DOCTORAL THESIS

Molecular signaling in the seed dormancy release and germination in arabidopsis

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Molecular Signaling in the Seed Dormancy Release and Germination in Arabidopsis

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Doctor of Philosophy

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Abstract

Seed germination is a complex process. Many signaling molecules and plant hormones participate in the breaking of its dormancy and initiation of its germination. Nitric oxide (NO) has been reported to be involved in breaking seed dormancy but its action mechanism is not clear. In this study we found that a rapid accumulation of NO induced an equally rapid decrease of abscisic acid (ABA) that is required for this action in the seed of Arabidopsis. Results of QRT-PCR and western indicate that 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 of the endosperm layer during imbibition. Evidently such response precedes the enhancement of ABA catabolism which is required for subsequent seed germination.

H_2O_2 is known as a signal molecule in plant cells and also plays a role in the regulation of seeds dormancy and germination. We found that H_2O_2 is involved in the seed dormancy and germination of Arabidopsis by regulating the ABA catabolism and GA biosynthesis during imbibition. Results of QRT-PCR showed that H_2O_2 up-regulated ABA catabolism gene *CYP707A*s, which decreased ABA content during imbibition. Such action required the participation of NO, another signal molecule. Results of QRT-PCR also indicate that H_2O_2 up-regulate GA biosynthesis at the same time. When ABA catabolism mutant, *cyp707a2*, and over-expressing plant, *CYP707A2-OE*, were used, ABA showed a negative correlation with GA biosynthesis. Exogenously applied GA overrode the inhibition of germination by low concentration of ABA, but the effect was not significant at high concentration of ABA. Together our results conclude that H_2O_2 mediates the upregulation of ABA catabolism, possibly through NO, and also the GA biosynthesis. High concentration of ABA also inhibits the GA biosynthesis but a balance of these two hormones jointly controls the dormancy and germination of Arabidopsis seeds.

Function of MAPK pathways on seed germination was also investigated. After analysis 10 *MPAKK*s mutants, we found that two of them (*MKK2* and *MKK10*) are involved in the ABA-regulated seed dormancy and germination. Our results indicate that both *mkk2* and *mkk10* show ABA-insensitive during germination. By measuring some ABA-regulated gene expression (*RD29A and RD29B*), MKK2 and MKK10 are likely to affect the expression of these genes regulated by ABA to participate in the regulation of seed dormancy and germination. Furthermore, we also found that MKK2 and MKK10 mediate ABA-suppressed GA biosynthesis since this suppression was not found in *mkk2* and *mkk10* mutant

In summary, hormones, signaling molecules and MAPK cascades pathways constitute a complex network mediate seed dormancy break and germination.

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