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The PEAPOD Pathway and Its Potential to Improve Crop Yield

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Highlights

- The TIFY proteins PPD1 and PPD2 recruit KIX8, KIX9 and NINJA to form repressor complexes which participate in the transcriptional machinery based on their binding partners.
- SAP-mediated proteasome-dependent degradation regulates PPD and KIX protein abundancies.
- The PPD/KIX/SAP module is widely conserved amongst higher plant species, excluding monocot grasses, and regulates leaf, flower, fruit and seed development in several crop species of agricultural importance.
- Emerging evidence suggests a broader role for the PPD pathway in controlling cell proliferation and developmental plasticity in a tissue-, developmental state- and/or environmental context-dependent manner.

Abstract

A key strategy to increase plant productivity is to improve intrinsic organ growth. Some of the regulatory networks underlying organ growth and development, and interconnections between these networks are highly conserved. An example of such a growth-regulatory module with a highly conserved role in final organ size and shape determination in eudicot species is the PEAPOD (PPD)/KINASE-INDUCIBLE DOMAIN INTERACTING (KIX)/STERILE APETALA (SAP) module. Here, we review the proteins constituting the PPD pathway and their role in different plant developmental processes and explore options for future research. Moreover, we speculate on strategies to exploit the knowledge of the PPD pathway for targeted yield improvement to engineer crop traits of agronomical interest, such as leaf, fruit and seed size.

JAZ and PPD Proteins: So Similar, Yet So Different

Various regulators of organ size, shape and differentiation, their targets, interacting proteins and the interconnections amongst them have been described [1-6]. One such pathway with highly conserved functions for the development of distinct plant organs in various eudicot species is the PEAPOD (PPD) pathway. PPD1 and PPD2 are transcriptional regulators that, together with the JASMONATE ZIM-DOMAIN (JAZ) proteins and TIFY DOMAIN PROTEIN 8 (TIFY8), constitute the plant-specific class II TIFY protein family [7-11]. JAZ proteins contain a ZINC-FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM (ZIM) domain, containing a core TIF[F/Y]XG motif and a jasmonic acid (JA)-associated (Jas) domain, and are well-described as negative regulators of JA signalling (Figure 1A) [9,12,13]. In the absence of JA, they bind and repress multiple transcriptional regulators, including MYC and R2R3 MYB transcription factors (Figure 1A) [9,14-19]. To function as transcriptional repressors, JAZ proteins act as adaptors to recruit the co-repressor TOPLESS (TPL) [9,20-22], which is, together with TOPLESS-RELATED (TPR) proteins, involved in several processes, such as meristem maintenance, hormone signalling and the control of flowering time [22-24]. Whereas JAZ5 to JAZ8 in *Arabidopsis thaliana* (arabidopsis) contain an ETHYLENE RESPONSE FACTOR (ERF)-ASSOCIATED AMPHIPHILIC REPRESSION (EAR) motif and can directly interact with TPL, the remaining JAZ proteins lack an EAR domain and interact via their ZIM domain with NOVEL INTERACTOR OF JAZ (NINJA), an EAR motif-containing adaptor protein

that recruits TPL to the JAZ repressor complex (Figure 1A) [18,21,22,25-30]. During specific JA responses, additional adaptor proteins can be recruited, such as the EAR MOTIF-CONTAINING ADAPTOR PROTEIN (ECAP) which negatively regulates JA-induced anthocyanin accumulation by connecting TPR2 to some JAZ proteins [31]. Upon treatment with 7-*iso*-(+)-jasmonoyl-L-isoleucine (JA-Ile), the bioactive form of JA [32], JAZ proteins interact with CORONATINE INSENSITIVE 1 (COI1), an F-box protein that is part of the SKP1/CULLIN1/F-BOX PROTEIN (SCF) E3 ubiquitin ligase complex (Figure 1A) [33-36]. The interaction results in the SCF^{COI1}-mediated ubiquitination and 26S-mediated proteasomal degradation of JAZ proteins, release of the JAZ-bound transcriptional regulators and induction of the JA response [9,12,33,34,36]. The interaction between JAZ proteins and a number of JA signalling regulators, such as MYCs, R2R3 MYBs and COI1, is mediated via the C-terminal Jas domain (Figure 1A) [9,15,16,18,33,37-39].

Similar to JAZ proteins, PPD proteins contain a central ZIM domain and a C-terminal Jas domain, though slightly modified (Jas*), mediating homo- and heterodimerisation with PPD, JAZ and NINJA proteins [10,11,40,41] and with transcriptional regulators (Figure 1B), such as the basic HELIX-LOOP-HELIX (bHLH) transcription factors MYC3/MYC4 and the chromatin remodelling complex member LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) [42,43]. Interestingly, binding affinities for MYC3 are predicted to be lower with PPD proteins compared with JAZ proteins, likely to be caused by a single amino acid substitution within the Jas domain [44]. This finding further confirms the close phylogenetic relationship between JAZ and PPD proteins, though also demonstrates that PPD and JAZ proteins might present mild differences in terms of interaction behavior [44]. In agreement, PPD proteins contain an additional N-terminal PPD domain that is indispensable for the interaction with KINASE-INDUCIBLE DOMAIN INTERACTING 8 (KIX8) and KIX9, also functioning as TPL-adaptor proteins (Figure 1B) [45]. Whereas JAZ protein stability is regulated by the F-box protein COI1 [33,34,36], the PPD/KIX-complex is poly-ubiquitinated by the F-box protein STERILE APETALA (SAP) containing SCF-complex (SCF^{SAP}), resulting in its proteasome-dependent degradation (Figure 1B) [46,47]. Whether SAP-mediated degradation of the PPD/KIX repressor complex results in the activation of downstream genes due to an abolished repression or the release of transcription factors, allowing their participation in the transcriptional machinery, influencing the expression of downstream genes indirectly, is currently largely unknown.

Moreover, it remains elusive how SAP activity is regulated and what triggers SAP-mediated degradation, which can be a developmental cue, a hormonal cue and/or based on environmental stimuli (Figure 1B).

In conclusion, PPD and JAZ proteins demonstrate high similarities in terms of protein structure, the molecular mechanisms in which they are involved and how their activity is regulated. Nonetheless, the exact protein partners and the biological processes in which PPD and JAZ proteins are involved, appear to be slightly distinct.

The PPD Pathway Has a Conserved Role in Eudicot Developmental Processes

Leaf Development

In arabidopsis, PPD1/2 and KIX8/9 limit asymmetric divisions of meristemoids, triangular stem-cell like cells that can divide reiteratively before differentiating into stomata [45,48-52]. Upon down-regulation of *PPD* or *KIX* or up-regulation of *SAP*, arabidopsis plants display propeller-like rosettes with enlarged dome-shaped leaves and an increase in meristemoid asymmetric cell divisions (Figure 2A) [45-47]. In addition to limiting meristemoid proliferation in leaves [45], referred to as the secondary cell cycle arrest, PPD proteins also control the shape of the primary cell cycle arrest front, denoting the arrest of pavement cell divisions [40]. In *ppd* and *ninja* mutants, the primary cell cycle arrest front is convex-shaped compared with a relatively straight front in wild-type leaves, leading to increased cell divisions in the central compared with the marginal leaf blade regions [40,53]. In concordance, *ppd*, *ninja*, *kix8-kix9* and *35S::SAP* mutants show an increased expression of the direct PPD2 target genes *CYCD3;2* and *CYCD3;3* [40,45-47]. Plants mutated in *NINJA* or constitutively overexpressing *CYCD3;2* also produce propeller-like rosettes with dome-shaped leaves, but lack the increase in the number of meristemoid amplifying divisions and in the leaf area [40,45,51]. These data demonstrate that the *ppd* leaf shape phenotype results, at least partially, from an increased CYCD3 activity, and that the PPD pathway is also involved in primary cell division [40]. Interestingly, SAP was previously identified SUPPRESSOR OF DA1 (SOD3) [46], with DA1 also being a regulator of organ size by limiting cell division [54]. Surprisingly, combining the mutant alleles *da1-1* or *enhancer of da1-1-2* (*eod1-2*) with an artificial miRNA line downregulating *PPD1* and *PPD2* (*ami-ppd*) results in plants bigger compared with the wild type, though smaller than the expected size for additive, hence

independent, effects [55]. This finding may indicate an interaction between the PPD and DA1 pathway, though no direct molecular link was established so far [55]. Moreover, not only the epidermal cell number, but also the palisade cell number is increased in *ppd* arabidopsis mutants compared with the wild type [42]. Previous findings demonstrate that epidermal and mesophyll cell proliferation are tightly coordinated, involving molecular mechanisms such as retrograde signalling [56] and the cell-to-cell movement of the transcriptional regulator *Arabidopsis thaliana* GRF-INTERACTING FACTOR 1 (AtGIF1)/ANGUSTIFOLIA3 (AN3) [57]. Similarly, also the arrest of meristemoid proliferation might be interlinked with the arrest of mesophyll and pavement cell division [51,56], though currently still largely unknown. In agreement with the leaf phenotypes observed in PPD signalling mutants, *PPD1* and *PPD2* are expressed throughout all leaf tissues [43]. In the epidermis, *PPD2* appears to be specifically expressed in stomatal guard cells [43], corresponding with the role of PPD2 in limiting meristemoid asymmetric cell division [45,51], putatively by signalling the transition from asymmetric to symmetric divisions. *PPD* genes are also proposed to be expressed at the shoot apical meristem (SAM), from which leaf primordia originate [43]. As increases in meristem size may contribute to an increase in final leaf area [1,58,59], PPD proteins might also be involved in controlling meristem size, through an unknown mechanism. Although these findings suggest that PPD proteins may act in a cell-autonomous manner, additional in-depth analysis will be required to study the expression patterns of the members of the PPD pathway at a higher spatiotemporal resolution to further elucidate their mode of action.

Production of enlarged leaves with uneven lamina is also observed upon down-regulation of *PPD* or *KIX* and up-regulation of *SAP* orthologues in *Medicago truncatula* (medicago; *BIG SEEDS 1*, *mtbs1-1*, *SMALL LEAF AND BUSHY 1* (*SLB1*)) [60,61], *Vigna mungo* (black gram; *MULTIPLE ORGAN GIGANTISM* (*MOG*), *VmPPD*) [62], *Pisum sativum* (pea; *ELEPHANT-EAR-LIKE LEAF 1* (*ELE1*), *PsPPD*, *BIGGER ORGANS* (*BIO*), *PsKIX*) [63], *Glycine max* (soybean; *BIG SEEDS 1/2* (*BS1/2*), *GmPPD1/2*, *GmKIX8-1*) [60,62,64,65], *Capsella rubella* (*CrSAP*) [66], *Cucumis sativus* L. (cucumber; *LITTLELEAF* (*LL*), *CsSAP*) [67] and *Populus tremula x P. alba* (*P. x canescens*, grey poplar; *BIG LEAF* (*BL*), SAP orthologue) [68], suggesting a highly conserved role for the PPD pathway in leaf growth regulation across rosid eudicot species (Table 1, Figure 3). More recently, also *Solanum lycopersicum*

(tomato) *Slkix8-kix9* plants were shown to produce enlarged and rippled leaves, demonstrating that the pathway is also conserved in asterid eudicots (Table 1, Figure 3) [69].

Transcription factors recruited to the PPD/KIX/NINJA complex during leaf development have not yet been described. Nonetheless, the expression of *GIF1/AN3* was significantly increased in young leaves of medicago (*MtGIF1/2*) and soybean (*GmGIF1*) upon down-regulation of *PPD* [60]. AtGIF1/AN3 acts together with AtGIF2 and AtGIF3 as a transcriptional co-activator that interacts with GROWTH-REGULATING FACTOR (GRF) proteins to control plant organ growth by positively regulating cell proliferation [42,70-75]. Since GRF5 and AtGIF1/AN3 promote primary cell division during leaf development [71,76,77], PPD proteins may also limit primary cell division in leaves by repressing the expression of *AtGIF1/AN3*, similar as during arabidopsis seed development (see '*Flower, Fruit and Seed Development*') [42].

Findings from pea, *Lotus japonicus* (lotus), medicago and arabidopsis suggest that WUSCHEL-RELATED HOMEOBOX (WOX) proteins may also be involved in PPD-mediated leaf development (Table 1) [63,78]. Besides with PPD orthologues (ELEPHANT EAR-LIKE LEAF 1, ELE1), KIX orthologues (BIGGER ORGANS, BIO) also interact with the WOX1 orthologue group in lotus (NARROW ORGANS 1, LjNAO1) and in pea (PsLATHYROIDES, PsLATH) [63]. Moreover, expression of *PsGRF5* in pea is dependent on both PsLATH and the PsBIO/PsELE1 module, and there are genetic interactions identified between WOX1, WOX3 and AtGIF1/AN3 in arabidopsis, and between the PsBIO/PsELE1 module and PsLATH to control organ development in pea [63,78]. Furthermore, *ASYMMETRIC LEAVES 1* (*AS1*) was identified as direct target gene of PPD2 [45] and plants constitutively expressing *AS1* also show propeller-like rosettes and dome-shaped leaves [79]. *AS1* represses distinct *KNOTTED1-LIKE HOMEOBOX* (*KNOX*) genes and is involved in the regulation of adaxial/abaxial leaf patterning [80-82], suggesting that also adaxial/abaxial leaf polarity might be affected in *PPD*/*KIX*/*NINJA*/*SAP* mutants. Altogether, these findings demonstrate a role for the PPD/KIX/NINJA/SAP module in controlling leaf size and shape determination by limiting cell division via a panoply of molecular mechanisms, some being still largely elusive (Figure 3).

Flower, Fruit and Seed Development

In multiple eudicot species, down-regulation of *PPD* or *KIX* or up-regulation of *SAP* orthologues also significantly affects other plant organs (Table 1, Figure 3) [42,46,47,51,60,62,64,65,68,83]. Arabidopsis Δ*ppd* mutants, in which both *PPD* loci are deleted, produce shorter and wider seed pods ('*pea pod-like*') with undulated silique walls (Figure 3) [51]. In agreement, *PPD1* and *PPD2* are highly expressed throughout early arabidopsis seed pod development [43,51,84]. Moreover, strong *GmPPD* soybean mutants display twisted pods [64] and *Slkix8-kix9* tomato plants produce enlarged fruits with an increased pericarp thickness (Figure 3) [69]. Also *ele1* and *bio* pea mutants display enlarged flowers [63], whereas down-regulation of the *SAP* orthologue in *Capsella rubella* causes the production of smaller petals due to a decrease in petal cell numbers [66]. In line with these observations, overexpression of the *SAP* orthologue in cucumber results in the production of bigger organs, including fruits, leaves and flowers (Figure 3) [67], and also in medicago, the SAP orthologue SLB1 regulates organ size by targeting the PPD soybean orthologue BS1 for degradation (Figure 3) [61].

Besides fruit size, disruption or overexpression of *PPD1*/*2*, *KIX8*/*9* or *SAP* also affects seed size and weight in arabidopsis [42,46,47,83], as well as in medicago (*BS1*, *mtbs1-1, SLB1*) [60,61], black gram (*MOG*, *VmPPD*) [62], pea (*ELE1*, *PsPPD*; *BIO*, *PsKIX*) [63], soybean (*BS1*, *GmPPD1/2; GmKIX8-1/-2*) [60,62,64,65] and cucumber (*LL*, *CsSAP*) [67] (Table 1, Figure 3). Whereas also soybean seed quality, more specifically the amino acid content, is significantly improved in *bs1* seeds compared with the wild type [60], the increase in seed size comes at the expense of seed number in black gram [62] and soybean [62,64]. Recently, it was shown that the increase in soybean seed and leaf size of *gmkix8-1* mutants can be uncoupled from each other [65]. Comparisons between hetero- and homozygous *gmkix8-1* plants revealed that whereas homozygous mutants produce enlarged leaves, heterozygous plants produce wild type-like leaves [65]. In contrast, both hetero- and homozygous *gmkix8-1* plants produce enlarged seeds, suggesting *GmKIX8-1* haploinsufficiency during seed, but not during leaf development [65]. These findings indicate that the increase in seed size observed in *gmkix8-1* plants does not result indirectly from an increased energy production due to the larger leaves, but that the PPD module restricts seed size in a direct manner [65]. During arabidopsis seed development, the PPD/KIX complex interacts with the bHLH transcription factors MYC3/MYC4 to directly bind and repress the expression of *AtGIF1/AN3*

(Figure 2) [42]. In agreement, the expression patterns of *KIX8*, *KIX9*, *PPD1*, *PPD2*, *MYC3*, *MYC4* and *AtGIF1/AN3* are partially overlapping during arabidopsis ovule and seed development [42], and seeds of *atgif1/an3* arabidopsis plants are significantly smaller compared with the wild type due to a decrease in cell numbers [42,74]. In contrast, however, enlarged embryos and increased embryonic, seed and cotyledon cell sizes were described for the *an3-4* allele [85,86]. These findings may indicate that mutant allele-specific effects could underlie the different reported effects of AtGIF1/AN3 on seed size, though requiring further investigation. In addition, it is currently unknown whether cell-to-cell mobility of AtGIF1/AN3, as reported for leaves [57], may also impact seed development. Upon down-regulation of the *PPD* orthologues, the expression of *GIF1/AN3* is also significantly increased in seeds of medicago (*MtGIF1*) and soybean (*GmGIF1*) [60]. Altogether, these findings demonstrate the involvement of the PPD/KIX/SAP module in controlling the growth and development of reproductive organs and seeds, putatively through similar conserved mechanisms [42,47,60,61,67]. Nonetheless, the *atgif1/an3* mutation complements the increase in seed size of arabidopsis *ppd*, *kix8-kix9* and *35S::SAP* plants only partially [42], suggesting the presence of additional molecular mechanisms underlying the seed size alterations in PPD signalling mutants (Figure 2) [83]. Among others, brassinosteroids (BRs) are required for normal seed size, shape and weight establishment [87] and plants overexpressing the BR-biosynthesis gene *DWARF 4* (*DWF4ox*) [88,89] or *BRASSINOSTEROID INSENSITIVE 1* (*BRI1*, *BRI1ox*), encoding the BR receptor [90], display an increased seed weight [83]. Interestingly, arabidopsis *BRI1ox-GFP* plants also produce rosettes with enlarged dome-shaped leaves, reminiscent of *ppd*, *kix8-kix9* and *35S::SAP* mutants [90-92]. Since PPD proteins were previously proposed to restrict BR biosynthesis and signalling [83], the increased size of arabidopsis *ppd*, *kix8-kix9*, *myc3/4* and *35S::SAP* seeds [42] could also result from increased BR signalling (Figure 2), though elusive so far.

Vascular Development

The plant's vascular network consists of phloem and xylem and is in arabidopsis leaves predominantly generated by the activity of (pro)cambium cells recruited from the mesophyll tissue layer [51,93-97]. Like meristemoids, (pro)cambium cells are referred to as dispersed meristematic cells and might thus be regulated in a similar manner [51]. Preliminary experimental

data suggest a potential role for the PPD pathway in regulating vascular development (Figure 2) [51,68]. Δ*ppd* plants exhibit a more complex venation pattern and increased procambium cell numbers in cotyledons [51,83]. In addition, xylem vessel numbers are increased compared with the wild type, whereas cotyledons of *PPD-OE* plants display reduced vascular growth [51,83]. In concordance, *PPD* expression is high in tissues that may correspond with the cotyledon and leaf vascular system [43,51] and overexpression of the *SAP* orthologue *BL* in grey poplar results in disturbed xylem development and reduced stem diameters [68], further supporting a putative involvement of the PPD pathway in vascular development. During vascular development, the TARGET GENE OF MONOPTEROS 5 (TMO5)–LONESOME HIGHWAY (LHW) complex directly induces the expression of the cytokinin biosynthesis genes *LONELY GUY 3* (*LOG3*) and *LOG4*, resulting in an increased (pro)cambium cell proliferation (Figure 2) [94,98-100]. TMO5–LHW activity can, however, be reduced by SUPPRESSOR OF ACAULIS-LIKE (SACL) transcription factors, as these can compete with TMO5 for binding with LHW [101,102]. *SACL* expression is strongly induced by the polyamine thermospermine, produced by ACAULIS 5 (ACL5) [103,104]. Interestingly, *ACL5* expression is reduced in Δ*ppd* arabidopsis plants [83] and PPD2 can directly bind to the promoter of *ACL5* in arabidopsis cell cultures [45,83], suggesting that PPD proteins may limit TMO5–LHW activity [83]. These findings suggest that the PPD/KIX/SAP module may limit meristemoid and (pro)cambial cell proliferation in eudicots to regulate the spacing of stomata and the vascular system, respectively, though subject for future research.

Flowering Time

Besides the effects on organ growth, arabidopsis *ppd* and arabidopsis and tomato *kix8-kix9* mutants display delayed flowering (Table 1) [69,83]. To induce the transition from vegetative to reproductive growth, the transcription factor CONSTANS (CO) is stabilised and activates the expression of *FLOWERING LOCUS T* (*FT*) in the leaf phloem companion cells [105]. FT is subsequently transported to the SAM to initiate flower formation [105]. In arabidopsis, CO activity is, amongst others, counteracted by SCHLAFMUTZE (SMZ), which represses multiple flowering time regulatory genes, including *FT* (Figure 2) [106]. In concordance with the delayed flowering phenotype of *ppd* and *kix8-kix9* plants, *SMZ* expression is increased in arabidopsis *ami-ppd* and *kix8-kix9* leaves [45,83]. Similarly, the expression of *APETALA2d* (*AP2d*), a *SMZ*

orthologue, is up-regulated in tomato *Slkix8-kix9* compared with wild-type leaves [69]. Besides *SMZ*, also several other genes encoding proteins affecting CO activity are differentially expressed in arabidopsis *ppd* compared with wild-type plants [45,83], including *NUCLEAR FACTOR Y SUBUNIT B2* (*NF-YB2*) [107], *TARGET OF EARLY ACTIVATED TAGGED 2* (*TOE2*) [108,109] and *SUPPRESSOR OF PHYA-105 1* (*SPA1*) [106,110,111], and the promoters of *SMZ*, *NF-YB2* and *TOE2* are directly bound by PPD2 in arabidopsis cell cultures (Figure 2) [45]. Taken together, these findings suggest a role for the PPD pathway in flowering time determination, though the exact underlying molecular networks are still largely elusive.

Hormone and Light Signalling

Amongst the genomic regions bound by PPD2, '*hormone metabolism*' is an overrepresented PageMan category [45,112] and '*hormone biosynthesis process*', '*regulation of hormone levels*' and '*response to light stimulus*' are amongst the significantly overrepresented gene ontology (GO) terms of genes differentially expressed in arabidopsis *ppd* seedlings [83,112]. These findings suggest a potential role for the PPD pathway in hormone and light signalling processes.

PPD proteins likely diverged from JAZ proteins, negative regulators of JA signalling [7-10], and both PPD and most JAZ proteins interact with the adaptor protein NINJA, also involved in JA signalling (Figure 1) [20,25,26,40,45]. Moreover, *DWARF IN LIGHT 1* (*DFL1*) is up-regulated in arabidopsis *ami-ppd* and arabidopsis and tomato *kix8-kix9* leaves and the promoter of *DFL1* is bound by PPD2 in arabidopsis cell cultures [45,69]. Amongst the nineteen GRETCHEN HAGEN 3 (GH3) proteins, described to be involved in maintaining auxin homeostasis by conjugating amino acids to excess auxin [113-115], GH3.3, GH3.5 and GH3.6 (*DFL1*) additionally conjugate amino acids to JA (Figure 2) [116,117]. Interestingly, also the expression of *GH3.3* is increased in arabidopsis *ami-ppd* compared with wild-type leaves [45]. Although these observations suggest a potential involvement of PPD proteins in JA signalling, *PPD* expression and PPD protein stability are not affected by JA treatment [20] and the phenotypes of *ppd* mutants are likely unrelated to JA signalling [40,45,51,83]. Whereas a direct involvement in JA signalling is unlikely, PPD proteins are proposed to be involved in several other hormonal pathways. It has been suggested that PPD proteins restrict BR biosynthesis and signalling (see '*Flower, Fruit and Seed Development*') and

arabidopsis *ppd* mutants show a differential response compared with wild-type plants for several other plant hormones, though mostly based on preliminary data [83].

Arabidopsis *ppd* plants also share phenotypes with shade-grown plants, including abscisic acid-insensitive seed germination, and hypocotyl and petiole elongation [51,83,118,119]. Moreover, PPD proteins are proposed to directly repress *SPA1* and activate *ATTENUATED FAR-RED RESPONSE* (*AFR)* [83], a negative and positive regulator of light signalling, respectively [111,120] and overexpression of *PHYTOCHROME B* (*phyBOE*) is epistatic to Δ*ppd* with regard to the hypocotyl length and the flowering time phenotypes [83]. Although these observations suggest a putative interaction between the PPD pathway and hormone and light signalling (Figure 2), additional research will be required to validate an interplay between the different signalling pathways and to identify the steering molecular players.

PPD1/PPD2 and KIX8/KIX9 Control Organ Growth in a Partially Redundant Manner

In arabidopsis, genomic transgenes of *PPD1* or *PPD2* can rescue the Δ*ppd* [51] and *ami-ppd* [43] mutant leaf size and shape phenotypes. In addition, overexpression of *PPD1* or *PPD2* results in the production of small seeds, whereas seed area, seed weight and leaf area are significantly increased in *ppd1 ppd2-cr* and *ppd1-cr ppd2* double mutants compared with the respective single mutants (Table 1) [42,83]. In agreement, *PPD1* and *PPD2* are expressed in an overlapping manner in arabidopsis, including young leaf primordia and during the early seed pod and seed developmental stages [42,43,51]. Similarly, overexpression of *KIX8* or *KIX9* results in the formation of small seeds compared with the wild type [42], and leaf and seed phenotypes (Table 1) and the increases in expression of the PPD2 target genes are most pronounced in *kix8-kix9* double mutants compared with the respective single mutants [42,45,69]. Although these findings indicate that PPD and KIX proteins control leaf, fruit and seed development in a redundant manner, PPD1, PPD2, KIX8 and KIX9 also appear to have specificities. Whereas *ppd2* mutants, for instance, display propeller-like rosettes with enlarged dome-shaped leaves and produce seeds with an increased area and weight, *ppd1* rosettes and seeds are indiscernible from those of wild type plants [42,43,46]. Analogously, *kix8* arabidopsis mutants exhibit mild propeller-like rosettes with dome-shaped leaves, enlarged seeds and a slight increase in expression of the PPD2 target genes compared with the wild type, whereas *kix9* plants are indistinguishable from

wild-type plants, both at a phenotypical and molecular level [42,45]. In concordance, whereas the *Slkix9* tomato mutant produces wild type-like leaves, the leaf phenotype of *Slkix8* mutants is intermediate of that of *Slkix8-kix9* and wild-type plants [69]. Altogether, these findings imply that although PPD and KIX proteins are functionally redundant, PPD2 and KIX8 appear to act as predominant players during leaf and seed development, likely to result from subtle differences in expression level and/or pattern [69]. In the future, it will be interesting to further investigate and compare the spatio-temporal expression of *PPD1/2* and *KIX8/9* to better understand tissue-specific interactions and their individual contribution to plant organ growth. The previous identification of KIX8-specific putative interaction partners [45] may even suggest PPD-independent functions for KIX proteins, though unexplored so far.

Why Are PPD/KIX/SAP Orthologues Absent in *Poaceae*?

Though being absent in non-photosynthetic eukaryotes and green algae, several TIFY protein family members have orthologues in monocot and eudicot species [7,8,41,121-127]. Interestingly, however, whereas highly conserved in both sequence and function across rosid and asterid species constituting most of the core eudicots [46,47,60-62,64,69], PPD/KIX/SAP orthologues appear to be absent in *Poaceae* (grasses) [43,45,46]. The phylogenetic distribution of PPD/KIX/SAP orthologues points towards a conserved function for the PPD/KIX/SAP module in processes that are intrinsically different between eudicots and monocot grasses [2,45,46]. In agreement, although cellular and molecular mechanisms governing leaf growth in eudicots and grasses are largely conserved [2,29,128,129], certain specificities remain. Whereas stomata in eudicots are distributed in a random manner, following the '*one-cell spacing rule*', monocot grasses show a linear stomatal organisation and lack stomatal precursor meristemoid cells [2,29,128-131]. Moreover, the venation pattern is reticulate in eudicot leaves, and parallel-like in monocot grasses [130]. Findings from lotus and pea indicate that the PPD/KIX module might interact, directly or genetically, with LjNAO1 and PsLATH, orthologues of AtWOX1 and MtSTENOFOLIA (STF) [63,132]. Overexpression of *MtSTF* in *Panicum virgatum* (switch grass), *Oryza sativa* (rice) or *Brachypodium distachyon* (Brachypodium) results in wider leaf laminae, in an increased leaf vein number and stem diameter, and, upon strong overexpression, in leaf blade twisting and curling [133]. Also in *Triticum aestivum* (wheat), overexpression of *MtSTF* results in

wider leaves, more leaf veins and increased epidermal cell divisions [134]. *WOX1* and its orthologues are described as regulators of leaf blade outgrowth and vascular development [135,136] and, unlike other *WOX* genes, lack orthologues in monocot grasses [136,137], in which WOX3 proteins predominantly regulate lateral organ development [138-141]. Several main players during vascular development, however, such as TMO5, LHW, ACL5, LOG and SACL (see '*Vascular Development'*), have orthologues in grasses, such as in rice and *Sorghum bicolor* (sorghum) [142]. These findings indicate that the PPD pathway is not required to modulate (pro)cambial cell proliferation in grasses, but that other proteins might be present to perform a similar function. Accordingly, combined with the lack of meristemoids, the PPD/KIX/SAP module might have become obsolete for leaf growth in grasses. Interestingly, however, PPD, KIX and SAP orthologues are present in several non-grass monocot species, such as *Musa acuminata* (banana) and *Elaeis guineensis* (oil palm) [43], as well as in the basal angiosperm *Amborella trichopoda* and even lycophytes [43], though it is currently unknown whether their function is conserved. In the future, it might be useful to perform more in-depth phylogenetic analyses to shed light on the conservation, functionality and evolutionary history of the PPD/KIX/SAP module.

Targeting the PPD Pathway to Improve Specific Plant Agronomic Traits

The significant increases in shoot, fruit and seed biomass in multiple eudicots upon down-regulation of *PPD* or *KIX* [42,45,51,60,62-64,69] or up-regulation of *SAP* genes [46,47,61,67,68] imply that the PPD pathway may hold great potential from a biotechnological point of view (Table 1, Figure 3). Increased fruit size, for instance, as observed in *Slkix8-kix9* tomato [69], *ele1* and *bio* pea [63] or *SAP*-overexpressing cucumber lines [67], was amongst the main selection criteria for nearly all fruit crops during domestication and still is today [143,144]. Moreover, the *ll* cucumber mutant, producing smaller organs proposed to result from an abolished interaction with and degradation of the KIX orthologues, was sold as a pickling cucumber variety and thus also considered interesting from an agronomic point of view [67]. Hence, the knowledge of the PPD pathway may be exploited to direct plant breeding or to enhance the success rate to generate crops with increased agronomical traits. Alterations in the size and/or shape of flowers and/or leaves might for instance be of interest to breed new varieties of ornamental plants. Besides organ size and shape, the PPD pathway also controls several other traits with major potential to

be explored in biotechnological engineering approaches. The increased amino acid content in soybean seeds upon down-regulation of *GmPPD* [60], for instance, suggests to exploit the PPD pathway to breed for crop varieties with an improved seed nutritional value. Moreover, overexpression of the *SAP* orthologue *BL* results in an impaired xylem formation and overall reduced stem diameter, pointing towards the potential or targeting the PPD/KIX/SAP module to improve wood development [68].

Targeting genes that act in multiple plant organs or at distinct developmental stages, as demonstrated for the PPD pathway, may however result in undesirable pleiotropic effects (Table 1) [145]. The delayed flowering time of arabidopsis *ppd* and *kix8-kix9* [83] and tomato *Slkix8-kix9* [69] plants and the fact that the increased seed sizes in black gram [62] and soybean [62,64] were, especially for strong alleles, at the expense of seed number, may for instance be unprosperous for farming purposes. Interestingly, many of the alleles selected during domestication are not severe gain- or loss-of-function alleles of genes involved in crop development, but result from mutations in *cis*-regulatory elements (CREs) [145-147]. Sequence variation in CREs can modify a specific aspect of the gene expression profile, leading to mild changes in gene expression level and/or pattern, including the developmental timing and tissue specificity [145,148]. Specific agronomic traits, such as fruit or seed size or seed quality, could for instance be improved by engineering specific CREs involved in the transcriptional regulation of PPD signalling members to modify a specific aspect of their expression profile [145,149-151]. Analogously, it was recently shown that soybean plants heterozygous for small deletions in the *GmKIX8-1* promoter produce enlarged seeds while maintaining wild-type leaf size [65], suggesting that this regulatory element might have great potential to increase seed size using a CRE-targeting approach. This hypothesis is further strengthened by the fact that the described mutation in the *GmKIX8-1* promoter is associated with the 'big seed' quantitative trait locus *qSw17-1* in multiple soybean populations [65]. Combining the knowledge of CREs involved in transcriptional regulation with the identification of upstream regulators or downstream target genes involved in specific PPD-regulated processes, could be another asset in fine-tuning biotechnological approaches. During arabidopsis seed development, for example, the PPD/KIX/MYC complex directly binds to a G-box sequence in the promoter of *AtGIF1/AN3*, encoding a positive regulator

of seed size and weight, and represses its expression (Figure 2) [42,72,152,153]. Besides the members constituting the PPD pathway and their functionality, also their transcriptional regulation may be conserved in a broad range of eudicot species. Accordingly, targeting specific CREs bound by the PPD/KIX complex during seed development could be a promising strategy to improve for instance seed size and quality in distinct crops of agricultural importance. Though of substantial applied interest and offering major potential to improve specific agronomic traits, CREs and their role in organ- or developmental-specific processes are currently still largely elusive for members constituting the PPD pathway.

Concluding Remarks and Future Perspectives

An increasing number of studies highlight a conserved role for the PPD pathway in regulating organ growth in various eudicot species, predominantly by restricting cell proliferation, and reveal interconnections with other growth-regulatory modules, though often preliminary and missing strong evidence. Nonetheless, these findings indicate that targeting the PPD pathway may hold great potential for targeted yield improvement of crops with a high agricultural and economical value. The considerable increase in knowledge of the members of the PPD pathway and their role during organ growth indicates that this pathway may act as a master switch controlling more aspects of plant growth and development than initially anticipated, leaving still much to be explored in the future (see '*Outstanding Questions Box*'). The complex phenotypes of PPD signalling mutants and the diversity in target genes may result from the fact that the intrinsic role of PPD/KIX proteins is to limit developmental plasticity, crucial to avoid excessive growth responses. As such, the PPD/KIX/NINJA complex might act as a general adaptor complex, interacting with various transcriptional regulators (i) to recruit the TPL machinery to specific DNA regions or (ii) to shield transcription factors from interactions with other proteins in a tissue-, developmental state- and/or environmental context-dependent manner. Whereas impressive biomass increases are obtained by modifying PPD signalling in various eudicot species, this is often accompanied with undesirable effects. In the future, a targeted engineering of the PPD pathway, for instance by targeting specific downstream target genes or specific CREs using modern genome-editing techniques, could harbour great potential to improve specific plant agronomic traits and generate higher-yielding crop varieties. Accordingly, it will be interesting,

from both a scientific and a more applied perspective, to further untangle the members constituting the PPD pathway, their transcriptional regulation and their involvement in distinct processes. This may render a better view on the variety of processes controlled by the PPD pathway and will allow to further unravel the interconnections amongst distinct growth-regulatory modules.

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Author contributions

MS and AB wrote the manuscript. NG, LP and DI complemented the writing. AB and DI supervised the writing.

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Outstanding Questions Box

- What are the structural and molecular differences between PPD and JAZ proteins enabling their involvement in different processes, despite their striking similarities?
- If the PPD/KIX/NINJA complex acts as a general adaptor complex, with which additional transcriptional regulators does it interact and does this occur in a process-, tissue- and/or cell-specific manner?
- What is the individual contribution of PPD1, PPD2, KIX8 and KIX9 in specific processes? This will require to interrogate their expression patterns at a high spatiotemporal resolution, putatively uncovering their tissue-specific interaction behaviour and/or their involvement in yet unexplored processes.
- How is SAP activity regulated and what triggers the SAP-mediated proteasome-dependent degradation of the PPD/KIX complex? Is there a ligand or trigger and is this then developmental, hormonal and/or based on environmental stimuli?
- Under which circumstances does crosstalk between the PPD pathway and light and hormone signalling occur and what is its biological relevance in a developmental and/or environmental context?
- What is the contribution of the PPD/KIX/SAP module to processes unexplored so far, such as flowering time and vascular development?
- Which mechanisms are involved in the transcriptional regulation of the PPD/KIX/SAP module and how could this be exploited for biotechnological applications?
- The PPD/KIX/SAP module is lost in *Poaceae* (monocot grasses), though retained in several non-grass monocot species as well as in the basal angiosperm *Amborella trichopoda* and even lycophytes, suggesting its involvement in processes that are intrinsically different in grasses. Which are these processes and are the functions of PPD/KIX/SAP orthologues retained in non-monocot grasses and more basal plants?

Figures

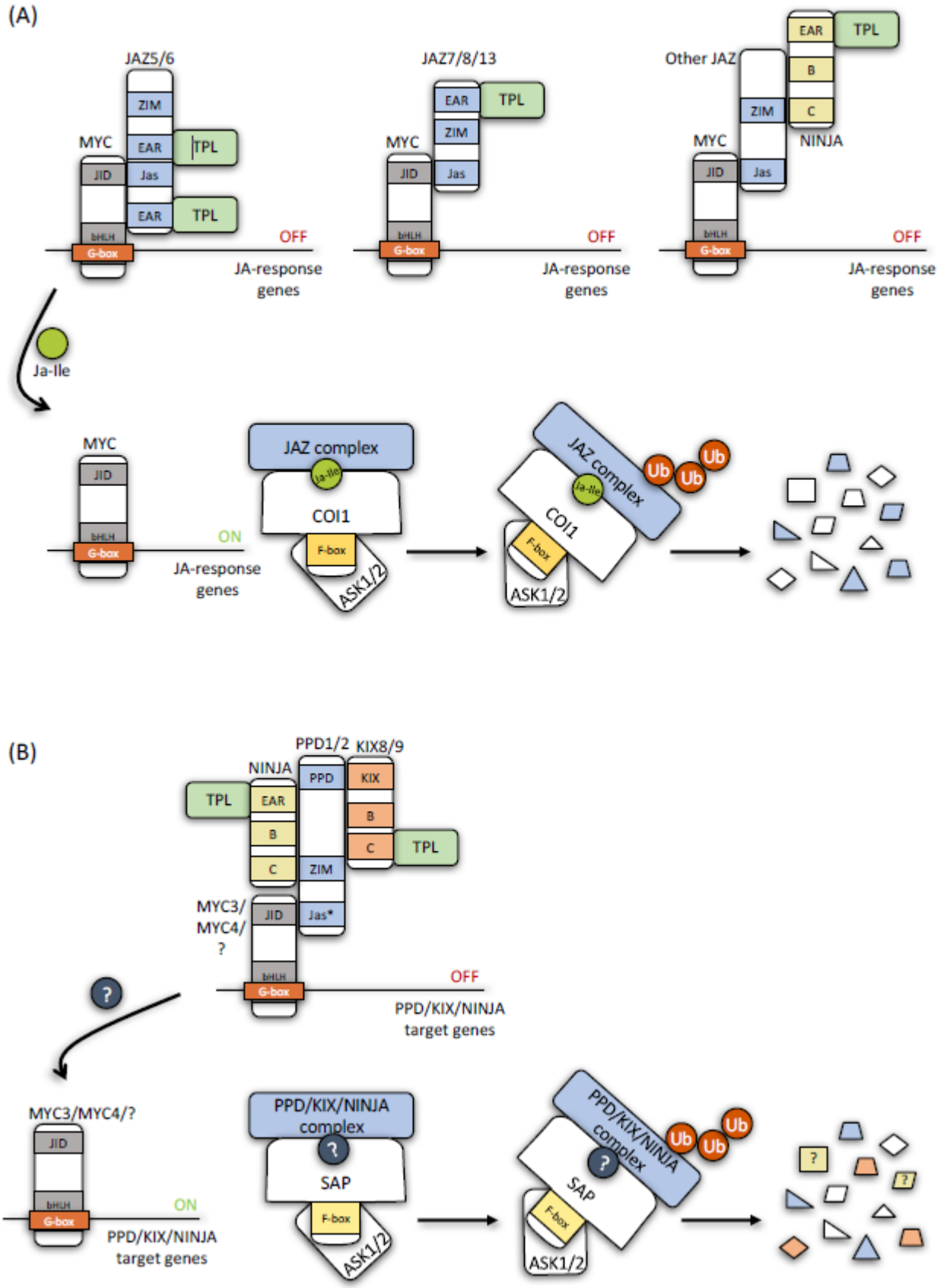
(A)
JAZ5/6
ZIM
EAR
TPL
MYC
JID
Jas
EAR
TPL
bHLH
G-box
OFF
JA-response genes
JAZ7/8/13
EAR
TPL
ZIM
MYC
JID
Jas
bHLH
G-box
OFF
JA-response genes
Other JAZ
EAR
TPL
B
ZIM
C
NINJA
MYC
JID
Jas
bHLH
G-box
OFF
JA-response genes
Ja-Ile
MYC
JID
bHLH
G-box
ON
JA-response genes
JAZ complex
Ja-Ile
COI1
F-box
ASK1/2
JAZ complex
Ub
Ub
Ja-Ile
COI1
F-box
ASK1/2
(B)
PPD1/2
KIX8/9
NINJA
PPD
KIX
TPL
EAR
B
B
C
TPL
C
ZIM
MYC3/
MYC4/
?
JID
Jas*
bHLH
G-box
OFF
PPD/KIX/NINJA target genes
?
MYC3/MYC4/?
JID
bHLH
G-box
ON
PPD/KIX/NINJA target genes
PPD/KIX/NINJA complex
?
SAP
F-box
ASK1/2
PPD/KIX/NINJA complex
Ub
Ub
?
SAP
F-box
ASK1/2
?
?

Figure 1. Similarities and Differences Between JAZ and PPD Proteins and Their Interaction Network. (A) In the absence of jasmonic acid (JA), JASMONATE ZIM DOMAIN (JAZ) proteins bind with MYC and R2R3 MYB transcription factors through their Jas domain and recruit the co-repressor TOPLESS (TPL) through their ETHYLENE RESPONSE FACTOR (ERF)-ASSOCIATED AMPHIPHILIC REPRESSION (EAR) motif. JAZ5 to JAZ8 and JAZ13 contain one or multiple EAR motifs and can therefore directly interact with TPL. The other arabidopsis JAZ proteins lack an EAR motif and recruit TPL by binding with their ZINC-FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM (ZIM) domain to the EAR motif-containing protein NOVEL INTERACTOR OF JAZ (NINJA). In all cases, this results in the repression of JA-response genes. Upon perception of JA-isoleucine (JA-Ile), the JAZ protein complex is poly-ubiquitinated by the F-box protein CORONATINE INSENSITIVE 1 (COI1)-containing SKP1/CULLIN1/F-BOX PROTEIN (SCF) E3 ubiquitin ligase complex, resulting in its degradation and the induction of the downstream JA-transcriptional response by JAZ-bound transcription factors. (B) PEAPOD (PPD) proteins have a specific PPD domain, that mediates the interaction with KINASE-INDUCIBLE DOMAIN INTERACTING PROTEIN 8/9 (KIX8/9), and a ZIM domain that interacts with NINJA, both recruiting TPL. Also the basic HELIX-LOOP-HELIX (bHLH) transcription factors MYC3 and MYC4 and potential other yet unidentified transcriptional regulators are recruited to the complex (MYC3/MYC4/?), likely via their slightly convergent Jas domain (Jas*). Poly-ubiquitination of the PPD/KIX complex by the F-box protein STERILE APETALA (SAP)-containing SCF complex, upon perception of a yet unknown signal (question mark), results in proteasome-dependent degradation of the PPD/KIX complex. Currently, it is unknown whether also NINJA is prone to SAP-mediated proteasome-dependent degradation (questions marks).

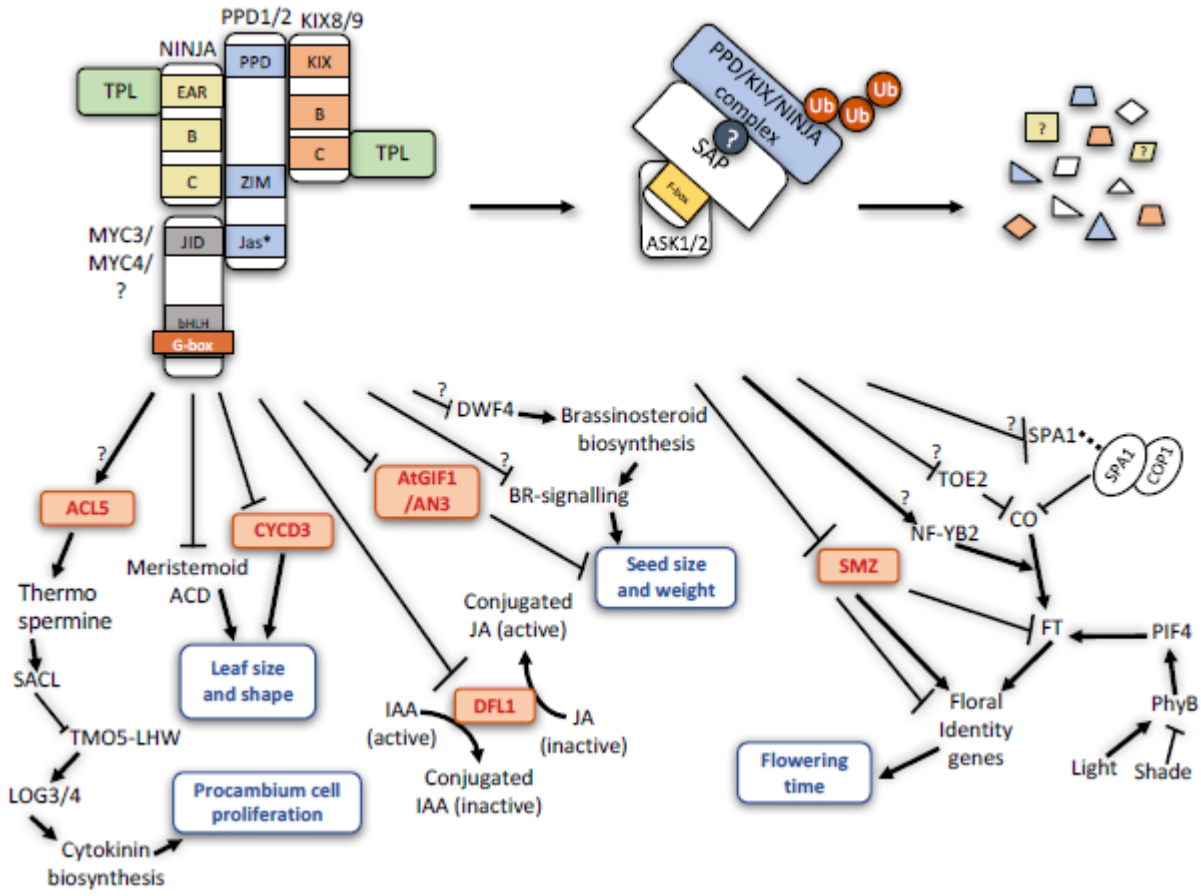

Figure 2. Schematic Overview of the PPD Pathway and Putative Connections with Developmental, Hormonal and Light Pathways. The PPD and ZIM domains of PEAPOD (PPD) proteins interact with KINASE-INDUCIBLE DOMAIN INTERACTING PROTEIN 8/9 (KIX8/9) and NOVEL INTERACTOR OF JAZ (NINJA), respectively, recruiting TOPLESS (TPL). Also the basic HELIX-LOOP-HELIX (bHLH) transcription factors MYC3 and MYC4 and potential other yet unidentified transcriptional regulators are recruited to the complex (MYC3/MYC4/?), likely via their slightly convergent Jas domain (Jas*). The protein complex directly represses the expression of distinct downstream target genes (red), including *DWARF IN LIGHT 1* (*DFL1*), *SCHLAFMUTZE* (*SMZ*), *CYCLIN D3s* (*CYCD3*) and *Arabidopsis thaliana GRF-INTERACTING PROTEIN 1* (*AtGIF1*)/*ANGUSTIFOLIA3* (*AN3*), to regulate distinct biological processes (blue). Also several other genes are proposed to be regulated by the PPD/KIX complex, including *ACAULIS 5* (*ACL5*), *DWARF 4* (*DWF4*), *NUCLEAR FACTOR Y SUBUNIT B2* (*NF-YB2*), *TARGET OF EARLY ACTIVATED TAGGED 2* (*TOE2*) and *SUPPRESSOR OF PHYTOCHROME A-105 1* (*SPA1*), though not yet validated (question marks). Poly-

ubiquitination of the PPD/KIX complex by the F-box protein STERILE APETALA (SAP)-containing SKP1/CULLIN1/F-BOX PROTEIN (SCF) complex results in its proteasome-dependent degradation. Currently, it is unknown whether also NINJA is prone to SAP-mediated proteasome-dependent degradation (questions marks). Abbreviations: ACD, asymmetric cell division; CO, CONSTANS; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; FT, FLOWERING LOCUS T; IAA, indole-3-acetic acid; LOG, LONELY GUY; LHW, LONESOME HIGHWAY; phyB, PHYTOCHROME B; SACL, SUPPRESSOR OF ACAULIS-LIKE; TMO5, TARGET GENE OF MONOPTEROS 5.

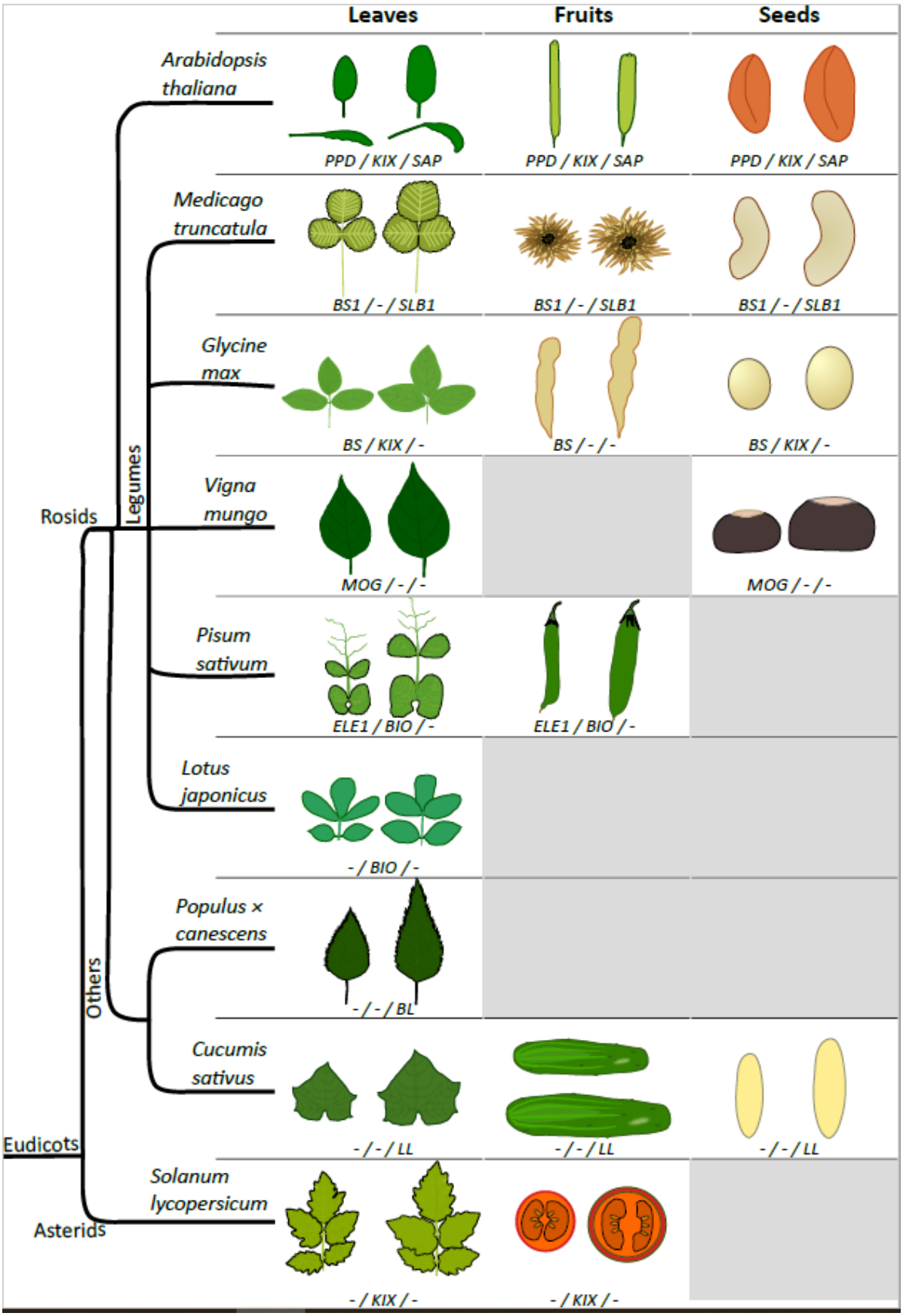
Leaves
Fruits
Seeds
Arabidopsis thaliana
PPD / KIX / SAP
PPD / KIX / SAP
PPD / KIX / SAP
Medicago truncatula
BS1 / - / SLB1
BS1 / - / SLB1
BS1 / - / SLB1
Glycine max
BS / KIX / -
BS / - / -
BS / KIX / -
Vigna mungo
MOG / - / -
MOG / - / -
Pisum sativum
ELE1 / BIO / -
ELE1 / BIO / -
Lotus japonicus
- / BIO / -
Populus × canescens
- / - / BL
Cucumis sativus
- / - / LL
- / - / LL
- / - / LL
Solanum lycopersicum
- / KIX / -
- / KIX / -
Legumes
Others
Rosids
Eudicots
Asterids

Figure 3. Organ Size Changes by Targeting the PPD/KIX/SAP Module in Various Plant Species. Schematic overview of leaf, fruit, and seed size alterations upon down-regulation of *PPD* and *KIX* or up-regulation of *SAP* or their respective orthologues in different plant species (PPD/KIX/SAP), unless no phenotype is reported for the respective orthologue (indicated by '-'). In each category, a representation of the wild-type and mutant organs is presented on the left and the right, respectively, except for the *Cucumis sativus* fruit, where the wild-type and the mutant organ is presented at the top and the bottom, respectively. Grey fields indicate that the effect of targeting the PPD/KIX/SAP module is currently unknown or irrelevant for the respective organ. The references corresponding to the phenotypes shown can be found in Table 1. *PEAPOD* (*PPD*); *PPD* orthologues: *BIG SEEDS* (*BS*); *MULTIPLE ORGAN GIGANTISM* (*MOG*); *ELEPHANT-EAR-LIKE LEAF 1* (*ELE1*). *KINASE-INDUCIBLE DOMAIN INTERACTING* (*KIX*)*; KIX* orthologue: *BIGGER ORGANS* (*BIO*). *STERILE APETALA* (*SAP*); *SAP* orthologues: *SMALL LEAF AND BUSHY 1* (*SLB1*); *BIG LEAF* (*BL*); *LITTLELEAF* (*LL*).

Table 1. Overview of PPD/KIX/SAP Mutant Phenotypes in Different Eudicot Species[a]

Gene name	*Arabidopsis thaliana orthologue*	Organism	Type	Shoot/leaf size phenotype	Seed/fruit phenotype	Additional phenotypes	Refs.
PPD1	*PPD1*	*Arabidopsis thaliana*	LOF	no difference	no difference		[42,43,51,83]
PPD1	*PPD1*	*Arabidopsis thaliana*	GOF	decreased	shorter, narrower, flatter siliques	reduced vascular system	[51,83]
PPD2	*PPD2*	*Arabidopsis thaliana*	LOF	increased	slightly increased		[40,43,51,83]
PPD2	*PPD2*	*Arabidopsis thaliana*	GOF	decreased	decreased	reduced number of stomata, reduced vascular network, shortened primary roots	[43,51,83]
PPD1/PPD2	*PPD1/PPD2*	*Arabidopsis thaliana*	LOF	increased	increased	increased number of guard and pavement cells, increased vascular network, delayed flowering	[40,42,43,45,51,83]
KIX8	*KIX8*	*Arabidopsis thaliana*	LOF	no difference	slightly increased		[42,45]
KIX9	*KIX9*	*Arabidopsis thaliana*	LOF	no difference	no difference		[42,45]
KIX8/KIX9	*KIX8/KIX9*	*Arabidopsis thaliana*	LOF	increased	increased	increased number of hypocotyl guard cells, delayed flowering, elongated hypocotyls	[42,45,83]
NINJA	*NINJA*	*Arabidopsis thaliana*	LOF	no difference			[40]
NINJA	*NINJA*	*Arabidopsis thaliana*	GOF			decreased JA sensitivty	[20]
MYC3	*MYC3*	*Arabidopsis thaliana*	LOF		increased		[42]
MYC4	*MYC4*	*Arabidopsis thaliana*	LOF		increased		[42]
SAP (SOD3)	*SAP (SOD3)*	*Arabidopsis thaliana*	LOF	decreased		small or absent flowers or flower parts	[42,46,47]
SAP (SOD3)	*SAP (SOD3)*	*Arabidopsis thaliana*	GOF	increased	increased	larger flowers	[42,46,47]

Gene name	*Arabidopsis thaliana orthologue*	Organism	Type	Shoot/leaf size phenotype	Seed/fruit phenotype	Additional phenotypes	Refs.
CYCD3;2	*CYCD3;2*	*Arabidopsis thaliana*	GOF	no difference		reduced stomatal index	[40]
CYCD3;3	*CYCD3;3*	*Arabidopsis thaliana*	GOF	decreased			[40]
AtGIF1/ AN3	*AtGIF1/AN3*	*Arabidopsis thaliana*	GOF	increased	increased		[42,71]
AtGIF1/ AN3	*AtGIF1/AN3*	*Arabidopsis thaliana*	LOF	decreased	Decreased, increased in *an3-4*	narrow petals	[42,70,71,86]
GRF5	*GRF5*	*Arabidopsis thaliana*	GOF	increased			[71]
GRF5	*GRF5*	*Arabidopsis thaliana*	LOF	slightly decreased			[71]
WOX1	*WOX1*	*Arabidopsis thaliana*	LOF	no difference			[135]
WOX1	*WOX1*	*Arabidopsis thaliana*	GOF	decreased		dwarfed phenotype, disturbed anther development, decreased fertility	[135]
MtSTF	*WOX1 (WUS/WOX family)*	*Brachypodium distachyon*	GOF	increased		higher number of leaf veins, thicker stems	[133]
CrSAP	*SAP*	*Capsella rubella*	LOF			decreased petal size	[66]
CsLL	*SAP*	*Cucumis sativus*	LOF	decreased	decreased	smaller fruits, smaller flowers, increased branching	[67]
CsLL	*SAP*	*Cucumis sativus*	GOF	increased	increased	bigger fruits, increased flower size	[67]
GmBS1/ 2 (GmPPD 1/2)	*PPD*	*Glycine max*	LOF	increased	increased	increased amino acid content in seeds, elongated petioles, increased ripening period	[60,62,64]
GmKIX8-1	*KIX8*	*Glycine max*	LOF	increased	increased		[65]
MtSLB1	*SAP*	*Glycine max*	GOF	increased	increased		[61]
LjBIO	*KIX8/KIX9 subfamily*	*Lotus japonicus*	LOF	increased		disrupted floral symmetry	[63]

Gene name	*Arabidopsis thaliana orthologue*	Organism	Type	Shoot/leaf size phenotype	Seed/fruit phenotype	Additional phenotypes	Refs.
LjNAO1	*WOX1 (WUS/WOX family)*	*Lotus japonicus*	LOF			small, narrow petals	[63]
MtBS1	*PPD*	*Medicago truncatula*	LOF	increased	increased	increased flower size, arrested axillary bud outgrowth	[60,61]
MtSLB1	*SAP*	*Medicago truncatula*	LOF	decreased		smaller flowers, increased lateral branching	[61]
MtSLB1	*SAP*	*Medicago truncatula*	GOF	increased	increased		[61]
MtSTF	*WOX1 (WUS/WOX family)*	*Medicago truncatula*	LOF	decreased		defective vascular patterning	[136]
NsLAM1	*WOX1 (WUS/WOX family)*	*Nicotiana sylvestris*	LOF	decreased			[136]
MtSTF	*WOX1 (WUS/WOX family)*	*Oryza sativa*	GOF	increased		higher number of leaf veins, thicker stems	[133]
MtSTF	*WOX1 (WUS/WOX family)*	*Panicum virgatum*	GOF	increased		higher number of leaf veins, enhanced regeneration, thicker stems	[133]
PsELE1	*PPD*	*Pisum sativum*	LOF	increased	increased	disrupted floral symmetry	[63]
PsBIO	*KIX8/KIX9 subfamily*	*Pisum sativum*	LOF	increased	increased	disrupted floral symmetry	[63]
PsLATH	*WOX1 (WUS/WOX family)*	*Pisum sativum*	LOF	decreased	(in strong alleles) small seed pods without seeds		[63,132]
PtBL	*SAP*	*Populus tremula x P. alba*	GOF	increased		more cells per leaf, increased adventitious rooting, reduced stem diameter and xylem formation	[68]
PtBL	*SAP*	*Populus tremula x P. alba*	LOF	decreased		decreased leaf cell number	[68]

Gene name	*Arabidopsis thaliana orthologue*	Organism	Type	Shoot/leaf size phenotype	Seed/fruit phenotype	Additional phenotypes	Refs.
SlKIX8	*KIX8*	*Solanum lycopersicum*	LOF	slightly increased			[69]
SlKIX9	*KIX9*	*Solanum lycopersicum*	LOF	no difference			[69]
SlKIX8 - SlKIX9	*KIX8/KIX9*	*Solanum lycopersicum*	LOF	increased	no difference	delayed flowering, increased fruit and pericarp size	[69]
VmPPD (MOG)	*PPD*	*Vigna mungo*	LOF	increased	increased	faster growth	[62]

[a]All comparisons are made to the corresponding wild type plants. GOF: Gain-of-function, LOF: Loss-of-function.