Seed dormancy back on track; its definition and regulation by DOG1

Seed traits like dormancy, germination rate, and longevity determine the start of the life cycle of plants. The desired values for these traits can be very different for plants growing under natural or agricultural conditions. For the first, germination should be postponed until the start of a favorable growth season and germination should be well balanced with the prevailing environmental conditions. For crop plants, germination should be immediate, uniform and fast.

Dormancy is defined as the absence of germination of a viable seed under conditions that are suitable for germination. Dormancy is usually measured on a population of seeds. Seed populations that do not fully germinate in conditions that favor germination can therefore be described as having a certain level of dormancy. Reduction in dormancy leads to a widening of the environmental conditions at which a seed can germinate and often to an increase in the rate of germination. However, we would advocate not to mix up germination rate with seed dormancy as we sometimes see in comparisons of mutant and wild-type seed batches.

Dormancy should be determined by analyzing germination under suitable conditions. Absence of germination or low germination percentages in populations of seeds indicate dormancy, which can be overcome either by extended storage in dry conditions (after-ripening) or imbibition at specific low or high temperatures (depending on the plant species; stratification). These dormancy releasing treatments should lead to an increased germination percentage. A typical experiment that allows identification of differences in dormancy level is a so-called after-ripening experiment. For this, seeds should be harvested from plants that are grown at the same time in the same conditions. Germination experiments are subsequently performed at increasing time intervals during seed dry storage. Generally it is seen that both germination percentage and germination rate increase with longer storage times (Ni & Bradford, 1993; Alvarado & Bradford, 2005; Meyer & Allen, 2009; Batlla & Benech-Arnold, 2015). To determine dormancy differences it is not required to evaluate germination percentage at multiple time-points after sowing, endpoint measurements are sufficient. The level of dormancy can be expressed in one value by calculating the Days of Seed Dry Storage required to reach 50% of germination (DSDS50 or AR50) (Alonso-Blanco et al., 2003) (Fig. 1a–d). Alternatively, a germination experiment at a fixed time-point after seed harvest also allows the identification of differences in dormancy levels (Fig. 1e, f). In such an experiment the seeds should be allowed enough time to germinate to ensure that a plateau phase is reached in order to avoid misinterpretation of differences in germination rate as dormancy. Apart from increasing after-ripening time, dormancy levels could also be determined by increasing stratification time (Kendall et al., 2011).

Differences in germination rate can be caused by genetic differences, as a response to the environment during seed production, or by the interaction between these two. This can often be observed in seed batches of crop plants (Li et al., 2011; Yang et al., 2015; Shim et al., 2020). Mutants that affect the sensitivity to environmental factors for germination can also show altered germination rates (Gualberti et al., 2002; Yu et al., 2019). Finally, germination rate can be associated with seed viability and aged seeds generally need more time to germinate (Bewley et al., 2013). However, differences in germination rate should not be confused with dormancy. To identify differences in germination rate, germination needs to be investigated at multiple time-points after sowing. Preferably such analyses should be performed in fully after-ripened seeds, since only then an effect of residual dormancy can be excluded. Germination rates can be visualized in graphs showing germination percentage on the $y$-axis and imbibition time (or time after sowing) on the $x$-axis. From such graphs the T50, which is the time in which half of the maximum germination percentage is reached, can be calculated (Fig. 1g,h). As germination rate can be derived from these graphs it is inappropriate to label the $y$-axis with ‘germination rate’ instead of ‘germination percentage’.

We see this frequently in the literature and would like to appeal to editors of scientific journals to prevent the interchange of ‘germination rate’ with ‘germination percentage’.

Seed dormancy is an adaptive trait, which is regulated by endogenous and environmental factors. These factors influence the levels of central dormancy regulators that, in their turn, control downstream mechanisms enabling the binary decision of a seed to germinate or not. Our current view is that the hormone abscisic acid (ABA) and the dormancy protein DELAY OF GERMINATION 1 (DOG1) are two of the most important central regulators of seed dormancy in Arabidopsis, as well as in other studied plant species. Both factors are necessary for seed dormancy and the absence of a single one of them leads to loss of dormancy. Several excellent recent reviews have extensively described the roles of these and other factors in dormancy (Chahtane et al., 2017; Finch-Savage & Footitt, 2017; Nonogaki, 2019). The intention of this Letter is not to repeat these reviews but to focus on the function of DOG1 because, in our opinion, recent publications did not always reflect the latest insights. DOG1 was originally identified as a seed dormancy quantitative trait locus (QTL) in Arabidopsis (Alonso-Blanco et al., 2003). The DOG1 gene was cloned 14 years ago and encodes a protein that lacks domains with a known function, which made it very difficult to obtain clues about its mode of action. In the first paper that
described the cloning of the DOG1 gene, it was suggested that DOG1 might have a direct role in transcriptional regulation based on a low level of homology to the wheat transcription factor HBP-1b. In addition, one of the three protein domains of DOG1 is also present in group D bZIP transcription factors, although other conserved domains of the bZIP proteins are missing (Bentsink et al., 2006). In the first 10 years after its cloning, no clear insights into its function were obtained and no confirmation for a role of DOG1 as a transcriptional factor was found. Nevertheless, it has been shown that the transcriptome of nondormant seeds discriminates already shortly after rehydration from that of dormant seeds (Dekkers et al., 2016a,b). Accordingly, seeds of the dog1 mutant showed altered transcription of hundreds of genes (Dekkers et al., 2016a,b). We think it is obvious that a mutant that has a strong impact on the dormancy level of seeds also causes major transcriptional changes, but this does not directly imply that it actually functions as a transcription factor.

The conserved and important role of DOG1 in seed dormancy was confirmed in various plant species like wheat (Ashikawa et al., 2014) and lettuce (Huo et al., 2016), and its function in several dormancy-related processes was revealed. This showed that DOG1 influences processes that are related to the regulation of germination and dormancy like endosperm weakening (Graeber et al., 2014) and controlling the level of microRNAs involved in phase transition (Huo et al., 2016). More direct insights into the mechanism by which DOG1 influences dormancy and germination were obtained recently when two laboratories independently showed that the DOG1 protein binds to and inhibits the action of specific members of clade A PP2C phosphatases in seeds. A genetic analysis showed that two of these phosphatases, ABA-HYPERSENSITIVE GERMINATION 1 (AHG1) and AHG3 are necessary for the function of DOG1 (Née et al., 2017; Nishimura et al., 2018). Interestingly, this group of phosphatases also has a central role in ABA signaling and therefore represents a converging point of these two important regulators of dormancy (Fig. 2). A protein that functions by inhibiting phosphatases is unlikely to have a direct role in transcriptional regulation. Therefore, the first ideas about the function of DOG1 as a
transcriptional factor are unlikely to be true. Further studies are required to identify the functional domains of DOG1, their exact molecular roles and downstream (transcription) factor(s).

With this Letter, we would like to advocate for a correct use of the terms seed dormancy and germination rate and for a proper reference to the latest insights of the actions of its most important regulators. We think that this is necessary to focus dormancy research in the right direction and to avoid following outdated leads.

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Author contributions

WJJS and LB wrote the manuscript. WJJS and LB contributed equally to this work.

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