Genetics of Tillering in Rice and Barley

Ahmed Hussien, Elahe Tavakol,* David S. Horner, María Muñoz-Amatriain, Gary J. Muehlbauer, and Laura Rossini

Abstract

Tillering, or the production of lateral branches (i.e., culms), is an important agronomic trait that determines shoot architecture and grain production in grasses. Shoot architecture is based on the actions of the apical and axillary meristems (AXMs). The shoot apical meristem (SAM) produces all aboveground organs, including AXMs, leaves, stems, and inflorescences. In grasses like rice (Oryza sativa L.) and barley (Hordeum vulgare L.), vegetative AXMs form in the leaf axil of lower leaves of the plant and produce tillers (branches). Tiller development is characterized by three stages, including (i) AXM initiation, (ii) bud development, and (iii) outgrowth of the axillary bud into a tiller. Each tiller has the potential to produce a seed-bearing inflorescence and, hence, increase yield. However, a balance between number and vigor of tillers is required, as unproductive tillers consume nutrients and can lead to a decreased grain production. Because of its agronomic and biological importance, tillering has been widely studied, and numerous works demonstrate that the control of AXM initiation, bud development, and tillering in the grasses is via a suite of genes, hormones, and environmental conditions.

In this review, we describe the genes and hormones that control tillering in two key cereal crops, rice and barley. In addition, we discuss how the development of new genomics tools and approaches, coupled with the synergy between the rice and barley genomes, are accelerating the isolation of barley genes underlying tillering phenotypes.
Tillers can develop inflorescences and contribute to grain yield, but tillers that grow late in the season will not produce any grain and will lower the overall harvest index (Sakamoto and Matsuoka, 2004; Mäkelä and Muurinen, 2011), indicating that a balance between number and vigour of tillers is required. A model plant with reduced height, low number of unproductive tillers, higher number of grains, and erect leaves has been proposed as the ideotype for cereal breeding (Donald 1968; Khush, 2001). Indeed, reduced tillering accompanied increased productivity in the domestication of maize (Doebely et al., 2006). Cereal varieties grown before the Green Revolution were responding to N fertilizer by profuse tillering and stem elongation, thus increasing biomass. Ideotype breeding led to shorter plants with more productive tillers and improved response to N, and increased harvest index to 0.5 (Khush, 2003). In rice, this ideotype is associated with certain alleles of the *wealthy farmer's panicle* (wfp)/ideal plant architecture1 (ipai) locus, which confer low tillering coupled with desired inflorescence features resulting in higher yield (Jiao et al., 2010; Miura et al., 2010). Reduced tillering has also been associated with improved kernel weight under terminal water deficit environments in wheat near-isogenic lines carrying the *tiller inhibition* (tin) gene (Atsmon and Jacobs, 1977; Kebrom and Richards, 2013; Mitchell et al., 2013).

In addition to being important crops, barley and rice are genetic model systems for the Poaceae family (Izawa and Shimamoto, 1996). Rice has a small genome (~430 Mb) with a high degree of chromosomal synteny with other major cereal crops (Bolot et al., 2009). Barley, with its diploid genome, represents another convenient model, particularly for Triticeae crops which share high genomic colinearity, providing a basis for genetic and genomic analyses in polyploid wheats (Bennetzen and Freeling, 1997; Hayes et al., 2003; Kumlehn and Hensel, 2009).

The conservation of synteny and integration of genomic tools among small grain cereals, particularly rice, barley, and wheat, allows the transfer of knowledge among these major crops. In the following sections, we will summarize progress in the identification and characterization of genes, hormones, and developmental pathways underlying tillering in rice and barley. Additionally, we highlight the emergence of new genomics resources and high-throughput technologies that are revolutionizing genetic studies in these cereal species, often by leveraging the high degree of colinearity that their genomes exhibit.

**Tiller Development in Rice and Barley**

Plant development is a continual process of organogenesis involving the activity of meristems, pluripotent stem cell populations present in different parts of the plant. Shoot architecture is ultimately determined by the activity and determinacy of the SAM and AXMs (Wang and Li, 2008). Tiller development in grasses comprises three main stages: (i) establishment of an AXM marked by formation of stem cell population in the leaf axil, (ii) production of leaf...
primordia from the AXM to form an axillary bud, and
(iii) outgrowth of the axillary bud to form a tiller (Fig. 1)
(Schmitz and Theres, 2005). Classically, shoot development
is considered to occur in repeated units called phytomers,
each consisting of an internode (stem segment), a node,
a leaf, and an axillary bud (Weatherwax, 1923; Sharman,
1942). Elaborations on this basic model have been pro-
posed for barley to account for floral organs and paired
structures present at branching points (e.g., prophyll and
palea) (Bossinger et al., 1992; Forster et al., 2007).

In barley and rice, the first AXMs are formed dur-
ing embryogenesis. The mature barley embryo generally
contains two axillary buds, one each in the axils of the
coleoptile and of the first leaf primordium (Kirby and
Appleyard, 1987), while the mature rice embryo gener-
ally contains one axillary bud in the axil of the coleoptile
(Saha, 1957). Upon germination, both plants continue to
produce more leaves and AXMs in an ordered and coor-
dinated progression, as described in detail in rice (Itoh
et al., 2005; Oikawa and Kyozuka, 2009). Leaves derive
from leaf founder cells at the flanks of the SAM, which
undergo a developmental transition from an indetermi-

ginate to a determinate cell fate as they are recruited into
incipient leaf primordia. The time interval between the
formation of two successive leaf primordia is called a
plastochron, and leaf developmental stages are identified
by their plastochron number (Pi), with the youngest vis-
ible leaf primordium indicated as P1, the next youngest
as P2, and so on (reviewed in Itoh et al., 2005). The AXM
develops in the leaf axil and stages of AXM formation
are indicated with Pi of the subtending leaves. In rice,
the first visible indication of AXM development is a

slight protrusion on the stem surface towards the P2 leaf
primordium (Oikawa and Kyozuka, 2009). Cell prolifera-
tion continues through the P3 and P4 stages when cells
undergo a critical transition acquiring meristematic fate.
The process is completed by the P5 stage when the new
AXM has initiated its own leaf primordia originating an
axillary bud (Oikawa and Kyozuka, 2009).

Depending on endogenous and environmental sig-
nals, an axillary bud may remain dormant or grow into a
tiller. Each tiller is a new axis of growth, organized
like the main culm in phytomer units. Each tiller har-
bors new axillary buds that may in turn develop new
tillers in a reiterative pattern (Fig. 1b). Tillers, therefore,
develop in acropetal succession with primary tillers

growing out of leaf axils of primary tillers, and so
on (Kirby and Appleyard, 1987). After the transition of
the main culm SAM from a vegetative to a reproductive
state, young tillers undergo senescence, possibly because
nutrients are routed away from developing tillers to the
elongating internodes (Mohapatra et al., 2011). The early
developing primary tillers benefit from higher sink/
source ratio, sink capacity, leaf area, spikelet number, and
filled grain percentage (Choi and Kwon, 1985).

Axillary meristem establishment and formation of
the axillary bud are mostly under genetic control, while
bud outgrowth is regulated by a complex network of
genetic, hormonal, and environmental factors (Kebrom
et al., 2013), making it highly responsive to environmen-
tal conditions, such as shading and nutrient availability
(Agusti and Greb, 2013). Being sessile, plant fitness
in varied environmental conditions depends on their

Figure 1. Tiller formation in barley and shoot architecture of barley and rice. (a) Longitudinal section of a barley shoot apex showing
a shoot apical meristem (SAM), an axillary meristem (AXM, the first stage in tiller formation) and axillary buds (AXB, the second stage
of tiller formation). (b) Barley plant with out growth of primary (1°T) and secondary tillers (2°T), the third stage of tiller formation. MC
= main culm. (c) Barley shoot architecture showing the MC and tillers (T). (d) Rice shoot architecture showing the MC and T. (e) Barley
two-rowed spike showing the flag leaf (FL), rachis (R), spikelet (S), and awn (AW). (f) Rice panicle showing the FL, R, primary branch
(PB), secondary branch (SB), and S.
ability to translate external signals into developmental responses. Several distinct signals are involved in the regulation of shoot branching, with key roles played by auxin, cytokinins (CK), and SL (Kebrum et al., 2013).

Upon transition to the reproductive phase, the SAM of each tiller is transformed into an inflorescence meristem and differentiates into an inflorescence, called a panicle in rice and spike in barley, organized around a main axis called a rachis (Fig. 1). In rice, each inflorescence meristem produces several AXMs that develop into rachis branches, and the AXMs generated subsequently may develop into the next order of rachis branches or lateral spikelets, resulting in a branched structure (Fig. 1) (Itoh et al., 2005; Oikawa and Kyozuka, 2009). In barley, AXMs arising from the rachis differentiate into spikelet triplet meristems, that in turn will develop into three spikelet meristems (Bossinger et al., 1992), one central and two lateral. In wild barley and two-rowed cultivars, only the central spikelet is fertile, while the lateral spikelets are sterile and remain underdeveloped. In six-rowed barley cultivars and mutants, all three spikelets mature to produce grains (Kirby and Appleyard, 1987; Komatsuda et al., 2007).

### Genetic and Hormonal Control of Tilling in Rice

Thanks to its importance as a crop and also as a model system for other cereals, the genetic basis of shoot branching in rice has received growing attention, and over the last decade, a number of genes involved in tiller development have been identified (Table 1, Fig. 2). Some of these genes specifically affect tiller development, while others affect other agronomically important traits such as inflorescence (panicle) architecture and plant height. Here we review the isolated and characterized genes involved in rice tillering and related hormonal pathways including auxin, CK, gibberellins (GA), and SL.

### Table 1. List of genes and mutants involved in rice tillering with their phenotypes, homologs, molecular function, and pathway.†

<table>
<thead>
<tr>
<th>Gene or mutant name, protein or gene family</th>
<th>Phenotype</th>
<th>Homologs</th>
<th>Molecular function or pathway</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>AXIN establishment and maintenance</strong></td>
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<tr>
<td><strong>MOC1—GRAS family</strong></td>
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<tr>
<td>reduced tillers†</td>
<td>tomato Lateral suppressor (Ls)</td>
<td>LAS</td>
<td>TF up-regulates OsTB1 and OSH1, works with LAX1 and LAX2 in AXM establishment; degraded by TAD1</td>
<td>Li et al., 2003; Xu et al., 2012; Lin et al., 2012</td>
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<tr>
<td>increased tillers and reduced plant height†</td>
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<td><strong>TAD1 and TF, Cdh1-type coactivators</strong></td>
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<tr>
<td>increased tillers and reduced plant height†</td>
<td>CCSS2A</td>
<td></td>
<td>APC/C, a multisubunit E3 ligase; forms a complex with OsAPC10; activates APC/C to target MOC1 for degradation by the ubiquitin-26S proteasome pathway</td>
<td>Xu et al., 2012; Lin et al., 2012</td>
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<td><strong>MIP1, Brefeldin A-sensitivity protein 1 (OsbRE1A and OsBre1B)</strong></td>
<td>increased tillers and reduced plant height†</td>
<td>Arabidopsis At_HUB1, Maize zm_Bre1</td>
<td>Bre1 protein family is E3 ubiquitin-protein ligase; At_HUB1 involved in chromatin modification and gene regulation; interacts with MOC1</td>
<td>Sun et al., 2010</td>
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<td><strong>LAX1, containing basic helix-loop-helix domain</strong></td>
<td>reduced tillers and panicule branches§</td>
<td>Arabidopsis AtBA1/LAX1, Maize BAT</td>
<td>TF; interacts with MOC1 and LAX2 in AXM establishment; maintenance</td>
<td>Komatsu et al., 2003; Oikawa and Kyozuka, 2009</td>
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<tr>
<td><strong>LAX2, Novel plant nuclear protein</strong></td>
<td>reduced tillers and panicule branches§</td>
<td>Arabidopsis DRIP1 and DRIP2 (although lack the amino acid stretches)</td>
<td>potentially works as coactivator of LAX1; interacts with MOC1 and LAX1 in AXM establishment; maintenance</td>
<td>Tabuchi et al., 2011</td>
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AUXIN

*OsPIN1-PIN1 family*

| increased tillers§ | Arabidopsis AtPIN1 | auxin efflux carrier/Transports Auxin | Xu et al., 2005 |

*OsPIN2-PIN2 family*

| increased tillers and tiller angle, reduced plant height§ | Arabidopsis AtPIN2 | auxin efflux carrier; transports auxin, down-regulates OsIAA1 (negative regulator of polar auxin transport) | Chen et al., 2012 |

*Increased number of Tiller/Enlarged Leaf Width (Dw) TLD1/Gretchen Hagen3 OsGH3.13-GH3*

| increased tillers, leaf angle and drought tolerance, reduced panicle length, plant height and spikelets no# | Arabidopsis WES1/GH3.5 | multienzyme acetyl amino synthetase; Conjugates IAA to amino acids; regulates negatively free IAA; Arabidopsis homologs respond to ABA and salicylic acid pathways | Zhang et al., 2009 |

*ASP1/Ramosa1 Enhancer Locus2-Transcriptional corepressor*

| increased primary tillers and reduced secondary tillers/disrupted phylloclade of tillers arrangement# | TPL, REL2 | transcriptional corepressor; derepression of auxillary bud growth; involved in auxin signaling | Kwon et al., 2012; Yoshida et al., 2012 |

*OsmiR395, NIRS393 noncoding miRNA*

<p>| increased tillers, early flowering, salt and drought susceptibility§ | Arabidopsis mir393 | represses OsTIR1 and OsAFB2, that further represses OsLUX1, auxin transport to auxillary buds and down-regulates OsTIR1 | Xia et al., 2012 |</p>
<table>
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<tr>
<th>Protein or gene family</th>
<th>Phenotype</th>
<th>Homologs</th>
<th>Molecular function or pathway</th>
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<tr>
<td><strong>Cytokinins (CK)</strong></td>
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<tr>
<td>OsIPT1, 2, 3, 4, 7, Adenosine 5'-phosphate isopentenyl transferase</td>
<td>overproliferation of axillary shoots</td>
<td>Arabidopsis Isopentenyl Transferase genes</td>
<td>catalyses prenylation of adenosine 5'-phosphates at the N6-terminus with dimethylallylphosphate (involved in CK biosynthesis)</td>
<td>Sakamoto et al., 2006</td>
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<td><strong>Gibberellin (GA)</strong></td>
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<tr>
<td>OsGA2ox5/6 oxidases</td>
<td>increased tillers and adventitious root growth, semidwarfing</td>
<td>AtGA2oxs, ZmGA2oxs</td>
<td>catalyse 2B-hydroxylation reaction/inactivate bioactive GA</td>
<td>Lo et al., 2008</td>
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<td><strong>Strigolactones (SL)</strong></td>
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<tr>
<td>D1/D17/HTD1, CCD7</td>
<td>increased tillers</td>
<td>MAX3</td>
<td>cleaves 3-carotene into 13-apo-3-carotenone/involved in SL biosynthesis/up-regulated by auxin</td>
<td>Ishikawa et al., 2005, Kebrom et al., 2013, Lo et al., 2008</td>
</tr>
<tr>
<td>D10/CCD8b, CCD8 family</td>
<td>increased tillers</td>
<td>MAX4</td>
<td>cleaves 3-carotene into 13-apo-3-carotenone/involved in SL biosynthesis/up-regulates by auxin</td>
<td>Ishikawa et al., 2005, Kebrom et al., 2013, Arite et al., 2007</td>
</tr>
<tr>
<td>D27, Iron-containing protein with isomerase activity</td>
<td>increased tillers</td>
<td>AtD27</td>
<td>produces 9-cis-3-carotene from all-trans-3-carotene in SL biosynthesis pathway/involved in SL biosynthesis</td>
<td>Ishikawa et al., 2005, Lin et al., 2009, Kebrom et al., 2013</td>
</tr>
<tr>
<td>D14, hydrolase/esterase</td>
<td>increased tillers</td>
<td>AtD14</td>
<td>involved in SL signaling, likely as SL receptor and SL catabolic enzyme/interacts with GA signaling regulator SLR1/regulated by OsMADS57</td>
<td>Ishikawa et al., 2005, Guo et al., 2013, Nakamura et al. 2013</td>
</tr>
<tr>
<td>D3, F-box leucin-rich repeat protein</td>
<td>increased tillers</td>
<td>Arabidopsis MAX2</td>
<td>mediates signaling of SL and karrikins; involved in SL biosynthesis</td>
<td>Ishikawa et al., 2005, Kebrom et al., 2013</td>
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<td><strong>SL pathway regulation and integration of different signals</strong></td>
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<tr>
<td>OsTEF1, EL1 superfamily</td>
<td>reduced tillers, seminal roots growth and salt tolerance</td>
<td>Arabidopsis Transcription elongation factor-like</td>
<td>involved in transcription elongation by RNA polymerase II; Induces cytochrome P450, potentially a member of SL pathway in Arabidopsis</td>
<td>Paul et al., 2012</td>
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<tr>
<td>OsTB1/FC1, class II proteins of TCP family</td>
<td>excessive tillers and thin culm</td>
<td>TB, BRC1/TBL1</td>
<td>TF; regulates SL signal transduction by interaction with OsMADS57 and reduces its inhibition for D14; target for repression by CK and GA; up-regulated by auxin</td>
<td>Minakuchi et al., 2010; Guo et al., 2013, Takada et al., 2003</td>
</tr>
<tr>
<td>OsMADS57, MADS-box domain protein</td>
<td>increased tillers, reduced tillers</td>
<td>involved in SL signaling by inhibiting D14; inhibits directly D14 and its inhibition activity reduced by interaction with OsTB1</td>
<td>Guo et al., 2013</td>
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<tr>
<td>OsMIR444a, MIR444 noncoding miRNA</td>
<td>reduced tillers</td>
<td>enhances the SL biosynthesis by repressing OsMADS57</td>
<td>Guo et al., 2013</td>
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<td>TH5, Class III lipase family</td>
<td>increased tillers, shorter plants, and less seed set</td>
<td>Arabidopsis PRLP8</td>
<td>may be involved in auxin and SL signaling</td>
<td>Liu et al., 2013</td>
</tr>
<tr>
<td>NSP1 and NSP2</td>
<td>reduced tillers</td>
<td>Legume NSP1 and NSP2</td>
<td>TF; down-regulates D27</td>
<td>Liu et al., 2011</td>
</tr>
<tr>
<td>OsSPL14/Os IPA1, WFP QTL–SPL family</td>
<td>reduced tillering, increased lodging resistance, and higher yield</td>
<td>AtSPLs and maize ZmSPLs</td>
<td>TF, regulates OsTB1</td>
<td>Miura et al., 2010, Chen et al., 2010, Lu et al., 2015</td>
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<td><strong>Brassinosteroid (BR)</strong></td>
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<tr>
<td>DL1/OsGRAS32, GRAS</td>
<td>reduced tillers and plant height</td>
<td>Arabidopsis AtGRAS8</td>
<td>TF, involved in inhibition of BR biosynthesis through interaction with OsBZR1 that perceives BR signal by OsBRI1</td>
<td>Tong et al., 2009, 2012</td>
</tr>
<tr>
<td>GS2, GS5 family</td>
<td>reduced tillers, dark green and dwarf plants</td>
<td>Arabidopsis GS53/SHAGGY-like kinase</td>
<td>Regulates DL1 gene by phosphorylation</td>
<td>Tong et al., 2012</td>
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</table>

*Table 1. Continued.*
MOCI was the first tillering gene to be identified in rice (Li et al., 2003). Plants carrying loss-of-function mutations in MOCI have only one main culm as a result of failure to establish AXMs, affecting both tiller and panicle branches (Li et al., 2003). MOCI encodes a GRAS (named after the first three members: Gibberellin-ACID INSENSITIVE, REPRESSOR of GA1 and SCARE-CROW) transcription factor homologous to tomato Lateral suppressor (Ls) (Schumacher et al., 1999), and Arabidopsis LATERAL SUPPRESSOR (LAS) (Greb et al., 2003). However, in lsl and las mutants, AXM defects are mainly observed in the vegetative phase, while mocl shows suppression of all types of AXM, indicating that AXM control mechanisms differ at least in part between monocots and eudicots. MOCI expression marks the initiation of the AXM and is maintained in the developing bud, consistent with a role in axillary bud initiation and outgrowth (Li et al., 2003). Recently, a protein mediating the degradation of MOCI protein was identified from analysis of the allelic mutants tad1 (Xu et al., 2012) and tillering enhancer (te; Lin et al., 2012). These mutants are characterized by high tillering and reduced plant height, similar to plants overexpressing MOCI (Li et al., 2003). The TAD1/TE gene encodes a Cdh1-type coactivator orthologous to the dicot CCS52A (Vinardell et al., 2003), which interacts with the anaphase-promoting complex (APC/C), a multisubunit E3 ligase. TAD1/TE forms a complex with APC/C-OsAPC10, targeting MOCI for degradation via the ubiquitin-26S proteasome pathway, thus repressing AXM initiation (Xu et al., 2012; Fig. 2). MOCI Interacting Protein 1 (MIPI) may be another player in this circuit (Sun et al., 2010).

When overexpressed, the MIPI gene causes enhanced tillering and semidwarf stature similar to MOCI overexpression (Li et al., 2003). MIPI is a member of the Brefeldin A-sensitivity protein 1 family, that includes At_HUB1, which encodes an E3 ligase involved in H2B (Histone 2B;
a subunit of the nucleosome) monoubiquitination in Arabidopsis (Fleury et al., 2007; Liu et al., 2007).

Soon after the first visible appearance of AXM formation, the LAX PANICLE1 (LAX1) gene plays a role in maintenance of AXM development. Plants carrying loss-of-function mutations in LAX1 are characterized by reduced numbers of rachis branches and spikelets on the panicle, and reduced number of tillers (Komatsu et al., 2001; Oikawa and Kyozuka, 2009). LAX1 encodes a putative basic-helix-loop-helix transcription factor required for formation of AXMs in both vegetative and reproductive phases (Komatsu et al., 2001; Oikawa and Kyozuka, 2009). LAX1 acts in a non-cell autonomous manner to maintain cell proliferation during AXM formation. Oikawa and Kyozuka (2009) proposed a two-step regulation of spatial and temporal LAX1 expression and activity. Spatially, LAX1 mRNA is specifically expressed in the boundary region at the adaxial side of the developing AXM, and later, LAX1 protein is trafficked toward the AXM. Temporally, LAX1 mRNA is expressed in leaf axils from P4 to later stages, while LAX1 protein movement is restricted to the P4 stage, accompanying the acquisition of meristematic fate. Plants carrying mutations in the LAX2 gene show similar phenotypes to lax1 mutants, with reduced branching in the vegetative and reproductive phases. LAX2 encodes a novel nuclear protein with a plant-specific conserved domain and was shown to physically interact with LAX1 (Tabuchi et al., 2011). Double mutant analyses suggest that MOC1, LAX1, and LAX2 function in partially independent but overlapping pathways to regulate AXM establishment and maintenance (Tabuchi et al., 2011) (Fig. 2).

**Auxin**

The main shoot apex suppresses the outgrowth of axillary buds via auxin that is produced in young expanding leaves and actively transported basipetally through the shoot, acting indirectly on axillary buds (Agusti and Greb, 2013). The movement of auxin from developing leaves to stem (auxin sink) is termed polar auxin transport (PAT) and depends on auxin efflux carriers of the adenosine triphosphate (ATP)-binding cassette B and the PIN-FORMED (PIN) protein families (Zázimalová et al., 2010). The PINs are integral membrane proteins with a topology similar to transporter proteins (Zázimalová et al., 2010). Auxin is transported to the organ initiation sites through the outermost epidermal layer of the shoot apex and is directed via the developing primordia into the basipetal stream of the main shoot. PIN1 proteins are localized in xylem parenchyma cells and maintain the auxin supplement to the basipetal stream of the main shoot, playing a role in PAT (Petrásek and Friml, 2009). In agreement with the role of PAT in inhibition of bud outgrowth, rice plants underexpressing OsPIN1b (previously known as REH1) showed increased number of tillers (Xu et al., 2005; Chen et al., 2012). Conversely, increased tillering and reduced stature were caused by overexpression of OsPIN2, suggesting that OsPIN1b and OsPIN2 play distinct roles in the control of shoot architecture (Chen et al., 2012).

Additional insight into the role of auxin comes from analysis of the ABERRANT SPIKELET AND PANICLE1 (ASPI; or rice RAMOSA1 ENHANCER LOCUS2) gene that encodes a transcriptional corepressor proposed to act in auxin signaling (Yoshida et al., 2012). A variety of developmental alterations were described for aspl
mutant plants, including derepression of axillary bud outgrowth and disturbed phyllotaxy in the vegetative phase, disorganized panicle branching and spikelet morphology, indicating a general role for ASPI in controlling meristem fate (Yoshida et al., 2012).

A member of the MIR393 miRNA family known to regulate expression of auxin receptors also affects tillering ability in rice. Plants overexpressing OsmiR393 showed reduced expression of the auxin receptors OsTIR1 and OsAFB2, which sequentially repress the auxin transporter OsAux1 (Xia et al., 2012). This ultimately down-regulates OstBI, a tillering repressor (see below), explaining the increased outgrowth of tillers in OsmiR393 overexpressing plants.

Another auxin-dependent pathway regulating rice tillering involves TLDI (increased number of Tillers/enlarged Leaf angles/Dwarfism), an indole-3-acetic acid (IAA)-amido synthetase that converts active auxin (IAA) to its inactive form via conjugation with amino acids (Zhang et al., 2009). The associated reduction of IAA concentration affects developmental traits and also influences plant resistance to biotic and abiotic stress via the abscisic acid, and salicylic acid pathways. Gain-of-function tld1-D mutants show pleiotropic phenotypes including increased number of tillers, reduced plant height, shorter panicle, and reduced number of spikelets (Zhang et al., 2009).

Cytokinins

Cytokinins are key regulators of many plant developmental processes, including cell division, activation of axillary buds, inhibition of root growth, and delay of senescence (Mok, 1994). Cytokinins are mainly synthesized in the root and transported upward along the xylem (Wang and Li, 2006). Cytokinin biosynthesis and signaling are affected by nutrient availability and environmental stresses, such as drought and high salinity (Krouk et al., 2011; Ha et al., 2012). The supershoot (sp5) mutants in Arabidopsis show massive shoot overproliferation and three- to ninefold increase in levels of Z-type CKs that indicates a role of SPS in suppression of AXM initiation and growth through the localized attenuation of CK levels at sites of bud initiation (Tantikanjana et al., 2001).

The fundamentals of CK biosynthesis were originally studied in Arabidopsis (Sakakibara, 2004) where the central rate-limiting step is prenylation of adenosine 5'-phosphates at the N6-terminus with dimethylallyldiphosphate at the central rate-limiting step is prenylation of adenosine 5'-phosphates at the N6-terminus with dimethylallyldiphosphate, catalysed by adenosine phosphate ISOPENTENYL TRANSFERASE (IPT). Auxin can down-regulate CK levels by inhibiting the expression of IPT genes (Ferguson and Beveridge, 2009). Other studies showed that the Arabidopsis KNOTTED-like homeobox (KNOX) protein SHOOTMERISTEMLESS (STM) induces expression of AtIPT7 (Jasinski et al., 2005; Yanai et al., 2005) and down-regulates GA biosynthesis genes, creating low-GA and high-CK status in the meristem, which may be essential for the maintenance of meristematic activity (Jasinski et al., 2005). Eight OsIPT genes have been identified and studied in the rice genome (Sakamoto et al., 2006). Transgenic rice plants overexpressing OsIPTs have increased axillary bud activity and reduced root formation, which are typical of CK overexpression. Also, OsIPT3 and OsIPT2 transformants showed elevated content in 12 different CK species, highlighting the overall stimulation of de novo CK biosynthesis (Sakamoto et al., 2006).

Gibberellins

Gibberellins interact with auxin in the regulation of stem elongation, with apically derived auxin regulating GA synthesis (O’Neill and Ross, 2002). In rice, GAs participate in controlling many plant developmental processes, positively regulating germination, stem and root elongation, and flower development, while negatively regulating OSH1 (a rice KNOX gene) and OstBI (see below) (Lo et al., 2008). The main catabolic pathway for GAs is the 2-β-hydroxylation reaction catalysed by C19- and C20-GA 2-oxidases (GA2oxs), which inactivate endogenous bioactive GAs, affecting GA-dependent developmental processes (Sakamoto et al., 2004). In total, 10 putative GA2ox genes were identified in the rice genome and rice lines overexpressing some C20-GA2oxs exhibit semidwarfing, early and increased tillering, and adventitious root growth (Lo et al., 2008).

The ERF protein associated with tillering and panicle branching (OsEATB) gene belongs to the APETALA2-like homeobox (KNOX) family of transcription factors. Transformed plants overexpressing OsEATB showed enhanced tillering, reduced height, enhanced panicle branching, and overall higher grain yield (Qi et al., 2011). These phenotypes were accompanied by decreased levels of different GAs, confirming the negative correlation between GA and tillering.

Strigolactones

Strigolactones have been recently identified as phytohormones which inhibit side branching in plants (Gomez-Roldan et al., 2008; Umehara et al., 2008). Strigolactones are synthesized in roots and move acropetally (Wang and Li, 2006). They interact with auxins in a dual-loop pathway to control axillary bud outgrowth, but the nature of this regulatory loop is unclear (Kebrom et al., 2013). Two hypotheses have emerged, mostly from studies in dicots: (i) SL act downstream of auxin (Foo et al., 2005; Bainbridge et al., 2005; Brewer et al., 2009; Hayward et al., 2009); (ii) SL negatively regulate auxin transport in the main stem, and inhibit the establishment of axillary bud PAT into the stem (Bennett et al., 2006; Crawford et al., 2010; Domagalska and Leyser, 2011). In rice, mutations in SL biosynthesis gene DWARF27 (D27) result in increased PAT (Lin et al., 2009), and exogenous auxin application can up-regulate expression of D10 and D17, two other genes involved in SL biosynthesis (Arite et al., 2007; Zou et al., 2006), indicating a complex interplay between auxin and SL. A scheme linking the SL pathway, shoot branching, and the rice genes characterized to date is presented in Fig. 2. More details regarding the SL pathway can be found
in some excellent reviews (Beveridge and Kyozuka, 2010; Domagalska and Leyser, 2011; Brewer et al., 2013).

**Brassinosteroids**

Along with GAs, brassinosteroids (BRs) are viewed as major players in the control of plant height. Brassinosteroids are phytohormones with a structure similar to animal steroid hormones, and mutants defective in BR biosynthesis and signaling generally exhibit a dwarf phenotype and other abnormalities in leaves and branches (Clouse and Sasse, 1998; Bishop and Koncz, 2002; Gendron et al., 2012). In contrast to the general negative correlation between plant height and tiller number (Hong et al., 2003; Booker et al., 2004), rice mutants impaired in the BR pathway, such as DWARF AND LOW-TILLERING (DLT), show reductions in both tillering and stature. The DLT (or OsGRAS32) gene encodes a GRAS transcription factor involved in feedback inhibition of BR biosynthesis (Tong et al., 2009, 2012). Brassinosteroids can down-regulate the expression of DLT via the OsBR1 receptor-like protein, which regulates both DLT and OsBZR1, a transcription factor that, in turn, regulates BR-responsive genes (Tong et al., 2009, 2012). OsBZR1 is also controlled by GS3/SHAGGY-like kinase (GSK2), which is the ortholog of Arabidopsis GSK2 BRASSINOSTEROID-INSENSITIVE2. Rice GSK2 overexpression results in dwarf plants with dark green leaves, compact structure, and fewer tillers (Tong et al., 2012).

**Strigolactone Pathway Regulation and Integration of Different Signals**

Recent studies are providing insight into the regulation of the SL pathway and its cross-talk with other hormonal pathways (Fig. 2). NODULATION SIGNALING PATHWAY1 and NODULATION SIGNALING PATHWAY2 are indispensable for SL biosynthesis through positive regulation of D27 (Liu et al., 2011; Ishikawa et al., 2005; Kebrom et al., 2013). Expression of D10, another SL biosynthesis gene, is increased in the rice this1 mutant (Liu et al., 2013). The this1 mutant is characterized by reduced height, infertile spikelets, and high tillering, due to the release of tiller bud outgrowth at the elongation stage (Arite et al., 2007; Ishikawa et al., 2005; Kebrom et al., 2013). The rice mutant fine culm1 (fc1) exhibits thin culm and excessive tillering due to a loss-of-function mutation of the OsTBI gene, which encodes a TBI/CYCLOIDEA/PCF (TCP) transcription factor homologous to TBI in maize, and Arabidopsis BRANCHED 1/TBI-LIKE 1 (Takeda et al., 2003; Choi et al., 2012). Thus, FC1/OsTBI functions as a negative regulator of lateral branching in rice, similar to maize TBI (Minakuchi et al., 2010). Based on insensitivity of the fc1 mutant to exogenous application of SL and epistatic interaction with d17, Minakuchi et al. (2010) proposed that FC1/OsTBI acts downstream of SL. Negative regulation of FC1/OsTBI expression by CK and GA (Lo et al., 2008) further led to the hypothesis that FC1/OsTBI may act as an integrator of multiple pathways controlling tillering (Minakuchi et al., 2010). More details about the interplay between OsTBI and SL have recently emerged. OsMADS57, a MADS-box domain protein was shown to interact with OsTBI and repress D14, a gene involved in SL signaling and possibly encoding a SL receptor (Guo et al., 2013). OsmiR444a, a member of the MIR444 miRNA family, posttranscriptionally regulates OsMADS57 transcript accumulation (Guo et al., 2013). In contrast to the function of FC1/OsTBI, genetic and molecular analyses of OsMADS57 indicate that it functions as a repressor of SL signaling and a positive regulator of tillering. Thus, interaction between the two transcription factors may act to fine-tune D14 expression and consequently tiller outgrowth (Guo et al., 2013). Recently, OsTBI was shown to be directly regulated by OsSPL14, a member of the SQUAMOSA Promoter Binding Protein gene family (Lu et al., 2013) previously associated with the semidominant WEALTHY FARMERS PANICLE/IDEAL PLANT ARCHITECTURE1 quantitative trait locus (QTL) (Jiao et al., 2010; Miura et al., 2010). Increased OsSPL14 expression results in reduced tillering, prolonged stalkochron duration, sturdier stems, increased number of primary branches in the panicle and, ultimately, higher grain yield, corresponding to the ideotype for rice breeding (Jiao et al., 2010; Miura et al., 2010). In the vegetative phase, OsSPL14 is specifically expressed in leaf primordia, indicating that OsSPL14 may affect tillering through regulation of stalkochron duration, an activity shared with the highly related Arabidopsis genes SPL9 and SPL15 (Luo et al., 2012). A genome-wide screen for OsSPL14 binding sites recovered various developmental genes including OsPIN1b, suggesting that OsSPL14 may also be involved in regulation of PAT (Lu et al., 2013). Negative control of OsSPL14 expression involves cleavage by OsmiR156, whose overexpression transformants show increased number of tillers, and reduced number of spikelets and grains per panicle (Luo et al., 2012). ABRANT PANICLE ORGANIZATION1 (APO1) and APO2 also act upstream of OsSPL14 (Luo et al., 2012). APO1 encodes the ortholog of an Arabidopsis F-box protein UNUSUAL FLORAL ORGANS, while APO2 is homologous to the Arabidopsis LEAFY gene, and the two proteins were shown to interact in vitro (Ikeda et al., 2007; Ikeda-Kawakatsu et al., 2012). Rice apos mutants have shorter stalkochron duration, increased number of tillers, and small panicles with reduced number of primary branches (Ikeda-Kawakatsu et al., 2012). Together, analyses of these genes indicate that regulation of stalkochron duration also affects the number of tillers formed by a plant as a result of the number of leaves and axils formed on the shoot (Wang and Li, 2011).

**Other Genes**

Other genes in the control of tillering have been recently characterized (Table 1). Future work will help to better understand if and how they interact with the aforementioned pathways. Among them, FRIZZY PANICLE (FZP) was initially identified as a repressor of AXM formation in the panicle, necessary to establish floral meristem
identity in rice spikelets (Komatsu et al., 2003). The FZP gene encodes an ethylene-responsive ERF transcription factor highly related to Branched Silkless1 that was shown to play a similar role in maize inflorescence development (Chuck et al., 2002). Noteworthy is a loss-of-function allele of FZP that shows defects in tillering, suggesting that FZP plays additional roles in controlling vegetative AXM development (Kato and Horibata, 2011).

In summary, since the first gene involved in tillering in rice was identified 10 yr ago (Li et al., 2003), many genes involved in tillering have been rapidly identified in this species, revealing a complex genetic and hormonal network regulating tiller development. The growing number of genes associated with the regulation of tillering in rice provides a molecular framework for this process, while opening new questions about the interplay of the different factors involved.

### Genetic Control of Tilling in Barley

Several barley mutants exhibiting tillering abnormalities have been identified and characterized providing some initial insight into the genetic and hormonal regulation of tillering in the Triticeae (Dabbert et al., 2010) (Table 2 and Fig. 3). These can be classified into four classes, according to their AXM activity. The first class includes mutants that fail to develop axillary buds, and consequently, develop no tillers. The mutant uniculm2 (cul2) is the best characterized example of this class with a phenotype similar to that of moc1 mutant in rice (Babb and Muehlbauer, 2003). The second class of mutants exhibit lower number of tillers due to compromised axillary bud outgrowth, including low number of tillers1 (Int1; Dabbert et al., 2010), absent lower laterals1 (als1; Dabbert et al., 2009) and uniculme4 (cul4) (Babb and Muehlbauer, 2003). The third group comprises mutants with modestly reduced tillering, such as the intermediate-b (int-b) and semibrachytic (uzu) mutants (Babb and Muehlbauer, 2003). The fourth class of mutants exhibits enhanced tillering, suggesting a defect in controlling tiller bud outgrowth. The mutants granum-a (gra-a), grassy tillers (grassy), intermediate-c (int-c), many noded dwarf1 (mnd1), and many noded dwarf6 (mnd6) represent the high tillering class (Babb and Muehlbauer, 2003; Druka et al., 2011). Additional loci altering inflorescence architecture may also affect tillering, although such defects have not been explored in detail in barley. One example is the barley INF-C gene, the ortholog of maize and rice TB1 genes, which acts in controlling spike row-type and also has an effect on seedling tiller number (Ramsay et al., 2011).

Uniculm2 (cul2) mutants possess one main culm without any tiller outgrowth (Babb and Muehlbauer, 2003). The cul2 mutation appears to be epistatic to low tillering mutations (Int1, als1, cul4, int-b, and uzu) and high tillering mutations (mnd1, mnd6, and gra). Barley cul2 mutants also show disorganization in the distal end of the developing inflorescence, and deviation from wild-type timing of reproductive developmental steps (Babb and Muehlbauer, 2003). Overall similarities between cul2 and the rice moc1 mutant include failure to develop axillary buds, reduced plant height, reduced inflorescence branching, and the epistatic effects to mutations in other loci. However, in contrast to moc1 where AXMs are not initiated, AXMs are present in cul2 leaf axils but do not progress to axillary

### Table 2. List of barley tillering mutants. For each mutant, the chromosomal position and relevant references are indicated, along with the corresponding (candidate) gene and rice ortholog when known.

<table>
<thead>
<tr>
<th>Barley mutant</th>
<th>Map position</th>
<th>Mutant phenotype</th>
<th>Gene (rice ortholog)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>uniculm2 (cul2)</td>
<td>6HL</td>
<td>no tiller, irregular INF</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003; Okagaki et al., 2013</td>
</tr>
<tr>
<td>low number of tillers1 (Int1)</td>
<td>3HL</td>
<td>few tillers, irregular INF</td>
<td>JoB ei2 (qSHT)</td>
<td>Dabbert et al., 2010</td>
</tr>
<tr>
<td>absent lower laterals1 (als1)</td>
<td>3HL</td>
<td>few tillers, irregular INF</td>
<td>ND</td>
<td>Dabbert et al., 2009</td>
</tr>
<tr>
<td>uniculme4 (cul4)</td>
<td>3HL</td>
<td>few tillers</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003; Rossini et al., 2006</td>
</tr>
<tr>
<td>intermediate spike-b (int-b)</td>
<td>5HL</td>
<td>reduced tillering, irregular INF</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003; Okagaki and Muehlbauer, unpublished data, 2013</td>
</tr>
<tr>
<td>semibrachytic (uzu)</td>
<td>3HL</td>
<td>reduced tillering and plant height, shorten spike</td>
<td>HvBR1 (OsBR1)</td>
<td>Babb and Muehlbauer, 2003; Osho et al., 2003; Nakamura et al., 2006</td>
</tr>
<tr>
<td>granum-a (gra-a)</td>
<td>3HL</td>
<td>high tillering, dwarf, shorten spike</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003</td>
</tr>
<tr>
<td>intermediate-c (int-c)</td>
<td>4HS</td>
<td>high tillering, Reduced lateral spikelet development</td>
<td>HvTB1 (OsTB1)</td>
<td>Ramsay et al., 2011</td>
</tr>
<tr>
<td>many noded dwarf1.a (mnd1.a)</td>
<td>7HL</td>
<td>high tillering, dwarf, irregular INF</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003; Nice and Muehlbauer, unpublished data, 2013</td>
</tr>
<tr>
<td>many noded dwarf6 / densinodosum 6 (den6)</td>
<td>5HL</td>
<td>high tillering, short spike</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003; Druka et al., 2011; Nice and Muehlbauer, unpublished data, 2013</td>
</tr>
<tr>
<td>semidwarf1 (swh1) / denso</td>
<td>3H</td>
<td>high tillering, reduced plant height</td>
<td>SD1</td>
<td>Jia et al., 2011</td>
</tr>
<tr>
<td>intermediate spike-m (int-m)</td>
<td>ND</td>
<td>high tillering, irregular INF</td>
<td>ND</td>
<td>Babb and Muehlbauer, 2003</td>
</tr>
</tbody>
</table>

1INFO, inflorescence.  
2ND, not determined.
buds, indicating that Cul2 acts at the stage of bud development. The cul2 locus was positioned on chromosome 6H near the centromeric region, but no candidate genes have been identified (Okagaki et al., 2013). Transcriptome analysis of cul2 mutants and corresponding wild-type plants using the Affymetrix Barley1 GeneChip (Close et al., 2004) indicated that CUL2 is necessary for coordinating signaling pathways and stress response and integrating them into AXM development (Okagaki et al., 2013).

The als1, Int1, and cul4 mutants typically develop only 1 to 3 tillers compared with their wild-type background (Dabbert et al., 2009, 2010; Babb and Muehlbauer, 2003). These loci, mapped at different positions on chromosome 3H, are epistatic to the high tillering mutants, based on the low tillering phenotype of the
double mutants (Dabbert et al., 2009, 2010; Muehlbauer, unpublished results, 2013). Transcriptomics studies showed that stress-related genes are up-regulated in the als1, but not in the lnt1 mutant. Gene expression, mapping, and sequence analyses supported the JuBel2 gene as a candidate for the Lnt1 locus (Dabbert et al., 2010). The JuBEL2 protein is a member of BELL-family of homeodomain transcription factors (Müller et al., 2001), sharing high similarity with qSH1, a rice QTL for seed shattering on chromosome 1 (Konishi et al., 2006) and the transcription factor Arabidopsis BELLRINGE (BLR, wild-type) in Arabidopsis (Smith and Hake, 2003; Byrne, 2003; Roeder et al., 2003). The mutant qSH1 allele in cultivated rice is a regulatory mutation that prevents qSH1 expression in the pedicle at the base of the rice seed, resulting in resistance to shattering (Konishi et al., 2006). No effect of this mutation was detected on expression of qSH1 in other tissues and no phenotypic effect on tillering was reported. However, a qSH1 loss-of-function allele has not been isolated in rice, and the qSH1 locus may control other traits in addition to shattering (Konishi et al., 2006). In Arabidopsis, blr mutants show reduced plant height, additional axillary branches and leaves, and disrupted phyllotaxy in vegetative and inflorescence units (Smith and Hake, 2003; Byrne, 2003; Roeder et al., 2003). A two-hybrid protein–protein interaction experiment showed that barley JuBEL2 can bind to class I KNOX proteins including Hooded/BKN3, which is a barley homolog of Arabidopsis STM (Müller et al., 2001). Similar results were obtained in Arabidopsis, where BLR protein interacts with the STM protein, assisting in its transportation to the nucleus (Cole et al., 2006; Rutjens et al., 2009). STM is required to maintain the meristematic identity of cells in both the SAM and AXM (Long and Barton, 2000). Plants mutated in BLR and two other related BELL-like genes, POUND-FOOLISH and ARABIDOPSIS THALIANA HOMEBOX GENE 1, lack a SAM, indicating that BELL-like proteins are essential for normal STM function (Rutjens et al., 2009). Together, these results suggest that interactions between JuBel2 and class I KNOX genes may promote AXM development and tiller development in barley.

Recessive mutations in the Int-b (located on chromosome 5H) and Uzu (wild-type, located on chromosome 3H) genes also reduce tiller number, but to a lesser extent compared with the mutants of the previous class (Babb and Muehlbauer, 2003). The Uzu gene encodes a putative BR receptor HvBR11 (Chono et al., 2003) and its use as a dwarfing gene has become widespread in Asian breeding programs (Hoskins and Poehlman, 1971; Tsuchiya, 1976; Zhang, 1994, 2000; Saisho et al., 2004). A similar correlation between tiller number and plant height has been associated with altered BR responses in rice DLT mutants (Tong et al., 2009, 2012), indicating BR plays a role in tillering and plant height in both barley and rice. Rice d61 is a mutant of the BRASSINOSTEROID-INSENSITIVE1 (OsBR1I) gene, the ortholog of barley HvBR1I. Reduced plant height in uzu and d61 mutants indicates that these BR-receptor genes play similar roles in regulation of stem elongation in barley and rice (Chono et al., 2003). However, no effect on tillering was reported for OsBR1I-defective mutants and severe alleles cause complete loss of fertility and malformed dark green leaves (Nakamura et al., 2006), pointing to diverse developmental roles of BRII genes in the two species.

In barley, a different link between hormones, plant stature, and tillering is supported by semidwarf1 (sdw1)/denso (mapped on chromosome 3H), an agronomically important dwarfing gene with pleiotropic effects on tillering (Jia et al., 2011). Compared with wild-type, Hv20ox2 expression is reduced four- and 60-fold in the denso and sdw1 mutants, respectively. These data indicate that low expression decreased plant height while increasing tillering (Jia et al., 2011) and are consistent with a negative correlation between GA and tillering observed in rice (see above). The rice genomic region collinear to the denso/sdw1 locus hosts the rice sd1/ Os20ox2 gene, the likely ortholog of Hv20ox2 (Jia et al., 2009). While denso/sdw1 and sd1 have similar phenotypic effects on plant height (Sasaki et al., 2002; Spielmeyer et al., 2002), we could not find information about the involvement of the rice gene in tillering.

Semidwarf phenotypes and excessive development of tillers also characterize plants carrying recessive mutations in the Gra-a (3H), Mnd1 (7HL), and Mnd6 (5HL) genes (Druka et al., 2011). In histological studies, gra-a mutants exhibit increased numbers of AXMs and axillary buds, with an occasional appearance of two shoot apical meristems (Babb and Muehlbauer, 2003), while the mnd6 mutant is characterized by the development of side branches from aerial nodes (Babb and Muehlbauer, 2003). Although the genes that correspond to gra-a and mnd mutations have not been identified, their phenotypes resemble those of rice mutants defective in SL biosynthesis and signal transduction pathways (Ishikawa et al., 2005; Zou et al., 2006; Arite et al., 2007). Characterization of these mutants may thus offer a foundation for the study of the SL pathway in barley.

Abnormal formation of lateral shoots from aerial nodes was also observed on virus-induced gene silencing of the P23k gene, involved in synthesis of (1,3;1,4)-β-D-glucan (Oikawa et al., 2009), suggesting a link between cell wall polysaccharide synthesis and branch development.

Beside these classical mutants, screening of novel mutagenized populations (see below) may uncover new loci and alleles conditioning tiller development. Screening of TILLMore—A population obtained from sodium azide mutagenesis of the reference genotype Morex (IBSC, 2012; Talamè et al., 2008; http://www.distafa.unibo.it/TILLMore/, verified 21 Jan. 2014)—identified a set of lines exhibiting decreased tillering that our group is currently characterizing.
In addition to mutants, QTLs involved in barley tillering have also been identified. Three QTLs for tiller number were mapped on chromosomes 1H bin 6-8, 2H bin 3, and 6H bin 10-11, each explaining 10.3 to 15.7% of the phenotypic variation in a cross between cultivated and wild barley (Gyenis et al., 2007), and also four QTLs for tiller number were detected on 1H, 2H, 3H, and 4H in a cross of a Syrian barley line, Arta, with a wild barley (H. vulgare ssp. spontaneum) (Baum et al., 2003).

In the following section we discuss how the development of new genomics tools and approaches, coupled with the synteny between the rice and barley genomes, can accelerate the isolation of barley genes underlying tillering phenotypes.

**Resources and Perspectives for Further Dissection of Tillering in Rice and Barley**

Monocots include three subfamilies of the grasses (Poaceae): the Panicoideae (e.g., *Sorghum bicolor*, Zea mays), Ehrhartioideae (e.g., *Oryza sativa*) and Pooideae (including *Brachypodium distachyon* and species of the tribe Triticeae such as barley and wheat) (Abrouk et al., 2010). They diverged from a common ancestor, with five proto-chromosomes around 60 to 75 million years ago (MYA), while rice and barley diverged 46 MYA, and wheat and barley diverged only 12 MYA (Petsko, 2002; Gaut, 2002; Bolot et al., 2009; Salse et al., 2009; Murat et al., 2010). Rice (*O. sativa ssp. japonica* ‘Nipponbare’) was the first cereal genome to be sequenced (International Rice Genome Sequencing Project, 2005). A recent release of the Os-Nipponbare-Reference-IRGSP-1.0 assembly (IRGSP-1.0; http://rapdb.dna.affrc.go.jp/ and http://rice.plantbiology.msu.edu/, verified 21 Jan. 2014) provides a valuable resource for cereal geneticists, and genomic platforms for functional analysis of rice genes were developed including high-quality genomic, physical, and transcriptome resources and integrated bioinformatics databases (reviewed by Yang et al., 2013). Additionally, various mutant libraries from T-DNA insertion, Ds/dSpm tagging, Tos17 tagging, and chemical or irradiation mutagenesis (reviewed by Jiang and Ramachandran, 2010; Yang et al., 2013; Wang et al., 2013) were developed to support both forward and reverse genetics approaches.

In total, 37,869 loci were annotated in rice by mapping transcript and protein sequences including 1626 loci, currently incorporating literature-based annotation data (Sakai et al., 2013). Among these loci, the rice genes related to tillering described above (Table 1) were isolated using these resources and represent a set of candidates for barley loci that still await molecular characterization.

Comparative genomics has become a fundamental approach for the identification of candidate genes, often in conjunction with map-based cloning (Goff et al., 2002; Droc et al., 2006; Zhang et al., 2006; Zou et al., 2006; Quraishi et al., 2009; Jaiswal, 2011; Matsumoto et al., 2011; Dibari et al., 2012; Sakai et al., 2013). Syntenic analysis among rice, wheat, *Brachypodium*, *sorghum*, and maize based on short conserved sequence regions indicated that wheat orthologs can be identified for 73% of rice genes, and on average, around 90% of cereal genomes can be considered part of macrosyntenic blocks (Abrouk et al., 2010). Several studies have focused on the high conservation of genomic arrangement in grasses to extract the maximum information from conserved syntenic associations. Numerous tools have been developed to compare plant genomes and tentatively identify orthologs (Salse et al., 2009). Such approaches were applied to identify candidate genes for a number of barley morphological mutants (Rossini et al., 2006; Druka et al., 2011; Ramsay et al., 2011). Because of the fact that many more genes controlling tillering have been identified in rice than barley, we were interested in determining the utility of rice–barley synteny to identify candidates for barley genes controlling tillering. We placed the known barley tillering mutants on the rice genome in relationship to the location of the rice genes that control tillering, and found that the majority of the known barley genes are not located in syntenic regions of the rice genome where genes controlling tillering are located (Fig. 4).

Considering also the different phenotypes associated to the rice homologues of known barley tillering genes (e.g., qSH1, OsBRI1), these results suggest that there may be differences in the genetic control between rice and barley, and that identifying additional barley tillering mutants will be necessary to obtain a better understanding of the commonalities and differences between the two species.

Recent progress in developing genomics tools is augmenting the power of comparative approaches between barley, rice, and other cereal genomes. For example, combining chromosome sorting, next-generation sequencing, array hybridization, and systematic exploitation of conserved synteny with model grasses including rice, the virtual chromosomal order of 21,766 barley genes (out of the estimated 32,000) was reconstructed and represented in tables known as “genome zippers” (Mayer et al., 2011). A deep whole-genome shotgun assembly and deep RNA sequence data supporting 26,159 “high-confidence” genes have become available (IBSC, 2012). Moreover, 22,651 representative full-length cDNA (???) sequences, including 17,773 novel barley full-length cDNAs, 1699 of which are barley-specific, are now available from 12 libraries from various organs or under different conditions (Matsumoto et al., 2011). Recently, these resources have been integrated with a new approach based on high-throughput put sequencing of reference mapping populations (Mascher et al., 2013), providing a much improved reference sequence resource for gene discovery and comparative genomics.

The implementation of efficient systems for high-throughput transformation has proved elusive for most cereals apart from rice, although some new methods have been proposed for activation tagging and insertional mutagenesis in barley (Ayliffe and Pryor, 2010). The development of barley genomics resources (see above) and the availability of new genomic technologies have accelerated the establishment of platforms.
for barley functional genomics. The recently developed barley hybridization-based exome capture tool (Mascher et al., 2013) provides a valuable instrument for the study of gene structure and variation in barley, while RNaseq technologies are offering new insight into transcriptome dynamics. Finally, bioinformatics tools to establish associations between genes, loci, and traits of interest from available barley transcriptome data have been developed (GeneNetwork, http://www.genenetwork.org, verified 21 Jan. 2014; Druka et al., 2008). These genomics tools can be applied to the systematic investigation of gene-trait relationships using classical mutant resources generated by physical and chemical mutagenesis and barley TILLING populations (Talamè et al., 2008; Gottwald et al., 2009; Druka et al., 2011).

The availability of complete or nearly complete genome sequences for many model organisms, in conjunction with the use of ultra-high-throughput sequencing technologies, has recently prompted the development of new forward genetic mapping approaches, based on the existing principle of Bulked Segregant Analysis (BSA) for the identification of recessive monogenic traits (Schneeberger and Weigel, 2011). In classical BSA, a cross or backcross between a mutant and wild-type plant is generated, and the phenotyped mutant and wild-type offspring are separately pooled and subjected to genotyping analysis (Michelmore et al., 1991). Only markers closely linked to a causal locus are expected to segregate consistently in mutant pools. Ultra-high throughput sequencing allows the combination of marker discovery and genetic mapping into a single step, potentially affording particularly high mapping resolution if bulk sizes, sequencing depth, and variability between individuals is high (Schneeberger and Weigel, 2011). Indeed, the basic principle has been applied in several species, both animal and plant, using statistical inference methods of varying degrees of sophistication (e.g., Blumenstiel et al., 2009; Schneeberger et al., 2009; Uchida et al., 2011; Austin et al., 2011; del Viso et al., 2012; Abe et al., 2012; Fekih et al., 2013; Krothapalli et al., 2013). In plants, different types of
of segregating populations have been used. Some studies were based on populations derived from crossing the mutant to a distinct genetic background, which segregate for a number of polymorphisms, as well as the causal mutation (Schneeberger et al., 2009; Galvão et al., 2012). A different approach was proposed for mutants derived from chemically mutagenized populations where the mutant is crossed to its own background, using only the mutagen-induced changes as segregating markers (Abe et al., 2012; Zhu et al., 2012; Hartwig et al., 2012; Fekih et al., 2013).

While the aforementioned approach is most reliable when a complete genome sequence is available, it can be adapted to incorporate information from synteny (Galvão et al., 2012), potentially allowing its use in organisms such as barley, taking advantage of the high resolution draft of the barley genome and its extensive EST and cDNA resources. Mutants isolated from chemically mutagenized populations are expected to be particularly amenable to mapping by this approach, as point mutations underlying developmental phenotypes are likely to be predominantly located in, or close to coding regions—the great majority of which have been sequenced in barley and are represented in an available exome capture array (Mascher et al., 2013). These considerations also negate the need for whole genome resequencing and would substantially reduce experimental costs.

An exciting variation on this theme has been employed in several systems with less well-characterized genomes. As a proxy for exome enrichment, deep sequencing of the transcriptome can be used to identify genes with an allele frequency close to one in mutant bulks (Trick et al., 2012; Liu et al., 2012; Hill et al., 2013). A possible limitation of this strategy is the difficulty in obtaining suitable sequencing coverage, particularly for genes expressed at low levels (Schneeberger and Weigel, 2011). On the other hand, some functional inferences regarding downstream effects of mutations under study might be obtained from identification of genes differentially expressed between mutant and wild-type bulks.

These new high-throughput approaches are expected to radically accelerate the identification of genes and causal mutations underlying tillering and other traits, particularly in grasses where extensive synteny exists. Beyond barley, other species, even those with large and complex genomes might be studied in this manner.

Concluding Remarks
In this review we have attempted to provide an overview of the known similarities and possible differences in the molecular and hormonal regulation of shoot branching, or tillering, in barley and rice. We have also dwelt on the ongoing development of genomic, comparative genomic, and postgenomic resources in these two species, with the intention of underlining how high-throughput approaches might complement classical genetic methodologies in coming years. Beside the feasibility of transferring knowledge between experimental systems, analysis of additional species beyond rice is expected to provide a more comprehensive understanding of the genetic basis and molecular evolution of tillering in cereals. A number of questions remain open. For example, how many genes are involved in controlling tillering in different grasses? How many of them are playing similar roles across species? In-depth comparisons based on phylogeny and functional characterization of genes identified from different species are needed to evaluate this. Even assuming overall conservation of regulation of tillering in grasses, recent gene duplications in various species are likely to lead to distinct degrees of functional redundancy in different points of tillering pathways—implying that different genes are likely to be identified from spontaneous or induced mutants in distinct systems. In this context, despite its relatively large and complex genome, barley represents an experimental system of great value, both intrinsically, and as a model for other Triticeae genomes. The availability of extensive genetic resources, ever-improving genomic and comparative genomic data, as well as novel and affordable high-throughput genotyping and sequencing platforms suggest that barley will continue to be a key system for the study of tillering and other developmental processes in grasses.

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Note Added in Proof

References


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