Predicting maternal auxin-signaling networks for seed dormancy regulation in Arabidopsis

Yixing Wang and Ming Yang (ming.yang@okstate.edu)
Department of Plant Biology, Ecology, and Evolution, Oklahoma State University, Stillwater, OK, USA

ABSTRACT

Auxin, like ABA, promotes seed dormancy, but how auxin promotes seed dormancy is not well understood. In studying seed dormancy regulation, we found that AUXIN SIGNALING F-BOX PROTEIN 1 (AFB1) and 5 maternally promoted seed dormancy and AFB1 had a stronger effect than AFB5. *AFB1* and *5* were expressed in the funiculus and the chalazal seed coat at the mature embryo stage, and *AFB1*, not *AFB5*, was also transiently expressed in a small chalazal seed coat region surrounding the remnant funiculus during seed imbibition. Analysis of publically available datasets for genes expressed in the funiculus and seed coat at the mature embryo stage allowed the assignment of the six *AFB*s into two groups: *TIR1*, *AFB1* and *4* as the first group with higher expression levels in the funiculus than in the chalazal seed coat, and *AFB2*, *3* and *5* as the second group with higher expression levels in the chalazal seed coat than in the funiculus. It was then assumed that auxin-upregulated and -downregulated genes associated with the first *AFB* group should be expressed at higher and lower levels in the funiculus than in the chalazal seed coat, respectively, and the reverse is assumed for those associated with the second *AFB* group. Three potential auxin-signaling networks including 30 genes were identified based on these assumptions and high linear correlation in expression within each group. These networks do not overlap in components and two-thirds of the genes are known or predicted to function in seed germination either positively or negatively. The presence of both positive and negative regulators in each of the networks is consistent with the plant's ability to either remain dormant or go into germination in response to environmental conditions. The identified components of the networks also suggest interactions of auxin with other hormones in seed dormancy regulation.

Introduction

Maintaining or breaking seed dormancy in appropriate environmental conditions is crucial to survival of plants. The molecular mechanism governing seed dormancy regulation is very complex as many proteins of diverse functions and multiple hormones have been reported to affect seed dormancy. In particular, the plant hormones abscisic acid (ABA) and auxin are well documented to play positive roles in seed dormancy regulation whereas gibberellins are known for inducing seed germination. In addition, a classical function of phytochrome photoreceptors is to promote seed germination. However, current understanding of seed dormancy regulation has not been established at the systems level. A systems-level understanding is especially relevant to seed dormancy regulation as seed dormancy and germination can be viewed as emergent properties of a complex system when responding to environmental conditions.

Results

Maternal AFB1 and AFB5 positively regulate seed dormancy

Table 1. T_2 Seed germination phenotypes and seedling sensitivity to IAA*

	Transgenic line	Seed germination	Sensitivity to IAA
AFB1:AFB1	2-2	Defective	More sensitive
	2-3	Normal	Less sensitive
	3-1	Defective	More sensitive
	3-3	Defective	More sensitive
	3-4	Normal	Normal
	3-5	Defective	More sensitive
	4-2	Defective	More sensitive
	5-1	Defective	More sensitive
	5-6	Normal	Normal
	5-7	Normal	Less sensitive
	6-1	Defective	More sensitive
	6-2	Defective	More sensitive
	6-3	Defective	More sensitive
	6-4	Defective	More sensitive
AFB5:AFB5	1-4	Normal	Less sensitive
	1-5	Defective	More sensitive
	2-7	Normal	Normal
	3-3	Defective	More sensitive
	4-5	Defective	More sensitive

*T_2 and Col-0 (control) seeds were planted on agar medium with 0.01 µM IAA to assess their sensitivity to IAA. The sensitivity levels were based on the comparison of root lengths between the T_2 plants and the Col-0 plants. Parallel experiments on agar medium without IAA were used to assess seed germination.

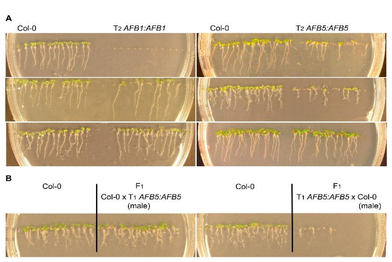

Fig. 1. Seed germination phenotypes. A) T_2 and wild-type (Col-0) seeds. Three independent T_2 lines are shown for *AFB1:AFB1* and *AFB5:AFB5*, respectively. B) F_1 and Col-0 seeds. The complete non-germination phenotype in some of the T_2 *AFB1:AFB1* seeds indicated that the *AFB1* transgene caused the seed dormancy phenotype in the maternal tissues. Reciprocal crosses in Fig. 1B also indicated that the *AFB5* transgene promoted seed dormancy in the maternal tissues.

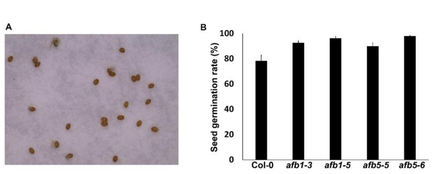

Fig. 2. Seed germination rates. A) Col-0 seeds after 72 hours on moist filter paper, showing the germination assay used in the investigation of seed germination rate. B) Germination rates of Col-0 and *afb1* and *5* mutants. Seeds of all mutant alleles germinated significantly faster than Col-0 seeds (≥150 seeds were counted for each sample, n = 10, t-test, $p < 0.05$)

References

Belmonte MF et al. Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed. Proc Natl Acad Sci USA, 2013, 110:E435–E444.
Khan D et al. Transcriptome atlas of the Arabidopsis funiculus--a study of maternal seed subregions. Plant J, 2015, 82:41-53.
Goda H et al. Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. Plant Physiol, 2004, 134:1555-73.

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Expression patterns of *AFB1* and *5* in mature fruit and seeds

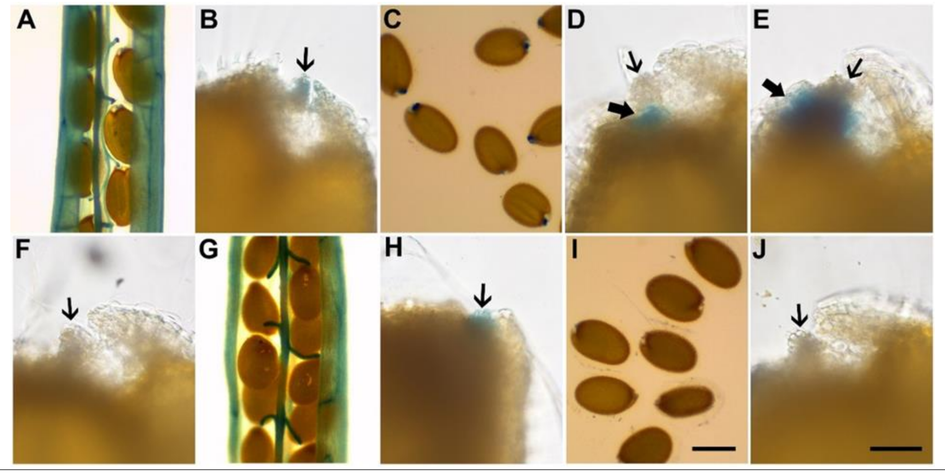

Fig. 3. GUS staining patterns in dehisced siliques and postharvest seeds in *AFB1:GUS* and *AFB5:GUS* lines. (A-F) *AFB1:GUS*. (G-J) *AFB5:GUS*. A) T_1 silique at dehiscence. B) An abscised T2 seed in A), showing GUS signal in the outer part of the hilum (arrow). C) Postharvest dry T_2 seeds directly placed in the GUS staining solution. D) A seed in C), showing the GUS signal in the seed coat region surrounding the hilum (thick arrow). The remaining funiculus was GUS-signal-free (thin arrow). E) T2 seed that was imbibed on an MS agar medium for six hours before GUS staining. F) T2 seed that was imbibed on an MS agar medium for 10 hours before GUS staining solution. G) and H) GUS signals in a dehisced T_1 silique and a T_2 seed from the silique, respectively. I) and J) Postharvest dry seeds were subjected to the same treatment as in C) and D). In summary, *AFB1* and *AFB5* were expressed in the vasculature and other cells in the fruit wall, the funiculus, and the hilum continuous with the funiculus. *AFB1*, not *AFB5*, was also transiently expressed in the chalazal seed coat surrounding the hilum in the early phase of imbibition. Scale bar in (I) for (A), (C), (G) and (I) = 400 µm, and scale bar in (J) for the remaining images = 50 µm.

Strategy for identifying auxin signaling networks in seed dormancy regulation

- Transcriptomic data used: 1) Genes expressed in the funiculus (FUN), chalazal seed coat (CZSC), and the distal half of the seed coat (SC) at the mature seed stage (Belmonte et al., 2013; Khan et al., 2015). 2) Auxin-up- and –downregulate genes (Goda et al., 2004)
- Identifying auxin-regulated genes in FUN and CZSC using the above transcriptomic data
- Comparing expression trends of *AFBs* from FUN to CZSC with those identified above: Auxin-upregulated genes and their associated *AFBs* should follow the same expression trends while auxin-downregulated genes and their associated *AFBs* should exhibit opposite trends
- Assigning identified genes into groups based on their associated *AFBs*, up- or downregulation by auxin, and expression trends

Predicting three auxin signaling networks in seed dormancy regulation

Following the above strategy, *in silico* searches uncovered that *AFB1*, *4*, and *TIR1* are expressed in a downward trend whereas *AFB2*, *3*, and *5* in an upward trend from FUN to CZSC. 118 genes, either down- or upregulated by auxin, were also identified, and were assigned into four groups based on the downward or upward expression trend from FUN to CZSC. Among the four groups, one with the downward expression trend and another with the upward expression trend were kept for subsequent analysis because they contained genes with well-known functions in seed dormancy or germination processes. To conduct linear correlation analysis of transcript values between gene pairs, a third data point, mRNA values in the distal half of the seed coat (SC) were first normalized (reduced) by a factor of 26.8 (the ratio of volume of SC to volume of CZSC, and assuming volume of CZSC ≈ volume of FUN). Linear correlation analysis using the three data points of FUN, CZSC, and SC further identified three sets of genes with high correlation coefficient values ($R^2 \geq 0.92$) that each is enriched for seed dormancy- or germination-related genes (Table 2). The two *AFB* groups also had high linear correlation coefficients between the members of the same group ($R^2 \geq 0.92$).

Table 2. Three predicted auxin signaling networks for seed dormancy regulation

AFB1-network A, downregulated by auxin, upward trend from FUN to CZSC	
Gene ID	**Function**
At2G28470[1]	BGAL8, β-galactosidase
At4g26320[1]	AGP13, arabinogalactan protein
At4g33720[4]	ATCAPE3, cysteine-rich secretory proteins
At2g40330[2]	PYL6, regulatory component of ABA receptor
At2g15370[4]	ATFUT5, fucosyltransferase
At5g42180[3]	PRX64, peroxidase
At1g09090[2]	ATRBOHB, respiratory burst oxidase homolog
AFB1-network B, downregulated by auxin, upward trend from FUN to CZSC	
Gene ID	**Function**
At4g20460[2]	NAD(P)-binding Rossmann-fold superfamily protein
At4g35060[4]	ATHMP39, heavy metal transport/detoxification
At4g40010[2]	SNRK2-7, kinase activated by salt and mannitol osmotic stress.
At4g17340[1]	TIP2;2, aquaporin
At1g15380[3]	GLYI4, ABA and JA crosstalk
At2g44790[2]	UCC2, suppressor of seed germination via PIF1-miR408
At4g26220[4]	Caffeoyl-coenzyme A O-methyltransferase
At1g05260[3]	PER3, cold-inducible cationic peroxidase
At1g78090[4]	homologous to trehalose-6-phosphate phosphatases

AFB5-network, upregulated by auxin, upward trend from FUN to CZSC	
Gene ID	**Function**
At5g57560[4]	ATXTH22, xyloglucan endotransglucosylase/hydrolase
At5g64100[2]	PRX69, Class III peroxidase
At1g51170[4]	Protein kinase, interacts with the transcription factor ATS
At4g30080[2]	ARF16, AUXIN RESPONSE FACTOR 16
At1g19050[1]	ARR7, regulator in response to cytokinin
At4g37900[2]	ATGRDP2, contains DUF1399 domain and RNA binding motif
At3g13380[4]	BRL3, brassinosteroid receptor
At2g34080[1]	Cysteine proteinase
At4g03140[2]	NAD(P)-binding Rossmann-fold superfamily
At2g28350[2]	ARF10, AUXIN RESPONSE FACTOR 10
At2g22420[3]	PRX17, cell wall-localized class III peroxidase
At1g23060[4]	MDP40, MICROTUBULE DESTABILIZING PROTEIN 40
At1g22880[1]	CELLULASE 5
At2g23060[4]	Acyl-CoA N-acyltransferase

[1]Known or predicted to promote seed germination according to others' work. [2]Known or predicted to promote seed dormancy according to others' work. [3]Predicted to either positively or negatively affect seed dormancy according to others. [4]Predicted to affect seed dormancy in this investigation.

Conclusions

- Experimental evidence supports or suggests two-thirds of the genes in the networks function in seed dormancy/germination
- The remaining one-third of the genes may also function in seed dormancy/germination
- The networks are inherently flexible as each of them consists of both positive and negative factors for seed germination