

## Advances in studies on physiological and molecular regulation of barley tillering

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**Abstract** Tillering is an important trait closely associated with yield potential and environmental adaptation in cereal crops, and it is regulated by the synergy of endogenous (genetic) and exogenous (environmental) factors. Physiological and molecular regulation of tillering has been intensively studied in rice and wheat, however, there are relatively little research on barley. In this review, we use the recent advances of bioinformatics to map all known and potential barley tiller development genes with their chromosomal genetic and physical positions, and many of them are the first time to be mapped. We also discuss the regulation of tillering at genetic, physiological and environmental levels. Moreover, we established a novel link between the genetic control of phytohormones and sugars with tillering. We also provide evidence of how the environmental cues and cropping system helps in optimizing tiller number. This comprehensive review will enable us to understand the physiological and genetic mechanisms controlling tillering and other developmental traits in barley.

**Keywords:** barley, development, genetic regulation, phytohormone, tillering<sup>1</sup>

### 1. Introduction

Barley (*Hordeum vulgare* L.) ranks as the fourth largest cereal crop in terms of planting area only after wheat, rice, and maize in the world (<http://faostat.fao.org>). The Green Revolution characterized by the introduction of semi-dwarfing genes into cereal crops results in a dramatic increase of grain yield via increasing tiller and spike number per plant, and, simultaneously, the reduction of plant height and lodging risk (Ye *et al.* 2019). Tillering is one of the major agronomic traits in cereal crops and closely related to grain yield formation and stability (Dockter and Hansson 2015). So-called tillers are the lateral branches that grow from the main shoot or basal meristem of non-elongated internodes and produce their own adventitious roots as well as spikes during their development (Beveridge and Kyozyuka 2010).

The development of tillers is regulated by a complex network of multiple factors involved in genetics, physiology, and environment. Currently, several mutants related to tillering have been identified in cereal crops, for example in barley; *many noded dwarf (mnd)* (Babb and Muehlbauer 2003) and *high number of tillers1 (hnt1)* (Ye *et al.* 2019) produce more tillers, while *uniculm2 (cul2)* (Okagaki *et al.* 2013), and *uniculme4 (cul4)* (Tavakol *et al.* 2015) have no tiller. Tillering is also influenced by many environmental factors, including water (Romina *et al.* 2014), nutrients (Tanaka and Nakano 2019), temperature (Wang *et al.* 2010) and light (Miralles and Richards 2000). There are a number of tillers initiated in cereal crops at the vegetative stage, while only a few of them could grow into the shoots

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with spikes and most of them stop their development and die before heading stage, becoming so-called non-productive tillers (Kebrom *et al.* 2013). These non-productive tillers compete with effective tillers for resource, such as light, nutrient and water (Islam and Sedgley 1981). Obviously the effective regulation of tiller development is crucial to optimize the number of productive tillers (Xing and Zhang 2010). The proposed ideotype of cereal plants consists of fewer unproductive tillers (Jiao *et al.* 2010). In wheat, mutant *tin* (*tiller inhibition*) produced fewer tillers which could develop into larger spikes under water-limited conditions (Mitchell *et al.* 2013), revealing that the potential tiller number should be ideal, and not too high or very low. In fact, the enhanced productivity of some cereal crops followed by domestication was complemented by fewer tiller, e.g., maize and foxtail millet (Doebley *et al.* 2006; Doust and Kellogg 2006). Particularly, the limitation of tiller development factors may lead to fewer tillers but higher productivity, e.g., *wealthy farmer's panicle* (*wfp*) in rice (Miura *et al.* 2010) and overexpression of a cytokinin dehydrogenase gene (*AtCKXI*) in the transgenic barley which resulted in more tillers but lower yield (Pospíšilová *et al.* 2016). However, a well-balanced number of tillers is the foremost requirement as unproductive tillers waste nutrients (Jiao *et al.* 2010). Thus, elucidating the molecular and physiological mechanisms associated with tillering is quite significant for increasing crop productivity.

A considerable progress has been made in deciphering the genetic basis of tillering development in barley. However, there is a gap in the combination knowledge of genetic improvement including genetics, genomics, and molecular physiology. It is important to compile and overview all new discoveries and gaps for an efficient breeding program. In the current review, we put steps forward for understanding tiller development and how genetics influence the process either separately or through interaction with other factor(s). We then discuss the molecular physiology including hormonal and sugar regulation of tiller development and highlight the potential of their manipulation to increase barley yield through regulating tillering development. As the first molecular evidence, the role of sugars in the development of lateral branches in barley is discussed because it acts as an important signal transducer and source of nutrients during tiller development. Moreover, we also provide a brief account of the environmental influence of tiller development and its association with barley yield. Finally, we provide a perspective on the applications of recent advances in molecular physiology with functional genomics-based approaches for optimizing tillering and thus grain yield.

## 2. Tiller formation and development

Tillering is determined by the activity of shoot apical meristems (SAMs) and axillary meristems (AXMs). Shoot development occurs continuously throughout the life of a plant in repeated stacked units called phytomers, which in barley consist of an upper and a lower half-node separated by a portion of the stem, an internode, with leaf primordia developing on the upper half-node, and axillary buds (AXBs) and root initials developing on the lower half node (Forster *et al.* 2007). Tillering in barley begins during germination, and new AXBs develop adjacent to the internode,

covered by the leaf sheath of the previously formed phytomer (Fig. 1-1). Usually, two AXBs are already formed in mature embryos, the first being in the axil of the coleoptile (T0 AXB) and the second (T1 AXB) in the axil of the first leaf (Kirby and Appleyard 1981).

Tiller development mainly consists of two phases: i) AXMs initiation and bud formation as shown in Fig. 1-1, and ii) bud growth into tiller (Fig. 1-2) (Schmitz and Theres 2005). Barley mutant *cul2* could not develop its axillary buds, having only one main culm with no-tillers due to losing the ability to initiate or maintain axillary buds (Babb and Muehlbauer 2003), while an increased number of tillers was observed in the *hnt1* mutant of barley with accelerated axillary bud initiation (Ye *et al.* 2019). On the other hand, bud growth fate is determined by a complex network of endogenous and environmental factors. The barley mutant of *low number of tillers1* (*lnt1*) produces fewer tillers due to weak axillary bud growth and suppressed formation of secondary tillers (Dabbert *et al.* 2010). The wheat mutant of *tin* and the rice mutant of *asp1* are also good examples of suppressed bud outgrowth (Domagalska and Leyser 2011; Yoshida *et al.* 2012).

During the seedling developmental phase, dozens of tiller buds are initiated while few pass from the first tiller phase (bud initiation and formation) to the second phase of tiller development and growth. The first developed tiller is usually visible when seedlings have three leaves (Okagaki *et al.* 2018) and the initiation of reproductive growth indicates the end of tillering in grasses (Fig. 1). Plants can develop primary tillers which arise from axillary buds of the main culm (Fig. 1-2: T1), and secondary tillers which arise out of leaf axils of primary tillers (Fig. 1-3:T2) (Kirby and Appleyard 1987). Some tillers will develop inflorescences called spikes, while other tillers fail to form spikes, becoming non-productive or vegetative tillers (Jones and Kirby 1977), as shown for T4 in 5th part of Fig. 1. The proportion of tillers that develop into spikes depends on endogenous factors and environmental conditions as well (Okagaki *et al.* 2018).

### 3. Genetic regulation of tiller development

Tillering is a highly complex trait and its genetic determinants have been well studied in rice and maize, while the relevant knowledge in barley is relatively limited (Hussien *et al.* 2014). The recent advancements in next-generation sequencing offer a great opportunity for the identification and mapping of causal mutations and genes that can be directly used in barley breeding programs. A number of QTLs involved in the regulation of tillering have been mapped, and some of them have been cloned using a map-based cloning strategy. To illustrate the progress in molecular physiology and genetics regulating tiller development, we listed the known genes and mutants of barley tiller development (Fig. 2; Appendix A). The genetic and physical map of tiller developmental genes/mutants will support the understanding of molecular mechanisms underlying tiller development.

Based on phenotypic observations, tillering mutants of barley could be categorized into four classes: 1) *uniculm2* (*cul2*) mutants having only one culm with no developed tillers (Babb and Muehlbauer 2003), 2) mutants with fewer

tillers, e.g., *Int1* (Dabbert *et al.* 2010), *absent lower laterals1 (als1)* (Agharkar *et al.* 2007), and *uniculme4 (cul4)* (Tavakol *et al.* 2015)), 3) mutants with moderate tiller numbers, e.g., *intermedium-b (int-b)* and *semibrachytic (uzu)* (Babb and Muehlbauer 2003), and 4) mutants with higher tiller number, e.g., *hnt1* (Ye *et al.* 2019), *granum-a (gra-a)*, *many noded dwarf1 (mnd1)*, *mnd6* and *Intermedium-c (Int-c)* (Babb and Muehlbauer 2003; Druka *et al.* 2011), *Grassy tillers* (Grassy; Druka *et al.* 2011), *HvD14* (Marzec *et al.* 2016), *Many noded dwarf1/5 (Mnd1/5)*, *Many noded dwarf3 (Mnd3)*; Franckowiak and Lundqvist 2002) and *many noded dwarf 4/6* (Mascher *et al.* 2014). Mutations are the primary source of genetic variations that are used to understand tiller development mechanisms. Therefore, uncovering the molecular genetics and physiology of the desirable and causative mutations have prospects for their utilization in breeding.

A number of studies have revealed the genetic and morphological characterization of certain tillering mutants. *Cul2* mutants, exhibiting only the main culm, is described to the suppression of axillary buds (Babb and Muehlbauer 2003). *Lnt1*, *cul4* and *als1* showed fewer tillers because of inability of axillary meristems to develop into primary tillers (Dabbert *et al.* 2009; Dabbert *et al.* 2010; Tavakol *et al.* 2015). Interestingly, when breeders crossed low tillering barley mutants with high tillering mutants, such as *als* or *cul2* with *gra-a* or *mnd1*, all hybrid plants produced a low tillering or unicum phenotype, suggesting epistatic behavior of low tillering to higher tillering mutants (Okagaki *et al.* 2013; Ye *et al.* 2019). Like the rice SL mutants, the barley *HvD14* mutant showed a dwarf phenotype with high tillering (Marzec *et al.* 2016). The *uzu* gene encodes an ortholog of *BR11* in *Arabidopsis* and *D61* in rice, which is associated with tillering regulation (Babb and Muehlbauer 2003). *CUL4* encodes a BROAD COMPLEX, TRAMTRACK, BRIC-À-BRAC (BTB)-ankyrin domain-containing protein homologous to *Arabidopsis* BLADE-ON-PETIOLE 1 (BOP1) and BOP2, which regulates certain tiller processes, including outgrowth of tillers and development of secondary buds (Babb and Muehlbauer 2003).

*VRS1* encodes the homeodomain-leucine zipper class I (HD-ZIP1) transcription factor which pleiotropically regulates tillering in barley by inhibiting bud outgrowth. It is found to be a homolog of *grassy tillers 1 (gt1)* in maize (Liller *et al.* 2015). *VRS5 (Int-C)* encodes a TEOSINTE BRANCHED 1/CYCLOIDEA/PCF1 (TCP) transcription factor, and is the homolog of TB1 in maize enhancing tiller number at early development stage but suppressing bud outgrowth at late development stage (Ramsay *et al.* 2011). *VRS4* is associated with trehalose-6-phosphate synthase (T6PS) and trehalose-6-phosphate phosphatase (T6PP), which are orthologues of maize RAMOSA2, encoding a LATERAL ORGAN BOUNDARIES (LOB) transcription factor promoting spikelet and floret determinacy (Koppolu *et al.* 2013). This suggests the potential roles of sugar pathways through T6PS and T6PP in determining plant stature developmental traits including tillering (as discussed below). *JUBEL2* encodes a BEL-like homeodomain transcription factor, which is an ortholog of *Arabidopsis* BELLRINGER (BLR) and the low tillering mutant, *low number of tillers 1 (Lnt1)*, suggesting correspondence of *LNT1* to JuBel2 (Müller *et al.* 2001). *ELI-A* encodes a conserved protein that may be a transposon. Although it has the ability to inhibit the *cul2* mutant phenotype, the single mutant with strong *eli-a* alleles produces fewer tillers, typically holding about half as many tillers as wild plants (Chatfield *et al.* 2000). High tillering phenotypes correspond with a mutation in *INT-C* and *MND*. *INT-C* is a member of *TB1* and an ortholog of the branching inhibitor of maize *TB1*, and loss-of-function mutants have a moderately high tillering phenotype (Ramsay *et*

*al.* 2011). *MND* encodes a cytochrome P450 in the CYP78A family homologous to rice *PLASTOCHRON1 (PLA1)*, and *pla1* mutants have a similar phenotype to *mnd* ones (Le Bris *et al.* 1999). *Gra-a* produced more axillary buds (Schmitz and Theres 2005) and similar to *mnd1*, showed excessive development of tillers and semi-dwarf phenotype (Druka *et al.* 2011). Comparatively differential expression of the genes in tillering mutants from those in non-mutants revealed that many up-regulated genes in low tillering mutants were involved in stress responses e.g., production of reactive oxygen species and calcium signaling, which are involved in organ development (Agharkar *et al.* 2007; Arend *et al.* 2009; Okagaki *et al.* 2013). Spike morphology in barley also affects tiller number, with two-rowed barley having higher tillers than the six-rowed barley (Tucker 1977). In addition, the mutation in the barley row-type *VRS1* gene affected tiller number pleiotropically (Liller *et al.* 2015). Genetic regulation of tiller development in barley revealed a significant variation in germplasm collection as well as the bi-parental population (Abeledo *et al.* 2004; Borrás *et al.* 2009). Taken together, these genes/mutants have a strong potential usage for improving barley grain yield through optimizing the productive tiller number. The molecular mechanism of the cross-talk between the genes regulating tiller development and spike development is still unexplored.

## 4. Physiological regulation of tiller development

### 4.1. Hormonal regulation

At present, auxin, strigolactones (SLs), cytokinins (CTKs), gibberellins (GAs), abscisic acid (ABA), and jasmonic acids (JAs) are reported to play essential roles in tiller development. Usually, the final phenotype of tiller development is a function of the interaction between a number of phytohormones.

**Auxin** Auxin is an important growth regulator that controls tiller development by regulating AXMs formation (Agusti and Greb 2013), and it is actively synthesized in the shoot apex and suppresses the axillary bud outgrowth indirectly (Agusti and Greb 2013). The auxin transportation downwards from shoot apex is termed as polar transport (PAT) and is determined by auxin efflux carriers of the adenosine triphosphate (ATP)-binding cassette B and the PIN-FORMED (PIN) protein families present in xylem parenchyma (Petrášek and Friml 2009; Zazimalov *et al.* 2010). The mutation affecting auxin transport could result in various abnormalities, such as the inability to establish axillary meristems in inflorescences (Yoshida *et al.* 2012). Recently, a new mutant *hnt1* of barley was reported to have more tillers due to accelerated bud formation and initiation. It was suggested that HNT1 may regulate PAT-related genes (Ye *et al.* 2019). It was reported that BA1, a homolog of LAX1, controls AXM formation by regulating auxin transport (Gallavotti *et al.* 2004, 2008). BA1 acts downstream and is a direct target of the protein kinase BIF2, a PINOID ortholog important for PAT (Skirpan *et al.* 2008, 2009). *Arabidopsis max1* mutants showed increased axillary branching because of higher expression of PIN1 and auxin transport (Shinohara *et al.* 2013). For example, mutations in the genes that specify organ boundaries, like CUP-SHAPED COTYLEDON (CUC) genes in *Arabidopsis*, disturb PAT, resulting in reduced branching, loss of leaf serration, and abnormal inflorescences (Vroemen *et al.* 2003; Nikovics *et al.* 2006; Raman *et al.* 2008; Bilsborough *et al.* 2011). Vegetative and reproductive axillary meristem formation is also inhibited in three

maize mutants with impaired PAT: *barren stalk 1 (ba1)*, *Barren inflorescence 1 (Bif1)*, and *bif2* (Mcstee *et al.* 2007). Several lines of evidence from *Arabidopsis* and other species suggest that the diverse phenotypes resulting from reduced or ectopic expression of class 1 KNOX genes are attributed to an increased or decreased PAT, respectively (Shinohara *et al.* 2013). Auxin could down-regulate CTK levels by inhibiting the expression of IPT (*ISOPENTENYL TRANSFERASE*) genes, resulting in suppressed AXM (Ferguson and Beveridge 2009). The exogenous application of auxin in barley plants inhibits tillering by suppressing bud formation (Woodward and Marshall 1988). However, the molecular mechanism for the regulation of barley tillering by auxin is still not completely clear.

**Cytokinins** Cytokinins are fundamental regulators of plant growth, including axillary bud activation and delay of senescence (Sakakibara 2006). In *Arabidopsis*, *supershoot (sps)* enhanced shoot propagation and AXM activity with CTK accumulation at bud initiation by suppression of a SPS gene (Tantikanjana *et al.* 2001). It was also reported that a particular *Arabidopsis* KNOTTED-like homeobox (KNOX) protein SHOOTMERISTEMLESS (STM) promoted expression of (IPT7) ISOPENTENYL TRANSFERASE7 (Jasinski *et al.* 2005) and down-regulated gibberellin biosynthesis genes, resulting in low GA and high-CTK contents in the meristem, which may be crucial for maintaining of meristematic activity (Jasinski *et al.* 2005). Transgenic rice plants overexpressing OsIPTs showed enhanced axillary bud activity by CTK overexpression (Sakamoto *et al.* 2006). In barley, the *HvCKX1* gene regulates the CTK status due to an enhanced cytokinin dehydrogenase activity that degrades CTK. *HvCKX1* knock-out mutants produced more tillers and grains than wild plants (Holubová *et al.* 2018), suggesting that the exploitation of CTK regulating genes in barley may be profitable for optimizing the number of productive tillers.

**Strigolactones** SLs are recently reported to function as growth regulators by inhibiting AXB internode elongation (Gomez-Roldan *et al.* 2008). SLs may also induce the expression of transcription factors orthologous to maize *TB1*, rice *FINE CULM1 (FC1)*, and *Arabidopsis BRANCHED1*, which act downstream of strigolactones to inhibit internode elongation (Minakuchi *et al.* 2010). Five barley genes, i.e., *HvD14*, *HvD27*, *HvMAX1*, *HvCCD7*, and *HvCCD8* are orthologous to genes in rice and *Arabidopsis*, and involved in SLs' function of inhibiting tiller development (Wang *et al.* 2018). In *Arabidopsis*, SLs are synthesized in the roots upon expression of *MAX* genes and then transported to AXB (Gomez-Roldan *et al.* 2008). The SLs interact with auxin in a dual-loop pathway to control axillary bud outgrowth, but the nature of this regulatory loop is still unresolved (Kebrom and Richards 2013). *Arabidopsis max1* mutants have increased axillary branching associated with overexpression of *PIN1* and enhanced auxin transport (Shinohara *et al.* 2013). The increased branching phenotype is dependent on the overexpression of *PIN1*, as *max1/pin1* double mutants exhibit fewer lateral branches (Bennett *et al.* 2006). In *Arabidopsis*, *max1* and *high tillering dwarf (htd)* in rice showed enhanced shoot branching regulated by SLs (Zou *et al.* 2006). Future research should be focused on the interactions of SLs and auxin in order to better understand the role of SLs' crosstalk with other phytohormones in affecting tiller development.

**Gibberellins (GAs)** GA has been reported to play a role in internode elongation in grasses. Bioactive GA is deactivated by an enzyme encoded by the *GA2ox1* gene. The deactivation of GA probably prevents GA reaching nodes under the shoot apex and results in inhibition of internode elongation (Sakamoto *et al.* 2001). It was observed that overexpression of *GA2ox* produced more tillers, suggesting that the increase in bioactive GA and less *GA2ox1* activity

inhibits tillering and permits internode elongation in rice (Lo *et al.* 2008). More tillers were observed in GA responsive mutant of turfgrass (Agharkar *et al.* 2007). In rice, *MOCI* (*MONOCULMI*), a tillering regulator is guarded by belting with the DELLA protein of *SLR1* (*SLENDER RICE1*). GA has a role in the degradation of *SLR1*, resulting in stem elongation and reduced tiller number (Liao *et al.* 2019). In barley, the *LNT1* (*LOW NUMBER OF TILLERS1*) gene encodes *JUBEL2* which is homolog to the KNOX protein regulating *GA2ox*, as reported in maize (Dabbert *et al.* 2009). The expression of *GA2ox1* in the *lnt1* barley mutant should be investigated in order to elucidate the regulation of tiller development by GAs.

**Abscisic Acid (ABA)** Previous studies indicated that ABA inhibits bud outgrowth as shown after exogenous ABA treatment in *Arabidopsis* (Chatfield *et al.* 2000). The possibility that ABA may control tiller development has been widely explored. Functional connections exist between the biosynthetic pathways of abscisic acid (ABA) and other phytohormones such as SLs. The effect of this connection on tillering has been proven in barley (Wang *et al.* 2018). In barley, two transgenic lines accumulating ABA as a result of RNAi-mediated down-regulation of HvABA 8'-hydroxylase 1 and 3 were developed. LC-MS/MS analysis confirmed higher ABA levels in stem base tissues in these transgenic lines. Both lines showed enhanced tiller formation and lower expression levels of HvD27, HvMAX1, HvCCD7, and HvCCD8, indicating that ABA suppresses SL biosynthesis, leading to enhanced tiller formation (Wang *et al.* 2018). Recently, a study has also shown that ABA-related gene expression increased in bud-containing tissues of *Arabidopsis* under low red light to far-red light exposure, which led to a general reduction in branch number, suggesting that a high red light to far-red light exposure could inhibit ABA effect (González-Grandó *et al.* 2013). Endogenous ABA exerts a direct effect on regulating axillary bud outgrowth in intact *Arabidopsis*, acting as a general inhibitor (Yao and Finlayson 2015). The biosynthetic pathways of ABA and SLs appear to be connected, but, so far, the mechanism has not been well explored.

**Jasmonic Acid (JA)** JA is one of the emerging endogenous growth regulators affecting many developmental processes in plants. It was previously known as a growth inhibitor but is now identified as a signal transducer related to stress responses. For example, JA may affect nutrient uptake and sugar transportation, leading to stress tolerance (Ruan *et al.* 2019). The very first investigation of JA's role in tillering in grass species was reported in sorghum where it was shown that exogenous application of JA promoted in situ bud growth (Liu and Finlayson 2019). The ERF109 transcription factor regulates auxin transport-related genes (Xu *et al.* 2020) and the sorghum homolog of ERF109 associated with JA GO terms was strongly induced by leaf removal (Liu and Finlayson 2019). Based on the results obtained in a study on sorghum, there is a possibility that JA application on buds induces ERF109, resulting in overexpression of the auxin transport-related genes, thus leading to bud growth acceleration (Liu and Finlayson 2019). It would be quite interesting to investigate the JA-Auxin interaction at the molecular level to elucidate JA's role in bud regulation related to tiller development.

## 4.2. Cross-talk of phytohormones in regulating tiller development

It is well documented that phytohormones interact in a complex network to regulate tiller formation. The individual as

well as the cumulative effect of different interacting phytohormones on tillering was shown in Fig. 3. For phytohormonal cross-talk in tiller development, the major and direct roles are played by CTKs and Auxin. CTKs promote tillering by boosting the auxin transport that enhances the bud outgrowth, while auxin inhibits CTKs which lowers or stops the auxin transport, resulting in auxin accumulation and bud dormancy. The other phytohormones have their indirect roles in the regulation of tiller development by inhibiting or promoting auxin transporters (or other phytohormones), resulting in low or high tillering phenotypes, respectively. SLs and JAs are the most recently identified phytohormones playing their roles in shoot branching. Previous studies revealed that SLs inhibited the bud outgrowth by blocking the auxin transport, while JA had an inhibitory effect on SLs and promoted the auxin transport, resulting in enhanced tiller development. Likewise, ABA and GAs inhibit the SLs, which indirectly stimulates bud outgrowth.

### 4.3. Regulation of tiller development by sugars

In addition to their metabolic roles, sugars can also act as a mediator in many important developmental processes of plants. Sugars are an energy provider and resource of carbon for protein synthesis. Sucrose functions as a signal to control growth and differentiation with its related products glucose and fructose (Ruan 2012; Lunn *et al.* 2014). Very few studies discussed the role of sugars in tiller development from a developmental and physiological viewpoint, and rarely showed the genetic regulation of sugar content and composition and its role in tiller development in cereals. In this section, we emphasize the role of sugars in regulating tiller development in barley.

In shoot branching, sugar is crucial for enhancing bud outgrowth. During the development of lateral branches, sugars seem to play a signaling role, notable through trehalose 6-phosphate, interacting with phytohormones. In wheat, the tin mutant showed a lower tiller number with low sucrose level in the inhibited buds due to down-regulation of sucrose inducible genes (Kebrom *et al.* 2012). Likewise, the defoliation causing bud inhibition was found to be associated with the up-regulation of sucrose starvation and down-regulation of sucrose inducing genes in dormant buds of sorghum (Kebrom and Mullet 2015), suggesting that the outgrowth may be dependent on the overall status of plant sugar. Recently, the molecular mechanism of regulation of shoot branching by sucrose has been reported in Arabidopsis and rice (Zafar *et al.* 2020; Fichtner *et al.* 2021). In barley, some reports shed light on the role of sugar-related genes specially *HEXOKINASE (HXK)*, *SUCROSE TRANSPORTER (SUC)* and trehalose 6-phosphate (Tre6P) for regulating shoot branching (Barbier *et al.* 2021; Fichtner *et al.* 2021), and three sugar-related QTLs (*HvSUT1*, *HvHXK9* and *HvHXK6*) associated with *HEXOKINASE* and *SUCROSE TRANSPORTER* genes were found to be related to the regulation of tillering in barley (Alqudah *et al.* 2016). Although the role of sugar in the regulation of shoot branching has been highlighted in very recent reports to our knowledge, no prior study has been conducted in barley to explore the role of sugars and the molecular mechanisms in the regulation of shoot branching (Fichtner and Lunn 2021). Therefore, we mapped around twenty sugar-related genes for the first time in barley. Among them, *HEXOKINASE* and Tre6P related genes are strongly supported by recent studies (Barbier *et al.* 2021), ultimately offering an opportunity to understand their molecular basis. These findings raise the importance of sugar-related genes in affecting



the most plastic traits, including tillering in grasses. Thus, it can be concluded that further investigations should be conducted at the molecular level on the regulation of tillers by sugars.

## **5. Regulation of tiller development by the environment-responding genes and agronomic factors**

Tillering is also influenced by many environmental factors, including temperature, photoperiod, water, and nutrient availability (Skinner and Simmons 1993; Miralles and Richards 2000; Romina *et al.* 2014). With respect to the global warming, the change of the phenological properties may be an efficient method for planting cereal crops, especially winter barley and wheat. Tillering was inhibited by high temperature, substantial vernalization and less photoperiod sensitivity (Wang *et al.* 2010). Tiller production in barley was shown to be significantly affected by the genes in response to environment, e.g., vernalization genes *VERNALIZATION-H1* (*Vrn-H1*) and *Vrn-H2*, and the photoperiod response gene *Ppd-H1* (Von Korff *et al.* 2006; Wang *et al.* 2010). Photoperiod affects tiller number by altering the duration of the vegetative growth. Barley genotypes carrying the photoperiod sensitive *Ppd-H1* allele had high expression levels of *Vrn-H3* (Campoli *et al.* 2012). Alqudah *et al.* (2016) reported that the barley accessions carrying the reduced photoperiod sensitivity (*Ppd-H1*) allele produced significantly more productive and non-productive tillers per plant than the accessions sensitive to photoperiod. In addition, the genes associated with barley flowering time, including *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3* exerted pleiotropic effects on plant development including tillering (Wiegmann *et al.* 2019). These pleiotropic effects were found to be strongly regulated by the response to environmental factors, such as day length and temperature (Herzig *et al.* 2018; Wiegmann *et al.* 2019). A number of quantitative trait loci (QTLs) that control tillering have been described in wheat (Naruoka *et al.* 2011; Yang *et al.* 2013; Xie *et al.* 2016). In a study, for the tillering traits, a number of QTLs were identified under the short day and artificially vernalized conditions, where the Ppd genes were active, and the QTLs were located on chromosomes 6B (QTLs 45, 48) and 4B (QTL 29), respectively (Giunta *et al.* 2018). Flowering-associated genes may influence wheat tiller number. Overexpression of TaZIM-A1 caused delayed heading and increased effective tiller number by regulating TaFT and *VERNALIZATION1* (*VRN1*) expression (Liu *et al.* 2019). The photoperiod-sensitivity gene *Ppd-1* influences tiller number in wheat (Dyck *et al.* 2004). The later-heading *vrn-A1* allele was associated with more tillers per plant in a wheat cultivar Cappelle-Desprez (Kato *et al.* 2000).

It may be suggested that the wild barley germplasm can be used to improve plant development for boosting grain yield. Several agronomic factors or agricultural management practices also influence tillering and productive tillers. For example, the cropping pattern can affect tillering, as cereal crops deplete nutrients from the soil, lowering the number of productive tillers in the next crop. There is a higher number of productive wheat tillers in a wheat-rice cropping pattern compared to a wheat-sunflower cropping pattern (Nawab *et al.* 2011). Cereal crops are exhaustive compared to legumes that facilitate the soil with nitrogen (N) and phosphorus (P) (Stagnari *et al.* 2017). In addition, a higher barley yield was observed when grown in a crop rotation including legumes rather than in a continuous barley crop sequence (Jones and Singh 2000). Water availability also regulates tillering, as water-limited conditions resulted

in fewer tiller formations (Chaturvedi *et al.* 1981). Improvements in soil moisture through the application of mulch reduced the number of non-productive tillers and increased yield in rice (Jabran *et al.* 2015). Excessive evaporation resulting in drought stress reduces the number of productive tillers in wheat and maize (Balwinder-Singh *et al.* 2016; Zhang *et al.* 2017). Nutrient management is also an important factor affecting tillering (Bakht *et al.* 2010). Although N and P are essential nutrients with regard to tillering in grasses, potassium (K) also plays a role in tiller regulation e.g. increasing tiller number in rice (Bahmaniar and Ranjbar 2007). Planting density or seeding rate also influences the morphology and number of tillers with an increased number of non-productive tillers at higher planting densities in barley (Kirby and Faris 1972). Similarly, a low tiller number was reported with dense seeding of spring wheat (Otteson *et al.* 2008). In short, the influence of the environmental and agronomic factors on tillering and productive tillers have been intensively studied, but the precise roles of all these factors are still not fully described. It is suggested that some integrated experiments should be conducted to understand the mechanisms of these factors towards the regulation of tiller initiation and development.

## 6. Conclusion and perspective

Tillering is a major yield determinant in cereal crops and is controlled by different endogenous and environmental factors. The misregulation of developmental genes may affect various physiological processes, resulting in higher or lower tiller number. Tiller development involves bud initiation and outgrowth. The bud initiation is severely inhibited in the *uniculm2* mutant with zero tillers. The mutants related to bud outgrowth are mainly determined by *lnt1*, *als1* and *cul4*, and the candidate gene of *als1* has not yet been identified. LNT1 encodes a JuBel2 homeodomain transcription factor and *cul4* encodes BOP-like BTB-ankyrin protein, which plays crucial roles towards a weak bud outgrowth of tillers. The mutants *gra-a*, *grassy*, *mnd1*, *mnd3*, *mnd5*, *aps1* and *int-m* enhance tiller production, whereas, *cst1*, *int-b* and *cul2* inhibit tillering in barley. However, the candidate genes for these mutants are yet to be identified. Some candidate genes of mutants that increase tiller production have been identified, e.g., *HNT1*, *INT-C*, *HvD14*, *MND6*, and *UZU1* which encode proteins to regulate tillering in barley. SDW1 regulates the biosynthesis of GA phytohormone and enhances tiller production. To date, only a few genetic studies have targeted and functionally characterized tillering genes in barley. Further investigations and functional validation of tillering genes are necessary for exploring more-natural tillering-enhancing alleles for crop breeding. Here, we provide an important foundation for uncovering the biological functions of sugar-related genes, suggesting that sugar-regulated genes potentially regulate tillering in barley and most likely other temperate cereals such as wheat. Targeting such genes by genome editing approaches such as an efficient *CRISPR/Cas9* system to produce heritable and desirable alleles are expected to significantly accelerate crop breeding. Abiotic stresses, for example, extreme temperatures, drought stress and phosphorus depletion inhibit the growth of lateral branches in barley, while nitrogen availability and photoperiod insensitivity promote barley tillering. For increasing grain yield, breeders prefer to select the lines with low non-productive tillering and high productive tillers (carrying spikes). It is also important to explore the tillering-enhancing alleles to maximize grain yield. A deep understanding of tillering as affected by genetic and agronomic factors will not only broaden our knowledge of

biological processes but will also probably allow breeders to better control and optimize tillering. Future studies should focus on the regulatory roles of phytohormones and their molecular interaction to define tillering and yield formation in barley and other cereal crops.

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### References

- Abeledo L G, Calderini D F, Slafer G A. 2004. Leaf appearance, tillering and their coordination in old and modern barleys from argentina. *Field Crops Research*, **86**, 23-32.
- Agharkar M, Lomba P, Altpeter F, Zhang H, Kenworthy K, Lange T. 2007. Stable expression of atga2ox1 in a low-input turfgrass (*Paspalum notatum*) reduces bioactive gibberellin levels and improves turf quality under field conditions. *Plant Biotechnology Journal*, **5**, 791-801.
- Agusti J, Greb T. 2013. Going with the wind—adaptive dynamics of plant secondary meristems. *Mechanisms of Development*, **130**, 34-44.
- Alqudah A M, Koppolu R, Wolde G M, Graner A, Schnurbusch T. 2016. The genetic architecture of barley plant stature. *Frontiers in Genetics*, **7**, 117.
- Arend M, Schnitzler J P, Ehling B, Hänsch R, Lange T, Rennenberg H, Himmelbach A, Grill E, Fromm J. 2009. Expression of the arabidopsis mutant abi1 gene alters abscisic acid sensitivity, stomatal development, and growth morphology in gray poplars. *Plant Physiology*, **151**, 2110-2119.
- Babb S, Muehlbauer G. 2003. Genetic and morphological characterization of the barley unculm2 (cul2) mutant. *Theoretical and Applied Genetics*, **106**, 846-857.
- Bahmaniar M, Ranjbar G. 2007. Response of rice cultivars to rates of nitrogen and potassium application in. *Pakistan journal of Biological Sciences*, **10**, 1430-1437.
- Bakht J, Shafi M, Zubair M, Khan M A, Shah Z. 2010. Effect of foliar vs. Soil application of nitrogen on yield and yield components of wheat varieties. *Pakistan Journal of Botany*, **42**, 2737-2745.
- Balwinder-Singh E H, Gaydon D, Eberbach P. 2016. Evaluation of the effects of mulch on optimum sowing date and

- irrigation management of zero till wheat in central punjab, india using apsim. *Field Crops Research*, **197**, 83.
- Barbier F, Cao D, Fichtner F, Weiste C, Perez-Garcia M D, Caradeuc M, Le Gourrierc J, Sakr S, Beveridge C A. 2021. Hexokinase1 signalling promotes shoot branching and interacts with cytokinin and strigolactone pathways. *New Phytologist*, **231**, 1088-1104
- Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O. 2006. The arabidopsis max pathway controls shoot branching by regulating auxin transport. *Current Biology*, **16**, 553-563.
- Beveridge C A, Kyojuka J. 2010. New genes in the strigolactone-related shoot branching pathway. *Current Opinion in Plant Biology*, **13**, 34-39.
- Bilsborough G D, Runions A, Barkoulas M, Jenkins H W, Hasson A, Galinha C, Laufs P, Hay A, Prusinkiewicz P, Tsiantis M. 2011. Model for the regulation of Arabidopsis thaliana leaf margin development. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 3424-3429.
- Borràs G, Romagosa I, Van Eeuwijk F, Slafer G A. 2009. Genetic variability in duration of pre-heading phases and relationships with leaf appearance and tillering dynamics in a barley population. *Field Crops Research*, **113**, 95-104.
- Le Bris M, Michaux-Ferrière N, Jacob Y, Poupet A, Barthe P, Guigonis J M, Le Page-Degivry M T. 1999. Regulation of bud dormancy by manipulation of aba in isolated buds of rosa hybrida cultured *in vitro*. *Functional Plant Biology*, **26**, 273-281.
- Campoli C, Shtaya M, Davis S J, Von Korff M. 2012. Expression conservation within the circadian clock of a monocot: Natural variation at barley ppd-h1 affects circadian expression of flowering time genes, but not clock orthologs. *BMC Plant Biology*, **12**, 97.
- Chatfield S P, Stirnberg P, Forde B G, Leyser O. 2000. The hormonal regulation of axillary bud growth in arabidopsis. *The Plant Journal*, **24**, 159-169.
- Chaturvedi G, Aggarwal P, Singh A, Joshi M, Sinha S. 1981. Effect of irrigation on tillering in wheat, triticale and barley in a water-limited environment. *Irrigation Science*, **2**, 225-235.
- Dabbert T, Okagaki R J, Cho S, Boddu J, Muehlbauer G J. 2009. The genetics of barley low-tillering mutants: Absent lower laterals (als). *Theoretical and Applied Genetics*, **118**, 1351-1360.
- Dabbert T, Okagaki R J, Cho S, Heinen S, Boddu J, Muehlbauer G J. 2010. The genetics of barley low-tillering mutants: Low number of tillers-1 (lnt1). *Theoretical and Applied Genetics*, **121**, 705-715.
- Dockter C, Hansson M. 2015. Improving barley culm robustness for secured crop yield in a changing climate. *Journal of Experimental Botany*, **66**, 3499-3509.
- Doebley J F, Gaut B S, Smith B D. 2006. The molecular genetics of crop domestication. *Cell*, **127**, 1309-1321.
- Domagalska M A, Leyser O. 2011. Signal integration in the control of shoot branching. *Nature Reviews Molecular Cell Biology*, **12**, 211.
- Donald C T. 1968. The breeding of crop ideotypes. *Euphytica*, **17**, 385-403.
- Doust A N, Kellogg E A. 2006. Effect of genotype and environment on branching in weedy green millet (*setaria viridis*) and domesticated foxtail millet (*Setaria italica*)(poaceae). *Molecular Ecology*, **15**, 1335-1349.

- Druka A, Franckowiak J, Lundqvist U, Bonar N, Alexander J, Houston K, Radovic S, Shahinnia F, Vendramin V, Morgante M. 2011. Genetic dissection of barley morphology and development. *Plant Physiology*, **155**, 617-627.
- Dyck J A, Matus - Cádiz M A, Hucl P, Talbert L, Hunt T, Dubuc J P, Nass H, Clayton G, Dobb J, Quick J. 2004. Agronomic performance of hard red spring wheat isolines sensitive and insensitive to photoperiod. *Crop Science*, **44**, 1976-1981.
- Ferguson B J, Beveridge C A. 2009. Roles for auxin, cytokinin, and strigolactone in regulating shoot branching. *Plant Physiology*, **149**, 1929-1944.
- Fichtner F, Barbier F, Annunziata M G, Feil R, Olas J, Mueller-Roeber B, Stitt M, Beveridge C A, Lunn J E. 2021. Regulation of shoot branching in arabidopsis by trehalose 6-phosphate. *New Phytologist*, **229**, 2135-2151.
- Fichtner F, Lunn J E. 2021. The role of trehalose 6-phosphate (Tre6P) in plant metabolism and development. *Annual Review of Plant Biology*, **72**, 737-760.
- Forster B P, Franckowiak J D, Lundqvist U, Lyon J, Pitkethly I, Thomas W T. 2007. The barley phytomer. *Annals of Botany*, **100**, 725-733.
- Franckowiak J D, and Lundqvist U. 2002. Current list of new and revised barley genetic stock descriptions. *Barley Genetics Newsletter*, **32**, 47.
- Gallavotti A, Yang Y, Schmidt R J, Jackson D. 2008. The relationship between auxin transport and maize branching. *Plant Physiology*, **147**, 1913-1923.
- Gallavotti A, Zhao Q, Kyojuka J, Meeley R B, Ritter M K, Doebley J F, Pe M E, Schmidt R J. 2004. The role of barren stalk1 in the architecture of maize. *Nature*, **432**, 630.
- Gomez-Roldan V, Fermas S, Brewer P B, Puech-Pagès V, Dun E A, Pillot J P, Letisse F, Matusova R, Danoun S, Portais J C. 2008. Strigolactone inhibition of shoot branching. *Nature*, **455**, 189.
- González-Grandó E, Poza-Carrión C, Sorzano C O S, Cubas P. 2013. Branched1 promotes axillary bud dormancy in response to shade in arabidopsis. *The Plant Cell*, **25**, 834-850.
- Giunta F, De Vita P, Mastrangelo A M, Sanna G, Motzo R. 2018. Environmental and genetic variation for yield-related traits of durum wheat as affected by development. *Frontiers in plant science*, **9**, 8.
- Herzig P, Maurer A, Draba V, Sharma R, Draicchio F, Bull H, Milne L, Thomas W T, Flavell A J, Pillen K. 2018. Contrasting genetic regulation of plant development in wild barley grown in two european environments revealed by nested association mapping. *Journal of Experimental Botany*, **69**, 1517-1531.
- Holubová K, Hensel G, Vojta P, Tarkowski P, Bergougnoux V, Galuszka P. 2018. Modification of barley plant productivity through regulation of cytokinin content by reverse-genetics approaches. *Frontiers in Plant Science*, **9**, 1676
- Hussien A, Tavakol E, Horner D S, Muñoz-Amatriaín M, Muehlbauer G J, Rossini L. 2014. Genetics of tillering in rice and barley. *The Plant Genome*, **7**, 10-32
- Islam T, Sedgley R. 1981. Evidence for a 'uniculum effect' in spring wheat (*triticum aestivum* L.) in a mediterranean environment. *Euphytica*, **30**, 277-282.
- Jabran K, Ullah E, Hussain M, Farooq M, Zaman U, Yaseen M, Chauhan B. 2015. Mulching improves water

- productivity, yield and quality of fine rice under water - saving rice production systems. *Journal of Agronomy and Crop Science*, **201**, 389-400.
- Jasinski S, Piazza P, Craft J, Hay A, Woolley L, Rieu I, Phillips A, Hedden P, Tsiantis M. 2005. Knox action in arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. *Current Biology*, **15**, 1560-1565.
- Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X. 2010. Regulation of *osspl14* by *osmir156* defines ideal plant architecture in rice. *Nature Genetics*, **42**, 541.
- Jones H, Kirby E. 1977. Effects of manipulation of number of tillers and water supply on grain yield in barley. *The Journal of Agricultural Science*, **88**, 391-397.
- Jones M, Singh M. 2000. Long-term yield patterns in barley-based cropping systems in northern syria. 2. The role of feed legumes. *The Journal of Agricultural Science*, **135**, 237-249.
- Kato K, Miura H, Sawada S. 2000. Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. *Theoretical and Applied Genetics*, **101**, 1114-1121.
- Kebrom T H, Chandler P M, Swain S M, King R W, Richards R A, Spielmeyer W. 2012. Inhibition of tiller bud outgrowth in the *tin* mutant of wheat is associated with precocious internode development. *Plant Physiology*, **160**, 308-318.
- Kebrom T H, Mullet J E. 2015. Photosynthetic leaf area modulates tiller bud outgrowth in sorghum. *Plant, Cell & Environment*, **38**, 1471-1478.
- Kebrom T H, Richards R A. 2013. Physiological perspectives of reduced tillering and stunting in the tiller inhibition (*tin*) mutant of wheat. *Functional Plant Biology*, **40**, 977-985.
- Kebrom T H, Spielmeyer W, Finnegan E J. 2013. Grasses provide new insights into regulation of shoot branching. *Trends in Plant Science*, **18**, 41-48.
- Kirby E, Appleyard M. 1987. *Cereal Development Guide*. 2nd ed. Arable Unit, National Agricultural Center, Stoneleigh. United Kingdom
- Kirby E, Faris D. 1972. The effect of plant density on tiller growth and morphology in barley. *The Journal of Agricultural Science*, **78**, 281-288.
- Kirby E M, Appleyard M. 1981. *Cereal Development Guide*. Stoneleigh, United Kingdom.
- Koppolu R, Anwar N, Sakuma S, Tagiri A, Lundqvist U, Pourkheirandish M, Rutten T, Seiler C, Himmelbach A, Ariyadasa R, Youssef H M, Stein N, Sreenivasulu N, Komatsuda T, Schnurbusch T. 2013. Six-rowed spike4 (*Vrs4*) controls spikelet determinacy and row-type in barley. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 13198-13203.
- Von Korff M, Wang H, L6on J, Pillen K. 2006. Ab-qtl analysis in spring barley: Ii. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*h. Vulgare* ssp. *Spontaneum*). *Theoretical and Applied Genetics*, **112**, 1221-1231.
- Liao Z, Yu H, Duan J, Yuan K, Yu C, Meng X, Kou L, Chen M, Jing Y, Liu G. 2019. *Slr1* inhibits *moc1* degradation to coordinate tiller number and plant height in rice. *Nature Communications*, **10**, 2738.

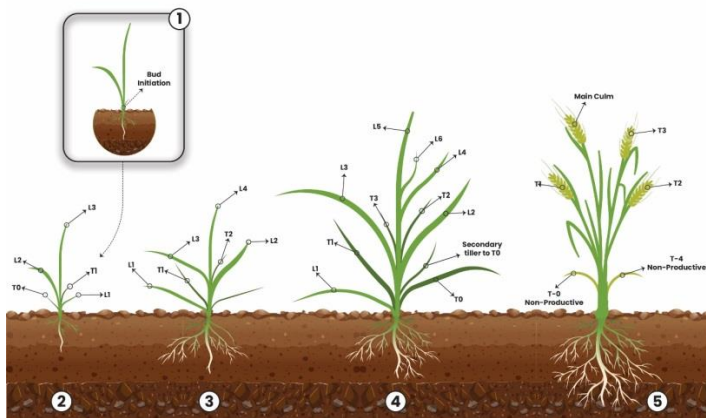
- Liller C B, Neuhaus R, Von Korff M, Koornneef M, Van Esse W. 2015. Mutations in barley row type genes have pleiotropic effects on shoot branching. *PLoS ONE*, **10**, e0140246.
- Liu H, Li T, Wang Y, Zheng J, Li H, Hao C, Zhang X. 2019. TaZIM-A1 negatively regulates flowering time in common wheat (*Triticum aestivum* L.). *Journal of Integrative Plant Biology*, **61**, 359-376.
- Xu, P, Zhao P X, Cai X T, Mao J L, Miao Z Q, Xiang C B. 2020. Integration of jasmonic acid and ethylene into auxin signaling in root development. *Frontiers in Plant Science*, **11**, 271.
- Liu R, Finlayson S A. 2019. Sorghum tiller bud growth is repressed by contact with the overlying leaf. *Plant, Cell & Environment*, **42**, 2120-2132.
- Lo S F, Yang S Y, Chen K T, Hsing Y I, Zeevaart J A, Chen L J, Yu S M. 2008. A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. *The Plant Cell*, **20**, 2603-2618.
- Lunn J E, Delorge I, Figueroa C M, Van Dijck P, Stitt M. 2014. Trehalose metabolism in plants. *The Plant Journal*, **79**, 544-567.
- Mascher M, Jost M, Kuon J E, Himmelbach A, Aßfalg A, Beier S, Scholz U, Graner A, Stein N. 2014. Mapping-by-sequencing accelerates forward genetics in barley. *Genome Biology*, **15**, 1-15.
- Marzec M, Gruszka D, Tylec P, Szarejko I. 2016. Identification and functional analysis of the *hvd14* gene involved in strigolactone signaling in *Hordeum vulgare*. *Physiologia Plantarum*, **158**, 341-355.
- Mcsteen P, Malcomber S, Skirpan A, Wu X, Kellogg E, Hake S. 2007. Barren inflorescence2 encodes a co-ortholog of the pinoid serine/threonine kinase and is required for organogenesis during inflorescence and vegetative development in maize. *Plant physiology*, **144**, 1000-1011.
- Minakuchi K, Kameoka H, Yasuno N, Umehara M, Luo L, Kobayashi K, Hanada A, Ueno K, Asami T, Yamaguchi S. 2010. Fine culm1 (*fc1*) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice. *Plant and Cell Physiology*, **51**, 1127-1135.
- Miralles D, Richards R. 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. *Annals of Botany*, **85**, 655-663.
- Mitchell J, Rebetzke G, Chapman S, Fukai S. 2013. Evaluation of reduced-tillering (*tin*) wheat lines in managed, terminal water deficit environments. *Journal of Experimental Botany*, **64**, 3439-3451.
- Miura K, Ikeda M, Matsubara A, Song X J, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M. 2010. *Osspl14* promotes panicle branching and higher grain productivity in rice. *Nature Genetics*, **42**, 545.
- Müller J, Wang Y, Franzen R, Santi L, Salamini F, Rohde W. 2001. *In vitro* interactions between barley tale homeodomain proteins suggest a role for protein-protein associations in the regulation of knox gene function. *The Plant Journal*, **27**, 13-23.
- Naruoka Y, Talbert L E, Lanning S P, Blake N K, Martin J M, Sherman J D. 2011. Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. *Theoretical and Applied Genetics*, **123**, 1043-1053.
- Nawab K, Shah P, Arif M, Ullah A, Khan M, Mateen A, Rab A, Munsif F, Ali K. 2011. Effect of cropping patterns, farm yard manure, k and zn on wheat growth and grain yield. *Sarhad Journal of Agriculture*, **27**, 371-375.

- Nikovics K, Blein T, Peaucelle A, Ishida T, Morin H, Aida M, Laufs P. 2006. The balance between the *mir164a* and *cuc2* genes controls leaf margin serration in arabidopsis. *The Plant Cell*, **18**, 2929-2945.
- Okagaki R J, Cho S, Kruger W M, Xu W W, Heinen S, Muehlbauer G J. 2013. The barley *uniculm2* gene resides in a centromeric region and may be associated with signaling and stress responses. *Functional & Integrative Genomics*, **13**, 33-41.
- Okagaki R J, Haaning A, Bilgic H, Heinen S, Druka A, Bayer M, Waugh R, Muehlbauer G J. 2018. Eligulum-a regulates lateral branch and leaf development in barley. *Plant Physiology*, **176**, 2750-2760.
- Otteson B N, Mergoum M, Ransom J, Schatz B. 2008. Tiller contribution to spring wheat yield under varying seeding and nitrogen management. *Agronomy Journal*, **100**, 406-413.
- Petrášek J, Friml J. 2009. Auxin transport routes in plant development. *Development*, **136**, 2675-2688.
- Pospišilová H, Jiskrova E, Vojta P, Mrizova K, Kokáš F, Čudejková M M, Bergounoux V, Plíhal O, Klimešová J, Novák O. 2016. Transgenic barley overexpressing a cytokinin dehydrogenase gene shows greater tolerance to drought stress. *New Biotechnology*, **33**, 692-705.
- Raman S, Greb T, Peaucelle A, Blein T, Laufs P, Theres K. 2008. Interplay of *mir164*, cup-shaped cotyledon genes and lateral suppressor controls axillary meristem formation in arabidopsis thaliana. *The Plant Journal*, **55**, 65-76.
- Ramsay L, Comadran J, Druka A, Marshall D F, Thomas W T, Macaulay M, Mackenzie K, Simpson C, Fuller J, Bonar N. 2011. Intermedium-c, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene teosinte branched 1. *Nature Genetics*, **43**, 169.
- Romina P, Abeledo L G, Miralles D J. 2014. Identifying the critical period for waterlogging on yield and its components in wheat and barley. *Plant and Soil*, **378**, 265-277.
- Ruan J, Zhou Y, Zhou M, Yan J, Khurshid M, Weng W, Cheng J, Zhang K. 2019. Jasmonic acid signaling pathway in plants. *International Journal of Molecular Sciences*, **20**, 2479.
- Ruan Y L. 2012. Signaling role of sucrose metabolism in development. *Molecular Plant*, **5**, 763-765.
- Sakamoto T, Kobayashi M, Itoh H, Tagiri A, Kayano T, Tanaka H, Iwahori S, Matsuoka M. 2001. Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. *Plant Physiology*, **125**, 1508-1516.
- Sakamoto T, Sakakibara H, Kojima M, Yamamoto Y, Nagasaki H, Inukai Y, Sato Y, Matsuoka M. 2006. Ectopic expression of knotted1-like homeobox protein induces expression of cytokinin biosynthesis genes in rice. *Plant Physiology*, **142**, 54-62.
- Schmitz G, Theres K. 2005. Shoot and inflorescence branching. *Current Opinion in Plant Biology*, **8**, 506-511.
- Shaaf S, Bretani G, Biswas A, Fontana I M, Rossini L. 2019. Genetics of barley tiller and leaf development. *Journal of Integrative Plant Biology*, **61**, 226-256.
- Sakakibara H. 2006. Cytokinins: Activity, biosynthesis, and translocation. *Annual Reviews of Plant Biology*, **57**, 431-449.
- Shinohara N, Taylor C, Leyser O. 2013. Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein pin1 from the plasma membrane. *Plos Biology*, **11**, e1001474.

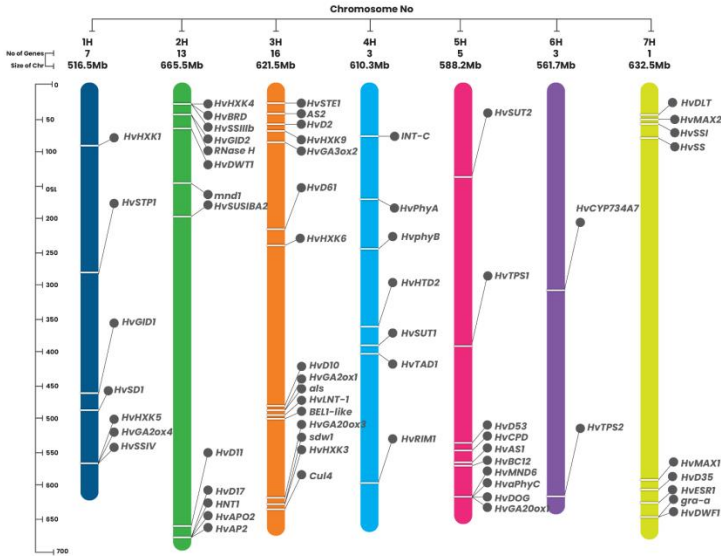


- Skinner R, Simmons S. 1993. Modulation of leaf elongation, tiller appearance and tiller senescence in spring barley by far - red light. *Plant, Cell & Environment*, **16**, 555-562.
- Skirpan A, Culler A H, Gallavotti A, Jackson D, Cohen J D, Mcsteen P. 2009. Barren inflorescence2 interaction with *zmpin1a* suggests a role in auxin transport during maize inflorescence development. *Plant and Cell Physiology*, **50**, 652-657.
- Skirpan A, Wu X, Mcsteen P. 2008. Genetic and physical interaction suggest that barren stalk1 is a target of barren inflorescence2 in maize inflorescence development. *The Plant Journal*, **55**, 787-797.
- Stagnari F, Maggio A, Galieni A, Pisante M. 2017. Multiple benefits of legumes for agriculture sustainability: An overview. *Chemical and Biological Technologies in Agriculture*, **4**, 1-13.
- Tanaka R, Nakano H. 2019. Barley yield response to nitrogen application under different weather conditions. *Scientific Reports*, **9**, 1-12.
- Tantikanjana T, Yong J W, Letham D S, Griffith M, Hussain M, Ljung K, Sandberg G, Sundaresan V. 2001. Control of axillary bud initiation and shoot architecture in arabidopsis through the supershoot gene. *Genes & Development*, **15**, 1577-1588.
- Tavakol E, Okagaki R, Verderio G, Shariati V, Hussien A, Bilgic H, Scanlon M J, Todt N R, Close T J, Druka A. 2015. The barley *uniculme4* gene encodes a blade-on-petiole-like protein that controls tillering and leaf patterning. *Plant Physiology*, **168**, 164-174.
- Tucker D. 1977. The effects of far-red light on lateral bud outgrowth in decapitated tomato plants and the associated changes in the levels of auxin and abscisic acid. *Plant Science Letters*, **8**, 339-344.
- Vroemen C W, Mordhorst A P, Albrecht C, Kwaaitaal M A, De Vries S C. 2003. The cup-shaped cotyledon3 gene is required for boundary and shoot meristem formation in arabidopsis. *The Plant Cell*, **15**, 1563-1577.
- Wang G, Schmalenbach I, Von Korff M, León J, Kilian B, Rode J, Pillen K. 2010. Association of barley photoperiod and vernalization genes with qtls for flowering time and agronomic traits in a bc 2 dh population and a set of wild barley introgression lines. *Theoretical and Applied Genetics*, **120**, 1559-1574.
- Wang H, Chen W, Eggert K, Charnikhova T, Bouwmeester H, Schweizer P, Hajirezaei M R, Seiler C, Sreenivasulu N, Von Wirén N. 2018. Abscisic acid influences tillering by modulation of strigolactones in barley. *Journal of Experimental Botany*, **69**, 3883-3898.
- Wiegmann M, Maurer A, Pham A, March T J, Al-Abdallat A, Thomas W T, Bull H J, Shahid M, Eglinton J, Baum M. 2019. Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues. *Scientific Reports*, **9**, 1-16.
- Woodward E, Marshall C. 1988. Effects of plant growth regulators and nutrient supply on tiller bud outgrowth in barley (*Hordeum distichum* L.). *Annals of Botany*, **61**, 347-354.
- Xie Q, Mayes S, Sparkes D L. 2016. Optimizing tiller production and survival for grain yield improvement in a bread wheat × spelt mapping population. *Annals of Botany*, **117**, 51-66.
- Xing Y, Zhang Q. 2010. Genetic and molecular bases of rice yield. *Annual Review of Plant Biology*, **61**, 421-442.
- Yamaguchi S. 2008. Gibberellin metabolism and its regulation. *Annual Reviews of Plant Biology*, **59**, 225-251.

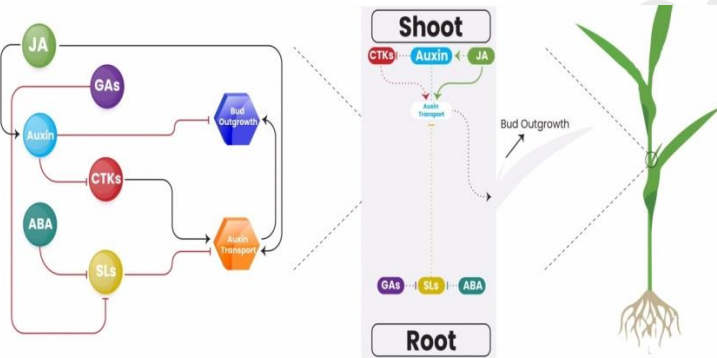
- Yang L, Shao H, Wu Q X, Yu J, Rang C F, Li L Q, Li X. 2013. QTLs mapping and epistasis analysis for the number of tillers and spike number per plant in wheat. *Journal of Triticeae Crops*, **33**, 875-882.
- Yao C, Finlayson S A. 2015. Abscisic acid is a general negative regulator of arabidopsis axillary bud growth. *Plant Physiology*, **169**, 611-626.
- Ye L, Wang Y, Long L, Luo H, Shen Q, Broughton S, Wu D, Shu X, Dai F, Li C. 2019. A trypsin family protein gene controls tillering and leaf shape in barley. *Plant Physiology*, **181**, 701-713.
- Yoshida A, Ohmori Y, Kitano H, Taguchi-Shiobara F, Hirano H Y. 2012. Aberrant spikelet and panicle1, encoding a topless-related transcriptional co - repressor, is involved in the regulation of meristem fate in rice. *The Plant Journal*, **70**, 327-339.
- Patil S B, Barbier F F, Zhao J, Zafar S A, Uzair M, Sun Y, Fang J, Perez-Garcia M, Bertheloot J, Sakr S, Fichtner F, Chabikwa T G, Yuan S, Beveridge C A, Li X. 2021. Sucrose promotes D53 accumulation and tillering in rice. *New Phytologist*. (<https://nph.onlinelibrary.wiley.com/doi/epdf/10.1111/nph.17834>)
- Zazimalov áE, Murphy A, Yang H, Hoyerova K, Hosek P. 2010. Auxin transporter-why so many? *Cold Spring Harbor Perspectives in Biology*, **3**, A001552.
- Zhang Q, Wang Z, Miao F, Wang G. 2017. Dryland maize yield and water-use efficiency responses to mulching and tillage practices. *Agronomy Journal*, **109**, 1196-1209.
- Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L. 2006. The rice high-tillering dwarf1 encoding an ortholog of arabidopsis *max3* is required for negative regulation of the outgrowth of axillary buds. *The Plant Journal*, **48**, 687-698.



**Fig. 1** Architecture of barley plant with its developmental stages of tillers (Shaaf *et al.* 2019). (1) The first stage of tiller development: Bud initiation, which can be seen after removal of the en-sheathing leaf. (2) The second stage of tiller development: Bud outgrowth into tiller: T1; from leaf axil & T0; from coleoptile. (3) & (4) Secondary tillers; (5) Productive tillers, T1 and T2 with grain bearing spikelets and Non-productive tillers; T0 and T4.



**Fig. 2** Chromosomal map of barley tillering related genes. Upside scale of shows the name/number of chromosome, number of genes mapped on each chromosome and the size of chromosome in Mbs. The white mark line indicates the physical location and the black bubble with black text is the name of specific gene.



**Fig. 3** Summary of hormonal cross-talk and their relationship in regulation of tillering. The arrow-shaped lines indicate the promoting effect and T-shaped arrows indicate the inhibitory effect of phytohormones.