation period at 60°C. The fluorescence was measured during the cycle to ensure single product amplification. Cycle threshold (Ct) values were determined automatically using the Sequence Detection Software ver. 1.2 (Applied Biosystems). Each reaction was conducted in triplicate. A ubiquitin gene (OsUBQ5; accession no. AK061988) was amplified using specific primers (UBQ5 fwd, 5’-ACCCTCGACTGCCACTACT-3’ and UBQ5 rev, 5’-ACGCTAAGCGTGTGT-3’) as an internal control. The ΔΔCt method was adopted for calculation of gene expression (User Bulletin #2, Applied Biosystems).

Histochemical analysis
Fresh hand-cut sections (approximately 100 µm-thick) were prepared from the third internodes of rice plants at the heading stage. For Wiesner staining, the sections were incubated for 10 min in phloroglucinol solution (1% in 70% ethanol), and treated with 18% HCl for 5 min. The sections were photographed under a light microscope (Olympus BX-51, Olympus, Tokyo, Japan) (Li et al. 2009; Zhang et al. 2006).

Milling of the tissues
Dried rice plants after removal of spikelets were separated to the following tissues [total fraction without spikelets (T), leaf blades (LB), leaf sheaths (LS), internodes without uppermost internodes (IN), uppermost internodes (U), tissues around ±5 mm from nodes (N), rachises and panicle branches without spikelets (R)]. These tissues were cut into about 5-mm pieces and dried in a desiccator at room temperature with silica gel. They were further dried in vacuo overnight at room temperature. Each dried sample (0.5 g) was ground in a ball mill (TissueLyser, Qiagen) with 20 mm stainless steel balls at 26 Hz for 3 min (T, U, N, R), 2 min (LB, LS), or 4 min (IN). The milled rice materials were put into glass bottles and stored in a desiccator with silica gel at room temperature until use.
Determination of enzymatic saccharification efficiency

Enzymatic saccharification efficiency was determined by the method of Hattori et al. (2012). Briefly, the starch in the tissue (T) was degraded using a Total Starch Kit (Megazyme, Co. Wicklow, Ireland). The starch-free residue (SFR) was separately subjected to acid saccharification and enzymatic saccharification using enzymes including Celluclast 1.5 L (cellulase), Novozyme 188 (celllobiase), and Ultraflo L (β-glucanase and xylanase with side activities of cellulase, hemicellulase, and pentosanase) (Novozymes, Bagsvaerd, Denmark). In the enzymatic saccharification of SFR, the amounts of glucose liberated were determined at 1, 2, 3, 6, 8, 10, 12, 14, 20, 24, and 48 h from the start. The enzymatic saccharification efficiency (%) was determined by the following equation:

\[
\text{Enzymatic saccharification efficiency (\%)} = \left( \frac{\text{glucan-derived glucose from SFR}}{\text{Glucan-derived glucose by acid saccharification from SFR}} \right) \times 100
\]

Preparation of coniferaldehyde dehydrogenation polymer

Synthetic lignin [dehydrogenation polymer (DHP)] was prepared by peroxidase-catalyzed polymerization of coniferaldehyde. Coniferaldehyde was prepared according to Nakamura and Higuchi (1976), 1H-NMR δ (CDCl3) 3.95 (3H, s), 6.59 (1H, dd, J 7.75, 15.80), 6.95 (1H, d, J 8.14), 7.06 (1H, d, J 1.82), 7.12 (1H, dd, J 1.83, 8.21), 7.40 (1H, d, J 15.79), 9.64 (1H, d, J 7.76), which was taken by Varian XL-200 FT-NMR spectrometer (Varian, Palo Alto, CA, USA). Coniferaldehyde thus obtained (10 mg) was dissolved in 0.1 ml of acetone, and mixed with 1 ml of 0.05 M acetate buffer (pH 5.2). Separately prepared 10 µl of horse radish peroxidase solution dissolved in 0.05 M acetate buffer (pH 5.2, 1 mg ml⁻¹) and 10 µl of 3% H2O2 were added to the reaction mixture. After 5 min at room temperature in the dark, 10 µl of 3% H2O2 was added and the reaction was continued for an additional 5 min in the same way. The reaction was stopped by adding 1.5 ml of ethyl acetate (EtOAc). The synthesized DHP was collected by centrifugation, washed with water for three times, and dried in vacuo.

Lignin analysis

Lignin content in each tissue (T), (LB), (LS), (U), (IN), (N), and (R) was determined using the thioglycolic acid lignin method (Suzuki et al. 2009). The aromatic ring composition of lignin was analyzed by a microscale protocol of the nitrobenzene oxidation method (Yamamura et al. 2010; Yamamura et al. 2011). Thioacidolysis of lignin and DHP was conducted as previously described (Nakatsuho et al. 2008; Yamamura et al. 2011). For detecting the cinnamaldehyde specific-indene compound, the thioacidolysis products from the hulls of gh2 mutant and DHP were individually separated by preparative silica gel TLC (EtOAc:n-hexane, 1:2), and submitted to GC-MS analysis. GC-MS was performed using a Shimadzu QP-5050A GCMS system (Shimadzu Co., Ltd., Kyoto, Japan). The GC-MS conditions were as follows: Shimadzu Hicap CBP10-M25-025 column (25 m×0.22 mm): carrier gas, helium; injection temperature, 230°C; oven temperature, 40°C at t=0 to 2 min, then to 230°C at 40°C min⁻¹; ionization, electron-impact mode (70 eV) (Nakatsuho et al. 2008; Yamamura et al. 2011).

Results

In the wild type of O. sativa L. ssp. japonica cv. Nipponbare (hereafter referred to as Nipponbare), cinnamyl alcohol dehydrogenase 2 gene [CAD2 (an ortholog of the GH2 in Zhefu802), Os02g0187800] was expressed widely in all the tissues tested (Figure 2). We screened the Tos17 mutant panel and isolated a plant that had a retrotransposon insertion in the second exon of the gene (Figure 1). Homozygous gh2 mutant exhibits obvious reddish-brown color in the panicles (hulls), internodes, and nodes at the heading stage (Figure 3), in which relatively high expression of OsCAD2 (=GH2) gene was observed (Figure 2). These phenotypes were typical of gh2 mutant of Zhefu802 (Zhang et al. 2006). Except for the reddish-brown coloration in these specific tissues, the gh2 mutant of Zhefu802 showed similar development to the wild type, and the bm phenotype was not reported (Zhang et al. 2006).

In sharp contrast, the present gh2 mutant clearly showed the bm phenotype as indicated in Figure 3
The reddish-brown pigment was observed in the sclerenchyma and vascular bundle cell walls in the leaf and culm (Figure 4C, I). In addition, histochemical staining with Wiesner reagent indicated enhanced red-purple coloration in the leaf midribs and internodes of the gh2 mutant compared with those of the control plants (wild-type and GH2 plants) (Figure 4). Furthermore, the RNAi technique successfully downregulated the...
When the Klason method is applied for rice lignin quantitation, it results in erroneous values due to high silica content (Lai and Sarkanen 1971). Hence, we employed the thioglycolic acid method (Suzuki et al. 2009). In all of the tissues tested, the gh2 mutant had 10–20% less lignin than control (GH2) plants (Figure 6). The gh2 mutant and control (GH2) plants were then subjected to lignin structural analyses using the thioacidolysis and nitrobenzene oxidation methods. The former method detected not only trithioethylphenyl compounds which are specifically formed from β-O-4 lignin substructures, but also the cinnamaldehyde specific-indene compound (Kim et al. 2002). The indene compound [trimethylsilyl-8G (Figure 7) described in Kim et al. (2002)] was detected from thioacidolysis products of hulls of gh2 mutant with mass spectra: MS, \(m/z\) (\%) 354 (M+, 83), 325 (28), 293 (100), 260 (58), 230 (32). On the other hand, the indene compound was not detected in the thioacidolysis products from the control (GH2) plants (Table 1). These results indicated the occurrence of coniferaldehyde residues in the lignin of the gh2 mutant. Nitrobenzene oxidation analysis showed that the aromatic composition of the gh2 mutant lignin was slightly modified, as evidenced by lower S/V and H/V values throughout the three organs: flag leaf, culm, and hull (Table 2).

Figure 8 shows the enzymatic saccharification efficiency in the tissue (T) of gh2 mutant and control (GH2) plants. The efficiency of saccharification after 48 h incubation was 35.0% in the control plants, while it reached to 40.6% in the gh2 mutants (Figure 8). The amounts of glucose liberated by acid saccharification
GH2

±

control

(1.0% cell wall residue).

In the present study we have demonstrated that a null mutant (30.1 ± 1.8% cell wall residue) did not differ significantly with those form mutant (31.0 ± 1.8% cell wall residue) did not differ significantly with those form mutant (31.0 ± 1.8% cell wall residue).

Discussion

from cell wall residues of gh2 mutant (31.0 ± 1.8% cell wall residue) did not differ significantly with those form control (GH2) plants (30.1 ± 1.0% cell wall residue).

of a gold hull and internode (gh)/brown midrib (bm) phenotype, and it was confirmed that this phenotype is due to the impaired OsCAD2 expression. This is the first description of a bm phenotype in a typical C3 grass, in this case rice.

The wild-type Nipponbare showed significant GH2 gene expression in the midrib (Figure 2), which was probably strong enough to cause the bm phenotype in the corresponding gh2 mutant. By contrast, in the wild-type Zhefu802, the expression of the GH2 gene in the midribs was lower than that in the hulls which exhibit a reddish brown pigment in the gh2 mutant, while the total CAD activity in the midribs was higher than that in the hulls (Zhang et al. 2006). These data suggest that OsCAD2 was not major in the midribs of the wild-type Zhefu802. This can account for the reason why no bm phenotype was observed in the gh2 mutant of the Zhefu802.

Histochemical staining with Wiesner reagent indicated the red-purple coloration was greater in the gh2 mutant than in the control plant, strongly suggesting the occurrence of cinnamaldehyde residues in the mutant, which is a typical characteristic of CAD-deficient mutants or transgenic plants (Barrière et al. 2004; Kim et al. 2003; Ralph et al. 2001; Sattler et al. 2010). This was further confirmed by the detection of a degradation fragment derived from the cinnamaldehyde structure; thioacidolysis of the gh2 mutant detected a degradation fragment derived from the cinnamaldehyde residues, which can account for the reason why no bm phenotype was observed in the gh2 mutant of the Zhefu802.

Table 2. Lignin aromatic ring compositions by nitrobenzene oxidation in the flag leaf, culm, and hull of GH2 and gh2 plants.

<table>
<thead>
<tr>
<th></th>
<th>Flag leaf</th>
<th>Culm</th>
<th>Hull</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanillin (V)</td>
<td>GH2</td>
<td>94.31±2.56</td>
<td>130.64±8.92</td>
</tr>
<tr>
<td></td>
<td>gh2</td>
<td>77.83±4.98*</td>
<td>114.54±3.38*</td>
</tr>
<tr>
<td>Syringaldehyde (S)</td>
<td>GH2</td>
<td>16.44±0.62</td>
<td>49.91±1.52</td>
</tr>
<tr>
<td></td>
<td>gh2</td>
<td>9.16±0.89*</td>
<td>30.72±2.61*</td>
</tr>
<tr>
<td>p-Hydroxybenzaldehyde (H)</td>
<td>GH2</td>
<td>20.05±0.31</td>
<td>57.83±8.25</td>
</tr>
<tr>
<td></td>
<td>gh2</td>
<td>14.99±0.50*</td>
<td>24.22±2.13*</td>
</tr>
<tr>
<td>S/V</td>
<td>GH2</td>
<td>0.17±0.01</td>
<td>0.38±0.02</td>
</tr>
<tr>
<td></td>
<td>gh2</td>
<td>0.12±0.01*</td>
<td>0.27±0.02*</td>
</tr>
<tr>
<td>H/V</td>
<td>GH2</td>
<td>0.21±0.01</td>
<td>0.44±0.04</td>
</tr>
<tr>
<td></td>
<td>gh2</td>
<td>0.19±0.01*</td>
<td>0.21±0.01*</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates±SD. Asterisks indicate significant difference from the GH2 plant (Student’s t-test, p<0.05). S/V and H/V: Molar ratios of S to V and H to V, respectively.

Figure 8. Enzymatic saccharification efficiency in the shoots of GH2 and gh2 plants over 48 h. Each value is the mean of three replicates±SD.
incubation for 48h, saccharification efficiency in the control (GH2) plants reached 35.0%, while in the gh2 plants was 40.6%, which is about 16.1% higher than that in the control plants. These results clearly indicate that rice gh2/bm mutants are promising for fodder and feedstock as an industrial fermentation substrate. Importantly, the mutation in the Nipponbare can be exploited to breed rice cultivars by cross-fertilization, especially forage paddy rice cultivars.

In conclusion, the cad2 (cinnamyl alcohol dehydrogenase 2) null mutant isolated from retrotransposon Tos17 insertion lines of Nipponbare showed the bm phenotype in addition to the gh phenotype. This is the first report of a bm mutant from C3 grasses. The gh2/bm mutant had higher enzymatic saccharification efficiency and lower lignin content compared with the control (GH2) plant. This mutation could be applied to breed forage paddy rice cultivars that are suitable for use as fodder and industrial feedstock.

Acknowledgements

The authors thank Ms. Aiko Morita, Ms. Kumiko Murata, Ms. Mayumi Inutsuka, and Ms. Sawako Ohtsu for their technical assistance. Thanks are due to Professor Ko Shimamoto, Nara Institute of Science and Technology, for the supply of pPANDA vector; and Novozymes, Bagsvaerd, Denmark, for the supply of Celluclast 1.5 L, Novozyme 188, and Ultraflo L. This research was partly supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, GMA-0006). A part of this study was conducted using the Development and Assessment of Sustainable Humansosphere/Forest Biomass Analytical System at the Research Institute for Sustainable Humansphere, Kyoto University, Japan.

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