Identification and characterization of the loci for the traits associated with lodging resistance in rice, using chromosome segment substitution lines

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Identification and characterization of the loci for the traits associated with lodging resistance in rice, using chromosome segment substitution lines

(染色体断片置換系統を用いた水稲の倒伏抵抗性に関連する形質の遺伝子座の特定とその作用機作)

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Chapter 1 General Introduction

Rice is the world's most important food crop and becomes major staple food for more than half world population (Peng et al., 2005). Rice yield potential was greatly improved since green revolution due to the development of semi-dwarf varieties and hybrid rice (Gu, 2010; Tang et al., 2010). It is harvested from over 163 million ha in more than 100 countries (Laborte et al., 2017). Rice is cultivated in various cropping systems and environment (Laborte et al., 2017; Maclean et al., 2013). The broad variation of rice cultivation area causes various of problems as well, including biotic and abiotic stress. Increasing rice productivity has been one of the main target to compensate population growth, and it will face with more complex problems related to climate change.

Lodging is one of the most important constrained factors on yield for cereal crops (Berry, 2013; Pinthus, 1974). It is a prominent problem that limits cereal productivity in developed and developing countries (Berry et al., 2004). Serious lodging could reduce grain yield up to 50% (Setter et al., 1997). Timing and duration of lodging also effect on the severe decline in yield. Lodging at the grain filing stage reduces grain yield 2.66 to 2.71% (Lang et al., 2012). Lodging occurs due to the interaction between susceptibility to the lodging of the plant and external force. Wind pressure and rain are the main factors of external force related to lodging in rice plant, which simply due to the unpredictable nature often hostile weather condition (Berry et al., 2003; Sterling et al., 2003).

When lodging occurs, the canopy structure is destroyed, and the photosynthetic rate and dry matter production are sharply reduced (Kashiwagi et al., 2005). In severe cases, it breaks stem or pull the root out, blocking the transportation of water, minerals and photoassimilates, leading to decline in yield and quality. The grain of lodged plant may also easily germinate, especially in variety with weak seed dormancy. Furthermore, it also has difficulties in harvest operation, increased expense in

grain drying that will increased production cost and in result reduced economical profit of agricultural endeavors.

Other study reported that continuous submerged condition increases lodging compared to aerobic sowing, and lodging resistance was improved due to better root anchorage (Tabbal et al., 2002). Severe lodging prevents the transport of water, nutrients and assimilates through the xylem and phloem, resulting in reduction assimilates for grain filling (Kashiwagi et al., 2005)

Bending type lodging occurs in different part of rice plant, from the base, in mid-tiller and upper part tiller as a result of heavy panicle (Bhiah et al., 2010). Lodging also happened, when plant canopy height was increased due to the bending of shoot. Lodging reduced photosynthesis by 60-80%, and every 2% of lodging caused a decrease of 1% in grain yield (Setter et al., 1997). Different growth stage may affect the yield reduction differently, lodging at the reproductive stage e.g., at anthesis or at grain filling stage (Piñera-Chavez et al., 2016). Moreover, lodging of rice plants during the ripening period results not only in reduction in yield, but also in decrease in grain quality, due to increase coloring of brown rice and flavor (Setter et al., 1997). Lodging at the early development stage has less influence on yield production (Berry et al., 2004).

In cereal crops, lodging can be classified into stem lodging and root lodging (Sterling et al., 2003). Stem lodging defines when stem bend or breaks, and roots are held firmly in a strong soil where the wind force buckles one of the lower internode of the shoot (Thomas, 1982). Stem lodging results from the interaction and the balance of forces which are, straw strength, environmental strength effecting straw strength, and external force (Setter et al., 1994). Furthermore, stem lodging could be specified into stem bending-type and stem breaking-type (Kono, 1995). Stem bending-type lodging occurs in entire internode, when they cannot withstand the bending pressure. It often causes by the increase in panicle weight during maturation or effects by strong wind and rain (Islam et al., 2007). Stem breaking is the type of lodging, which usually affects the lower internode (below

the third internode from the top) as a result of excessive bending pressure at the higher internode, and is determined primarily by the morphology and quality of culm (Ma et al., 2003; Zuber et al., 1999). Root lodging is easy to occur when the anchorage strength is reduced by weak soil or poorly developed anchorage roots (Ennos, 1991; Tams et al., 2004). The process of lodging result in the permanent displacement of cereal stem without any observable stem buckling. Crook and Ennos (1993) proposed that root lodging should be predominant, however Neenan and Spencer-Smith (1975) concluded stem lodging was the main failure mechanism. Furthermore Baker et al. (1998) illustrated both lodging, depended on particular crop characteristics. Types of lodging depend upon the environments at the time of lodging and during the growth of crop plants (Berry et al., 2003).

The severe impact of lodging depends on many plant traits and environmental conditions. Different plant traits govern plant stature and architecture, and contribute in enhance lodging resistance (Berry et al., 2004).

Plant height is an important trait in cereal crops, which not only determines plant architectures but also significantly influences grain yield and commonly associated with short, strong culm and high lodging resistance (Evans, 1998). Total plant height is aggerate of panicle length and length of internode above ground. It determines the resistibility of plant against lodging (Kong et al., 2013). Decrease in plant height resulted in high resistance to lodging due to low center of gravity and reduced upper fresh weight (Okuno et al., 2014). Some studies found correlation between plant height and lodging resistance (Kashiwagi et al., 2005; Yao et al., 2011). Plant height at the heading stage is an important trait for breeding lodging resistance in rice (Chen et al., 2005). Tall crops are easily flattened by wind and rain consequently caused yield loss. The longer elongated basal internode were responsible to higher plant stature and higher lodging index (Zhang et al., 2016). Reducing plant height through *sd-1* gene were able to increase lodging resistance due to decrease the effect of the upper part to the lower part of rice plant (Spielmeyer et al., 2002).

For long time, plant height has been considered as one of the main factor that effect lodging resistance and relate to crop yield (Wang et al., 2012; Wang and Li, 2005). It has been the main target for improving lodging resistance (Peng and Khushg, 2003). Nonetheless, depending only on plant height itself only can be done up to a certain limit. Reducing plant height also reduce photosynthetic capacity and leads to decrease in total biomass production, thus, restricting for further increased yield (Islam et al., 2007; Keller et al., 1999; Okuno et al., 2014). Plant height is not necessarily the most important factors determining lodging resistance in rice (Easson et al., 1993; Ookawa and Ishihara, 1992). Plant with a similar height differed in susceptibility to lodging (Terashima et al., 1992). Therefore, for continuous increased in crop yield, it is inevitable to increase lodging resistance by using novel approaches without reducing plant height that has negative effect on plant productivity.

Morphological traits and chemical composition of stem related to lodging, are very important for rice. Moderate plant height, large stem diameter, thick stem wall, high lignin and cellulose contents have been recommended as preferential traits for the improvement of lodging resistance (Berry, 2013; Mackill, 1996). Study by Zhang et al. (2010) showed significant differences in soluble sugar contents in culms and leaf sheaths in different lodging resistant varieties, and strong lodging resistant varieties had higher soluble sugar contents compared to weak varieties. The accumulation of polysaccharides could promote cellulose and hemicellulose synthesis and made culm wall thickening and flexibility enhancement (Zhou, 2006). Primary cell walls mainly consist of three polysaccharides: cellulose, hemicelluloses, and pectin (Somerville, 2006). And secondary cell walls include cellulose, different form of hemicellulose and lignin. Primary cell wall has relatively high water content, which is important to maintaining the ability of cell wall to expand. In contrast, the cellulose, hemicellulose and lignin structure of secondary cell wall densely packed, designed for strength and compression resistance (Taiz et al., 2015). These polysaccharides determine the cell shape and mechanical strength.

Cellulose is the most abundant plant polysaccharide providing mechanical support to individual cell and entire plant. Moreover, in addition to cellulose high accumulation of starch increase the bending stress and culm stiffness (Kashiwagi et al., 2006). The plant cell wall provides mechanical strength of culm that support by its composition. Lignin content and composition are important factors that affect the cell wall stiffness and the mechanical strength of plant.

Silicon is another important component associated with culm physical strength (Ma and Yamaji, 2006). Rice is a typical crop that has high silicon content and the deposition of silicon in plant cell walls can increase the mechanical strength of the organ (Gong et al., 2004). Silicon and potassium can promote lignification and silicification in thick-walled cells, thicken collenchyma cells and improve keratinocyte growth and increase cellulose content. Lodging resistant varieties showed significantly higher K and Si content (Zhang et al., 2010).

In plant breeding, the selection of natural variation existed is the basic activity. Since the beginning of rice domestication, the trait of rice plant has been continuously improved through introduction of naturally existed alleles by spontaneous and/or artificial crossing. Systematic plant breeding theory has been employed and widely practice. However, this approach has limitation in understanding genetic control. Many important traits in rice such as yield, culm length, heading date, eating quality and lodging showed continuous phenotypic variation. Each trait is controlled by multiple genes (Yano, 2001). It is difficult to identify these genes, known as quantitative trait loci (QTL), because the individual effect by each of these genes on phenotypes are relatively small. In rice, QTL analysis of complex traits has been conducted to detect genomic region associated with several traits exhibited complex inheritance (Jiang et al., 2008a; Lin et al., 1998; McCouch et al., 1988; Zhu et al., 2008).

During the past decade, many efforts have been made to dissect complex traits through QTL mapping which have contributed to a better understanding of genetic basis of wide range traits in rice

(Cai and Morishima, 2002; Li et al., 2001; Nagata et al., 2015; Wan et al., 2006; Yano and Sasaki, 1997). A number of QTL mapping studies for culm strength and thickness related to lodging resistance have been carried out using different rice segregating population (Kashiwagi and Ishimaru, 2004; Kashiwagi et al., 2008; Ookawa et al., 2010a; Yano et al., 2015). However, few information reported the QTL analysis for the traits associated with culm stiffness and cell wall materials such as cellulose and hemicellulose.

The identification of QTLs and candidate genes will have employed better understanding in the regulatory mechanism of lodging resistance. In this study, to find the superior allele for lodging resistance in rice, we compared the traits associated with culm thickness and stiffness and cell wall materials between *japonica* variety, Koshihikari and *indica* variety, Takanari using Chromosome Segment Substitution lines (CSSLs) derived from a cross between Takanari and Kosihikari, we estimated the QTLs location and their responsible genes.

This dissertation was organized into four chapter. Chapter 1, introduce the background of lodging, caused and mechanism of lodging, component and responsible traits affected lodging resistance in rice. Furthermore, in Chapter 2, we elucidated lodging resistance characteristic between *indica* variety, Takanari and *japonica* variety, Koshihikari. Chapter 3 reported genetic basis of bending- and breaking- type lodging resistance based on the QTLs estimated on CSSLs of Koshihikari segment in Takanari genetic background. In the last chapter we confirmed estimated QTLs for culm stiffness using reciprocal CSSLs and estimated the candidate gene by RNA-seq and real time RT-PCR.

Chapter 2 Comparison of the traits associated with breaking- and bending- type lodging resistance in Koshihikari and Takanari

2.1 Introduction

Lodging is a major constrain in rice production, especially high yielding varieties. Lodging lead reduction in the quality and quantity of yield. Yield reduction is mainly cause by the impaired translocation of water and nutrients, decreased interception of light, microclimatic change, and deterioration photosynthetic activity of the leaves (Kono, 1995). These problems will limit crop productivity either by interfering assimilation of dry matter or by impending crop harvesting (Berry and Spink, 2012).

Koshihikari is a Japanese premium rice variety (*Oryza sativa* L.). This variety has been widely cultivated in Japan, and also cultivated in various countries, including USA and Australia. Despite its good flavor, its yield is relatively low compared with that of modern Japanese high-yield varieties (Uehara et al., 1995; Yamauchi, 2001). Koshihikari has a long culm which usually prone to lodging (Hoshikawa and Wang, 1990).

Japanese *indica* varieties, Takanari was released in 1990, that showed a high rice yield under temperate climate. It descendent from high yielding varieties including IR 8 and Tongil (Takai et al., 2006), and from a cross between two Korean *indica* varieties, "Milyang 42" and "Milyang 25". The variety belongs to a moderate maturation group has extra-panicle weight type and short stiff culm, it shows high lodging resistance and high yield. Yield was 20% higher than standard *japonica* varieties, such as "Nipponbare" and "Musashikogane" (Imbe et al., 2004). Furthermore, Takanari has a short plant stature, and possesses the *sd1* gene (Takai et al., 2012), but it has large panicle and dark green and wide flag leaves (Takai et al., 2013).

Varietal differences in various lodging resistance trait has been observe between Koshihikari and Takanari. Previous study comparing traits associated with lodging resistance between rice varieties showed that Koshihikari possesses small section modulus (SM) but large bending stress (BS) (Ookawa and Ishihara, 1997). On the other hand, Takanari has large SM due to a large outer diameter and a small BS due to thin cortical fiber tissue (Ookawa et al., 2016).

It is important to identify the differences of lodging resistant traits between parents that contribute to increasing lodging resistance. By deeper understanding in the genetic factor that altered of the lodging resistance, the potential factors for higher resistance can be inferred from the varietal differences among Takanari and Koshihikari. The aims of this study are to characterize important traits of breaking- and bending- type lodging resistance in Takanari and Koshihikari rice varieties. Plant characteristics are responsible for each lodging resistance type. The dissection of component and responsible trait of each type elucidated into the scheme presented in Fig. 2.1.

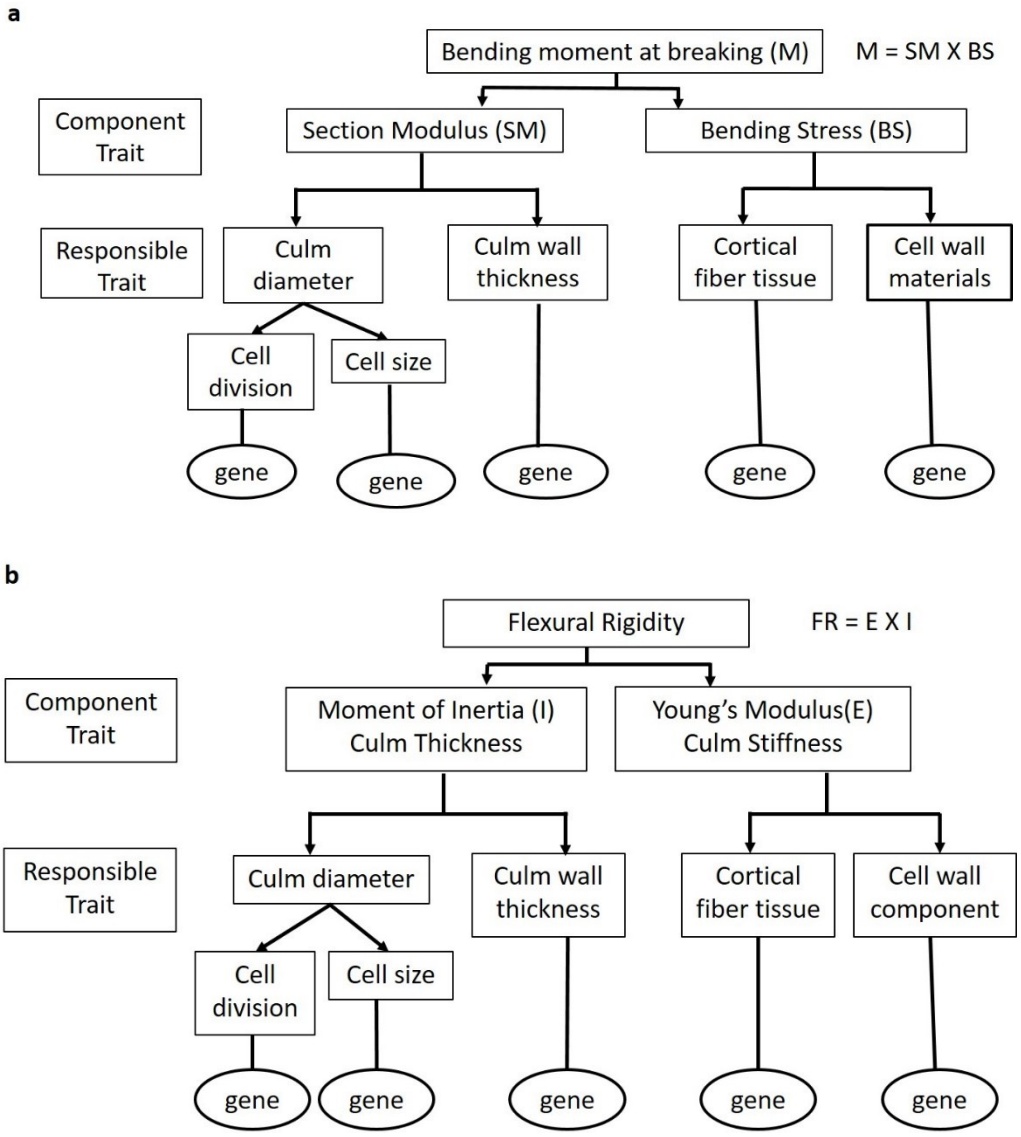


Fig. 2.1. The dissection of lodging resistance into component and responsible trait (a) breaking- and (b) bending- type.

2.2 Materials and methods

2.2.1 Plant material and cultivation

The rice (*Oryza sativa* L.) varieties Koshihikari and Takanari were used as parental lines in this experiment. Rice seeds were sown in nursery boxes. Seedlings were transplanted, at a density of one plant per hill, to a paddy field at the University farm in Tokyo on alluvial soil of the Tama River. The planting density was 22.2 hills m^{-2}, with a spacing of 15 cm × 30 cm. As a basal dressing, compound fertilizer was applied at rate of 5.0 kg $10a^{-1}$ for N and 6.0 kg $10a^{-1}$ for P_2O_5 and K_2O.

2.2.2 Measurements of culm strength

Morphological characters and breaking strength were measured in basal internode, the 4th internode from the panicle for Takanari and 5th internode from panicle for Koshihikari. Six culms from each plot were measure at 14 days after heading. Bending moment at breaking (M) and Young's modulus (YM) were measure at a distance 4 cm between supporting points by the method of Ookawa and Ishihara (1997) using Tensilon RTG-1210 universal testing machine (A&D, Tokyo, Japan) (Fig. 2.2).

The physical parameters of culm strength were used for precise phenotyping of breaking- and bending- type lodging resistance. Physical parameters for breaking type lodging resistance were calculated by the following formula:

$M = SM \times BS$

$$SM = \left(\frac{\pi}{32}\right) \times (a_1^3 b_1 - a_2^3 b_2)/a_1$$

where **a1** is the outer diameter of the minor axis in an oval cross-section, **b1** is the outer diameter of the major axis in an oval cross-section, **a2** is the inner diameter of the minor axis in an oval cross-section, and **b2** is the inner diameter of the major axis in an oval cross-section (Fig. 2.3).

In bending type lodging resistance**,** flexural rigidity describes the resistance of bending. Flexural rigidity (FR) is the product of Young's modulus (YM) and secondary moment of inertia (SMI). SMI and FR define by the following equation:

$$\text{SMI} = \left(\frac{\pi}{64}\right) \times (a_1^3 b_1 - a_2^3 b_2)$$

FR = YM x SMI

2.2.3 **Determination of cell wall materials**

The same samples as culm strength measurement, were used for determination of cell wall materials. Dry ground of culm samples -approximately 40mg-, was treated in 80% ethanol and then with 50% at 80℃ for 20 minutes for each ethanol treatment. The pellet after EtOH extraction was further extracted with heat-stable α-amylase 100 μl (12mg powder / ml in 200mM Pi buffer, 600U) from *Bazillus licheniformis* and 200mM Pi buffer to remove starch. After incubation in incubator at 85 ℃ for 30 min the samples were centrifuged, the supernatants were discarded, and the pellets were washed with deionized water. Pellets were dried at 80℃ for overnight, cooled in a desiccator and weighed repeatedly, until a constant weight was obtained. Pi buffer (pH 6.9) solution obtain from the mixture of 200mM NaH_2PO_4 H_2O and 200 mM Na_2HPO_4 $12H_2O$.

For the determinations of holocellulose and hemicellulose contents, 1.6ml $NaClO_2$ solution (400mg $NaClO_2$ / 60ml DW) and 0.2 ml of acetic acid were added to the starch-free residue to remove lignin and incubated at 80° C for 60 min. Samples were centrifuged, and the supernatants were discarded, repeated the treatment once. The pellets were washed with deionized water 2 times and acetone 1 times. Pellets were dried perfectly. Acid detergent extraction of bulk-hemicelluloses were conducted for cellulose and hemicellulose determination. 600µl ADS was added to the dried pellet,

and the samples were placed in an incubator at 95℃ for 1 h to hydrolyze hemicelluloses, and separated them from the remaining cell-wall components (cellulose). The pellet was washed with deionized water and acetone. Samples were dried perfectly, until a constant weight was obtained.

2.2.4 Statistical analysis

Statistical comparison of parental varieties is carried out by t-test.

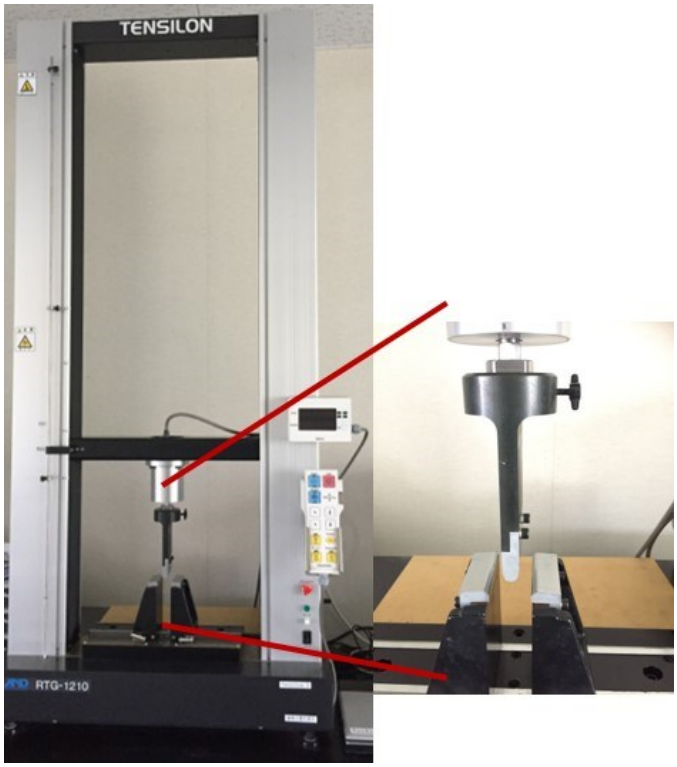


Fig. 2.2. Tensilon RTG-1210 universal testing machine (A&D, Tokyo, Japan).

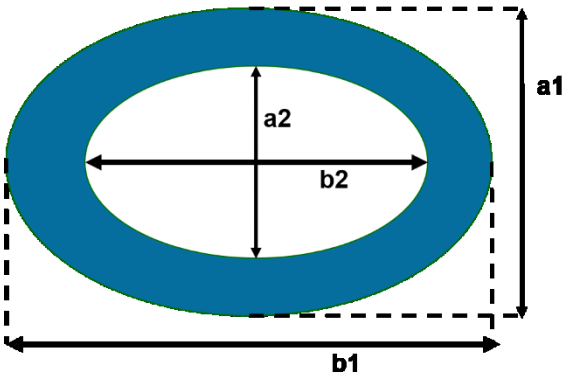


Fig. 2.3. Oval cross section of rice culm, where : **a1** is the outer diameter of the minor axis in an oval cross-section, **b1** is the outer diameter of the major axis in an oval cross-section, **a2** is the inner diameter of the minor axis in an oval cross-section, and **b2** is the inner diameter of the major axis in an oval cross-section.

2.3 Results

Varietal differences in lodging resistance were clearly observed between Takanari and Koshihikari on breaking- and bending-type lodging resistance parameters in 2015 and 2016. To understand the lodging resistance mechanism of each lodging type, the parental varieties (Takanari and Koshihikari) were evaluated for the component and responsible traits.

Phenotypical appearance of the basal culm of Takanari and Koshihikari showed clear differences in the culm diameter (Fig.2.4). This trait was responsible trait that effected directly to SM and SMI.

2.3.1 Physical parameters differences in breaking- and bending- type lodging resistance

In 2015 and 2016, Takanari showed a large bending moment at breaking (M) as compared with Koshihikari (Fig. 2.5a and 2.7a). Furthermore, M can be divided into SM and BS. Large significant difference in SM showed between Takanari and Koshihikari, in which Takanari exhibited larger SM to Koshihikari (Fig. 2.5b and 2.7b). On the other hand, BS in Takanari was smaller than that in Koshihikari (Fig. 2.5c and 2.7c). These results indicate that Takanari has a thick culm, but fragile as compare with Kosihikari.

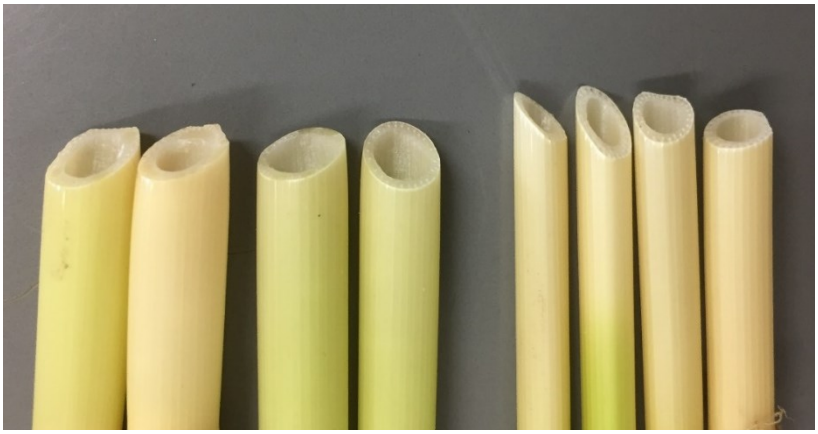
Based on the bending-type lodging resistance, Takanari showed significantly high flexural rigidity (FR) as compared with Koshihikari in 2015 and 2016 (Fig. 2.6a and 2.8a). Furthermore, the FR can be divided into two components, secondary moment of inertia (SMI) and Young's modulus (YM). The large FR in Takanari resulted from a large SMI (Fig. 2.6b and 2.8b). YM in Takanari was significantly higher than that in Koshihikari in 2015 but not the following year (Fig. 2.6c and 2.8c). Although in 2016 Takanari statically did not showed higher YM compare to Koshihikari, Takanari still had higher YM value.

2.3.2 Differences in cell wall component between Takanari and Koshihikari.

Beside numerous morphological traits of plants, some cell wall materials character such cellulose, lignin and soluble sugar are also important and contribute to lodging resistance. It is important to identify the differences of cell wall materials between parents.

The different densities of cell wall materials were shown between Takanari and Koshihikari at 14 days after heading. Cell wall materials contributed to culm stiffness. It is important to identify the component traits for cell wall materials to detect QTLs that affect the levels of these cell wall materials. In both years, the densities of cellulose and lignin in Takanari were higher than those in Koshihikari. There were no differences in the hemicellulose densities of Takanari and Koshihikari in 2015, but Takanari showed a high hemicellulose density as compared with Koshihikari in 2016 (Table 2.1).

From these result, Takanari showed significant differences compared to Koshihikari in most of observed traits in the study, except YM in 2016, and hemicellulose density in 2015. In breaking type lodging, Takanari exhibited higher M due to higher SM, in contrast Koshihikari had higher BS. In bending type lodging resistance and cell wall component density, Takanari showed higher value in each component as compare to Koshihikari.



Takanari Koshihikari

Fig. 2.4. Basal culm phenotypic appearance of Takanari (left) and Koshihikari (right).

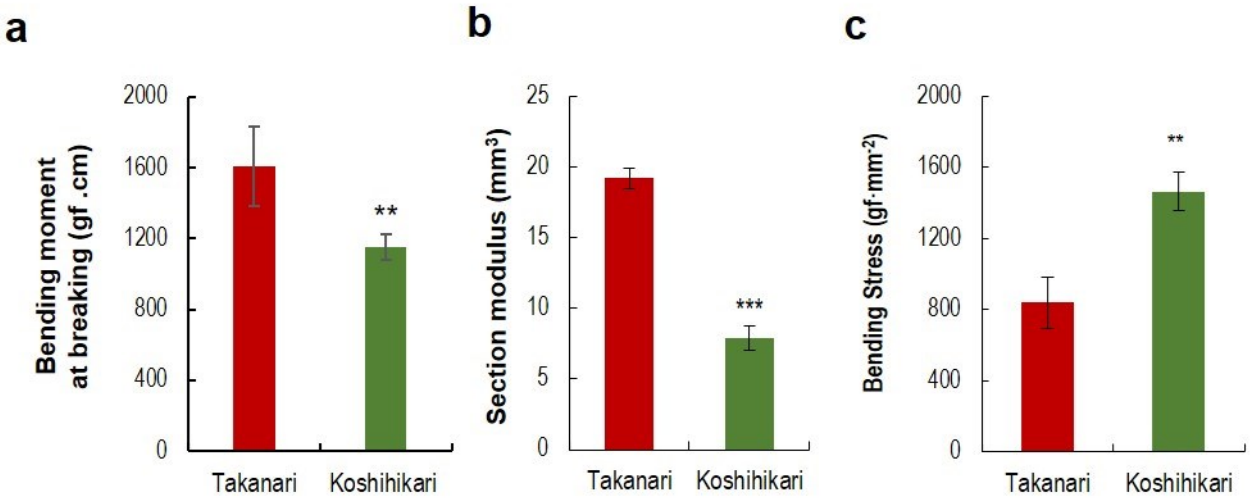


Fig. 2.5. Physical parameters associated with breaking-type lodging resistance of Takanari and Koshihikari in 2015: (a) bending moment at breaking, (b) section modulus, and (c) bending stress of the fourth internode. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively.

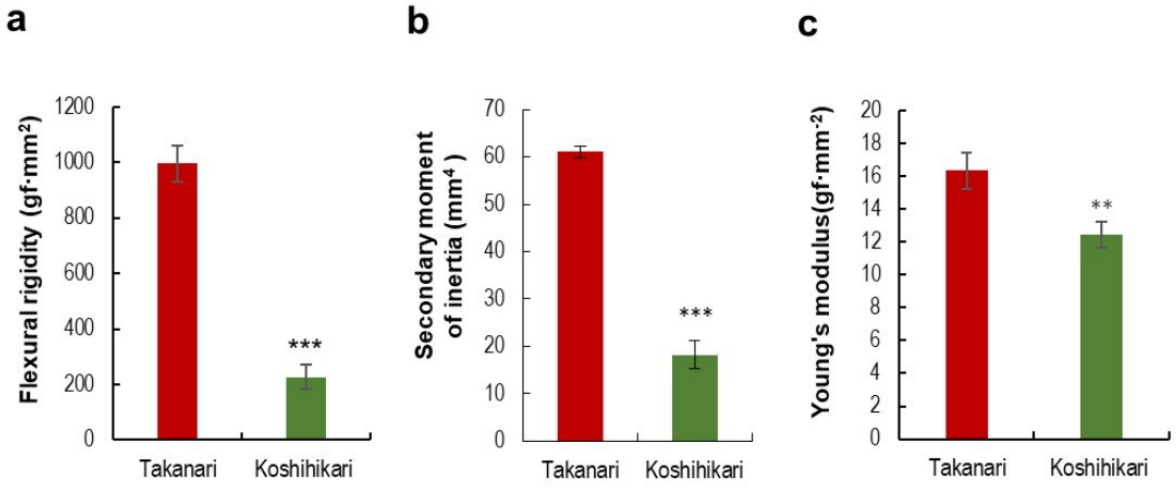


Fig. 2.6. Physical parameters associated with bending-type lodging resistance of Takanari and Koshihikari in 2015: (a) flexural rigidity, (b) secondary moment inertia, and (c) Young's modulus of the fourth internode. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively.

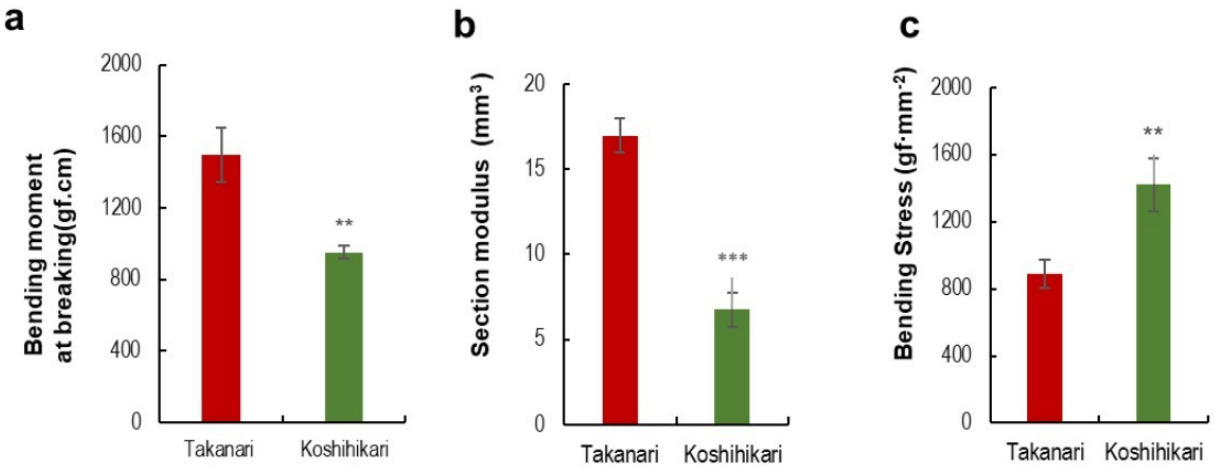


Fig. 2.7. Physical parameters associated with breaking-type lodging resistance of Takanari and Koshihikari in 2016: (a) bending moment at breaking, (b) section modulus, and (c) bending stress of the fourth internode. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively.

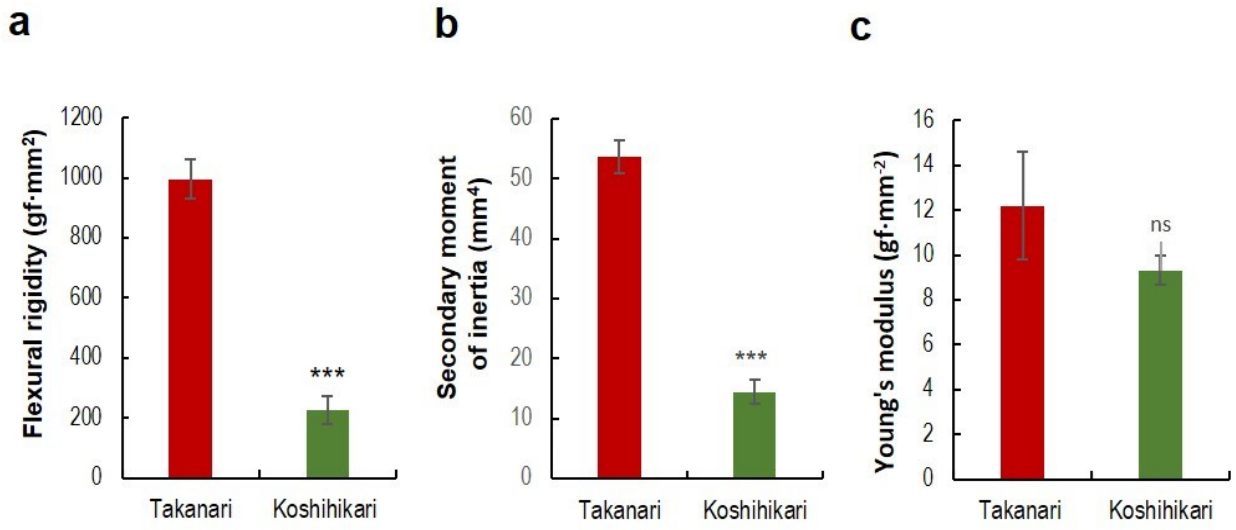


Fig. 2.8. Physical parameters associated with bending-type lodging resistance of Takanari and Koshihikari in 2016: (a) flexural rigidity, (b) secondary moment inertia, and (c) Young's modulus of the fourth internode. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively.

Table 2.1. Cell wall material density of Koshikari and Takanari in 2015 and 2016.

Variety	Holocellulose density	Lignin density	Cellulose density	Hemicellulose density
	$\mu g \cdot mm^{-3}$	$\mu g \cdot mm^{-3}$	$\mu g \cdot mm^{-3}$	$\mu g \cdot mm^{-3}$
2015				
Takanari	147.6	32.4	92.3	55.2
Koshihikari	107.7	26.1	66.2	41.4
	*	**	*	ns
2016				
Takanari	165.2	24.8	104.3	60.9
Koshihikari	123.3	9.8	85.8	37.6
	*	**	*	*

Note: ns, * and ** indicated, non-significant difference, significant at the level 5% and 1%.

2.4 Discussion

Previously the study to increasing lodging resistance emphasize on reducing plant height, but it only can be done up to a certain limit. The next breeding objective is to target the culm physical strength, rather plant height. Many researches have been performed on lodging resistance in various rice lines and variety. This enable to understand lodging mechanism. A choice of parental lines that showed wide phenotypic variation in the target traits is necessary for genetic study including QTL analysis, because the detection is based on natural allelic differences between parental lines. An optimum level of lodging resistance can be achieved through different combination of lodging resistance component trait. In this study, the parental lines, *indica* variety Takanari and *japonica* variety, Koshihikari exhibited large differences in most of the lodging traits observed. Takanari, an *indica* variety, have thick stem, short stature and high leaf sheath wrapping. Whereas Koshihikari tend to have thick stem wall and more flexible culm.

The parents of the CSSLs, Koshihikari and Takanari, have different characteristics for morphological phenotyping represent by differences in culm diameter (Fig. 2.4), which directly affect the bending load and resistance to breaking-type lodging. Higher M that indicated physical strength of culm on Takanari in the present study due to high SM (Fig. 2.5 and 2.7). The Large culm diameter of Takanari responsible for the large SM. Study by Hirano et al. (2014) showed the increases in culm diameter and culm wall thickness lead to high SM. Other study by Ookawa et al. (2010a) also reported that culm diameter had positive effects on the SM and M, leading to lodging resistance without any yield loss. Culm diameter is one of the traits that contribute to culm strength (Kashiwagi et al., 2008; Ma et al., 2002; Sarker et al., 2007; Zuber et al., 1999). As SM is determined by culm diameter, Takanari, which has a large culm diameter, exhibits a large SM.

In contrast with SM, Takanari exhibited lower BS compare to Koshihikari. Higher BS in Koshihikari indicated culm stiffness that demonstrated culm ability to bend without breaking.

Koshihikari has a small culm diameter and a high BS due to the accumulation of lignin and cellulose in culms (Ookawa and Ishihara, 1993), while the semi-dwarf *indica* variety, Takanari, has a large culm diameter (Ishikawa et al., 1999; Xu et al., 1997) and a small BS. Despite off low BS, Takanari expressed high cell wall material densities that related with culm stiffness (Table 2.1). These indicated the potential of improving lodging resistance through culm cell wall component. It has been reported cell wall component such cellulose, hemicellulose, lignin, silica provide the physical strength of plant (Ishimaru et al., 2008; Kashiwagi and Ishimaru, 2004). Further verification needed to confirm which component closely related to BS and QTLs identification responsible for these traits, in the next chapter we elucidated the genetic basis of the breaking-type lodging tolerance mechanism.

Bending-type lodging reflected on high FR in Takanari, due higher SMI and YM of the basal internode compare to Koshihikari (Fig.2.6 and 2.8). Increasing YM and SMI, significantly increased FR within genotypes (Kaack et al., 2003). FR as an indicator of bending type lodging resistance provides a measure of culm resistance to bending stress, hence it could reflect the susceptibility of cultivar to stem lodging (Niklas, 1990). Small FR in Koshihikari due to smaller SMI of area. The same result reported by San-Oh et al. (2001), in Niponbare that showed low FR. The higher FR at the basal internode decrease the curvature of culm on the region thereby lower the maximum stress. SMI depends on the culm configuration such as culm inner and outer diameters. Rice culm display efficient structure for covering FR, a hollow tube is more resistant to bending than a solid stem containing the same material (Silk et al., 1982).

The YM describes the relationship between stress and strain within the elasticity limit of a material (Niklas, 1989). It can serve as indicator of rigidity of rice stem. A variety that possess higher YM, require higher energy for the internode to break (Tavakoli et al., 2010). In this study high YM in Takanari showed higher bending resistance as compared with Koshihikari. The high elasticity, describe by YM, depend on the composition of the culm tissue. *Indica* variety, Takanari exhibited

higher cell wall density in almost all components in both year (Table 2.1). Kaack et al. (2003) reported, YM dependent significantly of the parenchyma vascular bundle area and the concentration lignin and cellulose.

The optimum stem structure not only provide sufficient strength, but also achieve the investment in biomass. Stem strength determined by the radius, wall width, and the material composition of the stem wall. Therefore, the density of the stem wall material can be used to predict the combination of stem characters that gives the optimum strength with the least stem biomass(Berry et al., 2007). Generally, lignin or cellulose determines physical strength, as lower lignin or cellulose contents cause the culm to be brittle (Jones et al., 2001; Kokubo et al., 1989; Ma et al., 2002). *Indica* variety, Takanari exhibited higher cell wall density in almost all components in both year but, it still has a small BS as compared with *japonica* variety, Koshihikari. Furthermore, the rigidity of basal culm may depend on carbohydrate component such as sugar, starch, cellulose and lignin.

Both varieties, Koshihikari and Takanari exhibited different responsible trait for lodging resistance. This observation indicates that lodging resistance in *indica* variety Takanari can be improved by incorporating the gene from *japonica* Koshihikari that increase culm stiffness. However, to understand the genetic mechanism underlaying responsible traits for lodging resistance between Takanari and Koshihikari, further study on QTLs identification are needed to be conducted.

Analysis of QTL can reveal the genetic basis of relationships among traits (Ebitani et al., 2005; McCouch and Doerge, 1995; Yano and Sasaki, 1997). Lodging resistance clearly determined by various factors, controlled by multiple QTLs with large and small effect.(Ishimaru et al., 2008; Ookawa et al., 2016). Identification of QTLs that control bending- and breaking- type lodging resistance will accelerate to obtain information concerning their basis, such as chromosomal location of genes, allelic effect, or epistasis interaction. Takai et al. (2014) has developed CSSLs from the cross between Koshihikari and the *indica*-type high-yielding variety Takanari. It will be useful tools

to give deeper understanding into the genetic mechanism underlaying lodging resistance trait. Through CSSLs, we will be able to uncover new alleles and dissect quantitative traits into genetic factors. For the next chapter, a set of CSSLs derived from cross between Takanari and Koshihikari were used, in order to comprehensively understand the relation between responsible trait for bending- and breaking- type lodging resistance and estimated related QTLs.

Chapter 3 Estimation of QTLs associated with lodging resistance based on breaking- and bending- type parameters using CSSLs

3.1 Introduction

In Chapter 2, it was confirmed that *indica* variety, Takanari and Koshihikari a *japonica* variety showed significant differences in almost all bending- and breaking- type lodging resistance component traits observed. Takanari and Koshihikari have different lodging resistance characteristic. Koshihikari has a small culm diameter and high BS, whereas Takanari has large culm diameter and high rigidity. In this chapter further study were conducted to identify QTLs responsible for those traits using CSSLs developed from Takanari and Koshihikari.

The agronomical traits of rice generally controlled by multiple gene and expressed in continuous phenotypic variation (Yano and Sasaki, 1997), including lodging resistance. Agronomic traits are influence by multiple genes, genes interaction, gene and environmental (G x E) interaction. The location of genes is generally referred to as QTLs. QTL is a part of the genome that influence a quantitative trait. Generally, they are multifactorial and are influenced by several polymorphic genes and environmental conditions, so one or many QTLs can influence a trait or a phenotype (Consortium, 2003). QTL analysis has been employed as a powerful approach to discover agronomically useful QTLs and their responsible genes (Jones et al., 2001; Kashiwagi and Ishimaru, 2004; Zhu et al., 2008).

QTL-based analysis can explain the genetic basis of relationships among traits and allows a comprehensive investigation of the genetic relationships among morphological, anatomical, and chemical traits. QTL associated with complex traits have been identified and have already delivered crucial information to accelerate our understanding of natural variations (Ashikari et al., 2005; Fujino et al., 2008; Ren et al., 2005; Yano et al., 2000). QTL with relatively large effects can be detected in

the genetic analysis or primary mapping population. However, some QTLs with minor effects and those with epistatic interaction with other loci might not be detected in QTL analysis (Yano and Sasaki, 1997) Some QTLs found to be masked by the QTLs with relatively large phenotypic effect or epistatic interaction (Lin et al., 2002; Yamamoto et al., 2000).

To address problems mentioned above, some novel mapping of introgression lines has been developed as chromosome segment substitution lines (CSSLs) (Ando et al., 2008; Ebitani et al., 2005; Kubo et al., 2002; Sobrizal et al., 1999). CSSLs are powerful tools for identifying the QTLs for agronomic traits (Ali et al., 2010). These lines are series of near-isogenic lines (NILs) containing the fragments of the whole donor genome (Zamir, 2001). A population of CSSLs ideally covers the entire donor genome, and these lines have been referred to as introgression lines (Eshed and Zamir, 1995). These are developed by repeated backcrossing, in conjunction with molecular markers, to define and track the introgressed chromosome fragments in each advancing backcross or selfed generation (Ebitani et al., 2005). The usefulness of the CSSL population lies in providing the starting materials to initiate fine mapping and Mendelisation of both large and small effect QTLs, which ultimately paves the path for map-based cloning of QTLs (Subudhi et al., 2015).

A CSSLs facilitates the mapping of QTLs that control trait differences between its parental lines. Some advantages of using CSSLs lines compared to traditional QTL mapping are: first, no crosses need to be performed and it can be used as a permanent mapping population for mapping traits with more precision and higher efficiency. Second, genotyping is not required, because the segregation of the genome among the lines is already known in the panel of CSSLs. Third, it allows the investigations of interactions between QTLs, which are difficult to evaluate in traditional mapping populations (Ali et al., 2010; Nadeau et al., 2000; Yamamoto et al., 2009). A set of CSSLs enable the genetic dissection of any quantitative trait by phenotyping progeny from each of the lines without

additional genotyping or generating crosses (Nadeau et al., 2000). Many phenotypes can be measured in parallel (Ookawa et al., 2016; Takai et al., 2014).

Beside numerous morphological traits of plants, some important biochemical contents are also important and significantly contribute to lodging resistance. Starch and soluble sugar are the non-constitutive carbohydrate in culms that play roles in maintaining the physical strength of culm. As the main component of cell wall, cellulose has a significant impact on maintaining stem mechanical strength (Xiang et al., 2010). Some researches on wheat and rice found that varieties with a high cellulose content in the stem were showed high resistance to lodging as compare with those of low cellulose content (Wang et al., 2006; Yang et al., 2009). Wu et al. (2008) reported that culm loses mechanical strength and easily lodge by decreasing in cellulose content. Cellulose usually constitutes 20-30% or 40-90% of the dry weight of primary or secondary cell wall, varying with the cell type (Taylor et al., 1999).

However, some studies suggested that lignin may also contribute to cell wall strength (Jones et al., 2001; Li, 2003). Nguyen et al. (2016) reported that lodging resistance tolerance against biotic and abiotic stress and feedstock quality of wheat biomass are closely associated with its lignin content. Lignin is a complex phenolic polymer closely linked with cellulose and hemicellulose, forming an important structural component of plant secondary cell wall. It provides plants with mechanical strength and vascular integrity (Vanholme et al., 2010). Lignin is a phenylpropanoid polymer deposited in the cell wall during secondary wall thickening (Brown et al., 2005), providing the plant body with strong mechanical support to enable the plant to grow upwards (Shen et al., 2009). Lignin content and composition are important factors that affect the cell wall stiffness and the mechanical strength of the plant body (Chabannes et al., 2001).

In this chapter, to identify putative QTLs responsible for breaking- and bending- type lodging resistance, CSSLs derived from a cross between *indica*-variety Takanari and *japonic*a-variety

Koshihikari were used. Koshihikari was the donor parent and Takanari was the recurrent parent. It was confirmed in the previous chapter that Takanari has higher lodging resistance except bending stress as compare to Koshihikari. These CSSLs were developed using PCR-based DNA makers (n=141) including developed gene markers (*GN1a, sd1*, and *APO1*) (Takai et al., 2014).

Moreover, previous studies have been conducted using the same CSSLs to explain gene activity based on QTLs for yield and yield component (Takai et al., 2014) and lodging resistance (Ookawa et al., 2016), but not elaborated its relation with cell wall material densities as responsible traits of culm stiffness. It has been reported that cell wall materials are important for lodging resistance trait. In this study, the relation of lodging resistance and its responsible trait were elucidated. Furthermore, the genetic basis underlaying this trait was investigated through the detected QTLs.

3.2 Materials and methods

3.2.1 Plant material and cultivation

Thirty-seven Koshihikari CSSLs in the Takanari genetic background (T-CSSLs) that were derived from a cross between Takanari (*Oryza sativa* L. spp *indica*) and Koshihikari (*Oryza sativa* L. spp *japonica*) were used for the estimation of QTLs. The graphical genotype of 39 T-CSSLs, presented in Fig 3.1. Each chromosome was covered by two or four overlapping segments. Most CSSLs carried only one chromosome segment. However, a small segment in SL 1315 was substitute in the genetic background. SL1321 also carried two heterozygous segments and one homozygous segment for Koshihikari. The parents, which serve as controls, were planted under the same conditions as the CSSLs in 2015.

Rice seeds were sown in nursery boxes. Seedlings were transplanted at a density of one plant per hill, to a paddy field at the University farm in Tokyo on alluvial soil of the Tama River. The

planting density was 22.2 hills m^{-2}, with a spacing of 15 cm × 30 cm. As a basal dressing, compound fertilizer was applied at rate of 5.0 kg $10a^{-1}$ for N and 6.0 kg $10a^{-1}$ for P_2O_5 and K_2O.

The method for culm strength measurement and determination of cell wall materials were conducted as describes in the method on Chapter 2.

3.2.2 Statistical analysis

Statistical comparison of multiple sets of data was carried out using the Dunnett's multiple comparison test with R software version 3.4. Coefficient correlation test was conducted using Jmp software ver.12.0.1.

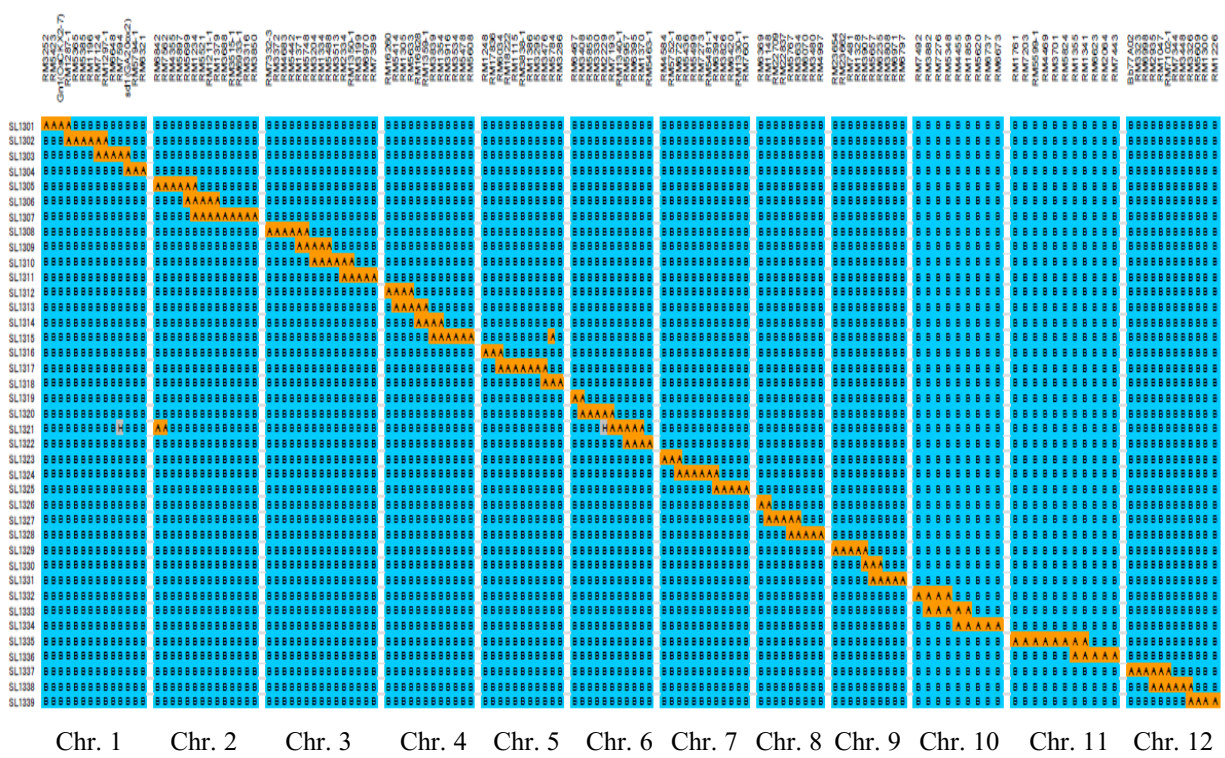


Fig. 3.1. Graphical genotypes of T-CSSLs. Orange regions indicate homozygosity for Koshihikari; blue regions indicate homozygosity of Takanari; grey region indicated heterozygosity.

3.3 Result

A set of 37 T-CSSLs of Koshihikari segments in the Takanari genetic background was used to identify QTLs related to bending- and breaking-type lodging resistance. Component traits of breaking-type and bending-type lodging resistance were compared with Takanari as the reference variety. QTLs were estimated in CSSLs when significant differences in related traits were detected between Takanari and the CSSLs.

3.3.1 Identification of QTLs related to breaking-type lodging resistance in T-CSSLs

To detect QTLs for the parameters associated with breaking-type lodging resistance, a total of 37 T-CSSLs were compared with Takanari. Three T-CSSLs showed significant differences in the M compared to Takanari. One line (SL 1307) had higher M value, and two lines (SL 1324 and SL 1327) showed lower M values than those of Takanari (Fig. 3.2a). The SM of Takanari was 19.2 mm^3 and ranged from 27.0 mm^3 to 13.5 mm^3 among CSSLs (Fig.3.2b). Significantly higher SM values were detected in SL 1302 and 1304, corresponding to substitutions of Koshihikari segment in Takanari background on chromosome 1. By contrast, SL 1336 had a lower SM value compared to Takanari. The BS in Takanari was 839.5 $gf \cdot mm^{-2}$; in CSSLs, BS values ranged from 583 $gf \cdot mm^{-2}$ to 1 451 $gf \cdot mm^{-2}$. The BS values in SL 1308, 1318, 1321, 1326, 1330, 1333, 1334, 1335, 1336, 1337, and 1338 were significantly larger than that in Takanari (Fig.3.2c).

3.3.2 Detected QTLs related to bending- type lodging resistance in T-CSSLs

To detect QTLs associated with bending-type lodging resistance, the basal culm of the fourth internode was used for the comparison of the traits associated with both type lodging resistance between T-CSSLs and Takanari. The FR in Takanari was 996.2 $gf \cdot mm^2$. In CSSLs, FR values were ranged from 393.6 $gf \cdot mm^2$ to 1847.3 $gf \cdot mm^2$. Significant differences were detected between two T-CSSLs and Takanari. The FR in SL 1302 was significantly higher than that in Takanari, whereas that in SL 1324 was smaller (Fig.3.3a). The SMI was 61.0 mm^4 in Takanari and range between 36.4 mm^4

and 107.5 mm^4 in the T-CSSLs. The SMI values in two CSSLs (SL 1302 and 1304) were significantly larger than that in Takanari and non-showed lower to Takanari (Fig.3.3b). The YM in Takanari was 16.3 $gf \cdot mm^{-2}$; in CSSLs, YM values ranged from 8.0 $gf \cdot mm^{-2}$ to 22.7 $gf \cdot mm^{-2}$. The YM was significantly smaller in SL 1324 and 1339 than that in Takanari, whereas the YM in SL 1307, 1334, 1335 and 1336 was larger (Fig.3.3c).

3.3.3 Detected QTLs related to Cell wall materials in T-CSSLs

The densities of cell wall materials were compared between Takanari and 37 CSSLs (Fig.3.4). The holocellulose density in Takanari was 147.6 $\mu g \cdot mm^{-3}$; in T-CSSLs and holocellulose densities ranged from 106.3 $\mu g \cdot mm^{-3}$ to 197.6 $\mu g \cdot mm^{-3}$ (Fig.3.4a). Lignin also contributes to increase stem strength. In this study, Lignin density in Takanari was 32.4 $\mu g \cdot mm^{-3}$ and ranged between 18.2 $\mu g \cdot mm^{-3}$ to 57.7 $\mu g \cdot mm^{-3}$ in T-CSSLs. The cellulose density in Takanari was 92.4 $\mu g \cdot mm^{-3}$, a value that was significantly higher than those in SL 1301, 1302, and 1311. One CSSL line, SL 1318, exhibited a significantly elevated cellulose density compared to that of Takanari (Fig.3.4c). There were significant differences in hemicellulose densities in five CSSLs. Notably, SL 1311 exhibited a low hemicellulose density. On the other hand, SL 1318, 1326, 1335 and 1336 had higher hemicellulose densities compared with that of Takanari (Fig.3.4d).

For further understanding the relationship between culm cell wall material density and culm stiffness component trait, the simple correlation coefficients were estimated based on Takanari and 37 CSSLs. As shown in Table 3.1, the correlation analysis demonstrated that the densities of holocellulose, lignin, cellulose and hemicellulose, had strong positive correlation with M and BS. On the other hand, it is negatively corelated with FR. Moreover, the densities of cell wall material did not showed correlation with YM. FR is the product of SMI and YM, the negative correlation between the densities of cell wall component and FR might be due to no correlation to YM. As mentioned in the Chapter 2 (Fig. 2.1.), cell wall components are the responsible trait for YM. Whereas ,SMI is

directly influenced by culm morphology such culm diameters and culm wall thickness. These results indicated that cell wall materials densities were closely related to culm stiffness in breaking-type lodging resistance, but not directly affecting bending-type lodging resistance.

3.3.4 Substitution mapping of QTLs for component traits of lodging resistance

To identify candidate regions for QTLs of cell wall materials associated with breaking- and bending-type lodging resistance, the substitution mapping of the QTLs was conducted. A total of 23 QTLs for breaking- and bending-type lodging resistance component traits were mapped. One QTL for M was mapped on chromosome 2 showed positive effects. Whereas, two QTL on chromosomes 7 and 8 showed negative effects of the Koshihikari segment in the Takanari genetic background. Substitution mapping confined QTLs for SM on chromosomes 1 and 11. Two QTLs on chromosome 1 showed positive effects, whereas one on chromosome 11 showed a negative effect of Koshihikari allele. Eight QTLs for BS were mapped; all showed positive effects with the Koshihikari allele. These QTLs were assigned to chromosomes 3, 5, 6, 8, 9, 10, 11, and 12 (Fig. 3.5a).

Two QTLs for FR were assigned to chromosomes 1 and 7, respectively, and contributed to increase and decrease in FR. Two QTLs for SMI that were assigned to chromosome 1 showed positive effect of the Kosihikari allele. Five QTLs were detected for YM. Three and two of these QTLs contributed to increase and decrease in YM, resulting from of the presence of the Koshihikari allele in the Takanari genetic background (Fig. 3.5b).

3.3.5 Substitution mapping of QTLs for the trait responsible for culm strength associated with cell wall materials

Based on the substitution mapping using T-CSSLs, a total of 12 QTLs were estimated for regions associated with holocellulose, lignin, cellulose, and hemicellulose densities. Holocellulose densities were increased by the substitution of Koshihikari segments on chromosomes 5 and 11. On the other hand, these were decreased on chromosomes 1 and 3. One QTL for lignin on chromosome

11 showed decreased by the substitution of Koshihikari segment. The presence of a Koshihikari segment in the Takanari genetic background contributed to decreased cellulose density for QTLs on chromosomes 1 and 3. In contrast, the cellulose density was increased by the placement of a Koshihikari segment on chromosome 5. Four QTLs were found in hemicellulose density, with three and one of these QTLs contributing to increase and decrease hemicellulose density, respectively (Fig.3.6).

QTLs for holocellulose, cellulose, and hemicellulose were estimated for the consistent regions with BS on chromosome 5, exhibited positive effects. These results suggested that the QTLs on chromosome 5 contributed to the increase in BS. Furthermore, on chromosome 11, QTLs for holocellulose, hemicellulose and lignin were detected at the consistent regions with BS and YM (Fig. 3.5 and 3.6). These results suggested that some QTLs for the densities of cell wall materials contributed to increase BS and YM in the Takanari genetic background.

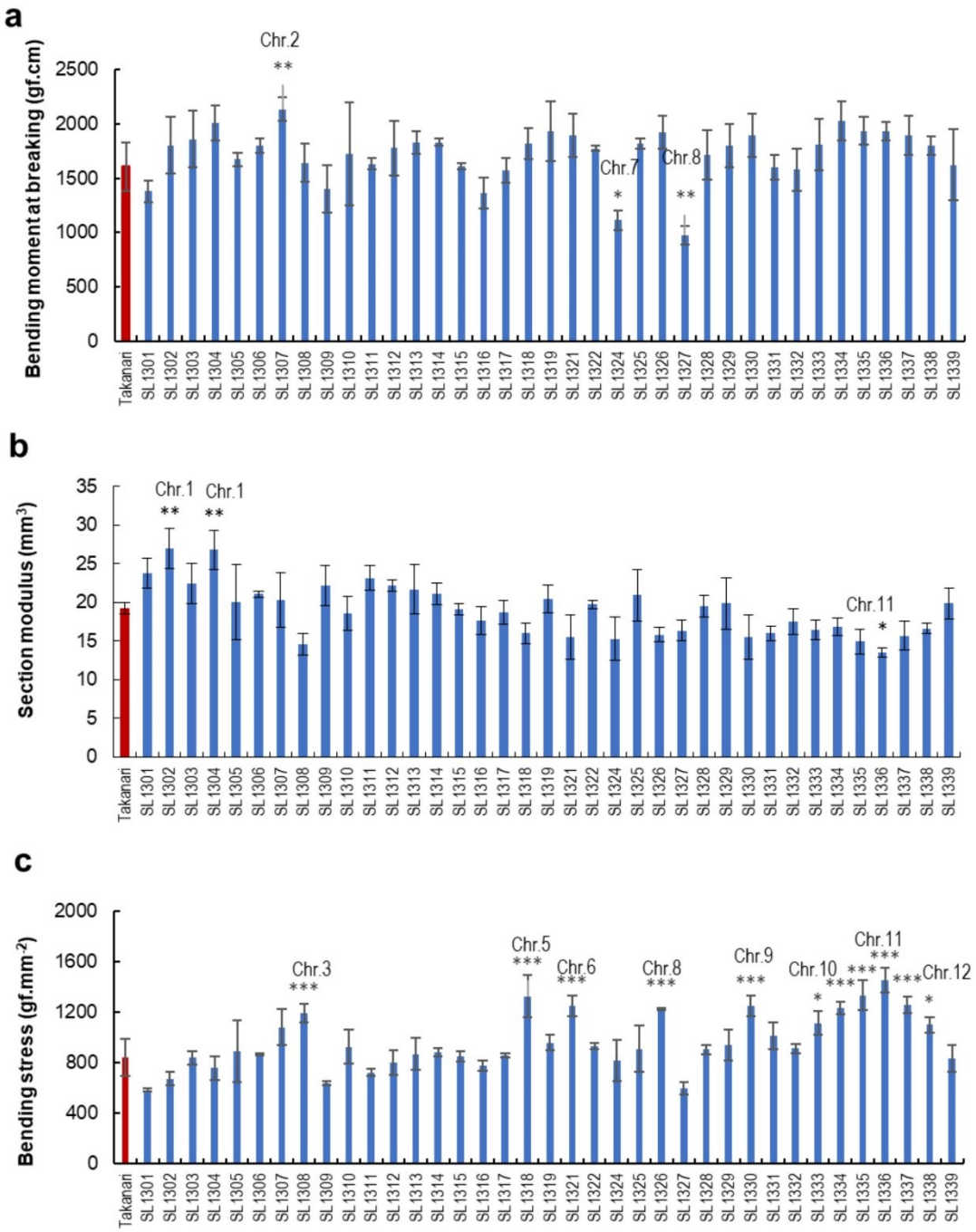


Fig. 3.2. Physical parameters associated with breaking-type lodging resistance in T-CSSLs in 2015: (a) bending moment at breaking, (b) section modulus, and (c) bending stress of the fourth internode. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively

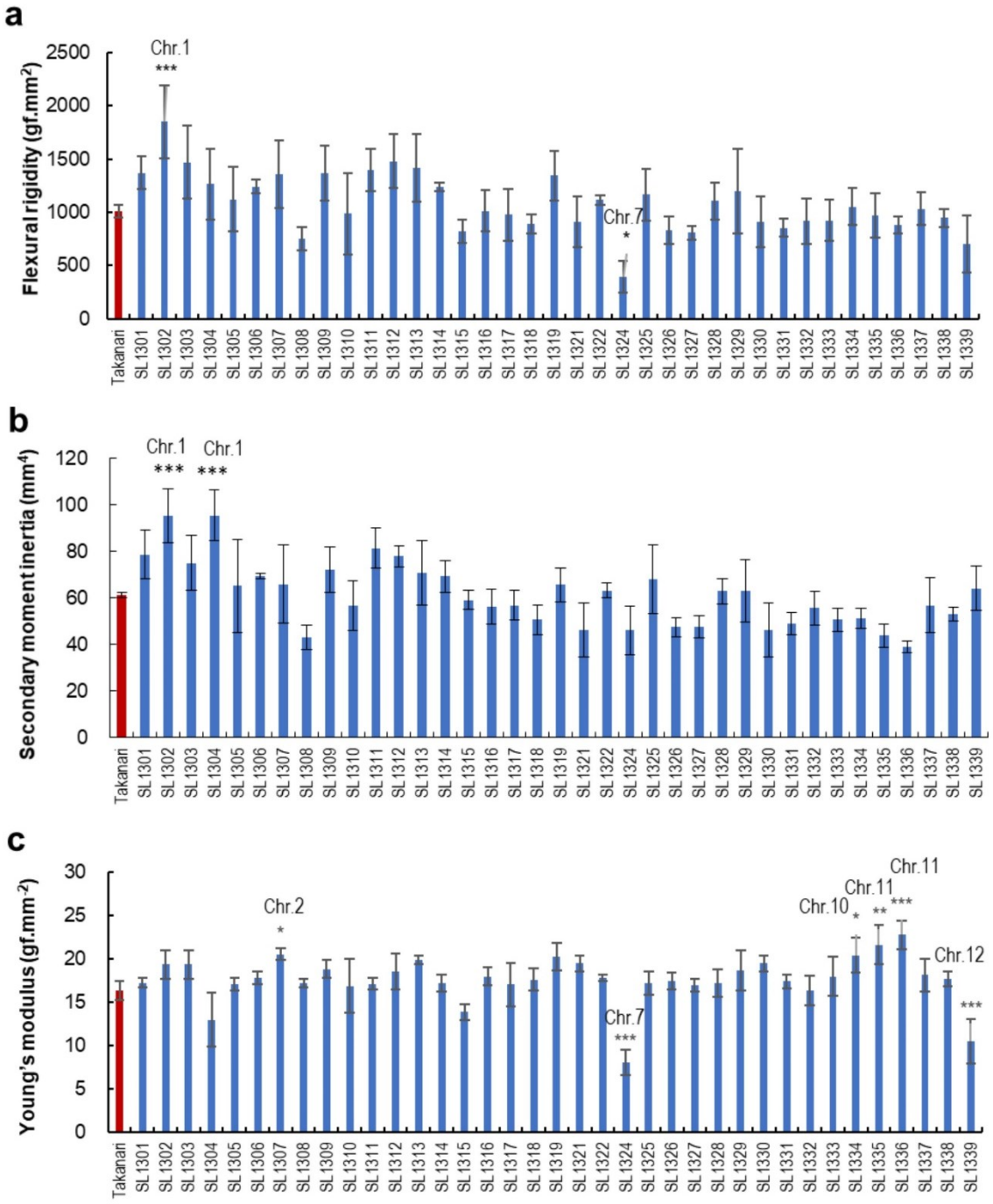


Fig. 3.3. Physical parameters associated with bending-type lodging resistance in T-CSSLs: (a) flexural rigidity, (b) secondary moment inertia, and (c) Young's modulus of the fourth internode. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively

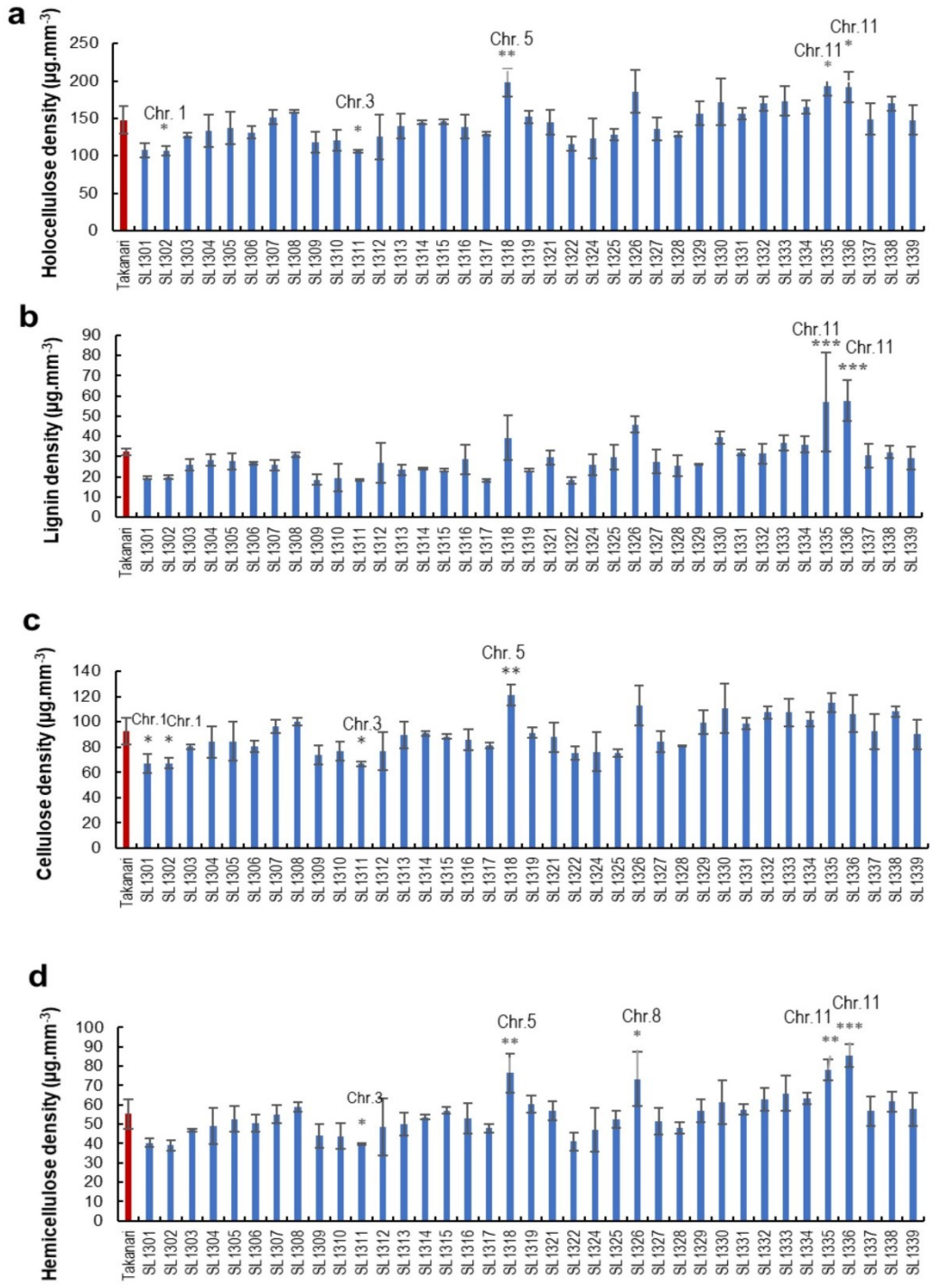


Fig. 3.4. Cell wall material density of the fourth internode in 2015: (a) holocellulose, (b) lignin, (c) cellulose, and (d) hemicellulose. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively.

Table 3.1 Coefficient of correlation between cell wall material density, culm strength and culm stiffness component traits.

Lodging resistance traits	Cell wall material density (μg·mm^{-3})			
	Holocellulose	Lignin	Cellulose	Hemicellulose
M	0.3852 *	0.3308 *	0.3827 *	0.3672 *
BS	0.8236 **	0.7685 **	0.7929 **	0.8190 **
FR	-0.4837 **	-0.4209 **	-0.4689 **	-0.4766 **
YM	0.2916	0.3180	0.2671	0.3082

*and ** indicate significant difference at the 0.05 and 0.01 levels, respectively.

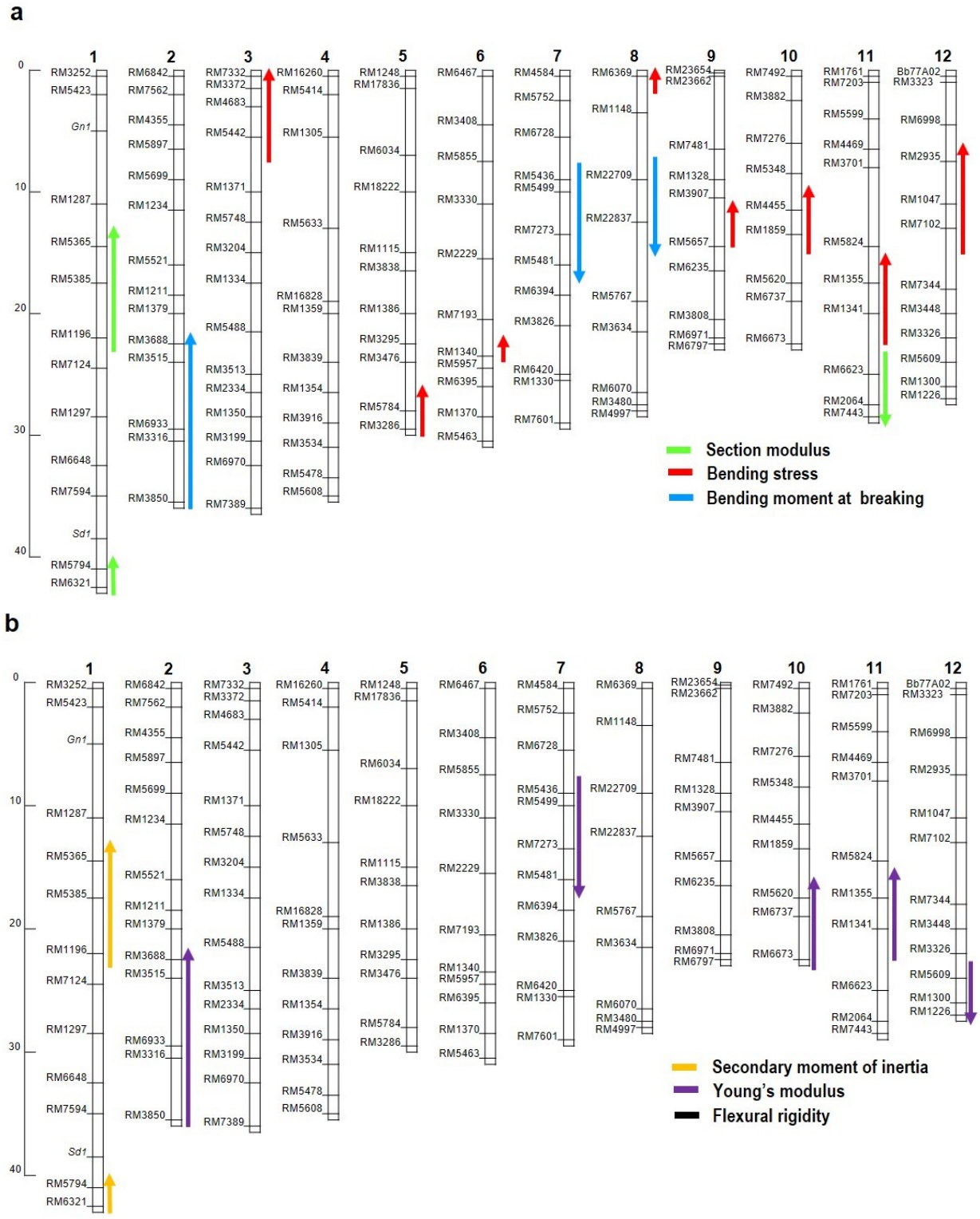


Fig. 3.5. Substitution mapping of QTLs by comparing overlapping segments among chromosome segment substitution lines (CSSLs) for: (a) breaking- and (b) bending-type lodging resistance. Upward arrows indicate positive effects, and downward arrows indicate negative effects, of Koshihikari segments in the Takanari background.

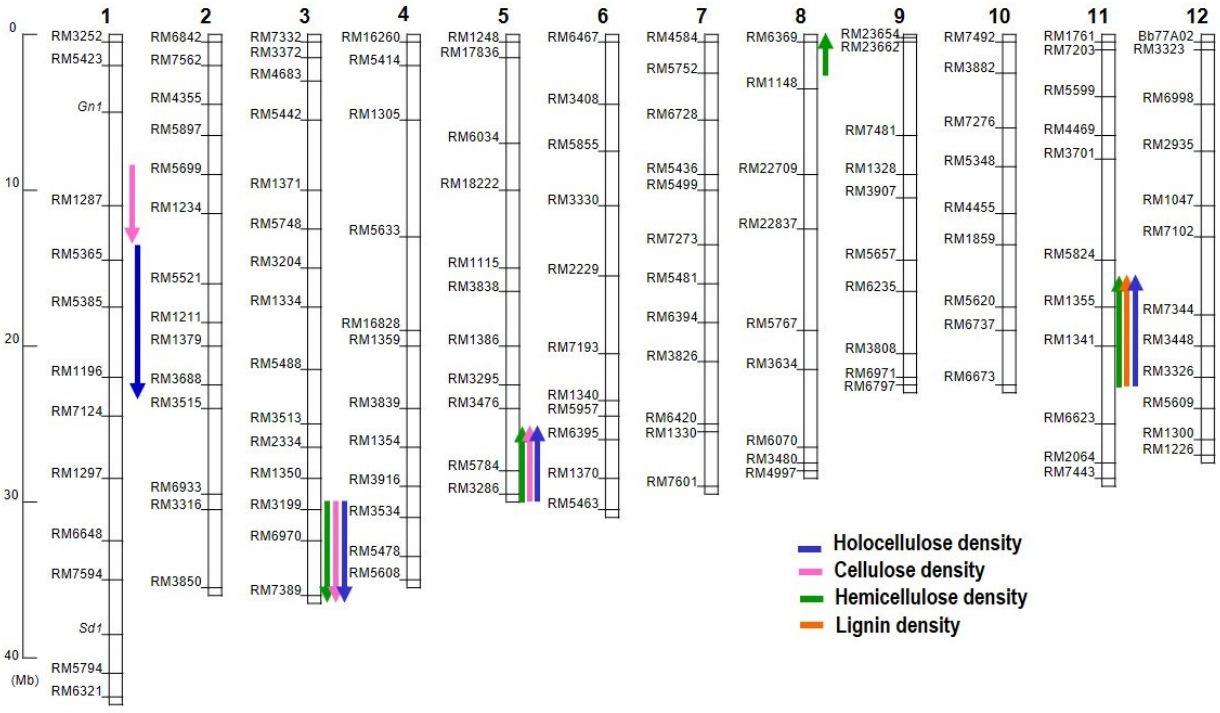


Fig. 3.6. Substitution mapping of QTLs by comparing overlapping segments among chromosome segment substitution lines (CSSLs) for cell wall materials densities. Upward arrows indicate positive effects, and downward arrows indicate negative effects, of Koshihikari segments in the Takanari background.

3.4 Discussion

CSSLs are ideal genetic populations in which to estimate the QTLs for target traits that are controlled by multiple responsible traits (Ando et al., 2008; Kubo et al., 2002; Nadeau et al., 2000). With CSSLs, it is possible to compare the phenotypic effects between alleles on substituted chromosome segments (Ebitani et al., 2005). Indeed, CSSLs have previously been used in rice to estimated QTLs that control important traits related to lodging resistance (Kashiwagi, 2014; Kashiwagi et al., 2016; Ookawa et al., 2016). In addition to an effective tool for introgressing valuable gene between commercial variety, CSSLs have been used to introgress genes between *japonica* and *indica* (Ebitani et al., 2005; Ishikawa et al., 2005; Wan et al., 2004). To identified genes related to lodging resistance we used a set CSSLs derived from *japonica* variety Koshihikari as donor parent into *indica* variety, Takanari as genetic background.

In the present study, QTLs for SM were detected on chromosomes 1 and 11 (Fig. 3.2b). In the previous study (Ookawa et al., 2016), a QTL for SM was detected on chromosome 1, and was located at the long arm in a region contained *SD1*. Thus, the QTL analysis showed that the genomic region containing the *SD1/sd1* gene on chromosome 1 contributes to the regulation of SM. NIL-SD1 has a significantly larger SM than NIL *sd1*.The *sd1* allele provides significant enhancement of lodging resistance by decreasing the moment of the entire plant through the repression of internode elongation (Murai et al., 2004). The QTL for SM was detected at the long arm region on chromosome 1 in this study (Fig. 3.2b), consistent with the results of previous study using the same reciprocal CSSLs (Ookawa et al., 2016). The Koshihikari allele of *SD1* contributes to the thick culm, although Koshihikari exhibits a fine culm. The thickness of cortical fiber tissues and culm weight are reported characteristics of high physical strength (Matsuda et al., 1983). These traits are key targets for strong culms when altering culm morphology. Whereas high SM in Takanari was caused by the large outer diameter (Ookawa et al., 2016).

QTLs for BS were identified on chromosomes 3, 5, 6, 8, 9, 10, 11, and 12. These QTLs contributed to an increase in BS, when Koshihikari chromosomal segment was substituted into the Takanari genetic background (Fig 3.2c). Using the same reciprocal CSSL, Ookawa et al. (2016) also detected QTLs for BS on chromosomes 6, 8, and 11. The results of both studies showed that these QTLs contributed to an increase in BS. Some QTLs for BS detected in this study were not detected in the previous study (Ookawa et al., 2016). This discrepancy might be caused by the complexity of the BS trait. BS is a complex trait controlled by many responsible traits such as morphological traits and cell wall materials (Matsuda et al., 1983) and by environmental factors. In Chapter 2, it was mentioned that higher BS value in Koshihikari due to the accumulation of cell wall component densities. Ookawa and Ishihara (1993) suggested elevated cellulose and lignin are responsible for higher BS in most *japonica* variety. Thus, optimum lodging resistance can be achieved through different combinations of lodging resistance component traits.

Identification of QTLs could enhance the optimum culm strength between breaking- and bending-type of lodging. A QTL for bending-type lodging resistance, *BSUC11* was identified from CSSLs between Koshihikari and Kasalath (Kashiwagi, 2014). The QTL also functions to prevent culm strength deterioration after grain filling (Kashiwagi et al., 2016). The QTLs related to bending-type lodging resistance were also detected in this study. Bending-type lodging resistance is defined by FR values indicating culm stiffness. FR is composed of YM and SMI. High SMI in chromosome 1 contribute to increasing FR, and low FR on chromosome 7 was caused by the low YM (Fig.3.3). YM depends on the composition of plant tissue, and SMI depends on the configuration of existing material, i.e., culm outer and inner diameters (Silk et al., 1982). Large differences in YM were found between the Koshihikari and Takanari (Chapter 2). YM is an indicator of the rigidity of rice culm (Ishimaru et al., 2008). YM was increased by the substitution of Koshihikari chromosomal segments on chromosomes 2, 10, and 11. Other QTLs that were identified on chromosomes 7 and 12 indicated

that Koshihikari alleles contribute to the decrease of YM in the Takanari genetic background (Fig. 3.5b). These results suggested that the detected regions include the genes responsible for the elevation of YM in Takanari.

Culm physical strength is determined by morphological traits and chemical components. Regarding cell wall chemical component, the structural carbohydrates cellulose and lignin generally determine the physical strength of a plant. In this study, the positive correlation between cell wall material densities with BS and M (Table 3.1) indicated the importance of cell wall material density contributed to culm stiffness that accelerated culm strength (Figs 3.5 and 3.6). It has been observed QTLs for significantly corelated traits usually had the same chromosome location (Brondani et al., 2002; Tian et al., 2006). Cellulose is soft and flexible in nature, and has good tensile strength, while lignin provides rigidity and mechanical support to plant tissues (Ma, 2009). High densities of cellulose and hemicellulose in the culms showed by high lodging resistance variety, Leaf Star (Ookawa et al., 2014). Moreover, Berry et al. (2003) reported that lignin and hemicellulose contents together increased the stem strength, and thus increased lodging resistant, while significant reduction of those component cause higher lodging index. Cellulose also has a qualitative effect on stem strength and increases stem rigidity(Reddy and Yang, 2005). In rice, higher accumulation of cellulose resulted in higher lodging resistance because of better stem strength against pushing force of wind (Yang et al., 2001). In addition, the study by Kashiwagi et al. (2016) showed increasing holocellulose through *BSUC11* function prevented culm strength deterioration. The genetic control of holocellulose content in culms could be a suitable strategy to resist lodging.

In this chapter, some QTLs for cell wall material densities detected together with BS on chromosome 5 (Fig. 3.5a and 3.6). Moreover, on chromosome 11, QTLs for the densities of holocellulose, hemicellulose, and lignin were detected at the same region with YM and BS (Fig. 3.5b and 3.6). Both regions indicated positive effect of Koshihikari segment on increasing BS and YM.

These results suggest that some QTLs for the densities of cell wall materials contribute to increase BS and YM and could be utilized to improve the lodging resistance for both types of breaking and bending in rice varieties. Previous study using CSSLs of *indica* x *japonica* demonstrated effectiveness for introgressing desirable alleles or allele combinations from distantly related donor into particular parent background (Bian et al., 2010). QTLs in the same chromosome location for various traits are possibly due to either the linkage of genes or the pleiotropic effect of a single locus. In order to confirm designated QTLs related to breaking- and bending-type lodging resistance, as well as to investigate the genetic mechanism, further study needs to investigate using reciprocal CSSLs. In addition, gene expression studies such as RNA-seq or real-time RT-PCR was performed to give preliminary information of the gene(s) function underlying it.

Chapter 4 Identification of QTLs and their responsible genes for culm stiffness by using introgression lines

4.1 Introduction

In Chapter 2 it was showed that *indica* rice variety Takanari and a *japonica variety*, Koshihikari have large significant difference in bending- and breaking- type lodging resistance. In Chapter 3, using the CSSLs of Koshihikari segment on Takanari background, some QTLs were estimated for the trait associated with bending- and breaking- type lodging resistance. The QTLs for holocellulose, cellulose and hemicellulose densities were detected at long arm region on chromosome 5 overlapped with BS, indicating positive effect of Koshikari segment for increasing BS. Thus, these suggested that some QTLs for cell wall material densities contributed to increase BS.

Reciprocal CSSLs between Koshihikari and the *indica*-type high-yielding variety Takanari have been developed to precisely identify QTLs for important agronomic traits (Takai et al., 2014). Recently, Ookawa et al. (2016) identified QTLs associated with the thickness of cortical fiber tissues, showing that these QTLs were located on chromosomes 2, 9, and 11. However, the QTLs for cell wall components associated with culm stiffness such as BS have not been reported. In the previous study, it was shown that Takanari had a large SM that contributed to M. On the other hand, Takanari had a small BS. It is expected that the substitution of the corresponding segment from Koshihikari into the Takanari genetic background would contribute to an increase in BS and M. The substitution of chromosome segment from a *japonica* variety Koshihikari with weak culm which includes a superior allele might be also utilized for improving lodging resistance in an *indica* variety, Takanari.

In 2005, rice genome sequence has completely become available (Sasaki, 2005), and the physical position of numerous genes could be determined based on the sequence information flanking markers. This has permitted the development of more comprehensive database in rice. The

introduction and identification of genes is notable in improving lodging resistance traits. Recent progress in the field of genomics and biotechnology, together with conventional breeding approach has the potential to find novel genes that able to improve physical strength of plant to resist against lodging without sacrificing grain (Ookawa et al., 2010a; Yano et al., 2015). The *sd1* allele is verified semi dwarf gene suitable for use to engineer improvements in lodging related to plant height (Asano et al., 2009; Spielmeyer et al., 2002). Given that extensive use of limited gene may disadvantage the diversification of rice varieties and hinder the genetic improvement process (Luh, 1980). Thus, the development of new genetic resources for lodging resistance is desirable.

Characterization of genes controlling quantitative traits is a major research purpose in terms for understanding important agronomic traits including rice lodging resistance. Once genes controlling QTL are identified, natural variation present within those loci can be better characterized and exploited. Identification of the physical position of a candidate gene is a major step towards the isolation of genetic factors that control quantitative traits. Stably expressed and effective QTLs provide a promising target for further genetic characterization and marker assisted breeding. A large number of QTLs for lodging resistance have been identified (Ishimaru et al., 2004; Kashiwagi, 2014; Ookawa et al., 2010a; Yano et al., 2015), however to our knowledge, there is no report on identification and mapping QTLs for culm stiffness related to cellulose on long arm region of chromosome 5 and the responsible gene controlling it.

Cellulose is the most abundant polysaccharide, and the majority produced in the secondary cell wall (McNeil et al., 1984; Ookawa et al., 2014). Cellulose is synthesized by CESA enzyme in the plasma membrane (McFarlane et al., 2013). Cellulose synthase in plants is encode by gene family named CESA (*Cellulose Synthase* A), which is a multigene family found in plants. The CESA gen family is part of a larger superfamily (cellulose synthase superfamily) that contains closely related CLS (*cellulose-synthase like*) gene family (Taiz et al., 2015). The number of CESA genes varies

among plant species with different set of CESAs required for cellulose synthesis (Carroll and Specht, 2011). Furthermore Wang et al. (2010) has characterized the CESA/CSL of rice and provide major roles of CESA/CSL, their potentially functional complement and their associations for cell wall synthesis.

The transcriptome is the complete set of RNA molecules in a cell and their quantity, for a specific developmental stage or physiological condition. It is necessary having better understanding the transcriptome for interpreting the functional element of the genome. Next-generation high-throughput RNA sequencing technology (RNA-Seq) is a recently developed method for discovering, profiling, and quantifying RNA transcripts with several advantages over other expression profiling technologies (Wang et al., 2009). RNA-Seq can reveal the precise location of transcription boundaries, to a single base resolution (Vera et al., 2008), RNA-Seq can also reveal the precise location of transcription boundary and sequence variations in the transcribed regions (Morin et al., 2008). Other advantage of RNA-seq is that it does not have an upper limit for quantification which correlated with the number of sequence obtained. In addition, RNA-seq also showed high levels of reproducibility, for both technical and biological replicates (Cloonan et al., 2008; Nagalakshmi et al., 2008). RNA-seq has been applied to the identification of stress-inducible transcript in rice such as phosphate or salinity (Mizuno et al., 2010; Oono et al., 2011).

In this chapter, the putative QTL region of long arm region of chromosome 5 was verified using reciprocal CSSLs, furthermore, the next step RNA-seq and real time RT-PCR was conducted to find the candidate genes related to lodging resistance on chromosome 5.

4.2 Materials and methods

4.2.1 Plant material and cultivation

Reciprocal CSSLs on chromosome 5 derived from a cross between Koshihikari (*Oryza sativa* L. spp *japonica*) and Takanari (*Oryza sativa* L. spp *indica*) were used to confirm QTLs regions responsible for physical parameters and cell wall materials on chromosome 5. Three CSSLs. SL 1218, SL 1219 and SL 1220 were used for Koshihikari genetic background, and SL 1316, SL 1317 and SL 1318 for Takanari genetic background. The parents, as controls, were planted under the same conditions as the CSSLs in 2016. Takanari, Koshihikari and SL 1318 were planted in 2017 to estimate candidate gene responsible for culm stiffness.

Rice seeds were sown in nursery boxes. Seedlings were transplanted at a density of one plant per hill to a paddy field at the University farm in Tokyo on alluvial soil of the Tama River. The planting density was 22.2 hills m^{-2}, with a spacing of 15 cm × 30 cm. As a basal dressing, compound fertilizer was applied at rate of 5.0 kg $10a^{-1}$ for N and 6.0 kg $10a^{-1}$ for P_2O_5 and K_2O.

The method for culm strength measurement and determination of cell wall materials were conducted as describes in the method on Chapter 2.

4.2.2 Statistical analysis

Means between parent and CSSLs on chromosome 5 were analysed using a *t*-test, when ANOVA showed significance at the level of 0.05 probability. ANOVA tests were conducted using Jmp software ver.12.0.1. The RT-PCR, were analysed using one-way analysis of variance followed by the Tukey-Kramer Multiple Range Test.

4.2.3 Estimation of candidate gene and gene expression analysis

RNA-seq Analysis

Sampling were conducted for the shoot apex meristem (SAM) on main culm at 40 days before heading (DBH) (Fig.4.1). The meristem was collected and frozen in liquid nitrogen. Furthermore,

RNA extraction was carried out, according to RNA extraction method of Iwate Bioengineering Research Center. RNA-seq was conducted at Iwate Bioengineering Research Center using Breath Adapter Directional sequencing (BrAD-seq) method. Genes with significant differences in the expression level at the 5% level among cultivars were detected using TCC analysis. Differentially Expressed Genes (DEGs) in the candidate region were estimated from RAP-DB (http://rapdb.dna.affrc.go.jp)

RT-PCR Analysis

Sampling for RNA extraction was conducted for the shoot apex meristem on main culm at 33, 26 and 20 days before heading (DBH). Sample were collected and frozen in liquid nitrogen. RNA was extracted from frozen samples, and reverse transcribed into cDNA. Primers for real-time RT-PCR were designed from the sequence of target genes using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). Primers used for real time RT-PCR of designated gene are F: GTGGCTTTGGTGGGAAAGA and R: CGGTTTTGGTGGTGGTAATG. Subsequently, real-time RT-PCR was performed using Step One Plus (Applied Biosystems). Gene expression analysis was performed on putative candidate gene.

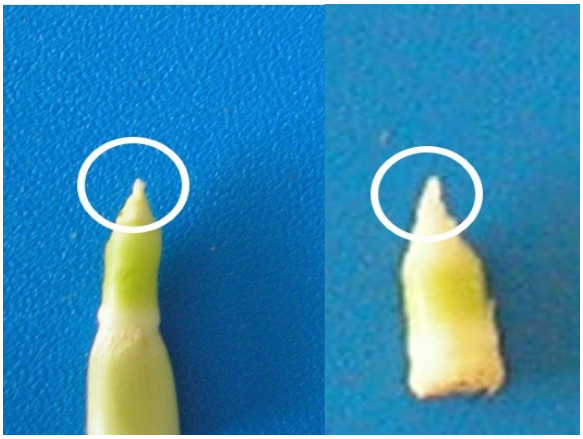


Fig. 4.1. The meristem of shoot apex on main culm at 40 days before heading (DBH)

4.3 Results

In the previous chapter, we detected QTLs for cell wall materials at the same region with BS on chromosomes 5 and 11, and in the same region with YM on chromosome 11. However, the performance of the CSSLs on chromosome 11 showed late maturity and abnormal growth compared with other lines (Table 4.1). Takai et al. (2014) reported that SL 1335 and 1336 showed a hybrid weakness such as delayed heading, dwarf plant stature, fewer spikelets, lower ripening percentage and lower yield. Plant abnormality stature might affect the accumulation of cell wall materials, indirectly. Therefore, we concentrated on the QTL on chromosome 5. The performance of reciprocal CSSLs at the long arm region of chromosome 5 in 2016 did not found any abnormality, as showing relatively similar heading date in each genetic background (Table 4.2).

4.3.1 Identification of QTLs for breaking- and bending- type lodging resistance on chromosome 5

To confirm the candidate regions of QTLs for the traits associated with BS and cell wall materials, reciprocal CSSLs of chromosome 5 were compared with the parent genetic background. The analysis of variance of the influence of lines on breaking type lodging and cell wall component densities on chromosome 5 in 2016 showed in Table 4.3. One QTL for M detected in K-CSSL, showed in SL 1218 (Fig. 4.2a). There were no QTL detected on chromosome 5 for CSSL with Takanari genetic background (Fig. 4.3a). Furthermore, QTLs for SM were detected in SL 1218, SL 1219 and SL 1220. Significantly higher SM were showed by K-CSSL as result of Takanari segment in Koshihikari back ground (Fig. 4.2b). While one T-CSSL, SL1316, SL1317 and SL1318 exhibited significant lower SM than Takanari (Fig.4.3b). Significant decreases in BS values were observed in K-CSSL upon the substitution of a Takanari segment into the Koshihikari genetic background. Two K-CSSLs, SL 1219 and 1220, exhibited smaller BS values than that of Koshihikari (Fig.4.2c). By contrast, SL 1317 and SL 1318, lines with a reciprocal substitution at the same region, had a

significantly higher BS than that of Takanari (Fig.4.3c). Thus, a reciprocal effect between K-CSSLs and T-CSSLs on chromosome 5 was detected for SM and BS (Fig. 4.2 and 4.3).

The QTLs for FR were identified on chromosome 5 in all K-CSSL which showed higher FR as compared with that in Koshihikari (Fig. 4.4a). Significant higher SMI also detected on chromosome 5 in all K-CSSL (Fig. 4.4b). Among the K-CSSLs, SL 1218 and SL1219 showed significant higher YM compared with Koshihikari (Fig. 4.4c).

The fourth internode from the base of culm were compared between Takanari and T-CSSLs. T-CSSLs did not showed significant difference in FR and YM parameters compare to Takanari (Fig. 4.5a and 4.5c). Two T-CSSL, which were SL 1316 and 1317 showed significant differences compared with Takanari in SMI (Fig. 4.5b). Koshihikari segment contributed to the decreasing of SMI in Takanari background.

Further investigations using reciprocal CSSLs were performed to confirm the results of QTLs for cell wall materials on chromosome 5. Two K-CSSLs, SL 1219 and 1220, showed low densities of holocellulose, cellulose, and hemicellulose, when these were compared with Koshihikari (Fig 4.6a). The cellulose densities in SL 1317 and SL 1318 were higher than that in Takanari (Fig. 4.6b). The detected QTL at the long arm region on chromosome 5 was consistent with the result of the previous year in the Takanari genetic background (Fig. 3.4c).

In previous chapter, it showed QTLs for holocellulose, cellulose, and hemicellulose were assigned to the same regions on chromosome 5 as those detected for BS-associated QTLs on chromosome 5 in 2015 (Fig. 3.5a and 3.6). In 2016, at the long arm region on chromosome 5, a reciprocal effect of T-CSSLs and K-CSSLs was observed for both BS and cellulose densities (Fig. 4.2c, 4.3c, 4.6a, and 4.6b). This showed that the QTLs for BS and cellulose density were detected at the same region (Fig. 4.7) for each CSSLs genetic background. These results suggested that the QTLs

for cellulose density on chromosome 5 contributed to the increase of BS in the Takanari genetic background.

Table 4.1. Heading date (date after sowing) of parent lines and T-CSSLs in 2015.

Lines	Heading (days after sowing)	Lines	Heading (days after sowing)
Koshihikari	96	SL 1319	107
Takanari	105	SL 1321	98
SL 1301	103	SL 1322	109
SL 1302	103	SL 1324	91
SL 1303	102	SL 1325	92
SL 1304	95	SL 1326	95
SL 1305	92	SL 1327	105
SL 1306	98	SL 1328	111
SL 1307	107	SL 1329	105
SL 1308	100	SL 1330	98
SL 1309	103	SL 1331	100
SL 1310	109	SL 1332	107
SL 1311	91	SL 1333	95
SL 1312	91	SL 1334	97
SL 1313	107	**SL 1335**	**127**
SL 1314	107	**SL 1336**	**131**
SL 1315	107	SL 1337	95
SL 1316	102	SL 1338	95
SL 1317	109	SL 1339	91
SL 1318	92		

Table 4.2. Heading date (date after sowing) of parent lines and reciprocal CSSLs of Chromosome 5 in 2016.

Lines	Heading (days after sowing)	Lines	Heading (days after sowing)
Koshihikari	97	Takanari	103
SL 1218	98	SL 1316	103
SL 1219	94	SL 1317	107
SL 1220	96	SL 1318	100

Table 4.3. Analysis of variance of the influence of lines on breaking type lodging and cell wall composition densities of chromosome 5 in 2016.

Source of variation	M (gf.cm)	SM (mm^3)	BS ($gf.mm^{-2}$)	Holocellulose density ($\mu.mm^{-3}$)	Lignin density ($\mu.mm^{-3}$)	Cellulose density ($\mu.mm^{-3}$)	Hemicellulose density ($\mu.mm^{-3}$)
Koshihikari and K-CSSLs	*	**	**	**	ns	**	*
Takanari and T-CSSLs	ns	**	**	ns	*	*	ns

*, ** significant at 0.05 and 0.01 probability level respectively; ns, not significant.

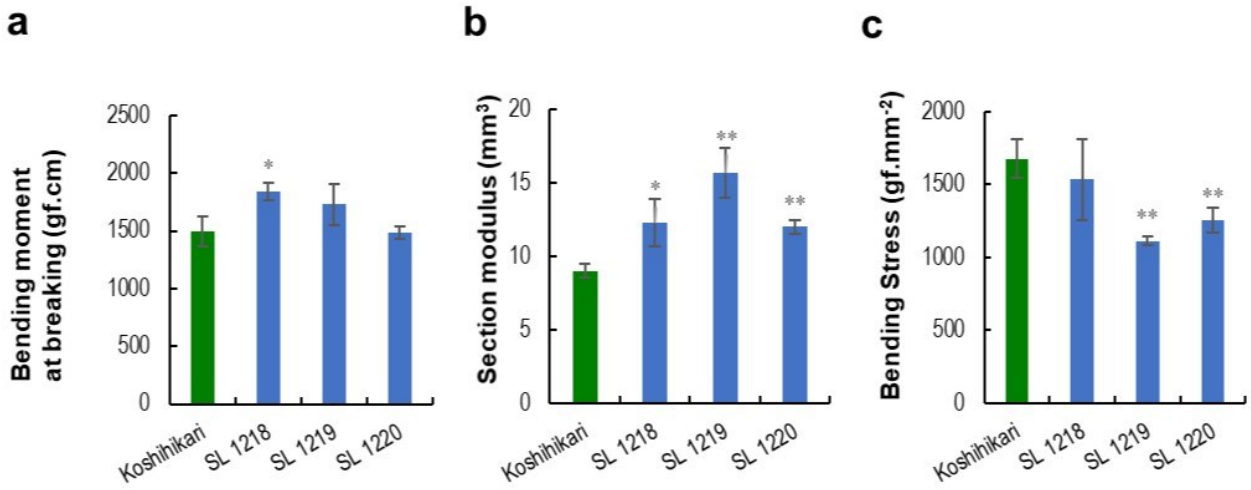


Fig. 4.2. Physical parameters breaking-type lodging resistance (a) bending moment at breaking, (b) section modulus and (c) bending stress of K-CSSLs on chromosome 5 in 2016. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively

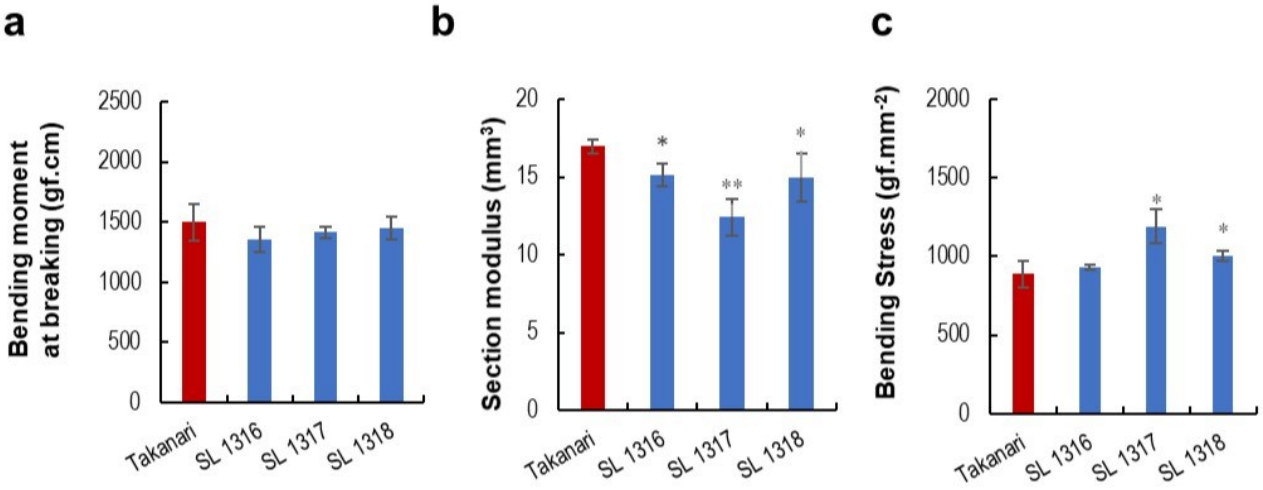


Fig. 4.3. Physical parameters breaking-type lodging resistance (a) bending moment at breaking, (b) section modulus and (c) bending stress of T-CSSLs on chromosome 5 in 2016. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively

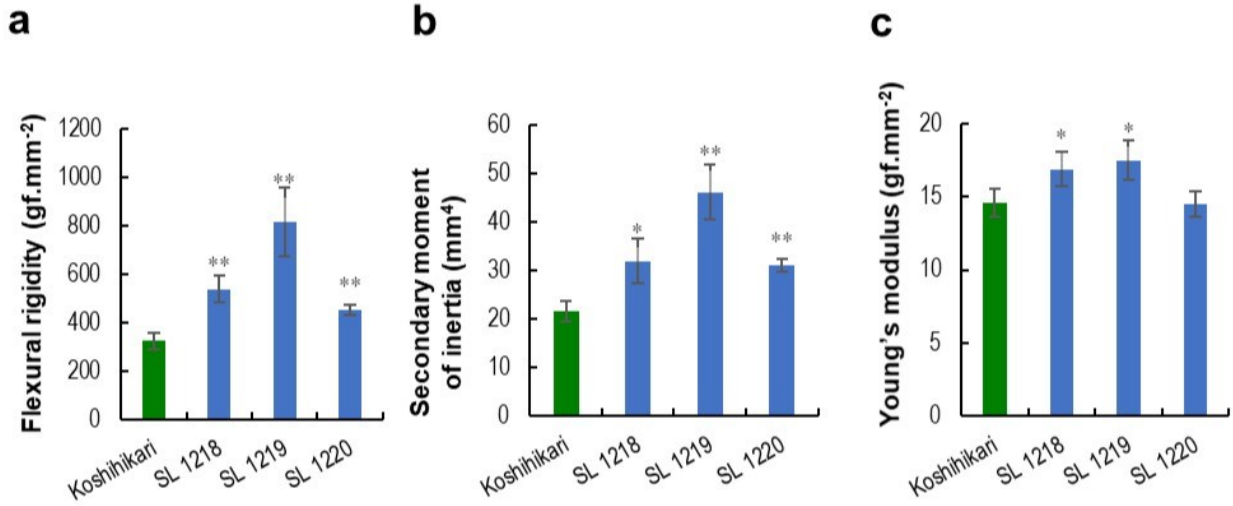


Fig. 4.4. Physical parameters bending-type lodging resistance (a) flexural rigidity (b) secondary moment of inertia and (c) Young's modulus of K-CSSLs on chromosome 5 in 2016. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively

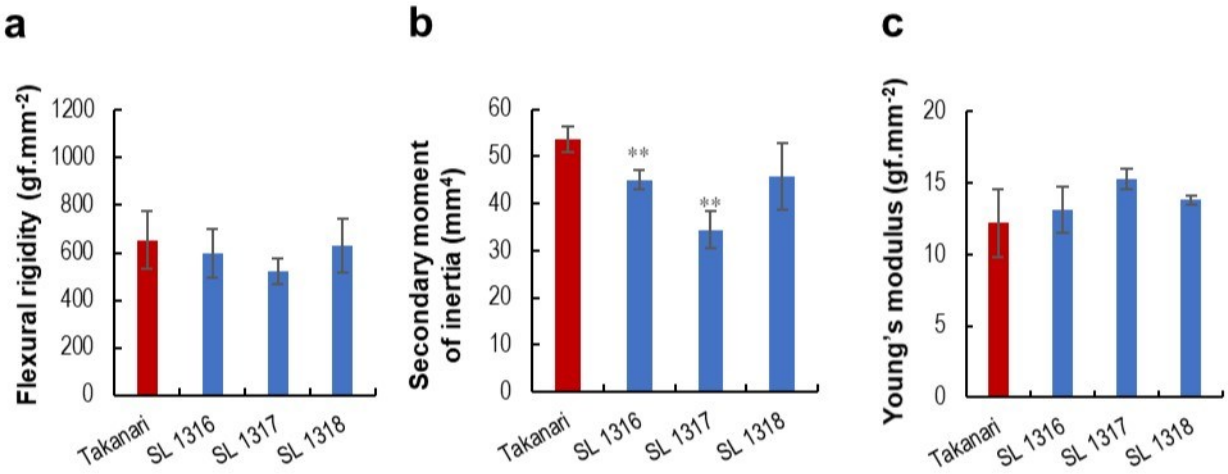


Fig. 4.5. Physical parameters bending-type lodging resistance (a) flexural rigidity (b) secondary moment of inertia and (c) Young's modulus of T-CSSLs on chromosome 5 in 2016. Data are expressed as the mean ± SD. *, **, and *** represent significant differences at 5%, 1%, and 0.1% level, respectively

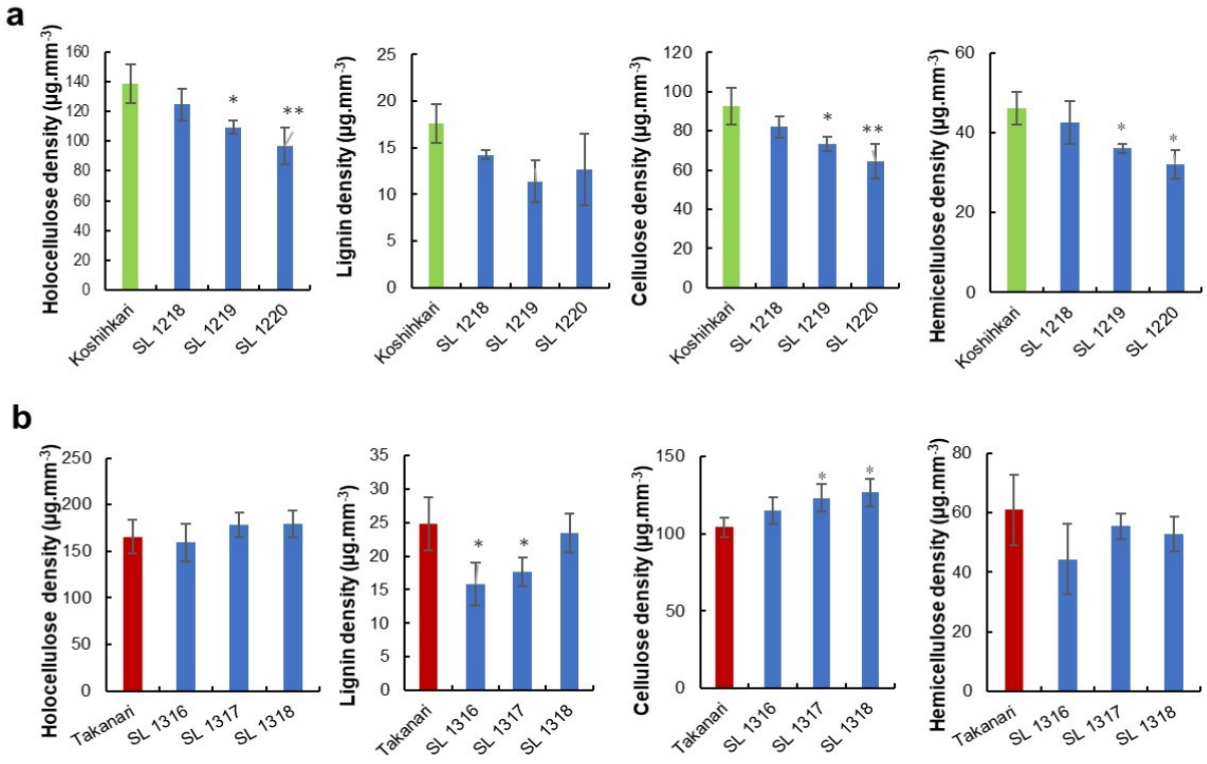


Fig. 4.6. Cell wall material densities in (a) T-CSSLs and (b) K-CSSLs of chromosome 5 in 2016. Data are expressed as the mean ± SD. *, and ** represent significant differences at 5%, and 1%, respectively

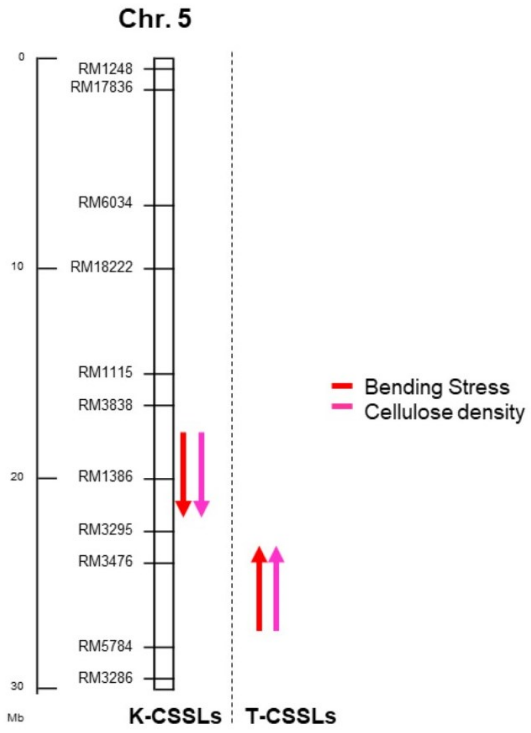


Fig. 4.7. Substitution mapping of QTLs by comparing overlapping segments among chromosome segment substitution lines (CSSLs) of K-CSSLs and T-CSSLs for: BS and Cellulose density on chromosome 5 in 2016.

4.3.2 Expression analysis of candidate genes for QTLs related to lodging resistance on chromosome 5

In Chapter 3, it was reported that some QTLs for holocellulose and cellulose were detected together with QTL for BS at the long arm region on chromosome 5 represented by SL1318. Moreover, those QTL were confirmed in both reciprocal CSSLs. RNA-seq was conducted to determined candidate gene(s) related to culm stiffness. In this chapter we perform RNA-seq analyses in Kosihikari, Takanari and SL 1318 to investigate gene expression responsible for culm stiffness.

Differential expressed genes (DEGs) analysis was conducted between parents, and between each parent with SL1318. The MA plot of each pair showed total expressed genes on the meristem at 40 days before heading (DBH). Total gene expression between Takanari and Kosihikari showed differences, and most of DEGs were more up-regulated transcripts (Fig 4.8a). Whereas, MA plot comparison between Koshihikari and SL 1318 showed the tendency of down-regulated (Fig 4.8c). MA plot showing the gene expression between Takanari and SL 1318 was more similar (Fig. 4.8b). This explained, because SL 1318 is CSSL with Takanari background.

The previous results showed that the QTLs of Cellulose and culm stiffness detected at the long arm region on chromosome 5, furthermore the study focused on the designated region. There were detected 67 genes intersects between Takanari and Koshihikari (Fig. 4.9a). Moreover 38 putative genes were detected intersect between Takanari and SL 1318 (Fig. 4.9b). In addition, detected intersect of putative genes between Koshihikari and Takanari were 943 genes (Fig. 4.9c). The description of the putative genes between two lines presented on Table 4.4 and 4.5. The comparison between Takanari and Koshihikari showed total 67 DEGs were detected. Among them 42 up-regulated and 25 down-regulated genes (Fig 4.10a). The comparison between Takanari and SL 1318 detected 38 DEGs, consists of 26 up-regulated and 12 down-regulated genes (Fig. 4.10b).

We identified a region on chromosome 5, as the putative location harboring the causal gene. Putative gene related to culm stiffness on the designated region obtained from RAP-DB. From the locus description, one putative gene which is Os05g0584600 encoding ATPase, AAA-type, core domain containing protein, and selected for real time RT-PCR analysis. The result showed there are differences in the expression level of Os05g0584600 whit in sampling time (DBH) (Fig. 4.11). At 33 DBH, Os05g0584600 showed different level of relative expression. Its expressed the highest on Koshihikari, while in Takanari and SL1318 relatively lower. Whereas on 26 and 20 DBH did not showed significant different level of gene expression between lines. These results showed 33 DBH is the most appropriate time to confirm the expression of Os05g0584600 between Takanari and Koshihikari. Despite of statically no significant difference between lines at 26 and 20 DBH, however the gene expression level exhibited similar expression trend. The expression level of OS05g0584600 higher on Koshihikari followed by SL 1318 and Takanari respectively. Similarly, phenotyping of BS showed the same trend (Fig 2.5c, 4.2c and 4.3c).

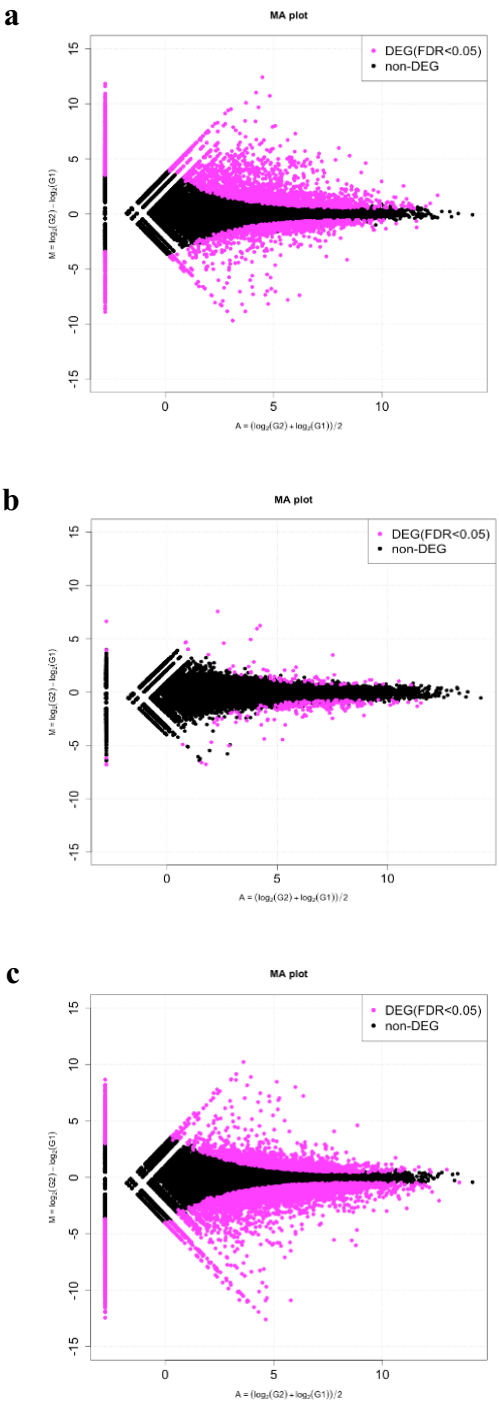


Fig. 4.8. MA plot showing differential expressed genes (magenta) in shoot apex meristem at 40 days before heading between (a) Takanari and Koshihikari, (b) Takanari and SL1318 and (c) Koshihikari and SL1318

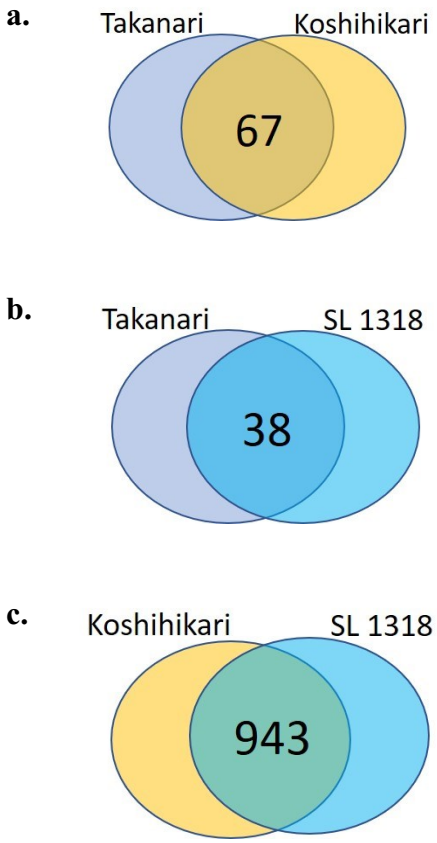


Fig 4.9. Venn diagram of intersection putative genes between (a) Takanari and Koshihikari, (b) Takanari and SL1318 and (c) Koshihikari and SL1318 on designated region of long arm region cromosome 5.

Table 4.4. List of putative genes of estimated QTLs on long arm region of chromosome 5 intersection between Takanari and Koshihikari.

No	Gene ID	Description
1	Os05t0484800-01	Protein of unknown function DUF567 family protein.
2	Os05t0486700-01	Ribosomal protein L24e domain containing protein.
3	Os05t0487300-02	Conserved hypothetical protein.
4	Os05t0488100-01	Conserved hypothetical protein.
5	Os05t0490500-01	Conserved hypothetical protein.
6	Os05t0490900-01	Conserved hypothetical protein.
7	Os05t0492500-01	Hypothetical protein.
8	Os05t0495300-01	Conserved hypothetical protein.
9	Os05t0501001-01	Hypothetical gene.
10	Os05t0503500-00	Non-protein coding transcript.
11	Os05t0507000-01	Similar to JHL23J11.5 protein.
12	Os05t0507400-01	Conserved hypothetical protein.
13	Os05t0508400-01	Mannose-binding lectin domain containing protein.
14	Os05t0511500-01	Similar to Lipoic acid synthase-like protein.
15	Os05t0513400-02	Protein of unknown function DUF803 family protein.
16	Os05t0521700-01	Conserved hypothetical protein.
17	Os05t0522600-01	Leucine-rich repeat, plant specific containing protein.
18	Os05t0526400-01	Reticulon family protein.
19	Os05t0531100-01	Protein of unknown function DUF584 family protein.
20	Os05t0537300-01	Similar to Unconventional myosin heavy chain.
21	Os05t0541750-01	Hypothetical gene.
22	Os05t0542900-01	Pectin lyase fold domain containing protein.
23	Os05t0546500-00	Similar to Cell death-related protein
24	Os05t0546800-01	Protein of unknown function DUF3615 domain containing protein
25	Os05t0547100-01	Conserved hypothetical protein.
26	Os05t0547600-01	Hypothetical protein.
27	Os05t0547800-01	Conserved hypothetical protein.
28	Os05t0547850-01	DNA-binding TFAR19-related protein family protein.
29	Os05t0548100-02	Similar to VIP1 protein.
30	Os05t0548900-01	Similar to Phosphoethanolamine methyltransferase.
31	Os05t0550300-01	Similar to Lipid transfer protein (Fragment).
32	Os05t0551350-00	Hypothetical conserved gene.
33	OS05T0551600-01	Hypothetical conserved gene.
34	Os05t0553400-01	Similar to Myb-related transcription factor-like protein (MYB transcription factor)
35	Os05t0553800-01	Similar to Anti-silencing protein-like (Anti-silencing function 1b) (Anti-silencing factor 1-like protein).
36	Os05t0554601-00	Conserved hypothetical protein
37	Os05t0555000-01	Conserved hypothetical protein
38	Os05t0556400-01	DOMON related domain containing protein
39	Os05t0557700-01	Ubiquitin-conjugating E2 enzyme, Phosphate homeostasis, Negative regulator of the inorganic phosphate (Pi)-starvation response-signaling pathway
40	Os05t0560000-01	Photosystem I reaction center subunit VI, chloroplast precursor (PSI- H) (Light-harvesting complex I 11 kDa protein) (GOS5 protein)
41	Os05t0560500-01	Conserved hypothetical protein
42	Os05t0560600-01	Homeodomain-like containing protein
43	Os05t0562000-01	Homeodomain-like containing protein.
44	Os05t0562300-01	Similar to DnaJ-like protein
45	Os05t0563950-01	Conserved hypothetical protein
46	Os05t0567600-00	Similar to SANT/MYB protein.
47	Os05t0568000-02	Similar to FAE1

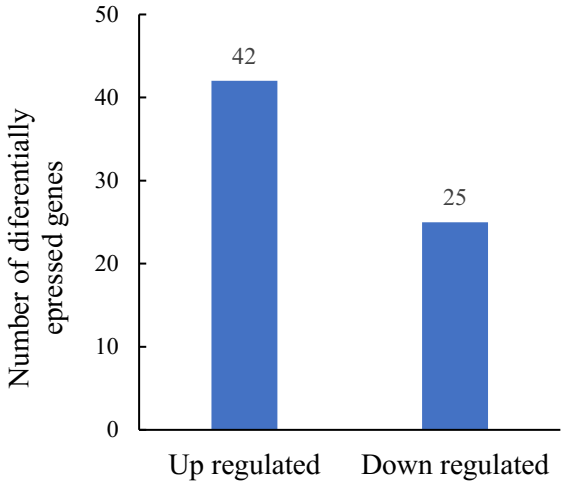
Table 4.4 Continued.

No	Locus id	Description
48	Os05t0568100-01	Similar to Iron sulfur cluster assembly protein 1, mitochondrial precursor (Iron sulfur cluster scaffold protein 1).
49	Os05t0569200-01	Protein of unknown function DUF3143 domain containing protein.
50	Os05t0571300-01	Conserved hypothetical protein.
51	Os05t0571800-01	Similar to H0402C08.3 protein.
52	Os05t0573200-00	Similar to Isocitrate dehydrogenase (Fragment)
53	Os05t0574400-01	Similar to Malate dehydrogenase
54	Os05t0576500-02	Hypothetical conserved gene.
55	Os05t0579600-01	Homeodomain-like containing protein.
56	Os05t0580200-01	Conserved hypothetical protein.
57	Os05t0581100-00	Hypothetical conserved gene
58	Os05t0581900-01	X8 domain containing protein
59	Os05t0583000-01	Similar to WRKY8
60	Os05t0584600-02	ATPase, AAA-type, core domain containing protein
61	Os05t0586600-01	Similar to SIGE (RNA polymerase sigma subunit E); DNA binding / DNA-directed RNA polymerase/ sigma factor/ transcription factor
62	Os05t0587600-01	Hypothetical conserved gene.
63	Os05t0588200-01	RuvA domain 2-like containing protein
64	Os05t0588800-02	Similar to Yarrowia lipolytica chromosome D of strain CLIB99 of Yarrowia lipolytica.
65	Os05t0589700-01	Similar to cDNA, clone: J090072P03, full insert sequence.
66	Os05t0591550-00	Conserved hypothetical protein
67	Os05t0593000-01	Phospholipase A2, active site domain containing protein

Table 4.5 List of putative genes of estimated QTLs on long arm region of chromosome 5 intersection between Takanari and SL1318.

No	Locus id	Description
1	Os05t0481500-01	K Homology domain containing protein
2	Os05t0485500-01	Similar to GAUT7 (GALACTURONOSYLTRANSFERASE 7); polygalacturonate 4-alpha-galacturonosyltransferase/ transferase, transferring glycosyl groups.
3	Os05t0486700-01	Ribosomal protein L24e domain containing protein
4	Os05t0490500-01	Conserved hypothetical protein
5	Os05t0492100-01	Hypothetical protein
6	Os05t0492500-01	Hypothetical protein
7	Os05t0500900-01	Similar to Indole-3-acetic acid-amido synthetase GH3.5 (EC 6.3.2.-) (Auxin- responsive GH3-like protein 5) (AtGH3-5).
8	Os05t0506000-01	Similar to MS5-like protein (Fragment)
9	Os05t0507400-01	Conserved hypothetical protein
10	Os05t0525900-01	Similar to Zing finger transcription factor PEI1.
11	Os05t0534500-01	Phospholipase C, phosphatidylinositol-specific , X region domain containing protein
12	Os05t0546500-00	Similar to Cell death-related protein
13	Os05t0546800-01	Protein of unknown function DUF3615 domain containing protein
14	Os05t0547100-01	Conserved hypothetical protein
15	Os05t0547600-01	Hypothetical protein
16	Os05t0547700-02	Conserved hypothetical protein
17	Os05t0547800-01	Conserved hypothetical protein.
18	Os05t0547850-01	DNA-binding TFAR19-related protein family protein
19	Os05t0548100-02	Similar to VIP1 protein.
20	Os05t0550300-01	Similar to Lipid transfer protein (Fragment)
21	Os05t0551600-01	Hypothetical conserved gene.
17	Os05t0553800-01	Similar to Anti-silencing protein-like (Anti-silencing function 1b) (Anti-silencing factor 1-like protein)
18	Os05t0554601-00	Conserved hypothetical protein.
19	Os05t0560500-01	Conserved hypothetical protein.
20	Os05t0563600-01	FAS1 domain domain containing protein
21	Os05t0566600-01	Similar to Negatively light-regulated protein
21	Os05t0567500-02	HhH-GPD domain domain containing protein
22	Os05t0568000-02	Similar to FAE1.
23	Os05t0569800-01	Tho complex subunit 7 domain containing protein
24	Os05t0575300-02	Similar to Translation initiation factor IF-2, chloroplast precursor (PvIF2cp)
25	Os05t0576500-02	Hypothetical conserved gene
26	Os05t0580200-01	Conserved hypothetical protein
27	Os05t0580500-01	Similar to 10A19I.14
28	Os05t0584600-02	ATPase, AAA-type, core domain containing protein
29	Os05t 0588200-01	RuvA domain 2-like containing protein
30	Os05t0589700-01	Similar to cDNA, clone: J090072P03, full insert sequence
31	Os05t0591550-00	Conserved hypothetical protein
32	Os05t0593900-01	Similar to C13 endopeptidase NP1 precursor
33	Os05t0481500-01	K Homology domain containing protein
34	Os05t0485500-01	Similar to GAUT7 (GALACTURONOSYLTRANSFERASE 7); polygalacturonate 4-alpha-galacturonosyltransferase/ transferase, transferring glycosyl groups.
35	Os05t0486700-01	Ribosomal protein L24e domain containing protein
36	Os05t0490500-01	Conserved hypothetical protein
37	Os05t0492100-01	Hypothetical protein
38	Os05t 0492500-01	Hypothetical protein

a.



b

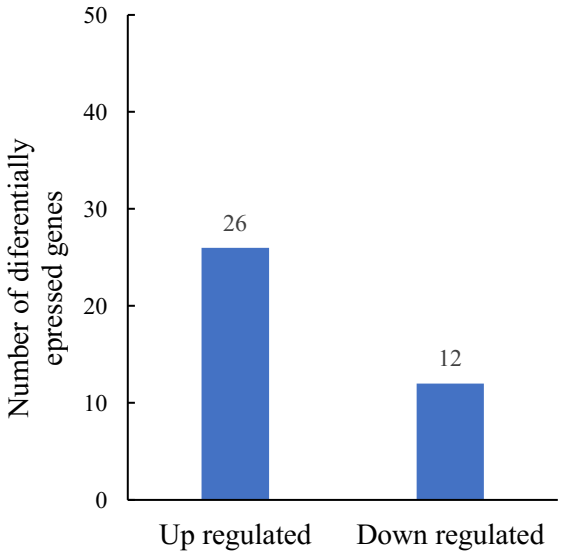


Fig 4.10. The numbers of up- and down- regulated DEGs detected on long arm region of chromosome 5 (estimated region between Os05t0486200 – Os05t0592800) (a) between Takanari and Koshihikari (b) between Takanari and SL 1318

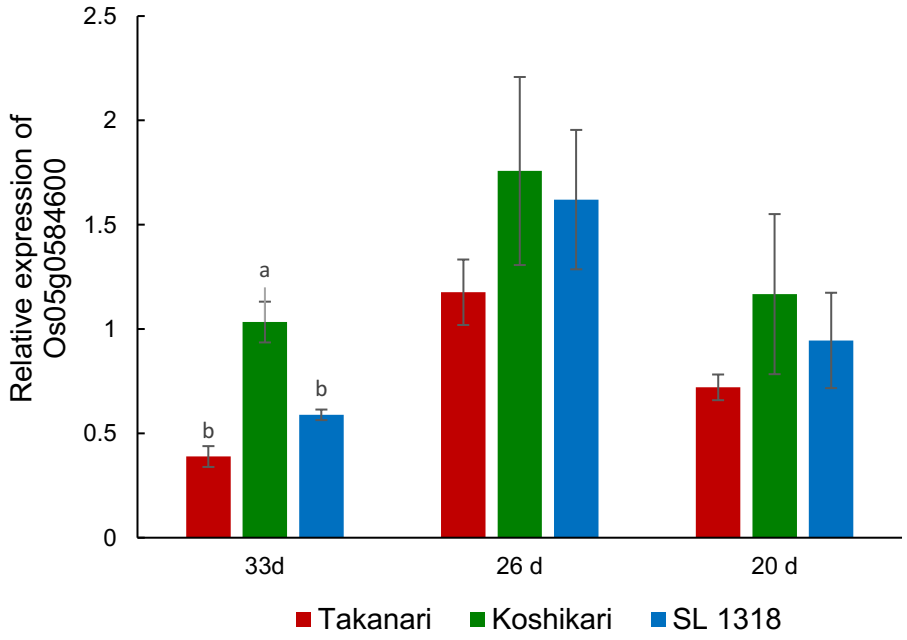


Fig. 4.11. Expression profiles by real time RT-PCR of Os05g054600 at 33, 26 and 20 days before heading (DBH). Results are presented as mean values of three technical replicates. Different letters above bars represent significant difference (P<0,01) among samples (Tukey-Kramer).

4.4 Discussion

As elaborate in previous chapter, QTLs for holocellulose, cellulose and hemicellulose were detected on chromosome 5 together with BS. Furthermore, QTL for lignin also detected at the same region with YM and BS on chromosome 11 (Fig. 3.5a, 3.5b and 3.6), but unfortunately, the CSSLs on chromosome 11 had the characteristic of late maturity and short stature compared with those in other lines. It indicated that this phenotype affected the accumulation of cell wall materials, indirectly. These phenotypes might cause by *hdb3* allele from Koshihikari located at long arm region on chromosome 11. This gene has a function of hybrid breakdown (Yamamoto et al., 2007). Hybrid breakdown (sterility or weakness in later generation) are commonly seen in crosses between *indica* and *japonica* (Matsubara et al., 2007; Oka, 1988; Yamamoto et al., 2007). In rice, hybrid breakdown causes reduced tiller numbers, retarded growth with short culm and panicle, chlorosis of leaves, poor seed set and retarded root growth (Jiang et al., 2008b; Sunohara et al., 2009). This result explains the reason why this study only focused on the QTLs for cell wall material on chromosome 5.

The introgression lines are useful genetic materials for the application of functional genomics tools to discover novel genes by focusing on differences in gene expression or protein expression to small chromosomal regions. We identified QTLs that increased SMI and YM in chromosome 5 of Koshihikari genetic background. SL 1219, which carried these QTLs and increased in FR up to 115% than Koshihikari (Fig. 4.2). These results indicated that combined QTL for both SMI and YM on chromosome 5 contributed to higher increase in bending type lodging showing by higher FR.

Reciprocal CSSLs confer the advantages of enabling evaluation of differences in allelic effect of QTLs in both genetic backgrounds. If detected QTLs in both genetic backgrounds show reciprocal effects, these loci should have no genetic interaction or epistasis with other background factors (Kubo et al., 2002). The SM-associated QTL on chromosome 5 showed a reciprocal effect at the same region in reciprocal CSSLs. SM in CSSLs was increased by placement of the Takanari allele in the

Koshihikari genetic background (Fig. 4.2b) and in the reciprocal lines. The Koshihikari allele reduced SM, when placed in the Takanari genetic background (Fig.4.3b). These results indicated that the same allele on chromosome 5 was responsible for regulating SM. A previous study using the same CSSLs showed that SM was increased by the Takanari allele at this region and decreased by the Koshihikari allele in the reciprocal lines (Ookawa et al., 2016). A similar reciprocal effect was observed by Takai et al. (2014) for yield component traits.

The same reciprocal effect also showed in SMI in which substitution of Takanari and Koshihikari allele increasing and decreasing SMI, respectively (Fig 4.4b and Fig.4.5b). Both parameters SM and SMI related to culm diameters, could be implied the gene controlled lodging resistance related to culm diameters. Study by Hirano et al (2014) showed that LRC1 and *smos1,* both had increased culm diameter and culm thickness, which result in a high SM value, and this explains the mechanism for their improved lodging resistance. While in FR, although in T-CSSLS did not showed significant difference, the reciprocal CSSLs showed the same tendency of the QTLs location.

Mapping of QTL also revealed the trade-off among parameters of lodging resistance component traits. In the allele of QTL with increased SM was associated decrease of BS on Koshihikari genetic background. The trade-off with an opposite effect showed on Takanari genetic background, decreasing SM with increasing BS. The same trade-off between SM and BS showed in previous study between *indica* variety Habataki and Koshihikari (Ookawa et al., 2010a). Hirano et al. (2014) suggested that might be a trade-off between SM and BS and that pronounced increase in culm thickness and diameter negatively affected culm quality that inevitably decrease BS. Thus, when developing new QTLs pyramiding line, it become important to carefully select the combination of alleles which most effectively enhance lodging resistance without inducing disadvantageous effect.

BS is highly affected by the component of the secondary cell wall such as cellulose, lignin and hemicellulose (Vogel, 2008), and high densities of cell wall materials are responsible for high

BS in Koshihikari (Ookawa et al., 2010b). Other study in superior lodging resistance variety reported that stiff culm in Leaf star caused by the higher cellulose and hemicellulose densities and thick cortical fiber tissue, irrespective of lignin densities of culm (Ookawa et al., 2014). Li et al. (2015) identified cellulose crystallinity as the major factor that negatively corelated to breaking-type lodging resistance. The QTLs for cell wall materials detected on chromosome 5 was confirmed on reciprocal CSSLs which QTLs for cellulose density and detected at the same region with BS (Fig. 4.6). This result indicated that the Koshihikari allele on chromosome 5 had a positive effect on cellulose density, even though the Koshihikari accumulated low level of cellulose compared with that in Takanari. A similar result was observed by Ookawa et al. (2016). It showed Koshihikari, a long-culm *japonica* variety, harbored the superior allele of the chromosome 1 *SD1* gene. Although the Koshihikari line had a fine culm, this line harbored a thick-culm *SD1* allele. These suggest that the *japonica*-variety Koshihikari has a hidden superior allele on chromosome 5 that contributes to the improved culm stiffness and lodging resistance of *indica* varieties such as Takanari. Although the genes associated with BS have not been determined yet, the results mentioned above suggest that the improvement of lodging resistance in *indica* variety could be conducted by incorporating superior allele from *japonica* type through the enhancement of cellulose density in culm.

In the present study, by using CSSLs we detected QTLs for strong culm traits related to breaking- and bending-type lodging resistance. We found a new QTL for cellulose density mapped to the long arm region on chromosome 5. This result suggested that it might be possible to improve the BS of the Takanari *indica* rice variety by introducing the allele for increased cellulose density from the *japonica*-variety Koshihikari. QTLs for BS and cellulose density were estimated at the same regions in K-CSSLs and T-CSSLs, respectively. However, the estimated region in K-CSSLs did not overlap with that in T-CSSLs (Fig. 4.7). Estimating the different regions of QTLs in the reciprocal

genetic backgrounds might be explained by the existence of multiple genes with the same function in the segment as a QTL cluster or epistasis with genetic background (Matsubara et al., 2016).

Despite for the advantages mention above, however there are some limitation on using CSSLs. In CSSLs the QTLs are initially assigned to an entire chromosome, rather than to a narrow region as in a cross population such as F_2 and RILs. In addition, CSSLs do not distinguish among multiple QTLs on the substituted chromosome (Nadeau et al., 2000). The mapping resolution of QTLs in CSSLs may be lower than primary mapping population, because it depends on the substituted chromosome segments in CSSLs. This disadvantage can be overcome by fine mapping of putative QTLs using CSSLs as based material. Developing NILs for target QTLs is required to map QTLs precisely (Ookawa et al., 2016). Further study will be needed to narrow down the region and identify the responsible genes. Such results will be expected to increase our understanding of the genetic factors controlling the development of rice varieties with improved bending- and breaking-type lodging resistance.

The use of strong culm genes in addition to semi dwarf gene is a promising new approach in improving lodging resistance. Characterization of *Brittle Culm3* (*BC3*), a gene encoding OsDRP2B, showed that *bc3* mutation reduces mechanical strength, which results from decreased cellulose content and altered secondary wall structure (Xiong et al., 2010). Wang et al. (2016) reported the utilization of RNA-seq successfully elucidated the mechanism of lodging resistance through the mechanism of *Reduce height 1* (*Rht1*) dwarfing gene in wheat and reduced the contents of lignin and cellulose. Previously, Wang et al. (2010) has mapped *OSCESA/CSL* cellulose synthase and cellulose synthase-like gene superfamily based on chromosome location in rice, and *OsCSL C7* gene was expressed on long arm region of chromosome 5.

RNA-seq was conducted to investigate the transcriptional level at the long arm region on chromosome 5 and lodging resistance in rice. Some DEGs were identified between designated region

which QTLs of cellulose and BS identified. One DEG, encoding Os05t0584600-02, considered to have important functions in physiological process (Table 4.4 and 4.5). Its encode ATPase, AAA-type, core domain containing protein. Adenosine triphosphates (ATPases) belonging to the AAA protein family (ATPases associated with several cellular activities) are involved in a wide range of activities such as proteolysis, protein folding, membrane trafficking, cytoskeleton regulation, organelle biogenesis, transcription control, and microtubule regulation (Santos, 2006). ATPases, play an important role in converting chemically stored energy into a biological activity. Candidate genomic region that encode ATPase (AAA type) induce *lrm* transcript that showed enhanced resistance to rice blast (Fekih et al., 2015). ATPase also participated on adaptation to salt stress of ice plant (Jou et al., 2006). Luptovčiak et al. (2017) reported mutant lack of KATANIN effected AAA ATPase domain protein. One of the KATANIN, mutant *fragile fiber 2 (fra2)* displayed fragility of all organs and particularly of stems. This fragility is accompanied by reduced cellulose deposition, resulting in shorter and thinner fibers and increased fragility due to the lower mechanical resistance (Burk et al., 2001).

In this study the expression level of Os05g0584600 of each sampling day showed the same trend between Takanari, Koshihikari and SL 1318 (Fig. 4.11) which also showed on phenotypic features of BS. However, it still has been unknown the precise mechanism ATPase (AAA type) involve in the mechanism underlaying culm stiffness, comprehensive explanation and research to provide biological system will be required to dissecting various aspect that may related to culm stiffness.

The effects of natural genetic variation on gene expression are a major determinant of phenotypic variability. SNP) is the simplest form of DNA variation among individual lines. These simple changes can be of transition or transversion type. SNPs may change the encoded amino acids (nonsynonymous) or can be silent (synonymous) or simply occur in the noncoding regions (Komar,

2007; Manning and Cooper, 2017). Therefore, the identification of numerous variations in SNPs may lead to a better understanding of their impact on protein function. Previous research in rice detected total of 1,226,791 SNPs between *indica* and *japonica*, Niponbare , that was average 3.2 SNPs/kb (Huang et al., 2009).

Here, SNPs between Koshihikari and Takanari on the exon regions responsible for the substitution in amino acid were detected. In the comparison of allele sequence for the putative gene Os05g0584600 in Koshihikari and Takanari , three SNPs were present in the exon 7, 12 and 15 (Fig 4.12, Table 4.6). The SNP on 29,096,829 bp in exon 7 caused the amino acid substitution mutation from isoleucine in Koshihikari to serine in Takanari, on 29,095,035bp in exon 12 from threonine to alanine, and on 29,094,152 in exon 15 from threonine to lysine.

SNPs may influence promoter activity for gene expression, messenger RNA (mRNA) conformation (stability), and subcellular localization of mRNAs and/or proteins (LeVan et al., 2001). Changes of protein might influence the physiological activity that leads to change in the phenotypic level. A gene contains two parts, exon and intron. Intron is removed during post transcriptional modification, but the exons are finally translated into amino acid sequence and produce enzyme. Therefore, the SNP in the exon part (coding region) is most important because they can affect the gene function. Kharabian-Masouleh et al. (2012) reported 66 functional SNPs were discovered in exon regions from 18 genes involved in starch synthesis in rice. SNPs in the coding region differentiated into synonymous and asynonymous type. Synonymous SNPs do not affect the amino acid sequence, on the other hand asynonymous SNPs change the amino acid sequence of protein and may influence the enzyme activity (Huq et al., 2016).

Although genes that responsible for culm stiffness have not been determined yet, the above-mentioned factors might help to provide a useful insight on how to carry out BS improvement. Thus, the lodging resistance of *indica* cultivar could be improved by introduction of genes from *japonica*

variety Koshihikari covering high BS through the increase of cellulose density in culm. Further research will be needed to identify the responsible genes and their physiological functions for candidate genes.

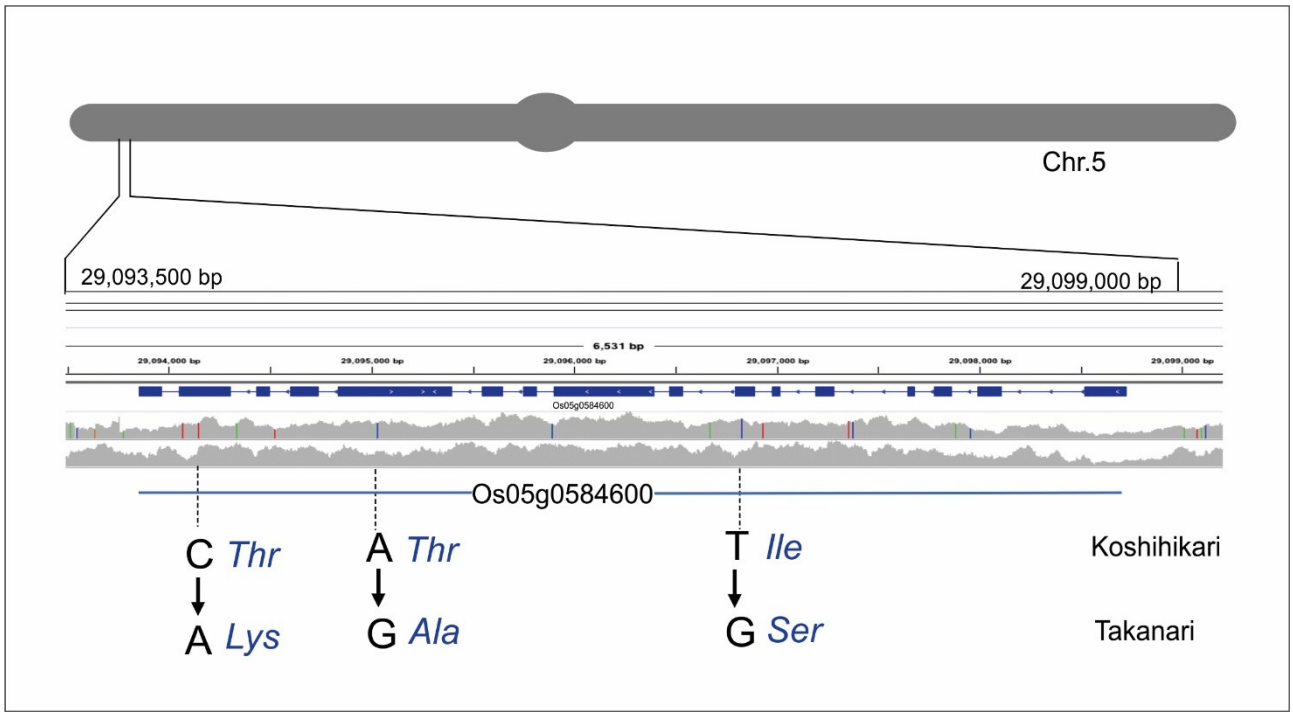


Fig. 4.12. Genetic map of Os05g0584600 at long arm region on chromosome 5, structure and amino acid mutations of AAA ATPase Os05g0584600 between Koshihikari and Takanari based on SNPs on exon region

Table 4.6. SNPs on the exon of candidate region

SNP coordinate on Chr 5	Koshihikari	Takanari	Amino Acid	
	Reference based	Altered base	Koshihikari	Takanari
29,094,152	C	A	T (Threonine)	K (Lysine)
29,095,035	A	G	T (Threonine)	A (Alanine)
29,096,829	T	G	I (Isoleucine)	S (Serine)

General discussion

1. Finding the superior allele of *japonica* variety to improve lodging resistance in Takanari

Lodging resistance is an important trait in to achieve high yield in rice production. It is an important determinant for lodging resistance to search the natural variations, and this led breeders to investigate the genetic markers for strong culm. To achieve this goal, the loci that influence this trait need to be identified to provide deeper understanding of their molecular mechanism. Since the advance of molecular marker technology, some QTLs for lodging resistance have been mapped in rice. The component of lodging resistance consists of various responsible traits, which mainly differ two major types in morphological (stem and root traits) and biochemical (Jones et al., 2001; Kong et al., 2013; Yadav et al., 2017).

In Japan, high yielding rice has been developed using *indica* and *japonica* varieties. The *indica* variety, like Takanari normally have short stature and thick culm diameter indicated strong culm, but easy to break, showed by low BS. Hence, the lodging resistance of these varieties has been improved by increasing strong culm traits such as culm diameters and short-stature (Kashiwagi et al., 2007). However, recent study revealed that dwarfism, also has some inadequate characteristic when related to crop productivity and plant biomass (Okuno et al., 2014). Koshihikari, dominant cultivated variety in japan, has thick culm wall and higher culm flexibility. In contrast Takanari, high yielding *indica* variety showed strong culm trait due to higher culm diameter. Thus, Koshihikari superior allele were able to contribute in lodging resistance enhancement in *indica* variety, Takanari.

In rice, culm morphological features contribute to culm strength, and some biochemical characteristics (such as the levels of cellulose, hemicellulose, holocellulose, and lignin) are also important for lodging resistance. Kokubo et al. (1991) reported that cellulose content correlated with BS in barley, and that the *brittle culm* (*bc*) mutant showed low cellulose content due to decreased cellulose biosynthesis in the cell wall. The accumulation of cellulose, hemicellulose, and lignin

improves cell wall thickness and flexibility (Kong et al., 2013). Ookawa et al. (1993) reported that the Koshihikari line and most *japonica* varieties have high BS, a phenotype caused by elevated levels of cellulose and lignin in the culm.

CSSLs attained to detect QTLs distributed throughout the genome with high sensitivity (Abe et al., 2013). It was demonstrated that CSSLs between *japonica* and *indica* were an effective tool for introgressing valuable genes from each varietal group (Ando et al., 2008; Ebitani et al., 2005; Takai et al., 2007). Major QTLs for grain size were detected together with many minor QTLs for heading date in CSSL between Asominori (*japonica*) and IR42 (*indica*) (Kubo et al., 2002).

Precise estimation of QTLs controlling lodging resistance has been reported by Ookawa et al. (2016). Major QTLs associated with strong culm traits such as culm thickness and culm stiffness were detected in reciprocal CSSLs and controlled independently by a single factor. In this study also demonstrated that candidate region of QTLs in introgression chromosome segment from *japonica* variety, Koshihikari in Takanari background enable to increased culm stiffness. It was confirmed that detected QTLs for cellulose density contributed to elevated BS in Takanari.

2. Improvement of lodging resistance in rice

Molecular genetic approaches and QTL mapping are established approaches in identifying useful genetic component. The identification of genes that are responsible for important agricultural traits has been mostly conducted by traditional molecular genetics (forward and reverse genetic screens) for discrete traits and by QTLs mapping for complex traits (Ashikari and Matsuoka, 2006; McCouch and Doerge, 1995). Increasing numbers of QTL analyses are providing enormous amounts of information about QTLs, such as the numbers of loci involved, their chromosomal locations and gene effects. Clarification of genetic bases of complex traits has a big impact not only on fundamental research on rice plant development, but it also has practical benefits for rice breeding

Identification of QTLs that control bending- and breaking- type of lodging resistance is a primary step in the efforts to enhance rice lodging resistance, by incorporating beneficial QTLs allele into superior genetic background. A good level of lodging resistance can be achieved through different combinations of lodging resistance component traits (Li et al., 2015; Ordonio et al., 2014; Yadav et al., 2017).

The detection of multiple QTLs for different traits might facilitate an improved strategy for developing lines with superior lodging resistance. Ookawa et al. (2010b) showed that the Leaf Star has superior lodging resistant characteristics, owing to culm thickness and culm stiffness traits. The combinations of multiple QTLs with different functions provide a better performance for lodging resistance. Notably, the pyramiding of the strong-culm genes in lines carrying *SCM2* and *SCM3* yielded much stronger culm performance than either QTL alone (Yano et al., 2015). For the trait of culm stiffness, Kashiwagi et al. (2016) reported that the combination of *BSUC11* and a QTL for non-structural carbohydrate (NSC) enabled increased lodging resistance, presumably by improving chemical component(s) in culm.

There has been good progress in gene identification by QTLs mapping for traits, some provide additional clue to improve specific traits such as lodging resistance. Studies that combine QTL mapping and association analysis might prove more successful than either strategy alone (Takeda and Matsuoka, 2008). In this study we use RNA-seq to identify putative gene in long arm region of chromosome 5. The designated region was in which the QTLs responsible for culm stiffness were detected. The DEG, encode ATPase, AAA-type, core domain containing protein that may play a part in cellulose biosynthesis.

3. Future research for high yielding and lodging resistance varieties

In molecular genetics, regulation and differential expression of gene play a key role in plant development and expression of complex trait. However, it remains a big challenge to link gene

expression with QTLs at the phenotypic level. Construction and detailed phenotyping of populations from some specific crosses, together with application large-scale variant discovery and genotyping, which now become more practical and economical (Huang et al., 2012; Li et al., 2014; Xu et al., 2012), therefore gene discovery related to lodging will be more comprehensive and efficient.

To transfer genetic information conferring advantageous traits to a cultivar of preference, both transgenic (genetic modification) and non-transgenic approaches can be used. The non-transgenic approach is based on hybridization of two varieties carrying advantageous QTLs or useful gene alleles, and subsequent marker-assisted selection of those genetic components. QTLs pyramiding can be used to selection of highly advantageous combination of QTLs (Ashikari and Matsuoka, 2006). Superior alleles from several or more different parental varieties can be introduced into an elite variety of interest (Takeda and Matsuoka, 2008). Yano et al. (2015) reported that pyramiding lines showed additive effect, significantly enhanced the thickness of the culm wall and culm physical strength. Other study by Ookawa et al. (2010a) demonstrated that QTL pyramiding of NIL-*SCM2*+*SCM3* were being able to attain superior SM having 20% higher grain yield over Koshihikari.

By contrast, the transgenic approached although does not require hybridization, it require identification of gene responsible for lodging resistance. In principle, combination of several beneficial genes can be transferred into the same line. However, this still have several limitations. In Rice some variety/lines are transformed only at low levels. Therefore, technical advance in gene manipulation will required for improvement of plant. Furthermore, the introduction of combination different beneficial genes into elite variety through transgenic approach also likely will provide an important direction to enhance lodging resistance with high yield in rice.

Abstract

Lodging has been an important constraint on rice production in monsoon Asia. When lodging occurs after a typhoon hits, the canopy structure is destroyed, and the capacities for photosynthesis and dry matter production are sharply reduced. In severe cases, lodging can result in breaking of the stem or pulling out the roots, blocking the transport of water, minerals, and photoassimilates and leading to declines in yield and quality. In cereal crops, stem lodging can be classified into two types: stem-breaking type and stem-bending type. To improve stem-lodging resistance, the strong culm traits of superior lodging-resistant varieties need to be characterized. The identification of quantitative trait loci (QTLs) and the corresponding genes associated with the parameters for bending moment at breaking (M) and flexural rigidity (FR) is expected to enable the efficient development of lodging-resistant varieties. A set of Chromosome Segment Substitution Lines (CSSLs) derived from the cross between Takanari and Koshihikari were used in this study to identify QTLs associated with lodging resistance.

The first part of this thesis was to identify the important traits of bending- and breaking- type lodging resistance between parents that contribute to increasing lodging resistance. Large differences were observed in the parameters of both breaking- and bending-type lodging resistance between Takanari and Koshihikari. The M of Takanari was larger than that of Koshihikari because of the larger section modulus (SM). The large culm diameter of Takanari was responsible for the large SM. In contrast, Takanari had a small bending stress (BS) compared with that of Koshihikari. The FR was larger in Takanari than that in Koshihikari. The large FR in Takanari resulted from a large secondary moment of inertia (SMI). Young's modulus (YM) in Takanari was significantly higher than that in Koshihikari in 2015 but not in the following year. The density of the stem wall material such lignin, holocellulose, hemicellulose and cellulose contributed to culm strength. Takanari exhibited

significantly high densities of holocellulose, lignin, cellulose and hemicellulose densities compared with Koshihikari.

The second part of this thesis was to estimate the QTLs related to breaking- and bending-type lodging resistance on CSSLs in Takanari genetic background. The QTLs for BS were assigned to chromosomes 3, 5, 6, 8, 9, 10, 11, and 12. Koshihikari alleles increased BS in these QTLs. The YM was increased by substitution of the Koshihikari chromosomal segments on chromosomes 2, 10, and 11. Other QTLs mapped to chromosomes 7 and 12, such that the Koshihikari alleles contributed to the decrease of YM. QTLs for cellulose density were assigned to chromosomes 1, 3, and 5, which were replaced by substitutions of Koshihikari segments. Some QTLs for cell wall material densities were detected together with BS on chromosome 5 Moreover, on chromosome 11, QTLs for the densities of holocellulose, hemicellulose, and lignin were detected at the same region with YM. Both regions indicated positive effect of Koshihikari segment on increasing BS and YM. These results suggested that some QTLs for the densities of cell wall materials contribute to increase BS and YM and could be utilized to improve the lodging resistance for both types of breaking and bending in rice varieties.

In the last part of this thesis, the putative QTL region at the long arm region on chromosome 5 was verified using reciprocal CSSLs, furthermore, RNA-seq and real time RT-PCR was conducted to find the candidate genes related to lodging resistance on chromosome 5. QTLs for BS and cellulose density were estimated at the same regions in K-CSSLs and T-CSSLs, respectively. The Koshihikari allele on chromosome 5 had a positive effect on cellulose density, although the Koshihikari accumulated cellulose to a level lower than that in Takanari. These results suggested that the *japonica*-variety Koshihikari has a hidden superior allele on chromosome 5 that contributes to the improved culm stiffness and lodging resistance of *indica* varieties such as Takanari. However, the estimated region in K-CSSLs did not overlap with that in T-CSSLs. Estimating the different regions

of QTLs in the reciprocal genetic backgrounds might be explained by the existence of multiple genes with the same function in the segment as a QTL cluster or epistasis with genetic background. The use of strong culm genes in addition to semi dwarf gene is a promising new approach in improving lodging resistance. Some candidate genes associated with cellulose deposition were found by RNAseq.

This study suggested that the lodging resistance of *indica* cultivar could be improved by introduction of genes from *japonica* variety Koshihikari covering high BS through the increase of cellulose density in culm.

Acknowledgement

In the name of Allah, the Most Gracious and the Most Merciful, Alhamdulillah, all praises to Allah for the strengths and bless in completing this thesis.

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Reference

Abe, T., Nonoue, Y., Ono, N., Omoteno, M., Kuramata, M., Fukuoka, S., Yamamoto, T., Yano, M., and Ishikawa, S. (2013). Detection of QTLs to reduce cadmium content in rice grains using LAC23/Koshihikari chromosome segment substitution lines. *Breeding science* **63**, 284-291.

Ali, M. L., Sanchez, P. L., Yu, S.-b., Lorieux, M., and Eizenga, G. C. (2010). Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from Oryza wild species into cultivated rice (O. sativa). *Rice* **3**, 218-234.

Ando, T., Yamamoto, T., Shimizu, T., Ma, X. F., Shomura, A., Takeuchi, Y., Lin, S. Y., and Yano, M. (2008). Genetic dissection and pyramiding of quantitative traits for panicle architecture by using chromosomal segment substitution lines in rice. *Theoretical and Applied Genetics* **116**, 881-890.

Asano, K., Hirano, K., Ueguchi-Tanaka, M., Angeles-Shim, R. B., Komura, T., Satoh, H., Kitano, H., Matsuoka, M., and Ashikari, M. (2009). Isolation and characterization of dominant dwarf mutants, Slr1-d, in rice. *Molecular Genetics and Genomics* **281**, 223-231.

Ashikari, M., and Matsuoka, M. (2006). Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends in plant science* **11**, 344-350.

Ashikari, M., Sakakibara, H., Lin, S., Yamamoto, T., Takashi, T., Nishimura, A., Angeles, E. R., Qian, Q., Kitano, H., and Matsuoka, M. (2005). Cytokinin oxidase regulates rice grain production. *Science* **309**, 741-745.

Baker, C., Berry, P., Spink, J., Sylvester-Bradley, R., Griffin, J., Scott, R., and Clare, R. (1998). A method for the assessment of the risk of wheat lodging. *Journal of theoretical biology* **194**, 587-603.

Berry, P., and Spink, J. (2012). Predicting yield losses caused by lodging in wheat. *Field Crops Research* **137**, 19-26.

Berry, P., Sterling, M., Baker, C., Spink, J., and Sparkes, D. (2003). A calibrated model of wheat lodging compared with field measurements. *Agricultural and Forest Meteorology* **119**, 167-180.

Berry, P., Sylvester-Bradley, R., and Berry, S. (2007). Ideotype design for lodging-resistant wheat. *Euphytica* **154**, 165-179.

Berry, P. M. (2013). Lodging Resistance cereal lodging resistance in Cereals cereal. *In* "Sustainable food production", pp. 1096-1110. Springer.

Berry, P. M., Sterling, M., Spink, J. H., Baker, C. J., Sylvester-Bradley, R., Mooney, S. J., Tams, A. R., and Ennos, A. R. (2004). Understanding and Reducing Lodging in Cereals. **84**, 217-271.

Bhiah, K., Guppy, C., Lockwood, P., and Jessop, R. (2010). Effect of potassium on rice lodging under high nitrogen nutrition. *In* "Proc 19th World Congress of Soil Sci, Soil Solutions for a Changing World, Brisbane, Australia", pp. 136-139.

Bian, J., Jiang, L., Liu, L., Xiao, Y., Wang, Z., Zhao, Z., Zhai, H., and Wan, J. (2010). Identification of *Japonica* Chromosome Segments Associated with Heterosis for Yield in *Indica* × *Japonica* Rice Hybrids All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher. *Crop science* **50**, 2328-2337.

Brondani, C., Rangel, P., Brondani, R., and Ferreira, M. (2002). QTL mapping and introgression of yield-related traits from Oryza glumaepatula to cultivated rice (Oryza sativa) using microsatellite markers. *Theoretical and Applied Genetics* **104**, 1192-1203.

Brown, D. M., Zeef, L. A., Ellis, J., Goodacre, R., and Turner, S. R. (2005). Identification of novel genes in Arabidopsis involved in secondary cell wall formation using expression profiling and reverse genetics. *The Plant Cell* **17**, 2281-2295.

Burk, D. H., Liu, B., Zhong, R., Morrison, W. H., and Ye, Z.-H. (2001). A Katanin-like protein regulates normal cell wall gBiosynthesis and cell elongation. *The Plant Cell* **13**, 807-827.

Cai, H., and Morishima, H. (2002). QTL clusters reflect character associations in wild and cultivated rice. *Theoretical and Applied Genetics* **104**, 1217-1228.

Carroll, A., and Specht, C. D. (2011). Understanding plant cellulose synthases through a comprehensive investigation of the cellulose synthase family sequences. *Frontiers in plant science* **2**, 5.

Chabannes, M., Ruel, K., Yoshinaga, A., Chabbert, B., Jauneau, A., Joseleau, J. P., and Boudet, A. M. (2001). In situ analysis of lignins in transgenic tobacco reveals a differential impact of individual transformations on the spatial patterns of lignin deposition at the cellular and subcellular levels. *The Plant Journal* **28**, 271-282.

Chen, Y.-D., Wan, B.-H., and Zhang, X. (2005). Plant ideotype at heading for super high-yielding rice in double-cropping system in south China. *Rice Science* **12**, 92-100.

Cloonan, N., Forrest, A. R., Kolle, G., Gardiner, B. B., Faulkner, G. J., Brown, M. K., Taylor, D. F., Steptoe, A. L., Wani, S., and Bethel, G. (2008). Stem cell transcriptome profiling via massive-scale mRNA sequencing. *Nature methods* **5**, 613.

Consortium, C. T. (2003). The nature and identification of quantitative trait loci: a community's view. *Nature Reviews Genetics* **4**, 911.

Crook, M. J., and Ennos, A. (1993). The mechanics of root lodging in winter wheat, Triticum aestivum L. *Journal of Experimental Botany* **44**, 1219-1224.

Easson, D., White, E., and Pickles, S. (1993). The effects of weather, seed rate and cultivar on lodging and yield in winter wheat. *The Journal of Agricultural Science* **121**, 145-156.

Ebitani, T., Takeuchi, Y., Nonoue, Y., Yamamoto, T., Takeuchi, K., and Yano, M. (2005). Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *indica* rice cultivar 'Kasalath'in a genetic background of *japonica* elite cultivar 'Koshihikari'. *Breeding Science* **55**, 65-73.

Ennos, A. R. (1991). The mechanics of anchorage in wheat Triticum aestivum L.: I. The anchorage of wheat seedlings. *Journal of Experimental Botany* **42**, 1601-1606.

Eshed, Y., and Zamir, D. (1995). An introgression line population of Lycopersicon pennellii in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* **141**, 1147-1162.

Evans, L. T. (1998). "Feeding the ten billion: plants and population growth," Cambridge University Press.

Fekih, R., Tamiru, M., Kanzaki, H., Abe, A., Yoshida, K., Kanzaki, E., Saitoh, H., Takagi, H., Natsume, S., and Undan, J. R. (2015). The rice (Oryza sativa L.) LESION MIMIC RESEMBLING, which encodes an AAA-type ATPase, is implicated in defense response. *Molecular Genetics and Genomics* **290**, 611-622.

Fujino, K., Sekiguchi, H., Matsuda, Y., Sugimoto, K., Ono, K., and Yano, M. (2008). Molecular identification of a major quantitative trait locus, qLTG3–1, controlling low-temperature germinability in rice. *Proceedings of the National Academy of Sciences* **105**, 12623-12628.

Gong, H., Chen, K., Wang, S., and Zhang, C. (2004). Advances in silicon nutrition of plants. *Acta Botanica Boreali-occidentalia Sinica* **24**, 2385.

Gu, M. (2010). Discussion on the aspects of high-yielding breeding in rice. *Acta Agron Sin* **36**, 1431-1439.

Hirano, K., Okuno, A., Hobo, T., Ordonio, R., Shinozaki, Y., Asano, K., Kitano, H., and Matsuoka, M. (2014). Utilization of stiff culm trait of rice smos1 mutant for increased lodging resistance. *PLoS One* **9**, e96009.

Hoshikawa, K., and Wang, S.-B. (1990). Studies on Lodging in Rice Plants: I. A general observation on lodged rice culms. *Japanese Journal of Crop Science* **59**, 809-814.

Huang, X., Feng, Q., Qian, Q., Zhao, Q., Wang, L., Wang, A., Guan, J., Fan, D., Weng, Q., and Huang, T. (2009). High-throughput genotyping by whole-genome resequencing. *Genome research*.

Huang, X., Kurata, N., Wang, Z.-X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., Lu, H., Li, W., and Guo, Y. (2012). A map of rice genome variation reveals the origin of cultivated rice. *Nature* **490**, 497.

Huq, M. A., Akter, S., Nou, I. S., Kim, H. T., Jung, Y. J., and Kang, K. K. (2016). Identification of functional SNPs in genes and their effects on plant phenotypes. *Journal of Plant Biotechnology* **43**, 1-11.

Imbe, T., Akama, Y., Nakane, A., Hata, T., Ise, K., Ando, I., Uchiyamada, H., Nakagawa, N., Furutachi, H., and Horisue, N. (2004). Development of a multipurpose high-yielding rice variety" Takanari". *Bulletin of the National Institute of Crop Science (Japan)*.

Ishikawa, S., Ae, N., and Yano, M. (2005). Chromosomal regions with quantitative trait loci controlling cadmium concentration in brown rice (Oryza sativa). *New Phytologist* **168**, 345-350.

Ishikawa, T., Fujimoto, H., Kabaki, N., Maruyama, S., and Akita, S. (1999). Dry matter production before heading and determination of number of spikelets of rice cultivar" Takanari". *Japanese Journal of Crop Science* **68**, 63-70.

Ishimaru, K., Ono, K., and Kashiwagi, T. (2004). Identification of a new gene controlling plant height in rice using the candidate-gene strategy. *Planta* **218**, 388-395.

Ishimaru, K., Togawa, E., Ookawa, T., Kashiwagi, T., Madoka, Y., and Hirotsu, N. (2008). New target for rice lodging resistance and its effect in a typhoon. *Planta* **227**, 601-609.

Islam, M. S., Peng, S., Visperas, R. M., Ereful, N., Bhuiya, M. S. U., and Julfiquar, A. W. (2007). Lodging-related morphological traits of hybrid rice in a tropical irrigated ecosystem. *Field Crops Research* **101**, 240-248.

Jiang, L., Xun, M., Wang, J., and Wan, J. (2008a). QTL analysis of cold tolerance at seedling stage in rice (Oryza sativa L.) using recombination inbred lines. *Journal of Cereal Science* **48**, 173-179.

Jiang, W., Chu, S.-H., Piao, R., Chin, J.-H., Jin, Y.-M., Lee, J., Qiao, Y., Han, L., Piao, Z., and Koh, H.-J. (2008b). Fine mapping and candidate gene analysis of hwh1 and hwh2, a set of complementary genes controlling hybrid breakdown in rice. *Theoretical and applied genetics* **116**, 1117-1127.

Jones, L., Ennos, A. R., and Turner, S. R. (2001). Cloning and characterization of irregular xylem4 (irx4): a severely lignin‐deficient mutant of Arabidopsis. *The Plant Journal* **26**, 205-216.

Jou, Y., Chiang, C.-P., Jauh, G.-Y., and Yen, H. E. (2006). Functional characterization of ice plant SKD1, an AAA-type ATPase associated with the endoplasmic reticulum-Golgi network, and its role in adaptation to salt stress. *Plant physiology* **141**, 135-146.

Kaack, K., Schwarz, K.-U., and Brander, P. E. (2003). Variation in morphology, anatomy and chemistry of stems of Miscanthus genotypes differing in mechanical properties. *Industrial crops and products* **17**, 131-142.

Kashiwagi, T. (2014). Identification of quantitative trait loci for resistance to bending-type lodging in rice (Oryza sativa L.). *Euphytica* **198**, 353-367.

Kashiwagi, T., Hirotsu, N., Madoka, Y., Ookawa, T., and Ishimaru, K. (2007). Improvement of resistance to bending-type lodging in rice [Oryza sativa]. *Japanese Journal of Crop Science (Japan)*.

Kashiwagi, T., and Ishimaru, K. (2004). Identification and functional analysis of a locus for improvement of lodging resistance in rice. *Plant physiology* **134**, 676-683.

Kashiwagi, T., Madoka, Y., Hirotsu, N., and Ishimaru, K. (2006). Locus prl5 improves lodging resistance of rice by delaying senescence and increasing carbohydrate reaccumulation. *Plant Physiol Biochem* **44**, 152-7.

Kashiwagi, T., Munakata, J., and Ishimaru, K. (2016). Functional analysis of the lodging resistance QTL BSUC11 on morphological and chemical characteristics in upper culms of rice. *Euphytica* **210**, 233-243.

Kashiwagi, T., Sasaki, H., and Ishimaru, K. (2005). Factors responsible for decreasing sturdiness of the lower part in lodging of rice (Oryza sativa L.). *Plant production science* **8**, 166-172.

Kashiwagi, T., Togawa, E., Hirotsu, N., and Ishimaru, K. (2008). Improvement of lodging resistance with QTLs for stem diameter in rice (Oryza sativa L.). *Theor Appl Genet* **117**, 749-57.

Keller, M., Karutz, C., Schmid, J., Stamp, P., Winzeler, M., Keller, B., and Messmer, M. (1999). Quantitative trait loci for lodging resistance in a segregating wheat× spelt population. *TAG Theoretical and Applied Genetics* **98**, 1171-1182.

Kharabian-Masouleh, A., Waters, D. L., Reinke, R. F., Ward, R., and Henry, R. J. (2012). SNP in starch biosynthesis genes associated with nutritional and functional properties of rice. *Scientific reports* **2**, 557.

Kokubo, A., Kuraishi, S., and Sakurai, N. (1989). Culm strength of barley. *Plant physiology* **91**, 876-882.

Kokubo, A., Sakurai, N., Kuraishi, S., and Takeda, K. (1991). Culm brittleness of barley (Hordeum vulgare L.) mutants is caused by smaller number of cellulose molecules in cell wall. *Plant physiology* **97**, 509-514.

Komar, A. A. (2007). Silent SNPs: impact on gene function and phenotype.

Kong, E., Liu, D., Guo, X., Yang, W., Sun, J., Li, X., Zhan, K., Cui, D., Lin, J., and Zhang, A. (2013). Anatomical and chemical characteristics associated with lodging resistance in wheat. *The Crop Journal* **1**, 43-49.

Kono, M. (1995). Physiological aspects of lodging. *Science of the rice plant* **2**, 971-982.

Kubo, T., Aida, Y., Nakamura, K., Tsunematsu, H., Doi, K., and Yoshimura, A. (2002). Reciprocal chromosome segment substitution series derived from *japonica* and *indica* cross of rice (Oryza sativa L.). *Breeding Science* **52**, 319-325.

Laborte, A. G., Gutierrez, M. A., Balanza, J. G., Saito, K., Zwart, S. J., Boschetti, M., Murty, M., Villano, L., Aunario, J. K., and Reinke, R. (2017). RiceAtlas, a spatial database of global rice calendars and production. *Scientific data* **4**.

Lang, Y.-z., Yang, X.-d., Wang, M.-e., and Zhu, Q.-s. (2012). Effects of Lodging at Different Filling Stages on Rice Yield and Grain Quality. *Rice Science* **19**, 315-319.

LeVan, T. D., Bloom, J. W., Bailey, T. J., Karp, C. L., Halonen, M., Martinez, F. D., and Vercelli, D. (2001). A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *The Journal of Immunology* **167**, 5838-5844.

Li, F., Zhang, M., Guo, K., Hu, Z., Zhang, R., Feng, Y., Yi, X., Zou, W., Wang, L., and Wu, C. (2015). High ‐ level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. *Plant biotechnology journal* **13**, 514-525.

Li, J.-Y., Wang, J., and Zeigler, R. S. (2014). The 3,000 rice genomes project: new opportunities and challenges for future rice research. *GigaScience* **3**, 8.

Li, Y. (2003). BRITTLE CULM1, Which Encodes a COBRA-Like Protein, Affects the Mechanical Properties of Rice Plants. *The Plant Cell Online* **15**, 2020-2031.

Li, Z.-K., Luo, L., Mei, H., Wang, D., Shu, Q., Tabien, R., Zhong, D., Ying, C., Stansel, J., and Khush, G. (2001). Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics* **158**, 1737-1753.

Lin, H., Ashikari, M., Yamanouchi, U., Sasaki, T., and Yano, M. (2002). Identification and characterization of a quantitative trait locus, Hd9, controlling heading date in rice. *Breeding Science* **52**, 35-41.

Lin, S., Sasaki, T., and Yano, M. (1998). Mapping quantitative trait loci controlling seed dormancy and heading date in rice, Oryza sativa L., using backcross inbred lines. *Theoretical and Applied Genetics* **96**, 997-1003.

Luh, B. S. (1980). "Rice: production and utilization," AVI Publishing Co.

Luptovčiak, I., Komis, G., Takáč, T., Ovečka, M., and Šamaj, J. (2017). Katanin: A Sword Cutting Microtubules for Cellular, Developmental, and Physiological Purposes. *Frontiers in plant science* **8**.

Ma, J., Ma, W., Tian, Y., Yang, J., Zhou, K., and Zhu, Q. (2003). The culm lodging resistance of heavy panicle type of rice. *Zuo wu xue bao* **30**, 143-148.

Ma, J. F., and Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in plant science* **11**, 392-397.

Ma, Q.-H. (2009). The expression of caffeic acid 3-O-methyltransferase in two wheat genotypes differing in lodging resistance. *Journal of Experimental Botany* **60**, 2763-2771.

Ma, Q. H., Xu, Y., Lin, Z. B., and He, P. (2002). Cloning of cDNA encoding COMT from wheat which is differentially expressed in lodging‐sensitive and‐resistant cultivars. *Journal of Experimental Botany* **53**, 2281-2282.

Mackill, D. J. (1996). "Rainfed lowland rice improvement," Int. Rice Res. Inst.

Maclean, J., Hardy, B., and Hettel, G. (2013). "Rice Almanac: Source book for one of the most important economic activities on earth," IRRI.

Manning, K. S., and Cooper, T. A. (2017). The roles of RNA processing in translating genotype to phenotype. *Nature Reviews Molecular Cell Biology* **18**, 102.

Matsubara, K., Ando, T., Mizubayashi, T., Ito, S., and Yano, M. (2007). Identification and linkage mapping of complementary recessive genes causing hybrid breakdown in an intraspecific rice cross. *Theoretical and applied genetics* **115**, 179-186.

Matsubara, K., Yamamoto, E., Kobayashi, N., Ishii, T., Tanaka, J., Tsunematsu, H., Yoshinaga, S., Matsumura, O., Yonemaru, J.-i., and Mizobuchi, R. (2016). Improvement of Rice Biomass Yield through QTL-Based Selection. *PloS one* **11**, e0151830.

Matsuda, T., Kawahara, H., and Chonan, N. (1983). Histological studies on breaking resistance of lower internodes in rice culm: IV. The roles of each tissue of internode and leaf sheath in breaking resistance. *Japanese Journal of Crop Science* **52**, 355-361.

McCouch, S. R., Kochert, G., Yu, Z., Wang, Z., Khush, G., Coffman, W., and Tanksley, S. (1988). Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics* **76**, 815-829.

McCouch, S. R., and Doerge, R. W. (1995). QTL mapping in rice. *Trends in Genetics* **11**, 482-487.

McFarlane, H. E., Watanabe, Y., Gendre, D., Carruthers, K., Levesque-Tremblay, G., Haughn, G. W., Bhalerao, R. P., and Samuels, L. (2013). Cell wall polysaccharides are mislocalized to the vacuole in echidna mutants. *Plant and Cell Physiology* **54**, 1867-1880.

McNeil, M., Darvill, A. G., Fry, S. C., and Albersheim, P. (1984). Structure and function of the primary cell walls of plants. *Annual review of biochemistry* **53**, 625-663.

Mizuno, H., Kawahara, Y., Sakai, H., Kanamori, H., Wakimoto, H., Yamagata, H., Oono, Y., Wu, J., Ikawa, H., and Itoh, T. (2010). Massive parallel sequencing of mRNA in identification of unannotated salinity stress-inducible transcripts in rice (Oryza sativa L.). *BMC genomics* **11**, 683.

Morin, R. D., Bainbridge, M., Fejes, A., Hirst, M., Krzywinski, M., Pugh, T. J., McDonald, H., Varhol, R., Jones, S. J., and Marra, M. A. (2008). Profiling the HeLa S3 transcriptome using randomly primed cDNA and massively parallel short-read sequencing. *Biotechniques* **45**, 81.

Murai, M., Komazaki, T., and Sato, S. (2004). Effects of sd1 and Ur1 (Undulate rachis-1) on lodging resistance and related traits in rice. *Breeding science* **54**, 333-340.

Nadeau, J. H., Singer, J. B., Matin, A., and Lander, E. S. (2000). Analysing complex genetic traits with chromosome substitution strains. *Nature genetics* **24**, 221-225.

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., and Snyder, M. (2008). The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science* **320**, 1344-1349.

Nagata, K., Ando, T., Nonoue, Y., Mizubayashi, T., Kitazawa, N., Shomura, A., Matsubara, K., Ono, N., Mizobuchi, R., and Shibaya, T. (2015). Advanced backcross QTL analysis reveals complicated genetic control of rice grain shape in a *japonica*× *indica* cross. *Breeding science* **65**, 308-318.

Neenan, M., and Spencer-Smith, J. (1975). An analysis of the problem of lodging with particular reference to wheat and barley. *The Journal of agricultural science* **85**, 495-507.

Nguyen, T. N., Son, S., Jordan, M. C., Levin, D. B., and Ayele, B. T. (2016). Lignin biosynthesis in wheat (Triticum aestivum L.): its response to waterlogging and association with hormonal levels. *BMC Plant Biol* **16**, 28.

Niklas, K. J. (1989). Nodal septa and the rigidity of aerial shoots of Equisetum hyemale. *American Journal of Botany*, 521-531.

Niklas, K. J. (1990). The mechanical significance of clasping leaf sheaths in grasses: evidence from two cultivars of Avena sativa. *Annals of Botany* **65**, 505-512.

Oka, H. (1988). Functions and genetic basis of reproductive barriers. *Origin of cultivated rice*, 181-209.

Okuno, A., Hirano, K., Asano, K., Takase, W., Masuda, R., Morinaka, Y., Ueguchi-Tanaka, M., Kitano, H., and Matsuoka, M. (2014). New approach to increasing rice lodging resistance and biomass yield through the use of high gibberellin producing varieties. *PLoS One* **9**, e86870.

Ookawa, T., Aoba, R., Yamamoto, T., Ueda, T., Takai, T., Fukuoka, S., Ando, T., Adachi, S., Matsuoka, M., Ebitani, T., Kato, Y., Mulsanti, I. W., Kishii, M., Reynolds, M., Pinera, F., Kotake, T., Kawasaki, S., Motobayashi, T., and Hirasawa, T. (2016). Precise estimation of genomic regions controlling lodging resistance using a set of reciprocal chromosome segment substitution lines in rice. *Sci Rep* **6**, 30572.

Ookawa, T., Hobo, T., Yano, M., Murata, K., Ando, T., Miura, H., Asano, K., Ochiai, Y., Ikeda, M., Nishitani, R., Ebitani, T., Ozaki, H., Angeles, E. R., Hirasawa, T., and Matsuoka, M. (2010a). New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. *Nat Commun* **1**, 132.

Ookawa, T., Inoue, K., Matsuoka, M., Ebitani, T., Takarada, T., Yamamoto, T., Ueda, T., Yokoyama, T., Sugiyama, C., Nakaba, S., Funada, R., Kato, H., Kanekatsu, M., Toyota, K., Motobayashi,

T., Vazirzanjani, M., Tojo, S., and Hirasawa, T. (2014). Increased lodging resistance in long-culm, low-lignin gh2 rice for improved feed and bioenergy production. *Sci Rep* **4**, 6567.

Ookawa, T., and Ishihara, K. (1992). Varietal difference of physical characteristics of the culm related to lodging resistance in paddy rice. *Japanese Journal of Crop Science* **61**, 419-425.

Ookawa, T., and Ishihara, K. (1993). Varietal difference of the cell wall components affecting the bending stress of the culm in relation to the lodging resistance in paddy rice. *Japanese Journal of Crop Science* **62**, 378-384.

Ookawa, T., and Ishihara, K. (1997). Genetic characteristics of the breaking strength of the basal culm related to lodging resistance in a cross between Koshihikari and Chugoku 117. *Japanese Journal of Crop Science* **66**, 603-609.

Ookawa, T., Todokoro, Y., and Ishihara, K. (1993). Changes in physical and chemical characteristics of culm associated with lodging resistance in paddy rice under different growth conditions and varietal difference of their changes. *Japanese Journal of Crop Science* **62**, 525-533.

Ookawa, T., Yasuda, K., Kato, H., Sakai, M., Seto, M., Sunaga, K., Motobayashi, T., Tojo, S., and Hirasawa, T. (2010b). Biomass production and lodging resistance in 'Leaf Star', a new long-culm rice forage cultivar. *Plant Production Science* 13 (1):58-66.

Oono, Y., Kawahara, Y., Kanamori, H., Mizuno, H., Yamagata, H., Yamamoto, M., Hosokawa, S., Ikawa, H., Akahane, I., and Zhu, Z. (2011). mRNA-Seq reveals a comprehensive transcriptome profile of rice under phosphate stress. *Rice* **4**, 50-65.

Ordonio, R. L., Ito, Y., Hatakeyama, A., Ohmae-Shinohara, K., Kasuga, S., Tokunaga, T., Mizuno, H., Kitano, H., Matsuoka, M., and Sazuka, T. (2014). Gibberellin deficiency pleiotropically induces culm bending in sorghum: an insight into sorghum semi-dwarf breeding. *Sci Rep* **4**, 5287.

Peng, S., and Khushg, G. (2003). Four decades of breeding for varietal improvement of irrigated lowland rice in the International Rice Research Institute. *Plant Production Science* **6**, 157-164.

Peng, S., Laza, R. C., Visperas, R. M., Khush, G. S., and Virk, P. (2005). Progress in breeding the new plant type for yield improvement: a physiological view. *Copyright International Rice Research Institute 2005*, 130.

Piñera-Chavez, F., Berry, P., Foulkes, M., Jesson, M., and Reynolds, M. (2016). Avoiding lodging in irrigated spring wheat. I. Stem and root structural requirements. *Field Crops Research* **196**, 325-336.

Pinthus, M. J. (1974). Lodging in wheat, barley, and oats: the phenomenon, its causes, and preventive measures. *Advances in agronomy* **25**, 209-263.

Reddy, N., and Yang, Y. (2005). Structure and properties of high quality natural cellulose fibers from cornstalks. *Polymer* **46**, 5494-5500.

Ren, Z.-H., Gao, J.-P., Li, L.-G., Cai, X.-L., Huang, W., Chao, D.-Y., Zhu, M.-Z., Wang, Z.-Y., Luan, S., and Lin, H.-X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature genetics* **37**, 1141-1146.

San-Oh, Y., Ookawa, T., Aizawa, N., and Hirasawa, T. (2001). Varietal differences in growth, lodging and related characters of rice plants broadcasted in submerged paddy field at different densities (agronomy). *Japanese Journal of Crop Science* **70**, 515-524.

Santos, L. (2006). Molecular mechanisms of the AAA proteins in plants. *Adv Agril Food Biotechnol* **37**, 1-15.

Sarker, Z., Shamsuddin, A., and Ara, R. (2007). Gene action of traits constributing to lodging resistance in wheat (Triticum aestivum L). *Bangladesh Journal of Plant Breeding and Genetics* **20**, 23-30.

Sasaki, T. (2005). The map-based sequence of the rice genome. *Nature* **436**, 793.

Setter, T., Laureles, E., and Mazaredo, A. (1997). Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis. *Field Crops Research* **49**, 95-106.

Setter, T., Peng, S., Kirk, G., Virmani, S., Kropff, M., and Cassman, K. (1994). Physiological considerations and hybrid rice. *In* "Breaking the yield barrier: Proceedings of a workshop on rice yield potential in favorable environments. International Rice Research Institute: Los Baños, Philippines", pp. 39-62.

Shen, H., Fu, C., Xiao, X., Ray, T., Tang, Y., Wang, Z., and Chen, F. (2009). Developmental control of lignification in stems of lowland switchgrass variety Alamo and the effects on saccharification efficiency. *BioEnergy Research* **2**, 233-245.

Silk, W. K., Wang, L. L., and Cleland, R. E. (1982). Mechanical properties of the rice panicle. *Plant Physiology* **70**, 460-464.

Sobrizal, K., Sanchez, P., Doi, K., Angeles, E., Khush, G., and Yoshimura, A. (1999). Development of Oryza glumaepatula introgression lines in rice, O. sativa L. *Rice Genet. Newsl* **16**, 107-108.

Somerville, C. (2006). Cellulose synthesis in higher plants. *Annu Rev Cell Dev Biol* **22**, 53-78.

Spielmeyer, W., Ellis, M. H., and Chandler, P. M. (2002). Semidwarf (sd-1),"green revolution" rice, contains a defective gibberellin 20-oxidase gene. *Proceedings of the National Academy of Sciences* **99**, 9043-9048.

Sterling, M., Baker, C. J., Berry, P. M., and Wade, A. (2003). An experimental investigation of the lodging of wheat. *Agricultural and Forest Meteorology* **119**, 149-165.

Subudhi, P. K., De Leon, T., Singh, P. K., Parco, A., Cohn, M. A., and Sasaki, T. (2015). A chromosome segment substitution library of weedy rice for genetic dissection of complex agronomic and domestication traits. *PloS one* **10**, e0130650.

Sunohara, H., Kawai, T., Shimizu-Sato, S., Sato, Y., Sato, K., and Kitano, H. (2009). A dominant mutation of TWISTED DWARF 1 encoding an α-tubulin protein causes severe dwarfism and right helical growth in rice. *Genes & genetic systems* **84**, 209-218.

Tabbal, D., Bouman, B., Bhuiyan, S., and Sibayan, E. (2002). Improving lodging resistance of direct wet-seeded rice. *Phil. J. Crop Sci* **27**, 55-64.

Taiz, L., Zeiger, E., Møller, I., and Murphy, A. (2015). Plant Physiology and Development, 6th Edn Sunderland. *MA: Sinauer Associates*.

Takai, T., Adachi, S., Taguchi-Shiobara, F., Sanoh-Arai, Y., Iwasawa, N., Yoshinaga, S., Hirose, S., Taniguchi, Y., Yamanouchi, U., and Wu, J. (2013). A natural variant of NAL1, selected in high-yield rice breeding programs, pleiotropically increases photosynthesis rate. *Scientific reports* **3**.

Takai, T., Arai-Sanoh, Y., Iwasawa, N., Hayashi, T., Yoshinaga, S., and Kondo, M. (2012). Comparative mapping suggests repeated selection of the same quantitative trait locus for high leaf photosynthesis rate in rice high-yield breeding programs. *Crop Science* **52**, 2649-2658.

Takai, T., Ikka, T., Kondo, K., Nonoue, Y., Ono, N., Arai-Sanoh, Y., Yoshinaga, S., Nakano, H., Yano, M., and Kondo, M. (2014). Genetic mechanisms underlying yield potential in the rice high-yielding cultivar Takanari, based on reciprocal chromosome segment substitution lines. *BMC plant biology* **14**, 295.

Takai, T., Matsuura, S., Nishio, T., Ohsumi, A., Shiraiwa, T., and Horie, T. (2006). Rice yield potential is closely related to crop growth rate during late reproductive period. *Field Crops Research* **96**, 328-335.

Takai, T., Nonoue, Y., Yamamoto, S.-i., Yamanouchi, U., Matsubara, K., Liang, Z.-W., Lin, H.-x., Ono, N., Uga, Y., and Yano, M. (2007). Development of chromosome segment substitution

lines derived from backcross between *indica* donor rice cultivar 'Nona Bokra'and *japonica* recipient cultivar 'Koshihikari'. *Breeding Science* **57**, 257-261.

Takeda, S., and Matsuoka, M. (2008). Genetic approaches to crop improvement: responding to environmental and population changes. *Nature Reviews Genetics* **9**, 444.

Tams, A., Mooney, S., and Berry, P. (2004). The effect of lodging in cereals on morphological properties of the Root-Soil Complex. *In* "Super soil 2004 3rd Aust. New Zealan Soils Conf", pp. 5-9.

Tang, S., Ding, L., and Bonjean, A. (2010). Rice production and genetic improvement in China. *Cereals in China* **15**.

Tavakoli, M., Tavakoli, H., Azizi, M., and Haghayegh, G. (2010). Comparison of mechanical properties between two varieties of rice straw. *Advance Journal of Food Science and Technology* **2**, 50-54.

Taylor, N. G., Scheible, W.-R., Cutler, S., Somerville, C. R., and Turner, S. R. (1999). The irregular xylem3 locus of Arabidopsis encodes a cellulose synthase required for secondary cell wall synthesis. *The plant cell* **11**, 769-779.

Terashima, K., Akita, S., and Sakai, N. (1992). Eco-physiological characteristics related with lodging tolerance of rice in direct sowing cultivation: I. comparison of the root lodging tolerance among cultivars by the measurement of pushing resistance. *Japanese Journal of Crop Science* **61**, 380-387.

Thomas, W. (1982). Plant growth regulators. *Yield of Cereals Course Papers* **1982**, 78-95.

Tian, F., Li, D. J., Fu, Q., Zhu, Z. F., Fu, Y. C., Wang, X. K., and Sun, C. Q. (2006). Construction of introgression lines carrying wild rice (Oryza rufipogon Griff.) segments in cultivated rice (Oryza sativa L.) background and characterization of introgressed segments associated with yield-related traits. *Theoretical and Applied Genetics* **112**, 570-580.

Uehara, Y., Kobayashi, A., Koga, Y., Uchiyamada, H., Miura, K., Fukui, K., Shimizu, H., Ohta, H., Fujita, Y., and Okuno, K. (1995). Breeding of a new rice cultivar "Dontokoi". *Bull. Hokuriku Natl. Agric. Exp. Stn* **37**, 107-131.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., and Boerjan, W. (2010). Lignin biosynthesis and structure. *Plant physiology* **153**, 895-905.

Vera, J. C., Wheat, C. W., Fescemyer, H. W., Frilander, M. J., Crawford, D. L., Hanski, I., and Marden, J. H. (2008). Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing. *Molecular ecology* **17**, 1636-1647.

Vogel, J. (2008). Unique aspects of the grass cell wall. *Current opinion in plant biology* **11**, 301-307.

Wan, X. Y., Wan, J. M., Jiang, L., Wang, J. K., Zhai, H. Q., Weng, J. F., Wang, H. L., Lei, C. L., Wang, J. L., Zhang, X., Cheng, Z. J., and Guo, X. P. (2006). QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. *Theor Appl Genet* **112**, 1258-70.

Wan, X. Y., Wan, J. M., Su, C. C., Wang, C. M., Shen, W. B., Li, J. M., Wang, H. L., Jiang, L., Liu, S. J., Chen, L. M., Yasui, H., and Yoshimura, A. (2004). QTL detection for eating quality of cooked rice in a population of chromosome segment substitution lines. *Theor Appl Genet* **110**, 71-9.

Wang, C.-Y., Dai, X.-L., Shi, Y.-H., Wang, Z.-L., Chen, X.-G., and He, M.-R. (2012). Effects of nitrogen application rate and plant density on lodging resistance in winter wheat. *Acta Agron Sin* **38**, 121-128.

Wang, J., Zhu, J., Lin, Q., Li, X., Teng, N., Li, Z., Li, B., Zhang, A., and Lin, J. (2006). Effects of stem structure and cell wall components on bending strength in wheat. *Chinese Science Bulletin* **51**, 815-823.

Wang, L., Guo, K., Li, Y., Tu, Y., Hu, H., Wang, B., Cui, X., and Peng, L. (2010). Expression profiling and intregative analysis of the CESA/CSL superfamily in rice. *BMC plant biology*, 10:282.

Wang, Y., and Li, J. (2005). The plant architecture of rice (Oryza sativa). *Plant molecular biology* **59**, 75-84.

Wang, Y., Xiao, X., Wang, X., Zeng, J., Kang, H., Fan, X., Sha, L., Zhang, H., and Zhou, Y. (2016). RNA-Seq and iTRAQ reveal the dwarfing mechanism of dwarf polish wheat (Triticum polonicum L.). *International journal of biological sciences* **12**, 653.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics* **10**, 57.

Wu, Q.-l., Wang, Z., and Yang, W.-y. (2008). Seedling shading affects morphogenesis and substance accumulation of stem in soybean. *Soybean Science* **26**, 868.

Xiang, D., Guo, K., Lei, T., Yu, X., Luo, Q., and Yang, W. (2010). Effects of phosphorus and potassium on stem characteristics and lodging resistance of relay cropping soybean. *Chinese J. Oil Crop Sci* **3**, 395-402.

Xiong, G., Li, R., Qian, Q., Song, X., Liu, X., Yu, Y., Zeng, D., Wan, J., Li, J., and Zhou, Y. (2010). The rice dynamin‐related protein DRP2B mediates membrane trafficking, and thereby plays a critical role in secondary cell wall cellulose biosynthesis. *The Plant Journal* **64**, 56-70.

Xu, X., Liu, X., Ge, S., Jensen, J. D., Hu, F., Li, X., Dong, Y., Gutenkunst, R. N., Fang, L., and Huang, L. (2012). Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nature biotechnology* **30**, 105.

Xu, Y.-F., Ookawa, T., and Ishihara, K. (1997). Analysis of the dry matter production process and yield formation of the high-yielding rice cultivar Takanari, from 1991 to 1994. *Japanese Journal of Crop Science* **66**, 42-50.

Yadav, S., Singh, U. M., Naik, S. M., Venkateshwarlu, C., Ramayya, P. J., Raman, K. A., Sandhu, N., and Kumar, A. (2017). Molecular Mapping of QTLs Associated with Lodging Resistance in Dry Direct-Seeded Rice (Oryza sativa L.). *Frontiers in plant science* **8**, 1431.

Yamamoto, E., Takashi, T., Morinaka, Y., Lin, S., Kitano, H., Matsuoka, M., and Ashikari, M. (2007). Interaction of two recessive genes, hbd2 and hbd3, induces hybrid breakdown in rice. *Theoretical and applied genetics* **115**, 187-194.

Yamamoto, T., Lin, H., Sasaki, T., and Yano, M. (2000). Identification of heading date quantitative trait locus Hd6 and characterization of its epistatic interactions with Hd2 in rice using advanced backcross progeny. *Genetics* **154**, 885-891.

Yamamoto, T., Yonemaru, J., and Yano, M. (2009). Towards the understanding of complex traits in rice: substantially or superficially? *DNA research* **16**, 141-154.

Yamauchi, M. (2001). Crop establishment and grain yield of direct sowing culture of rice [Oryza sativa] with recycled-paper mulch. *Japanese Journal of Crop Science (Japan)*.

Yang, J., Zhang, J., Wang, Z., and Zhu, Q. (2001). Activities of starch hydrolytic enzymes and sucrose‐phosphate synthase in the stems of rice subjected to water stress during grain filling. *Journal of Experimental Botany* **52**, 2169-2179.

Yang, S., Xie, L., Zheng, S., Li, J., and Yuan, J. (2009). Effects of nitrogen rate and transplanting density on physical and chemical characteristics and lodging resistance of culms in hybrid rice. *Acta Agronomica Sinica* **35**, 93-103.

Yano, K., Ookawa, T., Aya, K., Ochiai, Y., Hirasawa, T., Ebitani, T., Takarada, T., Yano, M., Yamamoto, T., Fukuoka, S., Wu, J., Ando, T., Ordonio, R. L., Hirano, K., and Matsuoka, M. (2015). Isolation of a novel lodging resistance QTL gene involved in strigolactone signaling and its pyramiding with a QTL gene involved in another mechanism. *Mol Plant* **8**, 303-14.

Yano, M. (2001). Genetic and molecular dissection of naturally occurring variation. *Current opinion in plant biology* **4**, 130-135.

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., and Nagamura, Y. (2000). Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. *The Plant Cell* **12**, 2473-2483.

Yano, M., and Sasaki, T. (1997). Genetic and molecular dissection of quantitative traits in rice. *Plant molecular biology* **35**, 145-153.

Yao, J., Ma, H., Zhang, P., Ren, L., Yang, X., Yao, G., Zhang, P., and Zhou, M. (2011). Inheritance of stem strength and its correlations with culm morphological traits in wheat (Triticum æestivum L.). *Canadian Journal of Plant Science* **91**, 1065-1070.

Zamir, D. (2001). Improving plant breeding with exotic genetic libraries. *Nature reviews genetics* **2**, 983-989.

Zhang, F.-z., Jin, Z.-x., Ma, G.-h., Shang, W.-n., Liu, H.-y., Xu, M.-l., and Liu, Y. (2010). Relationship between lodging resistance and cemical contents in culms and sheaths of *japonica* rice during grain filling. *Rice Science* **17**, 311-318.

Zhang, W.-j., Wu, L.-m., Ding, Y.-f., Weng, F., Wu, X.-r., Li, G.-h., Liu, Z.-h., Tang, S., Ding, C.-q., and Wang, S.-h. (2016). Top-dressing nitrogen fertilizer rate contributes to decrease culm physical strength by reducing structural carbohydrate content in *japonica* rice. *Journal of Integrative Agriculture* **15**, 992-1004.

Zhou, L. (2006). Effects of stem physiological properties on lodging resistance in hybrid rice. *Henan Agricultural Sciences* **10**, 20-23.

Zhu, L. H., Zhong, D. B., Xu, J. L., Yu, S. B., and Li, Z. K. (2008). Differential expression of lodging resistance related QTLs in rice (Oryza sativa L.). *Plant Science* **175**, 898-905.

Zuber, U., Winzeler, H., Messmer, M., Keller, M., Keller, B., Schmid, J., and Stamp, P. (1999). Morphological traits associated with lodging resistance of spring wheat (Triticum aestivum L.). *Journal of Agronomy and Crop Science* **182**, 17-24.

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