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Review Article

Leaf angle: a target of genetic improvement in cereal crops tailored for high-density planting

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Summary

High-density planting is an effective measure for increasing crop yield per unit land area. Leaf angle (LA) is a key trait of plant architecture and a target for genetic improvement of crops. Upright leaves allow better light capture in canopy under high-density planting, thus enhancing photosynthesis efficiency, ventilation and stress resistance, and ultimately higher grain yield. Here, we summarized the latest progress on the cellular and molecular mechanisms regulating LA formation in rice and maize. We suggest several standing out questions for future studies and then propose some promising strategies to manipulate LA for breeding of cereal crops tailored for high-density planting.

Introduction

Global food production is predicted to increase at least 100%–110% to be enough to feed the projected 10 billion people by 2050 (Tilman *et al.*, 2011; UN World Population Prospects, 2020). To meet this demand, annual cereal production will need to rise to about 3 billion tons from 2.79 billion in 2021 (FAO statistical databases, 2021). Therefore, new breeding technologies are urgently needed to boost crop yields. Growing evidence has shown that increasing planting density is an effective strategy to increase crop yield per unit area. For instance, the average plant density of maize in the Central Corn Belt of the United States has increased from ~30 000 plants per hectare in the 1930s to ~80 000 plants per hectare now, which contributed critically to the continuous yield increase in the past century (Duvick *et al.*, 2004; Lauer *et al.*, 2012). However, high-density planting could trigger shade avoidance responses (SAR) of plants, leading to increased extending growth and lodging rate, reduced branching or tillering, early flowering and senescence, thus great losses of the final biomass and grain yield. Therefore, to maximize crop yield per unit area, it is urgent to develop new cultivars harbouring traits that can alleviate the detrimental effects of SAR. Leaf angle (LA), the angle between the vertical stem of a plant and the midrib of a leaf blade (Figure 1a), is a key agronomic trait regulating plant architecture for dense planting. Upright leaves can enhance light perception, photosynthetic efficiency, ventilation and stress resistance in dense canopy, thereby allowing dense planting and increasing grain yield for cereal crops. For example, rice mutants of *OsDWARF4* have erect leaves and produce higher biomass and yields than wild type under dense planting (Sakamoto *et al.*, 2006). Similarly, an isogenic single-cross hybrid carrying the mutated *liguleless2* (*lg2*) gene displays erect leaves and could produce 40% more grain than its counterpart with horizontal leaves (Pendleton *et al.*, 1968). In addition, ample evidence indicates that more upright leaf is a selected trait during genetic improvement of crops (Duvick, 2005; Ma *et al.*, 2014; Wang *et al.*, 2020a). Therefore, identification of LA regulatory genes and elucidation of their functional mechanisms could provide promising targets and strategies for breeding elite cultivars tailored for high-density planting.

In this review, we summarize the latest progress on elucidating the molecular mechanisms regulating LA formation in cereal crops (mainly rice and maize). We also highlight some open questions for future in-depth LA studies. Finally, we propose some promising strategies to boost dense planting breeding through genetic modifications of LA and plant architecture in crops.

Cytological dynamics of LA development

The size of LA in grasses is largely determined by a structure called ligule (also called as lamina joint in rice) within leaf, which links the blade and sheath. The ligular region consists of an outgrowing ligule and a pair of wedge-like auricles, and bends the blade away from the vertical stem to form LA (Figure 1). Below, we use maize as the example to describe the cellular landscape of ligular development. As in other cereal crops, maize leaf is initiated through recruiting the founder cells at the flank of a shoot apical meristem. By the plastochron2 (P2) stage, the proximal–distal,

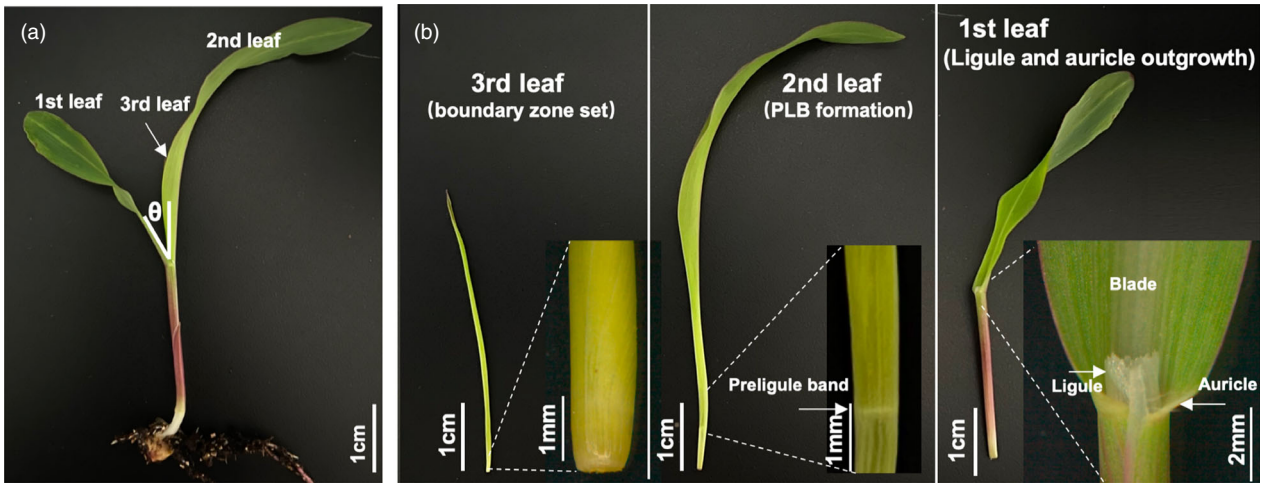

Figure 1 Developmental process of the ligular region in maize. (a) Leaf morphology of a young B73 maize seedling (4-day after germination, growth conditions:12 h light at 26 °C and 12 h dark at 20 °C). θ represents the angle between the vertical stem and the midrib of the leaf blade. The third leaf is still wrapped in the sheath of the second leaf and thus invisible. (b) The third leaf represents the boundary zone establishment stage (left), the second leaf represents the preligule band (PLB) formation stage (middle), and the first leaf is at the ligule and auricle outgrowth stage (right), respectively.

medial–lateral and adaxial–abaxial polarities have been sequentially defined, then a genetic programme initiates LA formation at the base of a developing leaf. Cytologically, LA formation can be broadly divided into three main sequential stages (Figure 1b): (i) the establishment of the blade–sheath boundary (BSB) zone that separates the future leaf blade and leaf sheath, (ii) the formation of a small cell band called the preligule band (PLB) within the boundary region and (iii) the development of ligule and auricle within the ligular region due to distinct cell division and expansion patterns (Sylvester *et al*., 1990). At the boundary zone formation stage, the cells giving rise to blade and sheath are indistinguishable, and the ligular structure per se is invisible (Becraft *et al*., 1990; Sylvester *et al*., 1990) (Figure 1b, left). The ligular region becomes first visible at the P5-P6 stages when a group of epidermal cells undergo rapid longitudinal and transverse anticlinal division, forming a PLB harbouring small cells (Figure 1b, middle). Later, at the P7 stage, a ligule emerges from the small cells at the base of preligule that divide periclinally and produce a ligular ridge in the adaxial side of the blade. After ligule initiation, a thin wedge of small cells behind the ligule begins to divide and enlarge and subsequently develops into the auricle structure to bend the blade (Figure 1b, right; Figure 2a,b). Transverse section analysis revealed that the maize ligular region and the rice lamina joint are mainly composed of vascular bundles, sclerenchyma cells and parenchyma cells (Figure 2c,d). Interestingly, the rice contains several aerenchymas within the lamina joint, while the maize contains a large number of colourless cells (also called 'clear cells') instead (Figure 2c,d). It is believed that the size of auricle, the mechanical strength of the midrib (which is mainly determined by sclerenchyma cells) and the ratio of adaxial to abaxial surfaces of the ligular region or lamina joint are largely responsible for the size of LA. In general, increasing the sclerenchyma cell layers at the abaxial and adaxial sides of the ligular region or lamina joint enhances mechanical strength of this part, thus resulting in erect leaves, whereas enhancing the proliferation and expansion of parenchyma cells in the adaxial side of the region enlarges LA (Figure 2) (Cao et al., 2020; Chen *et al*., 2018; Kong *et al*., 2017; Ning *et al*., 2011; Sun *et al*., 2015; Tian *et al*., 2019; Zhao *et al*., 2010).

Molecular regulation of LA formation

During the past 30 years, genetic-, molecular- and microdissection-based transcriptomic analyses have identified a few dozen genes and several classes of plant hormones that regulate the formation of ligule and auricle (Figures 3 and 4; Table 1 and Table S1). Below, we briefly summarize the molecular mechanisms regulating the three developmental stages of LA.

Regulation of the proximal–distal boundary between leaf blade and sheath

The initiation of ligular region marks the formation of a boundary between the leaf sheath and blade, and is key to the formation of the leaf proximal–distal axis and LA. Physiological and genetic studies have documented evidence for a role of auxin, brassinosteroids (BRs) and cytokinin (CK) in BSB formation. For example, applying inhibitors of polar transport of auxin in maize seedlings severely disrupts the formation of BSB (Tsiantis *et al*., 1999). Similarly, perturbation of BR synthesis or signalling transduction results in disrupted BSB, smaller ligule and abnormal auricle (Kir *et al*., 2015; Makarevitch *et al*., 2012). Furthermore, a semi-dominant *Hairy Sheath Frayed1* (*ZmHsf1*, encoding a CK receptor) mutant produces an abnormal outgrowth at the distal blade margin that resembles a leaf-like structure carrying auricle/ligule and sheath, indicating a role of CK in regulating BSB formation (Muszynski *et al*., 2020).

In addition, a series of studies revealed a conserved role of *KN1-LIKE HOMEOBOX* (*KNOX*) genes in BSB establishment in monocots and dicots, as ectopic expression in the gain-of-function mutants such as *Knotted1* (*KN1*), *Gnarley1* (*KNOX4*), *Liguleless3* (*LG3*) and *Liguleless4* (*LG4*) causes formation of ectopic BSBs in maize (Fowler and Freeling, 1996; Moreno 1999; Muehlbauer *et al*., 1999; Ramirez *et al*., 2009; Smith et al., 1992; Strable and Nelissen, 2021). It has been proposed that KNOX protein accumulated at the base of the leaf primordium might mediate efflux auxin from the base and boundary to maintain distal auxin distribution, possibly through direct regulation of

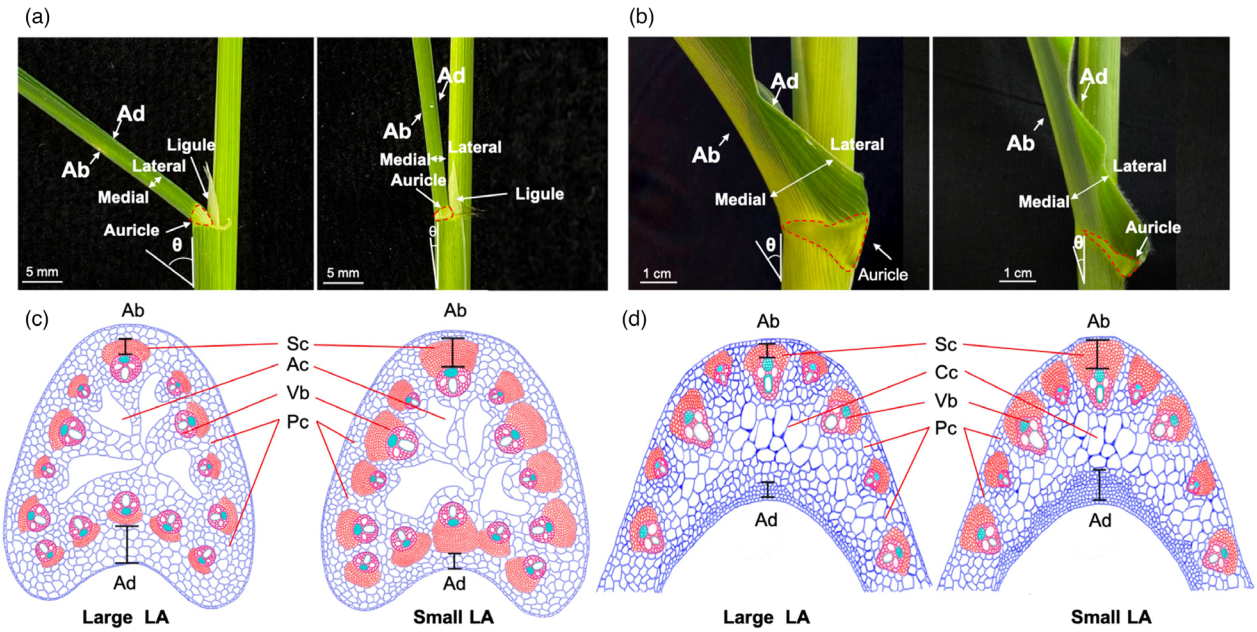

Figure 2 Morphology and cytology of the ligular region in rice and maize. (a) Morphology of the lamia joints of rice leaf with large (left) and small (right) leaf angle (LA). (b) Morphology of the ligular regions of maize leaf with large (left) and small (right) LA. (c) Schematic models of the cross-sections for the lamia joints of rice leaf with large (left) and small (right) LA. (d) Schematic models of the cross-sections for the ligular regions of maize leaf with large (left) and small (right) LA. The schematic models are drawn based on the cross-sections of the maize ligular region and the rice lamia joints stained with Fasga (Méchin *et al*., 2005), in which the sclerenchyma cells are shown in red, while parenchyma cells and colourless cells are shown in blue. The colourless cells (also called 'clear cells') are a group of cells intervening the abaxial vascular bundle and the adaxial sclerenchyma cells within the maize ligular region (Strable *et al*., 2017). The auricles are highlighted with red dashed circles in (a) and (b). Ad, adaxial; Ab, abaxial; Sc, sclerenchyma; Vb, vascular bundle; Pc, parenchyma; Cc, colourless cells; Ac, arenchyma.

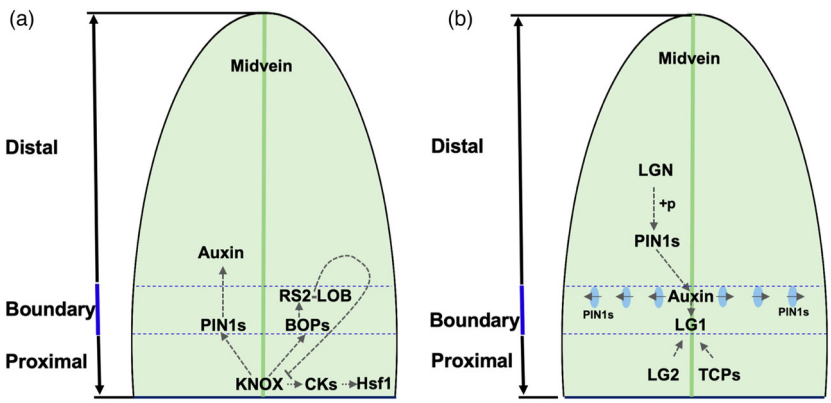

Figure 3 Models for blade–sheath boundary and preligule band formation. (a) A model for blade–sheath boundary (BSB) patterning. KNOX protein is accumulated at the base of the leaf primordium and might maintain distal auxin distribution through regulating PIN1s. KNOX protein might also promote CK accumulation at the proximal leaf primordium through activating CKs synthesis, and the CK signal could be further transduced by Hsf1. An intertwined regulatory network involving KONXs, BOPs, RS2 and LOB proteins likely operate in specifying BSB formation. (b) A model for preligule band formation. ZmLGN might phosphorylate and reorient ZmPIN1s to mediate transmission of auxin from midrib to the leaf margins. Two potential transcriptional modules (LG2-LG1 and TCPs-LG1) in regulating PLB formation are also shown.

PIN1s (Bolduc *et al*., 2012; Johnston *et al*., 2014). In addition, *KNOX* genes might promote CK accumulation at the base of leaf primordium possibly through activating CK synthesis; then, CK signalling could be transduced by *Hsf1* (see above) to promote sheath development (Muszynski *et al*., 2020; Sakamoto *et al*., 2006). These analyses collectively suggest that KNOX proteins function to maintain a distal auxin maxima and a proximal CKs level that is crucial for the BSB establishment. Besides, *KNOX* genes may also regulate a group of typical boundary genes to further define the BSB. For instance, it was reported that KN1 might directly regulate the expression of the maize homologs of *Arabidopsis* boundary gene *Blade-On-Petiole* (*ZmBOPs*) at the proximal leaf primordium to define BSB (Bolduc *et al*., 2012; Johnston *et al*., 2014). Consistent with this notion, the leaves of *Osbop1/2/3* triple mutant exhibit blade- but not sheath-characteristics, with the disruption of BSB and malformation of

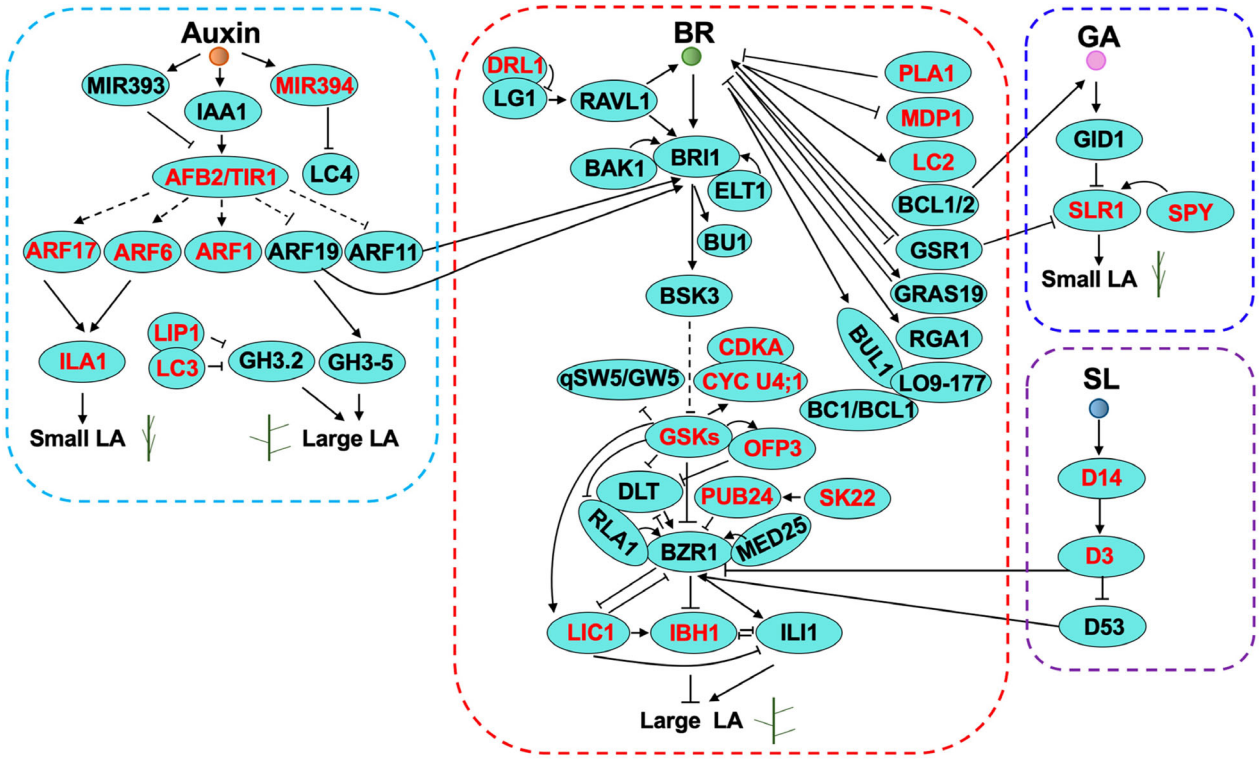

Figure 4 Model depicting the extensive cross-talks between the signalling pathways of BRs, Auxin, GA and SL in regulating leaf angle. Positive regulators which enlarge the size of leaf angle (LA) are shown in black, and the negative ones are shown in red. Black arrows indicate positive regulation, while black lines ended with perpendicular bars represent suppressive regulation. The dotted arrows and lines indicate undetermined relationships.

ligule and auricle (Toriba *et al*., 2019). Similarly, a barley *BOP* homolog *Uniculme4* (*HvCUL4*) also plays a role in ligule/auricle development (Tavakol *et al*., 2015). Interestingly, *BOP* genes may also regulate the expression of *KNOX* genes through a conserved feedback mechanism. In *Arabidopsis*, BOP proteins activate the *Lateral Organ Boundary* (*LOB*) transcription factors, and LOB in turn form a trimeric complex with Jagged Lateral Organs (JLO, LBD transcription factor) and Asymmetric Leaves1 (AS1, MYB transcription factor) to represses *KNOX* expression at the boundary (Ikezaki *et al*., 2010; Rast and Simon, 2012). Similarly, the maize *AS1* homolog *Rough sheath2* (*RS2*) and the *LOB* factor *Indeterminate Gametophyte1* (*IG1*) are co-expressed with *ZmBOPs* and inhibit the expression of *KNOX* genes (Evans, 2007; Schneeberger *et al*., 1998). These findings collectively indicate BSD formation entails an intertwined regulatory network of *KONX* genes, *BOP* genes and phytohormones, yet much remains to be elucidated regarding their detailed regulatory relationship (Figure 3a).

Regulation of PLB formation

The PLB produces small cells within the BSB region that undergo distinct division patterns, and ultimately give rise to ligule and auricle. It has been proposed that a signal originated from the midrib that transmits to the leaf margins triggers PLB formation (Becraft and Freeling, 1991). Limited evidence suggests that auxin might be the signal per se or plays a role in transmitting the preligule signal to guide PLB formation (Figure 3b). Interestingly, the semi-dominant *Lgn-R* maize mutant develops ligular patches next to midrib instead of a continuous ligule and displays diffuse blade–sheath boundaries with a discontinuous expression pattern for the auxin efflux transporter *ZmPIN1a* across the ligular region, in contrast with continuous expression pattern of *ZmPIN1a* in the wide type plant, suggesting that polar auxin transport plays a critical role in PLB formation (Moon *et al*., 2013). Further study showed that *ZmLGN* encodes a serine–threonine kinase and that it functions upstream of *ZmLG1*, hinting a possibility that *ZmLGN* might be involved in regulation of the phosphorylation status of *ZmPIN1a,* and subsequently influence transmission of auxin from midrib to the leaf margins (Figure 3b). However, further studies are required to validate this possibility.

Molecular genetic studies have also identified several transcription factors regulating PLB formation. Null mutants of maize *Liguleless2* (*ZmLG2,* which encodes a bZIP/DOG domain transcription factor) often lack or have incorrectly positioned ligule and auricles, and the BSB is diffuse (Walsh *et al*., 1998), whereas mutants of *Liguleless1* (*LG1*, which encodes a SQUAMOSA Promoter-Binding domain protein) in maize, rice, barley and wheat all have an evident BSB, but lack ligule and auricle, suggesting that *LG1* does not affect BSB formation, but is essential for PLB formation and outgrowth of ligule and auricle (Becraft *et al*., 1990; Lee *et al*., 2007; Moreno *et al*., 1997; Rossini *et al*., 2006; Sylvester *et al*., 1990; Yu, 2019). Interestingly, mosaic analysis indicated that the maize *lg2-R* phenotype is cell non-autonomous, whereas the *lg1-R* phenotype is cell autonomous. Spatiotemporal expression analysis further suggested that *ZmLG2* likely plays an early role prior to *ZmLG1* in specifying the formation of both BSB and PLB within the young leaf primordia (Walsh *et al*., 1998). Consistently, studies of the phenotypes of *OsLG2/2L* and *OsLG1* knockout lines, together

Table 1 Identified QTLs associated with leaf angle in maize and rice

Species	Parents	Pop.	Chromosomes (no. of QTLs)	PVE range	Candidate genes (maize and rice)	Reference
Zea mays	B73, Mo17	RILs	1(1), 2(2), 4(1), 5(2), 6(1), 7(2)	2%–28%		Mickelson *et al.* (2002)
Zea mays	Ye478, Dan340	$F_{2:3}$	1(2), 2(1), 3(2), 5(1)	2%–11%		Lu *et al.* (2007)
Zea mays	Yu82, Shen137	$F_{2:3}$	1(1), 2(1), 5(1)	7%–20%	*ZmDWF4, ZmYABBY15*	Ku *et al.* (2010)
Zea mays	NAM	RILs	1(5), 2(3), 3(4), 4(3), 5(4), 6(1), 7(3), 8(3), 9(2), 10(2)	0.5%–2%	*ZmLG1, ZmLG2*	Tian *et al.* (2011)
Zea mays	Yu82, Yu87-1	$F_{2:3}$	1(1), 2(1), 3(1), 7(1), 8(1)	7.34%–8.43%	*ZmDWF4, ZmLG1, ZmTAC1, ZmLG2, ZmLIC, ZmYABBY15*	Ku *et al.* (2012)
Zea mays	B73, Mo17	RILs	1(1), 5(1), 9(1)	10.4%–16.4%		Wassom, (2013)
Zea mays	Yu82, D132	$F_{2:3}$	4(1)	37%	*ZmCLA4*	Zhang *et al.* (2014)
Zea mays	Ye478, Ro8	$F_{2:3}$	1(4), 2(2), 3(2), 5(2), 9 (2)	1.28%–9%		Hou *et al.* (2015)
Zea mays	CY5, YL106	$F_{2:3}$, F_4	1(1), 3(1), 5(2), 10(2)	6%–85%	*ZmLG2*	Chen *et al.* (2015)
Zea mays	D276, A188, D72, Jiao51	F_1	1(3), 2(3), 4(2), 5(1), 7(2), 8(2), 9(1)	2.27%–7.75%	*ZmLG1*	Ding *et al.* (2015)
Zea mays	Huangzaosi, Huobai, Lv28, Weifeng322	RILs	1(4), 2(2), 3(1), 5(1), 6(2), 7(2), 8(1), 9(3), 10 (1)	2%–11%	*ZmLG1*	Li *et al.* (2015)
Zea mays	8984, GY220, 8622	$F_{2:3}$	1(4), 2(9), 3(5), 4(8), 5(8), 8(4), 9(2), 10 (8)	3.9%–16.4%		Yang *et al.* (2015)
Zea mays	Yu82, Zong3, Yu87-1, Shen137, Yu537A	RILs		5.6%–25.6%		Ku *et al.* (2016)
Zea mays	B73, K22, CI7, BY804, BY815, KUI3, BK, SK, DAN340, DE3, ZHENG58, B77, ZONG3, Yu87-1	RILs	1(14), 10(5), 2(19), 3 (3), 4(3), 5(10), 6(4), 7(7), 8(6), 9(6)	0.03%–0.23%		Pan *et al.* (2017)
Zea mays	Yu82, D683	RILs	1(1), 3(1), 7(1), 8(1)	8.41%–12.11%		Shi *et al.* (2017)
Zea mays	Zheng58, HD568	RILs	1(3), 2(4), 4(1), 5(4), 6(1), 7(3), 10(1)	3.02%–16.53%		Wang *et al.* (2017)
Zea mays	B73, Y804	RILs	1(12), 2(8), 3(2), 4(5), 5(10), 8(8), 7(10), 9(2), 10(5)	6%–12%		Zhang *et al.* (2017)
Zea mays	Langhuang 9, Chang7-2, TS141	F_2, $F_{2:3}$	1(2), 2(1), 4(1), 5(1), 6(1), 7(2)	3.61%–20.62%	*ZmGT1, ZmDWARF4, ZmPHYB1, ZmLCR, ZmTB1, ZmKN1-DL; ZmLG1, ZmTAC1, ZmGar1, ZmLG2, ZmCLA4; ZmIncw1, ZmDWARF1, ZmNCW4, ZmKNOX8, ZmBK2*	Zhao *et al.* (2018)
Zea mays	B73, Mo17, PHW30	$F_{2:3}$	1(3), 2(2), 3(5), 4(1), 8 (1)	7.54%–17.17%	*ZmDRL1, ZmLPA1, ZmPMK1, OsMDP1, ZmDWF4, ZmRAVL1, ZmLG1, ZmLG2, ZmIRS1, ZmLPA1, ZmDRL2, OsLG1*	Dzievit *et al.* (2019)
Zea mays	Lv28, H082183	$F_{2:3}$	1(2), 2(1), 5(2), 8(1)	4.92%–16.04%	*ZmDWF4*	Liu *et al.* (2019)
Zea mays	Ye478, Qi319	RILs	1(1), 2(1), 3(3), 4(1), 7(2), 10(1)	4.62%–11.49%		Zhang *et al.* (2020)
Zea mays	B73, ICAU1212	RILs		5.62%–20.14%	*ZmLG1, ZmLG2*	Tang *et al.* (2021)
Zea mays	350 inbred lines	Inbred	2(2), 5(1), 9(1), 10(1)		*ZmNAC16, ZmSBP18*	Wang *et al.* (2021)
Zea mays	P014, E1312	RILs	1(3), 2(1), 3(1), 6(1), 7(1), 9(1), 10(1)	3.21%–22.78%	*Zm00001d028164, hvZm00001d019053, ZmKNOX2, Zm00001d025352*	Zhang *et al.* (2021)
Oryza sativa	Lemont, Teqing	F_2, F_4	1(2), 2(2), 3(1), 5(2), 6(1), 7(1), 8(1), 9(2)	2.8%–47.5%	*OsTA*	Li *et al.* (1999)
Oryza sativa	863B, A7444	F_1, F_2, BC1	2(1), 8(1)	7.64%–13.28%		Hu *et al.* (2012)
Oryza sativa	Sasanishiki, Habataki,	CSSL	5(1), 8(1)	19.18%–37.71%		Bian *et al.* (2014)

Table 1 Continued

Species	Parents	Pop.	Chromosomes (no. of QTLs)	PVE range	Candidate genes (maize and rice)	Reference
Oryza sativa	529 O. sativa accessions	Inbred	1(1), 3(2), 6(1), 7(1), 8(1), 11(1), 12(1)	0.2%–26.5%	*OsbHLH174, OsbHLH173, OsbHLH153*	Dong *et al*. (2018)
Oryza sativa	Cheongcheong, Nagdong	DH	4(1), 11(3)	9%–51%		Ham *et al*. (2019)
Oryza sativa	Takanari, Koshihikari	BILs, NILs	1(2), 2(2), 4(1), 5(1), 9(1)	13.3%–23.4%		San *et al*. (2021)

Pop., population; PVE, percentage of variation; $F_{2:3}$, F_2-derived F_3; CSSL, chromosome segment substitution line; RILs, recombinant inbred lines; DH, double haploid.

with their spatiotemporal expression patterns also indicated that *OsLG2* and *OsLG2L* might function earlier than *OsLG1* in lamina joint position determination and lamina joint formation (Wang *et al*., 2021). Moreover, transcriptomic analyses showed that enrichment of auxin signalling output-related processes, including altered expression of *SAURs*, *AUX/IAA* family of genes and *ARF* genes, suggesting that *OsLG2/LG2L* and *OsLG1* likely act to regulate lamina joint development and thus LA size through regulating auxin signalling (Wang *et al*., 2021). In line with this notion, a recent study showed that TaSPL8, the ortholog of LG1 in wheat, regulates LA size via directly inducing the expression of *TaARF6* (Liu *et al*., 2019). Moreover, it was shown that expression of *ZmLG1* is promoted by ectopically expressed *Wavy Auricle in Blade* (*ZmWAB1*), which encodes a Teosinte Branched1, Cycloidea, PCF (TCP) transcription factor normally expressed in maize tassels (Hay and Hake, 2004; Lewis *et al*., 2014). Therefore, it was speculated that other TCP factors expressed in the ligule boundary region might trigger *LG1* expression to induce ligule formation (Lewis *et al*., 2014). These findings suggest two potential transcriptional modules (*LG2-LG1* and *TCPs-LG1*) regulating PLB formation, but the detailed regulatory relationship between them awaits further studies (Figure 3b).

Regulation of ligule and auricle formation

Leaf angle is a quantitative trait controlled by multiple genetic loci. Recent molecular genetic studies have identified dozens of QTLs and genes affecting LA in maize and rice (Figure 4; Table 1). Notably, most of the genes identified so far either encode transcriptional regulators or signalling components of various phytohormones. In particular, ample evidence indicates that BRs play a central role in regulating LA formation, while several other hormones, including auxin, gibberellins (GAs), strigolactones (SLs) and ethylene, also contribute to LA formation, likely through cross-talking with BR signalling. Below, we briefly summarize the signalling pathways of various hormones regulating LA formation.

BR regulation of LA formation

Numerous studies documented a positive role of BR in regulating LA formation (Figure 4; Table S1) (Li *et al*., 2020b; Luo *et al*., 2016). BR regulation of LA probably has been best studied in rice. Rice mutants deficient in BR biosynthesis (such as *Osbrd1*, *Osbrd2, Osd2, Osd4* and *Osd11*), BR perception (such as *Osbri1*) or signalling (*OsBZR1-RNAi*, *Osbsk2*, etc.) all display more erect leaves (Bai *et al*., 2007; Hong *et al*., 2003, 2005; Li *et al*., 2009; Mori *et al*., 2002; Nakamura *et al*., 2006; Sakamoto *et al*., 2006; Tanabe *et al*., 2005; Yamamuro et al., 2000; Zhang *et al*., 2016). It has been shown that OsBZR1 acts as a central transcriptional regulator in the OsBRI1-mediated BR signalling pathway to mediate various BR responses, including LA (Figure 4). For example, OsBZR1 represses the transcription of *OsDLT* (encoding a GRAS transcription factor, a positive regulator of LA) and *OsLIC1* (encoding a CCCH-type zinc-finger transcription factor, a negative regulator of LA) to positively regulate LA (Tong *et al*., 2012). In turn, OsDLT promotes the expression of *OsBZR1*. Moreover, OsLIC1 could repress the expression of *OsILI1* (encoding an HLH protein) but activate *OsIBH1* (encoding a bHLH protein that binds ILI1), and these two transcription factors jointly regulate the elongation of the adaxial cells in the lamina joint and LA size in rice via HLH/bHLH antagonism (Zhang *et al*., 2009, 2012). In the contrary, OsGSK2, a negative regulator of BR signalling in rice, can phosphorylate OsBZR1, DLT, OsLIC1, OsRLA1, OsOFP3 and qSW5/GW5 (a plasma membrane-localized calmodulin-binding protein) and consequently regulate their protein accumulation, thus modulating BR responses and reducing LA (Liu *et al*., 2017; Qiao *et al*., 2017; Tong *et al*., 2012; Xiao *et al*., 2020; Zhang *et al*., 2012). Interestingly, a recent study showed that when BR signalling is attenuated, BIN2 (a GSK3-like kinase) is activated, which inhibits BES1 activity to release the inhibitory effect on *CYC U4;1* transcription, and meanwhile, BIN2 can also phosphorylate CYC U4;1 to activate the CYC U4;1/CDKA complex, leading to enhanced abaxial sclerenchyma cell division and erect leaves (Figure 4; Sun *et al*., 2015). This study for the first time established a direct link between BR signalling and cell proliferation in the lamina joint during determination of LA size in rice.

In addition, it has been shown that a number of genes modulate LA through regulating BR biosynthesis or signalling (Figure 4). For example, OsRAV-Like1 (OsRAVL1, a B3 domain transcription factor) could directly bind to the promoters of *OsBRI1*, *OsDWARF2* (*OsD2*), *OsDWARF11* (*OsD11*) and *OsBRD1* to promote their expression, thus increasing the size of LA in rice (Je *et al*., 2010), whereas a transmembrane protein OsELT1 positively regulates LA size through stabilizing OsBRI1 (Yang *et al*., 2017). In addition, a mediator OsMED25 and an APETALA2 domain-containing transcription factor OsRLA1 regulate the size of LA through interacting with OsBZR1 and enhancing its activity, while a negative BR signalling regulator U-box E3 ubiquitin ligase OsPUB24 represses lamina joint inclination through ubiquitination and degradation of OsBZR1. The AtBIN2 homolog OsSK22 regulates the stability of OsPUB24 through phosphorylation (Min *et al*., 2019; Qiao *et al*., 2017; Ren *et al*., 2020a). Moreover, it was shown that *OsBU1* and *OsBUL1* (both of them encode typical HLH proteins) and *OsGRAS19* (encoding a homolog of DLT) positively regulate LA (Chen *et al*., 2013; Jang *et al*., 2017; Tanaka *et al*., 2009). Furthermore, it was shown that

OsBUL1, LO9-177 and OsBC1 form a tri-protein complex that positively regulates LA in rice through regulating BR signalling and cell elongation in the lamina joint (Chen *et al*., 2013; Jang *et al*., 2017). Furthermore, it was reported that *OsLC2* (encoding a VIN-3 like protein), OsPLA1 (encoding a cytochrome P450 protein) and *OsMDP1* (encoding a MADS DOMAIN-CONTAINING PROTEIN) negatively regulate LA through controlling expansion and division of adaxial parenchyma cells of the lamina joint (Duan *et al*., 2006; Xiong *et al*., 2021; Zhao *et al*., 2010). However, the detailed molecular mechanisms linking these genes to BR signalling remain to be elucidated in future studies.

Similarly, maize mutants defective in BR biosynthesis (such as *Zmna1, Zmna2 and Zmbrd1*) or signalling (such as *ZmBRI1-RNAi and Zmili1*) also display more erect leaves (Best *et al*., 2016; Hartwig *et al*., 2011; Kir *et al*., 2015; Makarevitch *et al*., 2012; Ren *et al*., 2020b), indicating a conserved positive role of BRs in LA regulation in maize. However, less is known about BR signalling mechanisms in maize, compared with rice. In a recent illuminating study, Tian *et al*. (2019) demonstrated that *ZmRAVL1* directly activates *ZmBRD1* expression, leading to increased BR levels and larger LA. Both ZmDRL1 (a YABBY protein with a C2C2 zinc-finger DNA-binding domain, a negative regulator of LA, Strable *et al*., 2017) and ZmLG1 can directly bind to the upstream regulatory sequence of *ZmRAVL1* (a positive regulator of LA). Moreover, ZmDRL1 can physically interact with ZmLG1 to suppress transcriptional activation of *ZmRAVL1* by ZmLG1, thus reducing LA. This study fills in a significant gap between LG1 and BR signalling in LA regulation.

Interestingly, two OsBRI1-independent BR signalling pathways regulating LA were also reported in rice. Oki *et al*. (2009) reported that a rice α-subunit of heterotrimeric G protein, OsD1/RGA1, mediates BR signalling independent of OsBRI1 and could enhance leaf inclination and grain size in rice. Similarly, Liu *et al*. (2016) demonstrated that *OsLPA1*, which encodes an INDETERMINATE DOMAIN protein, regulates lamina joint bending by suppressing auxin signalling that interacts with C-22-hydroxylated and 6-deoxo BRs in rice, in an OsBRI1-independent manner. They further showed that the expression levels of *OsPIN1a*, *OsPIN1c* and *OsPIN3a* are positively correlated with those of *OsLPA1*. Nevertheless, the detailed molecular mechanisms remain to be elucidated.

Regulation of LA formation by other hormones

Numerous studies suggest that auxin plays a negative role in regulating LA size (Figure 4), as a number of rice mutants defective in auxin biosynthesis or signalling (such as *Osfibs*, *OsIAA1-OE*, *OsGH3s-OE, Ostir1* and *OsAFB2-RNAi*) display enlarged LA (Bian *et al*., 2012; Du *et al*., 2012; Song *et al*., 2009; Yoshikawa *et al*., 2014). For example, a gain-of- function mutant for *OsGH3-1* encoding an indole-3-acetic acid–amido synthetase exhibits increased LA due to accelerated cell elongation at the lamina joint, likely caused by decreased free IAA levels (Zhao *et al*., 2013). At the mechanistic level, it has been shown that two positive regulators of auxin signalling, *OsARF6* and *OsARF17*, could directly bind to the promoter of *OsILA1* (encoding a Raf-like MAPKKK) and promote its expression, resulting in reduced mechanical tissue formation at the lamina joint, thus producing more upright flag leaf (Huang *et al*., 2021; Ning *et al*., 2011). In contrast, three negative regulators of auxin signalling, OsARF1, OsARF11 and OsARF19, were shown to promote *OsBRI1* and *OsGH3s* expression, thus enlarging the size of LA (Attia *et al*., 2009; Liu *et al*., 2018; Zhang *et al*., 2015). *OsLC3*, encoding a SPOC domain-containing transcription suppressor, also influences *OsGH3* expression to inhibit elongation of adaxial cells of the lamina joint, thus reducing LA size (Chen *et al*., 2018). In addition, auxin induces expression of *OsMIR393s* and *OsMIR394*, and *OsMIR393s* could enhance leaf inclination through suppressing their target genes *OsTIR1* and *OsAFB2*, while *OsMIR394* suppresses the expression of *OsLC4* (encoding an F-box protein and a target of OsMIR394), thus reducing LA through inhibiting elongation and expansion of adaxial parenchyma cells at the lamina joint (Bian *et al*., 2012; Qu *et al*., 2019). Moreover, a role of auxin in regulating LA in maize is supported by the observations that ZmPGP1, an auxin efflux carrier P-glycoprotein, is involved in the regulation of LA in maize (Li *et al*., 2019; Wei *et al*., 2018).

Several studies suggest that GA plays a promoting role in LA formation (Figure 4). For example, knockdown mutants of *OsSPY* (a negative regulator of GA signalling) have increased BR levels and hence increased LA (Shimada *et al*., 2006). In addition, a positive regulator of GA signalling, OsGSR1, positively regulates LA size through directly interacting with the BR biosynthetic enzyme OsBRD2 and enhancing its activity (Wang *et al*., 2009). Moreover, a recent study showed that transgenic rice plants expressing *OsBCL1* and *OsBCL2*, two homologous genes of *OsBC1*, under the control of *OsBUL1* promoter, exhibit larger LA and contain higher GA3 levels than the wild type plants, suggesting that both *OsBCL1* and *OsBCL2* promote cell elongation at the lamina joint at least partially through increased GA biosynthesis (Jang *et al*., 2021). However, another study showed that treatment with exogenous GA inhibits BR biosynthesis and signalling and hence reduces LA in plants, suggesting a negative role of GA in LA regulation (Tong *et al*., 2014). Therefore, the precise function of GA on LA regulation needs to be further clarified.

Several rice mutants defective in SLs biosynthetic and signalling (*d10*, *d17*, *d27*, *d3* and *d14*) display increased LA, indicating a negative role of SLs in LA regulation (Li *et al*., 2014). Accumulating evidence suggests that SLs may regulate LA through interacting with other hormone signalling pathways. For example, the SLs positive regulator OsDWARF3 (a F-box protein) was shown to interact with OsBZR1 and trigger its degradation, thus decreasing LA size, whereas the SLs negative regulator OsDWARF53 could enhance the transcriptional repression activity of OsBZR1 to increase LA size (Fang *et al*., 2020). In addition, a recent study showed that mutations in the ethylene biosynthetic gene *ZmACS7* and its closest paralog *ZmACS2* cause increased LA through promoting the longitudinal cell elongation of the auricle tissue in maize (Li *et al*., 2020a), suggesting a negative role of ethylene in LA regulation. Nevertheless, the detailed molecular mechanisms of SLs and ethylene regulation of LA formation remain to be further elucidated.

Perspective

As summarized above, despite the great progress made in recent years on elucidating the cellular and molecular mechanisms regulating LA formation in cereal crops (mainly in rice and maize), a number of pressing questions still stand out. First, the signal (s) that dictates the BSB formation and triggers the formation of PLB has not been unequivocally determined. Second, how the signal (s) is perceived and propagated from the midvein to the leaf margins still remain to be elucidated. Third, the detailed molecular mechanism regulating the outgrowth of ligule and auricle still

remain poorly understood. Fourth, from a view of plant breeding, despite a large number of LA-associated genes have been cloned (Table S1), the majority of them have not been exploited in plant breeding practice. A likely reason is that many LA genes pleiotropically regulate many other plant developmental processes besides LA. Therefore, changes, either loss-of-function or overexpression, in these LA genes often result in some unfavourable effects, such as plant dwarfism, reduced tillers and smaller grains (Hong *et al*., 2002, 2003, 2005; Tanabe *et al*., 2005; Tong *et al*., 2012). One approach to mitigate the negative effects of pleiotropic genes is to carefully manipulating their expression levels or patterns. For example, RNAi-*OsBRI1* transgenic plants obtained a 30% increased grain yield under dense planting conditions (Morinaka *et al*., 2006). In addition, identification and utilization of superior alleles in natural populations might be a preferred strategy. For instance, a recent study elegantly demonstrated that two QTLs that confer reduced LA, *Upright Plant Architecture1* (*UPA1,* encodes BRD1, an enzyme essential for bioactive BR biosynthesis) and *UPA2* (*UPA2* is controlled by a 2-bp nucleotide difference located 9.5Kb upstream of *ZmRAVL1*), can be successfully leveraged from its ancestor, teosinte, to maize to breed maize cultivars with more compact plant architecture and enhanced grain yields (Tian *et al*., 2019). Therefore, continued identification of new LA regulatory genes with minimal adversary effects on plant architecture may provide promising targets for genetic manipulation of LA and plant architecture for high-density planting. Moreover, with a better understanding of the regulatory network of LA formation, biotechnological approaches can be utilized to more precisely manipulate gene expression in a tissue- or developmental-specific manner to tailor plant architecture for high-density planting. The increased toolkits (such as genome editing) available now should greatly empower us to molecular design and create new germplasm that can better adapt to high-density planting and increased yield per unit land area in the years to come.

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Conflict of interest

The authors declare they have no conflict of interest.

Author's contributions

All authors contributed to the writing of this review article.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Blade-Sheath Boundary (BSB), preligule band (PLB) formation and leaf angle regulation related genes in maize and rice.