The reduction in lignin content is an important factor contributing to enhanced digestibility, and in this regard, a range of transgenic plants with reduced lignin content have been developed by the down-regulation of selected genes encoding lignin biosynthetic enzymes (Halpin 2019; Hisano et al. 2009) and transcription factors (Xie et al. 2018). However, plants with reduced lignin content are often characterized by undesirable agronomic traits (De Meester et al. 2020), such as dwarfism and the collapse of xylem vessel elements (Bensussan et al. 2015; Phitsuwan et al. 2013). Moreover, gains in fermentable sugar yields are often associated with lower biomass yields, even if the cellulose-to-glucose conversion efficiency is higher (Van Acker et al. 2014). Hence, other strategies have been suggested to alter lignin composition, without reducing the content (Pedersen et al. 2005).

In this latter regard, the expression of microbial cell
wall degradation enzymes in plants is a potential strategy that does not result in a reduction in lignin content. One such enzyme is CcAbf62A, a putative extracellular α-L-arabinofuranosidase, the encoding gene of which (CcAbf62A: DNA database accession no. AB557888) has been cloned from Coprinopsis cinerea (Hashimoto et al. 2011). C. cinerea is a mycotrophic basidiomycete that is often found on partially rotted grass, although not woody biomass. CcAbf62A targets arabinoxylan, the major hemicellulose of grasses (Kulkarni et al. 2012), cleaving the glycosidic bonds between the xylan backbone and arabinose sidechains (Hashimoto et al. 2011). The arabinoxylan in grass cell walls is acylated by hydroxycinnamates such as ferulate and p-coumarate at the 5′-hydroxy position of arabinosyl residues (Chiniquy et al. 2012; Sato-Izawa et al. 2020). Some of these hydroxycinnamate units subsequently undergo cell wall radical coupling reactions with other hydroxycinnamate units, monolignols, or oligolignols, thereby generating crosslinks between arabinoxylan chains and/or with lignin polymers (Eugene et al. 2020). Consequently, CcAbf62A activity contributes to reducing the number of links between lignin and cell wall polysaccharides, and degrades lignin carbohydrate complex (LCC). In the previous research, expression of fungal feruloyl esterase in tall fescue reduced the release of ferulic acid from the cell walls, and increased cell wall digestibility (Buanafit et al. 2008). In addition, knockout of xylan arabinosyl-transferase improved saccharification efficiency of rice straw (Chen et al. 2021). These results indicate that introduction of genes encoding for the enzymes which can cut the linkages between lignin and arabinoxylan into the grasses may cause increasing of the degradability of the cell wall materials. In this study, we introduced the CcAbf62A gene into rice with the aim of enhancing delignification efficiency and availability of lignocellulosic materials in the absence of a concomitant reduction in lignin content.

The DNA sequence encoding the signal peptide from prxA3a (DNA database accession no. D38050) (Osakabe et al. 1995), the gene of an anionic peroxidase of hybrid aspen, Populus sieboldii×P. grandidentata, was inserted into the 5′-flanking site to link the signal peptide to the amino terminus of mature CcAbf62A for secretion into the apoplast. The resulting DNA fragment was replaced with uidA encoding β-glucuronidase (GUS) in the downstream region of the CaMV35S promoter in a pIG121-Hm plasmid (Akama et al. 1992) and introduced into rice (Oryza sativa L. cv. Nipponbare) using the Agrobacterium-mediated transformation method mediated by the EHA105 strain of Rhizobium radiobacter. A total of 32 transgenic rice lines, denoted pG121-GUS(−)-Abf lines, were thus generated, among which, 13 showed markedly disrupted elongation growth (dwarf) and excessive tillering, similar to turf (Figure 1A, B, C, D). A further seven lines were characterized by delayed elongation growth (retarded-growth) (Figure 1C, E), and the remaining 12 showed no appreciable...
differences in plant height compared with the control plants (normal) (Figure 1D, E). The dwarf lines were also characterized by reduced acclimation ability and generally became withered soon after transfer from growth medium to soil (Pandey and Shukla 2015). Having thinned the copious tillers prior to transfer (Zou et al. 2006), only a single dwarf line remained viable after acclimation experiments (Figure 1F) and set seed. In our previous research, we introduced CcEST1 (DNA database accession no. AB540992) from C. cinerea (Hashimoto et al. 2010), the gene for an esterase which can cleave the feruloyl-ester bonds in LCC (Mayuzumi et al. 2016), and other genes into rice plants, but no transformant showed the markedly disrupted growth such as the dwarf lines transformed with CcAbf62A. Then, the strong dwarfism observed in the dwarf Abf lines are thought to be specifically caused by the expression of CcAbf62A. All plants were grown under growth chamber conditions (light period: 11 h, 28°C; dark period: 13 h, 25°C); however, although both normal and retarded-growth lines were also characterized by a larger number of tillers and spikelets than control plants, their seed setting rates were considerably lower (Table 1). The agronomic traits of dwarf T₀ lines could not be determined because most of them could not be acclimated. The only acclimated dwarf line also showed low seed setting ability (data not shown). All regenerated T₀ lines were screened for hygromycin resistance and subjected to genomic PCR analysis, and only those lines confirmed as transformants were used in subsequent experiments.

For the analysis of T₁ generation plants, lines showing typical dwarf, retarded-growth, or normal phenotypes was selected. The zygosity and copy numbers of CcAbf62A in the T₁ plants could not be determined because most of them could not be acclimated. The only acclimated dwarf line also showed low seed setting ability (data not shown). All regenerated T₀ lines were screened for hygromycin resistance and subjected to genomic PCR analysis, and only those lines confirmed as transformants were used in subsequent experiments.

The agronomic traits of dwarf T₀ lines could not be determined because most of them could not be acclimated.

Table 1. Agronomic traits of pIG121-GUS(−)−Abf lines (T₀).

| Line     | Phenotype          | No. of seeds | No. of spikelets | No. of productive tillers | No. of tillers |
|----------|--------------------|--------------|------------------|---------------------------|---------------|
| pActnos #1 | normal             | 112          | 147              | 6                         | 11            |
| pActnos #2 | normal             | 125          | 177              | 3                         | 12            |
| Abf-1     | normal             | 0            | 321              | 22                        | 41            |
| Abf-2     | normal             | 78           | 512              | 29                        | 36            |
| Abf-5     | normal             | 164          | 336              | 12                        | 28            |
| Abf-11    | normal             | 77           | 595              | 22                        | 58            |
| Abf-12    | normal             | 38           | 262              | 12                        | 36            |
| Abf-18    | normal             | 13           | 81               | 6                         | 11            |
| Abf-24    | normal             | 0            | 215              | 7                         | 37            |
| Abf-28    | normal             | 0            | 65               | 4                         | 12            |
| Abf-6     | retarded-growth    | 0            | 71               | 10                        | 23            |
| Abf-9     | retarded-growth    | 1            | 22               | 5                         | 15            |
| Abf-20    | retarded-growth    | 48           | 533              | 22                        | 34            |
| Abf-25    | retarded-growth    | 0            | 240              | 17                        | 20            |
| Abf-29    | retarded-growth    | 0            | 208              | 16                        | 23            |
| Abf-30    | retarded-growth    | 0            | 641              | 45                        | 72            |

All plants were harvested during the ripening stage. Spikelets include seeds and tillers include productive tillers. The agronomic traits of dwarf lines could not be determined because most of them could not be acclimated.
Dwarfism and increased tillering in rice producing CcAb62A

a sieve to remove the particulate fraction over 0.5 mm in size. Aliquots of the milled samples thus obtained were weighed (W1), and 0.25 g was placed in 100 ml medium bottles, to which 0.25 g of sodium sulfate, 50 ml of neutral detergent solution (30 g l−1 sodium dodecyl sulfate), 18.61 g l−1 EDTA-2Na, 6.81 g l−1 sodium tetraborate decaborate, 3.56 g l−1 disodium phosphate, and 1% (v/v) 2-ethoxyethanol) were added, followed by boiling for 1 h. The resulting residues were filtered using weighed glass filters, washed with three times with acetone, dried at 105°C, and weighed (W2). Thereafter, treated samples were transferred to medium bottles containing 40 ml of acid detergent solution (20 g l−1 cetyltrimethylammonium bromide in 500 mM sulfuric acid) and boiled for 1 h, with the residues obtained being similarly filtered, dried, and weighed (W3). The glass filters onto which samples had been collected were subsequently placed in 100 ml beakers, to which 10 ml of 72% sulfuric acid was added, and after 3 h at room temperature, the residues were filtered using weighed filter papers for quantification and washed with water until a filtrate with neutral pH was obtained. The residues were then dried and weighed (W4). Finally, the samples collected on filter papers were transferred to weighed crucibles and incinerated in a muffle furnace at 550°C for 2 h and subsequently weighed (W5). Relevant indices of cell wall components were calculated using the following formulae:

Neutral Detergent Fiber (NDFom) = (W2 − W5)/W1 × 100
Acid Detergent Fiber (ADFom) = (W3 − W5)/W1 × 100
Acid Detergent Lignin (ADL) = (W4 − W5)/W1 × 100

We found that normal and retarded-growth Abf lines were characterized by approximately the same NDFom values as the control line (pActnos), thereby indicating similar biomass contents (holocellulose and lignin) (Table 2). However, the retarded-growth Abf lines were found to have slightly lower NDFom values compared with the control line (93.9–99.2%), thereby indicating that the delay in elongation growth in these lines also had the effect of reducing biomass accumulation. The result that two (Abf-1 and Abf-2) of three normal Abf lines had significantly higher NDFom compared to two (Abf-20 and Abf-30) of three retarded-growth lines, also suggests a positive correlation between elongation growth and biomass content. Contrastingly, the ADLom value of the one normal Abf line (Abf-2) was observed to be significantly higher than those of other lines, thus indicating that this line contained higher amounts of cellulose and lignin. Given that the ADL content in Abf-2 did not differ significantly from that of the control lines, this normal line was assumed to have a higher cellulose content than that of the control. This higher cellulose content is presumed to be attributable to the preferable culm growth conditions, as Abf-2 was found to be characterized by more productive tillers than other normal Abf lines (Table 1).

As cell wall component analysis applicable to the dwarf lines, we conducted Infrared (IR) analysis. IR spectra were obtained using a PerkinElmer, Inc. Frontier system (Spotlight 200 Frontier) equipped with an ATR accessory. Leaves of the rice T0 generation were cut from the plants of three lines of each of the three phenotypes and freeze-dried. The spectra were recorded over wave number ranges of 4,000 to 500 cm−1, and all absorbance values were normalized to the band at 1,040 cm−1. The average spectra of three spectra recorded for each phenotype are shown in Figure 2.

In all three types of Abf line, a large proportion of the bands increased in intensity compared with those of the control line, and these increases were observed to be more prominent in the spectra obtained for dwarf (Abf-15) and retarded-growth (Abf-25) lines than in those of the normal Abf line (Abf-2). As the band at 1,040 cm−1 used as internal standard is associated with the C=O stretching vibration of cellulose, these observations are taken to be indicative of a dwarfism intensity-dependent reduction in the cellulose content of Abf lines (Figures 1, 2). We also detected a significant increase in the intensity of bands at 1,630 cm−1 and 1,508 cm−1 in the dwarf and retarded-growth Abf lines. Given that bands at 1,630 cm−1 are presumed to be the overlapping bands of conjugated C=O stretching vibrations (1,660 cm−1) and

| Line      | Phenotype       | NDFom ± SD  | ADFom ± SD  | ADL ± SD  |
|-----------|-----------------|-------------|-------------|---------|
| pActnos   | normal          | 64.0 ± 0.9 a| 33.9 ± 0.8 b| 2.3 ± 0.4 a|
| Abf-1     | normal          | 65.9 ± 1.3 a| 33.5 ± 0.3 b| 2.6 ± 0.5 a|
| Abf-2     | normal          | 66.5 ± 1.5 a| 38.9 ± 1.4 a| 2.9 ± 0.5 a|
| Abf-5     | normal          | 62.4 ± 1.1 b| 31.2 ± 1.2 b| 1.9 ± 0.3 a|
| Abf-20    | retarded-growth | 60.8 ± 1.3 b| 31.7 ± 1.0 b| 2.3 ± 0.2 a|
| Abf-25    | retarded-growth | 63.5 ± 0.1 a| 33.2 ± 0.4 b| 2.7 ± 0.4 a|
| Abf-30    | retarded-growth | 60.1 ± 0.5 a| 32.4 ± 0.4 b| 2.7 ± 0.0 a|

Values are the means ± standard deviation from triplicate analyses. Statistical analyses were performed by Tukey’s test. Different letters indicate significant differences at p<0.05. Quantification of cell wall components of dwarf lines could not be determined because they were too small to provide adequate sample amounts.
aromatic skeletal vibrations (1,600 cm\(^{-1}\)), whereas those at 1,508 cm\(^{-1}\) are assigned to aromatic skeletal vibrations (Horikawa et al. 2019), these bands are assumed to be related to the content of lignin or aromatic compounds. Bands between 1,240 cm\(^{-1}\) and 1,508 cm\(^{-1}\) were also increased especially in the dwarf line. Bands at 1,462 cm\(^{-1}\) are assigned to C-H bending vibrations in moieties of CH\(_2\) and CH\(_3\) in lignin. In particular, bands at 1,422 cm\(^{-1}\) are significantly increased in intensity in the dwarf line. These bands are not specific bands of lignin, but bands at 1,422 cm\(^{-1}\) are assigned to C-H bending vibrations in CH\(_3\) and are one of the characteristic peaks of lignin. Increases in intensity were similarly observed for bands at 1,730 cm\(^{-1}\) and 1,240 cm\(^{-1}\), which are related to the acetyl group in hemicellulose; however, the detected changes were smaller compared with those observed for the bands related to the contents of lignin or aromatic compounds. Accordingly, these observations would tend to indicate that the dwarf and retarded-growth lines are characterized by reductions in the contents of cellulose and hemicellulose and a corresponding increase in lignin content.

Total RNA was extracted from T\(_1\) plants using an RNeasy plant mini kit (Qiagen, Hilden, Germany) from T\(_1\) plants, from which cDNA was subsequently synthesized, with ReverTra Ace\(^{\circledast}\) (Toyobo, Osaka, Japan) and Taq DNA Polymerase being used for DNA amplification. Levels of CcAbf62A were compared using the Actin gene (DNA Data Base accession no. S44221) as an internal standard. The sequences of the specifically designed primer sets used for amplification are as follows: PrxA3a signal Forward 5′-GAA AGC TTT TCA GAA TGG TAG ATA AAG CAA TGC-3′ and Abf62A Reverse 5′-TTA ACA AGC AGA GTT GGT TTG AGT A-3′; and Actin Forward 5′-CGT CTT GGA TAA TGG AAC TGT-3′ and Actin Reverse 5′-TCT GGG TCA TCT TCT CAC GA-3′.

The expression of the transgene could be confirmed in all transgenic rice plants by RT-PCR (Figure 3). In comparison with Actin bands, the dwarf Abf line (Abf-26) seemed to have higher levels of CcAbf62A expression than either the retarded-growth (Abf-20) or normal (Abf-2) lines (Figure 3), thereby indicating that observed differences in the phenotypes of the three lines induced by CcAbf62A could be attributed to differences in the expression levels of this gene. In support of this assumption, we have previously demonstrated that all transgenic rice transformed with CcAbf62A driven by the rice Actin promoter showed no significant differences in phenotype compared with the empty vector controls (Mayuzumi et al. 2016). Although the rice Actin promoter is considered to have higher activity than the CaMV35S promoter in monocots (Schledzewski and Mendel 1994), including rice (Pérez Bernal et al. 2016), it is conceivable the CaMV35S promoter might drive extremely high expression levels of the transgene in the dwarf and retarded-growth lines examined in the present study. We thus speculate that the differences observed in the CaMV35S promoter-driven levels of CcAbf62A expression among Abf lines are attributable to positional effects, DNA methylation, or other factors.

To assess the extent of lignin deposition in the transgenic rice lines, we performed histochemical analysis.
using phloroglucinol staining (Sato-Izawa et al. 2020). Rice culms were cut from the roots at the vegetative, heading, and ripening stages at different internodes, and fixed in formalin : acetic acid : 50% ethanol (1 : 1 : 18). Cross-sections of the culms were obtained using a razor and placed on glass slides. Fifty microliters of phloroglucinol ethanol solution (20 mg ml⁻¹) was applied to the sections and left to stand for 1 min, after which 100 µl of 6 M HCl was applied. The preparations were then covered with a cover glass and observed immediately under an Olympus BX50 microscope.

At the vegetative stage, culms of the dwarf Abf line (Abf-26) were found to have lower amounts of lignin deposited in vascular bundles and epidermis compared with the control line (pIGnos) (Figure 4A, B). Although the delay in lignin deposition in the dwarf line continued to the heading stage (Figure 4C, D), deposition patterns similar to those in the control line were observed at the ripening stage (Figure 4E, F). However, we detected no evidence of irregular xylem tissue development, which is often observed in lignin-modified plants (Li et al. 2009). We suspect that the reduced acclimation ability of dwarf lines can be explained by increased water loss and reduced water conductivity that occur concomitant with delayed lignin deposition (Ke et al. 2019).

To evaluate the delignification efficiency, we conducted quantification of lignin content and alkaline treatment. As the normal (Abf-2) plants showed similar agronomic traits to the control plants, only retarded-growth (Abf-20) and dwarf (Abf-26) plants were selected from the transgenic lines for this analysis. The lignin content of tissues was determined using the acetyl bromide method (Yoshida et al. 2013). To prepare samples for analysis, T₁ generation rice plants were collected at the ripening stage, and having removed the seeds and roots, the culms and leaves were dried at 75°C, pulverized using a mill, and passed through sieves to remove particulate fractions greater than 0.5 mm. Aliquots of the milled samples were suspended in 80% ethanol and boiled at 100°C for 1 h. This process was repeated three times, after which the samples were dried overnight at 75°C. The following day, 1.0–2.0 mg samples were placed in screw-capped test tubes to which was added 0.5 ml of acetyl bromide reagent (25% acetyl bromide (v/v) in glacial acetic acid) followed by heating at 50°C for 2 h. The resulting solution was diluted with 1.5 ml of acetic acid and 0.2 ml of the supernatant was transferred to a 1.5-ml tube, to which was added 0.3 ml of 0.3 M NaOH, 0.5 ml of acetic acid,
and 0.1 ml of 0.5 M hydroxylammonium hydrochloride. Absorbance was measured at 280 nm, with lignin (Alkaline) (TCI, Tokyo, Japan) being used as a standard for lignin content.

In line with expectations, we detected no significant change in acetyl bromide lignin content, although we noted slight reductions in the retarded-growth and dwarf Abf lines compared with the control line (to 94.4% and 90.5%, respectively) (Figure 5A). These findings thus appear to contrast with the results obtained based on IR spectroscopy, which indicated that the retarded-growth and dwarf Abf lines contain lower amounts of cellulose and hemicellulose and seemingly have higher lignin contents (Figure 2).

Delignification efficiency was analyzed by alkaline treatment (Miyamoto et al. 2018; Park et al. 1999). Samples (50–60 mg) boiled with ethanol were suspended in 6 ml of degassed 1 M NaOH and shaken at 30°C and 75 rpm for 24 h, after which, the absorbance of the supernatant was measured at 280 nm. The rate of delignification was calculated based on the ratio of alkaline-soluble lignin to acetyl bromide lignin. Undissolved residues were filtered using weighed grass filters, washed with hot water, dried at 75°C, and the mass recovery rate calculated.

Contrary to expectations, we observed a slight reduction in the rate of alkaline delignification in the dwarf Abf and retarded-growth Abf lines (72.7% and 73.5%, respectively) compared to the control line (76.2%) (Figure 5B). In contrast, the mass recovery rate in the dwarf Abf line (51.6%) was found to be significantly lower than that in the retarded-growth Abf and control lines (58.2% and 59.4%, respectively) (Figure 5C). Generally, delignification and mass recovery rates show a negative correlation, and under alkaline conditions, ferulate-lignin cross-links (ester bonds) typically undergo cleavage, whereas the benzyl ether cross-links between lignin and polysaccharides are notably alkaline resistant (Grabber et al. 2008). Our findings tend to indicate that CcAbf62A reduces the number of ferulate-lignin cross-links by severing arabinose side chains from arabinoxylan and increasing the relative abundance of benzyl ether cross-links.

In this study, rice plants transformed with the gene CcAbf62A were found to show differing extents of dwarfism. However, the dwarfism observed in the Abf lines was not accompanied by a significant reduction in lignin content, but was instead characterized by delayed lignin deposition at the culms, which is assumed to be a dwarfism-related trait. Although we detected a significant reduction in the mass recovery rate of the dwarf line following alkaline treatment, in line with expectations, we found the rate of alkaline delignification to be slightly lower than that of the control line. This unanticipated finding would thus appear to indicate that CcAbf62A may have the effect of modifying the form of the linkages between lignin and cell wall polysaccharides. Further research on the relationships between the expression of CcAbf62A and morphological changes in transgenic rice plants will undoubtedly contribute to elucidating the unique role of arabinoxylan in grasses. To the best of our knowledge, this is the first study to observe excessive tillering in grass plants associated with the heterologous expression of arabinofuranosidase, and the relationship between the structure of lignocellulosic materials and tillering certainly warrants further investigation. Finally, although the detailed mechanisms whereby CcAbf62A contributes to pronounced dwarfism and excessive tillering in rice plants remain to be ascertained, we believe that this enzyme could potentially provide a basis for novel approaches to breeding plants with modified lignin contents, as well as high-yielding or lodging-resistant crops.

Acknowledgements

We thank Professors Makoto Yoshida and Yoshihiko Horikawa of the Graduate School of Agriculture, Tokyo University of Agriculture and Technology, for providing the CcAbf62A gene and for assistance regarding IR analysis, respectively.

References

Akama K, Shiraishi H, Ohata S, Nakamura K, Okada K, Shimura Y (1992) Efficient transformation of Arabidopsis thaliana: Comparison of the efficiencies with various organs, plant ecotypes and Agrobacterium strains. Plant Cell Rep 12: 7–11
Benussan M, Lefebvre V, Ducamp A, Trouverie J, Gineau E, Fortabat M-N, Guillebaux A, Baldy A, Naquin D, Herbette S, et al. (2015) Suppression of dwarf and irregular xylem phenotypes generates low-acetylated biomass lines in Arabidopsis. Plant Physiol 168: 452–463
Buanañfa MM de O, Langdon T, Hauck B, Dalton S, Morris P (2008) Expression of a fungal ferulic acid esterase increases cell wall digestibility of tall fescue (Festuca arundinacea). Plant Biotechnol J 6: 264–280
Chen C, Zhao X, Wang X, Wang B, Li H, Feng J, Wu A (2021) Mutagenesis of UDP-xylose epimerase and xylan arabinosyltransferase decreases arabinose content and improves saccharification of rice straw. Plant Biotechnol J 19: 863–865
Chiniquy D, Sharma V, Schultink A, Baidoo EE, Rautengarten C, Cheng K, Carroll A, Ulvskov P, Harholt J, Keasling JD, et al. (2012) XAX1 from glycosyltransferase family 61 mediates xylosyltransfer to rice xylan. Proc Natl Acad Sci USA 109: 17117–17122
De Meester B, Madariaga Calderón B, de Vries L, Pollier J, Goemminne G, Van Doorsselaere J, Chen M, Ralph J, Vanholme R, Boerjan W (2020) Tailoring poplar poplar lignin without yield penalty by combining a null and haploinsufficient CINNAMOYL-CoA REDUCTASE2 allele. Nat Commun 11; 5020
Eugene A, Lapierre C, Ralph J (2020) Improved analysis of arabinoxylan-bound hydroxycinnamate conjugates in grass cell walls. Biotechnol Biofuels 13: 202
Grabber JH, Hatfield RD, Lu F, Ralph J (2008) Coniferyl ferulate incorporation into lignin enhances the alkaline delignification
Dwarfism and increased tillering in rice producing CcAbf62A

and enzymatic degradation of cell walls. *Biomacromolecules* 9: 2510–2516

Halpin C (2019) Lignin engineering to improve saccharification and digestibility in grasses. *Curr Opin Biotechnol* 56: 223–229

Hashimoto K, Kaneko S, Yoshida M (2010) Extracellular carbohydrate esterase from the basidiomycete *Coprinopsis cinerea* released ferulic and acetic acids from xylan. *Biosci Biotechnol Biochem* 74: 1722–1724

Hashimoto K, Yoshida M, Hasumi K (2011) Isolation and characterization of CcAbf62A, a GH62 α-L-arabinofuranosidase, from the basidiomycete *Coprinopsis cinerea*. *Biosci Biotechnol Biochem* 75: 342–345

Hisano H, Nandakumar R, Wang Z-Y (2009) Genetic modification of lignin biosynthesis for improved biofuel production. In *In Vitro Cell Dev Biol Plant* 45: 306–313

Horikawa Y, Hirano S, Mihashi A, Kobayashi Y, Zhai S, Sugiyama J (2019) Prediction of lignin contents from infrared spectroscopy: Chemical digestion and lignin/biomass ratios of *Cryptomeria japonica*. *Appl Biochem Biotechnol* 188: 1066–1076

Kawai S (2014) Plant molecular breeding to energy crops as genetic modifications of biomass saccharification. In: Tojo S, Hirasawa T (eds) *Research Approaches to Sustainable Biomass Systems*. Elsevier Publishing, Oxford, Kidlington, pp 74–88

Ke S, Luan X, Liang J, Hung Y-H, Hsieh T-F, Zhang X-Q (2019) Rice OsPex1, an extensin-like protein, affects lignin biosynthesis and plant growth. *Plant Mol Biol* 100: 151–161

Kulkarni AR, Pattathil S, Hahn MG, York WS, O’Neill MA (2012) Comparison of arabinoxylan structure in bioenergy and model grasses. *Ind Biotechnol (New Rochelle NY)* 8: 222–229

Li X, Yang Y, Yao J, Chen G, Li X, Zhang Q, Wu C (2009) *FLEXIBLE CULM 1* encoding a cinnamyl-alcohol dehydrogenase controls culm mechanical strength in rice. *Plant Mol Biol* 69: 685–697

Mayuzumi Y, Maruyama R, Kawai S (2016) Expression of a lignin-carbohydrate complex degrading enzyme in plants (2). *Proceeding of the 61st Lignin Symposium*: 30–33 (In Japanese)

Miyamoto T, Yamamura M, Tobimatsu Y, Suzuki S, Kojima M, Takabe K, Terajima Y, Mihashi A, Kobayashi Y, Umezawa T (2018) A comparative study of the biomass properties of *Erianius* and sugarcane: Lignocellulose structure, alkaline delignification rate, and enzymatic saccharification efficiency. *Biosci Biotechnol Biochem* 82: 1143–1152

Osakabe K, Koyama H, Kawai S, Katayama Y, Morohoshi N (1995) Molecular cloning of two tandemly arranged peroxidase genes from *Populus takamatsensis* and their differential regulation in the stem. *Plant Mol Biol* 28: 677–689

Pandey V, Shukla A (2015) Acclimation and tolerance strategies of rice under drought stress. *Rice Sci* 22: 147–161

Park S-Y, Koda K, Matsumoto Y, Meshitsuka G, Iyama K (1999) Kinetic comparison between delignification and silica removal during alkaline pulping of rice straw. *Japan Tappi Journal* 53: 1492–1499 (In Japanese)

Pedersen IF, Vogel KP, Funnell DL (2005) Impact of reduced lignin on plant fitness. *Crop Sci* 45: 812–819

Pérez Bernal M, Abreu Remedios D, Valdivia Pérez O, Delgado Rigo M, Armas Ramos R (2016) Assessment three constitutive promoters for GUS expression in rice (*Oryza sativa* L., var. J-104). *Rev Colombo Biotecnologia* 18: 81–89

Phitsuwan P, Sakka K, Ratanakhanokchai K (2013) Improvement of lignocellulosic biomass in plants: A review of feedstocks, biomass recalitrance, and strategic manipulation of ideal plants designed for ethanol production and processability. *Biomass Bioenergy* 58: 390–405

Sato-Izawa K, Ito M, Nuoendagula, Kajita S, Nakamura SI, Matsumoto T, Ezura H (2020) Distinct deposition of extender-linked ferulic and p-coumaric acids to the cell wall of developing sorghum internodes. *Plant Biotechnol* 37: 15–23

Schledzewski K, Mendel RR (1994) Quantitative transient gene expression: Comparison of the promoters for maize polyubiquitin1, rice actin1, maize-derived *Emu* and CaMV 35S in cells of barley, maize and tobacco. *Transgenic Res* 3: 249–255

Simmons BA, Leque D, Ralph J (2010) Advances in modifying lignin for enhanced biofuel production. *Curr Opin Plant Biol* 13: 313–320

Van Acker R, Leple J-C, Aerts D, Storme V, Goeminne G, Ivens B, Legee F, Lapierre C, Piens K, Van Montagu MCE, et al. (2014) Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase. *Proc Natl Acad Sci USA* 111: 845–850

Van Soest P, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74: 3583–3597

Xie M, Zhang J, Tschaplinski TJ, Tuskan GA, Chen J-G, Muchero W (2018) Regulation of lignin biosynthesis and its role in growth-defense tradeoffs. *Front Plant Sci* 9: 1427

Yoshida K, Sakamoto S, Kawai T, Kobayashi Y, Sato K, Ichinose Y, Yaa K, Akio Kikusawa Sato H, Takamizo T, et al. (2013) Engineering the *Oryza sativa* cell wall with rice NAC transcription factors regulating secondary wall formation. *Front Plant Sci* 4: 383

Zhang W, McElroy D, Wu R (1991) Analysis of rice Act1 5′ region activity in transgenic rice plants. *Plant Cell* 3: 1155–1165

Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L (2006) The rice HIGH-TILLERING DWARF1 encoding an ortholog of *Arabidopsis* MAX3 is required for negative regulation of the outgrowth of axillary buds. *Plant J* 48: 687–696