A SQUAMOSA promoter binding protein-like transcription factor controls crop ideotype for high productivity in barley

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In cereal crops, erect leaf (small leaf angle) is a desirable trait that allows for dense planting through optimization of light capture, thus increasing photosynthesis efficiency and grain yield with a higher leaf area index. Therefore, understanding the genetic regulation of leaf angles is crucial in the development of high-yielding crop cultivars. In the present study, we cloned the Liguleless 1 (Lig1) gene in barley (Hordeum vulgare subsp. vulgare), which encodes SQUAMOSA promoter binding protein-like 8 (SPL8), elucidating a conserved mechanism underlying regulation of leaf angle in cereals.

Barley is the fourth most important cereal crop and a valuable model monocot for genetic research. A spontaneous mutant in an unknown cultivar, liguleless1 (lig1), is deficient in the formation of the ligule and auricle, exhibiting smaller leaf angles and a compact architecture (Figure 1a–c). An introduced mutant, BW483, was made by introgression of the lig1 mutation into the two-rowed cv. Bowman (Druka et al., 2011). On the single-plant basis, the near-isogenic pair of Bowman and BW483 showed similar yield and agronomic traits, with a slightly later heading in BW483 (Figures 1d and S1) (Druka et al., 2011). An erect leaf phenotype is generally coupled with undesirable agronomic traits, such as reduced grain size, fertility, tiller number, and tolerance to stress (Zhang et al., 2021), and plants with erect leaf phenotypes are only useful if elite yield components are well maintained. Therefore, lig1 provides a valuable resource for yield improvement with dense planting in barley.

To identify the Lig1 gene, we conducted map-based cloning using 257 F2 plants derived from the cross between Bowman and BW483. Of the tested F2 individuals, 196 showed normal plant architecture, and 61 were liguleless. The 3:1 ratio (χ² = 0.219, df = 1, and p = 0.64) suggested that the lig1 mutation is monofactorial recessive. For immediate gene localization and marker discovery, 46 F2 plants (23 each for wild type and liguleless) were genotyped with the barley 50k iSelect SNP Array. Consistent with the previous result (Rossini et al., 2006), the Lig1 gene was anchored to chromosome 2H, and linked SNPs were converted to semi-thermal asymmetric reverse PCR (STARP) markers for fine mapping (Table S1). Cosegregating with the SNP marker M8, the Lig1 gene was delimited within an /C24 290 kb region by M7 and M9 (Figure 1e). Annotation indicated that four protein-coding genes reside within the Lig1 region (Table S2). Of those, HORVU.MOREX.r3.2HG0202650 encodes a putative SQUAMOSA promoter binding protein-like (SPL) transcription factor sharing high similarity to AtSPL8, ZmLg1, OsLG1, and TaSPL8 (Figure S2) (Lee et al., 2007; Liu et al., 2019; Moreno et al., 1997; Unte et al., 2003). Therefore, we named HORVU.MOREX.r3.2HG0202650 HvSPL8.

SPL genes encode plant-specific transcription factors and play vital regulatory roles in various developmental processes, such as the transition from the vegetative to reproductive phase mediated by miR156. As a non-miR156 target, AtSPL8 is a tissue-dependent...
FIGURE 1  Map-based cloning and functional characterization of Lig1. A compact architecture caused by erect leaves was observed in BW483 (a). The auricle, ligule, laminal joint, and leaf midvein are missing in the mutant (b, c). Scale bars in (a)–(c) are 15, 1, and 1 cm, respectively. Heading date was slightly delayed in BW483 (d). Lig1 is located on 2H, delimited to an 0.8 cm region (e). Numbers above the linkage group indicate recombination breakpoints. Four protein-coding genes were identified in the Lig1 region spanning 290 kb (e). The HvSPL8 coding region contains three exons (rectangles) and two introns (straight lines) (f). Various primers indicated by arrows were used to analyze HvSPL8 alleles with two plants each for Bowman and BW483 (f). Sequencing 3′-RACE products revealed a large genomic deletion in the mutant, denoted by a red right-angle arrow (f). Functional validation of HvSPL8 was conducted using CRISRP-mediated mutagenesis. Targeted knockout of HvSPL8 phenocopied the lig1 mutant for the whole plant (g) and a single tiller (h). Localization of Lig1 to the nucleus apparent in overlay of DAPI and GFP images (i–k). Lig1 is highly expressed in the lamina joint (l). HvARF6 genes were downregulated (m, n), but HvD2 expression was unaffected in the mutant (o). Different letters on bar graphs indicate significance at 0.01 level by t test.
regulator affecting reproductive development, trichome formation on sepal, and stem filament elongation (Un et al., 2003). In wheat, TaSPL8 is involved in ligule and auricle development (Liu et al., 2019). OsLg1 also plays a critical role in shaping of a closed panicle and seed shattering during rice domestication, in addition to regulating lamina joint formation (Ishii et al., 2013). Therefore, SPL8 may distinctly function between monocots and dicots. The HvSPL8 gene contains three exons and two introns (Figure 1f). In BW483, we amplified only the first exon and part of intron 1, while the last two exons and the full-length coding region of Hvspl8 were not present (Figure 1f). To capture the mutation in Hvspl8, we conducted 3’-rapid amplification of cDNA ends (RACE) (Figure 1f). Sequencing of RACE products indicated that a ~10 kb genomic deletion (chr5H: 629747100-629757046) occurred at the first intron, and the last two exons were deleted in BW483 (Figure 1f). However, the neighboring gene, HORVU.MOREX.r3.2HG0202640 encoding a putative universal stress family protein, remains intact in the mutant (Figure S3). Therefore, the HvSPL8 gene was selected as the candidate of Lig1.

Using the barley genotype Golden Promise, we targeted the first exon of HvSPL8 for CRISPR-mediated mutagenesis with the Agrobacterium tumefaciens strain AGL1 and the JD633 vector (Debernardi et al., 2020; Harwood, 2014). All recovered T0 or M0 plants (n = 6) were defective in ligule and auricle formation and displayed a compact architecture (Figure 1g.h). DNA sequencing revealed five different mutant alleles, and at least one allele was discovered in each transgenic plant (Figure S4). All mutant alleles disrupted the HvSPL8 function completely. The liguleless phenotype was also confirmed in the T1 or M1 generation (n > 90 plants/transgenic line). The slightly delayed heading of the M1 plants validated that HvSPL8 facilitates reproductive phase transition, although it is not a target for miR156 (Figure S5). Therefore, we concluded that HvSPL8 is indeed the Lig1 gene.

To determine its subcellular localization, we fused HvSPL8 with a green fluorescent protein (GFP) under the CaMV 35S promoter using the pSite-2NB vector. Transient expression of HvSPL8-GFP in Nicotiana benthamiana revealed that the fusion protein was co-localized with 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei (Figure 1i–k), corroborating its role as a transcription factor. The BarTv1.0 transcript dataset has been constructed to spatiotemporally quantify gene expression in different barley tissues (Rapazote-Flores et al., 2019). Overall, low levels of HvSPL8 expression were shown by BarTv1.0 across all the tissues, but relatively higher expression levels were detected in inflorescence tissues, consistent with the involvement of HvSPL8 in reproductive development (Figure S6 and Table S3). Quantitative reverse transcription PCR (qRT-PCR) using 4-week-old seedlings showed that HvSPL8 is highly expressed in the lamina joint where the ligule and auricle form (Figure 1i). The finely-tuned local expression of HvSPL8 is likely the key to the compact architecture without causing other deleterious traits in BW483.

Genetic and functional studies revealed that various genes regulate leaf angle through phytohormone signaling pathways. In rice, disrupted biosynthesis of brassinosteroid (BR) results in a reduced leaf angle, and auxin is also involved in the regulation of leaf angle by modulating secondary cell wall biosynthesis in lamina joint tissues (Hong et al., 2005; Huang et al., 2021). OsARF6 is a critical player in auxin signaling, while OsD2 is a cytochrome P450 whose catalytic activity is required for BR biosynthesis. To determine if the SPL-mediated development of the ligule and auricle involves BR or auxin signaling, we analyzed the expression of HvARF6s and HvD2 (Table S4). Two potential orthologs of AtARF6 were identified in barley, and both of them were downregulated at the lamina joint in BW483 (Figure 1m,n). However, expression of HvD2 was not affected by the lig1 mutation (Figure 1o). Therefore, HvSPL8 may regulate leaf angle through controlling of the auxin signaling pathway, which is indispensable for secondary cell wall biosynthesis.

In summary, we cloned and functionally validated the Lig1 gene regulating leaf angle in barley. It was indicated that Lig1 encodes a plant-specific transcription factor, HvSPL8. Cloning of Lig1 provide a target for gene manipulation to increase spike numbers especially under dense planting conditions. Small leaf angle due to the loss of Lig1 function leads to efficient light interception, and erect leaves also allow increased light shedding to lower leaves, thereby improving canopy photosynthesis, which in turn facilitates crop productivity.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

S.Y. designed the experiments and wrote the first draft of the manuscript. S.Y. and M.O.C. developed the populations. M.O.C. conducted genetic mapping, gene validation, and gene function characterization. C.H.C. and J.D.F. contributed new reagents/analytic tools. S.Y. and M.C.O. analyzed the data. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

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