1149 Changes in Nitrogen Metabolism under Stressful Conditions

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Introduction: Drought tolerant plants can use several mechanisms to maintain productivity under stress conditions. Changes in nitrogen metabolism in the roots are of central importance under stressful conditions. Ammonia is assimilated into glutamine and glutamate by the enzymes GS and GOGAT under normal, non-stress conditions. Under stress conditions, nitrate reduction to ammonium and the assimilation of the latter into organic nitrogen compounds is increased in the root while the transport of nitrate to the shoot is blocked at the xylem transport level. This enhanced contribution of the root to the provision of organic nitrogen compounds to sustain plant growth under stress is typical of drought-tolerant RL-genotypes (varStepte) seeds were germinated then transferred to hydroponics in a greenhouse for root samples were extracted immediately after harvesting. Crude extracts for GS assay were obtained following the method described by Rhodes R.J., Assay and native gel electrophoresis for GDH were carried out as described by Pabich & Joy. Results and Discussion: Substantial changes in the levels of GS and GDH (amination) activities were noticed in drought-tolerant RL-genotypes under osmotic shock and salinity. Distinct opposite response on drought and salinity of nitrogen assimilation enzymes - GS, NADH-GDH, ADP were observed. Plants with increased GS and NADH-GDH activities were observed to be more tolerant to drought. The induction of FDGD and the activation/inhibition of PDFD under stress conditions is far more higher in stress tolerant plants. The unsalvageable conclusion seems to be that drought and salinity tolerant crops can and do use alternative variation mechanisms of nitrogen assimilation in roots to maintain productivity under stress conditions.

References:

1150 Catalytic Mechanism of a Family B3 β-glucosidase and Identification of His-169 as an Essential Residue for Activity

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A Family B3 β-glucosidase (EC 3.2.1.21) from Flavobacterium meningosepticum has been cloned and overexpressed. The chemical and physical properties of the recombinant protein are virtually identical to those of the enzyme purified from original microbe (Li, Y-K., and Lee, J-A., Enzym. Microb. Technol. 24, 144-150, 1999). By NMR spectroscopy study, the stereocchemistry of enzyme catalyzing the hydrolysis of p-nitrophenyl-β-D-glucopyranoside was unequivocally identified as occurring with retention of anomeric configuration, indicating that a double displacement mechanism was involved. Based on the kcat values of a series of arylglucosides with the leaving group pKs ranging from 4 to 10, the extended Bronsted relationship showed a concave-downward plot with a breakpoint near by pH 7. The Bronsted constant, β, for poor substrates (with pKs of leaving phenyls greater than 7) is ~0.85. Secondary deuterium kinetic isotope effects with 2,4-dinitrophenyl-β-D-glucopyranoside, o-nitrophenyl-β-D-glucopyranoside, and p-cyanophenyl-β-D-glucopyranoside as substrates are 1.17±0.01, 1.19±0.01, and 1.01±0.01, respectively. An S2,N,2-like mechanism for deglucosylation step and a S2,N,2-like mechanism for glucosylation step are proposed. The Km value of H169A mutant is comparable to that of wild type, whereas its kcat is at least 500-fold smaller. The wild-type enzyme shows a pH-profile with decreasing activity as the pH is increased (from 4 to 9). In contrast, the His-169 mutant exhibits a sigmoid pH-profile with higher activity in the higher pH-range. These results indicate that His-169 is likely to be one of the essential groups which functions as a general acid/base in catalytic mechanism.

1151 Imaging DNA-Looping by EcoRII: A Single Amino Acid Substitution Uncouples DNA Target Recognition from Cooperative DNA Interaction and Phosphodiester Bond Cleavage

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