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REVIEW: PART OF A SPECIAL ISSUE ON FLOWER DEVELOPMENT

The role of *WOX* genes in flower development

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• **Background** *WOX* (*Wuschel-like homeobOX*) genes form a family of plant-specific HOMEODOMAIN transcription factors, the members of which play important developmental roles in a diverse range of processes. *WOX* genes were first identified as determining cell fate during embryo development, as well as playing important roles in maintaining stem cell niches in the plant. In recent years, new roles have been identified in plant architecture and organ development, particularly at the flower level.
• **Scope** In this review, the role of *WOX* genes in flower development and flower architecture is highlighted, as evidenced from data obtained in the last few years. The roles played by *WOX* genes in different species and different flower organs are compared, and differential functional recruitment of *WOX* genes during flower evolution is considered.
• **Conclusions** This review compares available data concerning the role of *WOX* genes in flower and organ architecture among different species of angiosperms, including representatives of monocots and eudicots (rosids and asterids). These comparative data highlight the usefulness of the *WOX* gene family for evo–devo studies of floral development.

Key words: *WOX* genes, HOMEOBOX, flower development, WUSCHEL, PRS/WOX3, MAW/WOX1, EVERGREEN/WOX9, *Petunia* × *hybrida*, *Arabidopsis thaliana*, dicots, monocots, plant evo–devo.

WOX GENES ARE HOMEOBOX GENES

The >250 000 wild species of flowering plants display an incredible diversity of flower shapes (Krizek and Fletcher, 2005), whose architectural traits (such as fused versus free-standing petals and large versus narrow petals) can be very different from one species to another. Despite the fact that the genetic basis of organ identity in the flower is well understood nowadays, thanks to the development of the ABCE model of flower development, mainly based on the *MADS BOX* gene family (Bowman *et al*., 2012; Heijmans *et al*., 2012; Smaczniak *et al*., 2012), little is known about organ shape and the general morphology of the flower, for which a general model is still lacking. Interestingly, whereas *MADS BOX* genes are involved in organ identity at the flower level in plants, organ identity in animals is based on a completely different class of genes, the *HOMEOTIC BOX* (or *HOMEOBOX*) genes (Holland, 2013). First discovered in the fruit fly *Drosophila melanogaster* (Carroll, 1995; Castelli-Gair, 1998), *HOMEOBOX* genes derive their name from William Bateson's concept of homeosis, since mutations in these genes may lead to transformation of one part of the embryo into another during development (Robert, 2001). At the molecular level, HOMEOBOX proteins are characterized by the HOMEODOMAIN, composed of 60 amino acids on average and arranged in space with an *N*-terminal arm plus three α helixes able to bind DNA (Wolberger, 1996). At least 14 different classes of *HOMEOBOX* genes (where specific conserved domains, in addition to the shared HOMEODOMAIN, can be found) have been described in plants, from angiosperms to red algae, and many of them have been shown to play a role in plant development (Mukherjee *et al*., 2009).

Plant *HOMEOBOX* genes sharing sequence identity with the gene *WUSCHEL* (*At-WUS*) from arabidopsis are referred to as *WOX* ((*Wuschel-related homeobOX*) genes. *At-WUS* was identified as a central player in stem cell maintenance in the shoot apical meristem (SAM), although it is not required for SAM initiation (Laux *et al*., 1996; Liu *et al*., 2011). The name *wuschel* apparently derives from the bristled and bushy phenotype of the mutants, in which ectopic meristems are repetitively produced and prematurely terminated (Laux *et al*., 1996).

Since the discovery of *At-WUS*, several *WOX* genes have been characterized in different species. Other members of the *WOX* family are usually referred as '*WOX*' followed by an Arabic numeral (with a few exceptions), and can be grouped in different subfamilies or clades (van der Graaff *et al*., 2009; Vandenbussche *et al*., 2009).

WOX GENES PLAY DIFFERENT ROLES IN PLANT DEVELOPMENT

In arabidopsis, *WOX* genes have been shown to play a broad role in plant development, from stem cell maintenance at the meristem level (*WUSCHEL* in shoot meristem, *WOX4* in cambium, *WOX5* in root meristem) till embryo patterning (Laux *et al*., 1996; Haecker *et al*., 2004; Sarkar *et al*., 2007; Ji *et al*., 2010). We know from *Picea abies* that all the major *WOX* subfamilies, with the exception of the *MAW/WOX1* subfamily, probably

originated before the separation of angiosperms from gymnosperms (Hedman *et al.*, 2013). A relationship between *WOX* gene number and body pattern complexity among different species, all from the 'green lineage' (a grouping of land plants and green algae), has also been proposed. In fact, *WOX* genes can be divided into three different lineages that are supposed to reflect their ancestry: an ancient lineage (comprising *At-WOX10*, *At-WOX13*, *At-WOX14* and their homologues), an intermediate lineage (comprising *At-WOX8*, *9*, *11* and *12* and their homologues) and a new or 'WUS' lineage (comprising *AtWOX1–7*, including *WUSCHEL*, and their homologues) (Haecker *et al.*, 2004; Nardmann and Werr, 2012). Moreover, *WOX* genes from the WUS lineage are absent from green algae, bryophytes and ferns (with the exception of Leptosporangiatae). Further diversification, sub-functionalization and recruitment in different stem cell niches of these genes in angiosperms (but also gymnosperms), has been considered as contributing to the body plan diversity and evolutionary success of these groups (Nardmann and Werr, 2012). At the molecular level, the acquisition of repressive activity by proteins from the modern lineage, mainly due to an amino acid domain called the 'WUSCHEL box' (see red box on gene pictograms in Fig. 1), has been proposed to play a major role in this process (Lin *et al.*, 2013). In this review we will further focus on the implication of WOX gene function during floral development.

AT-WUSCHEL IS REQUIRED FOR STEM-CELL MAINTENANCE IN THE FLOWER (WUS SUBFAMILY)

WUSCHEL (*WUS*) is the founding member of the WOX family and is also representative of a clade, the 'WUS clade' (Fig. 1). *WUS* was initially isolated in arabidopsis (Laux *et al.* 1996) and its function has been thoroughly investigated in this model species.

WUS promotes the identity and maintenance of stem cells, a pool of undifferentiated and continuously dividing cells located in the central zone of both the SAM and the flower meristem (FM) (Laux *et al.*, 1996; Besnard *et al.*, 2011). Thus, on a *wus* genetic background, the SAM, instead of producing new organs throughout the life of the plant, stops functioning prematurely in an aberrant flat morphology. However, *wus* plants are still able to initiate a secondary meristem, but it fails to self-maintain, resulting in plants with a highly disorganized, bushy architecture. Similarly, *wus* flowers display many fewer stamens (usually one or two) and no carpels, consistent with precocious FM termination (Laux *et al.*, 1996). *WUS* is therefore necessary for meristem maintenance, but is not required for their initiation. Consistent with this function, *WUS* expression is restricted to a small domain, the organizing centre, located in the basal part of the central zone, beneath the L3 layer in the SAM and beneath the L2 layer in the FM (Mayer *et al.*, 1998). Mechanistically, it is known now that WUS acts non cell-autonomously to both promote stem cell identity and directly activate *CLAVATA3* (*CLV3*) expression within the central zone (Schoof *et al.*, 2000; Yadav *et al.*, 2011). In turn, the CLE peptide CLV3 diffuses outside of the central zone, binds to the CLV1 and CLV2/CORYNE receptor kinases and thus triggers the signalling pathway that eventually leads to the restriction of *WUS* expression within the organizing centre (Brand *et al.*, 2000; Schoof *et al.*, 2000; Lenhard and Laux 2003; Katsir *et al.*, 2011; Nimchuk *et al.*, 2011). Much evidence suggests that POLTERGEIST and POLTERGEIST LIKE1 are signalling intermediates between CLV3 perception and *WUS* regulation (Yu *et al.*, 2000; Song *et al.*, 2006). *WUS* is thus part of a negative genetic feedback loop that ensures the homeostasis of the meristem. Within this loop, it is interesting to note that WUS also directly represses *CLV1* expression (Busch *et al.*, 2010).

Identification of additional direct WUS targets, such as the A-type *Arabidopsis Response Regulator7* (*ARR7*), shed light on the way *WUS* specifies stem cell identity (Leibfried *et al.*, 2005). By repressing the expression of *ARR7*, WUS counteracts the inhibitory activity of ARR7 on cytokinin signalling in the centre of the SAM (To *et al.*, 2004; Leibfried *et al.*, 2005). WUS can therefore act both as an activator and a repressor of transcription (Ikeda *et al.*, 2009; Busch *et al.*, 2010), and the WUS box has been reported to be absolutely required for these two types of activity (Ikeda *et al.*, 2009). The role of WUS as a transcriptional repressor was further underscored by its interaction with two co-repressors, WSIP1/TOPLESS and WSIP2 (Kieffer *et al.*, 2006; Long *et al.*, 2006).

Under inductive conditions, the vegetative SAM can switch to an inflorescence SAM (iSAM or IM), which, instead of producing leaves on its flanks, generates FM. All the data gathered on WUS function cannot be generalized to the FM, as exemplified for instance with *TOPLESS RELATED1* and *2*, which are repressed by WUS in the SAM but activated in the FM (Busch *et al.*, 2010), further confirming the complex regulatory interaction reported earlier (Ikeda *et al.*, 2009). However, the majority of data are common to both meristems. This is especially true for the stem cell maintenance process and the WUS/CLV negative feedback loop, with some minor differences, such as reduced sensitivity to changes in CLV signalling in the FM compared with the SAM (Laux *et al.*, 1996; Clark *et al.*, 1997; Mayer *et al.*, 1998; Schoof *et al.*, 2000; Müller *et al.*, 2006; Yadav *et al.*, 2011). In arabidopsis, the WUS/CLV loop is absent in incipient floral primordia but it is rapidly set up, with activation of *WUS* expression at stage 1, followed by that of *CLV3* at stage 2–2½. However, and contrary to what happens in the SAM, stem cell maintenance is only transient in the FM (Prunet *et al.*, 2009). Indeed, once all floral organs have been initiated, activity of the FM stops and the flower becomes determinate. The mechanism controlling FM termination has been described mainly in arabidopsis. It has been shown to rely on a second genetic feedback loop that implies *WUS* and *AGAMOUS* (*AG*) (Lenhard *et al.*, 2001; Lohmann *et al.*, 2001). *AG* encodes a C-class MADS box protein that also controls the identity of stamens and carpels, the male and female reproductive organs, respectively (Yanofsky *et al.*, 1990; Bowman *et al.*, 1991). The feedback loop starts with the activation of *AG* transcription, at stage 3, by WUS together with LEAFY (LFY), which acts in a partially redundant way in this process (Yanofsky *et al.*, 1990; Lohmann *et al.*, 2001), and ends with the repression of *WUS* in the centre of the FM, at stage 6, concomitantly with or immediately after carpel initiation. This second part of the loop absolutely requires AG, making AG the main developmental switch to FM termination. Thus, on an *ag* genetic background, flowers are indeterminate and keep producing floral organs in their centre, and this phenotype coincides with the maintenance of *WUS* expression within the FM organizing centre (Bowman *et al.*, 1991; Lenhard *et al.*, 2001; Lohmann *et al.*, 2001). FM

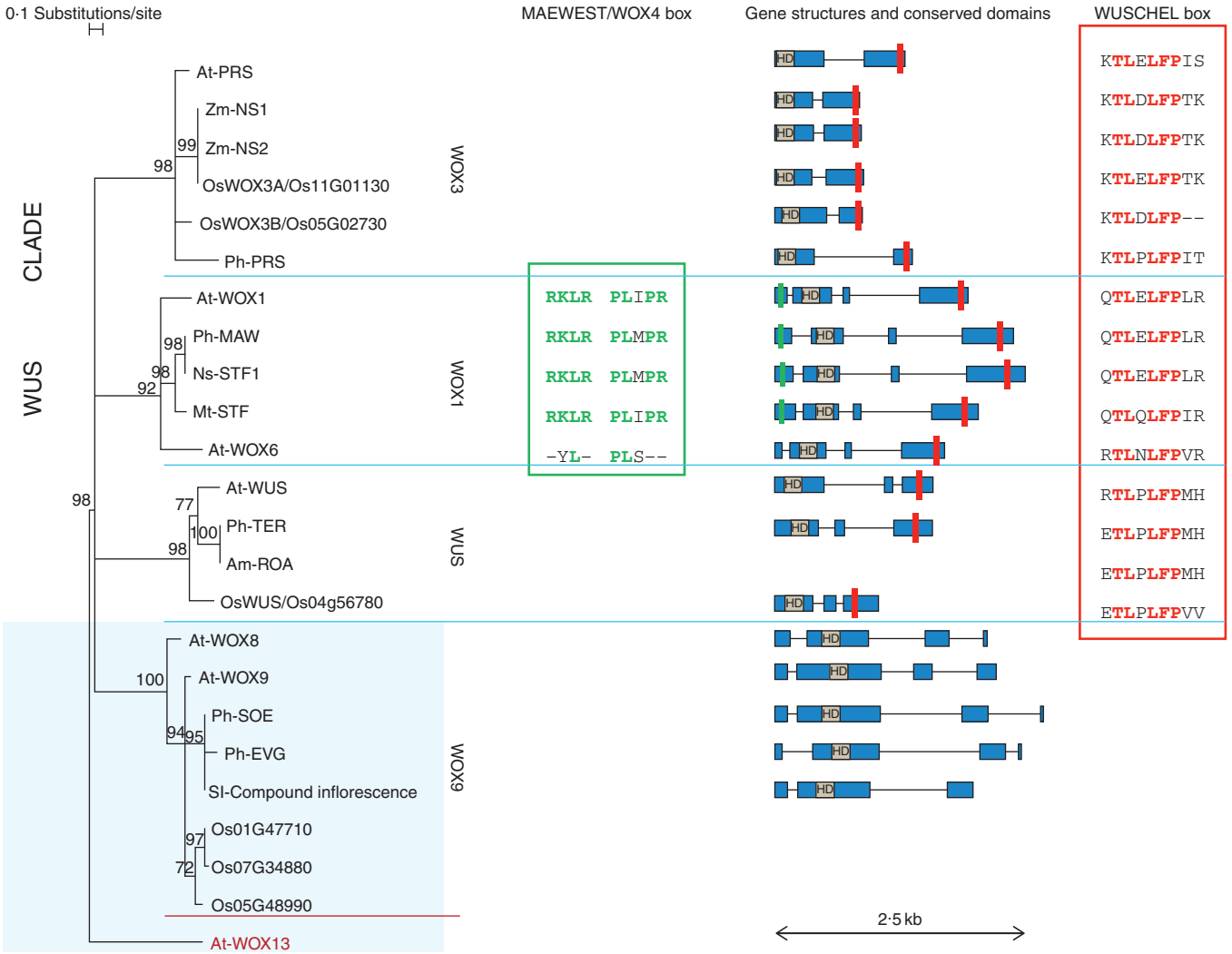

FIG. 1. Phylogenetic tree of WOX sequences. WOX sequences from different species (Am, *Antirrhinum majus*; At, *Arabidopsis thaliana*; Mt, *Medicago truncatula*; Ns, *Nicotiana sylvestris*, Ph, *Petunia* × *hybrida*; Os, *Oryza sativa*; Sl, *Solanum lycopersicum*; Zm, *Zea mays*) are clustered into different subfamilies (WOX3, WOX1, WUS, WOX9) after phylogenetic analysis. The phylogenetic tree is based on the HOMEODOMAIN sequences. The WOX13 sequence (dark red) from arabidopsis is used as outgroup. To support WOX relationships, 1000 bootstrap samples were obtained using the software TREECON (Van de Peer and De Wachter, 1994). Bootstrap values <65 % are not shown and corresponding branches are displayed as unresolved. On the right of the tree, gene structure is displayed for most of the *WOX* gene sequences (solid blue bars are used for exons and thin black lines for introns), also depicting conserved amino acidic boxes (HD, HOMEODOMAIN; red rectangle, WUSCHEL box; green rectangle, MAEWEST/WOX4 box, as in Vandenbussche *et al*., 2009). In addition, conserved amino acid residues are displayed for the MAEWEST/WOX4 box and the WUSCHEL box. Note that WOX sequences also display characteristic C-terminal motifs with functional properties (not shown), as illustrated in Vandenbussche *et al*. (2009). Where not specified, accession numbers are the same as in Vandenbussche *et al*. (2009); Mt-STF (AEL30892.1), Nt-STF1 (AEL30893.1) and Sl-Compound Inflorescence (NP_001234072.1) are from GenBank.

termination is therefore closely linked to initiation of carpel development. However, these two processes are not coincident and are uncoupled, although both are controlled by *AG* (Mizukami and Ma, 1995; Ji *et al*., 2011). Very interestingly, the fact that organizing centre cells retain a molecular identity distinguishable from that of surrounding cells even after the cessation of *WUS* expression further confirms the separation of the two processes but also demonstrates that organizing centre cells persist after FM termination and are not incorporated into carpels (Liu *et al*., 2011). Recently, two different mechanisms of repression of *WUS* by AG have been reported. They both explain why AG does not repress *WUS* expression from stage 3. In the first mechanism, AG represses *WUS* expression indirectly by activating *KNUCKLES* (*KNU*, a C2H2-type zinc finger transcription factor) expression, which in turn represses *WUS* expression directly or indirectly (Sun *et al*., 2009). In this model, *KNU* expression is blocked by repressive marks that are removed in an AG-dependent manner at stage 6. In the second mechanism, AG also directly represses *WUS* expression by recruiting polycomb group proteins to *WUS* (Liu *et al*., 2011). In this model, AG recruits polycomb group proteins to *WUS* earlier than in the first model, at stage 5. These two mechanisms are probably coordinated and act in parallel to each other in terminating floral stem cell maintenance.

From this detailed analysis in arabidopsis, it is clear now that to make a flower with a fixed number of floral organs it is of crucial

importance that *WUSCHEL* is switched off at very precise moments during development of the floral bud. Not all flowering species have such a rigidly controlled floral organ number within their flowers, which seems to be a character acquired later in angiosperm evolution. It would be interesting to investigate whether changes in the *WUS* regulatory network have occurred during evolution that might have led to increased robustness of the system, resulting in fully determined flower architectures. Likewise, one can question whether floral meristem termination occurs at the same moment in species with different placentation topologies (Colombo *et al*., 2008). For example, in *Petunia* and rice, which belong to the central placentation types, the floral meristem remains active after carpel primordia have been produced, because the placenta (and later on the ovules) develops directly from the floral meristem centre between the carpels. By contrast, in parietal placentation types such as arabidopsis, the placenta and ovules differentiate from the medial regions of the carpels after the FM has terminated. Interestingly, in both rice and *Petunia*, it has been shown that both D- and C-clade *AG*-like MADS box proteins participate in floral meristem termination (Dreni *et al*., 2011; Heijmans *et al*., 2012), with the D-lineage proteins being strongly expressed during placenta development, while the D-clade gene *STK* in arabidopsis does not seem to be involved at all in determinacy control (Pinyopich *et al*., 2003). This regulatory difference might be a direct consequence of different placentation topologies.

Besides arabidopsis, loss of function mutants for *WUS* orthologues have been described so far only in *Petunia* (*terminator*) and snapdragon (*rosulata*) (Stuurman *et al*., 2002; Kieffer *et al*., 2006), confirming their role in maintenance of the SAM. Unfortunately, *ter* and *roa* mutants never develop flowering branches, and the roles of *TER* and *ROA* in floral meristem control have therefore not yet been analysed. Expression studies of *WUS* orthologues are available for a wider range of species. Perhaps the most remarkable findings have been presented by Nardmann and Werr (2006), who showed in grasses that none of the isolated *WUS* orthologues exhibited an organizing centre-type expression pattern in the vegetative SAM, as in arabidopsis. Instead, it has been shown that the *WOX4* orthologue in rice, *Os-WOX4*, is involved in SAM maintenance, along with cytokinins (Ohmori *et al*., 2013). Moreover, in rice, mutant plants for the *LONELY GUY* gene, which codes for a cytokinin-activating enzyme, are also affected at the SAM and the inflorescence and floral meristems (Kurakawa *et al*., 2007). Taken together, these facts suggest major differences in *WUS* function in grass species compared with dicots.

THE PRS/WOX3 SUBFAMILY

The second *WOX* gene that was found to play a role in flower development is *Arabidopsis PRESSED FLOWER* (*At-PRS*, also called *WOX3*). Mutants for this gene have flowers with a flattened appearance (hence the name) because lateral sepal development is affected: they are usually smaller, sometimes with a filamentous appearance, or can be completely absent (Matsumoto and Okada, 2001). Although the size of the abaxial and adaxial sepals is normal, marginal regions show defects. *At-PRS* was shown to act independently of organ identity and meristem size. The expression of *At-PRS* was detected at the lateral regions of all lateral organs at very early stages, including leaves, flower primordia and floral organ primordia, despite the fact that phenotypic defects were much more restricted. Because of its expression pattern and mutant phenotype, *At-PRS* was proposed to regulate the lateral axis-dependent development of arabidopsis flowers (Matsumoto and Okada, 2001). Later, it was reported that arabidopsis *prs* mutants also lacked lateral stamens, and were additionally affected at the leaf level because of the absence of stipules at the leaf base (Nardmann *et al*., 2004).

Initially, floral mutant phenotypes had not been described for *PRS/WOX3* homologues in species other than arabidopsis. Instead, it was shown that the *NARROW SHEATH 1* and *2* genes in maize are *PRS/WOX3* homologues (Nardmann *et al*., 2004) and that they perform a crucial role in leaf margin development, with the *ns1 ns2* double mutant displaying a severely reduced leaf blade (Scanlon *et al*., 1996, 2000; Scanlon, 2000). A very similar leaf phenotype was found in *nal2 nal3* double mutants in rice, with *NAL2* and *NAL3* (*OsWOX3A*) being homologous to the maize *NS1* and *NS2* genes (Cho *et al*., 2013; Ishiwata *et al*., 2013). Interestingly, the widths of the lemma and palea were also significantly reduced in *nal2 nal3* mutants (Cho *et al*., 2013). Since the lemma and palea are considered to be equivalent to eudicot sepals, this indicates that the function of PRS/WOX3 proteins during floral development is conserved between monocots and dicots. Rice contains a third *WOX3* copy, called *OsWOX3B*/*DEP*, but this functions in the regulation of trichome formation in leaves and glumes (Angeles-Shim *et al*., 2012). It therefore seems that the *PRS/WOX3* subfamily in rice has further functionally diverged.

THE MAW/WOX1 SUBFAMILY

The evolutionary invention of petals, the usually brightly coloured organs of the flower, is generally believed to have played a major role in the evolution of pollination syndromes. In many taxa throughout the angiosperms, the petals fuse partly or completely to form a tubular structure, thereby creating a protective barrier enclosing the reproductive organs and nectaries in the centre of the flower. The *maewest* (*maw*) mutant in *Petunia* was isolated in a genetic screen for mutants with defects in petal fusion (Vandenbussche *et al*., 2009). Morphological analysis of *maw* flowers showed that petal fusion defects were mainly due to reduced lateral outgrowth of the initially separate petal primordia, which subsequently fail to fuse properly. Similar defects were found in carpels, resulting in partly unfused carpels, and sepals were narrower than wild-type. In addition, leaf blade outgrowth was considerably reduced along the lateral axis, as observed in floral organs, indicating that *MAW* plays a general role in the lateral outgrowth of organs. *MAW* was shown to encode a member of the *WOX1* subfamily of WOX transcription factors (Vandenbussche *et al*., 2009). Similar phenotypes in leaf and flower development were found for mutants of *MAW/WOX1* homologues in *Medicago truncatula* and *Nicotiana sylvestris* (McHale and Marcotrigiano, 1998; Lin *et al*., 2013; Tadege *et al*., 2011*a*). In addition, mutants for *MAW/WOX1* homologues in two other species, *narrow organs1* in *Lotus japonicus* and *lathyroides* in *Pisum sativum* (garden pea), have also been shown to be affected in lateral outgrowth of organs such as leaves and petals (Zhuang *et al*., 2012), further showing a broadly conserved role for *MAW/WOX1*

genes among different dicot species. In contrast, the dramatic *maw*/*wox1* phenotypes found in *Petunia*, *Medicago*, *Nicotiana*, *Lotus* and pea are absent in arabidopsis *wox1*, *wox6*, and *wox1 wox6* double mutants, showing that *WOX1* function is redundant (Vandenbussche *et al*., 2009), and that other factors can compensate for the loss of *WOX1/6* function in arabidopsis.

FUNCTIONAL OVERLAP BETWEEN *MAW*/*WOX1* AND *PRS*/*WOX3* SUBFAMILIES

Because mutants of members of both the *PRS*/*WOX3* and *MAW*/*WOX1* subfamilies in arabidopsis display a much less severe or no phenotypic difference compared with homologous mutants in other species (see the two previous paragraphs), and because *PRS* and *WOX1* overlap in expression pattern, it was hypothesized that arabidopsis *WOX1* and *PRS* genes might overlap in function. This was indeed confirmed by the phenotype of *wox1 prs* double mutants, consistent with their overlapping expression domains at the adaxial–abaxial boundary layer and at the organ margins (Vandenbussche *et al*., 2009; Nakata *et al*., 2012). In contrast to *prs* single-mutant flowers, all sepals (not only the lateral ones) in *prs wox1* flowers displayed reduced blade outgrowth, as was the case also for the petals. This phenotype was also found in leaf development, with *wox1 prs* leaves displaying obvious defects in blade outgrowth, while *prs* mutants were only lacking stipules. These results clearly indicate that, despite the fact that the *PRS*/*WOX3* and *MAW*/*WOX1* subfamilies are structurally different (Fig. 1), their proteins share a common function in organ development along the lateral axis. However, note that the carpel fusion defects found in *Petunia*, *Nicotiana* and *Medicago wox1* mutants (Vandenbussche *et al*., 2009; Tadege *et al*., 2011*a*) were not observed in *wox1 prs* mutants (Vandenbussche *et al*., 2009). So far, functional data for both the *PRS*/*WOX3* and the *MAW*/*WOX1* subfamily are only available in arabidopsis, and it will be interesting to investigate whether this functional overlap also exists in species in which *maw*/*wox1* single mutants do display a strong phenotype on their own. Along the same lines, loss of *wox3*/*prs* function in monocots results in severe leaf blade reduction, but, remarkably, grasses (including wheat, maize, rice and *Brachypodium*) do not have *WOX1* representatives (Nardmann and Werr, 2006; Nardmann *et al*., 2007; Vandenbussche *et al*., 2009), while all other WOX subfamilies are represented in their genomes. It would be very interesting to investigate whether the absence of the *WOX1* subfamily in grasses has developmental implications related to differences in leaf development between monocots and dicots.

In arabidopsis, *At-WOX1* and *At-PRS* have recently been proposed to define a so-called middle domain in leaf development, different from the classical adaxial and abaxial sides of the leaf, and able to drive blade outgrowth (Nakata *et al*., 2012). Furthermore, in this model *At-WOX1* and *At-WOX3* would be at the spatial and regulatory interface of adaxial (*HD-ZIPIIIs*, *ASYMMETRIC LEAVES1* and *2*)-, abaxial (*KANADIs*, *ARFs*)- or middle–abaxial (*FILAMENTOUS FLOWER*)-specifying genes (Nakata and Okada, 2012; Tsukaya, 2013). This may also imply the role of several hormones. For instance, *ASYMMETRIC LEAVES1* and *2* regulate the expression of *ARF3* (in both a direct and an indirect way) (Iwasaki *et al*., 2013), which probably controls the cytokinin biosynthetic pathway in its turn (Takahashi *et al*., 2013). At the same time, *KANADI1* is linked to plant hormone pathways and leaf morphology, usually in a way antagonistic to *HD-ZIPIII* genes (Reinhart *et al*., 2013), such as the auxin pathway (Huang *et al*., 2014), but probably also the cytokinin pathway, by binding to the *ASYMMETRIC LEAVES2* promoter (Merelo *et al*., 2013). On the other hand, a study of *stenofolia* (*stf*) mutants in *Medicago* and *Nicotiana* proposes a role in modulating phytohormone homeostasis and sugar metabolism, in this way playing a role in leaf development (Tadege *et al*., 2011*a*, *b*). Moreover, a role in cell proliferation along the adaxial–abaxial boundary has been shown for *STF* (Tadege *et al*., 2011), *WOX1* and *PRS* (Nakata *et al*., 2012), and a recent paper describes the interaction between *STF* and *ASYMMETRIC LEAVES2* with *TOPLESS* along the leaf margin (Zhang *et al*., 2014).

EVERGREEN IN *PETUNIA* IS INVOLVED IN INFLORESCENCE ARCHITECTURE (*WOX9* SUBFAMILY)

The *WOX9* subfamily is represented by two genes in both arabidopsis and *Petunia* (Fig. 2). The arabidopsis representatives are *STIMPY* (*STIP*, *WOX9*) and *STIMPY-LIKE* (*STPL*, *WOX8*) (Haecker *et al*., 2004; Wu *et al*., 2005), and the *Petunia* representatives are *EVERGREEN* (*EVG*) and *SISTER OF EVERGREEN* (*SOE*) (Rebocho *et al*., 2008). *EVG* in *Petunia* and *COMPOUND INFLORESCENCE* in tomato are essential for inflorescence development and architecture (Lippman *et al*., 2008; Rebocho *et al*., 2008). On an *evg* background, floral identity is not specified and apical floral meristems develop as inflorescence shoots instead (Fig. 3). Moreover, *evg* mutations display defects in the physical separation of the apical and lateral meristem, resulting in the formation of a fasciated meristem. *Petunia* displays a cymose inflorescence in which the apical meristem terminates by forming an FM and growth continues from the lateral or 'sympodial' meristem, which will generate a subsequent sympodial meristem before terminating in a flower. In *Petunia*, FM identity is mainly specified by *ABERRANT LEAF AND FLOWER* (*ALF*) and *DOUBLE TOP* (*DOT*), which are the homologues of *LEAFY* and *UNUSUAL FLORAL ORGANS*, respectively (Souer *et al*. 1998, 2008). Unexpectedly, *EVG* is not expressed in the apical floral meristem but in the sympodial incipient meristem (Rebocho *et al*., 2008). Mechanistically, the model assumes that *EVG* counteracts the effect of an unknown mobile factor that inhibits *DOT* expression in the FM, possibly indirectly by promoting proliferation of the lateral IM and separation from the apical FM.

In contrast, *WOX8*/*STPL* and *WOX9*/*STIP* in arabidopsis are required for embryo patterning and vegetative SAM maintenance but not for inflorescence development and architecture. *WOX8*/*STPL* and *WOX9*/*STIP* are expressed during the early stages of embryo development with overlapping and specific expression domains (Haecker *et al*., 2004). Briefly, only *WOX8*/*STPL* is expressed in the egg cell and zygote, whereas *WOX8*/*STPL* and *WOX9*/*STIP* are both expressed after the division of the zygote. However, their expression is restricted to the basal daughter cell, which will form the suspensor and the hypophysis (Haecker *et al*., 2004). Consistent with this expression pattern, weak *wox9*/*stip* alleles display fewer cells in the basal part of

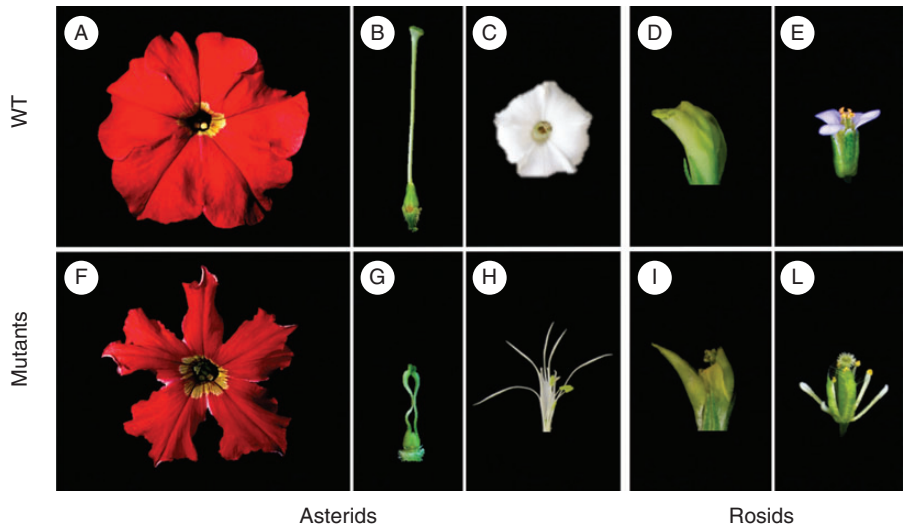

FIG. 2. Wox mutant flower phenotypes in petunia, *Nicotiana*, *Medicago* and arabidopsis (Vandenbussche *et al.*, 2009; Tadege *et al.*, 2011*b*; Lin *et al.*, 2013). (A, C, D, E) Wild-type (WT) flower phenotypes for *Petunia* × *hybrida*, *Nicotiana sylvestris*, *Medicago truncatula* and *Arabidopsis thaliana*, respectively. (B) A *Petunia* wild-type pistil. (F, H, I, L) Mutant flowers: *maw* in *Petunia* (F), *lam1* in *Nicotiana* (H), *stf* in *Medicago* (I) and *wox1 prs* in arabidopsis (L). (G) A strongly affected *maw* pistil (carpels unfused). (C, D, H, I) Courtesy of M. Tadege.

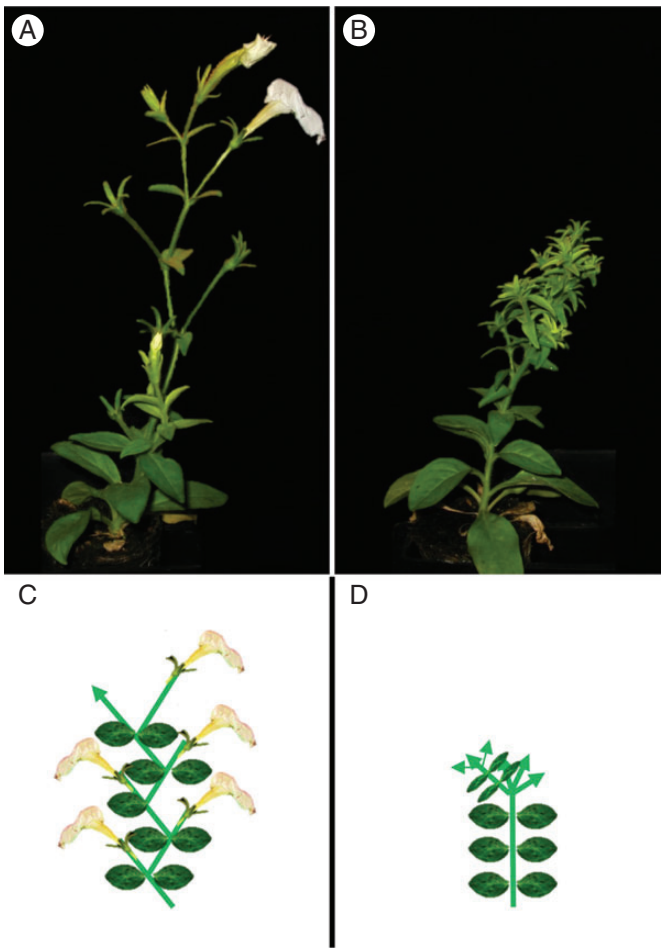

FIG. 3. *evergreen* in *Petunia* (Rebocho *et al.*, 2008). *Petunia* wild-type (A) and evg (B) inflorescences. Whereas the wild-type follows a typical zig-zag pattern, making a flower at each node and resulting in a cymose inflorescence (C), the *evg* mutant has a fasciated and bushy inflorescence (D), flowering only occasionally (terminal flowers).

the embryo while embryo development of strong alleles stops at the globular stage, both phenotypes being due to a reduction or a complete arrest of the cell cycle (Wu *et al.*, 2005, 2007). Interestingly, the *wox9*/*stip* phenotype can be rescued by the addition of exogenous sucrose, although developing carpels are not fully rescued, further confirming the role of *WOX9*/*STIP* in stimulating the cell cycle (Wu *et al.*, 2005). *WOX8*/*STPL* was shown to functionally overlap with *WOX9*/*STIP* in promoting embryonic cell division (Wu *et al.*, 2007; Breuninger *et al.*, 2008). Later during development, *WOX9*/*STIP* promotes the growth of the vegetative SAM and is required for the maintenance of *WUS* expression at the shoot apex. In this regulatory network, *WOX9*/*STIP* acts downstream of the cytokinin signalling pathways (Skylar *et al.*, 2010). More recently, *WOX8*/*STPL* has been shown to promote, along with the expression of *WOX2*, *CUC2* and *CUC3*, the establishment of the cotyledon boundary (Lie *et al.*, 2012).

It therefore seems that *EVG*, *WOX8*/*STPL* and *WOX9*/*STIP* have nothing in common. However, it is interesting to note that in both arabidopis and *Petunia* the constitutive expression of *WOX9*/*STIP* and *EVG* causes similar defects, suggesting that the proteins are functionally very similar and that diversification of *EVG* and *WOX9*/*STIP* might rely on alterations in their expression patterns.

SOE, the second member of the WOX9 clade in *Petunia*, displays an expression pattern very similar to those of *WOX8*/*STPL* and *WOX9*/*STIP* in arabidopsis (Rebocho *et al.*, 2008). Furthermore, the constitutive expression of *SOE* in *Petunia* phenocopies those of *EVG* and *WOX9*/*STIP*, further indicating that these proteins are functionally similar. It has therefore been proposed that *SOE* and *WOX8*/*STPL*–*WOX9*/*STIP* represent an ancestral gene and that *EVG* is a duplicated gene that acquired a new function in inflorescence development and a key role in the evolution of cymes (Rebocho *et al.*, 2008). This example illustrates how genes can be recruited upon duplication to undergo a neo- or sub-functionalization process.

EVOLUTION OF *WOX* GENE FUNCTION: PRIMARILY THROUGH CHANGES IN EXPRESSION PATTERNS?

Despite the fact that most of the different WOX subfamilies are structurally quite different from each other (differences in exon numbers, conserved peptide motifs specific for each subfamily, see Vandenbussche *et al*., 2009), proteins in a number of these subfamilies do seem to share some ancestral common function. A first example can be found in members of the WOX1 and PRS subfamilies in arabidopsis (Vandenbussche *et al*., 2009; Nakata *et al*., 2012). In this case, *WOX1* and *PRS* expression overlaps and the phenotype of *wox1 prs* double mutants clearly shows that they also functionally overlap. In a series of other examples, it turns out that the protein sequences of different family members have retained similar capacities, even though their expression patterns have completely diverged and do not overlap any more. For example, *WUS* is able to complement *prs* and *wox5* mutant phenotypes when expressed under their respective promoters (Sarkar *et al*., 2007; Shimizu *et al*., 2009). More recently, Lin and colleagues (2013) showed that arabidopsis *WUS*, *WOX1*, *WOX2*, *WOX3*, *WOX4*, *WOX5* and *WOX6* were all able to complement leaf blade and floral developmental defects in the *Nicotiana lam1* mutant (*lam1* is the *Nicotiana wox1* homologue mutant) when expressed under the control of the *Mt-STF* promoter [promoter of the *Medicago STENOFOLIA* gene (*WOX1* homologue)].

Together, this demonstrates that proteins of the WUS, MAW/WOX1, WOX2, PRS/WOX3, WOX4 and WOX5 subfamilies (together forming the WUS clade) still have some functional properties in common, despite their ancient origin. This further suggests that changes in *cis*-regulatory elements have constituted a major source of functional diversification within the WUS clade, obviously without excluding the possibility that changes in the protein sequence might also have contributed.

In contrast, *WOX7*, *WOX9*, *WOX11* and *WOX13* were not able to complement the *lam1* mutant phenotype. Interestingly, all WOX proteins that were able to complement possess the WUS box (WUS clade), whereas all others lack this motif (Vandenbussche *et al*., 2009; Lin *et al*., 2013), highlighting the importance of repressive activity linked to the WUSCHEL box for leaf blade expansion. This was further confirmed by the observation that chimeric WOX7, WOX9 and WOX13 proteins fused with either the WUS box or an SRDX repressor domain could complement the *lam1* phenotype (Lin *et al*., 2013). This shows that the acquisition of one or more transcriptional repressor domains in the members of WUS clade compared with the more ancient WOX9 and WOX13 clades has been instrumental in gaining their central function in organizing cell proliferation for meristem maintenance and lateral organ development.

WOX GENES AND FLOWER DEVELOPMENT: CONCLUDING REMARKS

Floral phenotypes thus far described for *wox* mutants include premature floral termination (*wus*), reduced lateral development of floral organs (MAW/WOX1 and PRS/WOX3 subfamilies), resulting in narrow organs with petal and carpel fusion defects, or the complete absence of flowering due to a defect in inflorescence meristem identity (*evergreen*, WOX9 subfamily). Except for the latter, the developmental defects found in the flower are part of a more general phenotype, which also includes defects in leaf blade expansion. Goethe (Goethe, 1790; Coen, 2001) proposed a long time ago that floral organs are in fact modified leaves, so it is perhaps not very surprising to find that *WOX* mutants are affected in both vegetative and floral development. Yet it is clear that nature has exploited *WOX* gene function during evolution for shaping floral architecture. Therefore, while the homeotic function of animal *HOX* genes is fulfilled in plant floral development by MADS box transcription factors, *WOX* genes contribute to general aspects of floral architecture and morphology. Classically, plant developmental biology has focused mainly on *Arabidopsis thaliana* as a model organism. Nevertheless, much of the progress made in our understanding of the function of different *WOX* genes comes from studies in different species. We consider this to be a strong argument in favour of the idea that plant developmental biology in general would benefit from reorientation towards a more multi-model approach.

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