Combined transcriptome and translatome analyses reveal a role for tryptophan-dependent auxin biosynthesis in the control of DOG1-dependent seed dormancy

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Summary

- The importance of translational regulation during Arabidopsis seed germination has been shown previously. Here the role of transcriptional and translational regulation during seed imbibition of the very dormant DELAY OF GERMINATION 1 (DOG1) near-isogenic line was investigated.
- Polysome profiling was performed on dormant and after-ripened seeds imbibed for 6 and 24 h in water and in the transcription inhibitor cordycepin. Transcriptome and translatome changes were investigated.
- Ribosomal profiles of after-ripened seeds imbibed in cordycepin mimic those of dormant seeds. The polysome occupancy of mRNA species is not affected by germination inhibition, either as a result of seed dormancy or as a result of cordycepin treatment, indicating the importance of the regulation of transcript abundance.
- The expression of auxin metabolism genes is discriminative during the imbibition of after-ripened and dormant seeds, which is confirmed by altered concentrations of indole-3-acetic acid conjugates and precursors.

Introduction

Seed dormancy, defined as the inability of a viable seed to germinate under optimal conditions, is an important adaptive trait for plants to survive in nature (Bewley, 1997). Seed dormancy is a complex trait for which substantial natural genetic variation is present in Arabidopsis thaliana. DELAY OF GERMINATION 1 (DOG1) is the major quantitative trait locus underlying this natural genetic variation (Alonso-Blanco et al., 2003; Bentsink et al., 2006, 2010; Huang et al., 2010). The gene encodes a protein with unknown but conserved function across plant species (Graeter et al., 2014). The strong dormancy phenotype of the Cape Verde Islands (Cvi) allele of DOG1 provides an ideal model system to investigate molecular pathways regulating seed dormancy.

Abscisic acid and GAs are two important hormones antagonistically regulating seed dormancy and germination (Koornneef et al., 2002; Liu et al., 2010). Seed dormancy can be relieved by after-ripening, which refers to a period of seed dry storage after seed harvest. During this period, transcription and metabolism are limited, because of the low moisture content and small nuclear size caused by chromatin condensation (Fait et al., 2006; van Zanten et al., 2011; Gao et al., 2013; Meimoun et al., 2014). Dry dormant and after-ripened seeds hardly show any difference in transcript patterns; however, transcriptional changes become visible when the seeds imbibe (Cadman et al., 2006; Finch-Savage et al., 2007). Moreover, differences in the abundance of individual proteins have been reported between dormant and after-ripened dry seeds (Chibani et al., 2006). Post-transcriptional regulation is proposed to regulate seed dormancy release, possibly through mRNA oxidation (Bazin et al., 2011; El-Maarouf-Bouteau et al., 2013), which inhibits protein translation in vitro (Bazin et al., 2011). Here, we investigated the role of transcription and translation during the imbibition of dormant and after-ripened seeds, making use of polysomal profiling and transcription inhibitors. Polysomal profiling uses a sucrose gradient-based fractionation method for separation of mRNAs based on their association with polysomes and thus identifies mRNAs that are being translated. These mRNAs can be identified by high-throughput profiling techniques such as microarray analysis and RNA sequencing (Moustroph et al., 2009b; Layat et al., 2014; Lin et al., 2014; Vragovic et al., 2015). The ratio between polysomal bound mRNA and total mRNA of a specific mRNA

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represents the polysome occupancy (PO) of that mRNA (Bai et al., 2016). Translation is primarily regulated at the initiation level, and therefore polysomal binding is a relevant proxy for translational activity or protein synthesis (Browning and Bailey-Serres, 2015). The importance of translational control during seed germination has previously been shown (Bastoulser-Seral et al., 2015; Bai et al., 2016). Here we tested the importance of translational control during DOG1-dependent dormancy and showed different transcriprional patterns in dormant and after-ripened seeds during seed imbibition. Moreover, inhibition of germination by DOG1 and the transcriptional inhibitor cordoncepin affected transcript abundance of genes involved in tryptophan (Trp)-dependent auxin and indole glucosinolate pathways.

Materials and Methods

Plant material and seed germination conditions
The Arabidopsis thaliana near-isogenic line carrying the Cvi introgression at the position of DOG1 in a Landsberg erecta genetic background (NILDOG1-1; here referred to as NILDOG1) was originally introduced by Alonso-Blanco et al. (2003) and was used in the current study for its high dormancy behavior. NILDOG1 plants were grown in three biological replicates, with four plants for each replicate, and seeds were harvested at maturity. Half of the freshly harvested dormant seeds were stored directly at 4°C to retain dormancy and the other half were after-ripened in ammonia at 20°C under continuous light (143 μmol m⁻² s⁻¹). Pictures were taken once a day for a period of 6 d using a Nikon D80 camera (Nikon, Tokyo, Japan) focused to a repro stand with a 60 mm macro objective. The camera was connected to a computer using Nikon CAMERA CONTROL PRO software, v.2.0. Clustering of seeds was prevented as much as possible. Germination was scored using the GERMINATOR package (Joosten et al., 2010). The effect of transcription inhibition on seed germination was tested by germinating the completely after-ripened seeds on different dosages (0.1, 1, 10, 100 and 1000 μM) of the transcription inhibitors cordoncepin and ά-amanitin (Sigma). Seed germination and seedling establishment were evaluated every day. For the ribosome isolation, both dormant and after-ripened seeds were spread on wetted papers filled with either 1 mM cordoncepin or water to ensure homogeneous spacing. The Petri dishes were wrapped with parafilm M (Bemis Company Inc., Neenah, WI, USA) to prevent water loss during seed imbibition. The experiment was carried out in a 22°C incubator under continuous light (143 μmol m⁻² s⁻¹). Seeds were sampled at 6 and 24 h after the start of imbibition (HAI). The harvested tissue was frozen in liquid nitrogen followed by freeze-drying. The dry material was stored at −80°C for further analyses.

Isolation of total RNA and polysomal RNA and polysome analysis
For the isolation of polysomal RNA, 400 mg of freeze-dried tissue was extracted with 8 ml of polysome extraction buffer (PEB; 400 mM Tris, pH 9.0, 200 mM KCl, 35 mM MgCl₂, 5 mM EGTA, 50 μg ml⁻¹ cycloheximide, 50 μg ml⁻¹ chloramphenicol) according to Subramanian (1978) and Mustroph et al. (2009a) with some modifications. The extracts were loaded on top of a sucrose cushion (1.75 M sucrose in PEB) and centrifuged (18 h, 90 000 g) using a Beckman Ti70 rotor for 18 h (Beckman Coulter, Brea, CA, USA). The resulting pellet was resuspended in wash buffer (200 mM Tris, pH 9.0, 200 mM KCl, 0.025 M EGTA, 35 mM MgCl₂, 5 mM dithiothreitol, 50 μg ml⁻¹ cycloheximide, 50 μg ml⁻¹ chloramphenicol), loaded on a 20–60% linear sucrose gradient, and centrifuged at 190 000 g for 1.5 h at 4°C using the Beckman SW55 rotor (Beckman Coulter). After ultracentrifugation, the gradients were fractionated into 20 fractions using a Teledyne Isco Density Gradient Fractionation System (Teledyne Isco, Lincoln, NE, USA) with online spectrophotometric detection of 254 nm. The polysomal fractions were pooled for polysomal RNA isolation. The ribosome abundance is reflected by the area under the curve and was calculated after subtracting the baseline obtained by measuring a blank gradient and normalizing to total area under the curve to account for possible uneven loading of the gradients.

Microarray hybridization and data analysis
Affymetrix Arabidopsis Gene 1.1 ST Arrays (Affymetrix, Santa Clara, CA, USA) were hybridized using the GeneChip® 3′ IVT Express kit (Affymetrix; cat. no. 901229) according to the manufacturer’s instructions. Hybridization data were analyzed and gene-specific signal intensities were computed using the R statistical programming environment (www.R-project.com), the BioConductor package affy (Gautier et al., 2004) and the Brainarray cdf file v.17.1.0 (http://brainarray.mbl.med.umich.edu/). DNA microarray data are available in Supporting Information Table S1 and in the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE75368. The limma and affy packages were used for RNA normalization (Irizarry et al., 2003) Probe set intensity signals that never exceeded the noise threshold (logExp < 4 in all samples) were removed. Signal distribution before and after RNA normalization and RNA degradation were evaluated using the affy package (Fig. S1). A linear model and Empirical Bayes methods were applied to assess differential expression (Smyth, 2005; Diboun et al., 2006). Gene enrichment analyses (Tables S2–S4) were performed using PANTHER Overrepresentation Test (release 20160715, P-value = 0.05) from the Gene Ontology Consortium (http://geneontology.org/page/go-enrichment-analysis).

Auxin metabolite profiling
Seeds were imbibed for 6 or 24 h, harvested in liquid nitrogen and freeze-dried; 1 mg freeze-dried material per replicate was
used for IAA metabolite profiling as described previously (Novak et al., 2012), with minor modifications (Novak et al., 2016). Two-way ANOVA and Tukey honest significant differences test were performed in R (v.13.4.0) for each metabolite at a significance level of 0.05.

**Results and discussion**

**Polysomal profiling reveals translational inhibition in dormant seeds**

Seeds of NIL*DOG1*-Cvi (Cape Verde Islands) were used to investigate the importance of translation during the imbibition of dormant seeds. The germination capacity of NIL*DOG1* seeds was followed during after-ripening. Freshly harvested seeds do not germinate, but dry storage (after-ripening) releases dormancy and the germination frequency gradually increases until it reaches 100% after 120 d (Fig. 1a). The freshly harvested dormant (D) and fully after-ripened nondormant seeds (AR) that present fully contrasting dormancy levels were used to determine the role of translation in dormant imbibed seed, using a translatomics approach. For that, polysomal profiles were run on dormant and after-ripened seeds at 6 and 24 HAI. At these time points, seeds do not yet show visible germination (Fig. 1b). The ribosomal profiles reveal no differences between dormant and after-ripened seeds at 6 HAI; however, at 24 HAI after-ripened seeds show a larger proportion of polysomes than do dormant seeds, indicating that after-ripened seeds are actively translating (Figs 1c,d, S2).

Inhibition of germination in dormant seeds is controlled by regulation of transcript abundance

The increase of polysomes in after-ripened seeds 24 HAI could be a result of increased specific translation of mRNAs or increased levels of translatable mRNAs. Both aspects play roles during germination and seedling establishment in Arabidopsis (Bai et al., 2016). To investigate this during seed dormancy regulation, total and polysomal-associated mRNAs of dormant and after-ripened seeds were compared using microarray analysis. This methodology does not allow a distinction to be made between changed transcription and changed mRNA stability. Therefore, genes that were expressed to relatively higher levels in dormant or after-ripened seeds during imbibition were defined as dormancy- or germination-associated genes, respectively. At 6 HAI, 304 and 300 genes were dormancy-associated and 315 and 258 genes were germination-associated in the total and polysomal mRNA fractions, respectively. At 24 HAI, numbers increased to 991 and 1136 for the dormancy-associated genes and 1691 and 2021 for the germination-associated genes.
1789 genes for the germination-associated genes in total and polysomal fractions, respectively (Fig. 2a). To address whether translational regulation occurs during the imbibition of dormant seeds, the PO for each mRNA was analyzed (relative level of mRNA in the polysomal fraction compared with its level in the total RNA pool). Our analysis showed that the dormancy state hardly affected the PO of the individual mRNAs (Fig. 2a). This is confirmed by the highly significant correlation between the change of specific mRNA species in the total mRNA in dormant vs after-ripened seeds and the change of the same mRNA in the polysomal mRNA (Fig. 2b). Overall, these analyses show that the observed increased translation in after-ripened seeds (Fig. 2d) depends on transcription and that the impact of translational regulation is relatively minor. This finding contrasts with conclusions of Basbouss-Serhal et al. (2015), where translational regulation during the imbibition of after-ripened and dormant Columbia seeds was suggested. The discrepancy observed may be caused by the different experimental systems we define dormancy release based on after-ripening time and use fully discriminative stages (zero (D) vs 100% germination (AR)), while previously Basbouss-Serhal et al. (2015) studied temperature-dependent germination that leads to a germination difference of 40% between the two dormancy stages. In addition, we imbibed the seeds in light, whereas the seeds in the study of Basbouss-Serhal et al. (2015) were imbibed in darkness. Light signaling has previously been shown to greatly impact translation in seedlings (Juntawong & Bailey-Serres, 2012; Gamm et al., 2014; Missra et al., 2015). Thus, the discrepancy in the results may be related to the different light or temperature conditions (Leon & Owen, 2003; Hofmann, 2014) or to other differences in experimental conditions, and further illustrates the complex multifactorial regulation of seed germination (Bentsink & Koornneef, 2008; Rajjou et al., 2012).

Role of transcription during the imbibition of dormant and after-ripened seed

Transcriptional regulation in the control of seed dormancy is important and was further investigated using transcriptional inhibitors. For this, two transcriptional inhibitors were used, α-amanitin and cordycepin. α-Amanitin was reported to inhibit seedling establishment but not germination, whereas cordycepin fully inhibited germination (Rajjou et al., 2004). In our study, both transcriptional inhibitors had a dosage-dependent effect on seed germination and seedling growth (Fig. 3a,b); however, 1 mM cordycepin completely abolished seed germination and seedling establishment. The difference in inhibitory effect between α-amanitin and cordycepin might be a result of differential uptake of these compounds by seeds. Cordycepin (1 mM) was used to inhibit transcription because it effectively blocked seed germination. Interestingly, the polysomal profiles of the cordycepin-treated seeds mimic those of the dormant seeds (Fig. 1c,d). Next, the effect of cordycepin on the total and polysomal-associated mRNAs was investigated. The number of genes differentially expressed at 6 HAI in cordycepin vs water is relatively low; 12 genes and one gene are induced and 19 and seven genes are inhibited by cordycepin on the total and polysomal mRNA levels, respectively (Figs 3, S3). This indicates that cordycepin hardly affects transcription at 6 HAI. Cordycepin blocks RNA polymerase II during mRNA chain elongation and it can also interfere with RNA 3’-end formation and polyadenylation, as suggested by Holbein et al. (2009). The lack of effect in early seed germination may be because cordycepin has not (yet) penetrated the seed or because the post-transcriptional effects of cordycepin dominate in the early stages as a result of limited transcriptional activity at this time point. At 24 HAI these differences have increased to 168 and 218 induced genes and 332 and 300

Fig. 2  The influence of dormancy level on the abundances of total mRNA and polysomal mRNA in Arabidopsis seeds. (a) The effect of dormancy on total RNA abundance (blue bars), polysomal RNA abundance (red bars) and polysome occupancy (PO = polysomal RNA abundance/total mRNA abundance; purple bars) at two stages of imbibition (6 and 24 h after the start of imbibition, HAI). Genes are considered as transcriptionally or translationally regulated when log₂(fold change) > 1 and P < 0.05 adjusted by the false discovery rate. Dormancy-associated genes are genes that are higher in dormant (D) than in after-ripened (AR) seeds, and after-ripening-associated genes are those that are higher in AR than in D seeds. (b) Correlation between total RNA changes (log₂AR/D) and polysomal RNA changes (log₂AR/D) of genes transcriptionally after-ripening-associated (red dots) and transcriptionally dormancy-associated (green dots) in AR seeds compared with D seeds at 24 HAI. Black dots represent genes that are not associated with either of the earlier-mentioned categories. Correlation coefficients are highly significant (P < 2.2E–16).

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inhibited genes in the total and polysomal associated mRNAs, respectively. We also provided the data for the cordycepin-treated dormant seeds; however, as these seeds cannot be separated phenotypically from the water-treated seeds (both are nongermination) we do not discuss these genes further. Although transcription is expected to be blocked by cordycepin, up-regulation of genes is observed. This suggests that cordycepin does not completely block transcription. Another explanation could be that the total pool of mRNA is reduced as a result of mRNA decay and that the genes identified as up-regulated are genes that are more stable. Investigating this possibility is of special interest, as a role for mRNA decay in the regulation of seed dormancy has recently been reported (Basbouss-Serhal et al., 2017). As an effect of global mRNA decay is hard to reveal using microarrays, as a result of global normalization, we performed quantitative reverse transcription polymerase chain reaction experiments for a selected group of genes (Table S7) that are revealed as up-regulated after cordycepin treatment (Fig. S5). The analyses confirmed the microarray data. The reference genes that we used for the normalization showed a stable expression and we therefore exclude an effect of global mRNA decay in these analyses. Overall, the minor effect of the inhibition of transcription at 6 HAI suggests that transcriptional activity at early time points does not determine whether seeds are able to germinate or not.

Role of the Trp-dependent auxin biosynthesis pathway in germination inhibition

In the presence of the transcriptional inhibitor cordycepin, dormant and after-ripened seeds do not germinate. As no PO changes were detected when comparing after-ripened (germinating) and nongerminating (dormant and cordycepin-inhibited) seeds, the overlap between the dormant and cordycepin-inhibited transcriptomes was investigated. Time-dependent changes from 6 to 24 HAI in dormant, nongerminating seeds were compared with the same time-dependent changes in after-ripened, germinating seeds. These analyses resulted in four groups of genes: those that are either up- or down-regulated from 6 to 24 HAI in the dormant seeds and those that are up- or down-regulated from 6 to 24 HAI in the after-ripened seeds. These four groups were compared with each other to select the genes that are dormancy-associated (899 genes that are dormancy up-regulated and after-ripening down-regulated from 6 to 24 HAI) and those that are after-ripening-associated (1266 genes that are after-ripening up-regulated and dormancy down-regulated from 6 to 24 HAI) (Fig. 4a). Gene ontology analysis revealed that the dormancy-associated genes are related to abiotic stress responses such as light, heat, oxidative stimulus and seed development, while a series of metabolic processes were after-ripening-associated (Table S3). Similarly, time-dependent changes from 6 to 24 HAI in cordycepin-treated seeds were compared with the same time-dependent changes in after-ripened, germinating seeds. These analyses resulted in four groups of genes: those that are either up- or down-regulated from 6 to 24 in the cordycepin-treated seeds and those that are up- or down-regulated from 6 to 24 HAI in the after-ripened seeds. These four groups were compared with each other to select the genes that are cordycepin-associated (93 genes that are cordycepin up-regulated and mock (imbibition of after-ripened seeds in water) down-regulated from 6 to 24 HAI) and those that are mock-associated (124 genes that are mock up- and cordycepin down-regulated from 6 to 24 HAI) (Fig. 4a). Among

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**Fig. 3 Cordycepin effect on transcription during two stages of Arabidopsis seed imbibition.** (a) Dosage-dependent germination inhibition by cordycepin. Bars represent means ± SD of three biological replicates. (b) Dosage-dependent germination inhibition by α-amanitin. (c) Number of transcripts influenced and inhibited by cordycepin in after-ripened seeds at 6 and 24 h after the start of imbibition (HAI) in total RNA and polysomal RNA, respectively. PO, polysome occupancy.
the cordycepin-associated genes were the hormone metabolism genes *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 5, 6 (NCED5, 6)* and *GIBBERELLIN 20-OXIDASE 3 (GA20OX3)*, confirming the importance of ABA and GA in the control of germination. Sulfur and indole-derived biosynthetic processes were mock-associated (Table S3). The overlap between the differentially expressed genes of the two types of germination inhibition experiments (after-ripened vs dormant seeds and after-ripened vs cordycepin-treated seeds) was significant (Fig. 4b; 41 genes up and 109 genes down in nongerminating seeds). This large overlap
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Fig. 4 The effect of after-ripening and cordycepin on the transcriptional changes from 6 to 24 h after the start of seed imbibition (HAI). (a) Dormancy and cordycepin are two independent factors that block seed germination in Arabidopsis (indicated by the blunt-ended lines). The upwards and downwards arrows indicate the dormancy- and after-ripening-associated genes or cordycepin- and mock (after-ripened seeds imbibed in water)-associated genes during 6–24 HAI. (b) Venn diagram comparing the genes influenced by dormancy and cordycepin during 6–24 HAI. (c) The enriched metabolic pathway for the genes influenced by both dormancy and cordycepin during seed imbibition. The genes presented in red are dormancy- and cordycepin-associated genes, and the underlined gene (NIT3) was associated with cordycepin only. The genes in black encode enzymes catalyzing metabolic conversions that were not detected in the current study. The metabolites in blue are significantly changed either by dormancy or during seed imbibition. The blunt-ended lines indicate inhibition of the pathway. The question mark indicates a likely, but unconfirmed, conversion. GSTP9, glutathione S-transferase PH19; SOT17, sulfortranseferase 17; SUR1, SUPEROOT 1; IGMT1, indole glucosinolate o-methyltransferase 1; TSA1, tryptophan synthase alpha chain; ASA1, anthranilate synthase alpha subunit 1; BGLU40, beta-glucosidase 40; PYK10, beta-glucosidase 23; CAD9, cinnamyl alcohol dehydrogenase 9; PAL3, phenyl alanine ammonia-lyase 3; PBP1, PYK10-binding protein 1; NIT3, NITRILASE3; IPGs, indole-3-glycerol-phosphate synthase; INS, indole synthase; CCoAOMT, caffeoyl-CoA O-methyltransferase; COMT, caffeic acid O-methyltransferase; Trp, tryptophan; Phe, phenylalanine. (d) Level of free IAA, IAA conjugates and IAA synthesis pathway intermediates during dormant and after-ripened seed imbibition. Ant, anthranilate; Tra, tryptamine; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IAOx, trans-cis-indole-3-acetaldoxime; IPyA, indole-3-pyruvic acid; IAA-glc, IAA-glucose; IAAsp, IAA-aspartate; IAGlu, IAA-glutamate; oxIAA, 2-oxoindole-3-acetic acid; oxIAA-glc, oxIAA-glucose. Data are normalized by seed DW.

Further strengthens our conclusion that seed dormancy is mainly controlled at the transcript level. Interestingly, many of the overlapping genes are related to both Trp-dependent auxin (IAA) and glucosinolate biosynthesis (Fig. 4c; Table S4). Trp-dependent auxin biosynthesis is linked to the indole glucosinolate pathway through the intermediate indole-3-acetaldoxime (IAOx; Zhao et al., 2002; Sugawara et al., 2009; Ljung, 2013). Several genes related to these pathways were down-regulated in the transcriptome of nongerminating seeds (Fig. 4c; Table S5). This includes transcripts coding for enzymes sequentially involved in the conversion of indole-3-glycerol phosphate (IGP) to indole (Ouyang et al., 2000), indole to Trp (Sun et al., 2009), Trp to IAOx (Zhao et al., 2002), and IAOx to S-alkyl-thiohydroximate and thiohydroximate (Barlier et al., 2000; Bak & Feyereisen, 2001; Klein & Papenbrock, 2009; Pfalz et al., 2011; Kim et al., 2015). Further, the glucosinolate biosynthesis pathway probably interacts with the phenylpropanoid biosynthetic pathway through IAOx (Hemm et al., 2003; Mach, 2015). Also enzymes involved in different steps in the phenylpropanoid pathway are down-regulated in nongerminating seeds (Fig. 4c; Table S5). These findings suggest that Trp-dependent auxin biosynthesis and related pathways are strongly repressed in nongerminating (dormant and transcriptionally inhibited) seeds.

To investigate whether the repression of Trp-dependent auxin biosynthesis led to metabolic differences, auxin biosynthesis pathway intermediates were quantified in the dormant and after-ripened seeds during imbibition (Fig. 4d; Table S6). IAA in dry seeds is mainly stored as conjugates, which are hydrolyzed during early imbibition to yield free IAA (Ljung et al., 2001). The hydrolysis of the IAA conjugates is a rapid process that allows immediate access of IAA to remove the need for long-distance transport. This process is followed by Trp-dependent auxin biosynthesis in developing tissues in the root and shoot apex (Ljung et al., 2001, 2005). Concentrations of free IAA and the conjugates IAA-Asp and IAA-Glu are rather low (50-fold lower) compared with the IAA precursors (indole-3-pyruvic acid (IPyA) and Trp). Free IAA concentrations were only marginally influenced by imbibition time (P = 0.048) and no difference was detected between the dormancy stages. The concentrations of Trp and derived IPyA, which are the main Trp-dependent auxin synthesis precursors (Ljung, 2013), were increased in the after-ripened seeds. Interestingly, the auxin influx carrier AUX1 was recently reported to have a positive role in seed germination regulated by histone H3K9K18 deacetylation (Wang et al., 2016). Moreover, the cell cycling-related Cyclin D-type (CYCD) genes that are downstream of AUX1 and are important for radial promotion (Wang et al., 2016) were also among the dormancy and cordycepin-inhibited genes in our analyses (Table S5). This might be a downstream effect of the auxin pathway as auxin is known to influence the cell division in both embryonic and vegetative stages (De Veylder et al., 2007).

In all, our data indicate the importance of auxin biosynthesis in seed germination; however, how the auxin synthesis pathway is activated remains unknown. In-depth studies on the regulation of seeds at different after-ripening stages could provide insights into this regulation.

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Author contributions

B.B., J.H. and L.B. designed the experiments. B.B. performed the experiments and conducted the analyses. O.N. and K.L. performed IAA metabolite profiling and analyzed the data. B.B., J.H. and L.B. wrote the manuscript. All authors commented on the manuscript.

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**Fig. S1** Gene 1.1 ST Genechip quality assessment and reproducibility.

**Fig. S2** Absorbance profiles of sucrose density gradient fractionated ribosomes at 0, 6 and 24 h after imbibition (HAI).

**Fig. S3** Bar graph representing the number of total and polysomal transcripts influenced and inhibited by cordycepin and seed dormancy.

**Fig. S4** Venn diagram comparing total and polysomal transcripts influenced by dormancy.

**Fig. S5** Confirmation of the cordycepin effect on changes in transcript abundance during seed imbibition.

**Table S1** The normalized and filtered dataset used for statistical analysis

**Table S2** Gene ontology for the total and polysomal RNA changes affected by seed dormancy

**Table S3** Gene ontology for the transcriptional changes during dormant seed imbibition in water and nondormant seed imbibition in cordycepin

**Table S4** Gene ontology for the genes affected by both dormancy and cordycepin

**Table S5** Genes affected by both seed dormancy and cordycepin

**Table S6** Concentrations of IAA and IAA precursors/metabolites in pg mg⁻¹ DW

**Table S7** Genes and primers used for qRT-PCR

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