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REVIEW

WUSCHEL: a master regulator in plant growth signaling

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Abstract

Key message **This review summarizes recent knowledge on functions of *WUS* and *WUS-related homeobox (WOX)* transcription factors in diverse signaling pathways governing shoot meristem biology and several other aspects of plant dynamics.**

Abstract Transcription factors (TFs) are master regulators involved in controlling different cellular and biological functions as well as diverse signaling pathways in plant growth and development. *WUSCHEL* (*WUS*) is a homeodomain transcription factor necessary for the maintenance of the stem cell niche in the shoot apical meristem, the differentiation of lateral primordia, plant cell totipotency and other diverse cellular processes. Recent research about *WUS* has uncovered several unique features including the complex signaling pathways that further improve the understanding of vital network for meristem biology and crop productivity. In addition, several reports bridge the gap between *WUS* expression and plant signaling pathway by identifying different *WUS* and *WUS*-related homeobox (*WOX*) genes during the formation of shoot (apical and axillary) meristems, vegetative-to-embryo transition, genetic transformation, and other aspects of plant growth and development. In this respect, the *WOX* family of TFs comprises multiple members involved in diverse signaling pathways, but how these pathways are regulated remains to be elucidated. Here, we review the current status and recent discoveries on the functions of *WUS* and newly identified *WOX* family members in the regulatory network of various aspects of plant dynamics.

Keywords Embryogenesis · Plant development · Shoot meristem · Transcription factor · Transformation · *WUSCHEL*

Abbreviations

AM	Axillary meristem
AGL15	*AGAMOUS-LIKE15*
ARR	ARABIDOPSIS RESPONSE REGULATOR
BBM	*BABY BOOM*
FM	Floral meristem
FUS3	*FUSCA3*
GFP	Green fluorescence protein
HDL	*HEADLESS*
IAA30	Indole acetic acid inducible 30
LEC	*LEAFY COTYLEDON*
LSY1	*LEAF LATERAL SYMMETRY1*
RAM	Root apical meristem
SE	Somatic embryogenesis
SAM	Shoot apical meristem
SERK	*SOMATIC EMBRYOGENESIS RECEPTOR KINASE*
TAA	*TRYPTOPHAN AMINOTRANSFERASE ARABIDOPSIS*
TFs	Transcription factors
WUS	*WUSCHEL*
WOX	*WUSCHEL-related homeobox*

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Introduction

Historically, the study of plant cell growth and development focused on the role of phytohormones (Darwin 1880; Skoog and Miller 1957). However, at the end of twentieth century, with the advent of molecular genetics and transcriptomics, plant growth and development became more focused on various transcription factors (TFs) and their diverse signaling pathways as well as their expression patterns. Several studies at the molecular level have suggested that plant cell dedifferentiation mainly depends on the sequential and proper expression of a number of TF genes which are required for morphogenesis (Zuo et al. 2002; Bouchabké-Coussa et al. 2013; Zhai et al. 2016; Rupps et al. 2016; Songstad et al. 2017; Jha and Kumar 2018; Kumar and Van Staden 2019; Gordon-Kamm et al. 2019; Kausch et al. 2019). More specifically, during embryogenesis, many TFs are involved in vegetative to embryo transition such as *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KNASE* (*SERK*) (Schmidt et al. 1997), *WUSCHEL* (*WUS*) (Zuo et al. 2002), *BABY BOOM* (*BBM*) (Boutilier et al. 2002), *LEAFY COTYLEDON1/2* (*LEC1/2*) (Harada 2001), *FUSCA3* (*FUS3*) (Luerûen et al. 1998), *EMBRYO MAKER* (Tsuwamoto et al. 2010), *ABAINSENSITIVE3* (*ABI3*) (Shiota et al. 1998), and *AGAMOUSLIKE15* (*AGL15*) (Harding et al. 2003). Among the different TFs which participate in the expression and regulation of SE, *WUS* gene is reported to play an essential role (Laux et al. 1996; Zuo et al. 2002; Meng et al. 2019).

WUSCHEL (*WUS*) gene encodes the homeodomain TF, originally identified as a master regulator required for shoot and floral meristem integrity in *Arabidopsis* (Laux et al. 1996; Mayer et al. 1998). Ectopic overexpression of *WUS* gene regulates cell fate during cell dedifferentiation including size of shoot meristem, somatic embryo, adventitious shoot and lateral leaf formation, by maintaining the pluripotent stem cells (Zuo et al. 2002; Gallois et al. 2004; Honda et al. 2018) (Fig. 1).

The expression of *WUS* TF gene is confined to a small group of cells in the central zone of the shoot apical meristem (SAM) and is required for the maintenance of stem cell fate in *Arabidopsis* (Mayer et al. 1998; Yadav et al. 2011). The SAM organization in *Arabidopsis* includes three different layers of stem cells (L1-L3), a central zone (CZ), a peripheral zone (PZ) and a rib zone (RZ). *WUS* gene is expressed in the organizing centre (OC) and promotes the expression of *CLAVATA3* (*CLV3*) in the stem cells (Schoof et al. 2000). Several reports reveal that the *WUS* TF gene can act both as a transcriptional repressor of cytokinin response genes and a transcriptional activator of the floral gene *AGAMOUS*, suggesting that its molecular mechanism is modified dependent on the developmental context (Lohmann et al. 2001; Leibfried et al. 2005; Kieffer et al. 2006; Ikeda et al. 2009). Likewise, *WUS* gene also acts as a positive regulator for the expression of *CLV3*, which negatively regulates the meristem size by suppressing *WUS* expression (Schoof et al. 2000; Reddy 2008; Ikeda and Ohme-Takagi 2014), although *WUS* gene product mainly acts as a transcriptional repressor to suppress *CLV3* expression (Ikeda et al. 2009).

In vitro plants can be regenerated via organogenesis or embryogenesis (Su and Zhang 2014; Gaillochet and Lohmann 2015; Kumar and Van Staden 2017), and *WUS* is the only TF that has been shown to be involved in regulation of both embryogenic (totipotent) and meristematic (pluripotent) stem cells to date. Although significant progress has been made in knowledge of *WUS* TF gene to understand its signaling pathway, the molecular mechanisms underlying embryo development and stem cell fate development still remain unclear. This review provides insights on the recent discoveries and state-of-the-art advances on *WUS* TF gene in the area of plant growth and development.

WUS-mediated regulation of the shoot meristem

The homeobox gene *WUS,* which is differentially expressed in the OC of the SAM, is required for stem cell identity and plays an important role in the regulation of shoot meristem (Laux et al. 1996; Mayer et al. 1998; Schoof et al. 2000). *WUS* is synthesized in the OC, then migrates into the CZ to directly activate *CLV3* by binding to its promoters (Yadav et al. 2011), and further *WUS-CLV* interactions create a feedback circuit between the OC and the stem cells to establish the shoot stem cell niche (Schoof et al. 2000; Zhou et al. 2015; Galli and Gallavotti 2016), as illustrated in Fig. 1. In this context, *WUSCHEL-RELATED HOMEOBOX* (*WOX*), a homologous of *WUS* gene, and *HAIRY MERISTEM* (*HAM*) TFs are transcriptionally downregulated via *CLV3* signal into the nucleus, while *WUS* interacts with *HAM1* and *HAM2* to regulate target gene expression and maintenance of stem cells (Zhou et al. 2015). Parallel to this, *WOX4* interacts with *HAM4* and is expressed in *Arabidopsis* procambial cells (Ji et al. 2010), while *WOX5* interacts with *HAM2*, it is expressed in the root apical meristem (RAM) and is involved in the control and maintenance of shoot and root stem cell niche (Sarkar et al. 2007; Zhou et al. 2015; Somssich et al. 2016).

A recent study in *Medicago truncatula* showed that *HEADLESS* (*HDL*), a homolog of *AtWUS*, is essential for shoot meristem regulation and leaf development (Meng et al. 2019), as *HDL* interacts with the transcriptional co-repressor *MtTPL* (*TOPLESS*) and acts as a transcriptional repressor in shoot meristem maintenance. Likewise, several other studies also suggested that *WUS* acts as a transcriptional repressor in the maintenance of shoot meristem through its

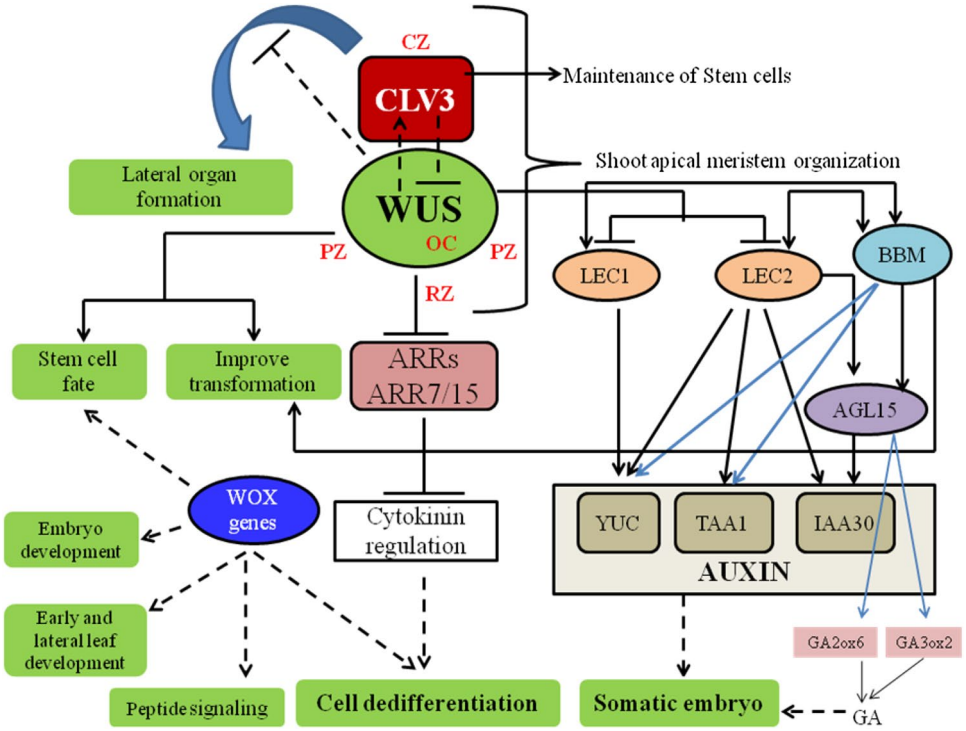

Fig. 1 A schematic model of the *WUSCHEL* (*WUS*) transcription factor gene showing the regulation of diverse functions in plant growth and development. As shown in Fig. 1, the stem cells are maintained in the apical shoot meristem by a regulatory feedback loop between the stem cells and organizing center (OC), via *CLV3* and *WUS* expression. The shoot apical meristem (SAM) organization with the central zone (CZ), peripheral zone (PZ) and rib zone (RZ) is shown. The stem cells (shown in red) express the *CLV3* signaling and OC (shown in green) induces the *WUS* expression. The *WUS* TF gene binds with *ARABIDOPSIS RESPONSE REGULATOR7* (*ARR7*) gene (Type-A response regulator which negatively regulates cytokinin signaling) to suppress its expression (To et al. 2004, 2007; Leibfried et al. 2005). However, the *ARR7/15* expression is regulated by *AUXIN RESPONSE FACTOR5/MONOPTEROS* activation (Zhao et al. 2010). The expression of *WUS* and *ARRs* is positively regulated by cytokinin signaling (Holt et al. 2014). On the other hand, during SE, the *WUS* TF gene transcriptionally regulates *LEC1*, *LEC2* and *AGL15* genes. The *LEC1* gene expressed the *YUC* gene (encodes a biosynthesis enzyme), whereas *LEC2* and *AGL15* activate the expression of *TAA1* (encodes an auxin biosynthesis enzyme) and *IAA30* (negative regulator of auxin) (Horstman et al. 2017). In addition, *AGL15* positively regulates Gibberellin (GA) degrading enzyme *GA2ox6*, and negatively regulates the biosynthesis gene *GA3ox2*, resulting in a reduced endogenous *GA* level (Ikeuchi et al. 2015).The *WOX* gene shows multiple functions including peptide signaling (Li et al. 2018), stem cell regulation (Dolzblasz et al., 2016), early and lateral leaf development (Honda et al. 2018; Yasui et al. 2018) and embryo development (Palovaara et al. 2010; Tvorogova et al. 2019) during plant growth development. In addition, *BBM* TF gene also binds to *YUC* and *TAA1* gene (Horstman et al. 2017). Arrows with a solid line show direct regulation and arrows with dotted lines indicate indirect regulation whose mechanisms are not clear as yet. *CZ* central zone, *GA* gibberellin, *PZ* peripheral zone, *RZ* rib zone, *OC* organizing center

interaction with *TPL* and *TPL*-related transcriptional co-repressor, which are employed by their conserved *WUS*-box (Ikeda et al. 2009; Dolzblasz et al. 2016; Meng et al. 2019). Furthermore, in their work using genetic analysis, Meng et al. (2019) also found that *HDL* and *STENOFOLIA* (*STF*), a master regulator of *M. truncatula* lamina outgrowth, regulate leaf blade development. Interestingly, the leaf blade outgrowth regulation by *STF* had been previously reported (Tadege et al. 2011; Lin et al. 2013; Zhang et al. 2014), implying that *WUS* would also be involved in the regulation of leaf blade growth.

As far as the relationship of *WUS* with auxins is concerned, in *A. thaliana*, *WUS* was shown to act rheostatically and restrict auxin signaling pathway to maintain the stem cell identity (Ma et al. 2019). This rheostatic activity of *WUS* is hypothesized to occur via regulation of histone acetylation and interference with HISTONE DEACETYLASES (HDAC) activity, which triggers auxin pathway in stem cells (Zhou et al. 2018). Loss of *WUS* action in the axillary meristem and SAM reduce shoot development, even if *WUS* overexpression encourages ectopic shoot growth development (Wang et al. 2017).

WUS involvement in floral and reproductive organ development

The regulation of *WUS* expression also involves an epigenetic mechanism network in the context of floral meristem development (Cao et al. 2015). In this context, Sun et al.

(2019) recently showed that *WUS* is repressed by *KNUCKLES* (*KNU*) through histone deacetylation in the floral meristem. Bimolecular fluorescence complementation (BiFC) assays indicated that *KNU* physically interacts at the nucleus with *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*), a *Polycomb Repressive Complex2* (*PRC2*) component, and thereby mediates the subsequent deposition of the epigenetic repression via histone H3K37me3 for the stable silencing of *WUS* (Sun et al. 2019).

In earlier work, in situ hybridization revealed that *WUS* is expressed in immature stomium cells and is involved in the anther development. As a result of this interaction, anthers of *wus* mutants had less and malformed lobes compared to the wild type, showing that *WUS* is essential for normal anther development (Deyhle et al. 2007). A recent study in *Chrysanthemum morifolium* also indicated that *WUS* interacts with CYCLOIDEA 2 (CYC2) TF and is involved in the regulation of reproductive organ (floral organs and pistils including style, ovary and stigma) development (Yang et al. 2019).

In future, it would therefore not be surprising to discover that *WUS* contributes to additional plant signaling pathways involved in other aspects of reproductive organ growth and development, and further research will undoubtedly provide novel insights for a better understanding of meristem biology in plant growth and development.

Relationship between *WUS* expression and shoot regeneration competence in vitro

WUS expression was shown to be essential to promote stem cell niche during shoot regeneration from cell and tissue culture studies (Meng et al. 2017; Zhang et al. 2017b). *WUS* is de novo activated and WUS^{+} expressing cells mark the shoot progenitor region during shoot regeneration in vitro (Zhang et al. 2017b).

A recent report indicated that the miR156-*SPL* (*SQUAMOSA PROMOTER BINDING PROTEIN-LIKE*) pathway directly or indirectly represses *WUS* expression to regulate the SAM size (Fouracre and Poethig 2019), confirming that *WUS* is specifically required for stem cell identity to regulate shoot meristem identity. Noteworthy, in *Arabidopsis*, *WUS*-related homeobox *WOX7* was also shown to regulate the lateral root development program in coupling with the sugar signaling, whereby *WOX7* acts as transcriptional repressor and inhibits lateral root formation in a sugar dependent manner, even if it has no effect during later stages of lateral root development (Kong et al. 2016).

In a breakthrough report by Zhang et al. (2017b), a two-step model for de novo activation of *WUS* has been proposed, which illustrates the molecular mechanism for shoot regeneration in *Arabidopsis*. Using genetic and time-lapsed imaging analysis, this study revealed that during shoot regeneration the WUS^{+} cells mark the regenerating region in *Arabidopsis*. Furthermore, upon transfer to cytokinin-rich shoot inducing medium (SIM), the *WUS* locus is subjected to an epigenetic reprogramming (Zhang et al. 2017b). However, the molecular mechanism by which cytokinins-rich SIM medium governs such epigenetic reprogramming at the *WUS* locus remains elusive. Several studies also suggest that *WUS* activation also depends on epigenetic regulation (Li et al. 2011; Cao et al. 2015; Zhang et al. 2017b; Wang et al. 2017; Sun et al. 2019). *WUS* expression during de novo shoot regeneration is associated with DNA methylation and histone modifications, and it has been shown that the removal of repressive histone H3 lysine trimethylation (H3K27me3) regulates shoot regeneration by modulating *WUS* expression in *Arabidopsis* (Li et al. 2011), with such H3K27me3 removal being division-dependent and essential for *WUS* induction (Zhang et al. 2017b). Furthermore, genetic analysis also confirmed that epigenetic changes at the *WUS* locus direct *WUS* expression during initiation of axillary meristems (Wang et al. 2017).

Crosstalk between cytokinin signaling and *WUS*

Phytohormones are essential for the maintenance of shoot stem cell homeostasis, and a crosstalk exists between cytokinin action and *WUS* function in the SAM. Cytokinin signaling plays a key role to maintain both cell proliferation and shoot meristem activity (Riou-Khamlichi et al. 1999; Werner et al. 2003; Gordon et al. 2009; Kieber and Schaller 2018), by acting via *Arabidopsis* histidine kinase (AHK) receptors which further pass on the signal to two classes of TFs: type-B *Arabidopsis* response regulators (ARRs) and type-A ARRs (Muller and Sheen 2007). The type-B ARRs activate transcription of cytokinin-induced genes, whereas, type-A ARRs form a negative feedback loop to reduce cytokinin responses. It has been shown that cytokinin signaling directly activates a dynamic pattern of *WUS* expression (Gordon et al. 2009). A positive feedback loop between cytokinin signaling and *WUS* function influences shoot meristem patterning (Leibfried et al. 2005; Gordon et al. 2009; Chickarmane et al. 2012; Wang et al. 2017), but the underlying molecular mechanism(s) remain to be elucidated. Some reports suggest that type-B ARRs (ARR1, ARR2, ARR10, and ARR12) directly bind to the *WUS* promoter and induce *WUS* expression (Wang et al. 2017; Zhang et al. 2017a, 2017b; Meng et al. 2017; Zubo et al. 2017). Supporting this hypothesis, chromatin immunoprecipitation (ChIP) analyses confirmed that type-B ARRs directly bind to the *WUS* promoter (Meng et al. 2017; Zhang et al. 2017b). Genetic analysis revealed that the function of type-B ARRs is essential for *WUS* expression for stem cell niche

during shoot regeneration (Zhang et al. 2017b; Meng et al. 2017). Interestingly, the expression of type-B ARRs was found 3 days prior to *WUS* in shoot regeneration (Zhang et al. 2017b). However, *WUS* expression can restore the shoot regenerative capacity in the *arr1* and *arr12* mutants (Zhang et al. 2017b). The type-B ARRs physically interact with the HD-ZIP III member of TFs and form a complex to activate *WUS* expression during shoot regeneration (Zhang et al. 2017b). In addition, ChIP-sequencing approach also elucidated *WUS* as a cytokinin-dependent direct target of ARR10 (Zubo et al. 2017). Cytokinin signaling also promotes de novo *WUS* expression to establish shoot stem cell niches during axillary meristem initiation (Wang et al. 2017). *WUS* overexpression was not found in the leaf axil in *arr1-4* mutant thereby indicating that ARR1 is required for *WUS* activation. ARR1 binds directly to the *WUS* promoter region and regulates axillary meristem development through *WUS* activation (Wang et al. 2017).

Furthermore, *WUS* has been shown to repress members of the type-A ARRs, which form a negative feedback loop to regulate cytokinin signaling (To et al. 2004, 2007; Leibfried et al. 2005; To and Kieber 2008). Type-A ARRs transcript levels are also responsive to several other factors. Thus, auxin induced ARR7 and ARR15 in the RAM (Muller and Sheen 2008), but repressed them in the SAM (Liebfried et al. 2005). In a breakthrough report, To et al. (2007) discovered that the type-A ARRs interact with other proteins in a phosphate-dependent manner to produce a negative regulation of the cytokinin signaling pathway.

Most recently, Kubalová et al. (2019) showed with *Arabidopsis* that inducing mutations in the biosynthetic pathway of tetrapyrrole uncouples the nuclear expression of *WUS* from de novo shoot development. Thus, such mutants exhibit quite contrasted responses to exogenous cytokinins, with highest *WUS* expression coupled with the lowest shoot regeneration competence during de novo organogenesis, demonstrating that the positive tight correlation between *WUS* expression and SAM activity is, at least partly, regulated by tetrapyrrole intermediates.

Taken together, these results suggest that a circular interaction between cytokinin signaling and regulatory genes provides a better understanding of the regulatory feedback loop networks. However, the underlying mechanism(s) by which type-A ARRs negatively regulate cytokinin signaling is yet to be clarified.

WUS is crucial for embryogenesis

Somatic embryogenesis (SE), the in vitro transition from vegetative to embryogenic phase in plants, was first reported 60 years ago by Reinert (1958) and Steward et al. (1958) and, since then, has gained momentum. In this respect, over the last two decades, many studies have shown that several candidate TFs are differentially expressed during conversion of somatic cells into embryogenic cells (Schrader et al. 1997; Schmidt et al. 1997; Boutlier et al. 2002; Zhai et al. 2016; Kumar and Van Staden 2019), most of them not being directly involved in the vegetative to embryogenic transition, but upregulated during late embryogenesis. The only exception is *SERK* gene, but its function remains elusive.

WUS TF gene is reported to play a key role in plant embryogenesis. It has been found that, during SE, *WUS* gene is positively upregulated in several plant species, such as *A. thaliana* (Zuo et al. 2002), *C. canephora* (Arroyo-Herrera et al. 2008), *G. hirsutum* (Bouchabké-Coussa et al. 2013; Zheng et al. 2014) and *M. truncatula* (Chen et al. 2009; Orlowska and Kepczynska 2018; Tvorogova et al. 2015; 2019). Table 1 lists *WUS*-related genes expressed during SE in *Arabidopsis*, while Table 2 shows their expression in other plant species.

In *A. thaliana*, ectopic expression of *WUS* was shown to be involved in vegetative-to-embryogenic transition in all tissues (leaf petiole, leaves, stem and root), without adding exogenous growth hormones (Zuo et al. 2002). Arroyo-Herrera et al. (2008) found that overexpression of *WUS* in *C. canephora* increased the somatic embryo production up to 400%, and significantly enhanced the SE in a heterologous system, but exogenous growth regulators were required for the induction of SE. In *G. Hirsutum* (cotton), *A. thaliana WUS* (*AtWUS*) significantly increased embryogenic callus formation (47.75%) when ectopically expressed (Zheng et al. 2014), and also positively upregulated *LEC1/2* and *FUS3* in such cotton embryogenic callus. Similarly, Bouchabké-Coussa et al. (2013) also revealed that overexpression of *WUS* gene significantly promoted (×3) embryogenic competence and triggered in vitro regeneration capacity in cotton when *WUS* was expressed ectopically. However, these authors also observed that overexpression of *WUS* resulted in the formation of abnormal embryo-like structures and that leaf-like structures developed on the embryos (Bouchabké-Coussa et al. 2013).

WOX gene family members are expressed in the zygote and regulate cell and tissue proliferation during early embryo patterning (Haecker et al. 2004; Wu et al. 2007; Breuninger et al. 2008; Ueda et al. 2011; Zhang et al. 2017c). *WOX8/9* expression is activated in the zygote and becomes restricted to the basal lineage, whereas *WOX2* expression is restricted to the apical lineage where it regulates gene expression programs (Wu et al. 2007; Breuninger et al. 2008; Ueda et al. 2011). However, it is poorly understood if both genes, *WOX8/9* and *WOX2*, are transcribed in the zygote or whether their mRNA is inherited from the egg cell. Similarly, *WOX8/9* and *WOX2* genes have been recognized as being expressed during early embryo development in conifers (Palovaara et al. 2010; Hedman et al. 2013; Zhu et al.

Table 1 *WUS* and *WUS*-related homeobox (*WOX*) transcription factors reported for *Arabidopsis thaliana* and their biological functions in plant growth regulation

Name of the gene	Biological function	References
WUS	Regulates stem cell fate in shoot meristem	Mayer et al. 1998
WUS	Maintenance of stem cell of shoot meristem	Schoof et al. 2000
WUS	Promotes the vegetative-to-embryonic transition during embryogenesis	Zuo et al. 2002
WUS	Induces ectopic organogenesis	Gallois et al. 2002
WUS	Is involved in the maintenance of shoot and floral meristem; Promotes stem cell fate	Sharma and Fletcher 2002
WUS	Plant ovule development	Gross-Hardt et al. 2002
WUS	Induces the shoot stem cell identity and developmental plasticity in the root meristem	Gallois et al. 2004
WOX2	Development of apical domain of somatic embryo	Haecker et al. 2004
WUS	Initiation of floral meristem	Xu et al. 2005
WUS	Controls meristem function	Leibfried et al. 2005
WUS	Is involved in the stem cell fate maintenance	Kwon et al. 2005
WUS	Regulates cell differentiation during male organogenesis	Deyhle et al. 2007
WUS	Renewal of embryonic stem cell during embryogenesis	Su et al. 2009
WUS	Regulates stem cell identity and floral patterning	Ikeda et al. 2009
WUS	Regulates stem cell homeostasis	Yadav et al. 2011
SlWUS	Development of inflorescence structures and flower identity	Xiang et al. 2012
WUS	Promotes cell division	Zhang et al. 2013
WUS	Promotes the initiation of axillary meristem	Wang et al. 2017
WUS	Shoot stem cell specification in roots	Negin et al. 2017
WUS	Maintenance of apical meristem and shoot development	Xie et al. 2018
WUS	Is involved in the maintenance of apical stem cell	Ma et al. 2019
WUS	Controls shoot apical meristem size	Fouracre and Poethig 2019

2014). Zhou et al. (2018) revealed a novel function of *WOXs* in regulating embryo patterning in tobacco, and confirmed by expression pattern analysis that *WOX2* and *WOX9* are crucial for early embryo patterning. In a very recent study with *M. truncatula*, Tvorogova et al. (2019) showed that the *WOX9* homolog, *MtWOX9-1*, participates in SE and its overexpression stimulates embryogenesis capacity by changing the expression levels of various SE-associated genes.

Taken together, these studies suggest that *WUS* and *WOX* family members have a significant impact on plant biology in improving the SE capacity in plant cells. In spite of these groundbreaking discoveries in elucidating *WUS* expression during embryo patterning, the regulatory signaling pathways responsible for its expression and regulation remain unclear. Further studies and refinements will improve the understanding of the molecular mechanisms by which *WUS* expression regulates embryo patterning.

WUS-mediated transformation

Plant genetic transformation is commonly mediated by *Agrobacterium*, whereas microprojectile bombardment and protoplast technology (applying electroporation or chemicals such as PEG to introduce plasmids carrying the transgene) have enabled new insights in the area of plant biology, allowing direct gene transfer. For a number of species, conventional plant genetic transformation is often hampered by a low efficiency, it is time consuming, and several technical bottlenecks exist for the recovery of transgenic plants (Newell 2000; Altpeter et al. 2016; Mookan et al. 2017). However, a few recent reports have provided promising solutions in the area of plant transformation where morphogenic regulators improved the transformation efficiency in several plants (Heidmann et al. 2011; Lowe et al. 2016, 2018; Mookan et al. 2017; Jones et al. 2019).

The overexpression of *WUS* gene has been implicated in several model and crop species to stimulate transformation efficiency (Lowe et al. 2016; 2018; Mookan et al. 2017; Jones et al. 2019). Thus, in experiments of co-transformation of *BBM* with *WUS2*, Lowe et al. (2016) provided evidence that maize TF *WUS2* stimulates high transformation frequency in several genotypes. Overexpression of maize *WUS2* gene improved transformation efficiency in several monocots including *Oryza sativa* (ssp. *indica*), *Saccharum officinarum* calli, and *Sorghum bicolor* somatic embryos (immature). *WUS* gene was transformed directly into immature embryos or leaf segments in five different pioneer inbred lines whereby pleiotropic effects such as thickened roots, wrinkled leaves, poor functioning and sterility were

Table 2 *WUS* and *WUS*-related homeobox (*WOX*) transcription factors gene reported from *other plant species* and their biological functions in plant growth regulation

Plant species	Family	Eudicot/Monocot	Gene type	Name of gene	Biological function	References
Antirrhinum majus	Plantaginaceae	Eudicot	*WUSCHEL*	*AtWUS*	Dedifferentiation and meristem maintenance	Kieffer et al. 2006
Beta palonga	Amaranthaceae	Eudicot	*WUSCHEL*	*WUS*	In vitro shoot morphogenesis	Sultana and Gangopadhyay 2018
Capsicum chinense	Solanaceae	Eudicot	*WUSCHEL*	*WUS*	In vitro morphogenesis	Solís-Ramos et al. 2009
Chryssanthemum morifolium	Asteraceae	Eudicot	*WUSCHEL*	*CmWUS*	Development of reproductive organs	Yang et al. 2019
Coffea canephora	Rubiaceae	Eudicot	*WUSCHEL*	*WUS*	Increasing somatic embryogenesis and ectopic morphogenesis	Arroyo-Herrera et al. 2008
Gossypium hirsutum	Malvaceae	Eudicot	*WUSCHEL*	*AtWUS*	Induces organogenesis and promotes somatic embryogenesis	Bouchabké-Coussa et al. 2013
Gossypium hirsutum	Malvaceae	Eudicot	*WUSCHEL*	*AtWUS*	Induction of embryogenic callus	Zheng et al. 2014
Larix decidua	Pinaceae	Conifer	*WUSCHEL-RELATED HOMEOBOX*	*LdWOX2*	Induction of somatic embryogenesis	Rupps et al. 2016
Ocotea catharinensis	Lauraceae	Eudicot	*WUSCHEL*	*OcWUS*	Induction of somatic embryogenesis	Santa-Catarina et al. 2012
Picea abies	Pinaceae	Conifer	*WUSCHEL-RELATED HOMEOBOX*	*WOX2* *WOX8/9*	Development of somatic embryo	Palovaara et al. 2010
Picea glauca	Pinaceae	Conifer	*WUSCHEL*	*AtWUS*	Involved in somatic embryogenesis and somatic seedling growth	Klimaszewska et al. 2010
Picea abies	Pinaceae	Conifer	*WUSCHEL-RELATED HOMEOBOX*	*WUS*; *WOX5*	Somatic embryo development	Hedman et al 2013
Picea abies	Pinaceae	Conifer	*WUSCHEL-RELATED HOMEOBOX*	*AtWOX8*; *AtWOX9*	Responsible for embryo patterning	Zhu et al. 2014

observed in transgenic plants, and removal of *WUS2* and *BBM* gene was essential to achieve normal transformants. For inbred line PHN46, a modest additional increase (4%) was found in callus transformation frequency after addition of *WUS2*. However, in inbred PH581, combination of *WUS2* and *BBM* elicited an increase from 0.4% to 25.3% in callus transformation. Interestingly, inbred PHP38 showed the highest transformation frequency (51.7%) when *WUS2* and *BBM* were used together. In line with these results, Mookan et al. (2017) found that co-expression of *WUS2* and *BBM* TF genes stimulated efficient transformation in sorghum (P898012 genotype) and a recalcitrant maize inbred line (B73) without use of a selectable marker gene. The PHP78891 expression cassette comprised *CRE:WUS2:BBM* with *lox* P sites. Transient expression of *GFP* was shown in shoots, early and late embryos, pollen and vegetative organs, by introduction of *Agrobacterium*-mediated transgenic PHP78891 vector. Furthermore, stable transgene integration and expression in regenerated sorghum P898012 and maize B73 were confirmed by PCR and southern blotting. An enhanced transformation frequency for P898012 genotypes (6.2%) and B73 (0 to 15%) was found without the use of any

selectable marker gene (Mookan et al. 2017). This selectable marker independent transformation approach may contribute to facilitate gene editing functions and overcome transformation barriers in recalcitrant genotypes. More recently, Lowe et al. (2018) showed that co-expression of *WUS2* and *BBM* showed transformation frequencies ranging from 9.1% to 62.5% in large numbers of immature embryos in PHR03, PHH5G and PH1V69 pioneer inbred maize lines.

These findings confirmed that plant transformation technology is moving towards a new era and that morphogenic regulators may overcome many transformation obstacles. In future, the role of *WUS* gene can therefore be used to enhance transformation frequency and would improve the information for unknown signaling pathways stimulating transformation. In this context, new discoveries and further refinements in this area, such as the use of TF genes like *BBM* and *WUS* are likely to improve the transformation efficiency of other recalcitrant species, including monocots.

WUS functions in crops

The blooming studies of *WUS* in *Arabidopsis* and several other plant species have significantly helped in elucidating *WUS* functions in major crops, and offered novel insights into improvements for crop agricultural practice (Table 3). In addition to the above mentioned *WUS* functions on transformation efficiency in rice, maize, sugarcane and sorghum, in other species *WUS* is implicated in stem cell formation, somatic embryo development and floral initiation process (Chen et al. 2009; Wong et al. 2011; Zhou et al. 2018; Kyo et al. 2018; Orlowska and Kepczynska 2018).

In situ hybridization analysis suggested that *MtWOX5* (*WUSCHEL*-related homeobox gene *WOX5*) is expressed in auxin-induced root primordia and meristems and is involved in pluripotent stem cell formation in *M. truncatula*, and RNAi analysis confirmed that overexpression of *MtWUS* is crucial for somatic embryo production (Chen et al. 2009). *MtWUS* expression was induced within 2 days and further peaked after 1 week in the presence of auxin and cytokinin. However, auxin alone did not induce expression, the enhanced *MtWUS* expression was cytokinin-dependent and this result is consistent with *WUS* and cytokinin relationship in *WUS*-regulation of the *Arabidopsis* meristem (Leibfried et al. 2005), as discussed above. Another study with the model legume *M. truncatula* suggested an involvement of *MtWUS* and *MtWOX5* during the initiation phase of SE, when they were key markers for cell dedifferentiation in leaf explants both in M9 (non-embryogenic) and M9-10a (embryogenic) lines (Orlowska and Kepczynska 2018). Thus, *MtWUS* expression on both lines was found to be maximum at day 2 (120-fold higher), but it decreased rapidly after 7 days. Meanwhile, an extreme upregulation of *WOX5* expression occurred between day 3 and day 14 and it remained unchanged thereafter (Orlowska and Kepczynska 2018). Similar to *M. truncatula*, in tobacco (*N. tabacum*) *WOX* genes are involved during early embryogenesis (Zhou et al. 2018), most of them exhibiting a cell type-specific and stage-specific expression pattern during embryogenesis. RT-qPCR (Quantitative real time reverse transcription PCR) analyses revealed that *WOXs* genes (*WOX2* and *WOX9*) are crucial for early embryo patterning (Zhou et al. 2018). A recent study also showed that coexpression of *WOXs* (*WOX2*, *WOX8* and *WOX9*) promotes remarkable regeneration from freely suspended cells and leaf segments of tobacco (Kyo et al. 2018).

In soybean (*Glycine max*), *WUS* spatial expression in the incipient floral primordia elucidated *WUS* function in the floral initiation process (Wong et al. 2011). RT-PCR analysis revealed that *GmWUS* (soybean ortholog of *WUS*) is expressed in the SAM and floral meristem, while in situ hybridization showed that *GmWUS* accumulates in incipient floral primordia. These observations are largely consistent with those reported earlier for the initiation of floral primordia in *Arabidopsis* (Wagner 2009). Interestingly, ectopic expression of *GmWUS* is sufficient to produce adventitious shoot formation on the petiole of a rosette leaf, whereas disruption in floral organ formation includes missing petals, defective floral buds and normal stamens and carpels (Wong et al. 2011). These results are also consistent with those reported for *Arabidopsis WUS* (Xu et al. 2005; Ikeda et al. 2009; Lohman et al. 2001).

It appears that *WUS* mediates the stress response and regulates early flowering in rice (Minh-Thu et al. 2018). RT-PCR analysis confirmed that *OsWOX13*, a homeodomain TF, was moderately upregulated under drought stress in leaf and root of rice. Overexpression of *OsWOX13* triggered floral development resulting in 7–10 days earlier flowering in rice (Minh-Thu et al. 2018). The *OsWOX4* member of *WOX* gene family regulates cellular activity in leaf development including tissue differentiation of both vascular development and midrib formation, as transcriptome profiling revealed that *OsWOX4* regulates the expression of several genes in leaf primordia and promotes cell proliferation, leading to leaf development (Yasui et al. 2018). The *WOX3* gene, *LEAF LATERAL SYMMETRY1* (*LSY1*), is involved in lateral organ development in rice by regulating adaxial-abaxial patterning at the edge of leaf primordia, and *LSY1* also regulates trichome initiation and function in the inflorescence by maintaining adaxial-abaxial identity in the stamens (Honda et al. 2018). Most recently, Hao et al. (2019) showed that the overexpression of *GmWOX18* significantly increased (more than 150-fold) adventitious shoot bud regeneration capacity in soybean under different abiotic stresses.

Table 3 *WUS* functions in crops

Plant species	Family	Eudicot/Monocot	Gene type	Name of gene	Biological function	References
Glycine max	Fabaceae	Eudicot	*WUSCHEL*;	*GmWUS*	Floral initiation process	Wong et al. 2011
Glycine max	Fabaceae	Eudicot	*WUSCHEL*;	*GmWOX18*	Increased the regeneration capacity	Hao et al. 2019
Medicago truncatula	Fabaceae	Eudicot	*WUSCHEL*; *WUSCHEL-RELATED HOMEOBOX*	*MtWUS\MtWOX5*	Induction of somatic embryogenesis Stem cell induction	Chen et al. 2009
Medicago truncatula	Fabaceae	Eudicot	*WUSCHEL-RELATED HOMEOBOX*	*MtWOX9*	Induction of somatic embryogenesis	Tvorogova et al. 2019
Medicago truncatula	Fabaceae	Eudicot	*WUSCHEL*; *WUSCHEL-RELATED HOMEOBOX*	*MtWUS\MtWOX5*	Induction of somatic embryogenesis	Orlowska and Kepczynska 2018
Nicotiana tabacum	Solanaceae	Eudicot	*WUSCHEL-RELATED HOMEOBOX*	*WOX2*; *WOX8*; *WOX9*	Promotes in vitro regeneration from leaf segments and embryos development from free cells	Kyo et al. 2018
Nicotiana tabacum	Solanaceae	Eudicot	*WUSCHEL-RELATED HOMEOBOX*	*WUS*; *WOX5*	Cell-type and stage-specific expression pattern during embryogenesis	Zhou et al. 2018
Oryza sativa	Poaceae	Monocot	*WUSCHEL-HOMEOBOX*	*WOX4*	Involved in the negative meristem maintenance	Ohmori et al. 2013
Oryza sativa	Poaceae	Monocot	*WUSCHEL*	*WUS2*	Stimulate transformation efficiency	Lowe et al. 2016
Oryza sativa	Poaceae	Monocot	*WUSCHEL-HOMEOBOX*	*OsWOX13*	Enhance the stress response (drought tolerance) and induces early flowering	Minh-Thu et al. 2018
Oryza sativa	Poaceae	Monocot	*WUSCHEL-HOMEOBOX*	*WOX3*	Regulates lateral leaf development	Honda et al. 2018
Oryza sativa	Poaceae	Monocot	*WUSCHEL-HOMEOBOX*	*OsWOX4*	Involves in early leaf development	Yasui et al. 2018
Pisum sativum	Fabaceae	Eudicot	*WUSCHEL*	*PsWUS*	Maintenance of shoot apical meristem	Ninan et al. 2017
Sorghum bicolor	Poaceae	Monocot	*Maize WUSCHEL*	*WUS2*	Stimulate transformation efficiency	Lowe et al. 2016
Sorghum bicolor	Poaceae	Monocot	*Maize WUSCHEL*	*WUS2*	Improved transformation frequency and promotes somatic embryogenesis	Mookan et al. 2017
Sachharum officinarum	Poaceae	Monocot	*WUSCHEL*	*WUS2*	Stimulate transformation efficiency	Lowe et al. 2016
Zea mays	Poaceae	Monocot	*WUSCHEL*	*WUS2*	Increase transformation frequency	Lowe et al. 2018
Zea mays	Poaceae	Monocot	*WUSCHEL*	*WUS2*	*Agrobacterium*-mediated transformation frequency	Jones et al. 2019

The summation of these discoveries on *WUS* and *WUS-related homeobox* genes provides novel mechanistic insights into the development of several crop species. However, further studies on the molecular regulation mechanisms underlying the functions of *WUS* and *WOX* genes will also facilitate an understanding of the diversification of *WUS* and *WOX* genes in different plant species, including monocots and eudicots for a better improvement of their sustainable agricultural performance.

Concluding remarks and perspectives

Our understanding of the molecular mechanisms which govern meristem regulatory networks and other plant signaling pathways is constantly increasing. A number of groundbreaking researches in *Arabidopsis* and various crop species have significantly increased our understanding of meristem biology. *WUS* expression has significant potential on plant biology research and other biotechnological applications. Several discoveries bridge the gap between *WUS* expression and plant signaling pathway by identifying different *WUS* and *WUS*-related homeobox genes during the formation of shoot (apical and axillary) meristems, vegetative-to-embryo transition, genetic transformation, and other aspects of plant growth and development.

The studies discussed above suggest that the *WUS* gene is required for meristem identity by recruiting transcriptional corepressors that induces differentiation and maintenance of stem cells. *WUS* was shown to be involved in vegetative-to-embryogenic transition without adding any exogenous growth hormones, when expressed ectopically. We also note that the *WOX* family of TFs comprises multiple members which are expressed in the zygote and involved in diverse signaling pathways during early embryo patterning. In addition, overexpression of *WUS* stimulates high transformation frequency in several genotypes, even if the rather limited information regarding transformation efficiency is due to the unknown signaling pathway which still remains unclear.

In addition to the above mentioned *WUS* functions, in crop species *WUS* and *WOX* genes are implicated in leaf development including tissue differentiation of both vascular development and midrib formation, lateral organ development, trichome initiation and function in the inflorescence by maintaining adaxial-abaxial identity in the stamens.

Further experiments should shed some light on how these regulatory members co-ordinate and control meristem biology and several aspects of plant dynamics. To decode these regulatory networks, a single-molecule imaging technology will be required to understand the diverse functions of individual *WOXs* in different signaling pathways. Together, structural studies of different *WOXs* may open new avenues for better understanding their signaling specificity and developmental plasticity.

During the past decade, new signaling pathways of *WUS* TF gene regulating diverse biotechnological functions of plant growth and development have been discovered. However, we are far from understanding the molecular mechanism(s) and complex network(s) of *WUS* TF signal(s) that still need to be deciphered, and how these TFs integrate cell-to-cell communication and regulate cell behavior in several plant growth responses. For future research, several tools have emerged, such as single-molecule imaging technology, that may help reveal important details of signaling pathways. In addition, further research will provide novel insights for a better understanding of meristem dynamics in plant growth regulation, which should in turn improve the modern biotechnological approaches for agriculture and crop productivity.

Recent discoveries have explored the multiple roles of *WUS* in diverse aspects of plant growth and development. However, a few outstanding questions still need to be clarified: (1) *WUSCHEL*-related homeobox (*WOX*) family contains multiple members which are involved in the diverse signaling pathways related to meristem regulatory network, but how these pathways are regulated remains unclear. (2) Individual *WOX* members regulate differentially to different signaling pathways. How is the specificity of these different *WOXs* members obtained? (3) Apart from known processes, what additional physiological and biological processes are regulated by *WUS*? (4) The underlying molecular mechanism by which *WUS* function and cytokinin signaling make a positive feedback loop and regulates shoot meristem patterning is still unclear.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

References

Altpeter F, Springer NM, Bartley LE, Blechl AE, Brutnell TP, Citovsky V, Conrad LJ, Gelvin SB, Jackson DP, Kausch AP, Lemaux PG, Medford JI, Orozco-Cardenas ML, Tricoli DM, Van Eck J, Voytas DF, Walbot V, Wang K, Zhang ZJ, Stewart CN Jr (2016) Advancing crop transformation in the era of genome editing. Plant Cell 28:1510–1520

Arroyo-Herrera A, Gonzalez AK, Moo RC, Quiroz-Figueroa F, Loyola-Vargas V, Rodriguez-Zapata L, Burgeff D'Hondt C, Suárez-Solís VM, Castano E (2008) Expression of *WUSCHEL* in *Coffea canephora* causes ectopic morphogenesis and increases somatic embryogenesis. Plant Cell Tissue Organ Cult 94:171–180

Bouchabké-Coussa O, Obellianne M, Linderme D, Montes E, Maia-Grondard A, Vilaine F, Pannetier C (2013) *Wuschel* overexpression promotes somatic embryogenesis and induces organogenesis in cotton (*Gossypium hirsutum* L.) tissues cultured in vitro. Plant Cell Rep 32:675–686

Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, Hattori J, Liu C, van Lammeren AAM, Miki BLA, Custers JBM, van Lookeren Campagne MM (2002) Ectopic expression of *BABY BOOM* triggers a conversion from vegetative to embryonic growth. Plant Cell 14:1737–1749

Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T (2008) Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo. Dev Cell 14:867–876

Cao X, He Z, Guo L, Liu X (2015) Epigenetic mechanisms are critical for the regulation of *WUSCHEL* expression in floral meristems. Plant physiol 168:1189–1196

Chen SK, Kurdyukov S, Kereszt A, Wang XD, Gresshoff PM, Rose RJ (2009) The association of homeobox gene expression with stem cell formation and morphogenesis in cultured *Medicago truncatula*. Planta 230:827–840

Chickarmane VS, Gordon SP, Tarr PT, Heisler MG, Meyerowitz EM (2012) Cytokinin signaling as a positional cue for patterning the apical–basal axis of the growing *Arabidopsis* shoot meristem. Proc Natl Acad Sci USA 109:4002–4007

Darwin C (1880) The power of movement in plants. John Murray, London

Deyhle F, Sarkar AK, Tucker EJ, Laux T (2007) *WUSCHEL* regulates cell differentiation during anther development. Dev Biol 302:154–159

Dolzblasz A, Nardmann J, Clerici E, Causier B, van der Graaff E, Chen J, Davies B, Werr W, Laux T (2016) Stem cell regulation by *Arabidopsis WOX* genes. Mol Plant 9:1028–1039

Fouracre JP, Poethig RS (2019) Role for the shoot apical meristem in the specification of juvenile leaf identity in *Arabidopsis*. Proc Natl Acad Sci USA 116:10168–10177

Gaillochet C, Lohmann JU (2015) The never-ending story: from pluripotency to plant developmental plasticity. Development 142:2237–2249

Galli M, Gallavotti A (2016) Expanding the regulatory network for meristem size in plants. Trends Genet 32:372–383

Gallois JL, Woodward C, Reddy GV, Sablowski R (2002) Combined SHOOT MERISTEMLESS and *WUSCHEL* trigger ectopic organogenesis in *Arabidopsis*. Development 129:3207–3217

Gallois JL, Nora FR, Mizukami Y, Sablowski R (2004) *WUSCHEL* induces shoot stem cell activity and developmental plasticity in the root meristem. Genes Dev 18:375–380

Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. Proc Natl Acad Sci USA 106:16529–16534

Gordon-Kamm B, Sardesai N, Arling M, Lowe K, Hoerster G, Betts S, Jones T (2019) Using morphogenic genes to improve recovery and regeneration of transgenic plants. Plants 8:38

Gross-Hardt R, Lenhard M, Laux T (2002) *WUSCHEL* signaling functions in interregional communication during *Arabidopsis* ovule development. Genes Dev 16:1129–1138

Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. Development 131:657–668

Hao Q, Zhang L, Yang Y, Shan Z, Zhou X (2019) Genome-wide analysis of the *WOX* gene family and function exploration of *GmWOX18* in soybean. Plants 8:215

Harada JJ (2001) Role of *Arabidopsis LEAFY COTYLEDON* genes in seed development. J Plant Physiol 158:405–409

Harding EW, Tang W, Nichols KW, Fernandez DE, Perry SE (2003) Expression and maintenance of embryogenic potential is enhanced through constitutive expression of *AGAMOUSLIKE15*. Plant Physiol 133:653–663

Hedman H, Zhu T, Von Arnold S, Sohlberg JJ (2013) Analysis of the *WUSCHEL-RELATED HOMEOBOX* gene family in the conifer *Picea abies* reveals extensive conservation as well as dynamic patterns. BMC Plant Biol 13:89

Heidmann I, de Lange B, Lambalk J, Angenent GC, Boutilier K (2011) Efficient sweet pepper transformation mediated by the *BABY BOOM* transcription factor. Plant Cell Rep 30:1107–1115

Holt AL, van Haperen JM, Groot EP, Laux T (2014) Signaling in shoot and flower meristems of *Arabidopsis thaliana*. Curr Opin Plant Biol 17:96–102

Honda E, Yew CL, Yoshikawa T, Sato Y, Hibara K, Itoh JI (2018) *LEAF LATERAL SYMMETRY1*, a member of the *WUSCHEL-RELATED HOMEOBOX3* gene family, regulates lateral organ development differentially from other paralogs, *NARROW LEAF2* and *NARROW LEAF3* in rice. Plant Cell Physiol 59:376–391

Horstman A, Li M, Heidmann I, Weemen M, Chen B, Muino JM, Angenent GC, Boutilier K (2017) The *BABY BOOM* transcription factor activates the *LEC1-ABI3-FUS3-LEC2* network to induce somatic embryogenesis. Plant Physiol 175:848–857

Ikeda M, Ohme-Takagi M (2014) *TCPs*, *WUSs*, and *WINDs*: families of transcription factors that regulate shoot meristem formation, stem cell maintenance, and somatic cell differentiation. Front Plant Sci 5:427

Ikeda M, Mitsuda N, Ohme-Takagi M (2009) *ArabidopsisWUSCHEL* is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning. Plant Cell 21:3493–3505

Ikeuchi M, Iwase A, Rymen B, Harashima H, Shibata M, Ohnuma M, Breuer C, Morao AK, de Lucas M, de Veylder L, Goodrich J, Brady SM, Roudier F, Sugimoto K (2015) PRC2 represses dedifferentiation of mature somatic cells in *Arabidopsis*. Nat Plants 1:15089

Jha P, Kumar V (2018) *BABY BOOM* (*BBM*): a candidate transcription factor gene in plant biotechnology. Biotechnol Lett 40:1467–1475

Ji J, Strable J, Shimizu R, Koenig D, Sinha N, Scanlon MJ (2010) *WOX4* promotes procambial development. Plant Physiol 152:1346–1356

Jones T, Lowe K, Hoerster G, Anand A, Wu E, Wang N, Arling M, Lenderts B, Gordon-Kamm W (2019) Maize transformation using the morphogenic genes Baby Boom and Wuschel2. In: Kumar S, Barone P, Smith M (eds) Transgenic plants. Methods in molecular biology, vol 1864. Humana Press, New York

Kausch AP, Nelson-Vasilchik K, Hague J, Mookkan M, Quemada H, Dellaporta S, Fragoso C, Zhang ZJ (2019) Edit at will: Genotype independent plant transformation in the era of advanced genomics and genome editing. Plant Sci 281:186–205

Kieber JJ, Schaller GE (2018) Cytokinin signaling in plant development. Development 145:pii:dev149344

Kieffer M, Stern Y, Cook H, Clerici E, Maulbetsch C, Laux T, Davies B (2006) Analysis of the transcription factor *WUSCHEL* and its functional homologue in *Antirrhinum* reveals a potential mechanism for their roles in meristem maintenance. Plant Cell 18:560–573

Klimaszewska K, Pelletier G, Overton C, Stewart D, Rutledge RG (2010) Hormonally regulated overexpression of *Arabidopsis WUS* and conifer *LEC1* (CHAP3A) in transgenic white spruce: implications for somatic embryo development and somatic seedling growth. Plant Cell Rep 29:723–734

Kong D, Hao Y, Cui H (2016) The *WUSCHEL* related homeobox protein *WOX7* regulates the sugar response of lateral root development in *Arabidopsis thaliana*. Mol Plant 9:261–270

Kubalová I, Zalabák D, Mičúchová A, Ikeda Y (2019) Mutations in tetrapyrrole biosynthesis pathway uncouple nuclear *WUSCHEL* expression from de novo shoot development in *Arabidopsis*. Plant Cell Tissue Organ Cult 139:395–401

Kumar V, Van Staden J (2017) New insights into plant somatic embryogenesis: an epigenetic view. Acta Physiol Plant 39:194

Kumar V, Van Staden J (2019) Multi-tasking of *SERK*-like kinases in plant embryogenesis, growth, and development: current advances and biotechnological applications. Acta Physiol Plant 41:31

Kwon CS, Chen C, Wagner D (2005) *WUSCHEL* is a primary target for transcriptional regulation by SPLAYED in dynamic control of stem cell fate in *Arabidopsis*. Genes Dev 19:992–1003

Kyo M, Maida K, Nishioka Y, Matsui K (2018) Coexpression of *WUSCHEL* related homeobox (*WOX*)*2* with *WOX8* or *WOX9* promotes regeneration from leaf segments and free cells in *Nicotiana tabacum* L. Plant Biotechnol 35:23–30

Laux T, Mayer KF, Berger J, Jürgens G (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development 122:87–96

Leibfried A, To JP, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU (2005) *WUSCHEL* controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 438:1172–1175

Li W, Liu H, Cheng ZJ, Su YH, Han HN, Zhang Y, Zhang SX (2011) DNA methylation and histone modifications regulate *de novo* shoot regeneration in *Arabidopsis* by modulating *WUSCHEL* expression and auxin signaling. PLoS Genet 7:e1002243

Li X, Hamyat M, Liu C, Salman A, Gao X, Guo C, Wang Y, Guo Y (2018) Identification and characterization of the *WOX* family genes in five *Solanaceae* species reveal their conserved roles in peptide signaling. Genes 9:260

Lin H, Niu L, Tadege M (2013) *STENOFOLIA* acts as a repressor in regulating leaf blade outgrowth. Plant Signal Behav 8:e24464

Lohmann J, Hong R, Hobe M, Busch M, Parcy F, Simon R, Weigel D (2001) A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell 105:793–803

Lowe K, Wu E, Wang N, Hoerster G, Hastings C, Cho MJ, Scelonge C, Lenderts B, Chamberlin M, Cushatt J, Wang L, Ryan L, Khan T, Chow-Yiu J, Hua W, Yu M, Banh J, Bao Z, Brink K, Igo E, Rudrappa B, Shamseer PM, Bruce W, Newman L, Shen B, Zheng P, Bidney D, Falco C, Register J, Zhao ZY, Xu D, Jones T, Gordon-Kamm W (2016) Morphogenic regulators *Baby boom* and *Wuschel* improve monocot transformation. Plant Cell 28:1998–2015

Lowe K, Rota ML, Hoerster G, Hastings C, Wang N, Chamberlin M, Wu E, Jones T, Gordon-Kamm W (2018) Rapid genotype "independent" *Zea mays* L. (maize) transformation via direct somatic embryogenesis. Vitro Cell Dev Biol-Plant 54:240–252

Luerûen H, Kirik V, Herrmann P, Miséra S (1998) *FUSCA3* encodes a protein with a conserved VP1/ABI3-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. Plant J 15:755–764

Ma Y, Miotk A, Sutikovic Z, Medzihradszky A, Wenzl C, Ermakova O, Gaillochet C, Forner J, Utan G, Brackmann K, Galvan-Ampudia CS, Vernoux T, Greb T, Lohmann JU (2019) *WUSCHEL* acts as a rheostat on the auxin pathway to maintain apical stem cells in *Arabidopsis*. Biorxiv. https://doi.org/10.1101/468421**(in press)**

Mayer KF, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T (1998) Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell 95:805–815

Meng WJ, Cheng ZJ, Sang YL, Zhang MM, Rong XF, Wang ZW, Tang YY, Zhang XS (2017) Type-B ARABIDOPSIS RESPONSE REGULATORs specify the shoot stem cell niche by dual regulation of *WUSCHEL*. Plant Cell 9:1357–1372

Meng Y, Liu H, Wang H, Liu Y, Zhu B, Wang Z, Hou Y, Zhang P, Wen J, Yang H, Mysore KS, Chen J, Tadege M, Niu L, Lin H (2019) *HEADLESS*, a *WUSCHEL* homolog, uncovers novel aspects of shoot meristem regulation and leaf blade development in *Medicago truncatula*. J Exp Bot 70:149–163

Minh-Thu P, Kim JS, Chae S, Jun KM, Lee GS, Kim DE, Cheong JJ, Song SI, Nahm BH, Kim YK (2018) A *WUSCHEL* homeobox transcription factor, *OsWOX13*, enhances drought tolerance and triggers early flowering in rice. Mol Cells 41:781–798

Mookan M, Nelson-Vasilchik K, Hague J, Zhang ZJ, Kausch AP (2017) Selectable marker independent transformation of recalcitrant maize inbred B73 and sorghum P898012 mediated by morphogenic regulators *BABY BOOM* and *WUSCHEL2*. Plant Cell Rep 36:1477–1491

Muller B, Sheen J (2007) Advances in cytokinin signaling. Science 318:68–69

Muller B, Sheen J (2008) Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. Nature 453:1094–1097

Negin B, Shemer O, Sorek Y, Williams LE (2017) Shoot stem cell specification in roots by the *WUSCHEL* transcription factor. PLoS ONE 12:e0176093

Newell CA (2000) Plant transformation technology: Developments and applications. Mol Biotechnol 16:53–66

Ninan AS, Shah A, Song J, Jameson PE (2017) Differential gene expression in the meristem and during early fruit growth of *Pisum sativum* L. identifies potential targets for breeding. Int J Mol Sci 18:428

Ohmori Y, Tanaka W, Kojima M, Sakakibara H, Hirano HY (2013) *WUSCHEL-RELATED HOMEOBOX4* is involved in meristem maintenance and is negatively regulated by the CLE gene *FCP1* in rice. Plant Cell 25:229–241

Orlowska A, Kepczynska E (2018) Identification of polycomb repressive Complex1, trithorax group genes and their simultaneous expression with *WUSCHEL*, *WUSCHEL-related Homeobox5* and *SHOOT MERISTEMLESS* during the induction phase of somatic embryogenesis in *Medicago truncatula* Gaertn. Plant Cell Tissue Organ Cult 134:345–356

Palovaara J, Hallberg H, Stasolla C, Hakman I (2010) Comparative expression pattern analysis of *WUSCHEL-related homeobox 2* (*WOX2*) and *WOX8/9* in developing seeds and somatic embryos of the gymnosperm *Picea abies*. New Phytol 188:122–135

Reddy GV (2008) Live-imaging stem-cell homeostasis in the *Arabidopsis* shoot apex. Curr Opin Plant Biol 11:88–93

Reinert J (1958) Morphogenese und ihre kontrolle an Gewebekulturen avs karotten. Naturwissen Schaften 45:344–345

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JA (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Science 283:1541–1544

Rupps A, Raschke J, Rümmler M, Linke B, Zoglauer K (2016) Identification of putative homologs of *Larix decidua* to *BABY BOOM*

(BBM), *LEAFY COTYLEDON1* (*LEC1*), *WUSCHEL-related HOMEOBOX2* (*WOX2*) and *SOMATIC EMBRYOGENESIS RECEPTOR-like KINASE* (*SERK*) during somatic embryogenesis. Planta 243:473–488

Santa-Catarina C, de Oliveira RR, Cutri L, Floh EI, Dornelas MC (2012) *WUSCHEL*-related genes are expressed during somatic embryogenesis of the basal angiosperm *Ocotea catharinensis* Mez. (Lauraceae). Trees 26:493–501

Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laux T (2007) Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. Nature 446:811–814

Schmidt ED, Guzzo F, Toonen MA, de Vries SC (1997) A leucine rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. Development 124:2049–2062

Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T (2000) The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell 100:635–644

Schrader S, Kaldenhoff R, Richter G (1997) Expression of novel genes during somatic embryogenesis of suspension-cultured carrot cells (*Daucus carota*). J Plant Physiol 50:63–68

Sharma VK, Fletcher JC (2002) Maintenance of shoot and floral meristem cell proliferation and fate. Plant Physiol 129:31–39

Shiota H, Satoh R, Watabe K, Harada H, Kamada H (1998) *C-AB13*, the carrot homologue of the *Arabidopsis AB13*, is expressed during both zygotic and somatic embryogenesis and functions in the regulation of embryo-specific ABA-inducible genes. Plant Cell Physiol 39:1184–1193

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 11:118–131

Solís-Ramos LY, Estrada T, Nahuath-Dzib S, Zapata-Rodriguez LC, Castano E (2009) Overexpression of *WUSCHEL* in *C. chinense* causes ectopic morphogenesis. Plant Cell Tissue Organ Cult 96:279–287

Somssich M, Je BI, Simon R, Jackson D (2016) *CLAVATA-WUSCHEL* signaling in the shoot meristem. Development 143:3238–3248

Songstad DD, Petolino JF, Voytas DF, Reichert NA (2017) Genome editing of plants. Cri Rev Plant Sci 36:1–23

Steward FC, Mapes MO, Mears K (1958) Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. Amer J Bot 45:705–708

Su YH, Zhang XS (2014) The hormonal control of regeneration in plants. Curr Top Dev Biol 108:35–69

Su YH, Zhao XY, Liu YB, Zhang CL, O'Neill SD, Zhang XS (2009) Auxin-induced *WUS* expression is essential for embryonic stem cell renewal during somatic embryogenesis in *Arabidopsis*. Plant J 59:448–460

Sultana M, Gangopadhyay G (2018) Early expression of *WUSCHEL* is a marker for in vitro shoot morphogenesis in tobacco and *Beta palonga*. Plant Cell Tissue Organ Cult 134:277–288

Sun B, Zhou Y, Cai J, Shang E, Yamaguchi N, Xiao J, Looi LS, Wee WY, Gao X, Wagner D, Ito T (2019) Integration of transcriptional repression and polycomb-mediated silencing of *WUSCHEL* in floral meristems. Plant Cell 31:1488–1505

Tadege M, Lin H, Bedair M, Berbel A, Wen J, Rojas CM, Niu L, Tang Y, Sumner L, Ratet P, McHale NA, Madueño F, Mysore KS (2011) *STENOFOLIA* regulates blade outgrowth and leaf vascular patterning in *Medicago truncatula* and *Nicotiana sylvestris*. Plant Cell 23:2125–2142

To JPC, Kieber JJ (2008) Cytokinin signaling: two-components and more. Trends Plant Sci 13:85–92

To JPC, Haberer G, Ferreira FJ, Deruere J, Mason MG, Schaller GE, Alonso JM, Ecker JR, Kiebera JJ (2004) Type-A *Arabidopsis* Response Regulators are partially redundant negative regulators of cytokinin signaling. Plant Cell 16:658–671

To JPC, Deruere J, Maxwell BB, Morris VF, Hutchison CE, Ferreira FJ, Schaller GE, Kiebera JJ (2007) Cytokinin regulates type-A *Arabidopsis* response regulator activity and protein stability via two-component phosphorelay. Plant Cell 19:3901–3914

Tsuwamoto R, Yokoi S, Takahata Y (2010) *Arabidopsis EMBRYOMAKER* encoding an AP2 domain transcription factor plays a key role in developmental change from vegetative to embryonic phase. Plant Mol Biol 73:481–492

Tvorogova VE, Lebedeva MA, Lutova LA (2015) Expression of *WOX* and *PIN* genes during somatic and zygotic embryogenesis in *Medicago truncatula*. Russ J Genet 51:1189–1198

Tvorogova VE, Fedorova YA, Potsenkovskaya EA, Kudriashov AA, Efremova EP, Kvitkovskaya VA, Wolabu TW, Zhang F, Tadege M, Lutova LA (2019) The *WUSCHEL*-related homeobox transcription factor *MtWOX9-1* stimulates somatic embryogenesis in *Medicago truncatula*. Plant Cell Tissue Organ Cult 138:517–527

Ueda M, Zhang Z, Laux T (2011) Transcriptional activation of *Arabidopsis* axis patterning genes *WOX8/9* links zygote polarity to embryo development. Dev Cell 20:264–270

Wagner D (2009) Flower morphogenesis: timing is key. Dev Cell 16:621–622

Wang J, Tian C, Zhang C, Shi B, Cao X, Zhang TQ, Zhao Z, Wang JW, Jiao Y (2017) Cytokinin signaling activates *WUSCHEL* expression during axillary meristem initiation. Plant Cell 29:1373–1387

Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H, Schmülling T (2003) Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant Cell 15:2532–2550

Wong CE, Khor SY, Bhalla PL, Singh MB (2011) Novel spatial expression of soybean *WUSCHEL* in the incipient floral primordial. Planta 233:553–560

Wu X, Chory J, Weigel D (2007) Combinations of *WOX* activities regulate tissue proliferation during *Arabidopsis* embryonic development. Dev Biol 309:306–316

Xiang W, Xin-guo W, Jiang-ping R, Ying MA, Jun YIN (2012) Characterization of tomato transcription factor *WUSCHEL* and functional study in *Arabidopsis*. J Integ Agr 11:1257–1265

Xie M, Chen H, Huang L, O'Neil RC, Shokhirev MN, Ecker JR (2018) A B-ARR-mediated cytokinin transcriptional network directs hormone cross-regulation and shoot development. Nat Commun 9:1604

Xu YY, Wang XM, Li J, Li JH, Wu JS, Walker JC, Xu ZH, Chong K (2005) Activation of the *WUS* gene induces ectopic initiation of floral meristems on mature stem surface in *Arabidopsis thaliana*. Plant Mol Biol 58:915–915

Yadav RK, Perales M, Gruel J, Girke T, Jonsson H, Reddy GV (2011) *WUSCHEL* protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex. Genes Dev 25:2025–2030

Yang Y, Sun M, Yuan C, Han Y, Zheng T, Cheng T, Wang J, Zhang Q (2019) Interactions between *WUSCHEL*- and *CYC2-like* transcription factors in regulating the development of reproductive organs in *Chrysanthemum morifolium*. Int J Mol Sci 20:1276

Yasui Y, Ohmori Y, Takebayashi Y, Sakakibara H, Hirano HY (2018) *WUSCHEL-RELATED HOMEOBOX4* acts as a key regulator in early leaf development in rice. PLoS Genet 14:e1007365

Zhai L, Xu L, Wang Y, Zhu X, Feng H, Li C, Luo X, Everlyne MM, Liu L (2016) Transcriptional identification and characterization of differentially expressed genes associated with embryogenesis in radish (*Raphanus sativus* L.). Sci Rep 6:21652

Zhang D, Wang X, Wang M, Li J, Guo X, Chong K, Xu Y (2013) Ectopic xepression of *WUS* in hypocotyl promotes cell division via *GRP23* in *Arabidopsis*. PLoS ONE 8:e75773

Zhang F, Wang Y, Li G, Tang Y, Kramer EM, Tadege M (2014) *STENOFOLIA* recruits *TOPLESS* to repress *ASYMMETRIC LEAVES2* at the leaf margin and promote leaf blade outgrowth in *Medicago truncatula*. Plant Cell 26:650–664

Zhang F, May A, Irish VF (2017a) Type-B ARABIDOPSIS RESPONSE REGULATORs directly activate *WUSCHEL*. Trends Plant Sci 22:815–817

Zhang TQ, Lian H, Zhou CM, Xu L, Jiao Y, Wang JW (2017b) A two-step model for de novo activation of *WUSCHEL* during plant shoot regeneration. Plant Cell 29:1073–1087

Zhang Z, Tucker E, Hermann M, Laux T (2017c) A molecular framework for the embryonic initiation of shoot meristem stem cells. Dev Cell 40:264–277

Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, Lohmann JU (2010) Hormonal control of the shoot stem-cell niche. Nature 465:1089–1092

Zheng W, Zhang X, Yang Z, Wu J, Li F, Duan L, Liu C, Lu L, Zhang C, Li F (2014) *AtWuschel* promotes formation of the embryogenic callus in *Gossypium hirsutum*. PLoS ONE 9:e87502

Zhou Y, Liu X, Engstrom EM, Nimchuk ZL, Pruneda-Paz JL, Tarr PT, Yan A, Kay SA, Meyerowitz EM (2015) Control of plant stem cell function by conserved interacting transcriptional regulators. Nature 517:377–380

Zhou X, Guo Y, Zhao P, Sun M (2018) Comparative analysis of *WUSCHEL*-related homeobox genes revealed their parent-of-origin and cell type-specific expression pattern during early embryogenesis in tobacco. Front Plant Sci 9:311

Zhu T, Moschou PN, Alvarez JM, Sohlberg JJ, Von Arnold S (2014) *WUSCHEL-RELATED HOMEOBOX 8/9* is important for proper embryo patterning in the gymnosperm Norway spruce. J Exp Bot 65:6543–6552

Zubo YO, Blakley IC, Yamburenko MV, Worthen JM, Street IH, Franco-Zorrilla JM, Zhang W, Hill K, Raines T, Solano R, Kieber JJ, Loraine AE, Schaller GE (2017) Cytokinin induces genome-wide binding of the type-B response regulator ARR10 to regulate growth and development in *Arabidopsis*. Proc Natl Acad Sci USA 114:5995–6004

Zuo J, Niu QW, Frugis G, Chua NH (2002) The *WUSCHEL* gene promotes vegetative-to-embryonic transition in *Arabidopsis*. Plant J 30:349–359