ENERGY ACCUMULATION AND UTILIZATION IN SUNFLOWER LINES

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INTRODUCTION

The rate of solar energy irradiated on a horizontal surface is 0 to 3,600. The maximum utilization of solar energy implies a high efficiency of the primary, photochemical processes and the reaction in the Calvin cycle. A high photochemical efficiency of the photosynthetic apparatus (measured by the rate of non-cyclic electron transportation and the rate of photophosphorilation) lowers the efficiency of other processes bringing about losses in the absorbed energy. The fluorescence of chlorophyll *a* as a mode of energy depletion may be used as a quick and non-destructive method of detecting changes and types of changes in the primary processes of photosynthesis and their interactions with the processes in the Calvin cycle.

However, the leaf is the site of not only photosynthetic processes but also oxidoreductive respiratory processes in the light and dark as wel as exoenergetic enzymic reactions which make use of the primary bound energy in carboxylation reactions and photosynthetic transformations of CO_2. About 25% of the primary bound energy are used for the primary fixation of CO_2. Therefore, the maximum gain of net photosynthesis is 260—270 mg $CO_2/dm^2/hr$ with the maximum coefficient of light energy utilization of 19—20%. Accordingly, the light curves of photosynthesis are not straight lines and their values are much below the theoretical ones.

It should be pointed out that plants are efficient users of light energy. They form complex canopies with a relatively high leaf area index (LAI), distributing the leaves in several layers. Arranged so, the leaves have the absorbing capacity of the leaf monolayer.

The best leaf area combines an optimum size with corresponding physiological activity. If we want to try to achieve it, we must first gain a more profound and a more subtile knowledge of the energy-accumulating efficiency of photosynthesis (Ničiporovič, 1979).

MATERIALS AND METHODS

We examined the following sunflower restorers : R-606, R-614, R-625, R-627, R-809, R-840, and R-842. They are highly divergent.

THE MEASUREMENT OF ENERGY IN PLANT TISSUE

Samples of plant tissue were dried, crushed, and molded into pills weighing about 0.305 grams. A piece of wire (7 cm long) was embedded in each pill to serve as the electric conduit for incineration of the sample in calorimetric chamber.

The calorimetric coefficient (C) was calculated by the following formula :

$$C = \frac{(6323 \text{ cal} \times \text{benzoic acid mass}) - \text{mass of the wire after incineration}}{\text{Difference in temperatures before and after incineration } (T_2 - T_1)}$$

One gram of benzoic acid releases 6,323 small calories.

$$C = \frac{6{,}323 \times 0.2810 \text{ g} - 5.5 \text{ cm}}{0.731} = 2{,}356 \text{ cal}$$

The caloricity of the plant tissue samples was calculated as follows :

$$\text{Cal/g} = \frac{(t_2 - t_1) - \text{remaining wire} \times \text{calorim. coeffic. (C)}}{\text{mass of plant tissue sample}} \parallel$$

$$= \frac{(1.565 - 1.088) - 7.2 \text{ cm} \times 2{,}354}{0.3057 \text{ g}} = 3{,}666 \text{ cal/g}$$

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THE MEASUREMENT OF THE PRIMARY CONVERSION OF LIGHT ENERGY

Sunflower leaves were kept at 4—10°C for 24 hours, with the petioles immersed in water. The leaves were then rinsed and left in water for 30 minutes. They were exposed to light during that period. Forty grams of the leaves were cut in pieces and homogenized in a mixer with 100 ml of solvent composed of 0.4 M sucrose, 10 mM Nacl, 1 mM $MgCl_2$, 1 mM EDTA, 50 mM sodium phyrophosphate, 0.6% Carbowax 4000, 0.05% cysteine, and 0.25% BSA, pH 7.8. The homogenate was strained through 10 layers of cotton and centrifuged at 1,000 rpm. for five minutes. The supernatant was decanted and the chloroplast sediment was resuspended in 30 ml of the solvent which did not contain Carbowax and cysteine while 50 mM tricine, pH 7.8, was added instead of pyrophosphate. The suspension was again centrifuged and the chloroplast sediment resuspended in a small amount of the solvent. The resultant suspension was used for further analyses.

The Hill reaction and the related photophosphorilation were followed in the isolated chloroplasts by means of an oxygen cathode, i.e. on the basis of oxygen release in the process of photosynthesis. One milliliter of the reaction mixture used contained 0.4 M sucrose, 50 mM tricine, pH 8.0, 3,000 catalase units, 10 mM phosphate, 1 mM $MgCl_2$, and 15 ug chloroplast chlorophyll.

THE MEASUREMENT OF THE LEAF FLUORESCENCE

Since the leaf fluorescence is affected by the treatment the plant receives before the measurement, we took care to treat equally the entire material. All leaves were exposed to red light for 10 minutes and 12 mm leaf disks were cut out from the same place, i.e. between the second and third lateral nerve, 1 cm to the right of the main nerve. The disks were turned face down on wet paper and covered with transparent plastic foil. Prior to the reading of the fluorescence emission spectrum, the disks were exposed to light for one minute, kept in dark for 20 minutes, and brought to light again to read their temporal kinetics of fluorescence for three minutes. The temporal kinetics was read at 685 nm (F 685) and the emission spectra at 650—800 nm, at 20°C.

The light source was a He-Ne lasser (= 632.8 nm) equiped with a filter for the same wavelength (Edmund Sci. Co.) C. Zeiss filters no. 5 and 6 were used to elliminate the excitant and the dispersed light. Fluorescence was detected with a photomultiplier EMI 9558 QB, C. Zeiss SPM 2 monochromator, and Sefram, Servotrace electronic equipment for the discrimination and magnification of impulses and a plotter.

RESULTS AND DISCUSSION

1. THE DYNAMICS OF SOLAR ENERGY ACCUMULATION AND THE DISTRIBUTION OF ACCUMULATED ENERGY IN PLANT PARTS

Budding stage

We analysed the accumulation of solar energy in the leaf, stem and head, expressing it per total plant and in calories per one gram of dry matter (Table 1).

Table 1

Solar energy bound in plant parts of sunflower restorers

Budding

Restorer	Leaf	Stem	Head	Total
	Calories/plant			
R-606	50 264	46 239	750	97 253
R-614	85 388	67 725	4 470	157 583
R-725	53 832	39 105	2 491	95 428
R-727	28 098	14 390	705	43 193
R-809	64 935	52 725	3 040	120 700
R-812	95 934	61 160	5 171	162 265
R-840	81 113	68 735	7 094	156 940
R-842	115 558	91 989	—	207 547
	Calories/gram			
R-606	3 515	3 443	3 749	10 707
R-614	3 546	3 482	3 450	11 478
R-725	3 004	3 348	3 460	9 812
R-727	3 365	3 142	3 523	10 030
R-809	3 330	3 107	3 897	10 334
R-812	3 252	3 236	3 830	10 318
R-840	3 437	3 273	3 941	10 561
R-842	3 246	3 339	—	6 585

R-842, R-812, R-614, and R-840 had the highest energy accumulation per plant, while R-727 had the lowest accumulation. The picture becomes radically different when expressing the accumulation in calories per gram of dry matter.

The data in Table 1 show that the examined restorers differed regarding the distribution of accumulated solar energy in plant parts. At budding stage, the largest amount of energy was accumulated in the leaf and then in the stem and head. If the amount of accumulated energy is expressed in calories per gram of dry matter, the head ranks first, the leaf second, and the stem third.

Flowering stage

At that stage, R-842 had the highest and R-727 the lowest accumulation of solar energy per plant. From the view point of accumulated

Table 2

Solar energy bound in plant parts of sunflower restorers

Flowering

Restorer	Leaf	Stem	Head	Total
		Calories/plant		
R-606	85 319	98 434	23 037	206 790
R-614	158 410	191 632	17 858	367 900
R-725	73 060	114 388	42 201	229 649
R-727	45 103	52 195	10 306	107 604
R-809	168 539	177 895	38 718	385 152
R-812	144 962	219 590	72 278	436 830
R-840	136 439	211 360	78 532	426 321
R-842	234 441	272 141	38 069	544 651
		Calories/gram		
R-606	3 643	3 783	3 938	11 364
R-614	3 588	3 939	3 079	10 606
R-725	3 521	3 871	3 827	11 219
R-727	3 404	3 715	3 964	11 083
R-809	3 295	3 461	3 911	10 667
R-812	3 468	3 746	3 745	10 959
R-840	3 415	3 641	4 017	11 073
R-842	3 342	3 785	3 945	11 072

energy expressed in calories per gram of dry matter, R-606 was the best performer and R-614 was the worst (Table 2).

Differences were observed among the restorers regarding the distribution of accumulated energy in plant parts. When expressed in calories per plant, the largest amount of energy was found in the stem, then in the leaf and head. Expressed in calories per gram of dry matter, the head stored the largest amount of energy.

Milk maturity

Table 3 shows that the largest amount of accumulated energy per plant was found in R-614 ; the lowest amount was found in R-727.

Table 3

Solar energy bound in plant parts of sunflower restorers

Milk maturity

Restorer	Leaf	Stem	Head	Seed	Total
			Calories/plant		
R-606	156 585	156 691	162 840	102 392	728 508
R-614	103 191	289 637	130 953	293 465	917 246
R-725	92 500	116 426	101 381	115 577	425 884
R-727	63 866	69 983	85 582	54 156	293 587
R-809	151 459	336 694	159 040	54 218	737 411
R-812	159 908	319 500	263 987	72 013	815 408
R-840	121 545	270 816	108 281	64 282	564 929
R-842	250 481	129 472	215 118	271 649	866 720
			Calories/gram		
R-606	3 353	3 783	4 001	5 622	16 759
R-614	3 272	3 692	3 733	5 247	15 944
R-725	3 223	3 627	3 551	5 105	15 506
R-727	3 419	3 646	3 269	4 982	15 316
R-809	2 729	3 713	3 680	4 426	14 548
R-812	3 099	3 683	4 096	5 299	16 177
R-840	2 943	3 640	3 895	4 999	15 477
R-842	3 199	2 574	3 883	5 211	14 687

At the stage of flowering, the accumulated solar energy was distributed unevenly in plant parts. Higher amounts were found in the head and grain than in the stem and leaf. If expressed in calories per gram of dry matter, the grain accumulated significantly larger amounts of bound energy than the vegetative plant parts.

Table 4 shows also that the amount of bound energy decreased in all vegetative parts but increased in the grain of all restorers. It means that an intensive translocation of high-molecular organic substances from the vegetative part to the grain takes place during the stage of milk maturity.

Table 4

Solar energy bound in plant parts of sunflower restorers per developmental stages

Restorer	Budding	Flowering	Milk maturity	Average
		Calories/plant		
R-606	97 253	206 790	728 508	357 875
R-614	157 583	367 900	917 246	480 910
R-725	95 428	229 649	425 884	250 320
R-727	43 193	107 604	293 587	148 128
R-809	120 700	385 152	737 411	414 421
R-812	162 265	436 830	815 408	471 501
R-840	156 940	426 321	564 929	382 730
R-842	207 547	544 651	866 720	539 639
		Calories/gram		
R-606	10 707	11 364	16 759	12 943
R-614	11 478	10 606	15 944	12 676
R-725	9 812	11 219	15 506	12 179
R-727	10 030	11 083	15 316	12 143
R-809	10 334	10 667	14 548	11 850
R-812	10 318	10 959	16 177	12 485
R-840	10 561	11 073	15 477	12 370
R-842	6 585	11 072	14 687	10 781

The tested restorers behaved specifically regarding the accumulation of energy. Table 5 shows differences in energy accumulated in the biological and agricultural yields per hectare of the restorers. Accordingly, their grain yields per hectare differed too.

Furthermore, it is evident that the amount of solar energy received by plants is tremendous. Its magnitude is expressed in millions of kilocalories per hectare per hour. However, a mere 1% of it is transformed into plant bulk by the process of photosynthesis.

If we take into consideration the energy value of the vegetative parts of sunflower, the question of its utilization immediately comes to mind. About 25% of the energy, in the form of harvest residues, are plowed under to maintain the physical soil properties. A technology should be developed which would make use of the remaining 75% of energy stored in the above-ground plant parts.

Table 5

Solar energy bound in grain yield per hectare of sunflower restorers

Restorer	Kg calories/ha in average biological yield	Kg calories/ha in grain yield	Grain yield kg/ha
R-606	14 315 000	8 095 680	1 440
R-614	19 236 400	11 738 600	2 237
R-725	10 012 800	4 623 080	906
R-727	5 925 120	2 166 240	435
R-809	16 576 840	2 168 720	490
R-812	18 860 040	2 880 520	544
R-840	15 309 200	2 571 280	514
R-842	21 585 560	10 665 960	2 080

2. THE PRIMARY CONVERSION OF LIGHT ENERGY

In the previous paragraphs we described the simple method of quantitative evaluation of photosynthetic efficiency based on the weight of the substrate and photosynthetic products contrasted against the amounts of accumulated energy. However, that method assesses the process only partially.

The contemporary concepts see the process of photosynthesis as the result of simultaneous processes of water photo-oxidation and hydrogen transfer (H^+ and e^- — two photosystems involved) and CO_2 reduction in the presence of the enzymes from the Calvin cycle. Therefore, the maximum energy — accumulating efficiency of the process of photosynthesis will be achieved if :

1) each eight absorbed quants of energy secure the transfer of four electrons through the reactive centers of the two photosystems and the formation of two molecules of NADP. H and three molecules of ATP, binding in them 126.5 kilocalories (G o v i n d j e e, 1975) ; 2) the formed molecules (2 of NADP. H and 3 of ATP) take part in the reduction of CO_2 molecules in Calvin cycle to the final accumulation of 112.5 kilocalories of energy.

The maximum energy-accumulating efficiency requires thus the synchronization of the following parameters : the structural organization of the leaf, the rate and intensity of the photosynthetic reaction.

The rate of electron transportation was measured in isolated chloroplasts, in the presence of an alien electron receiver (the Hill reaction). It was found that the Hill reaction, i.e. the rate of oxygen release under the effect of light and in the presence of potassium ferricyanide (2 mM) as the electron receiver, was stimulated by the presence of ADP (0.1 umols) and the decoupling reagent, ammonium chloride (5 mM) (Table 6).

The obtained rates of the Hill reaction indicated a normal photosynthetic activity of the isolated chloroplasts. The values of the basic and stimulated rate of the Hill reaction were mostly uniform, with the exception of

Table 6

Rate of oxygen release and ATP synthesis in sunflower chloroplasts (uMOLs/hr, mg HL)

Sample	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	52	140	102	0.82
R-727	51	78	140	148	0.85
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

higher rates in R-614, R-840, and R-842. The increased rates of electron transportation and the Hill reaction were followed by the correspondingly increased rates of photophosphorylation : the obtained values ranged between 100 and 250 umols of ATP per one mg of chlorophyll per hour.

3. THE LEAF FLUORESCENCE

Green leaves fluorescence when they are illuminated. The major portion of the fluorescence is released from chlorophyll *a* from photosystem II and it represents a part of the excitative energy which cannot be used in other ways, e.g. electron transportation to NADP. It is certain that the fluorescence has also to do with the assimilation of carbon. The fluorescence depends on many factors, primarily on the redox state of electron conveyers in the electron transportation chain, degree of acidification of the thylakoid membrane, and the phosphorylation of the membraneous proteins by ATP which is probably related to state 1—state 2 transitions.

There were four peaks in the emission fluorescence spectra measured at room temperature : at about 685, 700, 720, and 740 nm. The fluorescence emitted at 685 and 740 nm are known to belong to photosystem II (PS II) and photosystem I (PS I), respectively. The fluorescence at 720 nm is belived to originate from PS I.

Figure 1 shows the emission fluorescence spectra of the restorers R-727 and R-809, respectively. These spectra were different. In the case of the latter, the emission at 685 nm was less intensive in relation to that at 720 nm. It may signify either a lower activity of PS II or an increased energy transfer from PS II to PS I.

It is probable that the observed differences in the emission fluorescence spectra are attributable to the genetic diversity of the examined restorers. The differences may be also due to a disturbance in the photosynthetic apparatus generated by chemicals or stresses (high or low temperatures, drought). The

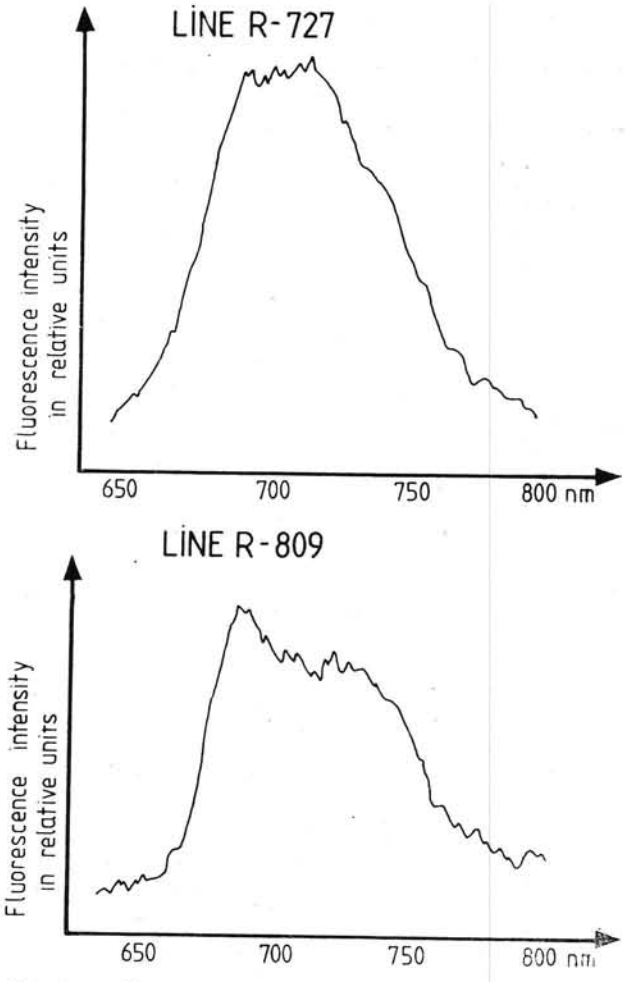

Fig. 1 — Fluorescence emission spectra of sunflower restorers R-727 and R-809

method of fluorescence reading may be very useful since it indicates disturbances in the photosynthetic apparatus quickly and without destroying the analysed sample.

CONCLUSION

We studied the energy-accumulating efficiency of sunflower restorers R-606, R-614, R-725, R-727, R-812, R-840, and R-842 by the methods of calorimetry, photophosphorylation, and fluorescence.

The analysed restorers were specific regarding the energy-accumulating efficiency of the leaf.

The accumulation of energy in tissue depended on the developmental stage and the plant parts examined. The accumulation of energy increased from budding to milk maturity in all restorers.

The restorers had insignificant rates of the Hill reaction in the presence of ferricyanide. The rates were considerably stimulated when ADP and ammonium chloride were added. The former additive increased the rate of electron transportation by 1.6 times.

Some restorers differed in the emission fluorescence spectra which was an indication of either a low activity of PS II or a high energy transfer from PS II to PS I.

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ACCUMULATION ET UTILISATION DE L'ÉNERGIE CHEZ LES LIGNÉES DE TOURNESOL

Résumé

L'efficacité de l'accumulation de l'énergie chez les lignées restauratrices de tournesol R-606, R-614, R-725, R-727, R-812, R-840 a été étudiée par les méthodes: calorimétrique, photophosphorylation et fluorescence.

Les lignées restauratrices se sont comportées d'une façon spécifique, concernant l'éfficacité de l'accumulation d'énergie par les feuilles. L'accumulation de l'énergie dans le tissu dépend du stade de développement et des parties examinées de la plante. Elle s'est accrue dans toutes les variantes, dès la phase de bouton floral jusqu'à la phase de maturité laiteuse.

Les lignées restauratrices ont eu des taux nonsignificatifs de la réaction Hill, en présence de la ferrycinide. Ces taux ont été considérablement stimulés en ajoutant ADP et chlorure d'ammonium. Ce premier composé a augmenté 1,6 fois le taux du transport d'électrons.

Certaines lignées restauratrices ont différé par rapport au spectre de la fluorescence émise, indiquant une activité réduite de PS II ou de l'énergie transférée de PS II à PS I. La méthode de la lecture de la fluorescence est particulièrement utile, celle-ci indiquant des différences et perturbations de l'appareil photosynthétique d'une façon rapide et sans détruire les échantillons analysés.

ACUMULACION Y EMPLEO DE ENERGIA EN LAS LINEAS DE GIRASOL

Resúmen

Fue estudiada la eficacia de la acumulación de energía en las líneas restauradoras de girasol R-606, R-614, R-725, R-727, R-812, R-842 por los siguientes métodos : calorimétrico, fotofosforilación y fluorescencia.

Las líneas restauradoras se han comportado específicamente en cuanto a la eficacia de la acumulación de energía por las hojas. La acumulación de energia en el tejido estuvo pendiente al estadio de desarrollo y a las partes de plantas examinadas. Ella aumentó en todas las variantes, desde la fase de botón floral hasta la fase de madurez en leche.

Las líneas restauradoras tuvieron cuotas nonsignificantes de la reacción Hill en presencia de la ferrycinidis. Dichas cuotas fueron considerablemente estimuladas al añadir ADP y clorura de amonio. El primer compuesto aumentó la cuota del transporte de electronos por 1,6 veces.

Algunas de las líneas restauradoras se distinguieron en 10 tocante al espectro de flurescencia emitida que es un indicio de la actividad reducida de PS II o bien de la energía transferada desde PS II hasta PS I. El método de leer la fluorescencia resulta particularmente útil, indicando diferencias y perturbanciones en el aparato fotosintético de modo ràpido y sin destruír las pruebas analizadas.