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Rapid accumulation of NO regulates ABA catabolism and seed dormancy during imbibition in Arabidopsis

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Nitric oxide's (NO) involvement in breaking seed dormancy has been demonstrated in previous research but its action mechanism remains to be clarified. We observed that a rapid accumulation of NO induces an equally rapid decrease of abscisic acid (ABA) that is required for the NO's action in Arabidopsis. In addition, the NO-induced ABA decrease correlates with the regulation of *CYP707A2* transcription and the (+)-abscisic acid 8'-hydroxylase (encoded by *CYP707A2*) protein expression. By analyzing *cyp707a1, cyp707a2* and *cyp707a3* mutants, we found that *CYP707A2* plays a major role in ABA catabolism during the first stage of imbibition. Fluorescent images demonstrate that NO is released rapidly in the early hours at the endosperm layer during imbibition. Evidently such response precedes the enhancement of ABA catabolism which is required for subsequent seed germination.

Key words: seed dormancy, germination, nitric oxide (NO), (+)-abscisic acid 8'-hydroxylase, ABA, *CYP707A2*

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Seed germination is a complex process and incorporates events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis.[1,2] Seeds of most angiosperms are dormant at maturity and this dormancy must be broken before germination can occur.[3] Seed dormancy is a very complicated problem and is described as "one of the least understood phenomena in seed biology" and remains confusing despite much recent progress.[3] It has been defined as the incapacity of a viable seed to germinate under favorable external conditions.[4] Seed dormancy can be explained as coat-imposed dormancy or embryo dormancy. In Arabidopsis it is controlled by both seed coat and embryo.[3,5-7]

ABA plays an important role in a number of physiological processes such as seed maturation, growth and developmental regulations, seed dormancy and adaptation responses to environmental stresses.[8-11] In addition, ABA has been shown as an important positive regulator of both the induction of dormancy during seed maturation and the maintenance of the dormant state in imbibed seeds following shedding.[12,13] Previous research indicates that seed dormancy is controlled by ABA and correlates with both ABA biosynthesis and catabolism.[31] In Arabidopsis, *CYP707A*s family plays an important role in ABA catabolism during imbibition.[30] Our results indicate that *CYP707A2* plays a major role in ABA catabolism during imbibition and regulates seeds dormancy.

NO acts as a signaling molecule and plays an important role in plants and animals.[14-16] In plants, previous studies have shown that NO can be emitted by plant cells and act as a growth regulator.[17,18] NO induces seed germination in replacement of red light,[17] affects growth and development,[19] increases iron homeostasis and accelerates plant cell senescence.[20,21] Furthermore, NO has been suggested to be involved in resistant responses to drought, salinity, heat stress, diseases, programmed cell death and ultraviolet-B radiation.[18,22] Some research also indicates that NO participates in seed dormancy and germination control.[17-19] Our results give evidences that NO regulates seed dormancy and germination and such action needs *CYP707A*s family's participation. NO can break the dormancy of wild type Arabidopsis seeds,

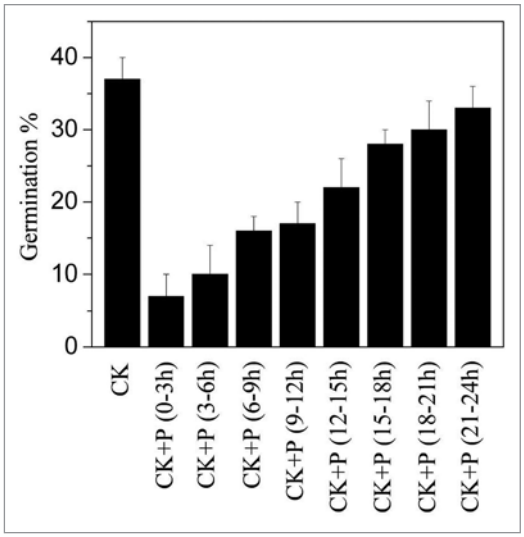

Figure 1. Effects of nitric oxide (NO) produced at different hours during imbibition on dormancy break. Seeds were imbibed with water and were scavenged for NO with c-PTIO at each 3 h at the first 24 h imbibition. Germination rate was detected after 7 d of imbibition.

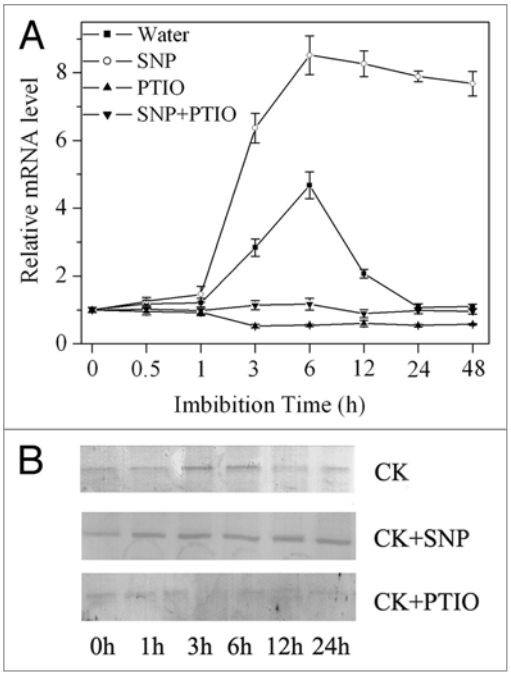

Figure 2. Effect of NO on *CYP707A2* gene expression and ABA 8'-hydroxylase expressions encoded by *CYP707A2*. (A) Changes of *CYP707A2* transcripts in freshly harvested WT seeds imbibed with water, SNP and c-PTIO. (B) western-blot analysis of ABA 8'-hydroxylase expressions in seeds of Arabidopsis during germination when treated with water (CK), sodium nitroprusside (SNP) or c-2-phenyl-4,4,5,5-tetramethyl imidazoline-1-oxyl-3-oxide (c-PTIO). Freshly harvested seeds were germinated under different treatments. Proteins were extracted and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were immunodetected with antibody (produced in rabbit) against ABA 8'-hydroxylase encoded by *CYP707A2*. Each treatment was immunodetected with antibody.

but it can't break *cyp707a2* mutant seed dormancy.

The relationship between NO signaling and ABA response has been demonstrated by some investigators.[23-26] For example, ABA-induced guard cell closure needs NO participation.[23,26-28] However the mechanism of NO affecting seed dormancy is not clear so far. Our study demonstrates that the rapidly accumulated NO induces an equally rapid ABA decrease that is required for seed dormancy break and germination in Arabidopsis. Our results indicate that seeds rapidly accumulate NO at first 3 h imbibition, and if NO was removed at this time the seed dormancy was enhanced. As shown in **Figure 1**, when NO was scavenged with PTIO at first 9 h of imbibition for 3 h, seed dormancy was enhanced substantially. NO induces ABA catabolism mainly by regulating the expression of ABA catabolism gene family *CYP707A*, especially by regulating the expression of *CYP707A2* and ABA 8'-hydroxylase encoded by *CYP707A2* (**Fig. 2A and B**).

We also observed that NO accumulation mostly visible at aleurone layer. Earlier study showed that aleurone layer responds to NO and the response is necessary for seed dormancy.[29] Although a lot of studies have indicated that NO is necessary for seed dormancy control, how NO is released during imbibition remains to be clarified. Whether it is by nitrate reductase, NOS or some other ways is not clear. In addition, if NO is not released by a single pathway, very possibly it is so, how these processes are regulated and/or coordinated are interesting questions. All of these need further studies.

In summary, our results demonstrate that *CYP707A2* and ABA 8'-hydroxylase encoded by *CYP707A2* play central role that is involved in ABA catabolism during imbibition in the seeds of Arabidopsis. NO acts crucially in regulating the transcription of *CYP707A2* and ABA 8'-hydroxylase encoded by *CYP707A2*. Our results also demonstrate that a rapidly accumulated NO at the first stage of imbibition is required for rapid ABA catabolism and seed dormancy break. The remaining question is how NO is rapidly accumulated at this stage is still vague. It needs more experimental evidence to illustrate this mechanism in the future work.

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