

PLANT PHYSIOLOGY

Second Hour Exam - Spring, 2006

1.

Vanlerberghe et al.

Plant Physiol. Vol. 109, 1995

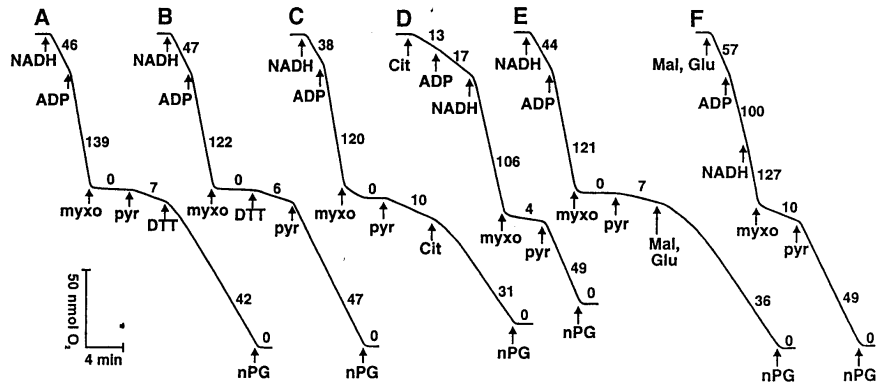


Figure 2. O_2 uptake by transgenic (S1) tobacco leaf purified mitochondria containing high levels of AOX protein (see Fig. 1). Where indicated, the following were added: 2 mM NADH, 1 mM ADP, 16 μ M myxothiazol (myxo), 1 mM pyruvate (pyr), 10 mM DTT, 100 μ M *n*-propyl gallate (nPG), 10 mM citrate (Cit), 10 mM malate (Mal), and 10 mM Glu. Numbers on traces refer to rates of O_2 uptake ($nmol O_2 mg^{-1} protein min^{-1}$). Representative results are shown.

What do the oxygen uptake recordings in the figure above demonstrate about the regulation of AOX activity? Be as detailed and specific as you can and cite specific traces (A-F) to support your conclusions.

They illustrate two factors that are required for maximum AOX activity, reduction and activation. The requirement for a reductant can be supplied by either DTT (traces A and B) or by citrate or malate + glutamate as seen in traces C-F.

The tracings also show that it doesn't matter when the reductant is supplied; it can be before the activator (traces B,D,F) or after the activator (traces A,C,E).

Pyruvate can act as the activator (traces A-F) and can be provided before or after the reductant (A-F).

2. In order to determine the role that the alternate oxidase (AOX) plays in resistance to infection by tobacco mosaic virus (TMV), Ordog et al. carried out the experiments illustrated in Fig. 1 and 4 below using transgenic tobacco plants.

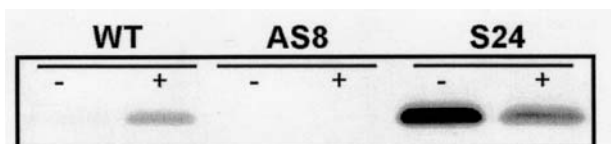


Figure 1. The level of leaf mitochondrial AOX protein in wild-type and transgenic (AS8 and S24) plants. AS8 plants contain an antisense AOX transgene that results in a silencing of leaf AOX expression, whereas S24 plants contain a sense AOX transgene that results in constitutive high levels of leaf AOX protein. Plants were either left untreated (-) or were treated for 12 h with 2 mM SA in their hydroponic medium prior to mitochondrial isolation. Mitochondrial protein (100 µg) was separated by SDS-PAGE, transferred to nitrocellulose, and probed with a monoclonal antibody against AOX. Typical results are shown.

- a) What is the effect of the anti-sense AOX transgene in AS8 plants? Why does it have this effect (i.e. what is the mechanism of action of an anti-sense gene)?

An anti-sense AOX gene prevents expression of the sense version of the gene by producing an anti-sense mRNA molecule that binds to the sense mRNA and causes it to be degraded. This results in no expression of the gene, even in the presence of salicylic acid.

- b) What is the effect of the sense transgene in S24 plants? What is the evidence for this?

The sense transgene is constitutively expressed meaning that it is always turned on and thus it results in much higher levels of expression of the AOX protein both in the presence and absence of salicylic acid.

The Western blot in Fig. 1 shows high levels of AOX present in S24, the sense line, in both the presence and absence of SA.



Figure 4. Summary of northern results establishing the presence or absence of TMV coat protein RNA in leaf tissue from wild-type (A), AS8 (B), and S24 (C) plants. Data represent the percentage of plants with detectable TMV coat protein RNA. Plants were grown in the absence (black bars) or presence (white bars) of SA and were inoculated with TMV, as explained in the legend to Figure 3. Total RNA was isolated from leaves 2 and 3 above the inoculated leaf, and 5 µg of RNA was separated on agarose gels containing formaldehyde and used for northern analysis with a radiolabeled DNA probe recognizing TMV coat protein RNA. This allowed plants to be readily scored for the presence or absence of TMV coat protein RNA. The M refers to a plant that was mock inoculated, in which case RNA was isolated at 10 DPI. Each bar represents data obtained from eight separate plants, each from a separate experiment.

c) What effect do each of these transgenes, when compared to wild type, have on the level of infection of tobacco plants with TMV at 10 days post-inoculation (DPI) in the **absence** of salicylic acid (SA)?

At 10 DPI neither transgene significantly affects the level of infection with TMV as compared to wild type when in the absence of SA

d) What effect do each of these transgenes, when compared to wild type, have on the level of infection of tobacco plants with TMV at 10 days post-inoculation (DPI) in the **presence** of (SA)?

At 10 DPI neither transgene significantly affects the level of infection with TMV as compared to wild type when in the presence of SA

e) What can you conclude about the role of AOX in the resistance of tobacco plants to infection with TMV?

Since over- and under-expression of both transgenes has no significant effect on the level of infection with TMV it appears that the AOX protein does not play any significant role in controlling infection.

3. What cell organelles are involved in the conversion of storage lipids to sucrose in fatty seeds such as castor bean (also called the glyoxylate cycle)?

glyoxysomes
mitochondria
oleosomes

What overall steps of this process occur in each organelle?

Glyoxysomes - fatty acids are oxidized to acetyl CoA and 2 acetyl CoAs are condensed to form succinate - malate is also produced

Mitochondria - succinate converted to malate which is transported into the cytoplasm where it is converted into PEP as the starting material for gluconeogenesis

Where does gluconeogenesis occur? Where does the synthesis of sucrose occur?

Cytoplasm

Cytoplasm

What part of this process that converts lipids to sucrose would be blocked if you treated the seeds with an inhibitor of PEP carboxykinase?

The conversion of OAA to PEP + CO₂ in the cytoplasm, as a prelude to gluconeogenesis

4. Most plants are continually subjected to changing illumination conditions that alter the quality and quantity of light they receive throughout the day. These changes could result in an imbalance in PSI vs PSII activity. Answer the following questions with regard to the mechanism by which plants are able to solve this problem:

a) What is the mechanism by which chloroplasts can detect that there is an imbalance in light energy absorbed by PSI vs PSII?

The reduction state of the plastoquinone pool

b) What components of the light-harvesting machinery are able to help redistribute incident light between PSI and PSII?

The mobile light-harvesting complexes (LHC)

c) Describe the mechanism that brings about their redistribution within the chloroplast membranes.

Phosphorylation by a membrane-bound protein kinase, which is activated by reduced PQ, will give a negative charge on the mobile LHC. They will then migrate out of the grana stacks and into the stroma lamellae where they donate light energy to PSI instead of PSII

d) How is this redistribution altered when light conditions return to more equal intensities for both PSI and PSII?

When PSI becomes active the PQ pool becomes more oxidized. The protein kinase becomes less active, the mobile LHC loses the phosphate groups due to the activity of a phosphatase and the mobile LHC migrates back into the grana stacks.

5. Carbon fixation in C_4 plants is known to be energetically more expensive than C_3 fixation because it requires two additional ATP's. How are these extra ATP's most likely generated? Describe the mechanisms in detail.

The extra ATP's could come from either cyclic or pseudocyclic electron transport.

Cyclic electron transport transfers electrons from P_{700} to an Fe-S center (primary electron acceptor of PSI) and back to P_{700} via the PQ pool and the cyt. b-f complex.

Pseudocyclic electron transport transfers electrons from water to O_2 generating water once again (hence pseudocyclic) using both PSI and PSII - transfer to O_2 may occur at the Fe-S center or ferredoxin associated with PSI.

6. C_4 plants show different responses to certain environmental factors such as light intensity and temperature. These responses are shown in the accompanying graph.

What does this graph tell you regarding the following parameters in C_3 and C_4 plants that are subjected to saturating CO_2 concentrations:

- a) rate of dark respiration
- b) light compensation points
- c) photochemical efficiency
- d) light saturation point

Mark these points on the graph and explain in detail the basis for the similarities or differences that you see.

a) the rate of dark respiration appears to be the same in C_4 and C_3 plants, presumably because the biochemical machinery of glycolysis and the mitochondrion is the same in both types of plants.

b) the light compensation point of the C_3 plant is lower than that of the C_4 plant because of the additional energy (2 ATP) needed by C_4 plants to fix one CO_2 . The C_4 plant is unable to generate lots of extra ATP's in low light. C_3 plants are not being limited by photorespiratory activity in saturating CO_2 so they are able to fix more CO_2 per photon than at ambient CO_2 and more than a C_4 plant.

c) the photochemical efficiency of C_3 plants is greater than C_4 plants for the same reasons as in (b)

d) the light saturation point may be reached in C_3 plants because of limitations of its photosynthetic machinery - overall capacity of phosphorylation and carboxylation reactions. C_4 plants can use all the additional light they can get to supply the extra ATP needed to drive their CO_2 pump.

7. We know that CAM plants are able to grow in xerophytic (dry) habitats and it has been observed that they have unusual patterns of stomatal movements and CO_2 uptake as illustrated in the graphs below:

Describe the metabolic reactions that are occurring in CAM plants and explain how they produce the patterns illustrated in the graphs. (Stomatal resistance is high when stomates are closed, low when they are open).

CAM plants fix CO_2 into malate which they store in their vacuoles. This is catalyzed by PEP carboxylase in the cytoplasm. The accumulation of malate at night causes the stomates to open and allow for more CO_2 to enter and be fixed at night. As light becomes available photosynthesis begins fixing free CO_2 which eventually leads to the decarboxylation of malate, after it diffuses out of the vacuole. As malate levels fall the stomates close due to loss of osmoticum and higher CO_2 concentrations. Additional malate continues to be decarboxylated in the light until it is all used up. This process accounts for the accumulation of malate in the dark and the depletion of malate in the light.

8. What effect does adding nitrogen fertilizer to a plant have on its carbohydrate levels? Explain this effect in detailed physiological terms.

It lowers carbohydrate levels

Assimilation of fixed nitrogen:

1) uses up TCA cycle intermediates which must be replaced by breakdown of carbohydrates

2) uses up reducing power from photosynthesis as electrons are shunted from PSI by ferredoxin in green tissues. This would prevent some CO_2 from being fixed into carbohydrate

or

if reducing power comes from NADH generated in respiration, it must be provided by the breakdown of carbohydrates

9. Answer the following questions regarding the nitrogen-fixing reaction in a root nodule of a leguminous plant:

a) end product - NH_3

b) enzyme and its location (be specific) - **nitrogenase - in cytoplasm of bacteroids**

c) role and location of leghemoglobin - **located in the cytoplasm of the host cell where it acts as an oxygen transporter to the bacteroids**

d) fate of the end product - **NH_3 leaves the bacteroid and enters host cytoplasm where it is incorporated into amino acids**

e) source of ATP and reducing power needed - **host-supplied carbohydrates are respired in the bacteroid to supply them**

f) wasteful reaction accompanying N_2 fixation - **reduction of H^+ to H_2**

10. If transpiration causes water movement in the xylem by a 'pull' from above, precisely where does that 'pull' originate? What forces produce the 'pull'?

The pull originates in the microcapillaries of the cell walls of leaf mesophyll cells. The forces of adhesion and cohesion produce the pull, replacing water molecules that are lost from the surface of the water column by evaporation.

What experimental evidence supports the existence of a 'pull'?

Changes in the girth of tree trunks (narrower during periods of high transpiration or 'pull') correlate with periods when evaporation and thus tension are highest

Fluid is drawn into bore holes in trunks of trees rather than being exuded as would happen if positive pressures existed in the xylem.

Explain the contribution this 'pull' makes to the Ψ of the xylem.

Ψ in the xylem is made more negative by the existence of 'negative pressures' or tension there. This force replaces numerically the positive wall pressures that exist in living cells with a negative value which, when added to the negative solute potential, makes Ψ more negative