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GAVIN PUBLISHERS

Journal of Tissue Culture and Bioengineering

Research Article

Balaur N, et.al J Tissue Cult Bioeng: JTCB-101.
DOI: 10.29011/JTCB-101.000001

The Peculiarities of Carbon Metabolism in the Ears of C_3 Cereals: CO_2 Exchange Kinetics, Chloroplasts Structure and Ultrastructure in the Cells from Photosynthetic Active Compo¬nents of the Ear

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Citation: Baluar N, Badicean D, Peterhaensel C, Mereniuc L, Vorontsov V, et al. (2018) The Peculiarities of Carbon Metabolism in the Ears of C_3 Cereals CO_2 Exchange Kinetics, Chloroplasts Structure and Ultra-Structure in the Cells from Photosynthetic Active Components of the Ear. J Tissue Cult Bioeng: JTCB-101. DOI: 10.29011/JTCB-101.000001

Received Date: 09 January, 2018; **Accepted Date:** 16 February, 2018; **Published Date:** 26 February, 2018

Abstract

The ear of C_3 cereals makes an important contribution to yield formation, but the mechanisms ensuring this phenomenon are not completely elucidated. Previously established peculiarities of carbon metabolism in the ear of cereals come in contradiction with the discovery of phenomenon of lack of apparent photorespiration in reproductive organs of the C_3 plants. In this article are presented the CO_2 exchange kinetics of the ear and compensation point; the structure and ultrastructure of chloroplasts from glume, lemma and awn in comparison with flag leaf (*Tr. durum, Triticale*), leaf and tassel of C_4 plants (*Zea mays*).

Was established, that CO_2 exchange kinetics of the ear is similar to the leaf and tassel of maize plants. In open measurement system the ear does not register a compensation point, but in the close system - does. The lack of apparent photorespiration in the tassel and presence of a compensation point is evidenced also for the maize plants, where it is formed from the released respired CO_2. The glume, lemma and awn, similar to maize leaf, have two types of cells: mesophyll cells and cells arranged around the vascular bundles, forming a “Kranz” type of crown. In these cells are present two types of chloroplasts: granal in the mesophyll cells and granal-agranal in the Kranz type of cells, with well-developed granas and weak developed lamellas.

The obtained results demonstrate that in the cereals ear, concomitantly are functioning structural and functional elements of C_3 and C_4 types of photosynthesis.

Keywords: Carbon Metabolism, Cereals Ear; Chloroplasts Ultrastructure; Compensation Point; CO_2 Kinetics; C_3 Plants; C_4 Plants, Photorespiration

Abbreviations:

A : Intensity of Net Photosynthesis

AP : Apparent Photorespiration

APP : CO_2 Assimilation after Light Turn off

FLC : First Layer of Cells, from the Crown, Surrounding Vascular Bundles

SLC : Second Layer of Cells, from the Crown, Surrounding Vascular Bundles

G : Compensation Point

MDH : Malate Dehydrogenase

NADP - ME : Malate Decarboxylase, NADP Dependent Malic Enzyme

PEPC : Fosfoenolpiruvat Carboxylase

PPDK : Piruvat Ortofosfat Dikinase

R : Dark Respiration (Mitochondrial)

Rubisco : Ribulozobifosfatcarboxylase

Introduction

All photosynthetic organs in C_3 and C_4 plants fix carbon to produce sugars and other organic substances. Beside the flag leaf, photosynthesis of the ear has an important role in grain formation by contributing 40-50% to the carbon stored in grains [1-3]. Earlier studies even demonstrated that the ear`s contribution to barley grain formation reaches 76% [4]. All components of the ear contribute to its photosynthetic efficiency: awn, glume, lemma and palea [2, 5-8]. Assimilation of CO_2 by the ear components vary, but only the awns respond for 40-80% of carbon metabolism from the entire ear, in different cereals [8,9]. Despite the important role of the cereal`s ear in yield formation, especially in drought conditions [8], the mechanisms of CO_2 assimilation are not completely elucidated yet. During the last two decades many investigations of CO_2 exchange in the ear and its photosynthetic active components were performed: CO_2 compensation point [10,11], CO_2 re-fixation [2,8,12,13], carbon isotopes discrimination [2,8], incorporation of labeled malate [10], C_4 enzymes activities and analysis of the corresponding gene expression levels [14-16], drought resistance of the ear photosynthetic active components [7,8]. In these studies, and many others that today are updated, scientific opinion is oriented towards the conclusion that only C_3 type of photosynthesis operates in the ear of cereals. In early investigations, based on compensation point and labeling experiments it was concluded that all ear components in general perform only C_3 type of photosynthesis [10]. The percentage of labeled CO_2 incorporated into C_4 products (malate and aspartate) was less than 10% and it was suggested that C_4 type of photosynthesis is not operational in these tissues. Despite these conclusions the authors cite many articles (23, published in the period of 1972-1993) regarding the evidence of structural and functional elements of C_4 syndrome in the photosynthetic active components of the ear. Based on these early results it was proposed that the existence of an intermediate type of C_3-C_4 or C_4 limited metabolism in the ear components of cereals is possible based on: 1) high activities of PEPC and PPDK; 2) labeled CO_2 incorporation into C_4 products; 3) CO_2 exchange and anatomical characteristics of the ear; 4) lack of O_2 photosynthesis inhibition in the rice ear; 5) the carbon isotope composition in the water-soluble fraction of ear components. After the above mentioned article [10] during the following 17 years of research on revealing the role of functional and structural C_4 elements in photosynthetic active components of the ear, especially in the cereals, did not stop. The main focus is the evidence of possible mechanisms that might explain the main role of the ear and its components, high levels of CO_2 assimilation compared to the flag leaf - key organ for carbon metabolism in C_3 plants [8,9,13-16]. Based on these articles it is first of all important to mention the main role of awn in the synthesis of sugars, compared to the flag leaf [9]. This conclusion is based on photosynthetic intensity, chloroplasts ultrastructure and high PEPC levels, that in all checked developmental stages (6 stages, with maximum detected levels during grain filling) was higher in the awn compared to the flag leaf.

Taking into account the cereals ear structure and spatial arrangement of its photosynthetic active components, the same works have studied re-fixation of CO_2 released during dark respiration. It was supposed that CO_2 re-fixation may be an adaptation mechanism, which improves photosynthesis and water use efficiency, especially in drought conditions, during grain formation and filling stages [8,9,13]. In a review [8] 95 scientific articles published during 1965-2007 were analyzed and the following conclusions were made regarding the photosynthetic activity of the ear: a) photosynthetic contribution of the ear for grain filling appears to be quite important, particularly when grain yield is source-limited (i.e., drought); b) awns, when present, seems to be the main photosynthetic organ of the ear, at least with regard to net fixation of atmospheric CO_2; c) re-fixation of respired CO_2 is a well-documented process in ears of C_3 cereals and represents a potentially important contribution for total photosynthesis; d) green pericarp and inner bracts (lemmas) are probably the main sites for CO_2 re-fixation: e) current evidence does not support the presence of C_4 or CAM metabolism in ears of C_3 cereals; f) the ear exhibits better photosynthetic performance under water stress conditions, resulting from a higher relative water content, and its capacity for osmotic adjustments compared to the flag leaf; g) delayed senescence (persistence of photosynthetic components) is a key process in maintaining ear photosynthesis, mainly under water stress.

The data presented by different authors show evidence of the contradictions regarding the mechanisms of CO_2 assimilation in the photosynthetic active ear components. Basically all these mechanisms are divided into four types of approaches: 1) in the ear is active only C_3 type of photosynthesis and C_4 type is absent; 2) in the ear of cereals a higher level of PEPC is present comparative to the flag leaf; 3) because of morphological structure, spatial arrangement of ear components and presence of PEPC, PPDK the ear is able to re-fix respired CO_2 at substantial rates; 4) photosynthetic active components of the ear have an important protection role against pathogens, high temperatures and water deficit especially during post-flowering and grain filling stages. These different approaches are the consequence of different used methods by different authors; studding the same morphological, physiological and biochemical indices of ear photosynthetic active components, but in different genotypes; under different ecological growing conditions; at different developmental stages of the cereals ear, etc.

In this context, the main aim of our article was to investigate the peculiarities of carbon metabolism in the ear of C_3 cereals. This was achieved through the studies of CO_2 exchange kinetics, compensation point, the structure and ultrastructure of chloroplasts in the same genotypes, growing conditions, developmental stages of the ear and using the same methods in comparison with the flag leaf of C_3 cereals and maize tassel/leaf (C_4 plants).

Materials and Methods

Study Objects and Plant Growth Conditions

C_3 plants of *Tr. durum* L. (variety Hordeiforme 335), *Triticale* (variety Ingen 93) and *Zea mays* (line 459 and hybrid RF7xW47) served as study objects. All these genotypes were grown in the experimental fields of the Institute of Genetics, Physiology and Plants Protection of the Academy of Science of Republic of Moldova. Biological material from maize (leaf and tassel glumes) were collected at the beginning of tassel flowering. In case of *Tr. durum* and *Triticale* biological samples were collected at two developmental stages (earning and milk/waxy (ripening) from four different tissues: flag leaf, awn, lemma and glume). All samples were collected in three biological repetitions and each repetition was composed by pooled material from three individual plants. The field grown plants were used for gas exchange analysis, anatomical structure and chloroplast ultrastructure determination.

CO_2 Exchange Kinetics and Compensation Point

Measurements of CO_2 exchange kinetics in photosynthetic active organs (flag leaf and ear of C_3 cereals, leaf and tassel of maize plants) were done at two developmental stages (earning and ripening) for C_3 cereals and at the stage of seven leaves/tassel appearance for maize plants. All measurements were performed on the intact plants. Components of CO_2 exchange (apparent photosynthesis, dark CO_2 assimilation, respiration, apparent photorespiration, compensation point) were determined using infrared gas analyzer (PP Systems, USA) integrated into the photosynthetic monitor PTM-48A (Bioinstrument S.R.L, Republic of Moldova) [17,18] in open and closed systems, according to manufacturer instructions.

Anatomical Structure and Chloroplasts Ultrastructure

For sections preparation the samples (leaf and tassel of maize plants; flag leaf, awn, lemma and glume for cereals) were fixed in glutaric aldehyde (2%) and post-fixed in osmium tetroxide (1%) [19]. Biological material was dehydrated in alcohol solutions (10%-100%) and embedded in Epon-812. All sectioning was done at BS-Y90A Tesla ultra-microtome. The sections for anatomical structure studies were stained in methylene blew (1%) and peronin-j (1%). Ultrafine sections were contrasted in Uranyl Acetate and Pb citrate 2% [20]. Anatomical structure of photosynthetic active organs was investigated on permanent preps using MezoPlant automated system (SIAMS, Russia). Chloroplasts ultrastructure was studied at the electronic microscope Tesla BS - 500.

Results and Discussion

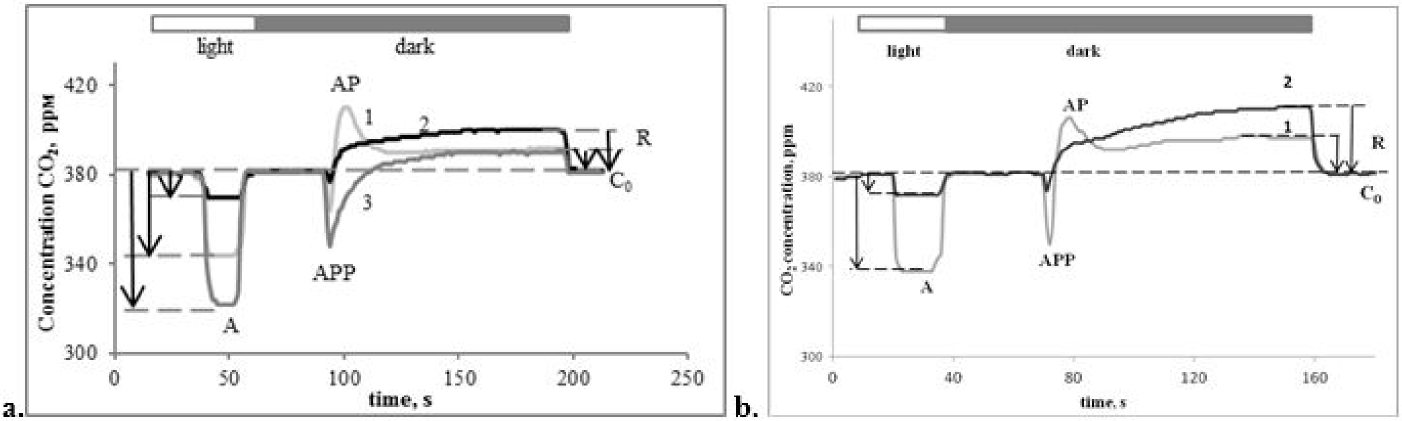
The necessity to update research and discussions in the field of CO_2 assimilation mechanisms and carbon metabolism in the ears of C_3 cereals appeared as a consequence of the last results regarding the CO_2 exchange in the ear [18]. In this article, it was demonstrated that CO_2 exchange kinetics in the ear of C_3 plants, in post-illumination phase, do not show apparent photorespiration - a phenomenon evidenced for the first time in 2007 [21]. Based on these results, it was confirmed that in C_3 cereals only the flag leaf performs C_3 photosynthesis, the rest of the photosynthetic active organs (mainly the ear) in post-illumination phase showed similarities with CO_2 exchange kinetics of the maize leaf (C_4 plant). These results are in contradiction with those mentioned in the introduction, that in the ear the degree of C_4 photosynthesis is equal to zero [10]. This essential contradiction, absence of apparent photorespiration similar to C_4 leaf and absence of C_4 metabolism in the ear as in C_3 flag leaf, turns all research in that field towards a new beginning: taking into account known and important role of the ear in yield formation of C_3 cereals [7], what is the ear of cereals from carbon metabolism point of view?

In order to get answer to this question we analyzed the same functional and structural indices that were reported previously in the literature. Our approach has three main peculiarities: 1) in order to have comparable results all analyses were performed on the same species, same photosynthetic active organs, at the same developmental stages, in the same ecological conditions; 2) to estimate similarities and differences in expression levels of C_3 and C_4 types of carbon metabolism in the ear comparative analyses with cereal flag leaf (C_3) and leaf/tassel of maize (C_4) were performed; 3) all measurements of CO_2 exchange kinetics in leaf, ear and tassel were carried in open and closed systems, on intact plants.

CO_2 Exchange Kinetics for Leaves of Cereals and Maize in Open System

Exchange kinetics of CO_2 is studied in two types of measurement systems: closed system, where the vegetative organ is separated from the plant and is placed in an isolated chamber that does not contact with atmospheric air; and open system, where vegetative organ is investigated on intact plant and atmospheric air freely circulates in the chamber [22]. The CO_2 exchange kinetics of leaf was studied in open system using PTM - 48A monitor [17]. Advantage of this system is in differential and parallel measurement of all the components of CO_2 exchange of the organs on the intact plants, while many of the methods used to study CO_2 exchange are destructive [22,23].

Comparative study was done between C_3 flag leaf, ear (*Triticale*, *Tr. durum*) and C_4 leaf (maize) (Figures 1a, b). Figure 1a shows representative CO_2 exchange profiles of *Triticale* flag leaves (1), *Triticale* ears (2) and maize leaves (3). After illumination, highest rates of net photosynthesis (A) were recorded for maize, intermediate rates for flag leaves, and lowest levels for ears. After darkening the samples, different CO_2 exchange profiles are observed: for the flag leaf, a transient CO_2 assimilation (APP) followed by a transient CO_2 release (AP) was observed. The latter had been used as an estimate of photorespiration in previous studies [18]. In contrast, maize leaves did not show CO_2 release in the APP phase, a well-known fact [24,25]. Interestingly, we observed the AP peak, but not the APP peak also for ears indicating that photorespiratory CO_2 release is absent in these tissues. After the two transient peaks, CO_2 release leveled out for all tissues and steady-state dark respiration (R) could be determined for all samples. R was highest for the ear sample and lower for the *Triticale* flag leaf and the maize leaf. Figure 1b shows a corresponding comparison of the *Tr. durum* flag leaf (1) and the ear (2). Again, the AP peak was observed in the leaf, but not for the ear sample.

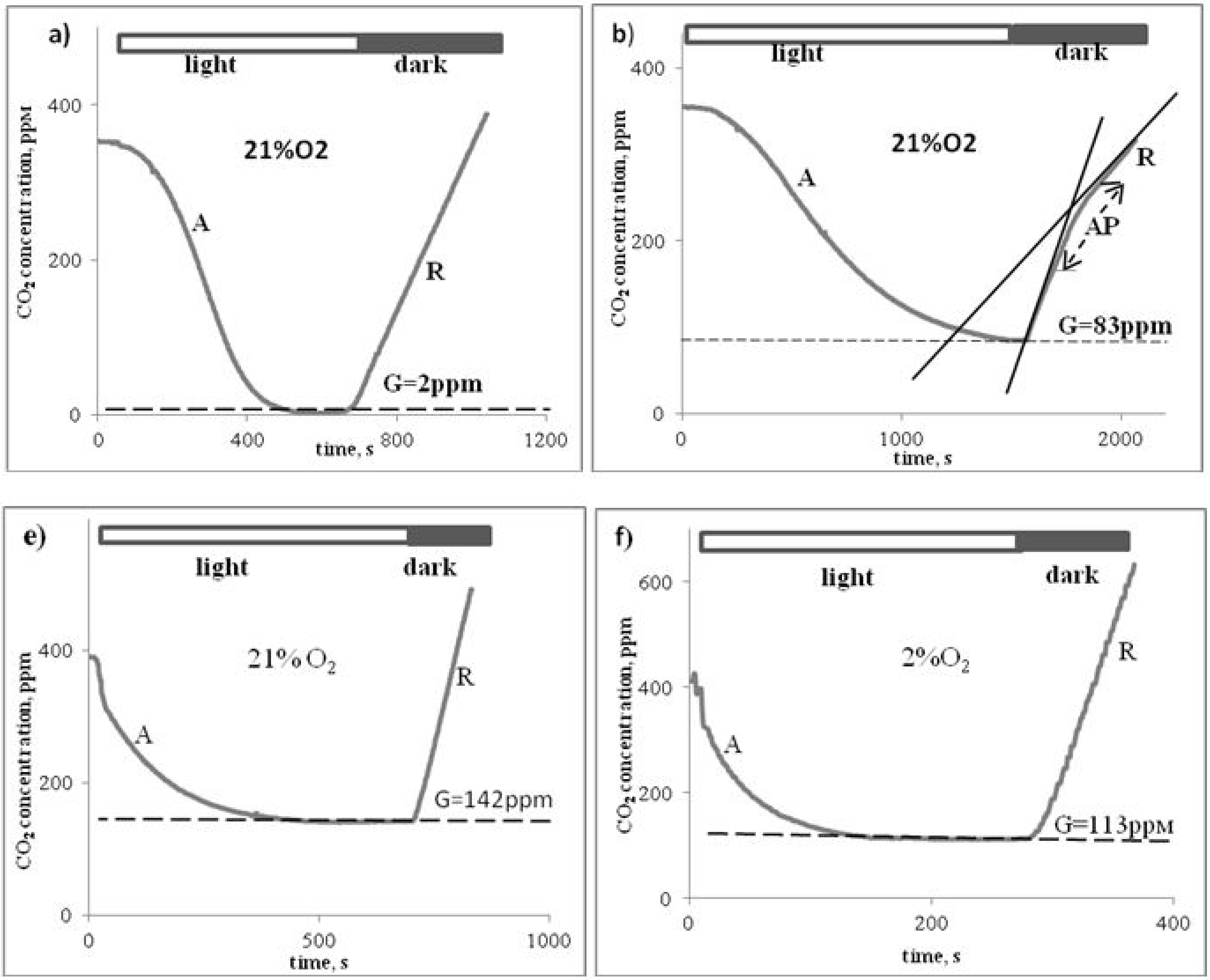


Figures 1(a-b): Typical and representative CO_2 exchange kinetics of C_3 (*Triticale* (a), *Tr. durum* (b)) and C_4 (maize (a)) in open measurement system. Presented curves are the result of CO_2 concentration measurement, per second, by infrared gas analyzer, where the air enters from illuminated chamber for 30sec (A) and darkened chamber for 180sec (APP, AP, R). Light and dark conditions are ensured by automatic open/close of transparent and dark chambers. The measurements were done on flag leaves or ear from field grown plants. A - Net photosynthesis; APP - dark CO_2 assimilation (post-illumination phase); AP - apparent photorespiration (CO_2 flux during post-illumination phase); R - respiration; C_0 - atmospheric CO_2 concentration; 1 - flag leaf of *Triticale* and *Tr. durum* at earning; 2 - ear of *Triticale* and *Tr. durum* at earning; 3 - maize leaf; s - seconds.

The difference between the ear, flag leaf of cereals and maize leaf is highlighted through lower intensity of photosynthesis, CO_2 assimilation in the dark, but on the other hand higher dark respiration. These peculiarities of CO_2 exchange kinetics of the ear, as going to be demonstrated below, have a special impact on compensation point.

CO_2 Compensation Point of Maize and Cereals Leaves in Open System

Compensation point represents the CO_2 concentration when a balance is established between assimilated and dissimilated CO_2. For maize leaf (Figure 2, a, line 1) it was around 2 ppm, very reduced and corresponds to literature data for compensation point in C_4 plants [25], confirming the absence of apparent photorespiration. For the flag leaf of C_3 *Triticale* and *Tr. durum* plants compensation points were 52,5 ppm and 85 ppm respectively (Figure 2, a, lines 2 and 3), values corresponding to literature data for C_3 flag leaf, where apparent photorespiration may be as high as 60% of the intensity of the photosynthesis [26]. The last three lines (4-6 in Figure 2 b) correspond to maize tassel and cereals ear respectively, where the compensation point is not registered - similar to maize leaf. These results indicate that in open system, in those photosynthetic active organs, where apparent photorespiration is not registered, the compensation point is also not registered and CO_2 exchange in the organs lacking apparent photorespiration does not depend of CO_2 concentration in the air.

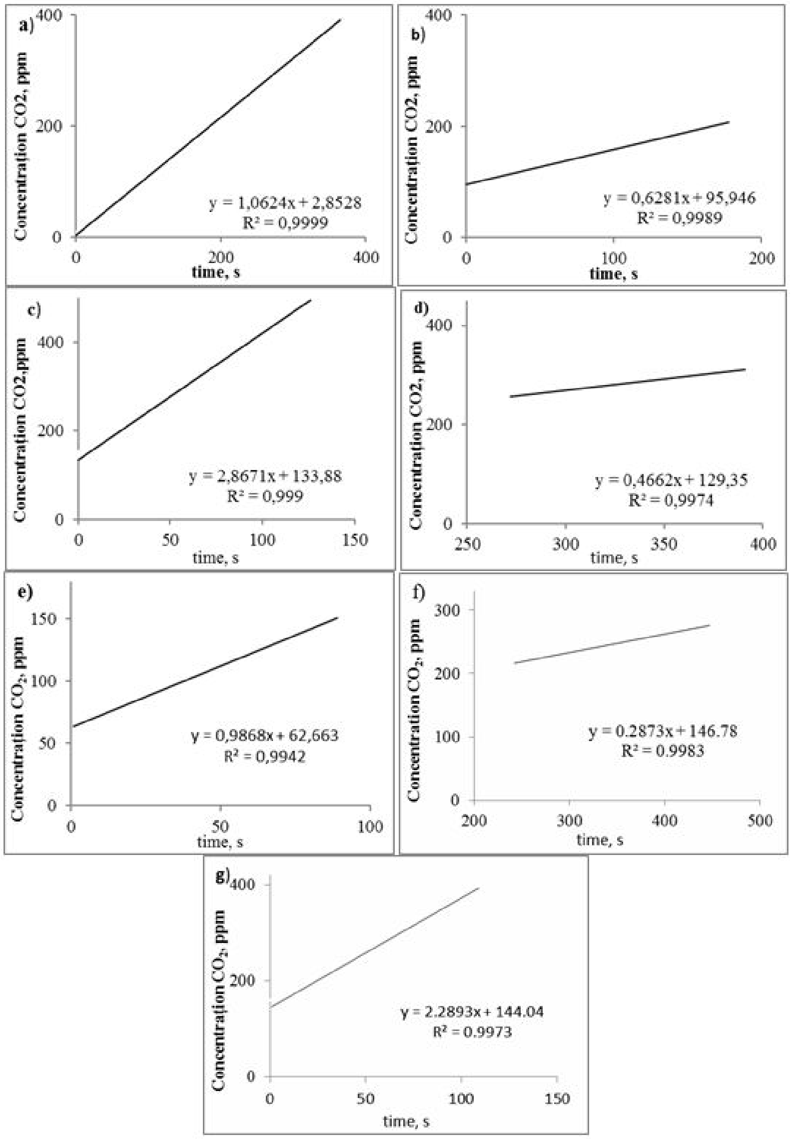


Figures 3(a-f): CO_2 exchange kinetics, compensation point in closed CO_2 measurement system and the expression of apparent photorespiration in the leaf, at 21% O_2: **a)** maize**; b**) *Triticale*; **c)** *Tr. durum* and the ears **d)** *Tr. durum;* **e)** *Triticale*; **f)** Triticale at 2% of O_2. The measurements were done on leaves and ears from field grown plants. A - net photosynthesis; G - Compensation Point; R - Regular Respiration; AP -Apparent Photorespiration; s - seconds

The flag leaf of cereals in closed system registers the same three components of CO_2 exchange as maize leaf (Figure 3b), but on the curve, that characterize respiration (R) appears a flux of CO_2 that changes line trajectory. This flux is the amount of CO_2 released during apparent photorespiration, characteristic to C_3 flag leaf (AP in Figure 1). In this way for cereals flag leaf in closed system are registered four components of CO_2 exchange: A, G, AP and R. The same peculiarities were registered for the flag leaf of *Tr. durum* plants (Figure 3 c) making them representative for the flag leaf of C_3 cereals.

CO_2 Compensation Point for the Ear of C_3 Cereals in Closed System

The ear of *Triticale* and *Tr. durum* plants, similar to maize leaf, in closed system registers only three components of the CO_2 exchange: A, G and R (Figure 3, d, e). Compared to the flag leaf of cereals the ear dos not register apparent photorespiration (AP). On the other hand the compensation point of the ear of *Triticale* and *Tr. durum* is around 142 ppm and 160 ppm respectively, similar high values for the ear was reported previously [10,11]. Comparative analysis of the ear, flag leaf of cereals and maize leaf evidenced one more peculiarity of the ear. The time to establish the equilibrium between CO_2 assimilation and dissimilation (compensation point) is around 8 min, similar to maize leaf. The flag leaf of cereals reaches the compensation point in 25 min. The CO_2 assimilation kinetics of the ear, having similarities with maize leaf, come in contradiction with the main peculiarity established in the literature - a photosynthetic active organ that does not register apparent photorespiration should have a reduced compensation point. And vice versa, the organ that registers high compensation point should have apparent photorespiration. In our case, for the ear that lack apparent photorespiration (Figure 2; Figure 3, d, e), was registered a compensation point that is twice as high as in the leaf and ten times higher than in the maize leaf. Even 2% O_2 concentration did not induce essential changes in the formation of compensation point of the ear (Figure 3, f). Calculation of regression curves for CO_2 exchange in maize leaf and flag leaf, ear of *Triticale* and *Tr. durum* (Figure 4) demonstrated the absence of apparent photorespiration in the ear.

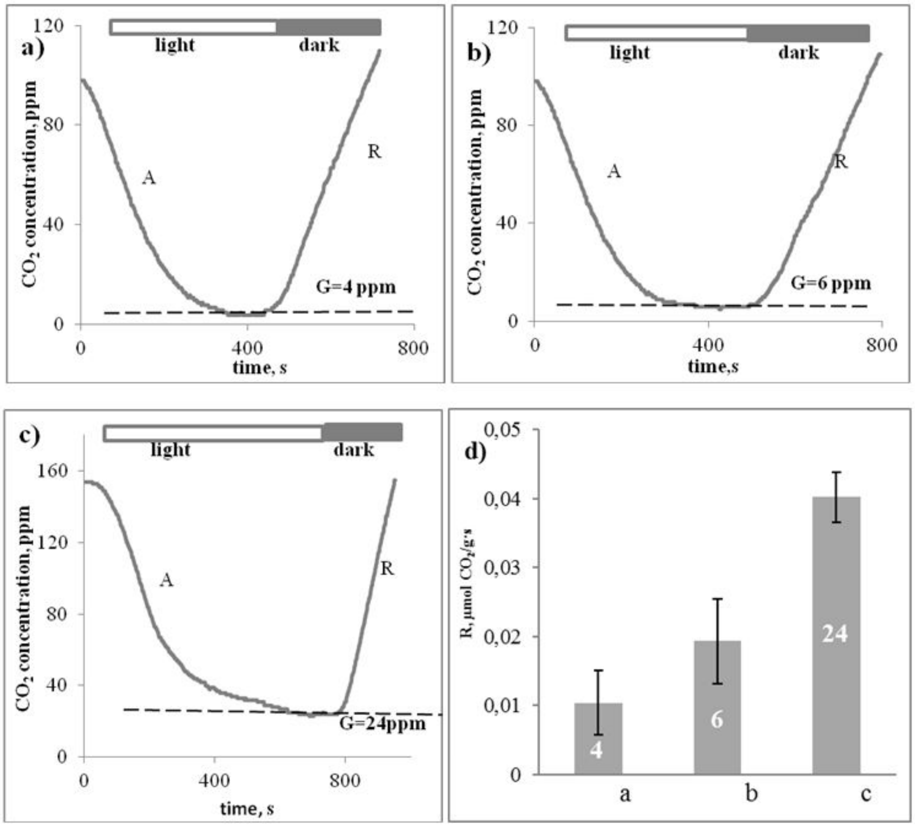


Figures 4(a-g): CO_2 exchange kinetics in closed measurement system, in post-illumination phase. **a)** maize leaf; **b)**, **d)** *Triticale* flag leaf; c) *Triticale* ear; **e), f)** *Tr. durum* flag leaf and ear **(g).** The measurements were done on leaves or ear from field grown plants. Y - equation for linear regression; R^2 - coefficient of determination; s – seconds.

The CO_2 exchange in the maize leaf (Figure 4a), the ear of *Triticale* (Figure 4c) and *Tr. durum* (Figure 4g) is described by linear equation of regression, demonstrating that in the post illumination phase, for these photosynthetic active organs, only one source of CO_2 exists - respiration. In case of the flag leaf of *Triticale* (Figure 4b, d) and *Tr. durum* (Figure 4e, f) the angle change of the regression curve is approximated through two equations of linear regression, with a high degree of confidentiality ($R^2 > 0.9$). The regression coefficients, before and after tilt angle change, differ essentially: for the flag leaf of *Triticale* - 0.628 and 0.466 respectively (the difference is 25.8%); for the flag leaf of *Tr. durum* - 0.987 and 0.287 respectively (the difference is 70.9%). These data show very clearly that in the C_3 flag leaf two sources of CO_2 release exists - photorespiration and respiration. But for the maize leaf and the ear of cereals only one source - respiration, based on which is formed the compensation point in the absence of apparent photorespiration. This conclusion is based on more in detail analysis of CO_2 exchange kinetics of the maize leaf.

CO_2 Compensation Point of Maize Leaf with Different Levels of Respiration

Because CO_2 infrared gas analyzer cannot distinguish between photo respired and respired CO_2 the work was focused on maize leaf that does not register apparent photorespiration but has a compensation point (Figure 2(1), 3(a)). Previously in our research, during measurements of compensation point of maize leaves, it was noticed that in some cases obtained values are greater than 10 $\mu mol^* mol^{-1}$ of CO_2, previously shown in literature [25]. This peculiarity may be linked with dark respiration, because apparent photorespiration is absent. In Figure 5 for maize leaves were registered compensation points, higher than 2 ppm, with maximum value of 24 ppm. This comes in contradiction with the fact that in closed measurement, in post-illumination phase apparent photorespiration was not registered as in case of cereals flag leaf (Figure 3b), on the other hand compensation point is registered and increases with intensification of respiration (Figure 5d).

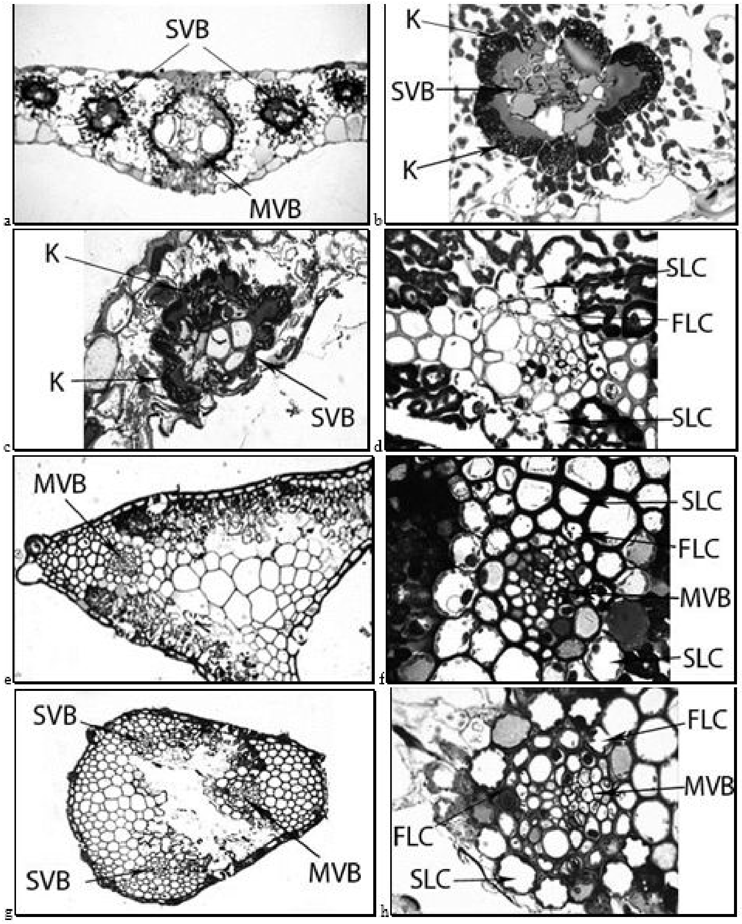


Figures 5(a-d): Dependence of compensation point and respiration values in the maize leaf. The numbers on bars in chart **d)** represent the values of compensation point for the different leaves (presented in charts **a-c**) that demonstrate the correlation between compensation point and intensity of the respiration. The measurements were done on leaves from field grown plants. A - net photosynthesis; G - compensation point; R -respiration; s - seconds. In chart d) is presented the average of 3 biological repetitions ± 95% confidence interval.

The highest compensation point corresponds to the highest level of regular respiration (24 ppm, Figure 5, c). The analysis of this correlation, between respiration and compensation point for maize leaf, demonstrates a strong positive correlation (R=0.81). As a result, for maize leaf lacking apparent photorespiration, the compensation point depends on concentration of CO_2 released from dark respiration and not from apparent photorespiration that is missing in C_4 plants. Taking all this into account one can make parallels with cereals ear, that manifest similarities with maize leaf in CO_2 exchange kinetics: registers an intensity of apparent photosynthesis, has a compensation point (established in the same time frame as in maize leaf) and is lacking apparent photorespiration. According to the same analogy the compensation point in the ear is formed from one source of released CO_2, from respiration. High values of compensation points for the ear (Figure 3d, e) correspond to the high levels of respiration (Figure 1, line 2).

Anatomical Structure of Photosynthetic Active Organs in C_3 and C_4 plants

Comparative analysis of CO_2 exchange kinetics of C_3 cereals flag leaves, ear and maize leaf confirmed previously obtained results [18,21] that similar to maize leaf the ear does not register apparent photorespiration. Absence of apparent photorespiration in C_4 leaf is ensured by C_4 syndrome. It is based on a specialized anatomical structure (Kranz anatomy) that allows an efficient assimilation of CO_2 released from decarboxylation of malate or aspartate [24,27]. In this context a comparative study was performed regarding the presence of structural elements of C_4 syndrome in the photosynthetic active components of the ear of C_3 cereals. From the literature it is known that C_4 syndrome is structurally presented in a layer of cells surrounding vascular bundles (in maize leaf). In the flag leaf and ear components of cereals a prototype of crown exists, that is formed by two layers of cells (Figure 6).



Figures 6(a-h): The structure of cells crown surrounding the vascular bundle in the photosynthetic active organs lacking apparent photorespiration. **a)** Kranz cells surrounding vascular bundle in the maize leaf (C_4 plants); **b)** Kranz cells surrounding secondary vascular bundle in the maize leaf; **c)** Kranz cells surrounding vascular bundles in the photosynthetic active components of the maize tassel; **d)** ”Kranz” type of cells surrounding vascular bundles in the *Triticale* leaf (C_3 plant); **e, f)** ”Kranz” type cells surrounding vascular bundles in the *Triticale* glume (C_3 plant); **g, h)** ”Kranz” type cells surrounding vascular bundles (primary and secondary) in the *Triticale* awn (C_3 plant); MVB - main vascular bundle, SVB - secondary vascular bundle, K - ”Kranz” cells, FLC - first layer of cells, SLC - secondary layer of cells.

The Kranz cell from maize leaf are bigger, contain more chloroplasts than mesophyll cells [24,27]. These peculiarities were evidenced also in the current study (Table 1). For maize leaf the volume of one Kranz cell is twice bigger than mesophyll cell and the volume, number of chloroplasts in one Kranz cell is higher than in mesophyll cell. The flag leaves of *Triticale* and *Tr. durum* plants have the same number of cells surrounding the vascular bundles but they lack above mentioned peculiarities. All indices of Kranz type cells in the flag leaf, containing chloroplasts, are smaller than the same indices in the mesophyll cells. Comparative analysis of these indices in the ear components (Table 1) demonstrated the same peculiarities as in maize leaf (Figure 6a, b, c): the same number of cells in the Kranz crown; the volume of one Kranz cell in glume and awn is bigger than in the mesophyll cells; number of the chloroplasts and the volume of one chloroplast are very similar to those in the maize leaf.

Variety	Studied organ	Nr of Kranz cells	Volume of one cell, μm^3 x10		Nr of chloroplasts in one cell		Volume of one chloroplast, μm^3	
			Kranz	mesophyll	Kranz	mesophyll	Kranz	mesophyll
earning phase (tassel appearance)								
Zea mays, Line 459	leaf	8 ± 2	22,8	10,2	10 ± 2,12	7 ± 2,02	58,4 ± 1,32	30,0 ± 2,07
Earning Phase								
Tr.durum,L. Hordeiforme 335	Flag leaf	9 ± 1	11,3	23,1	7 ± 3,07	13 ± 2,12	18,9 ± 0,78	23,9± 1,07
	Glume	4 ± 3	7,8	5,0	4 ± 1,87	8 ± 2,34	15,6 ± 1,32	16,0 ± 0,98
	Lemma	4 ± 2	6,0	5,6	3 ± 2,01	5 ± 1,09	14,7 ± 0,46	15,7 ± 0,47
	Awn	7 ± 2	6,2	6,8	6 ± 2,24	9 ± 1,43	13,3 ± 0,43	18,6± 0,62
Ripening Phase								
Tr.durum,L. Hordeiforme 335	Flag leaf	9 ± 2	12,2	24,2	8 ± 3,21	13± 2,22	20,1 ± 0,52	24,0 ± 0,98
	Glume	5 ± 2	8,0	5,7	5 ± 1,79	8 ± 2,17	16,7 ± 0,73	17,8 ± 0,73
	Lemma	5 ± 1	6,8	6,4	4 ± 2,17	6 ± 0,32	16,1 ± 0,43	18,1 ± 0,43
	Awn	8 ± 2	6,4	7,8	7 ± 2,43	9 ± 1,22	14,2 ± 0,94	19,3 ± 0,94
Earning Phase								

Triticale, Ingen 93	Flag leaf	8 ± 2	11,2	22,9	7 ± 0,78	16 ± 2,18	23,8 ± 0,21	29,4 ± 0,42
	Glume	8 ± 2	6,2	2,5	5 ± 0,82	6 ± 0,89	15,7 ± 0,72	16,2 ± 0,67
	Lemma	6 ± 2	3,0	2,2	5 ± 1,23	4 ± 1,02	15,0 ± 0,39	15,4 ± 0,72
	Awn	10 ± 2	3,2	5,2	5 ± 1,08	7 ± 1,52	20,2 ± 0,72	23,2 ± 0,43
Ripening Phase								
Triticale, Ingen 93	Flag leaf	8 ± 2	11,9	23,2	8 ± 1,23	18 ± 2,03	21,1 ± 1,08	31,7 ± 0,38
	Glume	9 ± 2	7,1	3,0	5 ± 1,89	7 ± 0,94	17,5 ± 0,89	18,8 ± 0,52
	Lemma	6 ± 2	3,6	2,7	6 ± 2,04	5 ± 1,08	15,3 ± 0,72	15,7 ± 0,34
	Awn	10 ± 2	3,8	5,5	6 ± 1,47	8 ± 1,23	21,3 ± 0,43	25,2 ± 0,77

Table 1: Comparative analysis of cells surrounding the vascular bundles and mesophyll cells of maize, *Tr. durum* and *Triticale* flag leaf and ear components.

In the last decade more and more attention is paid to the role of the cells that form the crown around the vascular bundles and have photosynthetic function [28-31]. For a C$_4$ leaf the distance between two vascular bundles, the number of mesophyll cells between them and the ratio between Kranz and mesophyll cells are much smaller compared with the C$_3$ flag leaf. Comparing the density of vascular bundles in different *Flaveria* species (C$_3$, C$_3$-C$_4$ and C$_4$) it was demonstrated that increased density is an evolutionary criteria for appearance of the C$_4$ photosynthesis [29]. Also it was suggested the necessity of genetic modification of anatomical structure of rice leaf (C$_3$ plants) as a way for photosynthetic productivity increase [28]. Taking into account the similarities in CO$_2$ exchange kinetics and anatomical structure between maize leaf and ear of cereals, demonstrated in the current study, we investigated the distances between vascular bundles. This parameter characterizes bundles' density per unit area and the number of mesophyll cells between them (Table 2).

Variety	photosynthetic active organs (average ± standard deviation)				
	Leaf	Tassel	Glume	Lemma	Awn
Zea mays, Line 459	49,4 ± 1,35	162 ± 27,31	-	-	-
Triticale, Ingen 93	90,7 ± 6,63	-	49,6 ± 5,58	115 ± 13,59	58,8 ± 6,32
Tr.durum L., *Hordeiforme* 335	84,9 ± 4,93	-	62,4 ± 5,94	158,3 ±14,94	58,9 ± 6,81

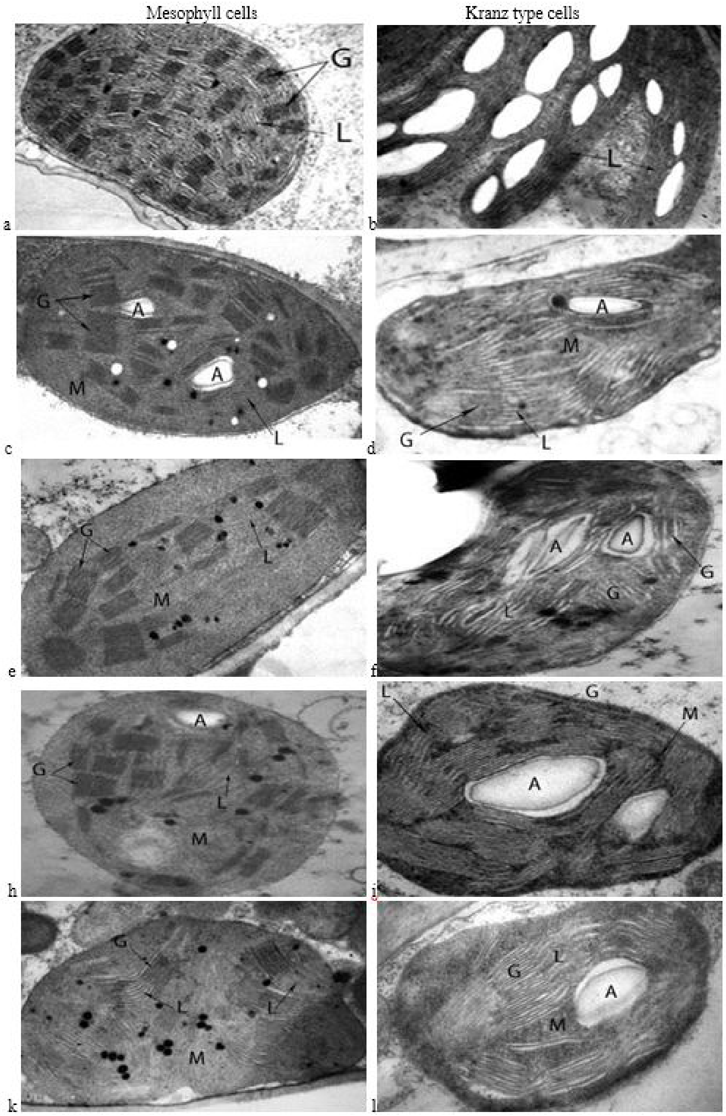
Table 2: Comparative analysis distances between vascular bundles in photosynthetic active organs of C$_3$ and C$_4$ plants (µm).

For the maize leaf we found a relatively small distance between two vascular bundles - 49,4 ± 1,35µm, data corresponding to the literature [30]. For the flag leaf of cereals that parameter is characterized by greater distances, for *Triticale* - 90,7 ± 6,63µm and for *Tr. durum* - 84,9 ± 4,93µm. From ear components, lemma has the largest distance between the vascular bundles (*Triticale* - 115,1 ± 13,6µm, *Tr. durum* - 100,3 ± 14,9µm), similarly to maize tassel. In glume and awn these distances are comparable to those in maize leaf (Table 2).

From anatomical structure point of view the cell surrounding vascular bundles in photosynthetic active components of cereals ear, lacking apparent photorespiration, have similar characteristics to the Kranz cells from maize leaf and tassel (Figure 6).

Chloroplasts Ultrastructure

It is known that C_4 type of photosynthesis is manifested in the two cells types (mesophyll and "Kranz" cells). Two types of chloroplasts and enzymes are localized there: in mesophyll cells - granal chloroplasts and PEPC, in Kranz cells - lamellar chloroplasts and Rubisco. As mentioned above in the photosynthetic active components of the ear (glume, lemma and awn) two types of cells are present: mesophyll cells and cells surrounding vascular bundles, forming a prototype of Kranz crown. This peculiarity imposed the necessity to study the ultrastructure of the chloroplasts from these two types of cells.



Figures 7(a-l): Ultrastructure of chloroplasts from mesophyll and Kranz type cells of C_3 and C_4 plants (second layer of cells - SLC). a) Mesophyll cells of maize leaf; c) cereals flag leaf; e) cereals glume; h) cereals lemma; k) cereals awn. b) Kranz type cells of maize leaf; d) cereals flag leaf; f) cereals glume; i) cereals lemma; l) cereals awn.

In Figure 7 is presented the ultrastructure of the most typical and representative chloroplasts from mesophyll and Kranz cells in maize leaf and cereals flag leaf, ear components. A comparative analysis demonstrates that in glume, lemma and awn of *Tr. durum* and Triticale plants, similar to the maize leaf, two types of chloroplasts in two types of cells are located. The ultrastructure of chloroplasts from mesophyll cells includes a well-organized membrane system of granas and lamellas, similar to chloroplasts from mesophyll cells of maize leaf (Figure 7 a, c, e, h, k). Chloroplasts ultrastructure in Kranz type of cells in glume, lemma and awn (Figure 7b, d, f, i, l) re¬vealed a membrane system more similar to lamellar one, that is characteristic for chloroplasts from Kranz cells in maize leaf and tassel. In Kranz type of cells in ear components it is possible to distinguish two types of chloroplasts: one type with lamel¬lar system of membranes (Figure 7f, l) and second one with more granas and less developed lamellas (Figure 7d, i). The chloroplasts from second layer of cells in the Kranz crown (Figure 6, FLC) are of granal type, with 4-5 granas, but lamel¬las are missing almost completely (Figure 8).

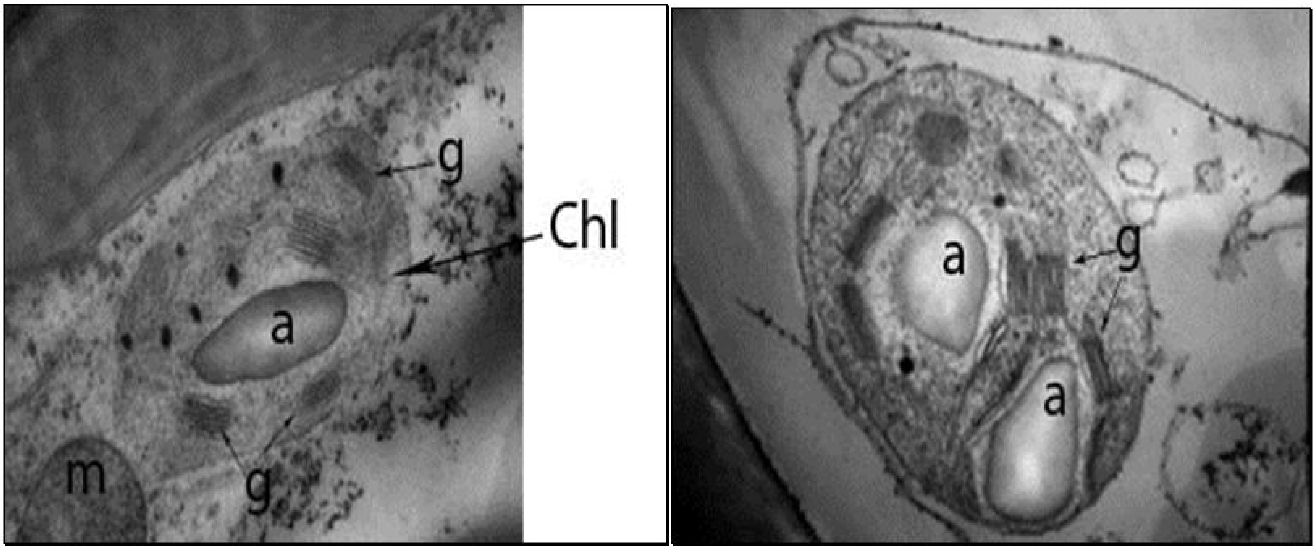


Figure 8: Ultrastructure of chloroplasts from Kranz type cells surrounding the vascular bundles in ear of cereals (first layer of cells - FLC). **Chl** - chloroplast; **m** - mytocondria; **g** - grana; **a** - starch.

Thus, was concluded that in mesophyll and Kranz type of cells from photosynthetic active components of the ear (glume, lemma and awn), similar to maize leaf, two types of chloroplasts are present: one type is granal, typical to mesophyll cells and the second one is lamellar, characteristic to Kranz type of cells. On the other hand, differently from maize leaf, in the Kranz cells of the ear components one more type of chloroplasts was detected - intermediate between granal and lamellar types, with weakly developed membrane system.

Summarizing all obtained results regarding anatomical structure and chloroplasts ultrastructure it is possible to conclude that around vascular bundles (MVB and SVB) of glume and awn (Figure 6 e, f, g, h) are present bigger cells than those from mesophyll cells. These cells form a crown that contains more and bigger chloroplasts (Table 1), arranged on all cell perimeter. Arrangement of these chloroplasts in the Kranz type of cells in the ear components is similar to the C4 plants (*Panicum maximum, Chloris gayana*) in which de-carboxylation of aspartate and phosphoenolpyruvate is done by PEPCK [24]. These types of plants have granal chloroplasts in their Kranz cells. Chloroplasts from Kranz cells of maize leaf (C4 - NADP subtype, where malate and pyruvate are decarboxylase by NADP malate dehydrogenase) have lamellar membrane system. These chloroplasts do not contain granas and are arranged on the perimeter of the cell wall opposite to the vascular bundle [24]. Comparing the literature and our results it is possible to conclude that in Kranz type of cells from the ear components, lacking apparent photorespiration, chloroplasts similar to those from C4 NADP-MDH subtype (lamellar chloroplasts) and PEPCK subtype (granal chloroplasts) are present.

Similar results regarding anatomical structure of Kranz cells and chloroplasts ultrastructure from these cells were described for plants with C3-C4 intermediate metabolism. It is considered that these plants have a decisive role in the understanding of the evolution of C4 plants [16,32-34].

Conclusion

1. The CO_2 exchange kinetics of the ear registers only three components (intensity of apparent photosynthesis, CO_2 assimilation in post illumination phase and respiration) from the total of four, registered in the flag leaf of cereals. The fourth component (apparent photorespiration) is not registered, similar to the tassel and leaf of maize plants. The cereals ear, maize leaf and tassel do not register a compensation point in the open CO_2 measurement system and the CO_2 exchange does not depend of CO_2 concentration in the

air. In the closed measurement system, the ear does register a compensation point, similar to the cereals flag leaf, but much greater. By analogy with the maize leaf, that lack apparent photorespiration but register a compensation point depending on the CO_2 released from the only source - respiration, the compensation point of the ear is probably formed also from the CO_2 released from respiration. Because the ear similar to the maize leaf does not register apparent photorespiration, needs the same amount of time to reach the compensation point and has similar CO_2 exchange kinetics.

2. Comparative analysis of the structure of photosynthetic active components of the ear (glume, lemma, awn), lacking apparent photorespiration, has registered two types of cells, similar to the maize leaf. The one is mesophyll cells and se second one is the cells surrounding the vascular bundles, forming a "Kranz" crown. These Kranz cells have similar structural characteristics with the Kranz cells surrounding the vascular bundles in the maize leaf and tassel, much bigger than the mesophyll cells, containing greater and bigger number of chloroplasts. The distance between the vascular bundles in glume and awn, that characterize their density on a unit area, is twice smaller than in the flag leaf and similar to the distance in maize leaf. In the mesophyll cells and "Kranz" type of cells of ear components (glume, lemma and awn) similar to the maize leaf are located two types of chloroplasts. In the mesophyll cells are located the granal type of chloroplasts, containing well developed system of granas and lamellas. In the "Kranz" type of cells is located the "Granal-agranal" (lamellar) type of chloroplasts: the "granal" type contains well developed granas and weak developed lamellas, but in the "agranal" type the situation is vice-verso.
3. The obtained results indicate that in the ear of cereals function concomitant, but at different level, structural and functional elements of C_3 and C_4 photosynthesis. The CO_2 exchange kinetics is similar to the leaf and tassel of maize plants; lack of apparent photorespiration and presence of the compensation point, formed from the respired CO_2, by analogy with maize leaf; presence of two types of chloroplasts with an intermediate ultrastructure between granal and agranal types.

Funding

This work was supported by Institutional Project 11.817.04.04F, financed by Academy of Science of Republic of Moldova and grant nr. 5386 from Science and Technology Center in Ukraine; also, partially by Visbi Postdoctoral fellowship to DB.

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