Review Article

The Dormancy Marker *DRM1/ARP* Associated with Dormancy but a Broader Role *In Planta*

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Plants must carefully regulate their development in order to survive a wide range of conditions. Of particular importance to this is dormancy release, deciding when to grow and when not to, given these varying conditions. In order to better understand the growth release mechanism of dormant tissue at the molecular and physiological levels, molecular markers can be used. One gene family that has a long association with dormancy, which is routinely used as a marker for dormancy release, is *DRM1/ARP* (*dormancy-associated gene-1/auxin-repressed protein*). This plant-specific gene family has high sequence identity at the protein level throughout several plant species, but its function *in planta* remains undetermined. This review brings together and critically analyzes findings on the *DRM1/ARP* family from a number of species. We focus on the relevance of this gene as a molecular marker for dormancy, raising questions of what its role might actually be in the plant.

1. Introduction

Plants are sessile; therefore, a number of mechanisms are elegantly managed in the plant to ensure its survival and optimisation in its given setting. Integral to this is the temporal and spatial determination of growth in relation to the surroundings. This growth may be vegetative, such as axillary budbreak and branch development, or floral, including flower and fruit development. To achieve this, cell division, cell elongation, or a combination of the two has been shown to play a pivotal role in plant growth regulation.

Dormancy is a means of ensuring a plant's long-term survival and is defined as the “transitory delay of visible growth that occurs in plant meristematic tissues” [1]. An example of this occurs in perennials over winter. When temperatures are low and daylight hours are limited, plants protect themselves by ceasing growth, waiting for the more favourable conditions of spring in which they focus their resources on growing and reproducing. As such, the key decisions around when to release dormant tissue from dormancy (dormancy

release/shoot outgrowth/shoot branching) are an important mechanism for plant biologists to understand.

Both perennial and annual plants (e.g., *Arabidopsis*) also undergo a type of dormancy known as apical dominance or paradormancy, where endogenous signals/hormones from the apical bud are received by lower axillary meristems, preventing their bud outgrowth. This phenomenon was first described in 1934 [2], when it was shown that axillary buds of decapitated plants were released from dormancy due to a lack of auxin flow down the stem. Since then, the interactions between auxin and other hormones including cytokinin (growth promoting) and strigolactone (branching inhibition) have been studied in-depth in order to better understand the complex cross-talk which is controlling this process [3].

Mutant lines exhibiting hyperbranching phenotypes have been integral to these dormancy release studies. These included characterisation of the *MAX/CCD/RMS* pathway involved in strigolactone biosynthesis and perception (reviewed by Seto et al. [4]); and more recently, additional hyperbranching mutants, the bud-specific dormancy release

signal integrator *brc1/tbl1*, have also been studied in order elucidate alternate pathways involved in this critical process. These studies relied heavily on the use of a genetic marker for dormancy status—*dormancy-associated gene 1 (DRM1)/auxin repressed protein (ARP)*. This review will present literature associated with *DRM1/ARP* from a number of species (summarised in Table 1) and will discuss the relevance of using *DRM1/ARP* as a molecular marker for dormant tissues.

2. *DRM1/ARP* and Dormancy

The first studied member of the *DRM1/ARP* gene family whose transcript expression was linked with dormancy physiology, *PsDRM1* (*dormancy-associated gene*)-1, was isolated from differential display performed with cDNA libraries from dormant and growing axillary buds of pea. To consider the relationship between expression of this gene and axillary bud dormancy, Stafstrom et al. [5] decapitated Alaska pea plants. By removing the apical buds of the pea plants, the bud can be released from paradormancy (apical dominance), allowing outgrowth of the axillary buds (Figure 1). *PsDRM1* expression was analysed by northern blot in the bud at various time points after decapitation and revealed that upon release of dormancy, *PsDRM1* levels decreased 20-fold with a concomitant increase in the expression of the cell cycle marker gene, *PsRPL27* [5].

Two homologues to *PsDRM1* have been identified in *Arabidopsis*: *AtDRM1* (*At1g28330*) and *AtDRM2* (*At2g33830*; formally known as *AtDRM1-homologue*). As in pea, a decapitation was conducted in *Arabidopsis* showing that *AtDRM1* and *AtDRM2* expression levels decreased with the release of paradormancy [6]. Subsequent expression levels increased from 24 h after decapitation, due to a putative apical dominance from the primary axillary outgrowth, leading to paradormancy in the lower buds.

Dormancy release experiments performed with *AdDRM1*, the *DRM1/ARP* homologue in *Actinidia deliciosa* (kiwifruit), using hydrogen cyanamide (commercially available as HiCane) as a dormancy release agent show the same expression profiles [7]. Treatment with hydrogen cyanamide was used to release the apical buds from endodormancy (compared with decapitation initiating release of apical buds from paradormancy in pea and *Arabidopsis*). Upon release of bud dormancy, the characteristic decrease in *AdDRM1* expression was seen. In the same experiment, the expression of *AdCDKB* (*cyclin-dependant protein kinase B*), a marker for growth from kiwifruit, was assessed. *Cyclin-dependent kinases* (CDKs) are known to be involved in the reinitiation of cell cycle progression through the G₁-S phase transition [8]. As *AdDRM1* levels decrease, *AdCDKB* expression increases [7].

Further evidence of an association of *DRM1/ARP* transcripts with periods of dormancy is seen in *PsARP* (*Paeonia suffruticosa auxin-repressed protein*), a gene identified as being associated with the release of dormant buds of tree peony. *PsARP* showed a sharp increase prior to bud break and then dropped to low levels as the buds grew [9].

In most cases, increases in *DRM1/ARP* expression are associated with reduced branching phenotypes: *phyb* [10], *tin*

FIGURE 1: The “decapitation experiment” used by Stafstrom et al. [5] to induce release of dormancy.

[11], and *CHR2 OX* [12]; and decreases in *DRM1/ARP* expression are associated with increased branching phenotypes: *axr1-12* [13], *max2* [13], and *chr12* [12] (Table 2). However, *DRM1/ARP* has also been used as a marker for dormancy in *Arabidopsis* in studies of *Teosinte branched 1-like* or *branched 1* (*TBL1* or *BRC1*) [13] with less dependability. Results showed that a reduction in mRNA expression of *DRM1/ARP* was not required for bud outgrowth leading to the conclusion at the time that *DRM1/ARP* was probably acting upstream of *TBL1* [13]. Alternatively, *DRM1/ARP* could be acting independently of *TBL1*. In the same study, there was no change in *DRM1/ARP* transcript levels in hyperbranching 35S:*YUCCA* where you may anticipate a reduction in expression, providing further evidence that *DRM1/ARP* may not be playing a role specifically in holding back bud outgrowth.

These findings weaken the argument for *DRM1/ARP* as a molecular marker for dormancy in axillary buds. Alternatively, these data suggest that *DRM1/ARP* is playing a role as an integrator only under a subset of specific dormancy-related conditions.

3. The *DRM1/ARP* Protein Family

Database searches show that the *DRM1/ARP* family is unique to plants, with homologues found in both monocotyledons and Dicotyledons [25]. Multiple protein sequence alignments show high conservation of the *DRM1/ARP* protein product, with two domains clearly identifiable at the amino-terminus and at the carboxy-terminus [7, 25]. This high conservation across species, and indeed monocotyledons and Dicotyledons, suggests a potential importance of the action of this protein in the normal development and physiology of plants. Moreover, the protein product is conserved in its size, with all 11 species analysed by Park and Han [25] between 11 and 14 kDa (between 109 and 126 amino acid residues). GFP fusion constructs of *EuNOD-ARPI* (*EuNOD-ARPI*; *Elaeagnus umbellata nodule auxin-repressed protein gene 1*), a *DRM1/ARP* homologue in the ornamental shrub *E. umbellata* (Japanese silverberry, Autumn-Olive), as well as GFP fusion constructs of *Brassica rapa* *DRM1* and *ARPI* suggest that it is cytosol-localised [16, 18]. However, *in silico*

TABLE 1: Summary of the characteristics and putative function of known DRM1/ARP family members.

Species	Protein identifier	Gene name	Possible function	Predicted protein length (aa)	Predicted protein size (kD)	Predicted pI	Predicted extinction coefficient	References
<i>Actinidia deliciosa</i>	FG458205	<i>Ad DRM1 ID/ AdDRM1</i>	Axillary bud dormancy maintenance and growth repression in mature fruit	115	12.722	10.58	19,480	Wood et al., 2013 [7]
<i>Arabidopsis thaliana</i>	NP_001154378	<i>AtDRM1</i>	Axillary bud dormancy maintenance and leaf maturation	122	13.406	10.71	19,480	Tatematsu et al., 2005 [6]
	NP_850220	<i>AtDRM2</i>	Axillary bud dormancy maintenance	108	11.500	10.32	19,480	
<i>Arachis hypogaea</i>	AAZ20292	<i>AhDRM1</i>	Drought stress response	121	13.459	10.19	22,460	Govind et al., 2009 [14]
<i>Brassica oleracea</i>	AAL674436	<i>BoDRM1</i>	Germination in suboptimal conditions	105	11.352	10.10	19,480	Soeda et al., 2005 [15]
<i>Brassica rapa</i>	ACQ90305	<i>BrDRM1</i>	Prevention of germination; prevention of hypocotyl elongation; temperature and salt stress response	128	14.035	9.35	18.115	Lee et al., 2013 [16]
	AAO32054	<i>BrARP1</i>		108	11.651	10.10	19,480	
<i>Capsicum annuum</i>	AAR8388.1	<i>CaARP1</i>	Cold and salt stress response	Only partial available				Hwang et al., 2005 [17]
<i>Elaeagnus umbellata</i>	AAC62104	<i>EuNOD-ARPI</i>	Pathogen response in root nodules	120	12.985	10.26	19,480	Kim et al., 2007 [18]
<i>Fragaria x ananassa</i>	Q05349	<i>FaλSAR5</i>	Growth repression in mature fruit	111	12.416	9.69	19,480	Reddy and Poovaiah, 1990 [19]
<i>Malus x domestica</i>	AAA71994	<i>MdAPI</i>	Growth repression in mature fruit	119	13.060	10.01	19,480	Lee et al., 1993 [20]
<i>Nicotiana benthamiana</i>	GW691612	<i>NbDRM3</i>	PTI immunity	127	13.623	10.36	16,960	Chakravarthy et al., 2010 [21]
<i>Nicotiana tabacum</i>	AAO21304	<i>NtARPL1;1</i>	Growth repression in mature pollen and flowers	137	14.636	11.39	20,970	Steiner et al., 2003 [22]
<i>Paeonia suffruticosa</i>	ABW74471	<i>PsARP</i>	Axillary bud dormancy maintenance	126	13.725	10.39	20,970	Huang et al., 2008 [9]
<i>Pisum sativum</i>	AAB84193	<i>PsDRM1</i>	Bud dormancy maintenance and growth repression in mature tissues	111	12.284	10.26	19,480	Stafstrom., 2000 [23]; Stafstrom et al., 1998 [5]

TABLE I: Continued.

Species	Protein identifier	Gene name	Possible function	Predicted protein length (aa)	Predicted protein size (kD)	Predicted pI	Predicted extinction coefficient	References
<i>Pyrus pyrifolia</i>	AGG19165	<i>PpARP1</i>	Growth repression in mature fruit	116	12.631	10.26	19,480	Shi et al., 2013 [24]
	AGG19166	<i>PpARP2</i>		139	14.273	10.58	9,970	
<i>Robinia pseudoacacia</i>	AAG33924	<i>RpARP</i>	Elongation repression in hypocotyls	115	12.641	10.22	19,480	Park and Han, 2003 [25]
<i>Solanum lycopersicum</i>	ABH07900	<i>SlDRM1</i>	Growth repression in “dormant” ovaries prior to fruit set	123	13.442	10.11	20,970	Vriezen et al., 2008 [26]

TABLE 2: Transcript expression profiles of *DRM1/ARP* family members in characterised mutants.

Species	Mutant	Mutant phenotype	Transcript expression profile in mutant <i>cf.</i> WT	Correlation between <i>DRM1/ARP</i> expression and reduced branching/enhanced dormancy?	Reference
<i>Arabidopsis thaliana</i>	<i>phyb</i>	Reduced branching, hyperelongation	↑	y	Finlayson et al., 2010 [10]
<i>Arabidopsis thaliana</i>	<i>tbl1/brc1</i>	Highly branched mutant	—	n	Finlayson, 2007 [13]
<i>Arabidopsis thaliana</i>	<i>axr1-12</i>	Highly branched mutant	↓ (buds)	y	Finlayson, 2007 [13]
<i>Arabidopsis thaliana</i>	<i>max2</i>	Highly branched mutant	↓ (buds)	y	Finlayson, 2007 [13]
<i>Arabidopsis thaliana</i>	35S:YUCCA	Reduced branching	—	n	Finlayson, 2007 [13]
Wheat (<i>Triticum aestivum</i>)	<i>tin</i>	Reduced tillering/branching	↑	y	Kebrom et al., 2012 [11]
Sorghum (<i>Sorghum bicolor</i>)	<i>phyb-1</i>	Reduced branching	↑	y	Kebrom et al., 2006 [27]; Kebrom et al., 2010 [28]
<i>Arabidopsis thaliana</i>	<i>chr12</i>	Reduced growth arrest when exposed to moderate stress	↓	Y	Mlynárová et al., 2007 [12]
<i>Arabidopsis thaliana</i>	<i>CHR12 OX</i>	Growth arrest of normally active primary buds, and reduced growth of primary shoot, only upon perception of stress conditions	↑	Y	Mlynárová et al., 2007 [12]

prediction shows AtDRM1 in the nucleus and mitochondria, possibly suggesting differing roles for family members from different species. No characterised localisation signals or DNA-binding domains are found in the protein sequence. Additionally, the PFAM motif (PF05564), “dormancy-/auxin-associated protein,” represents almost the entire protein, leaving few hints on the role of this protein *in planta*.

Recently evidence has emerged for DRM1/ARP family members as intrinsically disordered proteins (IDPs) [7]. IDPs, found in plants and animals, have a large proportion of their sequence yielding no fixed tertiary structure, yet still providing some biological function [29]. Signal transduction and transcriptional regulation are key processes within which structural disorder is prominent [30, 31]. A well-characterised example of IDPs in the plant kingdom is the late embryogenesis abundant (LEA) class of proteins, involved in abiotic stress tolerance [32, 33] as chaperones for other stress response genes [34–36]. ERD10 and ERD14 (early response to dehydration) are members of the dehydrin type of LEA proteins, shown to be both intrinsically disordered and

responding to high salinity, drought, and low temperature stresses in *Arabidopsis* [36]. Similarly, a (LEA)-like phosphoprotein (CDeT11-24) whose expression is associated with periods of dessication has been identified in the resurrection plant *Craterostigma plantagineum* [37]. Indeed with these findings in mind, DRM1/ARP family members may have roles in chaperoning proteins critical to the no-growth phase in tissues.

Despite the high degree of conservation across species, the biological significance of the DRM1/ARP protein family remains unknown.

4. Potential Role of *DRM1/ARP* in the Growth/No-Growth Decision outside of Meristematic Tissues

Despite being a commonly used marker for dormancy, *DRM1/ARP* is expressed in tissues other than dormant axillary buds with high expression often localised to nongrowing tissues.

Fruit development is an example of the growth/no-growth homeostasis in floral tissues (cf. vegetative tissues) and as such provides further evidence of *DRM1/ARP*'s association with nongrowing tissues. For example, one *DRM1/ARP* homologue, *API*, was first discovered in cortical tissue from ripe apple fruit [20]. In addition, Reddy and Poovaiah [19] showed a positive correlation between strawberry (*Fragaria ananassa*) fruit growth and repression of a homologue identified from a library of fruit starved for auxin, *λSAR5*, while being highly expressed in mature fruit which have stopped growing. More recently, *Pyrus pyrifolia ARP1* (*PpARP1*) and *PpARP2* expression was detected in the mesocarp tissue of Pear (*Pyrus pyrifolia*) fruit [24].

Similar data has been seen in kiwifruit with *AdDRM1* levels reaching a peak in ripe, mature fruit (unpublished: M. Wood), and in tomato where high levels of the gene transcript were localised to the ovaries, with a decrease exhibited after fruit set [26]. It is hypothesised that unpollinated ovaries are in a temporally dormant state, supported by expression of cell cycle genes induced after fruit set [26]. As these tissues may have included seeds and *in silico* expression profiles show seeds have high levels of *SIDRM1* expression, further analysis is required in these species to clarify whether this increase in expression is a result of increases in the maturing fruit flesh or rather in the associated maturing seeds.

Increased *DRM1/ARP* gene expression has also been described in tobacco flowers [22]; mature stems, leaves, and roots of pea [5]; dehiscent pollen of tobacco [22]; and root nodules of *E. umbellata* [18]. While not at particular high levels, *DRM1/ARP* transcript has also been detected in petioles, seeds, various floral organs, and across trunk wood of mature trees [5, 20, 22, 25, 38].

A classical example of tissue aging and transitioning into growth suppression is provided in the leaf senescence process. *Arabidopsis max2* mutants, already described earlier for their hyperbranching phenotype, also exhibit increased longevity during leaf senescence [39]. In *Arabidopsis*, *MAX2* transcript expression peaks during the early stages of senescence, while *AtDRM1* expression continually increases throughout senescence, reaching a maximum when yellowing begins, at which point expression remained reasonably constant [40]. These data suggest that *DRM1/ARP* is involved in leaf senescence, but later in the process than *MAX2*, raising the question; is *MAX2* controlling *DRM1/ARP*?

An interesting hypothesis given by Park and Han [25] is for a putative *in planta* role for *DRM1/ARP* family members in repression of cell elongation. The authors introduced an inverse correlation between expression of the *DRM1/ARP* homologue in black locust, *RpARP* (*Robinia pseudoacacia auxin-repressed protein*) and the elongation zone of 7-day-old hypocotyls. Possible contradictory evidence is found in the hyperelongated *phyb* mutant which has increased *DRM1* transcript expression in *Arabidopsis* axillary buds [10]. To clarify this, *AtDRM1* transcript expression would need to be measured in the hyperelongating hypocotyl tissue of the *phyb* mutant.

PsAD2, another gene identified as being associated with periods of dormancy, showed similar expression profiles outside of purely dormant meristematic tissues (accumulated

in axillary buds, roots, mature leaflets, and elongated stems) [41].

5. *DRM1/ARP* and Seed Dormancy

While characteristics of seed dormancy are not generally linked with bud dormancy, some striking similarities do exist. Seed dormancy is defined as the failure of a seed to germinate despite favourable conditions [42, 43]. Some chilling is often required for either seed germination or bud break, in perennial species at least. However, dormancy requires long-term exposure to low temperatures, while stratification of seeds is relatively short term [44]. Microarray expression data show that in *Arabidopsis*, high levels of *AtDRM1* transcript significantly reduce with imbibition [45], while transgenic *Arabidopsis* lines overexpressing *BrDRM1* or *BrARP1* exhibited delayed germination phenotypes, providing evidence that *DRM1/ARP* family members might be involved in seed dormancy maintenance. Assessment of relative *DRM1/ARP* levels in a seed germination microarray of barley showed that *DRM1* was unaffected [46].

Osmopriming of seeds is a process which initiates germination-related processes but does not allow radicle protrusion due the high osmolarity solutions used, limiting water availability. A study completed on *Brassica oleracea*, comparing the transcriptome of osmoprimed seeds with that of water germinated seeds, identified that *DRM1/ARP* was expressed in seeds germinated on water and not the osmoprimed seeds [15]. This finding conflicts with the reduction in transcript with imbibition seen in closely related *Arabidopsis* [45], suggesting that *DRM1/ARP*'s expression levels throughout germination are not integral to the process but might be indirectly linked. This profile in *B. oleracea* was shared with a number of stress-associated genes, including *cyclophilin*, *superoxide dismutase*, *GRP2*, and *glutathione-S-transferase* [15], providing evidence that *DRM1/ARP* might be playing a more general role in stress response in the seed.

6. Regulation of *DRM1/ARP* Family Gene Expression

Regulation of *DRM1/ARP* has been assessed in response to a number of treatments: hormonal, sugars, and abiotic and biotic across a number of plant species.

6.1. Hormonal Regulation of *DRM1/ARP*. As with many plant genes, *DRM1/ARP* is regulated hormonally. In particular, a number of often conflicting lines of evidence exist suggesting that *DRM1/ARP* family members are regulated by auxin, hence many family members being named ARPs (*auxin-repressed proteins*).

In 1990, a clone named *λSAR5* was identified from cDNA library of strawberry (*Fragaria ananassa*) fruit starved for auxin [19] and was later found to be a homologue of *DRM1/ARP*. Strawberry receptacles (the red-fleshed, seed/achene-coated organ) were used as a model for auxin action as the achenes are a source of auxin which controls the receptacle growth in a pollination-dependent manner. Receptacles can be de-achened and then auxin applied exogenously mimicking normal fruit development.

Within 6 h from the start of auxin treatment, λ SAR5 mRNA levels were significantly lower than those of receptacles with no auxin, suggesting that high levels of auxin may have a role in the downregulation of the *DRM1/ARP* gene. Repression was not shown with a weak acid control suggesting that any regulatory effects were not simply a result of auxin acid effect. The auxin analogue selected for these experiments was the synthetic naphthalene acetic acid (NAA), as opposed to the naturally occurring indole-3-acetic acid (IAA) for example. NAA can induce stronger effects than IAA due in part to being metabolised more slowly in the plant [47]; therefore any discrepancies observed between NAA and IAA may be a consequence of the nature of the two compounds, although this remains to be determined. Furthermore, NAA was applied at 1 mM, significantly greater than an accepted biologically relevant $\mu\text{mol gram}^{-1}$ fresh weight concentrations as determined in *Arabidopsis* and tobacco [48]. Pollinated fruit which have an endogenous source of auxin from the resulting achenes provide a more biologically relevant assessment of λ SAR5 mRNA levels in response to auxin. As expected, λ SAR5 mRNA levels are higher at 3 and 5 days after pollination (with auxin) than the unpollinated equivalents (no auxin), allowing the conclusion to be drawn that λ SAR5 is repressed during normal development of strawberry fruit in response to exogenous auxin. It is noteworthy that the process of de-achening, which was integral to this approach, would likely have induced a number of stress response pathways not specific to the exogenously applied auxin, which may have had separate effects on *DRM1/ARP* transcription.

A further auxin-repressed homologue from black locust, *RpARP*, was identified from an EST database of trunk wood from 10-year-old tree, with gene expression seen across all sections of the trunk wood [25]. *RpARP* levels decrease upon application of exogenous auxin in concentrations as low as $0.1 \mu\text{M}$. Upon removal of the source of auxin, *RpARP* levels increased again, showing a negative relationship between *DRM1/ARP* family member expression and auxin treatment. Unfortunately, this study also used the synthetic auxin isomer NAA and most of the experiments were completed with 1 mM auxin, beyond the accepted biologically relevant concentration.

Analysis of the roles of noncoding sequences showed that regulatory sequences for auxin repression were located in the UTR sequence as opposed to the promoter sequence for *RpARP* in transgenic *Arabidopsis* plants, supporting a possible posttranscriptional regulatory mechanism [25]. This regulation, however, is not mediated by the characterised mRNA instability sequences including *cis*-elements such as the small auxin upregulated transcript (SAUR) sequences found in the 3' UTR of several plant genes [49] or the mammalian AU-rich elements (AREs) [25, 50]. The data suggest a novel regulation mechanism that warrants further validation.

While *RpARP* and indeed λ SAR5 are showing some evidence for downregulation in their transcript levels in response to auxin, the timeframe in which this happens can provide insight into whether or not they are likely to be responding directly or indirectly to auxin. Gene expression

of primary auxin response genes occurs within a matter of minutes [51–54]. Those genes whose regulation by auxin takes longer than this are generally referred to as secondary auxin responders and are likely to be further downstream in the signalling cascade.

In contrast, analysis carried out in *E. umbellata* showed that transcript levels in field-grown leaves of a clone identified from a root nodule cDNA library, *EuNOD-ARP1*, were induced by exogenous auxin treatment after 6 h [18]. Furthermore, pear mesocarp discs exhibited an increase in transcript expression of *PpARP1* and *PpARP2* with IAA treatment [24]. These pieces of data contradict previous findings in strawberry [19], black locust [25], and in pea [23]. The type and age of the tissue assessed are a major difference between these studies which might explain these variations in auxin response.

A further conflicting finding has been described in *Arabidopsis*, where auxin overproducing 35S:*YUCCA Arabidopsis* lines displayed no change in *AtDRM1* expression compared to wild-type plants (Table 2) [13]. These mutants showed a reduced branching phenotype and approximately twice the amount of free auxin compared with WT plants [55]. As such Finlayson [13] argued that *DRM1/ARP* is unlikely to be affected directly by auxin, if at all.

More recently, transgenic *Arabidopsis* lines overexpressing the coding region of *BrDRM1* and *BrARP1*, which exhibit reduced hypocotyl growth in dark-grown seedlings, showed a compounded growth suppression with NAA application [16]. That these constructs used the coding region only and therefore do not contain any UTR sequence which would be required for an auxin response based on the *Robinia pseudoacacia* data [25] suggests that any auxin responses in this gene family might be regulated by different mechanisms in different species.

Overall these data question whether or not *DRM1/ARP* family members are regulated by auxin with conflicting data emerging from various experimental setups. Thorough analyses of auxin regulation *in planta* with a focus on biological relevance are therefore required.

Only scarce data are available pertaining to the effects of hormones other than auxin on expression of *DRM1/ARP* family members. In pea it was shown that *PsDRM1* is induced when samples were treated with ABA [23], while GA had no effect on transcript levels. Despite being the sole piece of published data on ABA regulation of *DRM1/ARP*, the gene has been used as a genetic marker for ABA response in studies of fruit set in tomato [26]. Vriezen et al. [26] argue that the decrease in *DRM1/ARP* after fruit set might be due to lower levels of ABA in the ovary after induction of fruit growth, although this remains to be assessed further.

Few data are available on any response of *DRM1/ARP* family members to cytokinin treatment; however, it would be expected that cytokinin treatment, which is generally associated with an increase in cell division, would lead to a decrease in transcript levels of *DRM1/ARP*. The only data available are from a transcriptomic approach comparing *Arabidopsis* seedlings treated with BAP with nontreated plants. A decrease in both *AtDRM1* and *AtDRM2* levels was observed

but further validation of this finding remains to be completed [56].

6.2. Regulation of *DRM1/ARP* Family Members by Sugars. A plant needs carbohydrates in the form of sugars to grow. In particular, sugar is required for expression of the cell cycle gene *CyclinD3* [57, 58]. Similarly, carbohydrate metabolism genes have been shown to change over the three distinct phases of dormancy in the buds of the perennial weed, leafy spurge [59], making a clear link between sugar levels and ratios to dormancy.

An association between sugar and *DRM1/ARP* was first described in black locust in 2003 [25]. In an attempt to further understand auxin regulation of *RpARP*, sucrose was analysed as a factor associated with auxin-mediated growth. Sugar deprivation led to an increase in *RpARP* gene expression. As discussed above in this paper's findings on auxin regulation, the age of this tissue, and therefore whether or not it was growing, was not mentioned in the text and age of the tissue may have an impact on the findings. Upon discovery of the sugar response, the upstream promoter sequence was screened and four sugar responsive elements (SREs), known to be responsible for sugar repression of the α -amylase gene in rice [60], were identified [61]. Furthermore, it was shown that the promoter sequence was required to mediate the sugar response [25].

A subsequent, in-depth study of sugar regulation of *DRM1/ARP*, amongst other genes, was carried out by Gonzali et al. [62]. In support of the findings of Park and Han [25], they described almost a complete repression of *AtDRM1* by sucrose, fructose, and glucose, but not by other sugars including turanose and mannitol. As modulation of *AtDRM1* was seen as a result of more than one sugar type, it was concluded that *AtDRM1* could be a low-specificity sensor of sugars [62].

More recently, studies of the reduced branching *tin* (tiller inhibition) mutant in wheat (*Triticum aestivum*) have drawn a link between sugar levels, dormancy status, and *DRM1/ARP* [11]. The dormant buds of *tin* show reduced sucrose content, transcriptional responses in both sucrose-inducible and sucrose starvation-inducible markers (downregulation and upregulation, resp.) and reduction in transcript expression of cell cycle markers leading to the hypothesis that the extensive bud dormancy seen in the mutant is due to cessation of cell division due to diversion of sucrose away from the buds. The dormancy status of the buds of *tin* plants was confirmed by *DRM1/ARP* and *TBL1* transcript expression assessment leading to an additional hypothesis that *DRM1/ARP* (as well as *TBL1*) are involved in dormancy responses specifically related to sucrose starvation [11]. Data from kiwifruit have shown that sucrose levels accumulate in dormant buds over winter and reduce again prior to bud break in spring [63]. Indeed, application of hydrogen cyanamide is associated with a rapid decrease in both detectable sucrose levels [63] and *DRM1/ARP* transcript levels [7].

Work in *Arabidopsis* showed a somewhat contradictory result [6]. A microarray was carried out allowing categorisation of genes categorised as either up- or downregulated in response to inflorescence decapitation. Both *AtDRM1*

and *AtDRM2* were downregulated in buds upon decapitation [6]. A search for regulatory elements enriched in promoter sequences of either group identified Sugar Responsive Elements (SREs) as being highly conserved in genes downregulated with decapitation. It must be noted, however, that this work only analysed the initial 500 bp upstream of the start codon and as such possibly neglected the majority of the promoter sequences. Furthermore, these motifs remain to be assessed biologically, which will inevitably provide a more robust basis for any hypotheses. These conflicting pieces of data suggest that *DRM1/ARP* family members are unlikely to be directly regulated by sugar levels.

6.3. Regulation of *DRM1/ARP* Family Members by Abiotic and Biotic Stresses. Across the plant kingdom, *DRM1/ARP* is increasingly being linked to stress responses, whether they are a result of abiotic or biotic factors.

Increases in *DRM1/ARP* transcript levels have been associated with the light signalling pathway mutant, *phyb*, in both *Arabidopsis* and *Sorghum* (*Sorghum bicolor*) [10, 27, 28] (Table 2). *phyb* mutants have reduced bud outgrowth, with a concomitant increase in *DRM1* expression [10, 27, 28], providing support for the hypothesis that *DRM1/ARP* is involved in growth suppression in bud and hypocotyls tissues.

Other abiotic stress factors have also been considered. An increase in *DRM1/ARP* expression with cold treatment or salt stress has been documented in pea [23], *Capsicum annuum* [17], and the close relative of *Arabidopsis*, *B. rapa* [16]. During times of eco-dormancy due to cold, it might be anticipated that *DRM1/ARP* expression is high, suggesting a role for *DRM1/ARP* in temperature signalling. An interesting set of experiments considered the effect of various growth regulators and abiotic stresses in conjunction with auxin application on *RpARP* gene expression [25]. Data showed that *RpARP* expression was induced either with or without auxin by cold stress providing evidence for cold stress overriding any auxin response. Other treatments showed no significant effect on transcript profiles. These data provide further evidence for *DRM1/ARP* family members not being directly regulated by auxin.

DRM1/ARP transcript expression has been assessed in response to further abiotic factors including drought conditions in peanut (*Arachis hypogaea*) [14] and heat shock in *B. rapa* [16], with both conditions leading to an increase in expression.

AtDRM1 and *AtDRM2* levels are increased in the *SNF2/Brahma-type AtCHR12* chromatin-remodelling gene overexpression lines, introduced earlier, which undergo arrest of axillary bud outgrowth and a reduction in inflorescence bolt growth, only in response to stress conditions [12]. Conversely, transcript levels of *AtDRM1* and *AtDRM2* are downregulated in *Atchr12* knock-out mutants [12]. These findings suggest that *AtDRM1/ARP* genes might be acting downstream of *AtCHR12* in a stress-modulated pathway.

As well as being involved in responses to abiotic factors, *DRM1/ARP* family members have also been linked to biotic stress responses. A cell death-based assay was undertaken using transient virus-induced gene silencing of *DRM3* (homologue of a closely related *DRM* family member

FIGURE 2: Schematic diagram of hypothetical *in planta* functions of DRM1/ARP. Mitogen activated protein (MAP) kinase, kinase, kinase (MKKK), MAP kinase, kinase (MKK), and MAP kinase (MK) are representative of a generalised signalling cascade resulting in expression of *DRM1/ARP*'s, in response to a range of abiotic, biotic, and endogenous factors. *DRM1/ARP* proteins are hypothesised to play a role in growth inhibition via a currently unknown pathway.

At1g56220) in *Nicotiana benthamiana*. Plants silenced for *NbDRM3* and challenged with a number of bacterial effectors were compromised in their pathogen-associated molecular pattern-triggered immunity (PTI) [21].

These data combined with the fact that DRM1/ARP proteins in kiwifruit have been shown to be IDPs [7], a class of proteins often associated with stress response pathways in plants, as well as multiple lines of evidence showing transcript expression outside of purely dormant tissue provide increasing evidence that DRM1/ARPs are involved in responding to suboptimal conditions, whether they be abiotic or biotic, and whether this is in meristematic tissue with (by definition) the potential to go dormant or not.

7. *DRM1/ARP* Mutants

Analysis of plant lines either overexpressing a gene and its resulting protein or downregulating expression is a powerful tool for characterizing a gene's role *in planta*.

Studies conducted in black locust [25] stated that overexpression of full length *RpARP* cDNA either with or without the UTRs resulted in *Arabidopsis* plants with no change in phenotype compared with wild-type plants. Similarly, overexpression of *EuNOD-ARPI* cDNA in *Arabidopsis* had no noticeable phenotype [18].

More recently, data have emerged claiming a number of alterations to the visible phenotype of *Arabidopsis* plants

overexpressing cDNA of either of two homologues of *DRM1* and *ARPI* from *B. rapa*. These included a reduction in vegetative growth (petiole length), as well as a reduction in seed productivity (silique length). A general delay in growth with no significant impact on bolting times was also reported, resulting in a reduction in the number of leaves associated with the vegetative-floral phase transition. In addition, transgenic plants exhibited a reduction in bolt length and a delay in seed germination. Double knockouts to *AtDRM1* and *AtDRM2* exhibited no change in phenotype [16]. These findings are of particular interest as they conflict with other species assessed thus far, providing further evidence of differing roles of *DRM1/ARP* family members across different plant species.

8. Conclusions and Future Directions

As highlighted in this review, *DRM1/ARP* transcript expression has generally been associated with dormant buds. Exceptions to this association are seen in *tbl1/brcl* and *35S:YUCCA* mutants, suggesting that *DRM1/ARP* is not playing a specific role in dormancy maintenance and as such may not be a reliable maker for dormancy release in future studies. Furthermore, the detection of transcripts outside of meristematic tissues, by definition the site of dormancy, often in nongrowing tissue means that the correlation between *DRM1/ARP* and dormant tissues is more likely representative of a link between the nongrowth status of these tissues, as opposed to dormancy *per se* (Figure 2).

This plant-specific gene and its resulting low molecular weight protein are highly conserved, and it may be negatively regulated by auxin. Ever since the identification of the dormancy-associated gene *DRM1/ARP* in pea more than a decade ago, and more recently in other species, its mode of action has still to be elucidated. Future work will require a multifaceted approach in order to draw conclusions on the function of *DRM1/ARP* in *planta*. Due to the conflicting findings around *DRM1/ARP* mutants, further analysis on a number of species is required to clarify the effect that either overexpression or downregulation of *DRM1/ARP* genes has on the plant. Transcriptome analysis of these various mutants will provide further information on the function of *DRM1/ARP*. Further physiological and transcriptional studies considering additional abiotic and biotic stress factors, as well as those already described in more depth, will be of interest. Antibodies would be helpful to use for protein localisation studies which could complement promoter: *GUS* analysis looking into the means of transcriptional regulation of this gene family. Finally, protein-protein interaction experiments, such as yeast-2-hybrid assays, could allow elucidation of binding partners.

Through identification of genetic pathways the genes are involved in; identification of the regulatory mechanisms of the genes; localisation studies at the protein level; and identification of binding partners, the function of *DRM1/ARP* will be clarified.

Conflict of Interests

No conflict of interests exists for any of the authors in relation to the work discussed in this paper.

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