



To germinate or not? Transcriptional gradients underlie the seed dormancy continuum

Dechang Cao ^{1,*}

¹ Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

*Author for correspondence: caodc@ibcas.ac.cn

Seed dormancy enables many plants to cope with harsh environments by delaying germination. Many plants across the phylogenetic spectrum produce seeds that are dormant at maturity. An extensive synthesis of over 13,000 species whose seed dormancy has been determined over the world revealed that plants producing dormant seeds predominate most ecosystems (Baskin and Baskin, 2014). There are several classes of seed dormancy that can arise for various reasons, including low growth potential of the embryo, impermeability of the seed coat, and underdevelopment (or undifferentiation) of the embryo. Among them, physiological dormancy, caused by low growth potential of the embryo, is the most widely occurring (Baskin and Baskin, 2014).

Dormancy obtained as seeds mature is called primary dormancy. Before the growing season, primary dormancy can be released and seeds become nondormant in preparation for germination. This process is reversible; nondormant seeds can become dormant again (secondary dormancy). In fact, dormant seeds can cycle back and forth between dormancy and nondormancy in soil seed banks. Dormancy does not begin nor end abruptly in the dormancy cycles, but instead there is a dormancy continuum, that is, seeds experience a series of gradually changing physiological states of conditional dormancy. The dormancy continuum allows inter-seed variation of dormancy depth within a population, which spreads the risk of germination in unfavorable seasons at the population level. However, the mechanisms underlying the dormancy continuum and the inter-seed variation of dormancy have long been elusive. In this issue of *Plant Physiology*, Krzyszton et al. (2022) performed comprehensive analyses of single-seed transcriptional germination competence to address this issue.

Detection of single-seed transcriptional heterogeneity is challenging due to high costs and small seed sizes. Krzyszton

et al. (2022) developed a time-saving and cost-effective protocol that skips RNA isolation and enables direct reverse transcription in crude seed extracts to profile single-seed transcriptomes using Illumina sequencing. By comparing to transcriptomes of pooled dry seeds, single-seed transcriptomic analyses revealed highly variable gene expression among seeds, which proved single-seed RNA-Seq as a useful approach to detect transcriptomic heterogeneity among individual seeds before they are distinguishable by any visible phenotypes.

Primary dormancy can be released by dry storage of the seeds at room temperature, a process generally referred to as “after-ripening.” Krzyszton et al. (2022) after-ripened fresh *Arabidopsis* (*Arabidopsis thaliana*) seeds for 6 months to alleviate dormancy, then stressed the seeds in a dark and high-temperature environment for 7 days to induce secondary dormancy. Residual variance of mRNA levels of specific genes in different seeds was used to evaluate the transcriptional heterogeneity within a population, which allowed Krzyszton et al. (2022) to distinguish high variable genes (HVGs) from those with homogeneous expression. Intriguingly, the number of HVGs decreased during the process of dormancy induction, suggesting a directional change in the transcriptomic landscape of single seeds in response to stress. Many of the HVGs encode seed storage materials or late embryo-abundant proteins, which are active in the process of dormancy induction. This result suggests that stress preferentially induced and prioritized pathways important for seed survival rather than germination.

The large datasets generated by single-seed RNA-Seq allowed credible identification of globally co-expressed gene clusters. There were two clusters of co-expressed genes showing unidirectional patterns during secondary dormancy induction. The first group showed a continuous decrease of

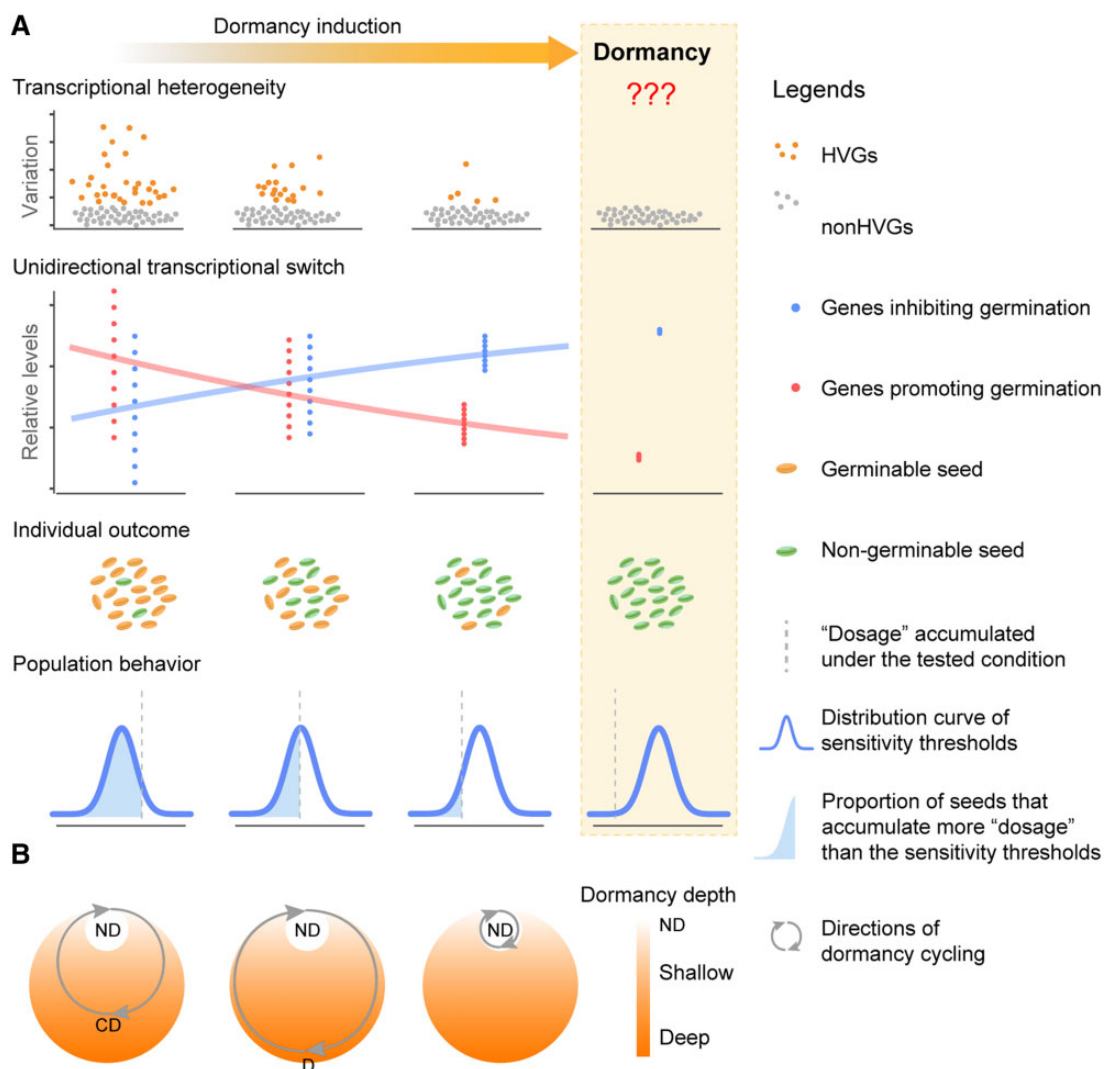


Figure 1 Gradual changes in single-seed transcriptional germination competence underlie the dormancy continuum. A, Different layers of regulating mechanisms underlying the dormancy continuum. During the process of secondary dormancy induction, there is a gradual decrease in the amount of HVGs. HVGs that inhibit germination generally increased, whereas those promoting germination gradually decreased. These transcriptomic switches in single seeds determine the binary outcome of their germination or not. The proportional distribution of individual outcome underlies germination percentages of the seed population in a specific germination condition. These different layers of changes finally lead to a gradual decrease in proportions of seeds that can germinate in the population (the blue shaded areas) after continuous dormancy induction. The yellow shaded column shows the possible situations of dormant seeds that are not covered in the Krzyszton et al.'s (2022) work. B, There are different kinds of dormancy cycles for seeds with physiological dormancy. Seeds exhibiting annual dormancy/nondormancy (D/ND) cycles differ with those having conditional dormancy/ND (CD/ND) cycles, in terms of dormancy depths of when they reach the deepest dormancy in the cycles. Also, seeds of some species maintain ND in soil seed banks. Changes in single-seed transcriptional germination competence of these different kinds of dormancy cycles will provide further insights into regulation of physiological dormancy in seeds. The cartoon seeds in the "Individual Outcome" of panel (A) were adopted from a free vector of www.vecteezy.com.

mRNA levels, while the second group exhibited the opposite pattern. Such unidirectional and continuous switch of transcriptional patterns occurred in accordance with the gradual decrease in transcriptional heterogeneity. These transcriptomic changes led to increasing homogeneity of seed physiological status, allowing the seed population to become gradually insensitive to environmental cues for germination.

The transcriptomic switch during stress treatment of *Arabidopsis* seeds revealed by Krzyszton et al. (2022) fit the population-based threshold (PBT) model, which has long

been used to quantitatively describe germination capacity (or dormancy status) of seed populations. In traditional population-based seed biology, physiological transition of seeds is viewed as a result of dosage effects. Seeds lose dormancy after accumulating individual specific dosages of time (for after-ripening) or hormonal levels (Bradford, 2018). Thus, there are specific "thresholds" for each seed, and the proportional distribution of the thresholds of the seed population can be described using PBT models. The transcriptional switch revealed by Krzyszton et al. (2022) during

dormancy induction added a “transcriptional dosage” to the PBT models of seed germination.

The genes with a decreasing expression level were enriched in translation- and ribosome-related GO terms (Krzyszton et al., 2022), similar to findings of Buijs et al. (2020). A similar study of the gymnosperm Chinese fir (*Cunninghamia lanceolata*) revealed genes regulating transcription and translation having reversible changes in dormancy cycles (Cao et al., 2016), suggesting that there could be a conserved mechanism of transcriptional and translational regulation underlying physiological dormancy in gymnosperms and angiosperms. Further, Krzyszton et al. (2022) performed a single-seed RNA-Seq on *A. thaliana* seeds with the mutated gene *DELAY OF GERMINATION 1* (*DOG1*) and found that these genes were highly affected by *DOG1*, suggesting potential roles of *DOG1* in regulating secondary dormancy.

The findings presented by Krzyszton et al. (2022) build a graceful bridge between the germination competence of single seeds and germination behaviors of seed populations at the transcriptional level. The authors showed that stress treatments gradually induced a decrease in inter-seed transcriptional heterogeneity along with directional changes in expression patterns of key genes associated with dormancy regulation (Figure 1A). The transcriptional germination competence of each seed determines its binary outcome of germination or not. The proportional distribution of binary outcome of all seeds in a population determines the germination percentage.

After the dormancy-inducing treatment, the fully stressed seeds germinated at the permissive temperature (12°C) showed almost 100% germination, despite low germination percentages (<40%) at 22°C and 27°C, indicating that the seeds were not totally dormant but conditionally dormant. Thus, situations of truly dormant, as well as nondormant seeds, require further investigation (Figure 1A), as does the question of whether dormant seeds maintain transcriptional homogeneity in response to imbibition. As previously

indicated, seeds with physiological dormancy may experience annual cycles of dormancy/nondormancy or dormancy/conditional dormancy in soil seed banks, and in some cases can maintain nondormancy (Cao et al., 2012). Future studies on single-seed transcriptional germination competence during dormancy/nondormancy or dormancy/conditional dormancy cycles will provide further insights into the transcriptional landscape associated with physiological dormancy of seeds (Figure 1B).

Funding

D.C. was supported by the Startup Funds for Recruited Young Talents from the Kunming Institute of Botany (KIB), Chinese Academy of Sciences, and a grant from the Germplasm Bank of Wild Species of KIB.

Conflict of interest statement. None declared.

References

- Baskin CC, Baskin JM (2014) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Ed 2. Academic Press/Elsevier, San Diego, USA
- Bradford KJ (2018) Interpreting biological variation: seeds, populations and sensitivity thresholds. *Seed Sci Res* **28**: 158–167
- Buijs G, Vogelzang A, Nijveen H, Bentsink L (2020) Dormancy cycling: translation-related transcripts are the main difference between dormant and non-dormant seeds in the field. *Plant J.* **102**: 327–339
- Cao D, Baskin CC, Baskin JM, Yang F, Huang Z (2012) Comparison of germination and seed bank dynamics of dimorphic seeds of the cold desert halophyte *Suaeda corniculata* subsp. *mongolica*. *Ann Bot* **110**: 1545–1558
- Cao D, Xu H, Zhao Y, Deng X, Liu Y, Soppe W, Lin J (2016) Transcriptome and degradome sequencing reveals dormancy mechanisms of *Cunninghamia lanceolata* seeds. *Plant Physiol* **172**: 2347–2362
- Krzyszton M, Yatusевич R, Wrona M, Sacharowski SP, Adamska D, Swiezewski S (2022) Single seeds exhibit transcriptional heterogeneity during secondary dormancy induction. *Plant Physiol* **190**: 211–225