DOCTORAL THESIS

Roles and regulatory mechanisms of proanthocyanidins during the seed germination in arabidopsis

Jia, Liguo

Date of Award:
2012

Roles and Regulatory Mechanism of Proanthocyanidins during the Seed Germination in *Arabidopsis*

JIA Liguo

A thesis submitted in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Prof. ZHANG Jianhua

Hong Kong Baptist University

June 2012

Abstract

Proanthocyanidins (PAs) are the main products of flavonoid biosynthetic pathway in seed but their biological functions in seed are still unclear. It was observed that seed germination was delayed with the increase of exogenous PAs concentration in *Arabidopsis*. Similar inhibitory effect occurred in peeled *Brassica napus* seeds by measuring its radicle elongation. Using abscisic acid (ABA) biosynthetic and metabolic inhibitor and gene expression analysis by real-time PCR, the inhibitory effect of PAs on seed germination is founded to due to its promotion of ABA *de novo* biogenesis, rather than any inhibition on its degradation. Consistent with the relationship between PAs contents with ABA accumulation in seeds, PAs-deficient mutants maintain a lower level of ABA compared with wild-type during germination. The data suggest that PAs distribution in the seed coat can act as a door keeper to determine seed germination. Its regulation on seed germination is mediated by ABA signaling pathway.

PAs' antioxidation function has long been proposed but how it is related to seed germination is not known. We studied PAs' anti-oxidation ability in seed coat and germination changes in PAs-deficient mutants of *Arabidopsis* under externally applied oxidative stresses. Germination of PAs-deficient mutant seeds was faster than that of wild type under low or no oxidative stress, suggesting a PAs-induced inhibition of germination. When the applied oxidative stress was high, germination of PAs-deficient mutants was lower than that of wild type, suggesting a loss of PAs-related anti-oxidation ability in the mutants. Using ABA signaling mutants, the studies demonstrated that both ABA signaling pathway and PAs are important for the response to serve oxidative stress during seed germination. However, the discrepancy of the response between *abi* mutants and PA mutants to oxidative stress suggests that ABA signaling pathway may not play a major role in PA's action in alleviating oxidative stress. Under low or no oxidative stress, germination was mainly determined by the ABA content in seed and the PAs-deficient mutant seeds germinated faster due to their lower ABA content than wild-type. However, oxidative injury inhibited germination when PAs-deficient seeds germinated under high oxidative stress. Wild-type exhibited higher germination under the high oxidative stress due to the PAs' anti-oxidation ability. Oxidative stress applied externally led to changes in endogenous PAs contents which coincided with the expression changes of PAs biogenesis genes. PAs modulated the activities of some key enzymes that controlled the levels of reactive oxygen species and the anti-oxidation capacity during the seed germination. This work suggests that PAs contribute to the adaptive mechanism that helps germination under environmental stresses by playing dual roles in both germination control and anti-oxidation reaction.

PAs in seed coat of *Arabidopsis* can function as antioxidant to remove superoxide radicle ($O_2^{\cdot -}$). However, we found that although PAs-deficient mutants have more accumulation of $O_2^{\cdot -}$, their hydrogen peroxide (H_2O_2) content is much lower when compared with wild-type. By examining three universal antioxidant enzymes, superoxide dismutase, catalase and peroxidase, we found peroxidase is much over activated in the mutant seeds. Biochemical staining experiment confirmed that the

high peroxidase activity in the mutant is mainly confined to the seed coat. There is no significant difference of peroxidase activity in the embryo between wild-type and the mutants. In fact some candidate peroxidase-encoding genes are down-regulated in terms of expressions in the embryo of PAs-deficient seeds. The results indicate that an enhanced peroxidase system in the coat of PAs-deficient mutant seeds is an adaptive mechanism for seed development and survival. A feedback regulation mechanism to H_2O_2 scavenging system in the embryo may also exist at the same time.

A new mutant with yellow seed coat was obtained in this study, which is called *transparent testa 20* (*tt20*). Histochemical analyses show *TT20* specifically regulates PAs accumulation but not mucilage distribution is *Arabidopsis* seeds. It also attenuates anthocyanins accumulation in seedlings under low temperature, water stress, sucrose and exogenous auxin treatments. Naringenin feeding assay together with RT-PCR expression analysis implies that PAs and anthocyanins accumulations have been determined before naringenin biosynthesis. Moreover, relatively more (-)-epicatechin as PAs oligomer distributed in mutant seeds suggest that *TT20* might involve in PAs polymerization. Higher germination speeds and lower anti-oxidation of *tt20* mutant are identified, which is consistent with the functions of PAs in Arabidopsis seeds. Although the gene locus of *TT20* is still not been localized, *CHI* should be the key candidate targets of *TT20* during PAs and anthocyanins biogenesis.

In summary, it has been found that PAs act as a biochemical regulator in the seed coat during the seed germination. *Arabidopsis* provides a unique platform for the study of their molecular mechanism. More research is needed to reveal how their accumulation is regulated during seed development.

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