Form 2

**Dissertation Abstract**

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| Dissertation title | Roles of sugar and ABA on stress tolerance in the liverwort *Marchantia polymorpha*（苔類ゼニゴケのストレス耐性における糖とアブシジン酸の役割） | | |


Abstract

***Objective***

Analysis of ABA insensitive mutants has led to identification of key regulatory genes require ABA for expression in response to a stress. Genes requiring ABA for expression are not induced in these mutants. So presence of stress tolerant genes responsive to ABA can be determined by using ABA insensitive mutants. Although many mutants have been reported, but information about ABA insensitive mutants of Marchantia and their response to stress are quite limited. It is thus important to analyze genetically the ABA insensitive mutants and transgenic of bryophytes for the further understanding of molecular mechanism of stress tolerance. In comparison to the studies on higher plants, information on the freezing behavior of bryophytes and factors that affect their freezing tolerance is quite limited. In this study the gemmae, which are vegetative clones produced on the thalli of *M. polymorpha,* have high sensitivity to ABA, and they are an ideal system to analyze ABA-induced changes in stress tolerance. It is thought that a number of factors can contribute to desiccation tolerance in plants, including specific proteins, antioxidants, sugars and other compatible solutes, though details of those in various species of bryophytes have not been clarified. To clarify mechanisms underlying physiological processes leading to development of stress tolerance in *M polymorpha* cells accumulation of low molecular weight soluble sugars in were analyzed with and without ABA treatment.

***Discussion***

The plant growth regulator abscisic acid (ABA) is involved in triggering responses to various environmental stresses such as freezing and desiccation in angiosperms, but little is known about its role in lower plants, especially in liverworts, representing one of the earliest land plant lineages. ABA-insensitive mutants and the transgenic have been isolated to compare their stress tolerance with that of wild type. For isolation of ABA-insensitive mutants, screening was carried out on gamma-radiated sporangium of Marchantia. Transgenic liverwort lines were developed by introducing MpABI1A encoding the group A protein phosphatase 2C, known as a negative regulator of ABA signaling. For analysis of ABA responses, I focused on gemmae, the

asexual reproductive form of liverworts which have higher degree of stress tolerance under natural condition but the underlying mechanisms are not understood. I found that sucrose that is accumulated in response to ABA plays a crucial role in stress tolerance in the liverwort gemmae. In a freezing test, survival rate after freezing of ABA-treated gemmae increased as the sucrose concentration in medium increased. In a desiccation test, the highest survival was observed in 0.3 M sucrose treated sample with ABA. It was thought that sucrose functions as a protectant during freezing and drying but accumulation of sucrose might vary in the presence or absence of ABA. Determination of sugar levels during culture with or without ABA revealed that the gemmalings accumulated more sugars in response to ABA. In contrast, it was found that mature thalli, which are sensitive to both freezing and desiccation, did not accumulate sugars in response to the ABA treatment. Results of TLC analysis indicated that the gemmalings accumulate sucrose by ABA treatment. Observation by a dissection microscope and area measurement indicated that growth of gemmalings was inhibited by 0.1 M and greater concentrations of sucrose. Significant growth inhibition was found when ABA was present in the medium. By northern blot analysis, I examined the expression of ABA-responsive genes isolated from ABA-treated gemmae and thalli. ABA treatment of gemmalings induced accumulation of transcripts for proteins with similarity to late embryogenesis abundant (LEA) proteins, which is accumulated in association with acquisition of desiccation tolerance in maturing seeds of angiosperms. It was found that expression of these transcripts was also induced by 0.1 M sucrose.

***Conclusion***

Experiments conducted in this study demonstrated that ABA plays a role in accumulation of sucrose and LEA-like proteins in gemmalings and enhances their stress tolerance in liverworts. Despite the experimental evidence showing growth inhibition and increased stress tolerance induced by exogenous ABA, the role of ABA in liverworts has been an issue of controversy among scientists for many years due to the lack of detailed physiological and structural studies. Results for *M. polymorpha* gemmalings obtained in this study provide convincing evidence for the role of ABA in environmental stress tolerance together with the results of our previous study showing the presence of molecular machinery for ABA signaling. It is proposed that freezing and desiccation stress signals affecting the ABA signaling process leading to accumulation of sugars and various transcripts such as those encoding LEA-like proteins are driven by conserved mechanisms operating in land plants required for stress tolerance, which will be revealed by molecular genetic studies.