

Adaptive responses to abiotic and biotic stresses in plants

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Plants are challenged by various environmental conditions and responded differently in terms of plants survival and crop productivity. These abiotic and biotic stresses result developing mechanisms to perceive stresses and having adaptive changes in plants. Three different stresses would be discussed in this talk along with introduction of the brief signal transduction pathway for each stress.

Many components in the signal transduction pathway for pathogen attack are found and protein kinases are the major role in pathogen recognition and activation of downstream defense mechanisms. After the signals for pathogen attack are perceived by plasma-membrane-bound receptor or extracellular and intracellular *R*-gene products, the signals transduce through downstream signaling components, such as calcium-dependent protein kinases MAP kinases, salicylic acid (SA), jasmonic acid (JA), etc. Activation of different kinases subfamilies and biosynthesis of SA and JA lead to changes in gene expression and the activation of defense mechanisms.

Chlamydomonas reinhardtii shows adaptive changes in order to survive in limiting CO₂ conditions because they live in quite variable conditions of CO₂ availability. We are trying to identify mutants defective in the acclimation response to limiting CO₂. Nuclear transformation to complement an Arg phenotype was used to generate insertional mutants, and *Cah1* expression and having high CO₂-requiring (HCR) phenotype were used as reporters for induction of genes involved in acclimation to limiting CO₂. Among a collection of insertionally generated mutants, a mutant has been isolated that showed no pCA1 protein and no *Cah1* mRNA. This mutant strain, designated *cah1-1*, has been confirmed to have a disruption in the *Cah1* gene caused by a single Arg7 insert. *cah1-1* is its lack of any significant growth phenotype, indicating pCA1 is not essential for function of the CO₂-concentrating mechanism or for growth of *C. reinhardtii* at limiting CO₂ concentrations. Eight independently isolated mutants of *C. reinhardtii* that require high CO₂ for photoautotrophic growth were

tested by complementation group analysis.

Two of the new mutants formed a single complementation group along with the previously described mutant *cia-5*, which appears to be defective in the signal transduction pathway for low CO₂ acclimation. The other mutations represent six additional, independent complementation groups. From approximately 7000 transformants screened for lack of *Cah1* expression, HCR90 was identified. This mutant shows a recessive HCR phenotype that co-segregates with the Arg⁺ phenotype and presence of 1 Arg insert. HCR90 does not show any allelism to the known HCR mutants. K_m (C_i) of HCR90 was found to be higher than that of wild type, suggesting HCR90 lacks a functional CCM. After 5' and 3' sequences flanking the Arg insert were obtained, genomic clones from a *C. reinhardtii* BAC library were identified by using both flanking DNA as a probe. The 5' and 3' flanking sequences identified non-overlapping sets of BACs, indicating a rather large disruption. Since HCR90 grows poorly in low CO₂, complementation of the mutation should be possible under direct selection in low CO₂. Transformations with a cDNA expression plasmid library for complementing of HCR90 phenotype are in progress.

Various abiotic stresses reduce crop yields and about 80% of wheat was lost by these abiotic stresses such as temperature, drought, flooding and salinity. Wheat survives subfreezing temperatures after a period of cold acclimation by regulating gene expression, stabilization of membranes and accumulation of sugars, other solutes and antifreezing proteins. Ice formation in the intercellular spaces subfreezing temperatures results in a decrease water potential outside the cell, indicating by movement of liquid water from intracellular to extracellular space. The active control of water flow may be crucial for adaptation to subfreezing temperatures, but the details of the mechanisms are not known. Water movement across the plasma and tonoplast membranes is mediated by aquaporins with various members of this gene family specific for development, stress and cellular distribution. We have isolated two different wheat aquaporin PIP (plasma membrane intrinsic protein) cDNA clones from cold-acclimated and subzero temperature-treated wheat crown cDNA library. One PIP, a PIP1 is a widely expressed vegetative aquaporin while the other, a PIP2 is upregulated by cold acclimation and exposure to freezing temperatures in crowns but not leaves and roots. The expression of the PIP2 correlates with water efflux from the crown in response of subfreezing temperature. Using yeast two hybrid system, a protein

interacting with PIP2 was identified from cold treated wheat crown library. The protein interacts with the PIP2 but not the PIP1. Functional analysis of the PIP2 interactive protein is in progress. Using yeast two hybrid system, PIP3 interacting with cold-upregulating and crown specific PIP2 (plasma membrane intrinsic protein) was identified from cold treated wheat crown library. Functional analysis of the PIP3 is in progress.

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