A new branch of understanding for barley inflorescence development

Kara A. Levin¹ and Scott A. Boden¹,²

¹ School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond 5064, SA, Australia
² The John Innes Centre, Department of Crop Genetics, Norwich Research Park, Norwich, NR4 7UH, UK.

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Moving beyond row-type architecture

Much of our knowledge of genes that regulate the arrangement of flowers in barley comes from investigating the development of spikelets, which are reproductive branches that form grain-producing florets (Gauley and Boden, 2019). In barley, spikelets are arranged in either two or six rows, with row-type determined by the lateral spikelets at each node being either sterile (two-row) or fertile (six-row) (Gauley and Boden, 2019). Wild barley species form two-row inflorescences, and defective alleles of at least five genes have been harnessed during breeding to improve fertility of the lateral spikelets, to generate six-rowed inflorescences (Gauley and Boden, 2019). These genes include VRS1, VRS2, VRS3, VRS4 and VRS5. VRS1 encodes a homeobox transcription factor that is expressed exclusively in lateral spikelets to suppress their development (Komatsuda et al., 2007). VRS3 and VRS4 influence lateral spikelet fertility by promoting VRS1 expression (Bull et al., 2017; Koppolu et al., 2013; van Esse et al., 2017). VRS5, also known as Intermediate-C (Int-C), encodes a TCP transcription factor that is orthologous to TEOSINE BRANCHED1 (TB1) from maize – it suppresses development and outgrowth of the lateral spikelets (Ramsay et al., 2011). TCPs form a large family of plant-specific transcription factors recognised by a bHLH motif, which are key regulators of differential plant form (Cubas et al., 1999). This study, together with similar analyses in maize, rice and sorghum, raises the prospect that other TCP transcription factors contribute to distinct aspects of inflorescence development beyond row-type architecture (Bai et al., 2012; Poursarebani et al., 2020; Shang et al., 2020; Yuan et al., 2009).

Shang et al. (2020) interrogated a barley mutagenesis population to identify a mutant that forms extended branches at the base of the inflorescence. These branches are unique from the outgrowth of lateral spikelets that occur in the six-rowed cultivars, as they involve the central spikelet meristem maintaining an indeterminate state to produce a short secondary inflorescence or fused multi-florets. These branches resemble those of the barley compositum2 (com2) mutant, where a mutation in an AP2/ERF transcription factor prevents termination of the spikelet meristem (Poursarebani et al., 2015). Mutants such as bdi1 and com2, therefore, provide an opportunity to investigate genetic pathways controlling the determinacy and fertility of the central spikelet within the triplet structure of barley, rather than lateral spikelets.

Linking inflorescence development with palea formation

The major cereals, including barley, rice, maize, wheat and sorghum, display remarkable diversity in the arrangement of spikelets and florets that form on the inflorescence. Our
understanding of the factors that contribute to inflorescence architecture diversity is improving as genes that control spikelet and floret development are discovered in different cereals, with certain genes shown to act as key regulators across multiple species. BDI1, for example, is an ortholog of RETARDED PALEA1/DEFECTIVE BODY OF PALEA (REP1/DBOP) in rice and BRANCH ANGLE DEFECTIVE1 (BAD1) from maize, which were discovered by studying mutants that influence palea formation and development of lateral branches on the tassel, respectively (Bai et al., 2012; Yuan et al., 2009). The palea, together with the lemma, helps form a protective envelope for the floral organs, and in some species, contributes to determining maximum grain size (Lombardo and Yoshida, 2015). Curiously, REP1 has no reported effect on inflorescence branching, nor does BAD1 on palea development (Bai et al., 2012; Yuan et al., 2009). Taken together, the work on BDI1 by Shang et al. (2020) and Poursarebani et al. (2020), who worked on the same gene that was named previously as COMPOSITUM (COM1), show that barley has potential to link the function of this TCP transcription factor in controlling palea formation and inflorescence branching across cereals (Poursarebani et al., 2020) (Box 1).

While the bdi1 mutant is not reported to show palea-related phenotypes, except some fused florets sharing one palea (Shang et al., 2020), certain com1 mutants in a two-rowed cultivar (α: Bowman) produced palea with increased cell size, cell wall thickness and more vascular bundles, relative to wild-type (Poursarebani et al., 2020). These differences in palea-related phenotypes may be attributed to the different cultivars used in each study. Interestingly, the fused-grain phenotype of the bdi1 mutant indicates that there may be a boundary organ defect during floret development, which might be consistent with the boundary signalling role described for COM1 (Poursarebani et al., 2020; Shang et al., 2020). The effect of com1 on palea cell size is the opposite of rep1 in rice, in which cells are smaller; however, barley, rice, sorghum and Brachypodium mutants all produced more vascular bundles in the palea (Poursarebani et al., 2020; Yuan et al., 2009). No palea phenotype was reported in maize bad1 mutants; however, the unique branched angle phenotype could be related to the positioning of the

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**Box 1. Depiction of inflorescence architecture (brown) and developing floret phenotypes controlled by BDI1/COM1 in barley and its orthologs in rice and maize**

(A) In wild-type barley, the developing inflorescence consists of one central determinate spikelet (CS), which contains a lemma (Le, blue) and a palea (Pa, red) that enclose floral organs. The bdi1 mutant forms an indeterminate CS with extended branches, and thickened cell walls form within the palea (Poursarebani et al., 2020). (B) In rice, a mutation in OsREP1 forms a smaller palea (Yuan et al., 2009), but no known effects on panicle architecture have been shown. (C) In maize, a mutation in ZmBAD1 causes defects in tassel branch angles, causing a clumped branching phenotype (Bai et al. 2012). No known effects of bad1 alleles on palea development have been shown.
palea, which is located on the same side as the lemma (Box 1) (Bai et al., 2012; Lombardo and Yoshida, 2015). Given the palea is proposed to act as a sink for auxin, and perturbed auxin distribution is associated with altered spikelet architecture, BD11/COM1 may influence spikelet architecture and floret development by modifying auxin-related processes during early inflorescence development (Bartlett et al., 2015; Boden, 2017; Yang et al., 2017; Youssef et al., 2017). BD11/COM1 function should therefore be investigated further to understand the link between palea development and spikelet architecture in cereals.

**Regulation of spikelet determinacy by cell walls and trehalose metabolism**

The transcriptional analysis performed by Shang et al. (2020) points towards molecular processes that contribute to the inflorescence branching of bdi1. During early inflorescence development, bdi1 mutants showed altered expression of genes involved in cell wall development, hormone signalling and carbohydrate-based metabolic processes (Shang et al., 2020). The effect on cell wall development genes is consistent with the phenotypes and transcription changes observed in com1 mutants, and indicates that cell boundary formation plays a key role in regulating spikelet meristem determinacy (Poursarebani et al., 2020).

Of the genes involved in carbohydrate metabolism, a gene encoding a trehalose 6-phosphate phosphatase (known as SISTER OF RAMOSA3) in mutants, and indicates that cell boundary formation plays a key role in regulating spikelet meristem determinacy (Poursarebani et al., 2020). The expression of SR3 and its association with inflorescence branching are consistent with the reduced spikelet meristem determinacy of ramosa3 and tpp4 mutants of maize (Claey s et al., 2019; Satoh-Nagasawa et al., 2006). Together, these results highlight the importance of further investigating cell wall development and trehalose metabolism to enhance our understanding of yield-related traits in barley.

In conclusion, the study by Shang et al. (2020) joins an emerging list of publications reporting the identification of genes controlling inflorescence development in barley, beyond those that control row-type architecture. These studies provide an ideal opportunity to compare gene function with that of the more-studied rice and maize, and to improve our understanding of the underlying genetic pathways contributing to the remarkable diversity of inflorescence architecture among cereals.

**Keywords:** barley, development, inflorescence, palea, spikelet, TCP transcription factor

**References**

Bai F, Reinheimer R, Durantini D, Kellogg EA, Schmidt RJ. 2012. TCP transcription factor, BRANCH ANGLE DEFECTIVE 1 (BAD1), is required for normal tassel branch angle formation in maize. Proceedings of the National Academy of Sciences, USA 109, 12225–12230.

Bartlett M, Williams S, Taylor Z, Deblasio S, Goldshmidt A, Hall D, Schmidt R, Jackson D, Whipple C. 2015. The maize PI/GLO ortholog Zmm16/stereile tassel silky ear1 interacts with the zymogamy and sex determination pathways in flower development. The Plant Cell 27, 3081–3098.

Boden SA. 2017. How hormones regulate floral architecture in barley. Nature Genetics 49, 8–9.

Bull H, Casco MC, Zwirik M, Flavell AJ, Thomas WTB, Guo W, Zhang R, Rapa zo-Flores P, Kyr iakidis S, Russell J, Druka A, Macaulay M, Waugh R. 2017. Barley SIX-PHOSPHATE SPIKE3 encodes a putative Jumonji C-type H3K9me2/me3 demethylase that represses lateral spikelet fertility. Nature Communications 8, 936.

Claeys H, Vi SL, Xu X, Satoh-Nagasawa N, Eveland AL, Goldschmidt A, Feil R, Beggs GA, Sakai H, Brennan RG, Lunn JE, Jackson DP. 2019. Control of meristem determinacy by trehalose-6-phosphate phosphatases is uncoupled from enzymatic activity. Nature Plants 5, 352–357.

Cubas P, Lauter N, Doebley J, Coen E. 1999. The TCP domain: a motif found in proteins regulating plant growth and development. The Plant Journal 18, 215–222.

Gaulay A, Boden SA. 2019. Genetic pathways controlling inflorescence architecture and development in wheat and barley. Journal of Integrative Plant Biology 61, 296–305.

Komatsuda T, Pourkeirandish M, He C, Azhaguvell P, Kamamori H, Perovic D, Stein N, Graner A, Wacker T, Tagiri A, Lundqvist U, Fujimura T, Matsuoka M, Matsumoto T, Yano M. 2007. Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proceedings of the National Academy of Sciences, USA 104, 1424–1429.

Koppolu R, Anwar N, Sakuma S, Tagiri A, Lundqvist U, Pourkeirandish M, Rutten T, Seiter C, Himmelbach A, Aryanadasa R, Kousha M, HM, Stein N, Sreenivasul N, Komatsuda T, Schnurbusch T. 2013. Six-rowed spike1 (Vrs4) controls spikelet determinacy and row-type in barley. Proceedings of the National Academy of Sciences, USA 110, 13198–13203.

Lombardo F, Yoshida H. 2015. Interpreting lemma and palea homologies: A point of view from rice floral mutants. Frontiers in Plant Science 6, 61.

Youssef N, Seidensticker T, Koppolu R, Trautewig C, Gawronsksi P, Bini F, Govind G, Rutten T, Sakuma S, Tagiri A, Wolde GM, Youssef HM, Battal A, Ciannarea S, Fusca T, Nussbaumer T, Pozzi C, Borner A, Lundqvist U, Komatsuda T, Salvi S, Tuberosa R, Uauy C, Sreenivasul N, Rossini L, Schnurbusch T. 2015. The genetic basis of composite spike form in barley and ‘miracle-wheat’. Genetica 201, 155–165.

Poursarebani N, Trautewig C, Melzer M, Nussbaumer T, Lundqvist U, Rutten T, Schmutzer T, Brandt R, Himmelbach A, Altschmied C, Koppolu R, Youssef HM, Sibout R, Dalmais M, Bendhamane A, Stein N, Xin Z, Schnurbusch T. 2020. COMPOSITUM 1 contributes to the architectural simplification of barley inflorescence via meristem identity signals. Nature Communications 11, 5138.

Ramsay L, Comardan J, Druka A, Marshall DF, Thomas WTB, McKim SM, Waugh R, Himmelbach A, Simpson C, Fuller J, Bonar N, Haynes PM, Lundqvist U, Franckowiak JD, Close TJ, Muehlbauer GJ, Waugh R. 2011. INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED1. Nature Genetics 43, 169–172.

Satoh-Nagasawa N, Nagasawa N, Malcolm B, Sakai H, Jackson D. 2006. A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature 441, 227–230.

Shang Y, Yuan L, Di Y, Jia Y, Zhang Z, Li S, Xing L, Qi Z, Wang X, Zhu J, Hua W, Wu X, Zhu M, Li G, Li C. 2020. A CYC/TB1 type TCP transcription factor controls spikelet meristem identity in barley (Hordeum vulgare L.). Journal of Experimental Botany 71, 7118–7131.

van Esse GW, Walla A, Finke A, Koornneef M, Pecinka A, van Korff M. 2011. Barley SIX-ROWED SPIKE3 encodes a putative Jumonji C-type H3K9me2/me3 demethylase that represses lateral spikelet fertility. Nature Communications 8, 936.

Youssef HM, Eggert K, Koppolu R, Alqudah AM, Poursarebani N, Fazeli A, Sakuma S, Tagiri A, Rutten T, Govind G, Lundqvist U, Graner A, Komatsuda T, Sreenivasul N, Schnurbusch T. 2017. VRS2 regulates hormone-mediated inflorescence patterning in barley. Nature Genetics 49, 157–161.

Yuan Z, Gao S, Xue DW, Luo D, Li LT, Ding SY, Yao X, Wilson ZA, Qian Q, Zhang DB. 2009. RETARDED PALEA1 controls palea development and floral zygomorphy in rice. Plant Physiology 149, 235–244.