Breeding Science 59: 664–670 (2009)

Gene silencing of barley P23k involved in secondary wall formation causes abnormal tiller formation and intercalary elongation

Ai Oikawa[1], Kazuya Nagai[1], Kiyoaki Kato[2] and Shin-ichiro Kidou*[3]

[1] *Cryobiosystem Research Center, Faculty of Agriculture, Iwate University*, Morioka, Iwate 020-8550, Japan
[2] *Department of Crop Science, Obihiro University of Agriculture and Veterinary Medicine*, Obihiro, Hokkaido 080-8555, Japan
[3] *Graduate School of Natural Sciences, Nagoya City University*, Mizuho, Nagoya, Aichi 467-8501, Japan

P23k is a monocot-unique protein that is highly expressed in barley. Our previous loss-of-function studies in barley leaves indicated that P23k, localized to tissues where cell wall polysaccharides accumulate, might contribute to secondary wall formation in the leaf. However, the *P23k* loss-of-function analysis was limited to the leaf, which is a vegetative organ. Considering the involvement of P23k in secondary wall formation, a dramatically altered phenotype is expected in the stem of *P23k* gene-silenced barley, where marked secondary wall deposition occurs during the reproductive growth stage. To test this hypothesis, *barley striped mosaic virus*-based virus-induced gene silencing of *P23k* was performed. Abnormal tiller formation and arrested intercalary elongation were observed in *P23k*-silenced barley. From these results, we speculated that cell wall architecture was altered by *P23k* gene silencing. Consistent with this idea, we observed a marked decrease in the amount of cell wall polysaccharides stained with calcofluor and down-regulation of the cellulose synthase-like *CslF6* gene involved in (1,3;1,4)-β-D-glucan synthesis. Taken together, these results suggest that P23k is possibly involved in determining secondary wall architecture and contributes to tiller formation and intercalary elongation in barley.

Key Words: *Hordeum vulgare*, P23k, virus-induced gene silencing, secondary wall formation, (1,3;1,4)-β-D-glucan, tiller, intercalary elongation.

Introduction

Tiller number is an important agronomic trait for grain production in cereals such as rice, wheat and barley. Although tillering (branching) is also found in dicots, it differs significantly from that of monocots: the tiller in monocots is derived from the lower, unelongated portion of the stem, while branching in dicots often occurs from the upper, elongated part of the stem. The formation of an axillary meristem, which enables tiller development at a late growth stage, involves changes in cell proliferation and differentiation at certain positions on the stem, accompanied by drastic and concerted modifications in gene expression (Talbert *et al.* 1995, Schmitz *et al.* 2002, Li *et al.* 2003, Sorefan *et al.* 2003, Takeda *et al.* 2003) and action of hormones such as auxins (Reinhardt *et al.* 2000, 2003, Benkova *et al.* 2003, Xu *et al.* 2005, Carraro *et al.* 2006). Despite progress in identification of genes expressed in the tiller or branch, our knowledge of their molecular basis is still fragmented. The reason for the restriction of the tiller to the lower, unelongated portion of the stem, especially in monocots, remains unclear.

A schematic representation of the stem architecture of the monocot barley (*Hordeum vulgare* L.) is shown in Fig. 1. In monocots, the internodes of the upper stem elongate following the development of an intercalary meristem just above each node in the reproductive growth phase. Therefore, the stem elongation is termed intercalary elongation. The cells produced by the intercalary meristem exhibit marked expansion in a specific region called an elongation zone during the intercalary elongation (Evans 1965, Fisher 1970, Bleecker *et al.* 1986). The first (uppermost) internode can elongate to a maximum of 30 cm and is the longest of the internodes, indicating that the stem length is determined by the degree of cell expansion from the intercalary meristem. In contrast, internodes of the lower, unelongated part of the stem do not have the ability to elongate since cell division in the intercalary meristem is repressed even when the shoot apical meristem passes into the reproductive phase. Thus, cell expansion in the elongation zone is considered to be the major determinant of the growth rate of the whole internode (Kende *et al.* 1998).

Plant cell expansion is regulated by the mechanical properties of the cell wall and cell wall synthesis (Taiz 1984). The plant cell wall is composed of an organized matrix of cellulose, hemicellulosic and pectic polysaccharides protein and lignin, and is conveniently considered to be of two types, a thin primary wall and a thicker secondary wall. The primary wall is the part of the wall laid down by young and

Communicated by T. Komatsuda
Received August 26, 2009. Accepted October 30, 2009.
*Corresponding author (e-mail: kidou@nsc.nagoya-cu.ac.jp)

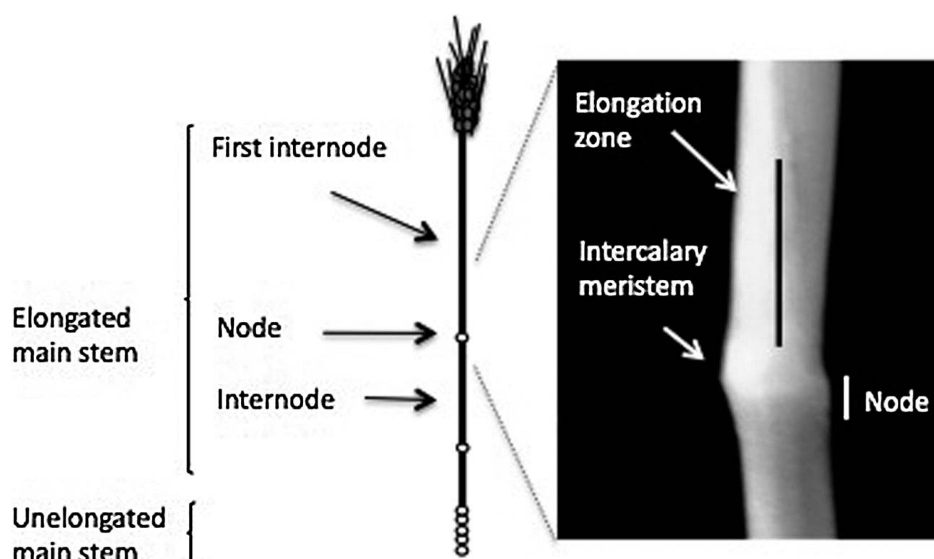

Fig. 1. Schematic representation of stem architecture of monocot barley.

undifferentiated cells such as parenchyma cells, while secondary wall deposition occurs after the cell has stopped growing, as in sclerenchyma cells (Talmadge *et al.* 1973). The cell wall is an intricate structure involved in the determination of cell size and shape, growth and development, and intercellular communication. By analyzing the direct attachment between cellulose and microtubules using live-cell imaging, Paredez *et al.* (2006) suggested that the cellulose-microtubule network might be important for intercalary elongation. Nemoto *et al.* (2004) reported that microtubules are involved in the rate and direction of cell expansion in intercalary elongation. Sauter and Kende (1992) also hypothesized that elevated levels of (1,3;1,4)-β-glucan (a hemicellulosic component) contribute to elongation growth of internodes by increasing the extensibility of the cell wall. However, evidence for a direct role of cell wall synthesis in intercalary elongation remains to be demonstrated in Gramineae, although it has recently been shown that mutation in the cellulose synthase-like family (Csl) F6, a (1,3;1,4)-β-D-glucan synthase, causes slightly shorter culms in barley (Tonooka *et al.* 2009).

Barley P23k was originally identified as a protein expressed in barley seedlings (Kidou *et al.* 2006). *P23k* is strongly expressed in the scutellum of imbibed barley seeds, and was synchronously down-regulated with consumption of the starchy endosperm. In addition, expression analyses of *P23k* mRNA in barley leaves showed up-regulation of *P23k* transcripts with increased photosynthetic activity (Oikawa *et al.* 2007). These results indicated that P23k might play a role in sugar metabolism. Furthermore, the P23k protein is also expressed abundantly in vascular bundles and sclerenchyma where secondary wall formation is active in vegetative leaves of barley (Oikawa *et al.* 2007). Virus-induced gene silencing (VIGS) of *P23k* in the vegetative growth stage led to abnormal leaf development, asymmetric orientation of the main veins, and cracked leaf edges caused by mechanical weakness (Oikawa *et al.* 2007). From these results, we speculated that P23k is also involved in cell wall synthesis.

In this study, based on *P23k* expression and the phenotype observed following *P23k* VIGS, we showed that P23k is involved in modulating secondary wall architecture and regulation of tiller formation in barley.

Materials and Methods

Plant material and growth conditions

Barley (*H. vulgare* cv. Minorimugi) seeds were surface-sterilized for 30 min with 2% (w/v) NaClO and then germinated inside wet towels in the dark at 25°C. The germinated seeds were transplanted into 1/5 Hoagland No. 2 liquid medium (pH 5.5) and placed in a cold room for six weeks with a 16 h light and 8 h dark cycle for vernalization treatment. The vernalized barley plants were grown in a greenhouse.

Preparation of infectious BSMV RNAs and plant inoculation

The *barley striped mosaic virus* (BSMV) plasmid vectors used in this study were described previously (Holzberg *et al.* 2002, Oikawa *et al.* 2007). Infectious BSMV RNAs were prepared from each plasmid by *in vitro* transcription using the mMESSAGE mMACHINE High Yield Capped RNA Transcription Kit (Ambion, Austin, TX, USA). Five microliters of each transcript, which included the BSMV RNAs α, β, and genetically modified γ, were combined with 40 μl FES buffer (Pogue *et al.* 1998) and inoculated into the first and second leaves of barley plants at the five- or six-leaf stage.

Expression analysis by RT-PCR and immunoblot analysis

To generate first-strand cDNA, 1 μg total RNA was annealed with 10 μM random hexamers in a 20 μl reaction

mixture, and extended using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (TOYOBO) at 42°C for 1 h. One microliter of each reverse transcription (RT) reaction was used as template in 20 µl PCR reactions containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 4 mM dNTPs, 0.2 units Ex Taq polymerase (Takara) and 1 µM primers. The primers used for gene amplification were as follows: *P23k* (5′-GGTACGGTAACGGAATAGC-3′ and 5′-TCGCCACACAAGCCTTTGATGTT-3′); *HvEF-1A* (5′-TTCAACGTCAAGAACGTGGCT-3′ and 5′-ACACAA ATAACCCAAGCGACTA-3′); *BSMVγ* (5′-GAATCCAAA AACCTGCTCAACC-3′ and 5′-CATTCGCTTTGAACTT ATCGGC-3′); *HvCslF6* (5′-CATACCTCGGGAGG CAAGCA-3′ and 5′-TTCAAACTTTGGCGGGCTCT-3′); *HvCesA1* (5′-ATGATGAAACTCGCCAGCCTC-3′ and 5′-TCATGCCTTAGAAACTAGGCC-3′); *HvGSL* (5′-CAT AAGTAGCTGGTTTATGGC-3′ and 5′-CAACGAGCGCA CTGTTTTCC-3′). Thermal cycling was conducted as follows: denaturing at 94°C for 1 min followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. The resulting products were separated by 2% agarose gel electrophoresis and visualized by EtBr staining. The specificity of the primers was tested by PCR amplification of the corresponding cDNA clone.

For immunoblot analysis, total proteins were extracted using a buffer consisting of 50 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.5 mM EDTA, 5 mM DTT, 1 mM PMSF, and 5% glycerol, and then subjected to 12% (w/v) SDS-PAGE following the procedure of Laemmli (1970). After transferal to a polyvinylidene fluoride membrane, immunoblot analyses with anti-P23k antibody were performed according to the manufacturer's (Amersham Pharmacia) instructions. Total protein was detected using Coomassie Brilliant Blue staining as a control.

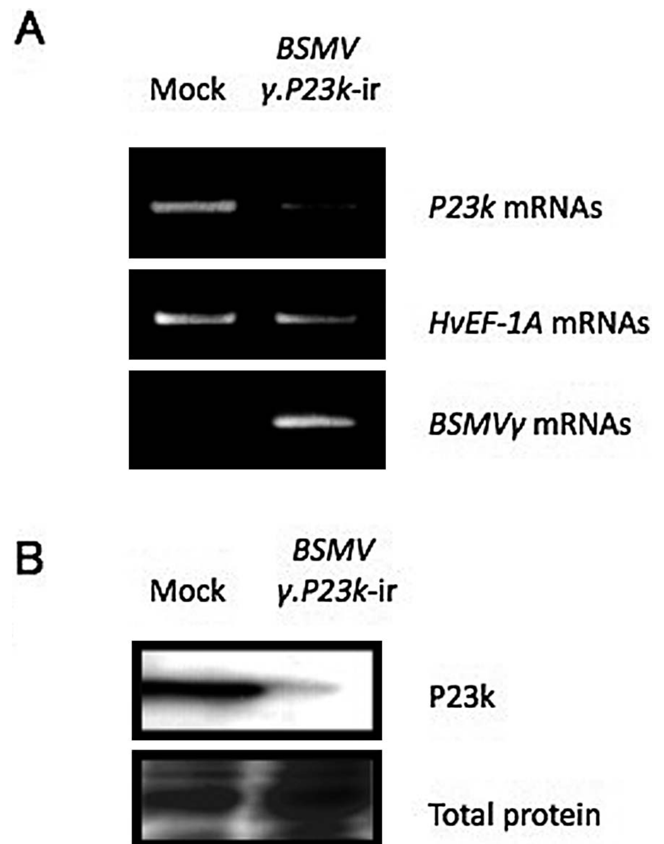

Fig. 2. Expression of *P23k* mRNAs and P23k proteins in *BSMV.γP23k*-ir systemic barley. FES buffer alone was used for mock inoculated controls. (A) RT-PCR analysis with gene-specific primers. *HvEF-1A* mRNAs were analyzed as a control. *BSMVγ* mRNAs were analyzed to investigate the infection of BSMV. (B) Immunoblot analysis using an anti-P23k antibody. These experiments were repeated three times with independent leaves.

Histochemical analysis by in situ hybridization

Selected BSMV uninfected barley tissues were fixed for 5 h at room temperature in a fixative solution (3.7% formaldehyde, 5% acetic acid and 50% ethanol). Fixed tissues were dehydrated in a series of ethanol and xylene solutions and embedded in paraffin (Paraplast Plus, Sigma). Embedded tissues were then sectioned at a thickness of 7 µm and placed on poly-L-lysine coated microslide glass (Matsunami). Sections were deparaffinized with xylene and rehydrated through a graded ethanol series. They were subsequently pretreated with proteinase K (Boehringer Mannheim) at 37°C for 30 min, dehydrated in a graded ethanol series, and dried under vacuum for 1 h. For the preparation of probes, a partial *p23k-1* cDNA clone (Kidou *et al.* 2006) was used. *P23k* sense and antisense probes were generated by *in vitro* transcription using a DIG RNA Labeling Kit (Boehringer Mannheim). Hybridization signals were detected using a DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Histochemical localization of cell wall polysaccharides

The deparaffinized sections were treated with 0.005% aqueous calcofluor (fluorescent brightener 28; Sigma) or 0.5% aqueous toluidine blue O, and visualized with a fluorescence microscope (Olympus, Japan).

Results

The construction and molecular assessment in VIGS of P23k

To address the relationship between P23k function and barley cell wall formation during the reproductive stage, we attempted to silence *P23k* expression in barley by BSMV-based VIGS. The efficiency of *P23k* VIGS based on its nucleotide sequence was previously confirmed by VIGS in barley leaves (Oikawa *et al.* 2007).

To gain better insight into the level of *P23k* gene silencing, we examined the expression of *P23k* at both the transcriptional and translational levels. RT-PCR analyses revealed the reduced expression of *P23k* mRNAs and the amplified *BSMVγ* mRNAs in *P23k* VIGS barley (Fig. 2A). Detection of the *BSMVγ* gene suggests that *BSMVγ.P23k*-ir is infected with *P23k* VIGS barley. Furthermore, immunoblotting using an anti-P23k antibody did not detect any P23k protein in

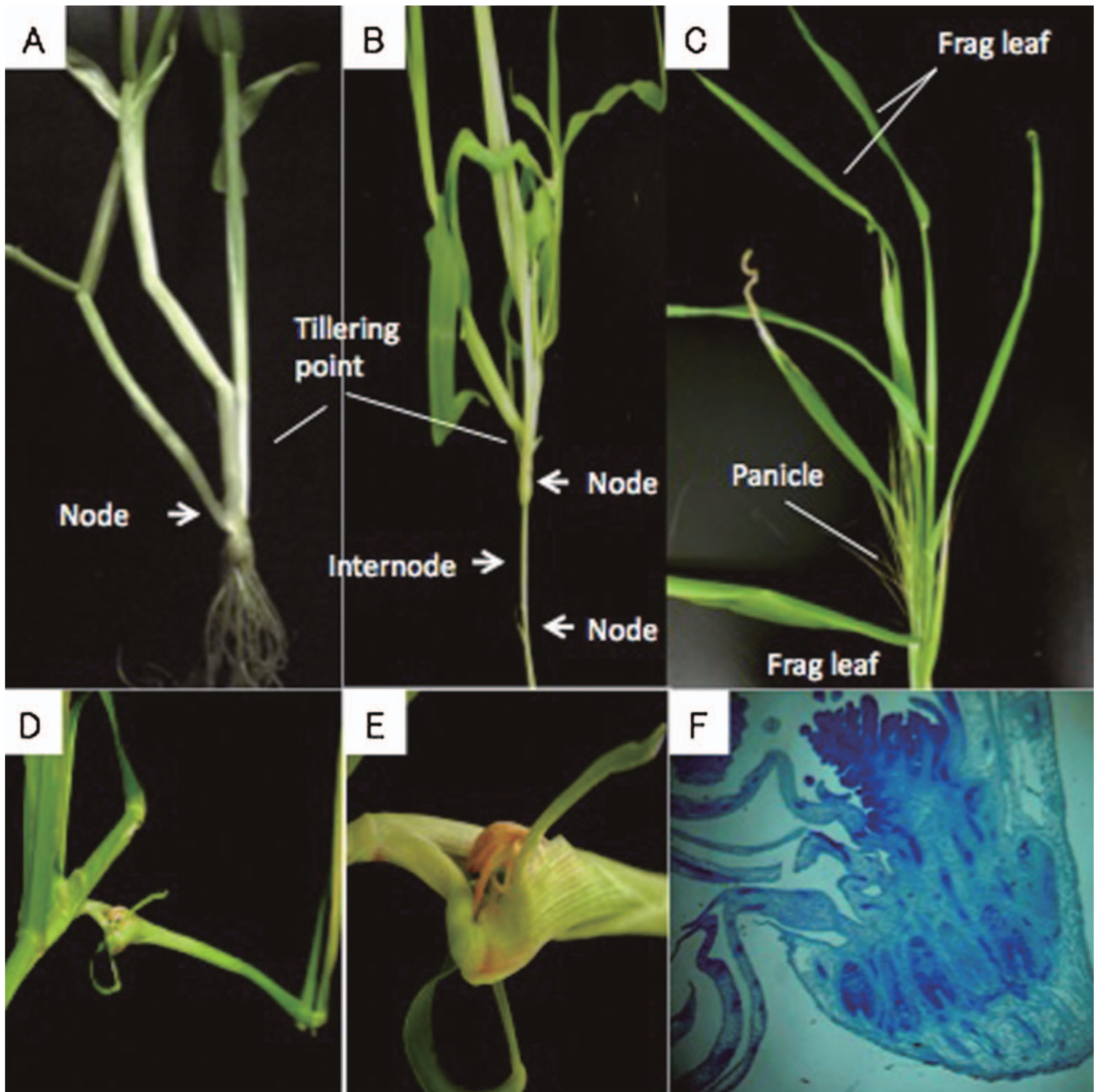

Fig. 3. *Barley striped mosaic virus*-based VIGS of *P23k* and phenotypic changes in barley. Virus symptoms were recorded at 20 to 40 days post-inoculation in *BSMV.γP23k*-ir. (A) Normal tillering of BSMV uninfected barley. (B) Abnormal tillering from the upper, elongated part of the stem. The leaf blades and leaf sheaths developed from below the elongated stem were removed to show the morphological abnormality. (C) Abnormal sequential tillering and arrested heading of the panicle. (D) Abnormal stem with growth-arrested leaves. (E) Higher-magnification image of the abnormal stem. (F) Micrograph of a longitudinal section through the abnormal stem.

the *P23k* VIGS barley (Fig. 2B). These results indicated that VIGS of *P23k* triggered by *BSMVγ.P23k*-ir led to the complete down-regulation of P23k expression at both the transcriptional and translational levels.

Formation of additional tillers in the upper region of the stem and suppression of intercalary elongation by P23k VIGS treatment

In monocots, tillers normally form only at nodes of the lower, unelongated stem region and fail to develop from the upper, elongated portion of the stem (Fig. 1 and Fig. 3A). However, formation of additional tillers was stimulated above the sixth internode by *P23k* VIGS treatment (Fig. 3B). Another phenotype observed in the heading stage is illustrated in Fig. 3C. Although some shoots produced flag leaves, subsequent heading of the panicle from the additional tiller was arrested. In addition, *P23k* VIGS resulted in development of an abnormal organ from some of the

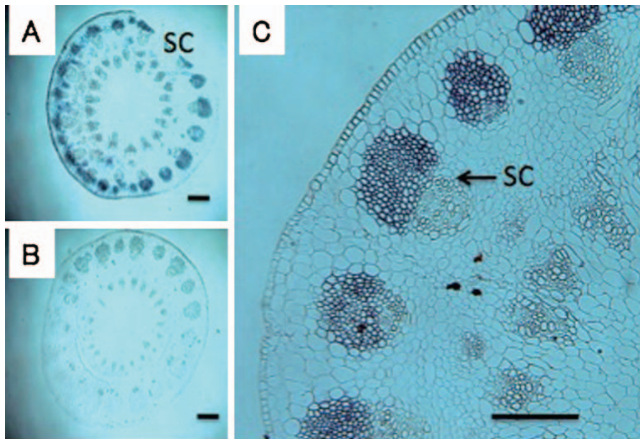

Fig. 4. Localization of *P23k* mRNA in the basal region of the internode of the upper, elongated part of the stem. *In situ* hybridization analysis using sense (B) and antisense (A, C) probes was carried out to detect *P23k* mRNA. (C) is a higher-power magnification of the image in (A). SC: sclerenchyma cell. Scale bar is 0.5 mm.

growth-arrested leaves (Fig. 3D and 3E). Figure 3F shows a micrograph of a longitudinal section of this organ. The spikelet was observed within the organ. However, subsequent intercalary elongation was inhibited in the spikelet. These phenotypes were observed in 10 out of 30 *P23k* VIGS barley plants.

Tissue-specific expression of P23k in the stem at the reproductive stage of BSMV uninfected barley

In situ hybridization analyses were performed to examine *P23k* expression in the upper, elongated portion of the stem. *P23k* mRNA was detected in developing sclerenchyma and vascular bundle cells in the basal region of internodes in elongated stems (Fig. 4A and 4C), but was not detected after hybridization using *P23k* sense probes (Fig. 4B). The basal region of internodes in elongated stems is known to be an area where cell wall deposition is active (Morrison *et al.* 1998), suggesting that *P23k* expression might be required for cell wall polysaccharide synthesis in the elongated stem.

Virus-induced gene silencing of P23k affects polysaccharide synthesis

The phenotype of *P23k* VIGS plants and the cellular expression pattern of *P23k* in the elongated stem prompted us to hypothesize that P23k contributes to intercalary elongation and tiller inhibition through modulating cell wall polysaccharide synthesis. To test this hypothesis, we histochemically compared the cell wall polysaccharide content in control and *P23k* VIGS barley with calcofluor, an authentic fluorescent dye that detects β-glucans (Fig. 5A). In *P23k* VIGS plants, a reduction in fluorescence intensity was observed compared with the control in transverse sections of the stem, suggesting that *P23k* VIGS causes a reduction in the amount of cell wall β-glucans.

Calcofluor stains β-glucans including cellulose, callose and (1,3;1,4)-β-D-glucan. To assess which type of polysaccharide synthesis was affected by *P23k* VIGS, we performed RT-PCR analyses with primers specific for polysaccharide synthesis-related genes. Interestingly, specific reduction in expression of the barley (1,3;1,4)-β-D-glucan synthase gene (*HvCslF*) was observed in the stem of *P23k* VIGS plants, while the expression of cellulose synthase (*HvCesA*) and callose synthase (*HvGSL*) were unaffected (Fig. 5B). These results indicate that P23k might be involved in (1,3;1,4)-β-D-glucan synthesis.

Discussion

Virus-induced gene silencing is a recently developed mRNA suppression technique used to characterize the function of plant genes. The generation of stable transformants is not required and the technique allows the characterization of phenotypes that might otherwise be lethal in stable transformants. Therefore, VIGS is particularly promising as a tool for the study of developmental and morphogenesis-related genes. *Barley striped mosaic virus*-based vectors have been used for VIGS in barley, and many genes have been successfully silenced in leaves (Holzberg *et al.* 2002, Hein *et al.* 2005, Scofield *et al.* 2005).

In our previous study, the *P23k* VIGS in barley leaves resulted in morphological changes involved in secondary wall

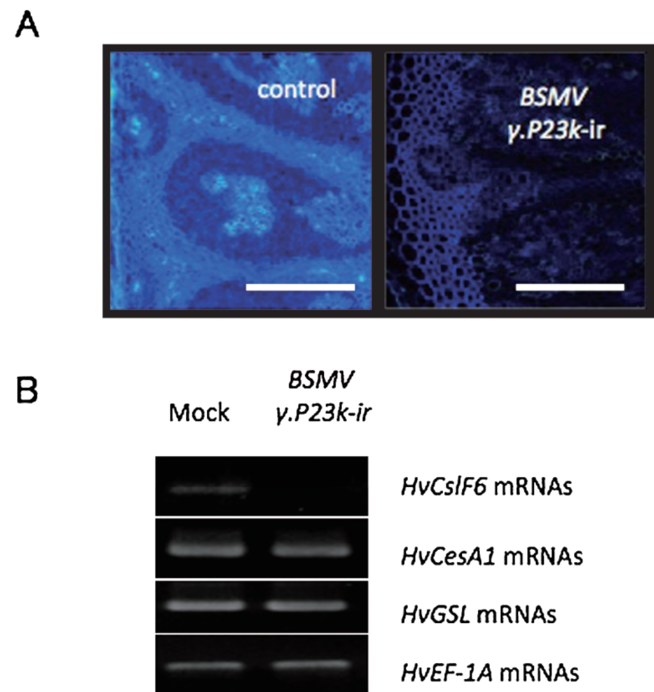

Fig. 5. Alteration of the polysaccharide content and expression of polysaccharide synthesis-related genes in barley infected with *BSMV.γP23k*-ir. (A) Histochemical staining of polysaccharides by calcofluor. Scale bar is 0.5 mm. (B) Expression patterns of cell wall polysaccharide-related genes. Gene-specific primers were used for the amplification of *HvCslF6*, *HvCesA1*, *HvGSL* and *HvEF-1A*. Products were visualized by EtBr staining. FES buffer alone was used for mock inoculated controls.

formation (Oikawa *et al.* 2007). In addition, histochemical analysis indicated that the distribution of P23k in leaves coincided with that of cell wall β-glucans. These results suggested that P23k might be involved in the synthesis of β-glucans and contributes to secondary wall formation in barley leaves. In the present study, we provide further evidence supporting the involvement of P23k in the synthesis of cell wall polysaccharides. First, the expression analyses showed that *P23k* mRNAs are localized in the sclerenchyma tissues where cell wall polysaccharides are abundant (Fig. 4). Second, the comparison of calcofluor fluorescence intensity showed that a significant decrease in the amount of β-glucans coincided with the silencing of *P23k* expression by VIGS (Fig. 5A). Third, additional tiller and spikelet formation and the inhibition of intercalary elongation in the newly formed spikelets were stimulated by *P23k* VIGS treatment (Fig. 3). Considering that the growth of inflorescence stems and leaves are highly influenced by cell wall synthesis (Somerville, 2006, Brown *et al.* 2007), the stunted intercalary elongation observed in *P23k* VIGS plants is consistent with the role of P23k in secondary cell wall synthesis. *Barley striped mosaic virus* infection alone did not cause these abnormal phenotypes. This is supported by the fact that no phenotypic alteration was observed in BSMV-infected VIGS-*GFP* experiments (Oikawa *et al.* 2007). Further control experiments may be required to evaluate the reliability of these results since we did not use an empty vector as a control in this VIGS experiment.

Plant cell walls are mainly composed of cellulose microfibrils and a diversity of polysaccharides collectively designated as hemicellulose. It is known that the type and content of hemicellulose differs among plants. In barley, (1,3;1,4)-β-D-glucan is a major component of hemicellulose in the primary walls and is also detected in secondary walls (Trethewey and Harris 2002). The (1,3;1,4)-β-D-glucan is unique to grasses (Gramineae), and is considered to act as a key component in both the construction and disassembly of cell wall architecture (Hoson *et al.* 2002, Buckeridge *et al.* 2004) in those plants. Carpita (1996) also reported that (1,3;1,4)-β-D-glucan is a key component in the regulation of development in grasses. Recently, cellulose synthase-like *CslF* and *CslH* genes were identified as key genes for the synthesis of cell wall (1,3;1,4)-β-D-glucan (Burton *et al.* 2006, Doblin *et al.* 2009). These authors generated transgenic *Arabidopsis* expressing *CslF* or *CslH* genes and confirmed (1,3;1,4)-β-D-glucan synthesis using specific monoclonal antibodies and enzymatic analysis. It provided direct evidence for the participation of rice *CslF* and *CslH* genes in (1,3;1,4)-β-D-glucan biosynthesis, as (1,3;1,4)-β-D-glucan is absent from the cell walls of wild-type *Arabidopsis*. However, with regard to *CslF* genes, the (1,3;1,4)-β-D-glucan content of cell walls in the transgenic *Arabidopsis* was considerably less than 0.1% (w/w). This result might indicate that other genes are necessary for the efficient biosynthesis of (1,3;1,4)-β-D-glucan regulated by *CslF* genes. Interestingly, the *P23k* gene is also specific to grasses, as with *CslF* genes. In addition, expression of the *P23k* gene is higher in barley than in other grasses, such as wheat and rice, in proportion to the (1,3;1,4)-β-D-glucan content in their endosperm walls (Stone and Clark 1992). Hence, we consider that P23k might participate in (1,3;1,4)-β-D-glucan biosynthesis.

The decreased β-glucan amount in *P23k* VIGS plants, as indicated by comparative calcofluor fluorescence intensity (Fig. 5A), and specific reduction of barley *CslF* gene expression in the same tissue (Fig. 5B) suggest that P23k might be involved in (1,3;1,4)-β-D-glucan synthesis. In rice seedlings, the molecular size and total amount of (1,3;1,4)-β-D-glucan affect coleoptile elongation (Chen *et al.* 1999). Sauter and Kende (1992) hypothesized that elevated levels of (1,3;1,4)-β-D-glucan contribute to elongation growth of internodes by increasing the extensibility of the cell wall in deepwater rice. We confirmed the similar distribution of *CslF6* gene expression with *P23k* in barley seedlings (data not shown), which suggested that *P23k* expression might be involved in the regulation of (1,3;1,4)-β-D-glucan synthesis at the transcriptional level and that it contributes to morphogenetic regulation unique to cereals, such as internode elongation of the stem, although expression of *P23k* does not seem to be correlated with intercalary elongation necessarily.

In this study, we demonstrated the efficiency and robustness of the BSMV-based VIGS system during reproductive development by silencing *P23k*. Virus-induced gene silencing of *P23k* resulted in abnormal tiller formation and inhibition of intercalary elongation. The morphological data, together with the *P23k* expression data, suggest that P23k is involved in cell wall polysaccharide synthesis, especially the synthesis of (1,3;1,4)-β-D-glucan, which is unique to grasses. Further study of the cell wall chemistry of *P23k* VIGS barley will provide greater insight into the specific relationship between P23k and (1,3;1,4)-β-D-glucan metabolism.

Acknowledgments

We thank Dr. G.P. Pogue for providing the BSMV γRNA-based vectors for VIGS. We also thank Dr. Kouki Yoshida (Taisei Co.) and Dr. Abidur Rahman (Iwate University) for useful discussions and advice. This work was supported by the Genomic Agricultural Innovation Program of MAFF (TRC1006).

Literature Cited

Benkova, E., M. Michniewicz, M. Sauer, T. Teichmann, D. Seifertova, G. Jurgens and J. Friml (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115: 591–602.

Bleecker, A.B., J.L. Schuette and H. Kende (1986) Anatomical analysis of growth and developmental pattern in the internode of deepwater rice. Planta 196: 490–497.

Brown, D.M., F. Goubet, V.W. Wong, R. Goodacre, E. Stephens, P. Dupree and S.R. Turner (2007) Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan

synthesis. The Plant J. 52: 1154–1168.

Buckeridge, M.S., C. Rayolio, B. Urbanowicz, M.S. Aurelio and N.C. Carpita (2004) Mixed linkage (1→3),(1→4)-β-D-glucans of grasses. Cereal Chem. 81: 115–127.

Burton, R.A., S.M. Wilson, M. Hrmova, A.J. Harvey, N.J. Shirley, A. Medhurst, B.A. Stone, E.J. Newbigin, A. Bacic and G.B. Fincher (2006) Cellulose synthase-like CslF genes mediate the synthesis of cell wall (1,3;1,4)-β-D-glucans. Science 311: 1940–1942.

Carpita, N.C. (1996) Structure and biogenesis of the cell walls of grasses. Annu. Rev. Plant Physiol. Plant Molec. Biol. 47: 445–476.

Carraro, N., C. Forestan, S. Canova, J. Traas and S. Varotto (2006) ZmPIN1a and ZmPIN1b encode two novel putative candidates for polar auxin transport and plant architecture determination of maize. Plant Physiol. 142: 254–264.

Chen, L., S. Kamisaka and T. Hoson (1999) Suppression of (1→3),(1→4)-β-D-glucan turnover during light-induced inhibition of rice coleoptil growth. J. Plant Res. 112: 7–13.

Doblin, M., F.A. Pettolino, S.M. Wilson, R. Campbell, R.A. Burton, G.B. Fincher, E. Newbigin and A. Bacic (2009) A barley *cellulose synthase-like CSLH* gene mediates (1,3;1,4)-β-glucan synthesis in transgenic *Arabidopsis*. Proc. Nat. Acad. Sci. USA 106: 5996–6001.

Evans, P.S. (1965) Intercalary growth in the aerial shoot of *Eleocharis acuta* R. Br. Prodr.: I. Structure of growing zone. Ann. Bot. 29: 205–217.

Fisher, J.B. (1970) Development of the intercalary meristem of cyperus alternifolius. Amer. J. Bot. 57: 691–703.

Hein, I., M. Barciszewska-Pacak, K. Hrubikova, S. Williamson, M. Dinesen, I.E. Soenderby, S. Sundar, A. Jarmolowski, K. Shirasu and C. Lacomme (2005) Virus-induced gene silencing-based functional characterization of genes associated with powdery mildew resistance in barley. Plant Physiol. 138: 2155–2164.

Holzberg, S., P. Brosio, C. Gross and G.P. Pogue (2002) Barley stripe mosaic virus-induced gene silencing in a monocot plant. The Plant J. 30: 315–327.

Hoson, T. (2002) Physiological functions of plant cell coverings. J. Plant Res. 115: 277–282.

Kende, H., E. Knaap and H.T. Cho (1998) Deepwater rice: a model plant to study stem elongation. Plant Physiol. 118: 1105–1110.

Kidou, S., A. Oikawa, N. Sasaki, H. Yasuda, T. Yamashita, H. Koiwa, K. Kato and S. Ejiri (2006) Identification of a 23 KD protein (P23k) related to the sugar supply in germinating seed. Plant Biotech. 23: 357–364.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Li, X., Q. Qian, Z. Fu, Y. Wang, G. Xiong, D. Zeng, X. Wang, X. Liu, S. Teng, H. Fujimoto, M. Yuan, D. Luo, B. Han and J. Li (2003) Control of tillering in rice. Nature 422: 618–621.

Morrison, T.A., H.G. Jung, D.R. Buxton and R.D. Hatfield (1998) Cell-wall composition of maize internodes of varying maturity. Crop Sci. 38: 455–460.

Nemoto, K., I. Nagano, T. Hogetsu and N. Miyamoto (2004) Dynamic of cortical microtubules in developing maize internodes. New Phytol. 162: 95–103.

Oikawa, A., A. Rahman, T. Yamashita, H. Taira and S. Kidou (2007) Virus-induced gene silencing of P23k in barley leaf reveals morphological changes involved in secondary wall formation. J. Exp. Bot. 58: 2617–2625.

Paredez, A.R., C.R. Somerville and D.W. Ehrhardt (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312: 1491–1495.

Pogue, G.P., J.A. Lindbo, W.O. Dawson and T.H. Turpen (1998) Tobamovirus transient expression vectors: tools for plant biology and high-level expression of foreign proteins in plants. *In*: Gelvin, S.B. and R.A. Schilperoot (eds.) Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 1–27.

Reinhardt, D., T. Mandel and C. Kuhlemeier (2000) Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12: 507–518.

Reinhardt, D., E.R. Pesce, P. Stieger, T. Mandel, K. Baltensperger, M. Bennett, J. Traas, J. Friml and C. Kuhlemeier (2003) Regulation of phyllotaxis by polar auxin transport. Nature 426: 255–260.

Sauter, M. and H. Kende (1992) Levels of β-glucan and lignin in elongating internodes of deepwater rice. Plant Cell Physiol. 33: 1089–1097.

Schmitz, G., E. Tillmann, F. Carriero, C. Fiore, F. Cellini and K. Theres (2002) The tomato Blind gene encodes a MYB transcription factor that controls the formation of lateral meristems. Proc. Natl. Acad. Sci. USA 99: 1064–1069.

Scofield, S.R., L. Huang, A.S. Brandt and B.S. Gill (2005) Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the Lr21-mediated leaf rust resistance pathway. Plant Physiol. 138: 2165–2173.

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

Sorefan, K., J. Booker, K. Haurogne, M. Goussot, K. Bainbridge, E. Foo, S. Chatfield, S. Ward, C. Beveridge, C. Rameau and O. Leyser (2003) MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. Genes Dev. 17: 1469–1474.

Stone, B.A. and A.E. Clark (1992) The chemistry and biology of (1→3)-β-D-glucans. Melbourne: La Trobe University Press.

Taiz, L. (1984) Plant cell expansion: regulation of cell wall mechanical properties. Annu. Rev. Plant Physiol. 35: 585–657.

Takeda, T., Y. Suwa, M. Suzuki, H. Kitano, M. Ueguchi-Tanaka, M. Ashikari, M. Matsuoka and C. Ueguchi (2003) The OsTB1 gene negatively regulates lateral branching in rice. The Plant J. 33: 513–520.

Talmadge, K.W., K. Keegstra, W.D. Bauer and P. Albersheim (1973) The structure of plant cell walls I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. Plant Physiol. 51: 158–173.

Talbert, P.B., H.T. Adler, D.W. Parks and L. Comai (1995) The REVOLUTA gene is necessary for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana*. Development 121: 2723–2735.

Tonooka, T., E. Aoki, T. Yoshioka and S. Taketa (2009) A novel mutant gene for (1-3,1-4)-β-D-glucanless grain on barley (*Hordeum vulgare* L.) chromosome 7H. Breed. Sci. 59: 47–54.

Trethewey, J.A.K. and P.J. Harris (2002) Localization of (1→3)- and (1→3),(1→4)-β-D-glucans in vegetative cell walls of barley (*Hordeum vulgare*) using immunogold labelling. New Phytol. 154: 347–358.

Xu, M., L. Zhu, H. Shou and P. Wu (2005) A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant Cell Physiol. 46: 1674–1681.