11 01

Analysis of the post-freezing stimulation of dark respiration in frost-hardened leaves of *Brassica napus* var. *oleifera* and *Hedera helix*

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In order to analyze the responses of dark respiration to non-lethal freezing stress, frost-hardened shoots of winter rape (*Brassica napus* var. *oleifera*) and ivy (*Hedera helix*) were exposed to -9°C overnight. After thawing the O_2 uptake of leaf slices was measured at 20°C with an oxygen electrode and the cytochrome and alternative pathways were estimated poisoning the respective pathways with CN^- and SHAM. Following freezing the total O_2 uptake was increased 1.5 times in rape and 1.8 times in ivy and the dark CO_2 evolution of intact ivy leaves exhibited a similar rise. The alternative pathway was more increased (3.8 × in rape and 2.6 × in ivy) than the cytochrome pathway (1.3 and 1.6 ×) now coming to about 20 % of the total respiration. There was no evidence of uncoupling of respiration after freezing. However, an enhanced supply of substrate may also contribute to the respiratory rise after freezing since the saccharose content of rape leaves was 2.9 times higher than before freezing.

11 02

Catalytic properties of malate dehydrogenase isoenzymes from castor bean endosperm

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In castor bean (*Ricinus communis* L.) endosperm different molecular forms of malate dehydrogenase (MDH; EC 1.1.1.37) localized in different cell compartments are present. We obtained homogenous preparations of MDH isozymes from the cytosol, mitochondria, glyoxysomes and etioplasts. The mitochondrial isozyme consists of two different types of subunits (42 and 35 kDa), whereas other MDH isozymes contain identical subunits. MDH isozymes are characterized by different K_m values for substrates and coenzymes. K_m (malate) is 5.8 mM for the mitochondrial isoform, 30 mM for the cytosolic form and 25 mM for the etioplast form. It is concluded that different isozymes of MDH localized in different compartments and connected with different malate pools possess specific physical, chemical and catalytic properties important for fine regulation of metabolic pathways of malate conversion in the plant cell.

11 03

Respiratory properties of mitochondria from *Chlamydomonas reinhardtii*

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Mitochondria were isolated from autotrophically grown *Chlamydomonas reinhardtii* cell wall less mutant CW 92. The isolated mitochondria oxidized malate, pyruvate, α-ketoglutarate, NADH and succinate. Respiratory control was obtained with malate (2.0) and pyruvate (2.4) as substrates. The respiratory properties were largely similar to those of mitochondria from higher plants. However, a much lower sensitivity to oligomycin indicates differences in the properties of the *Chlamydomonas* mitochondrial ATPase. Data on comparison of *Chlamydomonas* and potato mitochondrial ATPase will be presented.

11 04

The importance of mitochondrial functions in photosynthetic metabolism.

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The contribution of mitochondria to photosynthetic metabolism was studied in isolated protoplasts and intact leaves of barley and rye. Mitochondrial ATP production was active in a wide range of conditions such as: photorespiratory and non-photorespiratory conditions in both limiting and saturating light. In conditions of saturating light mitochondrial electron transport and/or ATP production were necessary for maximal rates of photosynthesis to be obtained. Mitochondria also play an important role during photosynthetic induction since inhibition of mitochondrial electron transport delays this process. Recent results also suggest that mitochondria play an important role in the frost hardening process in rye. It is concluded that an important function of leaf mitochondria in the light is to reoxidise excess redox equivalents from the chloroplasts and also in many situations to supply the cytosol with ATP

11 05

Functional components of respiration of plant organs

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The respiration of different organs was studied as related to their specific functions in sink-source system of a plant. In leaves the rate of dark CO_2 release was found to correlate with growth rate and to depend on a leaf development from sink to source status. The growth respiration coefficient of leaves was 0.40 g g^{-1}, the maintenance coefficient was 0.030 - 0.040 g g^{-1} per 24 h at 20 °C. In mature leaves about 45 % of respiratory CO_2 was associated with photoassimilate export (the loading of assimilate from mesophyll to minor veins). For potato plants it was established that the 6 - 8 % of exported assimilates may be used in respiration required for assimilate translocation. In stems only a small part of respiratory CO_2 was associated whith phloem transport and assimilate storage in phloem parenchyma. The respiration rate in specific storage organs (potato tubers) declined by more than two three times if their assimilate import was ceased. The values of tubers respiration coefficients were ten times lower than of potato leaves coefficients. We concluded that in plants the respiratory cost of the leaves growth and maintenance of its function as source photoassimilate are most expensive.

11 06

The ratio of leaf conductance for CO_2 and H_2O

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The ratio of leaf conductance for CO_2 and H_2O molecules g'/g at various values of g was investigated. Gas exchange of CO_2 and H_2O was measured in atmosphere with 1 % O_2 and 0.023 % CO_2 at transition "darkness - light", reduction CO_2 concentration from 0.2 to 0.023 % autooscillation of leaf gas exchange in the light. The attached sugar beet and sunflower leaves which have been untreated and treated with ABA were the objects of investigation. It was supposed that transient processes 3 - 5 min after alteration of conditions were determined only by changes of g. The conductance of mesophyll in every experiment was calculated at minimum values of g. It has been shown that value of k decreased from 0.65 to 0.22 when g decreased from 2.27 to 0.02 μmol m^{-2} s^{-1}. This can be explained by the effect of patchy stomatal closure. The k (g) function is described by formula: $k' = g / (0.072 + 1.5\ g)$.

11 07

Glyoxysomal pathway of succinate oxidation in scutella of cereal seedlings

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The enzymatic system of succinate oxidation distinct from mitochondrial succinate dehydrogenase (SDH) was revealed in scutella of maize and wheat seedlings. It is insensitive to SDH inhibitors thenoyltrifluoroacetone and malonate and localized in glyoxysomal membranes. The products of glyoxysomal succinate oxidase complex (GSOC) are malate and H_2O_2. GSOC is flavine containing system which is most active during the intensive operation of the glyoxylate cycle. K_m value of GSOC is 18±4 mM being much higher than K_m of SDH. Succinate oxidation catalyzed by GSOC is connected with its further metabolization in amino acids, carbonic acids and CO_2. GSOC was not detected in cotyledons of sunflower and soybean. It is concluded that the alternative system of succinate oxidation is glyoxysomes of cereal seedlings is necessary for its rapid conversion without control from the tricarboxylic acid cycle.

11 08

Determination of photorespiration enzymes activity (glycolate oxidase and catalase) as a test for selection of sugar beet plants

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Sugar beet plants originating from initial population and their progeny were selected on low glycolate oxidase activity and high catalase activity. These activities were measured in 3-month-old plant leaves. The purpose of experiments was to obtain lines with a higher productivity. Plants with lover glycolate oxidase activity and higher catalase activity in relation to initial population were selected. A control group with high activity of both enzymes was chosen, as a reference for estimating effectiveness of the selection. The progeny was obtained by intercrossing within selected group of plants. High variation of the tested characters was found in initial material and its progeny plants. Correlation between activities of glycolate oxidase and catalase was stated. The results obtained so far indicate on the possibility of selection plants with required traits. In the end of the studies the obtained lines will be crossed with MS - lines in order to evaluating their productivity.

11 09

Developments in respiration research: the state of the art

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Information will be presented on the quantitative significance of respiration for a plant's carbon budget as dependent on environmental conditions. Special attention will be given to the non-phosphorylating alternative path. I will discuss recent developments on the regulation of the partitioning of electron flow through the cytochrome and alternative pathways. In this context, the role of the state of reduction of ubiquinone, the substrate common to both pathways, will be discussed. Finally, new insights into the physiological significance of the alternative path will be discussed. It is concluded that an old hypothesis, namely that the alternative path allows respiration to proceed when NAD(P)H is rapidly produced during the synthesis of organic acids, now has to be rejected.

11 10

Influence of glycine and malate oxidation on pyruvate dehydrogenase complex activity in isolated mitochondria

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The pyruvate dehydrogenase complex (PDC) located in the mitochondrial matrix catalyses the conversion of pyruvate to acetyl-CoA, under the reduction of NADH. PDC is regulated both by product inhibition and by reversible phosphorylation. It has been proposed that light has an indirect inhibitory effect on PDC activity. A possible mediator of this inhibition is photorespiratory glycine oxidation. By simultaneously measuring mitochondrial substrate uptake and PDC activity, it was possible to follow changes in PDC activity during oxidation of glycine and malate. When 1 to 20 mM glycine was oxidized an addition of 100 µM ADP (state 3) reduced PDC activity with 50 %. This reduction was maintained as the mitochondrial oxidative phosphorylation entered state 4 (ADP limiting). When low concentrations of malate were oxidized there was no inhibition of PDC. On the other hand, malate concentrations higher than 5 mM, increased PDC activity. The results are discussed with respect to the influence on PDC activity of NADH and ammonium produced in glycine oxidation in contrast to metabolites produced during oxidation of malate.

11 11

Operation of main steps of dark respiration in light in monocotyledon ephemeroids leaves

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Operation of oxidative pentose phosphate cycle (OPPC), glycolysis and Krebs cycle was investigated in the mature cells zone of leaves of monocotyledon ephemeroid species *Chionodoxa luciliae* Boiss. and *Scilla sibirica* Haw. in light. It was shown that the rate of $^{14}CO_2$ evolution during ^{14}C-glucose and ^{14}C-fumarate oxidation under high CO_2 concentration (1%) was equal in light and darkness. It is suggested that the investigated steps of dark respiration are not limited in light during photosynthesis. It was shown previously that OPPC and glycolysis were inhibited in light in the zone of mature cells of barley leaves. Functioning of OPPC, glycolysis and Krebs cycle in the leaves of monocotyledon ephemeroid species may be connected with low temperature of their habitat and specific strategy of their life.

11 12

The effect of phosphate deficiency on respiration and energy status of bean leaves

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The phosphate level influenced dark respiration of the leaves. Respiration of leaf slices from phosphate sufficient (+P) and phosphate deficient (-P) leaves was measured by oxygen electrode. The energy status of the leaves was characterized by measuring ATP, ADP and AMP levels in the leaves during the light and dark period. The respiration of -P leaves during the dark period was lower and more resistant to cyanide than control leaves. In the darkness ATP and ATP/ADP ratios in -P leaves were about 50% lower than control. Less pronounced differences were observed during the light period. In -P leaves ATP/ADP ratio was about 30% less than control. Photosynthesis and total respiration were only slightly lower and small differences in participation of alternative pathway were observed.

11 13

The dependence of reconstruction of mitochondrial activity *in vitro* on proteins and energy

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The isolated maize (*Zea mays* L.) mitochondria were inactivated *in vitro* after 30 min. The energetic activity of the mitochondria may be reconstructed. The process of the reconstruction depended on the thermostable protein factor obtained from maize seedlings. The number of low molecular mass heat shock proteins was found in the thermostable protein fraction. The successful reconstruction was associated not only with the protein factor but with ATP and substrate of respiration. Thus, the reconstruction of the energetic activity of mitochondria is an active and energy dependent process.

11 14

Citrate metabolism and operation of electron transport systems in higher plants

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Interaction between 6-^{14}C-citrate metabolism and operation of electron transport systems in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) was investigated. The most potent pathway of citrate metabolism took place via aconitase and isocitrate dehydrogenase reaction. This fact was supported by the strong decrease of $^{14}CO_2$ escape in conditions of inhibition of isocitrate dehydrogenase activity. Radiochromatographic and inhibitory analyses suggest that citrate metabolism in plants is connected with different electron transport systems: cyanide and rotenone sensitive and cyanide and rotenone resistant which depends on physiological conditions. Ascorbate in the presence of glutathione stimulated CO_2 release during citrate metabolism via activation of NADP-isocitrate dehydrogenase (E.C.1.1.1.42) and interaction of its function with the cytosolic NAD(P)H-oxidizing ascorbate oxidase systems.

11 15

Determination of the diurnal course of root respiration, enlargement of the alternative pathway and RQ-values of some grass species

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In our previous studies on root respiration, measured as oxygen consumption during the light period, it was found that slow-growing species respire relatively fast, considering their slow rate of growth and ion uptake (1). The aim of this study was to investigate whether root respiration values obtained during the light period only, are representative for the whole diurnal course, i.e. light and dark period. To see if fast- and slow-growing species behave differently in this respect, total root respiration, the engagement of the alternative pathway and the respiratory quotient (RQ) were determined at regular intervals during 24 hours. In the grasses investigated, results ranged from total root respiration decreasing 20 percent during the dark period to no differences between light and dark period. The decrease was totally accounted for by a lower activity of the alternative path in the dark. RQ-values varied between 1 and 1.6. It is concluded that the relatively high rates of root respiration of slow-growing species are not associated with an unusual RQ or alternative path engagement.

(1) Poorter,H., Van der Werf,A., Atkin, O.K. & Lambers,H. 1991. Physiol. Plant. 83: 469-475.

11 16

Dark respiration and grape frost resistance under the extreme humidity

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It has been established that dark respiration components of grape leaves depend on the stage of plant development. During grape blossom time, when the strong growth of biomass, including the generative organ formation, takes place, the growth and maintenance respiration are approximately equal. In September after the crop maturity during the period of plant preparation to unfavourable winter conditions the dark respiration rate of grape decreases, mainly, due to the growth respiration fall. At the end of vegetation the maintenance respiration components are 2 - 4 times higher than that of the growth respiration. Frost and drought resistant varieties are characterized by greater expenditure on the maintenance respiration and less one on the growth respiration. Under extreme humidity the maintenance respiration increases.

11 17

Carboxyatractyloside restores the palmitate-induced uncoupling in sunflower mitochondria

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The content of mitochondrial ATP/ADP antiporter was evaluated in several plant species. Sunflower hypocotyl mitochondria contained a higher level of ATP/ADP carrier than those from pea stem, maize root and soybean hypocotyl. On these mitochondria, ADP, atractyloside and carboxyatractyloside inhibited the O_2 consumption stimulated by low concentrations of palmitate. This effect was associated to the ability of ADP and carboxyatractyloside to restore the palmitate-collapsed electrical potential. It is suggested that the ATP/ADP translocator is involved in the free fatty acid-induced uncoupling of oxidative phosphorylation in plant mitochondria, only when its level is sufficiently high and the concentration of the fatty acid is low to collapse only partially the electrical potential.

11 18

Measurements of in vivo Q reduction levels in plant cells

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In recent years mitochondrial research has focused attention on the role of the Q-pool in the regulation of mitochondrial respiration. Studies on the kinetics of the ubiquinol oxidizing pathways reveal that the inhibitor titrations which are generally used to determine the relative contributions of the cytochrome and alternative pathway may not necessarily produce the correct image of the distribution. Scientists, involved in the study of plant respiration in vivo have until now neglected to incorporate the results of these techniques (eg. Q reduction data) in their studies and interpretations.
Recently in our group a method is developed to determine *in vivo* Q reductions, a method with which engagement of the alternative pathway can be predicted without the use of inhibitors. It is shown that (1) both ubiquinone and ubiquinol can be extracted from *Petunia hybrida* suspension cultures and detected using HPLC, (2) that all Q detected in the cell extract originates from the mitochondria, (3) that the reduction levels vary with different treatments of the cells.
This method could be very useful in order to predict a possible engagement of the alternative pathway in total respiration without the use of inhibitors.

11 19

Purification of a pea stem mitochondrial H^+-PPiase

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A membrane-bound protein exhibiting PPiase activity was purified by electroendosmotic preparative electrophoresis from etiolated pea stem mitochondria. The enzyme elutes as a single peak and is relatively pure, because only a very limited number of polypeptides are detectable by SDS-PAGE of the active fractions. The PPiase is associated to a band with a molecular size of 35 kDa, has a specific activity of 0.7 mmol Pi mg^{-1} prot min^{-1} (37 °C, pH 8.0) and Km value of 200 μM. The hydrolytic activity is Mg^{2+}-requiring, is inhibited by imidodiphosphate, Ca^{2+}, F^- and stimulated by phospholipids and detergents. Of the phospholipids tested, cardiolipin, phophatidylcholine and phosphatidylethanolamine have the maximal activating effect. The isolated protein is very similar to the catalytic subunit of PPiases isolated from rat liver (β-subunit) and *Saccharomyces cerevisiae* mitochondria.