Form 2

Dissertation Abstract

Report no.	(Course-based) No.991	Name	TOTAN KUMAR GHOSH
Dissertation title	Abscisic Acid Responses in Basal Land Plants Reveal Molecular Mechanisms Crucial for Stress Adaptation in Embryophytes (基部陸上植物のアブシジン酸応答から明らかとなる有胚植物のストレス適応に必須の分子メカニズム)		

Abstract

Abscisic acid (ABA), a ubiquitous phytohormone existing in a wide variety of organisms and regulating many of the important physiological processes of plants like growth, development, stomatal conductance, seed maturity and dormancy, senescence etc. Nowadays, it has been reflected as the stress hormone which triggers responses to various environmental stresses such as desiccation and freezing. Bryophytes comprising liverworts, mosses and hornworts are being postulated as earliest lineage of extant land plants and occupying most critical stage of land plant phylogeny. A lot of research efforts have been made with ABA response and its signaling pathways in angiosperms. In contrast, very few claims have been reported on basal land plants; bryophytes. Hence, the efforts were made in the present study to reveal the ABA signaling molecules conserved in embryophytes using two widely used model bryophytes, moss *P. patens* and liverwort *M. polymorpha*. Carotenoid mediated ABA biosynthesis, and its role in higher plants are well documented today. Contrary, this information is still unclear in basal land plants due to lack of mutant lacking ABA biosynthesis enzyme. The *PpABA1* encoding zeaxanthin epoxidase crucial enzyme for carotenoid mediated ABA biosynthesis was found in *P. patens* and *ppaba1* line lacking *PpABA1* was developed by the efforts of gene targeting. The *ppaba1* lines accumulated very small amount of endogenous ABA and showed reduced osmotic acclimation capacity in correlation with reduced accumulation of *LEA*-like genes indicating conserved role of endogenous ABA in hyperosmotic acclimation of mosses. Whereas, less effect on cold acclimation capacity of *ppaba1* indicated minor role of endogenous ABA in cold acclimation of mosses. The results suggested that moss comprises both ABA-dependent and independent signaling during acclimation to environmental stresses.

Recently, couple of demonstrations proposed that the PP2C mediated negative ABA

regulation is conserved in bryophytes. In contrast, positive regulation mediated by SnRK2 is yet to be clarified in this plant group. Hence, for getting new clue and diversified information of positive ABA response, further we engaged liverwort *M. polymorpha* for comparative functional analysis with moss *P. patens*. However, in compared with moss, very few demonstrations of ABA response in liverwort were emphasized though it is known as sister group to all land plant lineages. We analyzed *MpARK* of liverwort *M. polymorpha* as the ortholog of *PpARK*, mutation of which causes a strong ABA insensitive phenotype in AR7 mutant of *P. patens*. Restoration of ABA sensitivity in AR7 by the cDNA of *MpARK* indicated the presence of positive ABA regulator in liverwort. Overexpression of *MpARK* in *M. polymorpha* resulted the enhancement of ABA induced growth inhibition as well as accumulation of *LEA*-like transcripts in transgenic lines expressing *MpARK-GFP* indicates conserved positive ABA regulation process in liverworts.

Later on, we demonstrated ABA-induced *LEA* gene expression and analyzed promoter elements triggering ABA response. ABA-induced gene expression is generally mediated by ACGT-core sequences recognized by bZIP transcription factors. But functional activation of these promoter elements are still unknown in liverworts. Therefore, we analyzed promoter elements for ABA response in *liverwort M. polymorpha* by transient assay using endogenous *dehydrin* and heterologous wheat *Em* promoter fused with *GUS* reporter. The assays resulted that ACGT-core motifs proximal to the transcription initiation site contribute the major role in ABA-mediated gene expression. The RY sequence recognized by B3 domain of ABI3 also exhibited responsible for ABA induced gene expression in liverwort *M. polymorpha*. The results suggested that the presence of common *cis*-acting ABA responsive elements in liverworts. Finally, it can be claimed that the signaling molecules essential for regulating ABA responses are conserved in embryophytes and the mechanisms were developed during early stage of land plant evolution and adaptation.