E219 Water Stress Responses in the leaves of *Selaginella tamariscina*

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In order to investigate the tolerance limit under water stress, an individual *S. tamariscina* was grown in each pot and its morphological change was monitored. After *S. tamariscina* was given water stress, the protein patterns of its leaves were also examined. *S. tamariscina* was found to resurvive once it was rehydrated after 90 days of water stress. *S. tamariscina* leaves under water stress for 7 days showed no specific proteins induced by water stress. But the amount of 53kDa specific protein was increased after 90 days of water stress and was decreased during recovery periods. Based on the immunoblot using anti-dehydrin antibodies, this 53 kDa protein turned out to be a dehydrin-like protein. Acid phosphatase activity was known to be changed by water stress, and in the case of *S. tamariscina* leaves, total acid phosphatase activity was increased 40% by water stress. These results suggested that *S. tamariscina* could minimize and overcome the effect of water stress by increasing the the amount of 53 kDa stress protein

E220 Cytochrome P450 Mediated Reactions During Brassinosteroids Biosynthesis in *Marchantia polymorpha*

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We have previously demonstrated that *Marchantia* cells contain enzymes responsible for the conversion from teasterone to typhasterol via 3-dehydroteasterone, a partial biosynthetic sequence of the early C6-oxidation pathway. Recently, we identified castasterone and its biosynthetic precursor in the late C6-oxidation pathway, 6-deoxocastasterone from *M. polymorpha*. These indicated that both biosynthetic pathway, the early and late C6-oxidation pathway for brassinosteroids biosynthesis are present in *M. polymorpha*. To confirm that, we examined conversions of 6-deoxocastasterone to castasterone, typhasterol to castasterone and castasterone to brassinolide by *in vitro* enzymatic reactions. All three conversions were successfully catalyzed by microsomal enzymes prepared from *M. polymorpha*. Furthermore, the enzymatic reactions were strongly inhibited by cytochrome c, a specific inhibitor of cytochrome P450 proteins, indicating that the three enzymes, namely 6-deoxocastasterone oxidase, typhasterol 2α -hydroxylase and castasterone oxidase (lactonase) are cytochrome P450 enzymes. In this presentation, *in vivo* conversions of 6-deoxocastasterone to castasterone and typhasterol to castasterone established by feeding experiment using deuterium labelled([$^2H_6$]) substrates are also discussed.