for light microscopy also showed higher concentrations of peroxidase in induced cucumber leaves, though localization to a cell type was not possible. Diaminobenzidine staining for the electron microscopic localization of peroxidase suggested that peroxidase was concentrated between the electron-dense cell walls and the plasmalemma.

AGRICULTURE, PLANT PHYSIOLOGY

Blue light perception in higher plants. Studies on ATPase activities and redox components at the plasma membrane level. Asard, Han, Ph.D. *Universitaire Instelling Antwerpen (Belgium)*, 1991. 123pp. Major Professor: J. A. De Greef
Order Number DA9129653

Plasma membranes were isolated from several higher plant tissues to characterize ATPase and redox properties. Membrane purification procedures included density gradient centrifugation and aqueous-polymer two-phase partitioning. The purity of fractions from different tissues was addressed through analysis of marker enzyme activities. Glucan synthetase II activity (plasma membrane marker) was significantly enriched and specific activities of markers for tonoplast, ER and mitochondria were strongly reduced.

Enzymatic properties of the plasma membrane ATPase were studied in detail. Vanadate is a potentially specific inhibitor when used at low concentrations and combined with a phosphatase inhibitor.

Three redox components were characterized in the purified plasma membranes. A duroquinone-stimulated NADH-dehydrogenase cosedimented with the vanadate-sensitive ATPase. Apparently two binding sites for duroquinone were demonstrated. The plasma membrane of at least three plant species also contains a specific b-type cytochrome. This component has a redox mid-pointpotential around +110 mV to +160 mV and an α-band maximum at 560.7 nm. It is considered specific for the plasma membrane. The reduction of this cytochrome through irradiation with actinic blue light suggests the presence of a flavo-protein. The flavin and cytochrome are probably the major constituent of a short electron transfer chain in the plasma membrane.

Factors affecting the lipid content of developing pea embryos. Bettey, Mary, Ph.D. *University of East Anglia (United Kingdom)*, 1990. 273pp. Order Number BRDX93154

Available from UMI in association with The British Library. See inside back cover for order information.

The aim of this work was to discover why pea (*Pisum sativum* L.) embryos recessive at the r locus (wrinkled embryos) have a higher lipid content than embryos dominant at this locus (round embryos). The r locus is a gene encoding starch branching enzyme. Wrinkled embryos have a lower activity of this enzyme than round embryos, and a reduced rate of starch synthesis. The higher lipid content of wrinkled embryos must be a consequence of this, but is not due directly to differences in the availability of substrate for lipid synthesis as a consequence of different rates of starch synthesis, or to differences in the capacity for malonyl CoA synthesis.

The different lipid contents of round and wrinkled embryos arise primarily from considerable structural differences between them, which in turn are a consequence of differential accumulation of sucrose, the primary substrate for starch synthesis. Most of the lipid in developing embryos is polar, structural lipid, and polar lipid accounts for much of the difference in lipid content between round and wrinkled embryos. This difference must reflect differences in rates of lipid synthesis and/or degradation.

Acetyl CoA carboxylase has extremely low measurable activity in pea embryos, and is probably rate-limiting for fatty acid synthesis. It is likely to be important in mechanisms leading to a different rate of lipid synthesis in round and wrinkled embryos. Comparison of the kinetic properties of the purified enzyme from round and wrinkled embryos revealed no significant differences. It was inhibited by physiological concentrations of ADP, CoA, and malonyl CoA, and stimulated by magnesium. Inhibition of the enzyme by malonyl CoA might provide a means of regulating flux into fatty acid synthesis. Differential regulation of the enzyme, arising from different demands for polar lipid, might account for the different lipid contents of round and wrinkled embryos.

Expression of the murine ornithine decarboxylase gene in transgenic *Nicotiana tabacum* var. *xanthi*. DeScenzo, Richard Anthony, Ph.D. *University of New Hampshire*, 1991. 234pp. Director: Subhash C. Minocha Order Number DA9151283

Ornithine decarboxylase (ODC), arginine decarboxylase (ADC), and S-adenosyl-methionine decarboxylase (SAMDC) are the three key regulatory enzymes for polyamine (putrescine, spermidine, and spermine) biosynthesis. In order to gain more insight into the relationship between polyamine metabolism and other physiological processes, research was undertaken to obtain increased putrescine biosynthesis in tobacco by overexpression of a murine ODC cDNA.

Both a full-length and a truncated murine ODC cDNA were cloned into a binary expression vector containing the Cauliflower Mosaic Virus (CaMV) 35S promoter. Using standard leaf-disc transformation procedures, transgenic tobacco plants containing either the full length or the truncated ODC cDNA were obtained. Presence of the murine ODC cDNA as well as transcription were confirmed via Southern and Northern blotting. Western blot analysis identified a polypeptide unique to the transformed plants which immunoreacted with anti-ODC antibody.

A series of enzyme assays were done to differentiate between native and murine ODC activity. Assays were run at the pH optima for native ODC (pH 8.2) and murine ODC (pH 6.8). At pH 6.8, there was very little activity in the control plants, but a significantly higher activity in the transformed plants. Difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC activity, completely inhibited ODC activity in the transformed plants at pH 6.8. However in the control plants at pH 6.8 and both the control and transformed plants at pH 8.2, DFMO only inhibited ODC activity by approximately 30–50%. Almost 100% inhibition of ODC activity by immunoprecipitation of ODC protein with anti-ODC antibody was observed in the transformed plants, at pH 6.8. The results show clearly that the activity of murine ODC can be detected and quantified even in the presence of the plant ODC. The transgenic plants containing the truncated ODC cDNA always had several fold higher activity than those containing the full-length cDNA.

Transgenic plants containing the truncated ODC cDNA contained 10–12 times the levels of putrescine than the control plants. Transgenic plants containing the full-length cDNA contained 4–5 times the level of putrescine as compared to the control. In addition to increased levels of putrescine, there was an amine-containing compound unique to the transformed plants with a retention time very similar to putrescine.

The behaviour of coconut and wheat leaf explants in tissue culture: A comparative study. Jesty, James Henry Frederick, Ph.D. *University College, Cardiff (Wales) (United Kingdom)*, 1990. 416pp. Order Number BRDX93116

Available from UMI in association with The British Library. See inside back cover for order information.

A comparative study of cell division in immature leaf tissue of the coconut palm, *Cocos nucifera* L. cv. Malayan Dwarf x Rennell and wheat, *Triticum aestivum* L. cv. Chinese Spring, *in vivo* and *in vitro* was conducted.

There was no basipetal gradient of cell division, or cellular protein content in basal leaf segments of coconut. Cells were arrested in the G1-phase of the cell cycle. Cell and nuclear areas were greater in segments proximal to the leaf base. In wheat, the mitotic index declined, cells arrested in G1, and cellular protein increased, with increasing cell age (i.e. basipetally).

Coconut leaf explants expanded when cultured on semi-solid Y3 medium or liquid 4A then semi-solid 4B medium. Neither leaf segment position, nor 2,4-dichlorophenoxyacetic acid concentration had a significant effect on the growth of coconut leaf explants on Y3. Following culture initiation on Y3, the nuclear: cellular ratio decreased, as did the mitotic index, concurrent with G1 cell arrest. After 3 months *in vitro* growth was re-initiated, cells traversed the G1/S transition; callus formed at this time.

In wheat, the callusing frequency on Murashige and Skoog medium declined with increasing cell age. Callusing was associated with the presence of cells in S phase. In mature, less responsive leaf segments, cells were released from a G1 block; similar to the cellular behaviour of coconut explants.

A major cell cycle control point in G1 operated in coconut and wheat callus. Gradients of esterase isoenzyme activity existed in the leaves of both species. Measurements of cellular protein and isoenzymes in

vivo and *in vitro* were used as markers of proliferative and organogenic potential in cultures of both species. A hypothesis was developed which related the initiation of growth in coconut and wheat cultures to the mediation of an auxin signal by the PI response.

Expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in maize as influenced by light and bleaching herbicides. Ji, Wan, Ph.D. *Virginia Polytechnic Institute and State University,* 1991. 114pp. Chairman: Kriton K. Hatzios

Order Number DA9126878

The activity of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR, EC 1.1.1.34) is highly expressed in 4-day-old etiolated seedlings of normal ('DeKalb XL72AA'), dwarf (d_5), and albino (lw_3) maize (*Zea mays* L.). HMGR activity of maize seedlings appeared to be exclusively associated with the microsomal rather than the plastidic fraction of maize cells. Maize tissues with high meristematic activity such as germinating seeds, leaf bases, root tips, and the site of origin of lateral roots contained high levels of HMGR activity. Results suggest that HMGR may play an important role in cell division and that light may regulate HMGR activity indirectly by increasing cell differentiation. Under conditions of strong light pretreatment with the bleaching herbicides clomazone, norflurazon, fluridone and acifluorfén stimulated by 4- to 7-fold the activity of HMGR extracted from 'DeKalb XL72AA' maize seedlings. The *in vivo* activity of maize HMGR was not stimulated by any of the four bleaching herbicides when herbicide-treated maize seedlings were grown under continuous dark conditions. Results suggest a possible interaction between mature chloroplasts and the nucleus of maize cells in regulating the expression of HMGR activity. A cDNA sequence from 'DeKalb XL72AA' maize was amplified by Polymerase-catalyzed chain reaction (PCR) procedures using two oligonucleotide primers that were based on conserved regions of the sequence of the HMGR gene from tomato (*Lycopersicon esculentum* Mill.). Sequencing analysis revealed that this cDNA sequence was exactly the same as that of the tomato HMGR gene. This indicates that the cDNA might be that of tomato resulting from contamination of the PCR apparatus by tomato nucleic acids. Southern blot analysis, showed that this sequence hybridizes to both maize and tomato genomic DNAs under high stringency conditions. Use of this cDNA sequence as a probe for Northern blot analysis revealed a great discrepancy between the levels of HMGR mRNA and the expression of HMGR activity in maize. The nature of the isolated cDNA sequence is presently unclear. Further research is needed to clarify these results. (Abstract shortened with permission of author.)

Investigation into the mechanism of action of coronatine—a phytotoxin. Kenyon, Janette, Ph.D. *University of East Anglia (United Kingdom),* 1990. 357pp.

Order Number BRDX93159

Available from UMI in association with The British Library. See inside back cover for order information.

Coronatine is a non-specific, chlorosis-inducing toxin, produced by several pathovars of *Pseudomonas syringae.* The aim of this work was to investigate the mechanism of action of coronatine, which, despite considerable research effort, was unknown.

Coronatine induced many physiological changes in plant tissues. Photosynthesis declined, in parallel with loss of chlorophyll. Levels of many amino acids in tobacco leaves altered. Protein synthesis was reduced in cereal root tips and tobacco leaves. Phenolic compounds increased. However, these changes followed, rather than preceeded chlorosis, and required higher doses of coronatine than that required for chlorosis. It was therefore concluded that these observed changes were secondary effects, arising from a still-unknown primary action.

Coronatine stunted the growth of wheat seedling roots more than it stunted shoots. This selective effect suggested a hormone-like action. The effect of coronatine was compared with those of the plant hormones. Of all the hormones tested, only the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) induced chlorotic lesions indistinguishable from those due to coronatine. ACC and coronatine induced very similar physiological and ultrastructural changes in tobacco leaves.

Coronatine stimulated ethylene production by tobacco leaves sooner, and at lower doses, than it induced chlorosis. Coronatine and ACC induced stomatal opening in detached epidermal strips of tobacco, which was blocked by an inhibitor of ethylene action. I concluded that coronatine-induced symptoms were due to ethylene.

Radiolabelling experiments revealed that coronatine induced ethylene biosynthesis, and was not degraded to ethylene. ACC synthase, the rate-limiting enzyme in ethylene biosynthesis, was stimulated by coronatine in etiolated mungbean hypocotyls. I suggest that the primary action of coronatine is to increase the activity of ACC synthase. This would lead to increased ethylene production, which would cause the symptoms associated with coronatine.

Purification and properties of ATP sulfurylase and adenosine 5′-phosphosulfate sulfotransferase from *Euglena.* Li, Jiayang, Ph.D. *Brandeis University,* 1991. 148pp. Chairman: Jerome A. Schiff **Order Number DA9129745**

ATP sulfurylase (ATPS) and adenosine 5′-phosphosulfate sulfotransferase (APSST) catalyzing the first two reactions in assimilatory sulfate reduction have been purified to homogeneity from *Euglena gracilis* var. *bacillaris* (aplastidic mutant W_{10}BSmL) and extensively characterized. Two forms of ATP sulfurylase, one predominantly bound to mitochondrial membrane (ATPSm) and one mostly soluble (ATPSc) are obtained and yield different cyanogen bromide cleavage patterns indicating different primary structures. Both forms are monomeric, ATPSc is 52.3 kDa and ATPSm is 55 kDa. The pI is 7.9 for ATPSc and 5.8 for ATPSm. ATPSc is more sensitive to APS inhibition than ATPSm in the forward (SO_4^{2-} incorporation) reaction; in the reverse reaction ATPSc requires much higher concentrations of PPi and $MgCl_2$ than ATPSm. A novel assay for the two forms in the presence of each other based on bulk DEAE adsorption in presence and absence of NaCl is described.

Various kinetic parameters have been determined for ATPSm and are reported. Using nitrate as a dead-end inhibitor in the molybdolysis reaction, the mechanism of the ATPSm catalyzed reaction appears to be ordered Bi Bi (ATP adding first, PPi releasing first). Phosphate inhibition of the ATPSm-catalyzed sulfate to APS reaction varies with ATP, sulfate and phosphate concentrations; phosphate inhibition is most effective at low ATP and sulfate concentrations.

APS sulfotransferase (APSST) is a tetramer of 102 kDa (with multiple forms from pI 5.0–5.5) held together by covalent (probably S-S) bonds. The apparent Km for APS is 0.1 μM using dithiothreitol (DTT) as the thiol. Activity is stimulated by Mg^{2+}, Ca^{2+} or Ba^{2+}; the enzyme uses any organic thiol but DTT and dithioerythritol give the highest activity. In the absence of APS, the enzyme is inactivated (and is rendered monomeric) by thiols but is protected from thiol inactivation by AMP, adenosine 5′-phosphoramidate (APA) or adenosine 5′-monosulfate (AMS), which also inhibit APSST activity; AMP inhibition is competitive with APS but APA inhibition is uncompetitive. APSST resists inactivation by sodium dodecyl sulfate (SDS) in absence of thiols; SDS stimulates APSST activity at low concentration but is inhibitory at high concentration.

Incubation of APSST with AP^{35}S leads to formation of labeled enzyme. Part of this label is bound to a cysteine residue of the enzyme in the form of S-sulfocysteine and part is found in a low molecular weight compound bound non-covalently to the enzyme which may serve as a sulfo-carrier. A mechanism is proposed for the initial reactions catalyzed by APSST.

Growth, mechanical stress responses and nutation of sunflower seedling hypocotyls. Peacock, Kenneth, Ph.D. *University of Aberdeen (United Kingdom),* 1990. 194pp.

Order Number BRDX93229

Available from UMI in association with The British Library. See inside back cover for order information.

The aims of this thesis were four-fold: (1) To characterise the patterns of growth underlying established nutations of etiolated sunflower seedling hypocotyls. (2) To characterise the growth patterns and events surrounding the initiation of nutation in etiolated sunflower seedling hypocotyls. (3) To investigate the effects of applied compression and tension forces on the straight growth of etiolated sunflower seedling hypocotyls. (4) To investigate the relationship between auxin and the responses to mechanical stress in etiolated sunflower seedling hypocotyls.