

Modulation of Rice Leaf Angle

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Leaf angle, the inclination between the leaf blade midrib and the stem, is one of the most important canopy parameters in plant architecture that influence light interception, photosynthetic efficiency, and planting density. Thus, ideal plant architecture with an erect leaf angle and optimum leaf orientation allows for more efficient light capture during photosynthesis and better wind circulation under dense planting conditions.

Generally, rice leaf angle is determined by the elongation and/or division of lamina joint cells. Newly isolated rice genes (*OsBCL1* and *OsBCL2*) are positive regulators in the elongation of lamina joint cells and their potential for regulating rice leaf angle has been examined in this research.

Keywords: bHLH ; cell elongation ; leaf inclination ; lamina joint ; transcription factor ; rice

1. Overview

Leaf angle and grain size are important agronomic traits affecting rice productivity directly and/or indirectly through modulating crop architecture. *OsBC1*, as a typical bHLH transcription factor, is one of the components comprising a complex formed with LO9-177 and *OsBUL1* contributing to modulation of rice leaf inclination and grain size. In the current study, two homologues of *OsBC1*, *OsBCL1* and *OsBCL2* were functionally characterized by expressing them under the control of *OsBUL1* promoter, which is preferentially expressed in the lamina joint and the spikelet of rice. Increased leaf angle and grain length with elongated cells in the lamina joint and the grain hull were observed in transgenic rice containing much greater gibberellin A₃ (GA₃) levels than WT, demonstrating that both *OsBCL1* and *OsBCL2* are positive regulators of cell elongation at least partially through increased GA biosynthesis. Moreover, the cell elongation was likely due to cell expansion rather than cell division based on the related gene expression and, the cell elongation-promoting activities of *OsBCL1* and *OsBCL2* were functional in a dicot species, *Arabidopsis*.

2. Rice Leaf Angle

Rice leaf angle, the degree of bending between the leaf blade and culm, is a critical factor affecting plant architecture and grain yield ^{[1][2]}. In general, crops with erect leaves have increased photosynthetic efficiency and nitrogen storage for grain filling and are suitable for dense planting ^[3]. Many genes or *quantitative* trait loci (QTLs) such as *D61/OsBRI1*, *IL11*, *LC2*, *ILA1*, *RAV6*, *OsARF19*, and *SLG* have been reported to control leaf angle ^{[1][4][5][6][7][8][9]}. Most rice mutants identified with altered leaf inclination have abnormal cell division and/or expansion and altered cell wall composition at the lamina joint ^{[1][5][10][11]}. In addition, phytohormones occupy an important place in the regulatory layers for rice leaf inclination ^[12]. In general, brassinosteroid (BR) affects grain size, leaf angle, and yield potential in rice. Moreover, leaf inclination is a distinctive BR-responsive architectural trait and BR-deficient or -insensitive mutants produce erect leaves in rice ^{[4][13][14][15]} while the exogenous application of BR or the genetic enhancement of BR signaling results in increased leaf inclination ^{[16][17][18]}. BR is known to affect cell elongation and/or cell division and both cell number and cell size are key factors mainly determining the size of each organ during plant development ^{[19][20]}.

In rice, BR and gibberellin (GA) are two major phytohormones affecting plant height and leaf angle by regulating cell growth ^{[21][22]} and the crosstalk between the two phytohormones is mediated by complex networks; they interact at the signaling level as well as at the biosynthesis regulation level and the crosstalk can be distinct based on hormone concentrations, developmental stages, and different tissues even within a species ^{[21][23]}. For example, BR signaling mutants are impaired in GA biosynthesis ^[24] while GA regulates BR biosynthesis at the transcriptional level in rice. A negative regulator of the GA signaling pathway, *OsSPY* represses BR biosynthesis ^[13] and may also negatively regulate BR signaling by enhancing DELLA-BZR1 interaction ^[24]. Furthermore, a positive regulator of GA signaling, *OsGSR1* activates BR synthesis through direct interaction with the BR biosynthesis enzyme, DWF1 ^[25]. *Oryza sativa Dicer-like 3a* (*OsDCL3a*) involved in the GA pathway has also been identified to regulate leaf inclination in rice ^[26] and impaired *OsDCL3a* expression by RNA interference caused increased leaf angle by modulating the expression of GA and BR

associated genes, including *OsGSR1* and *BRD1*. Recent evidence emerging from a rice microRNA studies suggests that *OsmiR396d* also supports the notion that BR-GA co-regulation is implicated in leaf inclination [27].

Basic-helix-loop-helix (bHLH) proteins form the second largest family of transcription factors in plants, where they play key roles in critical metabolic, physiological and developmental processes [28]. In particular, 167 bHLH proteins have been identified in rice. These proteins can be divided into two groups, the atypical non-DNA-binding and the typical DNA-binding bHLH family based on to their DNA-binding activity [29], and they can be positive or negative growth regulators by interacting in an antagonistic and redundant manner to regulate various biological processes involved in growth including cell elongation, biosynthesis, stress resistance and signal transduction pathways [2][5][30][31][32][33][34].

It has also been reported that some bHLH proteins are involved in hormone biosynthesis and/or signaling in plants [2][34][35][36][37]. *Arabidopsis* PIF3, PIF4, PIF5, and PIF3-LIKE 5 (PIL5) are involved in the GA biosynthesis and signaling pathway [35][37] and the function of rice *OsbHLH073* is associated with GA biosynthesis [34]. Other bHLH proteins play important roles in controlling BR signaling: *Arabidopsis* BEE1, BEE2, and BEE3, as products of early response genes are required for full BR response [35] and AIF2 interacts with BIN2 to participate in the BR signaling pathway [38]. In rice, INCREASED LAMINAR INCLINATION (ILI) and ILI1 BINDING bHLH1 (IBH1), regulate cell elongation in the lamina joint, affecting leaf bending in rice under BR induction [5]. Moreover, a trimeric complex formed by BRASSINOSTEROID UPREGULATED1-LIKE1 (*OsBUL1*), an atypical bHLH protein and *OsBUL1* COMPLEX1 (*OsBC1*), a typical bHLH protein bridged by a small KxDL motif-containing protein, LO9-177 responds to BR signaling and regulates leaf inclination in rice [2]. Recently, *OsBLR1* (known as *OsbHLH079*) has been identified as a positive regulator of BR signaling for determining leaf angle and grain shape [11][39].

Here, we found that *OsBC-Like1* (*OsBCL1*, Os08g42470, *OsbHLH080*; [40]) and *OsBC-Like2* (*OsBCL2*, Os02g47660, *OsBLR1*, *OsbHLH079*; [11][39][40]), homologues of *OsBC1* (Os09g33580, *OsbHLH081*; [2][40]) function as positive regulators in cell elongation of laminar joints and grains in rice via, at least, increased GA biosynthesis. In spite of high sequence similarity between *OsBCL1* and *OsBCL2* at the protein level, distinct protein interaction patterns with putative partners are observed in the yeast two-hybrid system. Overexpression of *OsBCL1* and *OsBCL2* under the control of *OsBUL1* promoter driving lamina joint- and panicle-preferential gene expression, caused increased leaf angle and grain size. Furthermore, ectopic expression of the two genes in the dicot plant, *Arabidopsis*, resulted in narrow leaves with elongated epidermal cells confirming that *OsBCL1* and *OsBCL2* contribute to cell elongation in both monocot and dicot plants.

3. Conclusions

Ectopic expression of *OsBCL1* and *OsBCL2* under the control of *OsBUL1* promoter [2], which drives the gene expression to seedlings, lamina joints, nodes and panicles preferentially caused increased leaf angle in rice through the elongation of cells in lamina joints. In addition, increased length of internodes and grains has also been observed in transgenic rice plants. These phenotypic alterations are reminiscent of transgenic rice plants containing *pOsBUL1:OsBC1* [2]. Moreover, their spatiotemporal patterns of transcript abundance and GUS expression, particularly in the lamina joints and floral organs, are similar to the previous results gained from *OsBC1*. Thus, these results support the notion that *OsBC1*, *OsBCL1* and *OsBCL2* may play a critical and, at least, partially redundant role in cell elongation throughout rice development. In particular, the highest sequence similarity detected between *OsBC1* and *OsBCL1* at the protein level and their overlapping expression patterns strongly indicate that *OsBCL1* is the closest homologue of *OsBC1*. Based on the elongation of cells in the lamina joint and the lemma/palea in transgenic rice plants containing *pOsBUL1:OsBC1* and *pOsBUL1:OsBCL2*, endogenous levels of BR and GA₃, two predominant phytohormones regulating plant cell elongation have been measured. A significant increase in GA₃ was detected only in transgenic rice seedlings with *pOsBUL1:OsBCL1* and *pOsBUL1:OsBCL2*, while BR was not detectable in either WT or transgenic plants. It seems that the BR level in rice plants is below the limit of the detection system we used. Expression level of genes involved in GA biosynthesis was indeed higher in the transgenic seedlings compared to WT. Furthermore, expression level of genes involved in cell expansion including *OsEXPA3* and *OsEXPA4* was significantly increased in the transgenic plants compared to the WT whereas expression level of cell cycle genes such as *OsCDC6* and *OsMCM3* was indistinguishable from that of WT, supporting the notion that the larger leaf angles are mostly due to cell elongation/expansion rather than the increased number of cells in the lamina joint. Of note, however, only *OsBCL1* was upregulated by exogenous GA₃ treatment for 24 h, indicating that distinct regulation of gene expression can be attained by exogenously applying GA₃ among the three homologous genes, *OsBC1*, *OsBCL1* and *OsBCL2* [2].

References

1. Zhao, S.-Q.; Hu, J.; Guo, L.-B.; Qian, Q.; Xue, H.-W. Rice leaf inclination2, a VIN3-like protein, regulates leaf angle through modulating cell division of the collar. *Cell Res.* 2010, 20, 935–947.
2. Jang, S.; An, G.; Li, H.-Y. Rice Leaf Angle and Grain Size Are Affected by the OsBUL1 Transcriptional Activator Complex. *Plant Physiol.* 2017, 173, 688–702.
3. Sakamoto, T.; Morinaka, Y.; Ohnishi, T.; Sunohara, H.; Fujioka, S.; Ueguchi-Tanaka, M.; Mizutani, M.; Sakata, K.; Takatsuto, S.; Yoshida, S.; et al. Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nat. Biotechnol.* 2006, 24, 105–109.
4. Yamamuro, C.; Ihara, Y.; Wu, X.; Noguchi, T.; Fujioka, S.; Takatsuto, S.; Ashikari, M.; Kitano, H.; Matsuoka, M. Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. *Plant Cell* 2000, 12, 1591–1606.
5. Zhang, L.Y.; Bai, M.Y.; Wu, J.; Zhu, J.Y.; Wang, H.; Zhang, Z.; Wang, W.; Sun, Y.; Zhao, J.; Sun, X.; et al. Antagonistic HLH/bHLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and Arabidopsis. *Plant Cell* 2009, 21, 3767–3780.
6. Ning, J.; Zhang, B.; Wang, N.; Zhou, Y.; Xiong, L. Increased Leaf Angle1, a Raf-Like MAPKKK That Interacts with a Nuclear Protein Family, Regulates Mechanical Tissue Formation in the Lamina Joint of Rice. *Plant Cell* 2011, 23, 4334–4347.
7. Zhang, X.; Sun, J.; Cao, X.; Song, X. Epigenetic Mutation of RAV6 Affects Leaf Angle and Seed Size in Rice. *Plant Physiol.* 2015, 169, 2118–2128.
8. Zhang, S.; Wang, S.; Xu, Y.; Yu, C.; Shen, C.; Qian, Q.; Geisler, M.; Jiang, D.A.; Qi, Y. The auxin response factor, OsARF19, controls rice leaf angles through positively regulating OsGH3-5 and OsBRI1. *Plant Cell Environ.* 2015, 38, 638–654.
9. Feng, Z.; Wu, C.; Wang, C.; Roh, J.; Zhang, L.; Chen, J.; Zhang, S.; Zhang, H.; Yang, C.; Hu, J.; et al. SLG controls grain size and leaf angle by modulating brassinosteroid homeostasis in rice. *J. Exp. Bot.* 2016, 67, 4241–4253.
10. Mantilla-Perez, M.B.; Salas Fernandez, M.G. Differential manipulation of leaf angle throughout the canopy: Current status and prospects. *J. Exp. Bot.* 2017, 68, 5699–5717.
11. Seo, H.; Kim, S.H.; Lee, B.D.; Lim, J.H.; Lee, S.J.; An, G.; Paek, N.C. The Rice Basic Helix-Loop-Helix 79 (OsBHLH079) Determines Leaf Angle and Grain Shape. *Int. J. Mol. Sci.* 2020, 21, 2090.
12. Zhou, L.-J.; Xiao, L.-T.; Xue, H.-W. Dynamic Cytology and Transcriptional Regulation of Rice Lamina Joint Development. *Plant Physiol.* 2017, 174, 1728–1746.
13. Shimada, A.; Ueguchi-Tanaka, M.; Sakamoto, T.; Fujioka, S.; Takatsuto, S.; Yoshida, S.; Sazuka, T.; Ashikari, M.; Matsuoka, M. The rice SPINDLY gene functions as a negative regulator of gibberellin signaling by controlling the suppressive function of the DELLA protein, SLR1, and modulating brassinosteroid synthesis. *Plant J.* 2006, 48, 390–402.
14. Tong, H.; Liu, L.; Jin, Y.; Du, L.; Yin, Y.; Qian, Q.; Zhu, L.; Chu, C. DWARF AND LOW-TILLERING Acts as a Direct Downstream Target of a GSK3/SHAGGY-Like Kinase to Mediate Brassinosteroid Responses in Rice. *Plant Cell* 2012, 24, 2562–2577.
15. Sun, S.; Chen, D.; Li, X.; Qiao, S.; Shi, C.; Li, C.; Shen, H.; Wang, X. Brassinosteroid signaling regulates leaf erectness in *Oryza sativa* via the control of a specific U-type cyclin and cell proliferation. *Dev. Cell* 2015, 34, 220–228.
16. Wu, C.-y.; Trieu, A.; Radhakrishnan, P.; Kwok, S.F.; Harris, S.; Zhang, K.; Wang, J.; Wan, J.; Zhai, H.; Takatsuto, S.; et al. Brassinosteroids regulate grain filling in rice. *Plant Cell* 2008, 20, 2130–2145.
17. Hong, Z.; Ueguchi-Tanaka, M.; Fujioka, S.; Takatsuto, S.; Yoshida, S.; Hasegawa, Y.; Ashikari, M.; Kitano, H.; Matsuoka, M. The Rice brassinosteroid-deficient dwarf2 mutant, defective in the rice homolog of Arabidopsis DIMINUTO/DWARF1, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, dolichosterone. *Plant Cell* 2005, 17, 2243–2254.
18. Li, D.; Wang, L.; Wang, M.; Xu, Y.Y.; Luo, W.; Liu, Y.J.; Xu, Z.H.; Li, J.; Chong, K. Engineering OsBAK1 gene as a molecular tool to improve rice architecture for high yield. *Plant Biotechnol. J.* 2009, 7, 791–806.
19. Potter, C.J.; Xu, T. Mechanisms of size control. *Curr. Opin. Genet. Dev.* 2001, 11, 279–286.
20. Sugimoto-Shirasu, K.; Roberts, K. “Big it up”: Endoreduplication and cell-size control in plants. *Curr. Opin. Plant Biol.* 2003, 6, 544–553.
21. Tong, H.; Xiao, Y.; Liu, D.; Gao, S.; Liu, L.; Yin, Y.; Jin, Y.; Qian, Q.; Chu, C. Brassinosteroid regulates cell elongation by modulating gibberellin metabolism in rice. *Plant Cell* 2014, 26, 4376–4393.

22. Zhang, C.; Bai, M.-y.; Chong, K. Brassinosteroid-mediated regulation of agronomic traits in rice. *Plant Cell Rep.* 2014, 33, 683–696.
23. Tong, H.; Chu, C. Reply: Brassinosteroid Regulates Gibberellin Synthesis to Promote Cell Elongation in Rice: Critical Comments on Ross and Quittenden's Letter. *Plant Cell* 2016, 28, 833–835.
24. Ross, J.J.; Quittenden, L.J. Interactions between Brassinosteroids and Gibberellins: Synthesis or Signaling? *Plant Cell* 2016, 28, 829–832.
25. Wang, L.; Wang, Z.; Xu, Y.; Joo, S.H.; Kim, S.K.; Xue, Z.; Xu, Z.; Wang, Z.; Chong, K. OsGSR1 is involved in crosstalk between gibberellins and brassinosteroids in rice. *Plant J.* 2009, 57, 498–510.
26. Wei, L.; Gu, L.; Song, X.; Cui, X.; Lu, Z.; Zhou, M.; Wang, L.; Hu, F.; Zhai, J.; Meyers, B.C.; et al. Dicer-like 3 produces transposable element-associated 24-nt siRNAs that control agricultural traits in rice. *Proc. Natl. Acad. Sci. USA* 2014, 111, 3877–3882.
27. Tang, Y.; Liu, H.; Guo, S.; Wang, B.; Li, Z.; Chong, K.; Xu, Y. OsmiR396d Affects Gibberellin and Brassinosteroid Signaling to Regulate Plant Architecture in Rice. *Plant Physiol.* 2018, 176, 946–959.
28. Pires, N.; Dolan, L. Early evolution of bHLH proteins in plants. *Plant Signal. Behav.* 2010, 5, 911–912.
29. Li, X.; Duan, X.; Jiang, H.; Sun, Y.; Tang, Y.; Yuan, Z.; Guo, J.; Liang, W.; Chen, L.; Yin, J.; et al. Genome-wide analysis of basic/helix-loop-helix transcription factor family in rice and Arabidopsis. *Plant Physiol.* 2006, 141, 1167–1184.
30. Jung, K.-H.; Han, M.-J.; Lee, Y.-S.; Kim, Y.-W.; Hwang, I.; Kim, M.-J.; Kim, Y.-K.; Nahm, B.H.; An, G. Rice Undeveloped Tapetum1 is a major regulator of early tapetum development. *Plant Cell* 2005, 17, 2705–2722.
31. Oikawa, T.; Kyoizuka, J. Two-Step Regulation of LAX PANICLE1 Protein Accumulation in Axillary Meristem Formation in Rice. *Plant Cell* 2009, 21, 1095–1108.
32. Ogo, Y.; Itai, R.N.; Nakanishi, H.; Kobayashi, T.; Takahashi, M.; Mori, S.; Nishizawa, N.K. The rice bHLH protein OslRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. *Plant J.* 2007, 51, 366–377.
33. Yang, X.; Ren, Y.; Cai, Y.; Niu, M.; Feng, Z.; Jing, R.; Mou, C.; Liu, X.; Xiao, L.; Zhang, X.; et al. Overexpression of OsbHLH107, a member of the basic helix-loop-helix transcription factor family, enhances grain size in rice (*Oryza sativa* L.). *Rice* 2018, 11, 41.
34. Lee, J.; Moon, S.; Jang, S.; Lee, S.; An, G.; Jung, K.-H.; Park, S.K. OsbHLH073 Negatively Regulates Internode Elongation and Plant Height by Modulating GA Homeostasis in Rice. *Plants* 2020, 9, 547.
35. Friedrichsen, D.M.; Nemhauser, J.; Muramitsu, T.; Maloof, J.N.; Alonso, J.; Ecker, J.R.; Furuya, M.; Chory, J. Three redundant brassinosteroid early response genes encode putative bHLH transcription factors required for normal growth. *Genetics* 2002, 162, 1445–1456.
36. Lee, S.; Lee, S.; Yang, K.-Y.; Kim, Y.-M.; Park, S.-Y.; Kim, S.Y.; Soh, M.-S. Overexpression of PRE1 and its Homologous Genes Activates Gibberellin-dependent Responses in *Arabidopsis thaliana*. *Plant Cell Physiol.* 2006, 47, 591–600.
37. Paik, I.; Kathare, P.K.; Kim, J.I.; Huq, E. Expanding Roles of PIFs in Signal Integration from Multiple Processes. *Mol. Plant* 2017, 10, 1035–1046.
38. Kim, Y.; Song, J.H.; Park, S.U.; Jeong, Y.S.; Kim, S.H. Brassinosteroid-Induced Transcriptional Repression and Dephosphorylation-Dependent Protein Degradation Negatively Regulate BIN2-Interacting AIF2 (a BR Signaling-Negative Regulator) bHLH Transcription Factor. *Plant Cell Physiol.* 2017, 58, 227–239.
39. Wang, K.; Li, M.-q.; Chang, Y.-p.; Zhang, B.; Zhao, Q.-z.; Zhao, W.-l. The basic helix-loop-helix transcription factor OsBLR1 regulates leaf angle in rice via brassinosteroid signalling. *Plant Mol. Biol.* 2020, 102, 589–602.
40. Wei, K.; Chen, H. Comparative functional genomics analysis of bHLH gene family in rice, maize and wheat. *BMC Plant Biol.* 2018, 18, 309.