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Influence of Sucrose on *In Vitro* Growth, Photosynthetic Pigment and Rubisco/Rubisco Activase in Tobacco

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The influence of sucrose on *in vitro* growth, chlorophyll, and rubisco/rubisco activase were studied in tobacco. After 7 weeks of treatment of 1-5% sucrose concentrations, the most pronounced effect on *in vitro* growth and chlorophyll was found at 4% sucrose. Rubisco activity increased with increasing concentrations of sucrose, but a point was reached beyond which increasing concentrations of sucrose cause inhibition of this enzyme. Rubisco content showed patterns of change similar to rubisco activity. These data suggest that rubisco activity was associated with an amount of rubisco protein, and that sucrose can be both a positive effector and negative effector. The degree of intensity of 55 and 15 kD polypeptides identified as the large and small subunit of rubisco by SDS-PAGE analysis at 4% sucrose was significantly higher than that at other treatments, indicating sucrose had an effect on both subunits. Under the assumption that effects of sucrose on rubisco may be related to rubisco activase, in addition to, its activity and content were determined. The rubisco activase activity at 4% sucrose was more increased than the others. A similar change pattern was also observed in content of rubisco activase. The intensity of two 52 and 51 kD polypeptide bands at 4% sucrose was higher than that of corresponding bands at the others. These results suggest that the stimulatory effects of the activation of rubisco by sucrose seem to be caused by rubisco activase.

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Nucleoside Diphosphate Kinase 2 Interacts with Two Oxidative Stress-activated Mitogen-activated Protein Kinases to Regulate Cellular Redox State and Enhances Multiple Stress Tolerance in Transgenic Plants

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Nucleoside diphosphate kinases (NDPKs) are multifunctional proteins that regulate a variety of eukaryotic cellular activities. However, much less is known about the functional significance of NDPKs in plants. We show here that NDPK is associated with H₂O₂-mediated MAPK signaling in plants. H₂O₂ stress strongly induces the expression of the NDPK2 gene in *Arabidopsis thaliana* (AtNDPK2). Proteins from transgenic plants overexpressing AtNDPK2 showed high levels of autophosphorylation and NDP kinase activity, and they have lower levels of reactive oxygen species (ROS) than wildtype plants. Mutants lacking AtNDPK2 had higher levels of ROS than wildtype. H₂O₂ treatment induced the phosphorylation of two endogenous proteins whose molecular weights suggested they are AtMPK3 and AtMPK6, two H₂O₂-activated A. thaliana MAPK. In the absence of H₂O₂ treatment, phosphorylation of these proteins was slightly elevated in plants overexpressing AtNDPK2 but markedly decreased in the AtNDPK2 deletion mutant. Yeast two-hybrid and *In Vitro* protein pull-down assays revealed that AtNDPK2 specifically interacts with AtMPK3 and AtMPK6. Furthermore, AtNDPK2 also enhances the myelin basic protein phosphorylation activity of AtMPK3 *In Vitro*. Finally, constitutive overexpression of AtNDPK2 in *Arabidopsis* conferred an enhanced tolerance to multiple environmental stresses that elicit ROS accumulation *in situ*. Thus, AtNDPK2 appears to play a novel regulatory role in H₂O₂-mediated MAPK signaling in plants.

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Chilling-induced Enhancement of Fatty Acid Unsaturation of Phosphatidylglycerol in Rice Plants

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We analyzed the fatty acid compositions of membrane lipids of rice plants from the detached leaves or intact ones, respectively, that had been exposed to low temperatures in combination with light for certain periods of time. When the detached leaves of rice plants were chilled in light for 48h, lipid classes such as MGDG (monogalactosyl diacylglycerol), DGDG (digalactosyl diacylglycerol), SQDG (sulfoquinovosyl diacylglycerol) and PG (phosphatidylglycerol) hardly showed any significant changes in their fatty acid compositions. The results indicated that chilling of rice plants did not lead to the regulatory development of omega-3 fatty acid desaturase, at least on the level of detached leaves. By contrast, when rice plants were acclimated to cold for several days by shifting down the temperature from 15°C to 4°C, and then the intact leaves were subjected to fatty acid analysis, it was found that ratio of 18:3 to 18:2 of PG increased. The observations suggest that chilling of intact rice plants induced enhancement in the expression and/or activity of omega-3 fatty acid desaturase. The fatty acid compositions of other lipid classes such as MGDG, DGDG were not significantly affected by chilling, which indicates that omega-3 fatty acid desaturase might act exclusively on the specific classes of membrane lipids. SQDG was noted for its alteration in the fatty acid composition in response to chilling. We propose that acclimation of rice plants to chilling is associated with increase in the degree of fatty acid unsaturation of PG.

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Maximum Growing of *Dunaliella salina* in Mixotrophic Growth

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To obtain the economic benefits of biomass production, the maximum growth rate of *Dunaliella* was induced by use of the mixotrophic culture. Initially, heterotrophic capability of *Dunaliella* has been investigated with 3 different *Dunaliella* strains including *D. bardawill*, *D. salina* (UTEX 1644) and *D. salina*-155. *D. salina*-155 has been selected from mutated *D. salina* (UTEX 1644). Three different strains grow autotrophically with the same culture condition described in (Jin *et al.* 2003 in Biotech. and Bioengin.: 81: 115-125). However, when the carbon source, sodium bicarbonate was replaced with sodium acetate, only *D. salina*-155 grew actively in acetate containing media and reached same final cell density as other strains grow autotrophically. When *D. salina*-155 was subjected to mixotrophic growth condition, final cell density of this strain was higher by 50% than that of control. This mixotrophic growth (acetate media or glucose media with light) of *D. salina* is discussed in terms of its potential utilization by the algal biotechnology industry for production of high-value bioproduct.