HHS Public Access

Author manuscript

Curr Biol. Author manuscript; available in PMC 2018 February 06.

Published in final edited form as:

Curr Biol. 2017 February 06; 27(3): 437–444. doi:10.1016/j.cub.2016.12.016.

Plant stress tolerance requires auxin-sensitive Aux/IAA transcriptional repressors

Eilon Shani[1,2,#], **Mohammad Salehin**[1,#], **Yuqin Zhang**[2], **Sabrina E. Sanchez**[3], **Colleen Doherty**[4], **Renhou Wang**[1], **Cristina Castillejo Mangado**[1], **Liang Song**[5], **Iris Tal**[2], **Odelia Pisanty**[2], **Joseph R. Ecker**[5], **Steve A. Kay**[3], **Jose Pruneda-Paz**[1], and **Mark Estelle**[1,*]

[1]Section of Cell and Developmental Biology and Howard Hughes Medical Institute, University of California San Diego, 9500 Gilman Dr. La Jolla, CA 92093

[2]Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978, Israel

[3]Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

[4]Department of Molecular and Structural Biology, North Carolina State University, Raleigh, NC 27695

[5]Genome Analysis Laboratory, Howard Hughes Medical Institute and The Salk Institute for Biological Studies, La Jolla, CA 92037

Summary

The Aux/IAA proteins are auxin-sensitive repressors that mediate diverse physiological and developmental processes in plants [1, 2]. There are 29 *Aux/IAA* genes in *Arabidopsis* that exhibit unique but partially overlapping patterns of expression [3] (Figure S1A). Although some studies have suggested that individual *Aux/IAA* genes have specialized function, genetic analyses of the family have been limited by the scarcity of loss-of-function phenotypes [4]. Further, with a few exceptions, our knowledge of the factors that regulate *Aux/IAA* expression is limited [1, 5]. We hypothesize that transcriptional control of *Aux/IAA* genes plays a central role in the establishment of the auxin-signaling pathways that regulate organogenesis, growth, and environmental response. Here we describe a screen for transcription factors (TFs) that regulate the *Aux/IAA* genes. We identify TFs from 38 families including 26 members of the DREB/CBF family. Several DREB/CBF TFs directly promote transcription of the *IAA5* and *IAA19* genes in response to abiotic stress. Recessive mutations in these *IAA* genes result in decreased tolerance to stress

*Correspondence to: mestelle@ucsd.edu.
#These authors contributed equally to this work.

Author Contributions

E.S., S.M., S.E.S., C.D., M.E. designed the experiments
E.S., S.M., Y.Z., S.E.S., C.D., I.T., O.P. conducted the experiments
E.S., S.M., S.E.S., C.D., M.E. analyzed and interpreted the data
E.S., S.M., L.S., J.R.E., M.E., drafted or revised the article
C.C.M, R.W., L.S., J.R.E., S.A.K., J.P-P., contributed unpublished essential reagents

conditions demonstrating a role for auxin in abiotic stress. Our results demonstrate that stress pathways interact with the auxin gene regulatory network (GRN) through transcription of the *Aux/IAA* genes. We propose that the *Aux/IAA* genes function as hubs that integrate genetic and environmental information to achieve the appropriate developmental or physiological outcome.

Keywords

auxin; Aux/IAA; plant hormone; abiotic stress; repressor

Results

A screen for regulators of the *Aux/IAA* genes identifies diverse transcription factors

Auxin regulates diverse aspects of growth and development in response to both genetic and environmental inputs [1, 2, 6]. Since the *Aux/IAA* genes have a central role in auxin response, we reasoned that transcriptional regulation of these genes might act to integrate environmental inputs into the auxin GRN. To identify novel factors that function in the auxin GRN we implemented a yeast-1-hybrid (Y1H) approach. We cloned the first ~500 bp of 15 different *Aux/IAA* promoters (Table S1) with at least one promoter from each of the major subclades (Figure 1A) and screened them against 1,956 *Arabidopsis* TFs [7] (see Supplemental Experimental Procedures). The screen identified 433 different interactions, with an average reproducibility of 41% between two screens (Figure 1B, dataset S1) in line with previous genome-scale yeast screens [8–10]. The 433 interactions are a sum of 173 different TFs that fall into 38 TF families. 83 TFs bound to several *pIAAs* while 90 TFs interacted with a single *pIAA* (Figure 1B, Table S2). The ARF proteins, which are expected to bind some of *IAA* promoters directly [11], were not recovered, possibly because full length ARFs are not expressed well in yeast [7]. Most *IAA* promoters displayed specificity for particular TF families (Figure 1C, Table S2). For example, 13 TCP proteins bound to *pIAA10*, consistent with earlier *in vivo* data [12].

Interestingly, the DREB/CBF family had high enrichment scores across most of the *IAA* promoters (Figure 1D, Figure S1B). Because a connection between the DREB/CBFs and auxin signaling has not been described, we elected to focus on these TFs. The *DREB/CBF* gene family consist of 56 members in *Arabidopsis*, divided into subfamilies A1-6 [13, 14]. DREB/CBF proteins from subfamilies A2, A3 and A6 displayed a robust interaction with most of the *pIAAs*, while proteins from the subfamilies A1, A4 and A5 clades showed specific interaction to *pIAA3* and *pIAA5* (Figure 1D).

Stress conditions inhibit auxin response

The DREB/CBF proteins are key factors in stress response [15]. Members of these families regulate the expression of large numbers of genes in response to stresses such as drought, cold, and high salinity, and when overexpressed confer increased drought tolerance in *Arabidopsis* and other species [16–18]. Although some DREB/CBF target genes are known to function in stress tolerance, our knowledge of the greater stress regulatory network remains quite limited [19].

To investigate whether DREB/CBF binding to the *pIAAs* is physiologically relevant we first asked if abiotic stresses affect auxin response using the *DR5:3XVENUS-NLS* auxin reporter. The results in Figure 2A and Figure S2A show that desiccation of seedlings or growth on medium containing PEG dramatically suppressed auxin response. In addition, we found that PEG strongly inhibited growth of the hypocotyl in response to auxin (Figure S2A). To explore this effect further we used Nanostring technology to test the transcriptional response of 57 auxin-related genes to a 30 min desiccation stress. The results show that desiccation strongly induced the 6 *DREB/CBF* genes that showed strong binding to *pIAA5* (Fig 2B). Expression of the auxin co-receptors *TIR1*, *AFB2* and *AFB4* was not affected while expression of *AFB3* and *AFB1* was reduced (Figure S3B). Several auxin biosynthetic genes were also affected (Figure S2C). Expression of many of *Aux/IAA* genes was affected by the treatment (Figure 2C and S2D).

Because the largest fold-induction was observed for *IAA5* (3.5-fold change) we elected to focus on this gene and its close relatives *IAA6* and *IAA19*. The *IAA19* gene was induced 1.7 fold by desiccation (Figure 2C). First, we retested the interaction between *pIAA5* and CBF2 and DREB2A by Y1H. We also included the well-characterized CBF1 and DREB2B as well as two additional DREBs, At1G19210 and At2G44940 in this experiment. All six proteins interacted with *pIAA5* (Figure 2D). Since *IAA19* was not included in the original screen, we tested this promoter against the 6 DREB/CBFs. Similar to *pIAA5*, each DREB/CBF protein showed strong binding to *pIAA19* (Figure S3A).

The DREB/CBF proteins bind the conserved DRE/CRT element [15, 17]. At least one DRE element is present within 500 bps upstream of *IAA2, IAA5, IAA6, IAA9,* and *IAA19* (Figure S3B). To characterize the DRE elements adjacent to *IAA5,* we mutated both elements and repeated the Y1H assay with selected TFs (Figure 2D and E). The DREB/CBFs displayed dramatically reduced binding to the mutated *pIAA5* (*pIAA5m*) (Figure 2D) compared to the wild-type promoter, indicating that the DRE elements are essential for DREB/CBF binding to *pIAA5* in yeast.

To further investigate the activity of the *IAA5* promoter, we generated plants that express LUC under control of the *IAA5* promoter (*pIAA5:LUC*) as well as a variant promoter in which both DRE/CRT elements were mutated as described above (*pIAA5m:LUC*). Plants with the wild-type construct displayed a ~3-fold induction after a 30 min desiccation period (Figure 2F). Interestingly, the *pIAA5m:LUC* line had a significantly reduced basal level of LUC signal in 3 independent lines, suggesting that the DRE elements are important even in the absence of stress (Figure 2F). Further, the response of the mutant construct to desiccation was dramatically reduced compared to the wild type confirming that the DREs are required for stress induction of *IAA5*. Finally, plants expressing *iaa19-dII-dIV-3xYPet* under control of the *IAA19* promoter (*pIAA19:iaa19--dII-dIVxYPet*) [20] showed a significant increase in YPet florescence in the stele following a 1 hour desiccation treatment (Figure 2G) [20].

The *IAA19* gene (also known as *MASSUGU2*) has been previously characterized [21]. To learn more about the *IAA5* gene, we generated *pIAA5:GUS, pIAA5:IAA5-GUS,* and *pIAA5:IAA5dII-GUS* lines. The *dII* mutation is an amino acid substitution in the degron sequence that is expected to stabilize the protein. The results in Figure S4 show that *IAA5*

transcription is induced by auxin and that the IAA5dII protein accumulates to a greater extent than the wild-type protein. In addition, expression of the mutant protein confers a phenotype that is very characteristic of a gain-of-function *aux/iaa* mutant[22]. Thus, *IAA5* is a typical member of the *Aux/IAA* family.

CBF1 and DREB2A directly regulate *IAA5* and *IAA19* expression

At this point we elected to focus on regulation of the *Aux/IAA* genes by CBF1 and DREB2A. To determine if these two TFs bind the *Aux/IAA* promoters *in vivo*, we performed chromatin immunoprecipitation (ChIP) experiments with *35S:CBF1-YFP* and *gDREB2A:DREB2A-Ypet-His-FLAG* lines [23]. For CBF1 ChIP, chromatin was isolated from light grown whole seedlings while for DREB2A ChIP, chromatin was isolated from roots dissected from light-grown whole seedlings that had been subjected to a 1 h desiccation treatment to increase DREB2A protein. The *IAA5* promoter has two *DRE* motifs in the −160 to – 69 bp interval while *pIAA19* has a single *DRE* element within the −281 to −161 bp fragment. Both of these sequences were enriched after ChIP with either CBF1-YFP or DREB2A-Ypet (Figure 3A, B). We used a *COR78* (also known as *RD29*) promoter fragment containing a *DRE* element as a positive control, which showed enrichment in both ChIPs, consistent with a previous report [23]. Taken together the data from Y1H and ChIP experiments confirm that DREBs/CBFs bind to *DRE* elements in the *IAA5* and *IAA19* promoters in yeast and in plants.

Next, we asked if the DREBs regulate *IAA5* and *IAA19* in accordance with the ChIP data. We generated a β-Estradiol inducible *DREB2A* line in which the *G1090* promoter in the *pER-GW* promoter was fused to the *DREB2A* CDS and treated seedlings with β-Estradiol for 4 h (Figure 3C). This treatment resulted in a substantial increase in *IAA5* and *IAA19* RNA levels confirming that these genes are targets of DREB2A. Interestingly we also found that *IAA6* was slightly induced by DREB2A overexpression suggesting that this gene may also be a DREB/CBF target. To further examine the transcriptional regulation of *IAA5* and *IAA19* by DREBs we utilized the *CBF2-DN* line that overexpresses a C-terminal truncated and inactive version of CBF2 [24]. This truncated protein acts to repress DREB-mediated transcription. We found that basal expression of *DREB2A, IAA5,* and *IAA19* was similar in the *CBF2-DN* and control lines. However, as reported previously, stress induction of *DREB2A* was compromised in the *CBF2-DN* line (Figure 3D) [24]. Similarly, we found that induction of both *IAA5* and *IAA19* was reduced in the transgenic lines. Finally, induction of *IAA19-YPet* in *pIAA19:IAA19-3xYPet* after a 70 m desiccation period was reduced in *CBF2-DN* (Figure S5).

As a *cbf1 dreb2a* mutant was not available, we used a *dreb2a dreb2b* double mutant to further examine the role of these proteins in stress-induction of the *Aux/IAA* genes. qPCR analysis showed that accumulation of *IAA5* and *IAA9* transcript was reduced after desiccation in the *dreb2a dreb2b* double mutant (Figure 3E). These results confirm that the DREB/CBF proteins are required for stress-induced expression of these two *Aux/IAA* genes. The effects of both the *CBF2-DN* transgene and *dreb2a dreb2b* double mutant on *IAA6* levels were minimal and variable and therefore not included here.

The *IAA5* and *IAA19* genes are required for stress tolerance

Because the DREB/CBF and auxin GRNs are directly linked through *IAA5* and *IAA19* we wondered if these genes might contribute to stress response. We also examined the role of the closely related *IAA6* gene. The recessive *iaa5-1, iaa6-1,* and *iaa19-1* mutants do not have an obvious phenotype when grown under optimal conditions [4]. However, when we applied a 75-min desiccation treatment to 7-day-old seedlings, followed by rescue on MS plates, we found that all three single mutants, as well as the *iaa5 iaa6 iaa19* triple mutant, were hypersensitive compared to wild type with a pronounced effect on survival and subsequent growth (Figure 4A, B, and C). To determine if the genes were also required for tolerance to other stresses, we grew the mutants on medium supplemented with polyethylene glycol-8000 (PEG-8000, Fisher Scientific) (−1.0 Mpa) for 9 days. All four lines displayed PEG hypersensitivity with a 45 to 50% reduction in primary root length after transfer to PEG (Figure 4D, E), and ~ 50% reduction in fresh weight compared to unstressed plants (Figure 4F). To confirm that these defects are due to mutations in the *IAA* genes, we introduced the wild-type sequence into the mutant line under control of the native promoter. Both *IAA6* and *IAA19* restored the wild-type level of stress tolerance confirming that the mutations are causative.

Discussion

Genetic studies of the *Aux/IAA* genes have long been hampered by the lack of loss-of-function phenotypes [4]. There have been a few reports describing the effects of recessive *aux/iaa* mutations on growth and development [5, 25–29], but in general investigators have relied on gain-of-function stabilizing mutations to discern the function of individual members of the family. Our discovery that recessive mutations in *IAA5, IAA6*, and *IAA19* confer a stress phenotype should encourage the careful examination of loss-of-function mutations in other *IAA* genes. The identification of novel conditional phenotypes will increase our knowledge of the function of these genes.

The Aux/IAA proteins are known to function as transcriptional repressors. This implies that stress tolerance requires repression of a subset of auxin-regulated genes. The identity of these genes is presently unknown. One possibility is suggested by studies which show that stress tolerance is associated with growth inhibition [30, 31]. Gibberellic acid has been implicated in this inhibition but it is possible that auxin is also involved[32]. If stress-induced growth inhibition involves repression of auxin-responsive genes, loss of the *IAAs* may reduce this response. Since the ultimate consequence of reduced tolerance is decreased growth, the initial lack of growth inhibition may not be apparent. Alternatively, it is possible that Aux/IAA target genes that are not directly associated with growth are involved in stress tolerance, since many auxin responsive genes are not obviously related to growth [33]. In fact, a recent study in the moss *Physcomitrella patens* demonstrates that over a third of the genes in genome are regulated by the Aux/IAA proteins [34].

It is interesting that the phenotype of the triple mutant is similar to that of each of the single mutants. This may be because the three genes have distinct patterns of expression in the root. Indeed, examination of publically available expression data shows that the three genes exhibit distinct expression patterns in the root (http://bar.utoronto.ca) [35](Figure S6). *IAA5*

is expressed primarily in the epidermis. Both *IAA6* and *IAA19* are expressed in the stele, but the *IAA6* expression zone extends further upwards towards the base of the root. Alternatively, it has been suggested that Aux/IAAs function in higher order complexes that could include many Aux/IAA proteins [36, 37]. It is possible that several different Aux/IAA proteins must be present in these complexes for activity. It is also interesting to note that some of the *Aux/IAA* genes are expressed at a higher level in whole seedlings than *IAA5, IAA6*, and *IAA19* (Fig. S2D). However, at cellular resolution the situation can be quite different. For example, *IAA7* is expressed at a much higher level than *IAA6* or *IAA19* in seedlings, but at a lower level than in the stele [35].

It is clear that plant responses to abiotic stress are complex and involve multiple signaling pathways that regulate many aspects of growth and physiology. Here we show that an auxin GRN is directly integrated into the DREB/CBF stress pathway through regulation of *Aux/IAA* genes. Although we have focused on *IAA5, IAA6,* and *IAA19*, it is possible that other members of the family are also regulated by DREB/CBF proteins. Indeed, in our analysis 11 *Aux/IAAs* are affected by desiccation treatment in addition to *IAA5* and *IAA19.* Further, mining of transcriptome data reveals that other *Aux/IAA* genes respond to abiotic stress (For examples see Fig S1). In the future, it will be vital to determine if these genes also contribute to stress tolerance and to identify the Aux/IAA targets that contribute to this stress response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

MS would like to thank Estelle lab members for helpful discussions and Paul Chen for assistance. Thanks to Jose Alonso and Anna Stepanova for sharing the *IAA19:iaa19-dII-dIV-3xYPet* line. This work was supported by a grant by the NIH (GM43644 to M E., R01GM067837 and R01GM56006 to S.A.K.), the NSF (MCB1024999 to J.R.E.), the Gordon and Betty Moore Foundation (GBMF3038 to ME., GBMF3034 to J.R.E.), the Vaadia–BARD Postdoctoral Fellowship (FI-431-10 to ES) and the Israel Science Foundation (1832/14 and 2158/14 to ES). M.E. and J.R.E. are investigators of the Howard Hughes Medical Institute..

References

1. Salehin M, Bagchi R, Estelle M. SCFTIR1/AFB-Based Auxin Perception: Mechanism and Role in Plant Growth and Development. The Plant cell. 2015; 27:9–19. [PubMed: 25604443]
2. Weijers D, Wagner D. Transcriptional Responses to the Auxin Hormone. Annual review of plant biology. 2016; 67:539.
3. Bargmann BO, Vanneste S, Krouk G, Nawy T, Efroni I, Shani E, Choe G, Friml J, Bergmann DC, Estelle M, et al. A map of cell type-specific auxin responses. Mol Syst Biol. 2013; 9:688. [PubMed: 24022006]
4. Overvoorde PJ, Okushima Y, Alonso JM, Chan A, Chang C, Ecker JR, Hughes B, Liu A, Onodera C, Quach H, et al. Functional genomic analysis of the AUXIN/INDOLE-3-ACETIC ACID gene family members in Arabidopsis thaliana. The Plant cell. 2005; 17:3282–3300. [PubMed: 16284307]
5. Dello Ioio R, Nakamura K, Moubayidin L, Perilli S, Taniguchi M, Morita MT, Aoyama T, Costantino P, Sabatini S. A genetic framework for the control of cell division and differentiation in the root meristem. Science. 2008; 322:1380–1384. [PubMed: 19039136]
6. Salehin M, Estelle M. Ethylene prunes translation. Cell. 2015; 163:543–544. [PubMed: 26496600]

7. Pruneda-Paz JL, Breton G, Nagel DH, Kang SE, Bonaldi K, Doherty CJ, Ravelo S, Galli M, Ecker JR, Kay SA. A genome-scale resource for the functional characterization of Arabidopsis transcription factors. Cell reports. 2014; 8:622–632. [PubMed: 25043187]
8. Gaudinier A, Zhang L, Reece-Hoyes JS, Taylor-Teeples M, Pu L, Liu Z, Breton G, Pruneda-Paz JL, Kim D, Kay SA, et al. Enhanced Y1H assays for Arabidopsis. Nat Methods. 2011; 8:1053–1055. [PubMed: 22037706]
9. Vermeirssen V, Deplancke B, Barrasa MI, Reece-Hoyes JS, Arda HE, Grove CA, Martinez NJ, Sequerra R, Doucette-Stamm L, Brent MR, et al. Matrix and Steiner-triple-system smart pooling assays for high-performance transcription regulatory network mapping. Nat Methods. 2007; 4:659–664. [PubMed: 17589517]
10. Reece-Hoyes JS, Diallo A, Lajoie B, Kent A, Shrestha S, Kadreppa S, Pesyna C, Dekker J, Myers CL, Walhout AJ. Enhanced yeast one-hybrid assays for high-throughput gene-centered regulatory network mapping. Nat Methods. 2011; 8:1059–1064. [PubMed: 22037705]
11. Lau S, De Smet I, Kolb M, Meinhardt H, Jürgens G. Auxin triggers a genetic switch. Nature Cell Biology. 2011; 13:611–615. [PubMed: 21478855]
12. Koyama T, Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M. TCP transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during differentiation of leaves in Arabidopsis. The Plant cell. 2010; 22:3574–3588. [PubMed: 21119060]
13. Nakano T, Suzuki K, Fujimura T, Shinshi H. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant physiology. 2006; 140:411–432. [PubMed: 16407444]
14. Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K. DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration-and cold-inducible gene expression. Biochemical and biophysical research communications. 2002; 290:998–1009. [PubMed: 11798174]
15. Shinozaki K, Yamaguchi-Shinozaki K, Seki M. Regulatory network of gene expression in the drought and cold stress responses. Current opinion in plant biology. 2003; 6:410–417. [PubMed: 12972040]
16. Mallikarjuna G, Mallikarjuna K, Reddy MK, Kaul T. Expression of OsDREB2A transcription factor confers enhanced dehydration and salt stress tolerance in rice (Oryza sativa L.). Biotechnol Lett. 2011; 33:1689–1697. [PubMed: 21528404]
17. Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. The Plant cell. 2006; 18:1292–1309. [PubMed: 16617101]
18. Wang H, Wang H, Shao H, Tang X. Recent Advances in Utilizing Transcription Factors to Improve Plant Abiotic Stress Tolerance by Transgenic Technology. Front Plant Sci. 2016; 7:67. [PubMed: 26904044]
19. Yoshida T, Mogami J, Yamaguchi-Shinozaki K. ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Current opinion in plant biology. 2014; 21:133–139. [PubMed: 25104049]
20. Zhou R, Benavente LM, Stepanova AN, Alonso JM. A recombineering-based gene tagging system for Arabidopsis. The Plant journal : for cell and molecular biology. 2011; 66:712–723. [PubMed: 21294796]
21. Tatematsu K, Kumagai S, Muto H, Sato A, Watahiki MK, Harper RM, Liscum E, Yamamoto KT. MASSUGU2 encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in Arabidopsis thaliana. The Plant cell. 2004; 16:379–393. [PubMed: 14729917]
22. Liscum E, Reed JW. Genetics of Aux/IAA and ARF action in plant growth and development. Plant molecular biology. 2002; 49:387–400. [PubMed: 12036262]
23. Chow BY, Sanchez SE, Breton G, Pruneda-Paz JL, Krogan NT, Kay SA. Transcriptional regulation of LUX by CBF1 mediates cold input to the circadian clock in Arabidopsis. Curr Biol. 2014; 24:1518–1524. [PubMed: 24954045]

24. Park S, Lee CM, Doherty CJ, Gilmour SJ, Kim Y, Thomashow MF. Regulation of the Arabidopsis CBF regulon by a complex low-temperature regulatory network. The Plant journal : for cell and molecular biology. 2015; 82:193–207. [PubMed: 25736223]
25. Koenig D, Bayer E, Kang J, Kuhlemeier C, Sinha N. Auxin patterns Solanum lycopersicum leaf morphogenesis. Development. 2009; 136:2997–3006. [PubMed: 19666826]
26. Ben-Gera H, Shwartz I, Shao MR, Shani E, Estelle M, Ori N. ENTIRE and GOBLET promote leaflet development in tomato by modulating auxin response. The Plant journal : for cell and molecular biology. 2012; 70:903–915. [PubMed: 22332729]
27. Tian Q, Reed JW. Control of auxin-regulated root development by the Arabidopsis thaliana SHY2/IAA3 gene. Development. 1999; 126:711–721. [PubMed: 9895319]
28. Swarup K, Benkova E, Swarup R, Casimiro I, Peret B, Yang Y, Parry G, Nielsen E, De Smet I, Vanneste S, et al. The auxin influx carrier LAX3 promotes lateral root emergence. Nat Cell Biol. 2008; 10:946–954. [PubMed: 18622388]
29. Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latche A, Pech JC, Bouzayen M. The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. The Plant cell. 2005; 17:2676–2692. [PubMed: 16126837]
30. Naser V, Shani E. Auxin response under osmotic stress. Plant molecular biology. 2016:1–12.
31. Skirycz A, Inze D. More from less: plant growth under limited water. Curr Opin Biotechnol. 2010; 21:197–203. [PubMed: 20363612]
32. Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P. The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. The Plant cell. 2008; 20:2117–2129. [PubMed: 18757556]
33. Chapman EJ, Greenham K, Castillejo C, Sartor R, Bialy A, Sun TP, Estelle M. Hypocotyl transcriptome reveals auxin regulation of growth-promoting genes through GA-dependent and -independent pathways. PloS one. 2012; 7:e36210. [PubMed: 22590525]
34. Lavy M, Prigge MJ, Tao S, Shain S, Kuo A, Kirchsteiger K, Estelle M. Constitutive auxin response in Physcomitrella reveals complex interactions between Aux/IAA and ARF proteins. Elife. 2016; 5
35. Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science. 2007; 318:801–806. [PubMed: 17975066]
36. Korasick DA, Jez JM, Strader LC. Refining the nuclear auxin response pathway through structural biology. Current opinion in plant biology. 2015; 27:22–28. [PubMed: 26048079]
37. Korasick DA, Westfall CS, Lee SG, Nanao MH, Dumas R, Hagen G, Guilfoyle TJ, Jez JM, Strader LC. Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:5427–5432. [PubMed: 24706860]

Highlights

1. The *Aux/IAA* genes of Arabidopsis are regulated by diverse TFs.
2. The *Aux/IAAs* function as hubs that integrate signals from diverse pathways.
3. The CBF1 and DREB2A TFs directly regulate two *Aux/IAA* genes, *IAA5* and *IAA19*.
4. The *IAA5, IAA6,* and *IAA19* are required for stress tolerance.

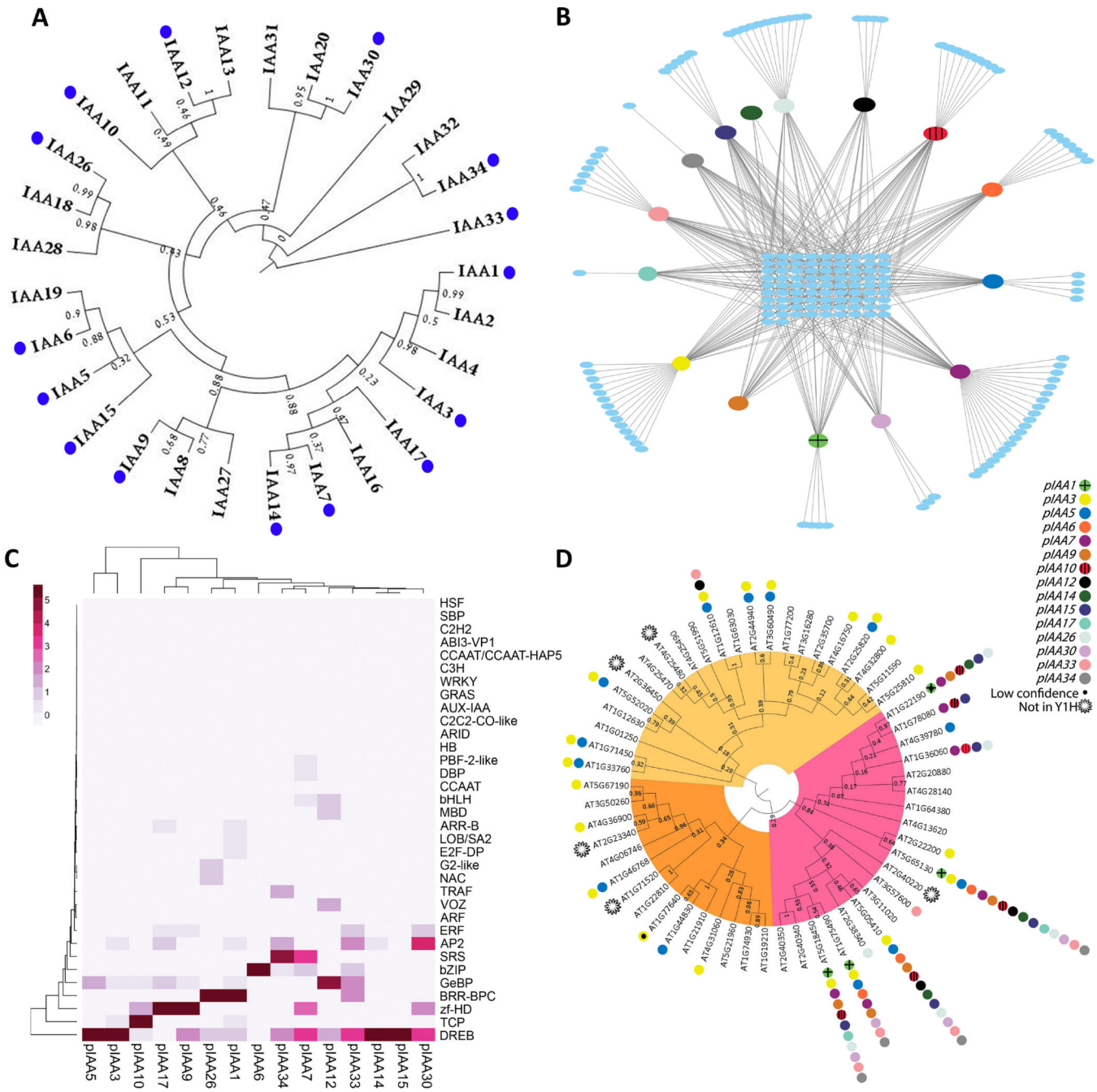

Figure 1. Y1H screen for regulators of *Aux/IAA* genes

(A) Phylogenetic tree of *Arabidopsis Aux/IAA* proteins. Blue dots mark *Aux/IAA* promoters cloned for the Y1H screen. **(B)** *IAA* promoter - TF interaction network. The *IAA* promoters are indicated by colored coded ovals; interacting TFs are blue ovals. 83 TFs interacted with several *pIAAs* while 90 TFs interacted with a single *pIAA*. **(C)** Heatmap showing enrichment of TF families for each promoter fragment. The enrichment score for each TF family on the promoter fragments was calculated by Fisher's exact test. The −log10 p-value of this enrichment score is shown above with darker colors indicating a greater enrichment score. **(D)** Phylogenetic tree of the *Arabidopsis* DREB/CBF proteins. Color-

coding sub-families A1 and A4 (yellow); sub-families A2, A3 and A6 (pink); sub-family A5 (orange). Interactions between DREB/CBF proteins and *IAA* promoters (color-coded circles) are plotted next to gene number. See also Figure S1, and Tables S1 and S2.

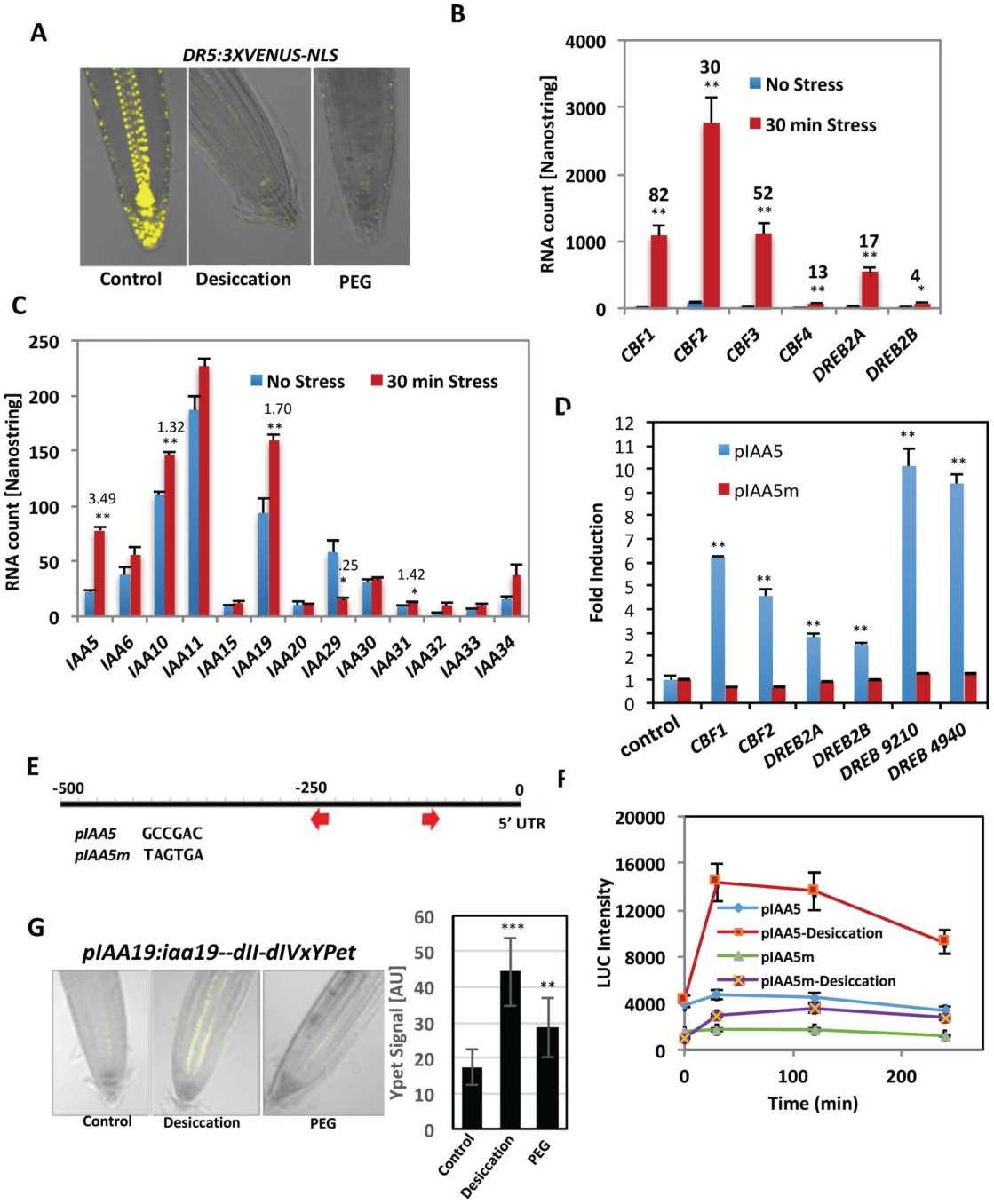

Fig 2. The DRE element is required for *IAA5* and *IAA19* expression

(A) *DR5:3XVENUS-NLS* seedlings after 30 min of desiccation. **(B–C)** Expression level in response to a 30 min desiccation period. $n = 3$ biological replicates. (**B**) Selected *DREB*s. The numbers above the bars represent fold change; (**C**) Selected *IAAs*. **(D)** Y1H interaction assay for selected DREB/CBF proteins with *pIAA5* and *pIAA5m*. Bar graph is the mean of 4 biological replicates. **(E)** Representation of the *IAA5* promoter (first 500 bp). Arrows indicate the position of DRE/CRT motifs. In *pIAA5m* the two DRE/CRT motifs are mutated as indicated. **(F)**. Expression of the *pIAA5:LUC* and *pIAA5m:LUC* reporters in response to

desiccation. Shown are averages (+/− SE) for three independent *pIAA5* and *pIAA5m* lines (n = 56 seedlings). Results are representative of at least three independent experiments. **(G)** Roots of *iaa19-dII-dIV-3xYPet* seedlings in response to desiccation (1h) and PEG (3h). YPet florescence shown in yellow. The graph at right is the quantification of YPet florescence in Arbitrary Units (AU). For all panels, differences are significant at $p<0.05$ (*) and $p<0.01$ (**) Student's t-test. See also Figures S2, S3, S4, and S5.

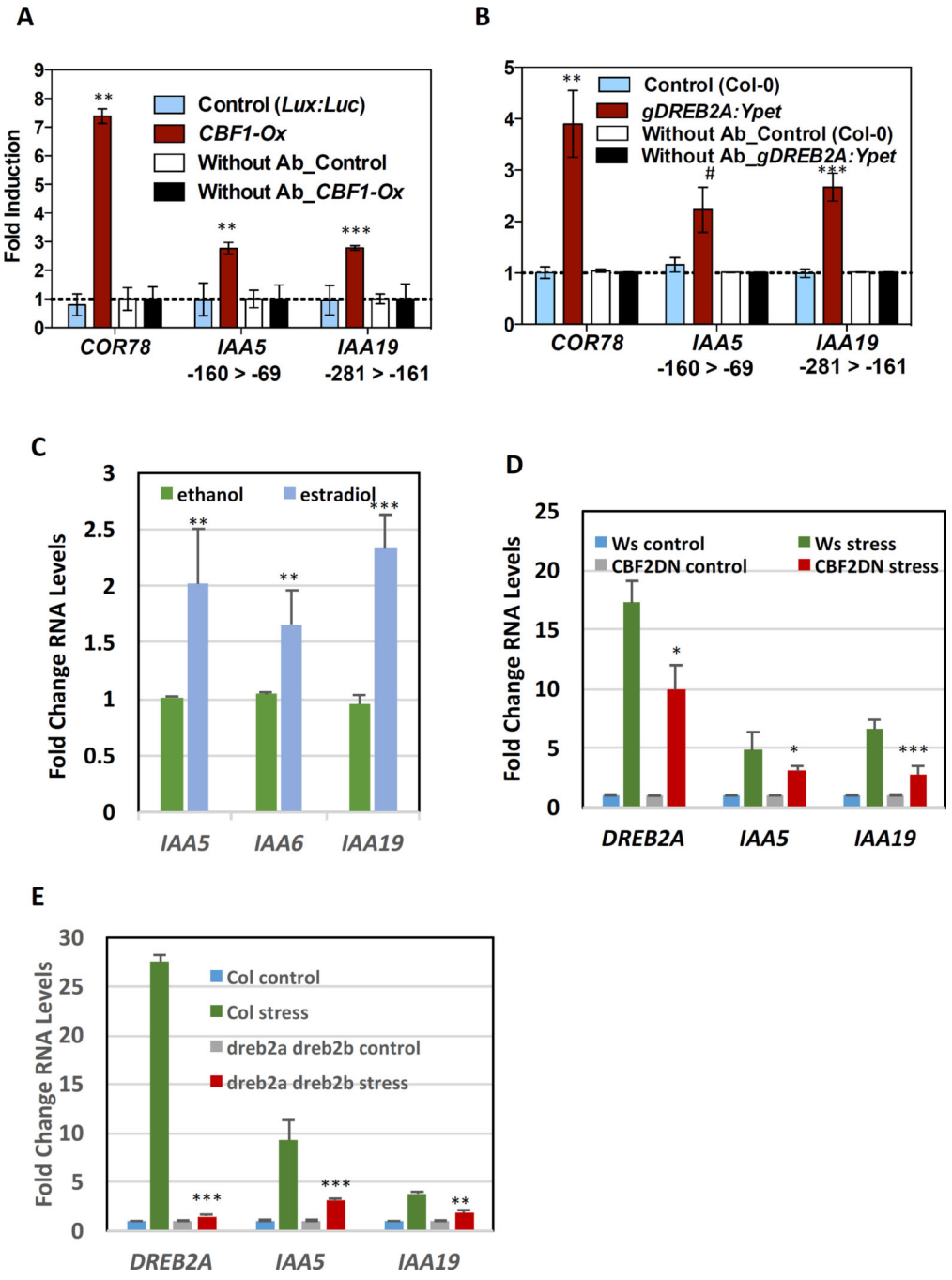

Fig 3. DREB/CBFs regulate transcription of *IAA5, IAA6,* and *IAA19*

(A–B) CBF1 and DREB2A bind to the promoter of *COR78*, *IAA5* and *IAA19*. **(A)** CBF1 binds to the promoter of *COR78*, *IAA5* and *IAA19* in 10-day-old light-grown whole seedlings. **(B)** DREB2A binds to the promoter of *COR78*, *IAA5* and *IAA19* in the roots of 10-day-old light-grown seedlings that were desiccated for 1h. Sonicated chromatin was immunoprecipitated either with or without anti-GFP antibody. Fold induction was normalized to no antibody and wild-type controls. Data represents mean with standard error from two independent biological replicates with two technical replicates for CBF1 and three

independent biological replicates for DREB2A. Differences are significant at $p<0.01$(**), $p<0.005$ (***), and $p<0.08$ ($^{\#}$). **(C, D, E)** CBF1 and DREB2A directly regulate *IAA5* and *IAA19* transcription. **(C)** Estradiol regulated DREB2A overexpression (*Est.≫DREB2A*) induces *IAA5*, *IAA6* and *IAA19* expression. Data represent means with standard error from three independent biological replicates. Significant at $p<0.005$ (***) and $p<0.05$ (**). **(D)** Expression of *DREB2A*, *IAA5* and *IAA19* in *CBF2DN* line after 1 h desiccation. Differences are significant at $p<0.05$ (*) and $p<0.005$ (***). **(E)** *IAA5 and IAA19* levels in *dreb2a-dreb2b* double mutant after 1 h desiccation. Differences are significant at $p<0.005$ (***) and $p<0.01$ (**) Student's t-test.

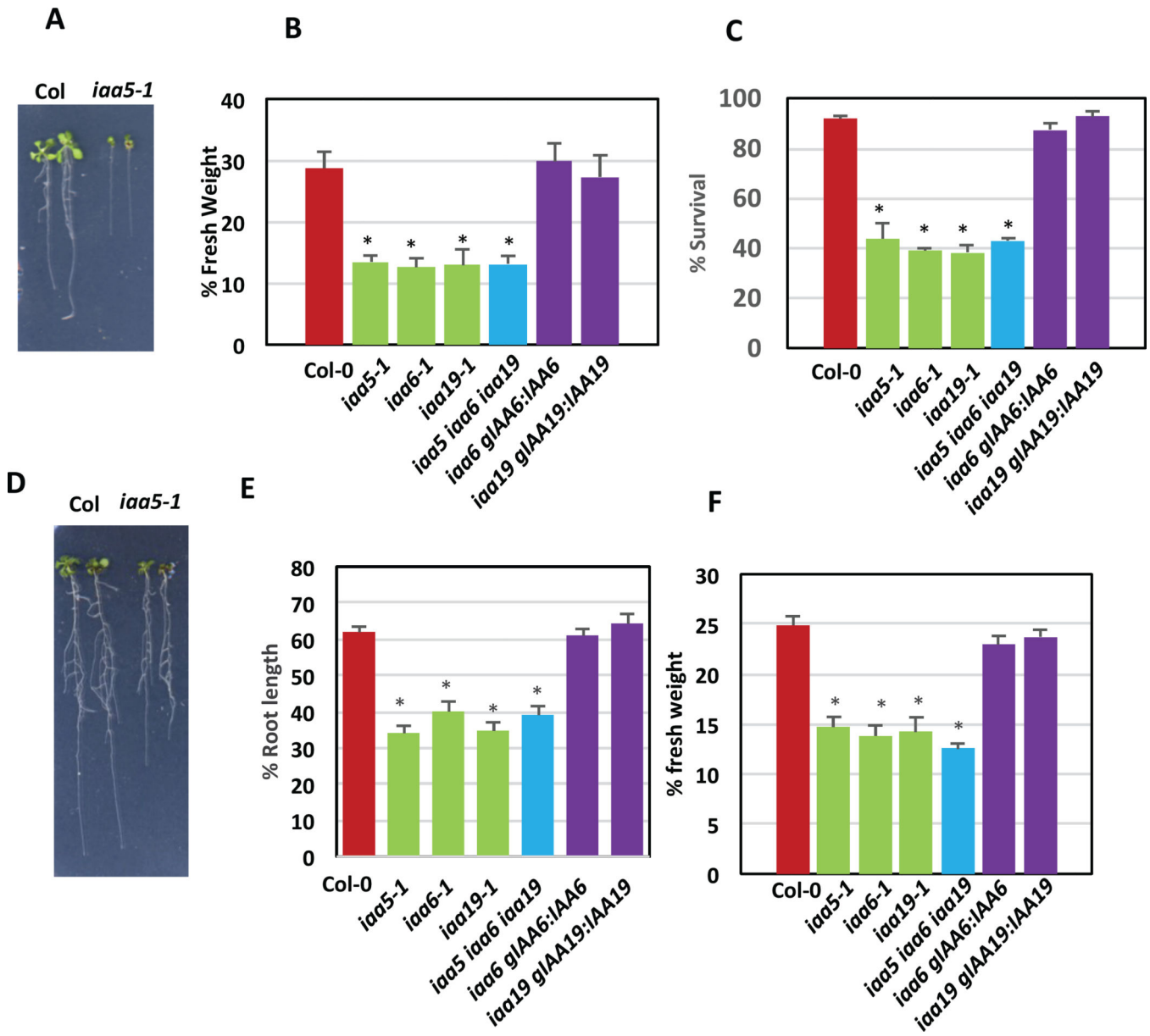

Fig 4. The *IAA5, IAA6,* and *IAA19* genes are required for stress tolerance

(A–C) Response of wild-type and mutant seedlings to desiccation. 7 days-old seedlings were placed on parafilm for 75 min and transferred to ½ MS plates for 10 days. **(A)** Representative seventeen-day-old Col-0 and *iaa5-1* seedlings after recovery from desiccation stress. **(B)** Fresh weight after desiccation stress relative to respective control plants. **(C)** Survival rate after desiccation. Data represents mean +/− standard error from one of the three independent biological replicates. n=12–15 seedlings for each genotype**.** Differences are significant at p<0.001 (*) Student's t-test for all mutants compared to Col-0. **(D–F)** Response of wild-type and mutant seedlings to growth on medium containing PEG-8000 (−1.0 Mpa). Seedlings were grown on ½ MS for 5 days and transferred to PEG-infused plates for a further 10 days **(D)** Representative fifteen-day-old Col-0 and *iaa5-1* seedlings after PEG stress. **(E)** Primary root growth of seedlings after PEG stress compared to the

respective control plants. **(F)** Fresh weight of seedlings after PEG stress relative to controls. Data represents mean +/− standard error from one of the three independent biological replicates. n=12–15 seedlings for each genotype. Differences are significant at p<0.001 (*) Student's t-test for all mutant genotypes compared to Col-0. See also Figure S6.