ENERGY - ACCUMULATING EFFICIENCY OF THE LEAF OF SOME SUNFLOWER RESTORERS

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Abstract

Sunflower lines R-606, R-614, R-725, R-727, R-809, R-812, R-840, and R-842 were studied for energy - accumulating efficiency by methods of calorimetry, photophosphorylation, and fluorescence.

The lines reacted specifically regarding the energy-accumulating efficiency of the leaf. Energy accumulation in tissue depended in the examined lines on development stage and plant part. Energy accumulation had an increasing trend in all lines from budding to milk maturity.

With all lines, the rate of the Hill reaction was insignificant in the presence of ferricyanide. The reaction was considerably stimulated by AOP as well as by ammonium chloride. The presence of ADP accelerated the electron transportation 1.6 times over the basic rate.

Some lines differed in the emission spectra of fluorescence. That phenomenon indicated either a lower activity of photosystem II or an increased energy transfer from photosystem II to photosystem I.

Introduction

An efficient utilization of solar energy implies high efficiency of the primary, photochemical, processes and reactions of the Calvin cycle. A high photochemical efficiency of the photosynthetic apparatus (measured by the rate of non-cyclic electron transportation and the rate of phosphorylation) lowers the efficiency of other processes causing losses in the absorbed energy. The fluorescence of chlorophyll a, one of the processes which use the absorbed energy, may be used as an efficient indicator of changes and types of change in the primary photosynthetic processes and their interaction with processes in the Calvin cycle.

However, the leaf is the site of not only photosynthetic processes but also the oxidoreductive processes of transpiration in light

and dark as well as exoenergetic enzymic reactions which use the primarily bound energy, i.e., carboxylation and photosynthetic transformation of CO_2. About 25% of the primarily bound energy is used for the primary fixation of CO_2.

Materials and Methods

The research objects were highly diverse sunflower restorers R-606, R-614, R-725, R-727, R-809, R-812, R-840, and R-842.

Plant tissue energy was measured by the method of calorimetry, after Ćupina (1982, 1983, 1984).

Energy absorption by chloroplasts was determined as follows. Sunflower leaves were kept for 24 hours at 4-10°C with their petioles immersed in water then rinsed, immersed in water and illuminated for 30 minutes. Fourty grams of the leaves were chopped up and homogenized in a mixer with 100 ml of a solution for homogenization consisting of 0.4M of surcrose, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM EDTA, 50 mM sodium pyrophosphate, 0,6% Carbowax 4000, 0.05% cysteine, and 0.25% BSA, pH 7.8. The homogenate was strained through 10 layers of gauze and centrifuged at 1000 rpm. for 5 minutes. The supernatant was thrown away and the chloroplast sediment resuspended in 30 ml of a solution for rinsing, i.e., the homogenization solution without Carbowax and cysteine, but with 50 mM of tricine instead of pyrophosphate, pH 7.8. Centrifuging was then repeated. The obtained chloroplast sediment was again resuspended in a small amount of the rinsing solution and used for analysing.

The Hill reaction and the related photophosphorylation were followed in isolated chloroplasts by an oxygen cathode, on the basis of oxygen release in the course of the process of photosynthesis. One milliliter of the reaction mixture contained 0.4 M of sucrose, 50 mM tricine, pH 8.0, 3000 catalase units, 10 mM phosphate, 1 mM $MgCl_2$, 15 µg chloroplast chlorophyll.

The intensity of fluorescence was measured in whole leaves. Since fluorescence is affected by the treatment of plants before measurement, all plants were treated in the same manner: the leaves were exposed to red light for 10 minutes and leaf disks, 12 mm in diameter, were cut out from the right side of the leaves, between the second and the third lateral nerve, about 1 cm away from the main nerve. The disks were placed face down a thermostat-equipped leaf holder, on wet paper, and covered with transparent plastic foil. Before reading the emission spectra, the disks were illuminated for one minute, shaded for 20 minutes, and illuminated again to read fluorescence kinetics during the first three minutes. The kinetics was read at 685 nm (F 685), the emission spectra at 650--800 mm. All readings were made at 20°C.

The disks were illuminated by a He-Ne laser (λ = 632,8 nm), with a laser filter of the same wavelength (Edmund Sci.Co.) fitted to

the light port. C.Zeiss filters No. 5 and 6 were used to neutralize exciting and dispersed light. Fluorescence was detected by EMI 9558 QB photomultiplicator, using C.Zeiss SPM 2 monochromator, electronic equipment for impulse discrimination and amplification, and a Sefram Servotrace plotter.

Results

Photochemical activity of chloroplasts

The rate of electron transportation was measured in isolated chloroplasts, in the presence of an external electron receiver (the Hill reaction). It was found that the Hill reaction, i.e., the rate of oxygen release under the effect of light in the presence of potassium ferricyanide (2 mM) as electron receiver was stimulated by adenosindiphosphate (ADP : 0.1 millimols) and ammonium chloride (5 mM) as the decoupling reagent.

The rate of oxygen release and ATP synthesis in sunflower chloroplasts (μ-mols/hr, mg chl.).

RESTORER	O_2			ATP	ADP/O
	FeCN	+ ADP	+ NH_4Cl		
R-606	42	60	134	98	0.82
R-614	43	72	170	222	1,54
R-725	45	62	140	102	0.82
R-727	51	78	140	148	0.65
R-809	34	58	140	117	1,00
R-812	39	63	178	129	1,02
R-840	60	98	225	193	0,98
R-842	52	82	220	165	1,00

The measured rates of the Hill reaction indicated a normal photosynthetic activity of the isolated chloroplasts. The values of the basic and stimulated rates were about equal except in R-614, R-840, and R-842 which had higher values. The increased rates of electron transportation and the Hill reaction were followed by increased rates of photophosphorylation. The measured rates ranged between 100 and 250 μ-mols of ATP/mg chlorophyll/hour.

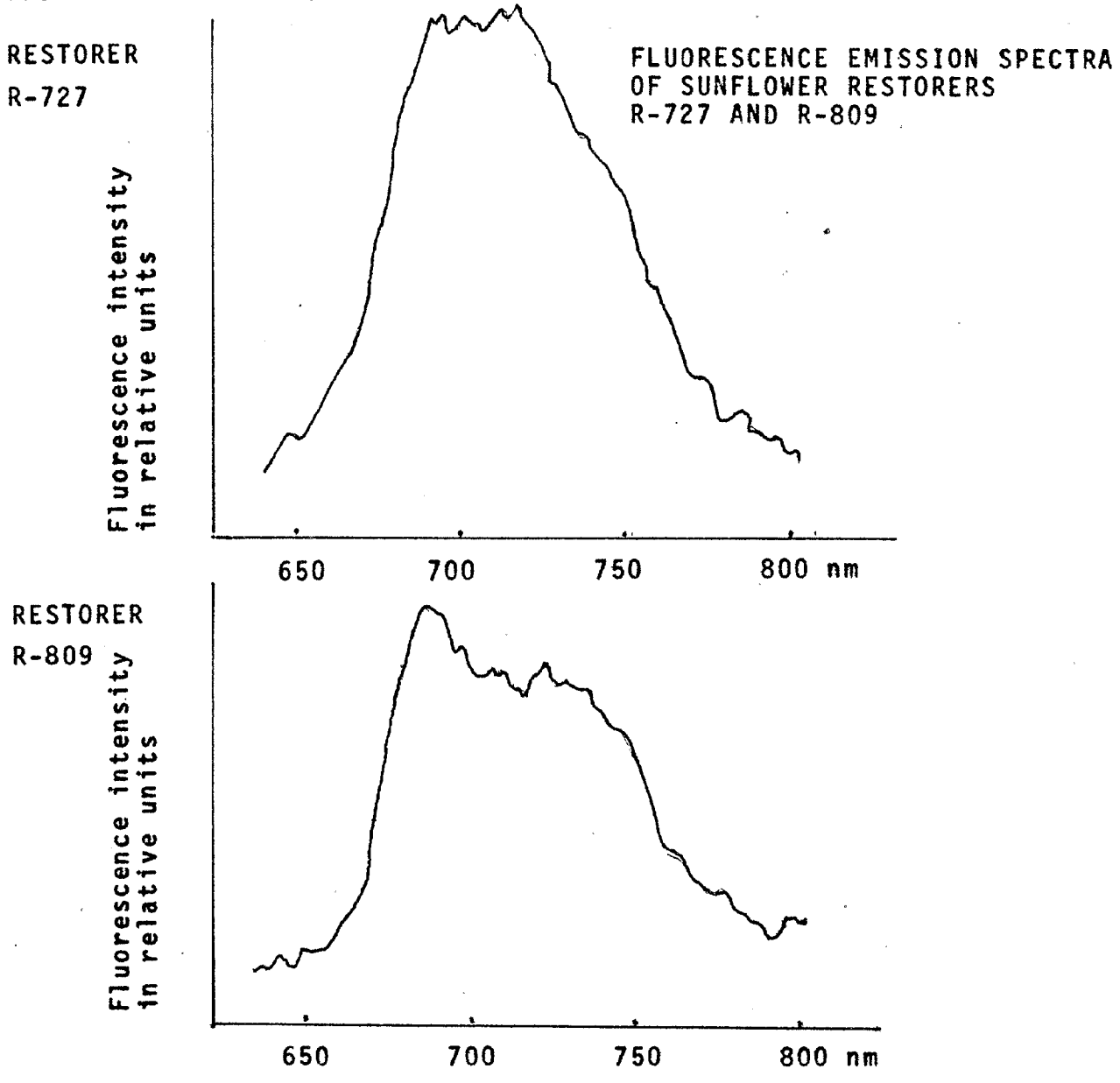
Fluorescence of leaves

When illuminated, green leaves fluoresce. The major portion of fluorescence is released from chlorophyll a of photosystem II, representing the part of excitation energy which cannot be used in any other way, e.g., for the electron transportation to NADP.

Fluorescence is beyond doubt related to carbon assimilation. It depends on a number of factors but primarily on the redox state of electron transmitters in the electron transportation chain, the degree of acidification of the thylakoid membrane, and the phosphorylation of membrane proteins by ATP which are probably related to the transitions state 1 - state 2.

Four maximums were found in the emission spectra of fluorescence, at room temperature: at about 685, 700, 720, and 740 nm. The fluorescence emissions at 685 and 740 nm are known to belong to photosystems II (PS II) and I (PS I), respectively. The origin of the maximum at 720 nm is uncertain, but it is belived to originate from PS I.

The graphs show the emission spectra of fluorescence for the restorers R-727 and R-809. Differences among them are evident. With the former, the emission at 685 nm is lower than that at 720 nm, indicating either a low activity of PS II or a high energy transfer from PS II to PS I.



The differences among the restorers regarding their emission spectra of fluorescence are attributable to their genetic diversity. However, the differences may also be attributed to disturbances in the photosynthetic apparatus caused by chemicals or stress (e.g., extreme temperatures or drought). The method of fluorescence may be very useful in that respect because it may provide indications of disturbances in the photosynthetic apparatus quickly and non-destructively.

Discussion

Contemporary concepts picture the process of photosynthesis as the result of concurrent photooxidation and hydrogen transfer involving two photosystems and CO_2 reduction in the presence of enzymes from the Calvin cycle. According to Govindjee (1979), the two photosystems provide the transition of four electrons resulting in the formation of two molecules of NADP-H and three molecules of 126.5 kilogram - calories.

The energy-accumulating efficiency of sunflower leaves may be followed on the basis of the fluorescence of whole leaves and chloroplasts. Green sunflower leaves fluoresce when illuminated. The major part of the fluorescence is released by chlorophyll a from photosystem II representing a part of the excitation energy which cannot be used in any other way, e.g., for electron transportation to NADP. It is also assumed that fluorescence is related to CO_2 assimilation.

Lloyd et al. (1977) studied the intensity of photosynthesis in different sunflower genotypes and found large variability in that respect. Similar results were obtained by Ćupina (1964) who studied net assimilation of some sunflower genotypes in field conditions. Differences in the intensity of photosynthesis in sunflower genotypes were discussed by Rawson et al. (1980). Terbea (1982) analysed the productivity of photosynthesis in a number of sunflower lines and hybrids and found that the accumulation of biomass was in correlation with the photosynthetic potential at the leaf area index below 3, and no correlation when the index exceeded 3. She also found that mother forms were dominant donors of the photosynthetic indicators. Ćupina (1982, 1983) studied the energy value of some NS sunflower hybrids and found hybrid specificity regarding energy accumulation. He found a similar regularity in sunflower restorers (Ćupina, 1984).

The obtained results indicate the restorers' specificity in respect to the utilization of solar energy in the process of photosynthesis.

According to the obtained rates of the Hill reaction, the photosynthetic activity of isolated chloroplasts was normal. The values of normal and stimulated rates were mostly uniform, except in R-614, R-840, and R-842 which had increased rates. The increased rates of electron transportation and the Hill reaction were followed by

increased rates of photophosphorylation, ranging from 98 to 222 micromols ATP/milligram chlorophyll/hour in R-606 and R-614, respectively.

The differences in the emission spectra of fluorescence were evident and probably related to differences in the activity of PS II and PS I.

The methods of absorption and fluorescence of chloroplasts may be very useful when estimating the energy-accumulating efficiency of leaves of different sunflower lines and hybrids.

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