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Preliminary observations on the integration of photosynthesis with solute transport. I. Elodea densa Planch as a suitable experimental material

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Fisiologia vegetale. — *Preliminary observations on the integration of photosynthesis with solute transport.* I. Elodea densa *Planch as a suitable experimental material* (*). Nota di FRANCESCO ALBERGONI (**), MARIA TERESA MARRÈ (**) ed ERASMO MARRÈ (***), presentata (****) dal Corrisp. E. MARRÈ.

RIASSUNTO. — Viene discusso il problema della integrazione nelle piante superiori delle funzioni di fotosintesi e di trasporto di soluti a livello cellulare. Vengono presentati i risultati di ricerche preliminari che indicano come meteriale adatto a ricerche di questo tipo le foglie isolate di *Elodea densa* Planch, in cui fotosintesi e trasporto di soluti, operati dalle stesse cellule, sono determinabili in condizioni sperimentali strettamente comparabili, e in cui viene dimostrata, in assenza di fotosintesi, la presenza di un sistema di estrusione elettrogenica di protoni dipendente dalla presenza di K^+ nel mezzo, inibito dal vanadato e fortemente stimolato dalla fusicoccina.

INTRODUCTION

From a physiological-finalistic point of view, photosynthesis and mineral nutrition must be integrated: growth as new synthesis of living matter requires both carbohydrate synthesis and mineral nutrition. In the chloroplast-containing cells photosynthetic metabolism involves the transport of a number of inorganic as well as organic solutes, including photosynthesis substrates and products. On the other hand, the redistribution of solutes in the organism (obviously involving transport at cell level and translocation) is a necessary requirement in differentiated organisms (higher plant), due to the different capacities of either photoorganication of CO_2 or of organic compounds and mineral salt transport in the different territories of the plant.

The problem thus arises of the analysis of this apparently necessary integration and of the elucidation of its mechanism. Obviously enough, a fruitful approach to this problem is conditioned by the choice of an experimental material suitable for this type of studies. It seems reasonable to assume that such a material should satisfy at least the following requirements:

1) *For photosynthesis.* The material should allow the easy and accurate measurement and control of the rates of O_2 and CO_2 exchanges as a func-

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tion of O_2 and CO_2 (or HCO_3^-) concentration, of H^+ and other ions concentrations at the cell surface, and also of electrophysiological parameters known either to influence or to be influenced by photosynthesis, respiration and solute transport.

2) *For solute transport.* Exposure of the maximum amount of the surface of all cells to the medium.

3) *For both photosynthesis and transport.* Maximum physiomorphological homogeneity. In particular, all cells—and possibly the same cells—should be capable of both photosynthesis and solute transport.

4) *Other general requirements.* Easiness to dispose of a large amount of material grown in controlled environmental conditions, and in well defined developmental stages.

Most of these requirements are conveniently met by the leaves of the submerged aquatic macrophyte *Elodea* (= *Egeria*) *densa* Planch. All cells of the elaves of this species, living in a liquid environment, are the site of both active photosynthesis and solute uptake. Each leaf presents a single longitudinal (phloematic) vascular bundle, dividing the leaf blade into two equal halves, each consisting of two layers of chloroplastrich cells; these belong to two well differentiated types: very large, highly vacuolated cells, the layer at the lower side, much smaller cells, that at the upper leaf side (fig. 1).

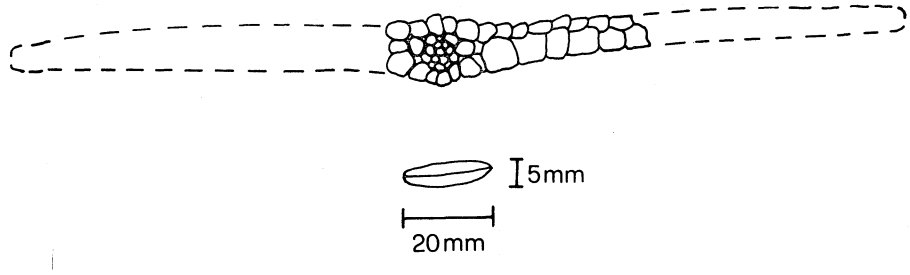

Fig. 1. - Schematic representation of the structure of a *Elodea densa* leaf (transversal section).

This material has already been fruitfully utilized by other authors for studies on various aspects of photosynthesis and in particular of CO_2 and HCO_3 uptake, transmembrane electrical potential changes [1, 3, 4] establishment of an electrical potential across the leaf blade [8, 9, 10], biochemical nature of the electrogenic mechanism [2]. In those studies the experimental conditions adopted optimised the photosynthetic rate (in particular, high pH and thus high level of HCO_3 in the medium), while for our purposes the necessity of following in parallel both photosynthesis and ion (in particular H^+) movements made it useful to work also at a consistently lower pH (for example at pH 6, where the CO_2/HCO_3 ratio is about 7/3). In the following section we present some results dealing with the photosynthetic rate (in the light) and the electrogenic H^+ ex-

trusion (considered as the main mechanism providing energy for solute transport) measured in our experimental conditions.

MATHERIALS AND METHODS

Almost fully grown young leaves were excised from actively growing *Elodea* shoots (cultivated in large tanks in a greenhouse) which had been pre-incubated for a night in the dark in 0.5 mM $CaSO_4$ at 20 °C. The excised leaves were randomized, and pre-incubated for about 2 h in 0.5 mM $CaSO_4$, in the light (10.000 lux) in Erlenmayer flashs (25 leaves equal to about 150 mg fr w in 10 ml medium), then used for the various experiments, in the conditions described in the legends of the figures.

Photosynthesis and respiration were measured polarographically as O_2 evolution or consumption, by means of a Clark electrode (Radiometer PO_2 5047) the leaves (usually 4 leaves) being arranged perpendicularly to the light flux in a closed chamber (6 cc volume) thermoregulated and illuminated at the desired light intensity by a glass fibre system.

Electrophysiological measurements. For the measurement of the transmembrane (actually vacuole-out) electrical potential difference (PD), two leaves of *Elodea* were set in a Plexiglas cuvette, perfused with a solutions containing 0.5 mM $CaSO_4$, 0.25 mM K_2SO_4, 1 mM MES/BTP (MES = 2-(N-Morpholino) ethane-sulfonic acid; BTP = 1,3 bis[tris(Hydroximetryl)-Methylamino]-Propane) at pH 6 at a flow rate of 10 ml min^{-1} 20 °C. Micropipettes (R tip 10-20 MΩ) were used as microsalt bridges to Ag/AgCl electrodes and inserted vertically in the tissue by means of a Leitz micromanipulator. PD was recorded with a high impedance electrometer amplifier (WPI KS-700) and a chart recorder.

RESULTS

I. *Photosynthesis by E. densa leaves.*

Fig. 2 shows the respiratory (O_2 uptake) and the photosynthetic (O_2 evolution) rates measured at pH 6, in agitated medium. It is seen that white (but not green) light induces a rapid efflux of O_2, which is further increased by the injection of 1 mM (final) $NaHCO_2$ in the closed chamber. The addition of the photosynthesis inhibitor dichlorophenyldimethylurea (DCMU) (blocking electron transfer from system I to system II) induced in about 2 minutes a complete block of O_2 evolution, so that O_2 uptake became equal to that observed in the dark. The DCMU concentration here used is relatively high; we observed, however, that at lower concentrations some photosynthetic activity was still present when light intensity was high (above 10.000 lux). As shown, 5 ×

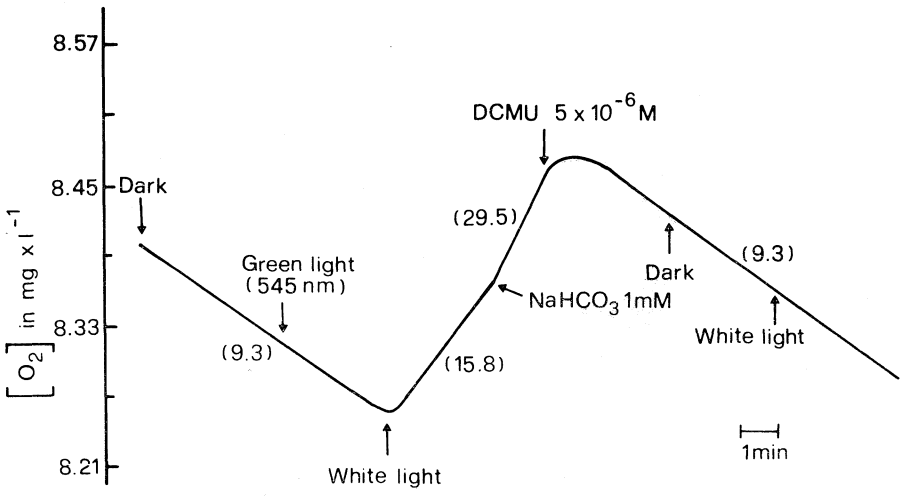

Fig. 2. – O_2 consumption or evolution by *E. densa* leaves as a function of illumination (green or resp. white light), and of the addition to the medium of 1 mM $NaHCO_3$, or 5×10^{-6}DCMU. The basal medium contained 0.5 mM $CaSO_4$, 25 mM K_2SO_4, 50 mM MES/BTP pH 6. The rate of O_2 consumption or evolution in $\mu mol \cdot g\ fr\ w^{-1} \cdot h^{-1}$ are indicated in brackets.

$\times 10^{-6}$ M DCMU did not apparently influence in any way the respiratory O_2 uptake.

Fig. 3 shows the dependence of photosynthetic O_2 evolution on white light intensity. It is shown that the compensation point (with CO_2 equilibrated with air, pH 6) corresponded to 1200 lux, and the maximum O_2 evolution rate to 50.000 lux.

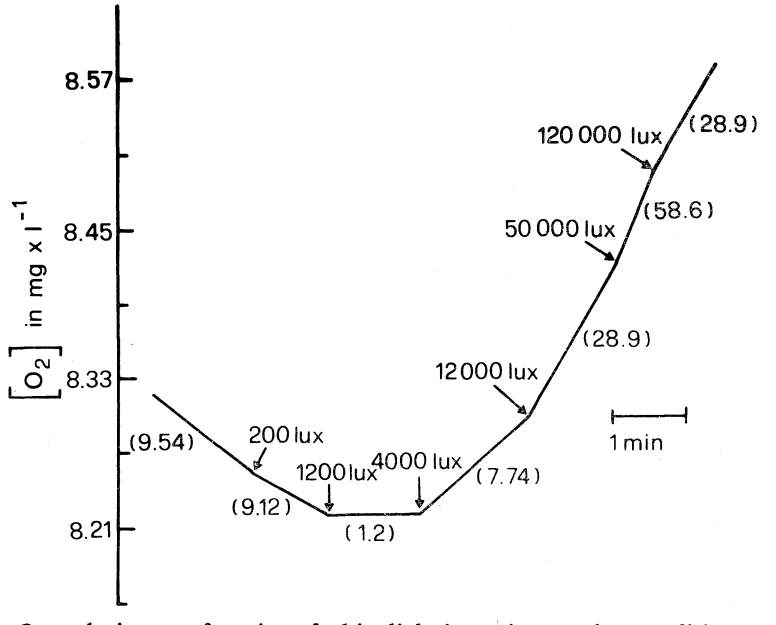

Fig. 3. – O_2 evolution as a function of white light intensity. Other conditions as in fig. 2.

II. *Demonstration of an electrogenic proton pump in E. densa leaves.*

The operation of an electrogenic H^+ pump at the plasmalemma of the cells of many higher plant species is well established. Either ATP hydrolysis or redox energy [2] have been proposed as sources of energy for H^+ extrusion by the pump. At the moment, the existence and the importance of redox-driven H^+ pump is still open to investigation, while many data demonstrate the presence at the plasmalemma of an electrogenic H^+ translocating ATPase characterized by its sensitivity to vanadate as an inhibitor [5, 6]. Fusicoccin (FC), a toxin known to strongly activate electrogenic H^+ extrusion *in vivo* has been recently shown to stimulate in native plasmalemma vesicle systems the ATP-driven, vanadate-sensitive H^+ transport [11]. FC-activated, vanadate sensitive, electrogenic H^+ extrusion is strongly dependent on K^+ uptake [5, 7]. Moving from these data, in our experiments we attempted a preliminary characterization of *Elodea densa* leaves by investigating: *a*) its dependency on K^+ concentration in the medium; *b*) its sensitivity to vanadate; *c*) its sensitivity to FC.

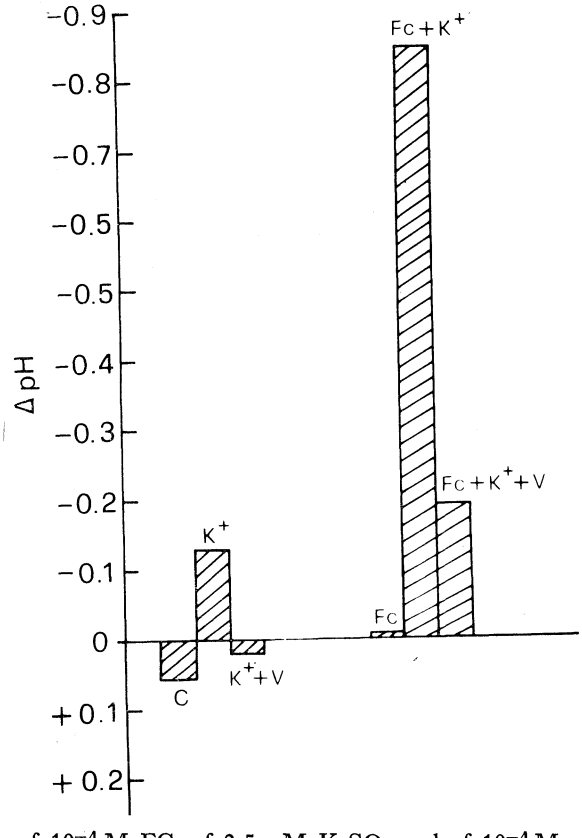

Fig. 4. – Effects of 10^{-4} M FC, of 2.5 mM K_2SO_4 and of 10^{-4} M sodium vanadate on the pH of the incubation medium of *E. densa* leaves, in the dark. Basal medium as in fig. 2 except that MES/BTP was 0.5 mM; pH 6; 25 leaves = 140 mg fr *w* in 7 ml of medium per flask, agitation 60 strikes per min, temperature 20 °C.

The data of fig. 4 show that *E. densa* leaf cells are indeed endowed of a K^+-dependent, vanadate-inhibited and strongly FC-stimulated mechanism of

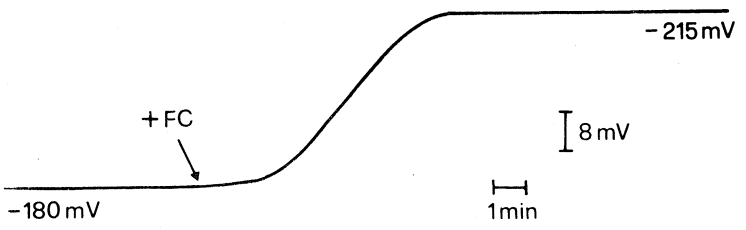

Fig. 5. - Effect of 10^{-5} M FC on the transmembrane electrical potential (PD) of *E. densa* leaves, in the dark. Basal medium as in fig. 4, except for K_2SO_4 which was 0.25 mM in this experiment.

H^+ extrusion. The data of fig. 5 also show that activation of H^+ extrusion by FC is associated with a consistent hyperpolarization of the transmembrane electrical potential.

Conclusions

The results reported above can be summarized as follows:

I) Isolated *Elodea densa* leaves provide a convenient experimental system allowing the parallel study of photosynthetic activity and of ion (in particular H^+) transport. This system appears therefore suitable for the study of the interrelationships between the two functions.

II) *Elodea densa* leaves are endowed with a H^+ extruding mechanism, which also operates in the dark, which is strongly dependent on the presence of K^+ in the medium, is activated by FC, and is almost completely inhibited by vanadate. The stimulation of this mechanism by FC is associated with a marked syperpolarization of the transmembrane electrical potential. These results huggest that, at least in the absence of photosynthesis, an ATP-driven proton pump is the main mechanism carrying out electrogenic H^+ extrusion in this material. Whether this same mechanism is influenced by photosynthesis will be the object of a further communication.

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