THE PHENOMENON OF ABSENCE OF APPARENT PHOTORESPIRATION IN C_3 - PLANT REPRODUCTIVE ORGANS

FENOMENUL ABSENȚEI FOTORESPIRAȚIEI APARENTE ÎN ORGANELE REPRODUCTIVE ALE PLANTELOR DE TIP C_3

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Abstract. *It has been found for the first time that the reproductive organs (spikes, pods) of C_3– plants lack the phenomenon of absence of apparent photorespiration. The absence of CO_2 elimination at postillumination stage has been established for 31 genotypes, including 18 ones of four cereal species (Triticum aestivum L., Tr. durum L., Secale cereale L.,and Triticale); six genotypes of two species from the Leguminosae family (Pisum sativum L. and Glycine max L.), and seven wild and slightly cultivated cereal species such as Tr. boeoticum Boiss; Tr. dicocoides L; Koern; Tr. dicoccum Shuebl; Tr. spelta L; Tr. compactum L; and Tr. monococcum Tr. sphaerococcum Persiv. For the first time, it has been established that concomitantly with the absence of apparent photorespiration, the photorespiration cycle is present and active in the reproductive organs (spikes, pods) of C_3 – plants demonstrated by a high activity of glycolate oxidase – a key enzyme of the glycolate cycle. In this context, the phenomenon of absence of apparent photorespiration in the reproductive organs (spikes, pods) of C_3 – plants is not a result of inactive glycolate metabolism or its inhibition by endo- and exogenous factors. These facts (absence of apparent photorespiration, presence of glycolate cycle, high activity of glycolate oxidase) indicate the existence of two different mechanisms of carbon metabolization for C_3 – plants: one is located in leaves and the other in reproductive organs.*

Key words: photorespiration, glycolate oxidase, reproductive organs, C_3 plants.

Rezumat. *Lucrarea evidențiază în premieră fenomenul absenței fotorespirației aparente în organele reproductive (spice, păstăi) la plante de tip C_3. Absența eliminării de CO_2 în faza post iluminare a plantelor a fost stabilită pentru 31 genotipuri: 18 – de cereale din cadrul a 4 specii – Triticum aestivum L., Tr. durum L., Secale cereale L., Triticale; 6 – de leguminoase din cadrul a 2 specii- Pisum sativum L., Glycine max L şi 7 specii sălbatice şi puțin cultivate de cereale – Tr. boeoticum Boiss; Tr. dicoccoides Koern; Tr. dicoccum Shuebl; Tr. spelta L; Tr. compactum L; Tr. monococcum L; Tr. sphaerococcum Persiv. S-a stabilit în premieră că în organele reproductive (spice, păstăi)* ale *plantelor de tip C_3, concomitent cu absența fotorespirației aparente, este prezent şi activ ciclul fotorespiratoric, care a fost demonstrat prin activitatea înaltă a glicolat oxidazei – enzimă cheie a ciclului glicolatic. În acest context, fenomenul absenței fotorespirației aparente în organele reproductive la plante de tip C_3 nu rezultă dintr-un metabolism inactiv al glicolatului sau inhibiția sa de către factorii endogeni sau exogeni. Aceste fapte (absența fotorespirației aparente, prezența ciclului glicolatic* şi *activitatea înaltă a glicolat oxidazei) indică existența a două*

mecanisme diferite *de metabolizare a carbonului la plante de tip C_3: unul – localizat în frunze, celălalt– localizat în organele reproductive.*

Cuvinte cheie: fotorespirația, glicolat oxidaza, organe reproductive, plante de tip C_3.

INTRODUCTION

Photosynthesis is a well known process and in spite of this a lot of problems remain still unelucidated. The photorespiration (glycolate cycle) represents one of them as its role is not still definitively established (2). But it is well known, that about 50 % of C_3 plants photosynthesis products are consumed in photorespiration. In this context, it is hypothesized that if the lost of energy and the products, synthesized in photosynthesis could be reduced, the productivity of C_3 plants would be doubled ((5, 16, 7, 4, 15). The scientific investigations carried out in this direction during 30 years with the scope to increase productivity of C_3 plants at the expense of photorespiration level decrease did not succeed (16, 7, 6, 15). But these investigations have arrived at a conclusion that photorespiration is an essential component part of the production process (6) and acts as a physiological mechanism of photosynthetic apparatus protection. Experimental data on the presence of photorespiration and glycolate cycle activity in reproductive organs have not been described in the literature.

This work reports new results concerning the photorespiration phenomenon in reproductive organs of C_3 plants.

MATERIALS AND METHODS

Thirty one genotypes of thirteen species from *Poaceae* family, including *Tr. aestivum* L. (three genotypes), *Tr. durum* L. (five genotypes), *Secale cereale* L.(two genotypes), *Triticale (*eight genotypes), *Tr. boeoticum* Boiss, *Tr. dicoccum* Schuebl, *Tr. dicoccoides* Koern, *Tr. monococcum* L., *Tr.compactum* L., *Tr. spelta* L., *Tr. sphaerococcum* Persiv and six genotypes of *Fabaceae* family, including three genotypes of *Glycine max* L and other three of *Pisum sativum* L. were used to study CO_2 exchange for C_3 plants. The above-mentioned genotypes were grown under field conditions during 2007 and 2008. A part of the investigated genotypes, including three ones of *Tr. aestivum* L., three genotypes of *Glycine max* L., three ones of *Pisum sativum* L and seven wild and slightly cultivated cereal species were cultivated under control conditions of greenhouse and used in comparative analysis with those from field conditions.

Studies on CO_2 exchange and activity of key enzyme of photorespiratory cycle were performed for field cereals at heading complete – flowering period, for cereals of greenhouse control conditions at the heading complete – flowering stage, ripening (milky – wax development in kernel) stages and for *Fabaceae* plants at the flowering and pod formation stages. CO_2 exchange (photosynthesis intensity, respiration, apparent photorespiration) measurements were done for cereal flag leaves and legumunaceae leaves, and for reproductive organs – cereal spikes and leguminaceae pods

CO_2 exchange was studied on the basis of a novel monitoring technology (3) of this process using a modern apparatus – monitor of photosynthesis and transpiration (PTM-48A) of Bioinstruments SRL company, Chisinau, Republic of Moldova. Resolution capacity of the apparatus is 0, 0002 μmoli $CO_2 \cdot g^{-1} \cdot s^{-1}$.

The presence and activity of photorespiratory cycle (glycolatic) was detected on the basis of activity determination of the glycolatic cycle key enzyme - glycolate

oxidase (10) in the leaves and components of reproductive organs: spikes – glume, lemma, awns; pods – intact pods, valves and seeds.

RESULTS AND DISCUSSIONS

With the discovery of Calvin (C_3) and Hatch - Slack (C_4) (C_4) (7, 9, 13) cycles, the vegetal world was divided into plants with C_3 – and C_4 - types of photosynthesis and CAM (crassulacean acids metabolism) plants. These plant groups differ not only on the mechanisms of CO_2 assimilation by photosynthetic apparatus, but on the presence or absence of photorespiration. C_3 plants are characterized by the release of CO_2 at the post-illumination stage (photorespiration), which is provided by carbon metabolism in the glycolate cycle. CO_2 elimination under the influence of light is absent in C_4 plants. The CO_2 released during malate decarboxilation in Kranz anatomy cells is included again in Calvin cycle. The specialty literature explains the photorespiration absence suggesting that the glycolate cycle is not characteristic of C_4 plants, or its activity is reduced by the endogenous factors that lead to inhibition of this cycle (7) in spite of the fact that glycolate oxidase - the key enzyme of the glycolate cycle is active in C4 plant leaves and has the same function as in the cells of C_3 plants (8, 11).

The photosynthesis process accompanied by photorespiration in C_3 plants is also characteristic of reproductive organs, especially those of wheat, the photosynthesis level of which achieves 60 % of leaf photosynthesis intensity. From this point of view, the photosynthesis of reproductive organs have been studied in details (14, 13, 7, 1, 12), while photorespiration of reproductive organs is not recorded and described in specialty literature.

The results of our studies on CO_2 exchange for thirty one genotypes of C_3 plants, including thirteen cereal species of the *Poaceae* family and two ones of the *Fabaceae* family demonstrate the absence of CO_2 elimination under the light influence (apparent photorespiration) in reproductive organs – spikes, pods**.**

Reliability of this new fact is determined by the resolution capacity of the apparatus PTM-48A, which is, as mentioned above, 0.0002 μmoli $CO_2 \cdot g^{-1} \cdot s^{-1}$, that is, if carbon metabolism in the glycolate cycle resulted in release of any quantity of CO_2, its value is so low, that practically, it can be considered that the CO_2 elimination in the glycolate cycle does not occur – the apparent photorespiration is absent. It should be mentioned for comparison, that apparent photorespiration for the leaves of the genotypes studied is recorded at a quite wide range from 0.15 to 1.55 μmoli $CO_2 \cdot g^{-1} \cdot s^{-1}$. Results on the photosynthesis share in the intact spikes of wheat plants of the photosynthesis in leaves present another argument in favor of the reliability of the absence of apparent photorespiration in reproductive organs. As mentioned, this part can achieve a level of 60 %. Our data confirm this fact: the photosynthesis share in spikes of the photosynthesis level of cereal plant leaf (*Tr. aestivum* L., *Tr. durum* L., *Secale cereale* L., *Triticale* and seven wild and slightly cultivated) varies from 6.8% to 64.2%. This fits for the genotypes of the *Glycine max.* L. and *Pisum sativum* L species: the photosynthesis share in pods of that in leaves ranges from 3.6% to 43.4%.

The phenomenon of apparent photorespiration absence in the reproductive organs of C_3 plants is demonstrated and by the comparative studies of kinetic registrations of CO_2 exchange components (CO_2 assimilation – photosynthesis, typical mitochondrial respiration, apparent photorespiration) for the leaves and reproductive organs of C_3 plants.

The data presented in fig. 1 (a, b, c) show that leaves of C_3 plants (fig.1a) are characterized by registration of CO2 assimilation – photosynthesis (A), apparent photorespiration (PR) and typical mitochondrial respiration – R, while for reproductive organs – intact spike and pod (fig. 1b, c) only photosynthesis (A) and typical mitochondrial respiration (R) are registed. So, apparent photorespiration (PR) in reproductive organs is not evidenced, that means that the CO2 elimination during the post-illumination period does not take place – it is absent.

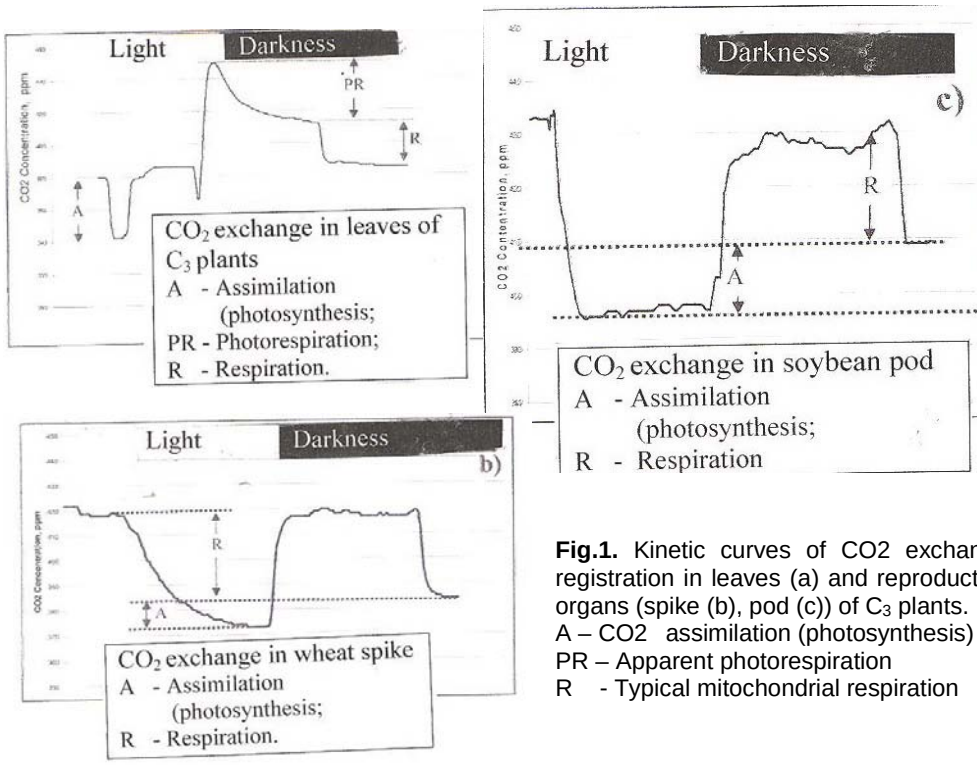

Fig.1. Kinetic curves of CO2 exchange registration in leaves (a) and reproductive organs (spike (b), pod (c)) of C_3 plants.
A – CO2 assimilation (photosynthesis)
PR – Apparent photorespiration
R - Typical mitochondrial respiration

The evidence of apparent photorespiration phenomenon absence in the reproductive organs of C_3 plants has led to the necessity to study the key enzyme of photorespiratory cycle – glycolate oxidase for establishment whether this phenomenon is connected with the absence or limitation of glycolate cycle activity, or the phenomenon is based on other mechanisms (ways) of carbon metabolization.

The results on glycolate oxidase activity presented in table 1 show a considerable enzyme activity in both leaves and reproductive organs. The glycolate oxidase activity in spike is much higher in comparison with that of cereal leaves.

In pods of legume plants, the activity level of glycolate oxidase is high enough

and does not differ so much from that of leaves. So, we can conclude that the high activity of glycolate oxidase in the reproductive organs of C_3 plants demonstrates that the absence of apparent photorespiration is not a result of glycolate metabolism inactivity or its inhibition by the endogenous and exogenous factors.

These results (the absence of apparent photorespiration, presence of glycolate cycle, high level of glycolate oxidase) indicate the existence of two different mechanisms of carbon metabolization for C_3 plants: one is located in leaves, and the second - in reproductive organs.

Table 1

Glycolate oxidase activity in leaves and the reproductive organs of C_3 plants

№	Species, cultivars	Character of reproductive organs	Activity of glycolate oxidase; μmoli glioxilic acid · g^{-1} · min^{-1}		
			leaves	spikes	pods
1	*Tr. Aestivum* L.	spikes			
1.1	*Balada*	awned	224,4±3,1	393,4±6,4	-
1.2	*Dana*	awned	178,4±5,3	253,2±591	-
1.3	*Belițcaia*	awned	162,4±1,7	417,7±11,4	-
1.4	*Belicanca 7*	awnless	238,8±4,0	120,3±3,8	-
2	*Glycine max* L.	pods			
2.1	*Aura*	pods	440±3,8	-	390,0±2,7

Totalizing the presented results we mention that regarding the description, for the first time, of phenomenon of apparent photorespiration absence in reproductive organs we insist on the necessity to introduce in scientific circulation the précised term for photorespiration – the formula "apparent photorespiration", which characterizes only the CO_2 elimination during the post-illumination stage of CO_2 exchange comparing to the term "photorespiration", which includes both CO_2 elimination and the oxidation reactions under the light influence, carbon metabolism in glycolate cycle and whole metabolism of photosynthesis products.

CONCLUSIONS

A new phenomenon has been detected, which previously was not known for C_3 plants: the phenomenon of absence of apparent photorespiration at the presence of the glycolate cycle in the reproductive organs of C_3 plants, characterized by the fact that in the leaves of C_3 plants as a result of glycolate metabolization, synthesized in photosynthesis process and subsequently oxidized in peroxisomes by glycolate oxidase, the release of CO_2 takes place under the light influence, while in the reproductive organs of C_3 plants (spikes, pods) at the presence of glycolate and high glycolate oxidase activity level, CO_2 elimination does not take place under the light influence – the apparent photorespiration is absent.

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